UNITED STATES DISTRICT COURT

WESTERN DISTRICT OF WASHINGTON

AT SEATTLE

JESSE VARGISON and Rachael Forbis, individually and on behalf of themselves and all others similarly situated,

Plaintiffs,

v.

PAULA'S CHOICE, LLC,

Defendant.

Case No.

COMPLAINT—CLASS ACTION

JURY DEMAND

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I. INTRODUCTION

1. Consumers should be able to trust a company's promises and statements about its products, and companies must keep their promise of cruelty-free products, ensuring that their team performs no animal testing. If companies do not keep their promise, the law holds companies accountable by recognizing that a company's promises and statements are part of the contract between the parties, and consumers deserve protection if the product fails to meet the promises on the label.

2. Defendant Paula's Choice, LLC ("Paula's Choice") was founded on the principle that "Beauty Begins with Truth." One of the "truths" that Paula's Choice tells its customers is that it is "cruelty-free, always." Since 1995, Paula's Choice has publicized and reinforced this sentiment with its customers, always expressly emphasizing that its skincare products were never tested on animals and Paula's Choice never performs animal testing anywhere in the world.

3. Paula's Choice's promise that its products were never tested on animals can be found on its products themselves, as well as in all its other media, publicity, and public relations materials, including advertisements, websites, marketing campaigns, and interviews. Over the years, Paula's Choice's product labels promise that its skincare products are 100% cruelty-free. Those promises include: "Never Animal Tested," and carry the Leaping Bunny certification logo, which tells consumers the company and product are cruelty-free.

4. Yet, despite claiming Paula's Choice was always cruelty-free and repeating that promise for over 28 years, Paula's Choice prioritized its profits over its principles. Paula's Choice has not honored its promises, allowing animal testing on numerous products just to gain access to one of the world's biggest consumer marketplaces, China.

5. Paula's Choice claimed it was always cruelty-free and through its Leaping Bunny certification, that it did not conduct animal tests anywhere in the world. While portraying itself in the United States as always being cruelty-free, Paula's Choice opted to import and sell its products in China where testing on animals was mandatory for companies like Paula's Choice during the class period. *See infra*.

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6. When a company agrees to perform animal testing to gain access to the Chinese market—while claiming the opposite in advertising, to the public, and on every product that it sells—consumers who purchased products with false representations about the characteristics of the products are harmed.

7. Because the marketplace disdains cosmetic products affiliated with animal testing, the members of the Class were damaged at the point of sale by overpaying for cosmetics that were in fact actually tested on animals despite assurances on the product and its packaging stating otherwise.

8. Consequently, Plaintiffs bring this as a class action on behalf of purchasers of any Paula's Choice skincare products. Plaintiffs claim on behalf of the Class that this conduct breached the express warranties given to Plaintiffs and the Class; violated the implied warranties of merchantability given to Plaintiffs and the Class, violated the Magnuson-Moss Warranty Act, and violated the Washington Consumer Protection Act.

II. PARTIES

9. Plaintiff Jesse Vargison is a resident of Seattle, Washington who purchased Paula's Choice products.

10. Plaintiff Rachael Forbis is a resident of Renton, Washington who purchased Paula's Choice products.

11. Paula's Choice, Inc. was registered on November 30, 1994 as a Washington corporation. Thinking Forward Concepts, LLC was registered on November 15, 2012, as a Washington limited liability company. On November 16, 2012, Paula's Choice, Inc. merged with and into Think Forward Concepts, LLC, and the amended name of the limited liability company became Paula's Choice, LLC. Paula's Choice, LLC's current principal place of business is 700 Sylvan Ave, Englewood Cliffs, NJ 07632-3113. Through at least May 16, 2022, Paula's Choice, LLC's principal place of business was 705 5th Ave. S, Suite 200, Seattle, WA, 98104-4425.

III. JURISDICTION AND VENUE

12. This Court has subject-matter jurisdiction over this action under 28 U.S.C. § 1332(d)(2) because at least one class member is of diverse citizenship from Defendant, there are

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over 100 class members, and upon information and belief, the aggregate amount in controversy exceeds $5,000,000.

13. This Court has personal jurisdiction over Plaintiffs because they are residents of this District, or they submit to this Court's jurisdiction.

14. This Court has personal jurisdiction over Defendant Paula's Choice because it is a Washington limited liability company, has conducted and continues to conduct business in Washington.

15. Venue is proper in this District under 28 U.S.C. § 1391 because the events that gave rise to the claims occurred in substantial part in this District, and Paula's Choice has a choice of venue provision in its Terms of Use selecting King County, Washington.

16. Upon information and belief, before November 2022, Paula's Choice maintained headquarters in the State of Washington.

17. Upon information and belief, Paula's Choice developed, determined, and disseminated its cruelty-free claims at and from its headquarters in Washington.

18. Upon information and belief, all marketing and advertising decisions related to Paula's Choice's cruelty-free claims were made and disseminated from its headquarters in Washington.

19. Upon information and belief, Paula's Choice developed and determined the cruelty-free labels, promises, representations, and logos placed on all its bottles and packaging from its headquarters in Washington.

20. Upon information and belief, all decisions related to Paula's Choice's cruelty-free claims were made at and disseminated from its headquarters in Washington, including its decision to sell products in China, which required animal testing, while simultaneously telling its consumers it was not performing animal testing anywhere in the world.

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IV. FACTS

A. History of Paula's Choice

21. Paula's Choice is a manufacturer of professional skincare products, founded by Paula Begoun ("Begoun").

22. Begoun began working in the beauty industry in the 1970s, working as a makeup artist, esthetician, and opening her own cosmetics store.

23. In 1985, Begoun published her first book, *Blue Eyeshadow Should be Illegal*, which gained Begoun national attention and multiple appearances on the Oprah Winfrey Show.

24. Begoun also began writing a syndicated column where she provided advice on beauty products.

25. In 1992, Begoun wrote *Don't Go to the Cosmetics Counter Without Me*.

26. At the same time, Begoun began working with a team of cosmetic chemists to develop her own skincare products.

27. In 1994, Paula Begoun founded Paula's Choice, Inc. in Washington, which later became Paula's Choice, LLC.

28. In 1995, Paula's Choice began selling skincare products exclusively online.

29. Begoun acted as the face of Paula's Choice, being featured in the advertising for the company.

30. Paula's Choice is currently only sold at PaulasChoice.com or at authorized retailers, which include Sephora (online or in-store), Amazon.com, and Dermstore.com.

31. It is estimated that Paula's Choice has annual revenues of over $300 million per year.

B. Paula's Choice's "Cruelty-free" Promise

32. Since its formation, a key component of Paula's Choice's brand was that Paula's Choice's products were cruelty-free or never tested on animals.

33. "Cruelty-free" is a term used in the skin care industry that means products and the ingredients in those products are not tested on animals.

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34. Throughout its history, Paula's Choice maintained that it was a cruelty-free company and that its products were never animal tested, including these commitments on its products and packaging, on its website, and in its advertising.

35. Paula's Choice includes its representations and promises about being cruelty-free on its products, product labels, and packaging.

36. Paula's Choice products state on the bottles and packaging that they are "NEVER ANIMAL TESTED."

37. Since at least 2013, Paula's Choice products also show that they are Leaping Bunny Certified, displaying the following symbol on the bottle:

38. To become Leaping Bunny Certified a company must promise that it does not and will not conduct any animal testing, including on the formulations and ingredients in those products, anywhere in the world.[1]

39. Leaping Bunny certification also requires the company to promise that it "shall not allow Animal Testing to be performed by or for submission to regulatory agencies in foreign countries."[2]

[1] The Corporate Standard of Compassion for Animals ("The Standard"), LEAPING BUNNY PROGRAM, https://www.leapingbunny.org/about/corporate-standard-compassion-animals-standard (last visited Mar. 12, 2024).

[2] *Id.*

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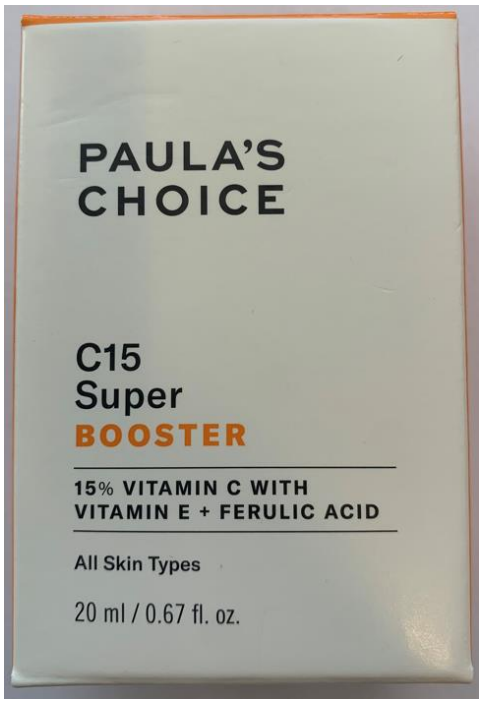
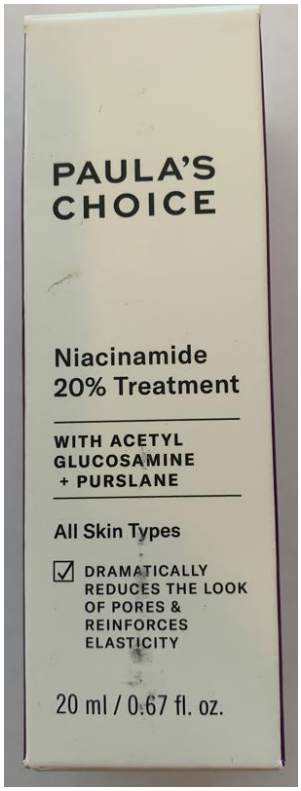
40. Some examples of those labels on the bottles and packaging include:





41. Paula's Choice bottles also state that the product was Manufactured in the USA for Paula's Choice, LLC.

42. Upon information and belief, the decision to put Paula's Choice's cruelty-free claims on all its bottles and packaging was made from its headquarters in Washington.

43. Over the years, Paula's Choice has represented in various ways on its website that it is a cruelty-free company that does not perform animal testing on its products.

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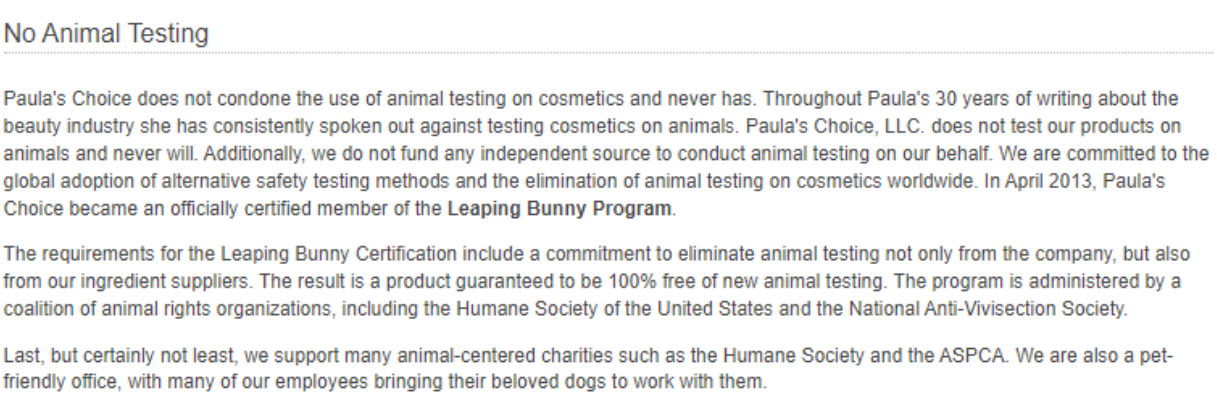
44. In 2013, Paula's Choice represented on its website:[3]



No Animal Testing

Paula's Choice does not condone the use of animal testing on cosmetics and never has. Throughout Paula's 30 years of writing about the beauty industry she has consistently spoken out against testing cosmetics on animals. Paula's Choice, LLC. does not test our products on animals and never will. Additionally, we do not fund any independent source to conduct animal testing on our behalf. We are committed to the global adoption of alternative safety testing methods and the elimination of animal testing on cosmetics worldwide. In April 2013, Paula's Choice became an officially certified member of the **Leaping Bunny Program**.

The requirements for the Leaping Bunny Certification include a commitment to eliminate animal testing not only from the company, but also from our ingredient suppliers. The result is a product guaranteed to be 100% free of new animal testing. The program is administered by a coalition of animal rights organizations, including the Humane Society of the United States and the National Anti-Vivisection Society.

Last, but certainly not least, we support many animal-centered charities such as the Humane Society and the ASPCA. We are also a pet-friendly office, with many of our employees bringing their beloved dogs to work with them.

45. Paula's Choice made the same commitment on its website in 2015 and 2016:[4]

NO ANIMAL TESTING

Animal testing is not okay with us. We're part of the Leaping Bunny Program, which means not only have we eliminated animal testing from our company, but also from our ingredient suppliers. We love animals so much our employees bring their dogs to work. Keeps things real.

46. In 2014, Paula's Choice's website advertised "NO ANIMAL TESTING":[5]

[3] About Paula's Choice, PAULA'S CHOICE, https://web.archive.org/web/20130501172337/http://www.paulaschoice.com/who-we-are/about-paulas-choice/?= (last visited Mar. 12, 2024).

[4] Our Story, PAULA'S CHOICE, https://web.archive.org/web/20150621211049/http://www.paulaschoice.com/who-we-are/about-paulas-choice and https://web.archive.org/web/20160730004741/http://www.paulaschoice.com/who-we-are/about-paulas-choice/ (last visited Mar. 12, 2024).

[5] Who We Are, PAULA'S CHOICE, https://web.archive.org/web/20140628110642/http:/www.paulaschoice.com/who-we-are/ (last visited Mar. 12, 2024).

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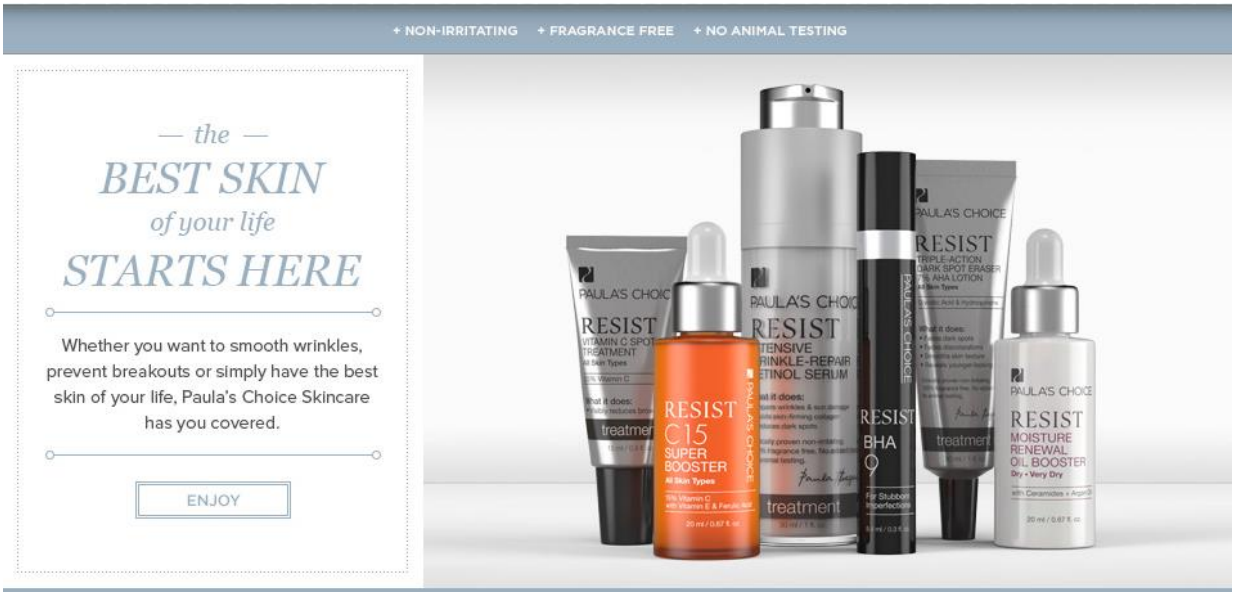
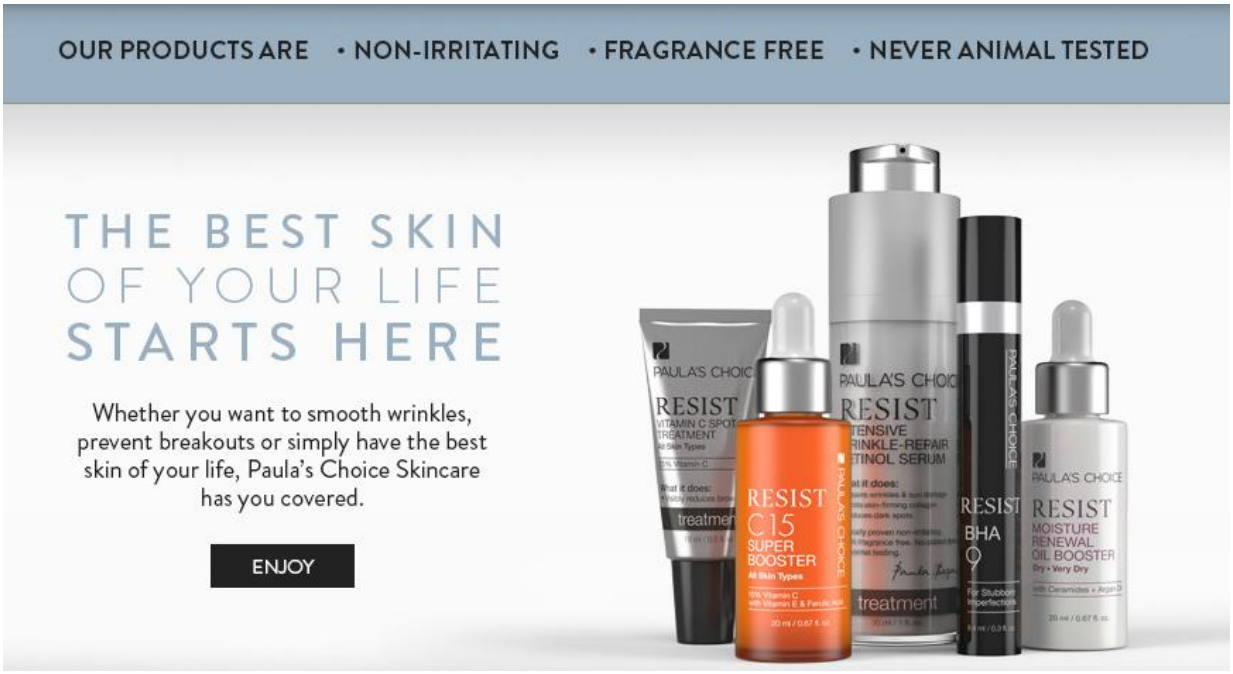
47. Paula's Choice continued to advertise that it performed no animal testing in 2015 and 2016:[6]





[6] Who We Are, PAULA'S CHOICE, https://web.archive.org/web/20150623020742/http://www.paulaschoice.com/who-we-are and https://web.archive.org/web/20160730010946/http://www.paulaschoice.com/who-we-are/ (last visited Mar. 12, 2024).

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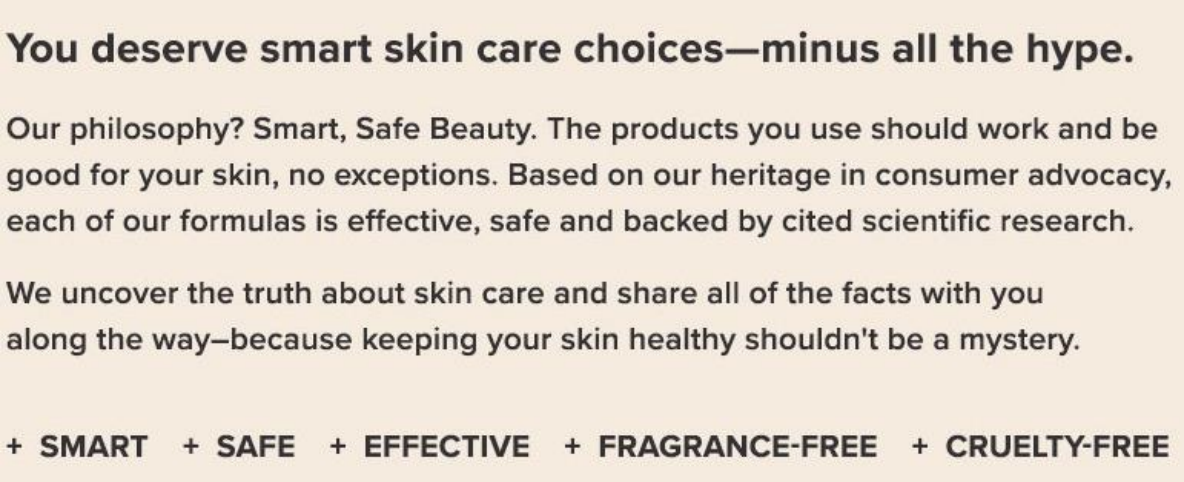
48. Paula's Choice's website in 2019–2022 advertised under its "BEAUTY BEGINS WITH TRUTH" banner that it was "cruelty-free":[7]



49. In 2019–2022, Paula's Choice also represented on its website that it was "Cruelty-Free Always."[8]

[7] About Us, PAULA'S CHOICE, https://web.archive.org/web/20191218102216/https://www.paulaschoice.com/who-we-are/about-us, https://web.archive.org/web/20200815010527/https://www.paulaschoice.com/who-we-are/about-us, https://web.archive.org/web/20211030202527/https://www.paulaschoice.com/who-we-are/about-us, and https://web.archive.org/web/20220409210946/https://www.paulaschoice.com/who-we-are/about-us (last visited Mar. 12, 2024).

[8] About Us, PAULA'S CHOICE, https://web.archive.org/web/20191120232457/https://www.paulaschoice.com/who-we-are/about-us, https://web.archive.org/web/20200815010527/https://www.paulaschoice.com/who-we-are/about-us, https://web.archive.org/web/20211105165129/https://www.paulaschoice.com/who-we-are/about-us, https://web.archive.org/web/20220409210946/https://www.paulaschoice.com/who-we-are/about-us (last visited Mar. 12, 2024).

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50. Today, Paula's Choice continues to promote its cruelty-free stance.

51. Paula's Choice currently represents on its website that it has never tested on animals:[9]

ANIMAL TESTING AND BY-PRODUCTS

Do you test on animals?

No. Paula's Choice has never tested on animals at any stage of product development and never will. We also do not contract with any third parties to conduct animal testing on our behalf. We're part of the Leaping Bunny Program, which means not only have we eliminated animal testing from our company, but also from our ingredient suppliers.

Paula's Choice supports many charities focused on animal welfare such as the Humane Society, ASPCA and NAVS. Paula's Choice is a dog-friendly office, with most of our employees bringing their canine companions to work each day.

52. Upon information and belief, Paula's Choice's claims about animal testing were developed and issued from its headquarters in Washington.

53. Paula's Choice's official Instagram account promotes that it is cruelty-free.[10]

[9] Animal Testing and By-Products, PAULA'S CHOICE, https://help.paulaschoice.com/hc/en-us/articles/360035072173-Animal-Testing-and-By-Products (last visited Mar. 12, 2024).

[10] Paula's Choice (@paulaschoice), INSTAGRAM, https://www.instagram.com/paulaschoice/ (last visited Mar. 12, 2024).

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54. In a recent Instagram post, Paula's Choice reiterated that its products were not tested on animals:

55. That Instagram post included the following caption:[11]

[11] Paula's Choice (@paulaschoice), INSTAGRAM (September 30, 2023), https://www.instagram.com/p/Cx0cLeZNtV8/ (last visited Mar. 12, 2024).

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56. In a similar post, Paula's Choice reiterated it was cruelty-free:

57. The post was captioned:

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58. A similar post appeared on Paula's Choice's Facebook page.[12]

59. Upon information and belief, the representations and content of Paula's Choice's website and social media pages were developed and issued from its headquarters in Washington.

60. People for the Ethical Treatment of Animal's ("PETA") has a certification program called "Beauty Without Bunnies," which requires companies to sign a statement of assurance verifying it does not test on animals anywhere in the world.

61. Paula's Choice appears on PETA's list of companies that does not test on animals anywhere in the world, including China.[13]

[12] Paula's Choice Post (@PaulasChoice.Inc), FACEBOOK (September 24, 2023), https://www.facebook.com/reel/853079449637680 (last visited Mar. 12, 2024).

[13] Paula's Choice (Unilever), PETA, https://crueltyfree.peta.org/company/paulas-choice/ (last visited Mar. 12, 2024).

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62. On April 1, 2021, Paula's Choice and Paula Begoun were featured as a cruelty-free, women-owned brand on PETA's website.[14]

C. Animal Testing in the Cosmetic Industry

63. Animal testing has been used in the cosmetics industry in the past to ensure that products were safe for consumers to use.

64. Testing performed on animals in the cosmetics industry include various toxicity and irritancy tests.

65. Acute toxicity tests are used to determine the danger of exposure to a chemical by mouth, skin, or inhalation and is usually performed on mice or rats.[15]

66. LD50, also known as Lethal Dose 50, is a type of acute toxicity test where animals are dosed with a test chemical to determine the dose at which half of the test animals die.[16]

67. Fixed dose method is another type of acute toxicity test, but it does not use death at the endpoint. The testing will be stopped when the animal demonstrates signs of ailment or distress.[17]

68. Other acute toxicity tests include the up-and-down procedure and acute toxic class methods. While these tests do not result in the death of the animal, the animals will often endure intense pain, convulsions, loss of motor function, and seizures.[18]

69. The animals are killed when all the testing is complete so a necropsy can be performed to determine internal damage.[19]

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[14] Rebecca Maness, *These Women are Leading the Charge for Cruelty-Free Products*, PETA, https://www.peta.org/living/personal-care-fashion/animal-test-free-women-owned-beauty-brands/ (last visited Mar. 12, 2024).

[15] Animals in Science, AMERICAN ANTI-VIVISECTION SOCIETY, https://aavs.org/animals-science/how-animals-are-used/testing/ (last visited Mar. 12, 2024); Earnest Oghenesuvwe Erhirhie, Chibueze Peter Ihekwereme, & Emmanuel Emeka Ilodigwe, *Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance*, Interdisciplinary Toxicology, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6117820/ (last visited Mar. 12, 2024).

[16] *Id.*

[17] *Id.*

[18] *Id.*

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70. The Draize test is a test devised in 1944 by John H. Draize and Jacob M. Spines, toxicologists at the FDA, to assess how chemicals cause eye and skin irritation. The Draize test is generally performed on rabbits, particularly albino rabbits, although the testing can be performed on other animals as well.[20]

71. During the testing a chemical is placed in the eye or on the skin of a conscious and restrained animal, left on for a set amount of time, rinsed off, and its effects then recorded.[21]

72. The animals are then observed for up to fourteen days looking for signs of erythema and edema in the skin or redness, swelling, discharge, ulceration, hemorrhaging, cloudiness, or blindness in the tested eye.[22]

73. The animals are killed after the testing if the test causes irreversible damage to the eye or skin. If the test does not cause permanent damage, the animas are typically used again once all traces of the tested product have dispersed from the testing site.[23]

74. Skin sensitization tests are used to determine if a chemical causes an allergic reaction.

75. One type of skin sensitization test is the Guinea Pig Maximization Test, where a chemical is injected into the guinea pig, along with a chemical adjuvant to boost the immune reaction. Multiple doses are given until the animal develops an allergic reaction.[24]

76. Another skin sensitization test is the Buehler test, which is similar to the Guinea Pig Maximization Test, but no adjuvant is used to boost the immune reaction.[25]

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[19] *Id.*

[20] Draize test, WIKIPEDIA, https://en.wikipedia.org/wiki/Draize_test (last visited Mar. 12, 2024).

[21] *Id.*

[22] *Id.*

[23] *Id.*

[24] Animals in Science, AMERICAN ANTI-VIVISECTION SOCIETY, https://aavs.org/animals-science/how-animals-are-used/testing/ (last visited Mar. 12, 2024).

[25] *Id.*

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77. In both the Buehler test and the Guinea Pig Maximization test the animals are killed after testing.[26]

78. A more recent and commonly used skin sensitization test is the Local Lymph Node Assay, where test chemicals are applied to the surface of the ears of mice. The mice are then killed and then their lymph node cells are removed and analyzed.[27]

D. Consumers' Attitudes Toward Animal Testing

79. Consumers around the world have called for the end of animal testing for cosmetics. Too many consumers are opposed to it.[28]

80. Most U.S. consumers prefer cosmetic products that have not been tested on animals.

81. In a recent poll from 2019, 79% of Americans support a federal law that would end animal testing on cosmetics.[29]

E. Regulations on Animal Testing for Cosmetics in the U.S. and Globally

82. The FDA has the authority to regulate cosmetics under the Federal Food, Drug, and Cosmetic Act ("FD&C Act"), related statutes, and regulations promulgated under the FD&C Act.[30]

83. "The FD&C Act does not specifically require the use of animals in testing cosmetics for safety, nor does the Act subject cosmetics to FDA premarket approval."[31]

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[26] *Id.*

[27] *Id.*; Local lymph node assay, WIKIPEDIA, https://en.wikipedia.org/wiki/Local_lymph_node_assay (last visited Mar. 12, 2024).

[28] Kerry Postlewhite, *'Brands can no longer ignore the 8.3 million people who want end to animal testing'*, REUTERS EVENTS, https://www.reutersevents.com/sustainability/brands-can-no-longer-ignore-83-million-people-who-want-end-animal-testing (last visited Mar. 12, 2024).

[29] New Poll Reveals US United Against Cosmetics Animal Tests, CRUELTY FREE INTERNATIONAL, https://crueltyfreeinternational.org/latest-news-and-updates/new-poll-reveals-us-united-against-cosmetics-animal-tests (last visited Mar. 12, 2024).

[30] Animal Testing & Cosmetics, U.S. FOOD & DRUG ADMINISTRATION, https://www.fda.gov/cosmetics/product-testing-cosmetics/animal-testing-cosmetics (last visited Mar. 12, 2024).

[31] *Id.*

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84. The FDA goes beyond not requiring animal testing, also suggesting that "consideration should be given to the use of scientifically valid alternative methods to whole-animal testing."[32]

85. Because the practice of animal testing is found to be objectionable and cruel by so many, several states and other countries have gone further and banned the practice.

86. In 1988, the United Kingdom was the first country to ban animal testing on cosmetics.

87. The European Union also banned animal testing on cosmetics in a phased approach that was completed in 2013.

88. Other countries including Israel, India, Turkey, Brazil, New Zealand, and Norway also have bans on animal testing.

89. Eleven states have passed laws that ban or limit the sale of cosmetic products tested on animals, including Virginia (Va. Code Ann. § 59.1-572), California (Cal. Civ. Code § 1834.9.2), Louisiana (La. Stat. Ann. § 51:772), New Jersey (N.J. Stat. Ann. § 4:22-59), Maine (Me. Rev. Stat. tit. 10, § 1500-M), Hawaii (Haw. Rev. Stat. Ann. § 321-30.4), Nevada (Nev. Rev. Stat. Ann. § 598.993), Illinois (410 Ill. Comp. Stat. Ann. 620/17.2), Maryland (Md. Code Ann., Health-Gen. § 21-259.30), New York (N.Y. Gen. Bus. Law § 399-AAAAA), and Oregon (Or. Rev. Stat. Ann. § 646A.009).

90. The legislative history of some of these laws reveals public support for banning animal testing.

91. Nev. Rev. Stat. Ann. § 598.993 prohibits the sale of cosmetics that have been tested on animals on or after January 1, 2020.

92. The legislative history of Nev. Rev. Stat. Ann. § 598.993 shows that the motivations for the bill were, in part, to meet the demand of and protect consumers: "Consumers ***overwhelmingly*** are starting to reject products tested on animals. Statistically, businesses that have

[32] *Id.*

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eliminated their animal testing policies have been successful and profitable. On an anecdotal level, I can tell you that I have had dozens of people come to me since I introduced this bill and say, I only use products that are not tested on animals; I always check the label; and it is so hard to know if a product has been tested on animals." Nevada Assembly Committee Minutes, 5/15/2019 (emphasis added).

93. Cal. Civ. Code § 1834.9.5, prohibits manufacturers from selling cosmetics in California if the cosmetic was tested on animals on or after January 1, 2020.

94. The legislative history of Cal. Civ. Code § 1834.9.2 (S.B. 1249) shows that over 4,000 individuals contacted the legislature to voice support of the bill. California Bill Analysis, S.B. 1249 Sen., 8/28/2018. In contrast only four entities (and no individuals) voiced opposition to the bill. *Id.*

95. The bill analysis also provided: "The bill has received an ***intense groundswell of support from concerned citizens, animal welfare groups, and many companies in the cosmetic industry*** that are strongly committed to a vision of a truly "cruelty-free standard" for cosmetic products sold in California. The bill is also supported by a coalition of approximately 80 cosmetic companies who attest that they are proof that a company can be profitable but also committed to manufacturing products without any reliance on animal testing whatsoever." California Bill Analysis, S.B. 1249 Assem., 6/26/2018 (emphasis added).

96. That analysis further noted: "The Committee has received over 6,500 letters in support of the bill from individuals providing a California address, and has taken note of an online petition signed by more than 150,000 persons from around the world, voicing support for this bill." California Bill Analysis, S.B. 1249 Assem., 6/26/2018.

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F. Beauty Industry in China

97. According to one industry report, as of 2020, the Chinese cosmetics market is the second largest in the world after the United States, which includes hair care, skin care, and other toiletries.[33]

98. China has an increasing demand for "higher quality, premium brand products."[34]

99. More than half of Chinese cosmetics consumers prefer foreign brands over local ones.[35]

100. The market size of cosmetics in China was more than 455 billion yuan in 2021, equivalent to over $63 billion USD at the current exchange rate.[36]

101. According to the United States Department of Commerce in 2016, China is the 10th largest market for U.S. personal care and cosmetics exports" and "China is expected to become the largest market for personal care and cosmetics products globally in the next five to ten years." Excerpts from the Asia Personal Care & Cosmetics Market Guide, 2016, United States Department of Commerce, International Trade Administration, Exhibit 1 at 10.

102. Until 2021 it was mandatory for foreign manufacturers and distributors who wanted to sell products in China to obtain a specific approval issued by the National Medical Products Administration ("NMPA") (formerly the China Food and Drug Administration ("CFDA")).

103. Skincare products, such as moisturizers, cleansers, and toners, are classified under Chinese law as ordinary or "non-special use cosmetics."

104. Skincare products containing sunscreen are classified under Chinese law as "special use" cosmetics.

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[33] Cosmetics market size in China from 2015 to 2023 with forecasts until 2025, STATISTA, https://www.statista.com/statistics/875794/china-cosmetics-market-size/ (last visited Mar. 12, 2024).

[34] *Id.*

[35] *Id.*

[36] *Id.*; Chinese Yuan to United States Dollar, GOOGLE FINANCE, https://www.google.com/finance/quote/CNY-USD?hl=en (last visited Mar. 12, 2024).

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105. Starting in 1990, the NMPA required all imported special and non-special use cosmetics to be tested on animals in Chinese designated and certified laboratories before they could be approved for importation and distribution in the Chinese market.

106. From 1990 to the present, all foreign-produced special use cosmetics need to be registered with and approved by the NMPA before they could be imported and sold in China.

107. From 1990 to November 7, 2018, all foreign-produced non-special use cosmetics needed to be registered with and approved by the NMPA before they could be imported and sold in China.

108. After November 7, 2018, non-special use cosmetics only require a premarket registration and can be imported after registration has been completed. That premarket registration requires the same animal testing as the earlier registrations but changes the timing for when a product can be imported into China.

109. To receive NMPA registration on foreign-produced special or non-special use cosmetics, a company must appoint and register a domestic responsible agent in China.

110. The domestic responsible agent must file an application with the NMPA on behalf of the company that includes an examination and testing report issued by an NMPA-designated examination and testing institution.

111. All NMPA-designated testing institutions are in China.

112. This means the domestic responsible agent must hire a laboratory in China to perform the required testing. These laboratories are designated and certified by the Chinese government.

113. The examination and testing report is governed by Chinese specific regulations, with standards issued in 2002, updated in 2007, and updated again in 2015.

114. From 2007 to 2014, China's Hygienic Standards for Cosmetics (2007) dictated the required examination and testing report. A translation of those standards is attached as Exhibit 2.

115. Those standards provide that the examination and testing report include multiple skin irritation tests for cosmetics used daily, acute skin irritation tests for cosmetics rinsed after use, and acute eye irritation tests for products that may come into contact with eyes.

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116. The specifications for those tests are described in detail in the Hygienic Standards for Cosmetics.

117. The acute skin irritation test includes applying the test substance to the shaved skin of the animal, leaving the product on for 2 hours or longer, and then observing skin reactions at 1, 24, 48, and 72 hours after the product is removed. Ex. 2, Part II at 107.

118. The multiple skin irritation test includes the same procedure as the acute skin irritation test, but the product is applied to the animal every day for 14 days, shaving the animal's skin before each application. Ex. 2, Part II at 107.

119. The 2007 Standards note regarding the skin irritation tests: "Animals should be humanely executed if they show signs of severe depression and distress at any stage of the test." Ex. 2, Part II at 105.

120. The acute eye irritation test involves applying the test substance in the conjunctival sac of one eye of the animal and not rinsed for at least 24 hours, but the substance is only rinsed if deemed necessary. The eyes are examined at 1, 24, 48, and 72 hours after the substance is applied. If no irritation is found, the test is terminated. If irritation is found, the test continues, and the eyes of the animals are examined again at 4 and 7 days. Ex. 2, Part II at 113–14.

121. The 2007 Standards note regarding the acute eye irrigation test: "Animals that show signs of severe depression and distress at any stage of the test should be humanely put to death and the subject evaluated appropriately in the light of the test. Animals that show corneal perforation, corneal ulceration, corneal 4 points for more than 48h, lack of light reflex for more than 72h, conjunctival ulceration, gangrene and decay, which are usually signs of irreversible damage, should also be humanely executed." Ex. 2, Part II at 111.

122. Since 2015, the examination and testing report is governed by the Safety and Technical Standards for Cosmetics (2015). A translation of those standards is attached as Exhibit 3.

123. Those standards provide that the examination and testing report must include the acute dermal irritation test and the acute eye irritation test.

124. The specifications for those tests are similar to the 2007 tests and are described in detail in the Safety and Technical Standards for Cosmetics, but they include placing the product to

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be tested in the eye of the animal or on the shaved skin of the animal, leaving that product in the eye or on the skin, and observing its effects at 1, 24, 48, and 72 hours after application. Ex. 3 at 20–35.

125. Those regulations also provide: "If animals show severe depression and pain at any stage of the trial, they should be executed humanely." Ex. 3 at 22, 28.

126. If a product is deemed special use because it contains UV protection, a skin phototoxicity test must be performed, which requires shaving off four patches of hair from a white rabbit or albino guinea pig, as shown in the following diagram (Ex. 2. Part II at 125):

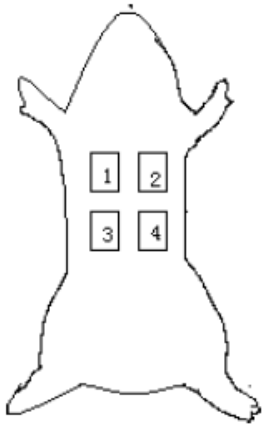

Figure 1 Schematic diagram of the location of the skin debridement area of the animal

127. The UV product is then applied to patches 1 and 2, with patches 3 and 4 left bare. Aluminum foil is then taped to the animal to cover patches 1 and 3, while patches 2 and 4 are irradiated with UV light. Ex. 2, Part II at 123–26.

128. The animal's skin reactions are then observed and scored at 1 hour, 24 hours, 48 hours, and 72 hours after UV exposure. *Id.*, Part II at 125.

129. This test is identical in the 2007 and 2015 Standards. Ex. 3 at 44–49.

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130. The process to import products into China is also described by the United States Department of Commerce in its "Asia Personal Care & Cosmetics Market Guide, 2016" (Ex. 1 at 16), which states:

> Cosmetics in China are categorized as ordinary and special use cosmetics. Perfume, skin care, shampoo and color cosmetics fall into ordinary products and special use products refer to hair dye, hair perm, hair-growing, sunblock, anti-spot, slimming, breast-beautifying, depilatories and deodorant etc.
>
> According to the CFDA (China Food and Drug Administration), all foreign cosmetic product manufacturers must complete a safety and health quality test, and obtain a hygiene permit before they are allowed to sell in the Chinese market. Application for this pre-market approval process can only be carried out by a Chinese legal entity. Overseas cosmetics manufacturers without legal representation in China are thus required to apply for the permit through agent services. The Manufacture [sic] needs to sign a "Letter of Authorization" confirming that it authorizes a Chinese company to be the registration responsible party in mainland China for the products.

131. The Department of Commerce also describes the required testing and the application procedures (*Id.* at 16–18):

> **Safety and Health Quality Test**
> This test is performed by designated laboratories appointed by the CFDA and are listed on the CFDA website. All these labs have different testing capabilities designated for testing against specific conditions, such as microbiology, hygienic chemistry, toxicology test (which includes animal testing) or conducting safe-for–human-use trials (for special use cosmetics). The test normally takes 2-3 months for ordinary cosmetics and 3-8 months for special-use cosmetics, while costs vary from $700 to $6,000 depending on the types and complexity of the products.
> . . .
> **Hygiene Permit for Imported Cosmetics**
> Once testing is completed, the designated laboratory will issue a test report which needs to be submitted together with the other required documents for the application of the Hygiene Permit from CFDA. A committee under CFDA convenes to technical review and evaluate of [sic] imported cosmetics. The technical review time will be 3 months generally. If one application has been approved, a certificate will be issued by the CFDA. Companies

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need to submit the following documents (all translated in Chinese and notarized by a Chinese notarization company):

Application form for the cosmetic product to be imported

- Chinese product name and nomenclature;
- Product formula;
- Product quality and safety control file (The product info such as appearance, flavor, batch no and shelf life is required. Other quality control index like heavy metals and microbiology should be provided as well);
- Original product packaging including labelling information and product information sheet;
- Testing report and relevant data from testing organization certified by CFDA;
- Safety assessment report of cosmetics containing potential risk substances;
- Stamped copies of power of attorney and business license of Chinese responsible agent;
- Statement from manufacturer guaranteeing that materials used meet the requirements of BSE free regions.
- Free Sale Certificate at production country (region) or country (region) of origin
- Brief description and diagram of production process
- Technical requirements for cosmetic products in text
- Other relevant information which can support the application

The applicant will be notified by the CFDA within 5 days confirming whether the application is accepted or not. If the application is not accepted, the CFDA will provide explanation of discrepancies or missing documentation allowing the application to be resubmitted.

The Hygiene Certificate is valid for 4 years, and foreign manufacturers are required to renew it at least 4 months before it is expired.

132. Foreign-produced cosmetics also must be imported through China in accordance with its customs regulations.

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133. Through 2021, a company would have had to provide a copy of the NMPA registration when going through customs, along with other information required under Chinese law.[37]

134. An imported cosmetic must include a product label, which must list the manufacturer, the domestic responsible agent, and the NMPA registration number.

135. Up until 2018, a registration for non-special use cosmetics with the NMPA lasted four years, meaning that every foreign-produced cosmetic sold in China would have to be registered every four years and undergo animal testing every four years.

136. After 2018, non-special use product registrations do not expire, but starting on January 1, 2022, the registrant must provide an annual report to the NMPA. If an annual report is not filed, the NMPA may cancel the registration.

137. Registrations for special-use cosmetics only last for four years, meaning that every foreign-produced special use cosmetic sold in China would have to be registered every four years and undergo animal testing every four years.

138. In addition to the pre-market animal testing, all foreign-produced special and non-special use cosmetics can be subjected to post-market safety testing by Chinese authorities. This post-market testing includes animal testing.

139. On February 26, 2021, the NMPA promulgated the Administrative Provision for Cosmetics Registration and Filing Documents ("2021 Provision"), which allows foreign manufacturers of non-special use cosmetics to receive an exemption from animal testing. Starting on May 1, 2021, as a guarantee of safety, the NMPA can accept a specified certification and product safety assessment from the country of manufacture, instead of requiring animal testing.

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[37] These requirements are found in the Measures for the Supervision and Administration of Inspection and Quarantine of Imported and Exported Cosmetics (formerly General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China) (Order No. 143, revised according to Orders No. 238, No. 240, and No. 243 of the General Administration of Customs), the Announcement from General Administration of Customs (Announcement No. 99 of 2020), and Announcement on Adjusting the Supervision Requirements for Some Imported and Exported Goods (Announcement No. 99 of 2020).

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140. This exemption does not apply to special use cosmetics.

141. Since the 2021 Provision came into effect, several foreign manufacturers, including at least one based in California, have been granted approvals without requiring animal testing.

142. Since 2014, a cosmetic company can also get an exemption from animal testing by setting up or using domestic manufacturing facilities in China.

143. This exemption does not apply to special use cosmetics.

144. Domestic manufacturers of non-special use cosmetics can receive an exemption from animal testing, although they can still be subject to post-market testing by Chinese authorities. This post-market testing includes animal testing.

G. Paula's Choice and the Chinese Market

145. Paula's Choice began selling skincare products in China in 2009.

146. Paula's Choice registered at least sixty products, including one special use cosmetic with sunscreen, with the NMPA in China from 2009 to 2020. A list of Paula's Choice's NMPA registrations is attached as Exhibit 4.

147. Each of those product registrations would have required a testing report, meaning that Paula's Choice selected and retained a Chinese laboratory to perform animal testing.

148. The NMPA maintains a database, listing each of the registrations.

149. Each product received an NMPA registration number, meaning Paula's Choice's domestic responsible agents submitted an application for each product that included the examination and testing report as outlined in the Hygienic Standards for Cosmetics (2007) and Safety and Technical Standards for Cosmetics (2015).

150. Each examination and testing report required Paula's Choice's products (all 60 of them) to be tested on animals by the respective Chinese lab selected during the registration process.

151. Each product also lists Paula's Choice or its agent as the manufacturer of the product.

152. Paula's Choice also had to provide a copy of the NMPA registration or application when going through customs, along with other information required under Chinese law. *See supra*.

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153. Paula's Choice could not have imported foreign cosmetics into China, or gone through Chinese customs, without an NMPA registration, each of which would require retaining a certified laboratory to do animal testing for that product.

154. At least by 2009, Paula's Choice engaged Shanghai Yingwen Economic and Trade Co., Ltd. ("Shanghai Yingwen") as its distributor in China.

155. Shanghai Yingwen was established by Ying Yang ("Yang") on August 18, 2006.

156. Shanghai Yingwen is wholly owned by Yang.

157. Shanghai Yingwen continued to act as Paula's Choice's domestic responsible agent in China through 2020.

158. Each of the sixty of Paula's Choice's NMPA registrations from 2009 to 2020 lists Shanghai Yingwen as the registered agent. Paula's Choice's NMPA registrations, Ex. 4.

159. Shanghai Yingwen established and maintained the Paula's Choice China website www.paulaschoice.com.cn.

160. While the Paula's Choice China website is no longer active, it was active and selling products in China from at least December 19, 2009,[38] to March 22, 2012.[39]

161. In addition to selling products on the Paula's Choice China website, Paula's Choice sold in China through Amazon China, Paula's Choice Tmall site, and Little Red Book.

162. In 2010, Paula Begoun travelled to China to promote Paula's Choice's entry into China.

163. In 2010, Paula's Choice posted on its Facebook page: "Looks like I'll be going to China in September for media interviews. Paula's Choice is available in China at www.PaulasChoice.com.cn. –Paula."[40]

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[38] Homepage, PAULA'S CHOICE CHINA, https://web.archive.org/web/20091219102503/http://www.paulaschoice.com.cn/ (last visited Mar. 12, 2024).

[39] Homepage, PAULA'S CHOICE CHINA, https://web.archive.org/web/20120322162205/http://www.paulaschoice.com.cn:80/ (last visited Mar. 12, 2024).

[40] Paula's Choice Post, FACEBOOK (June 8, 2010), https://www.facebook.com/PaulasChoice.Inc/posts/looks-like-ill-be-going-to-china-in-september-for-media-interviews-paulas-choice/117169221660237/ (last visited Mar. 12, 2024).

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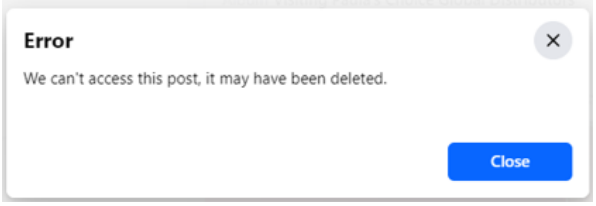
164. On August 10, 2010, Paula's Choice posted on its Facebook page that Begoun would be visiting Paula's Choice's distributor in China:

165. Upon information and belief, that post has since been removed from the Paula's Choice Facebook page.

166. On September 20, 2010, Paula's Choice's Facebook page posted that Begoun was visiting with its global distributors, attaching photos from her trip to China:[41]

[41] The post itself is visible on Paula's Choice's Facebook page, but the URL could not be accessed, as clicking on it results in a message which states: "**Error** We can't access this post, it may have been deleted."



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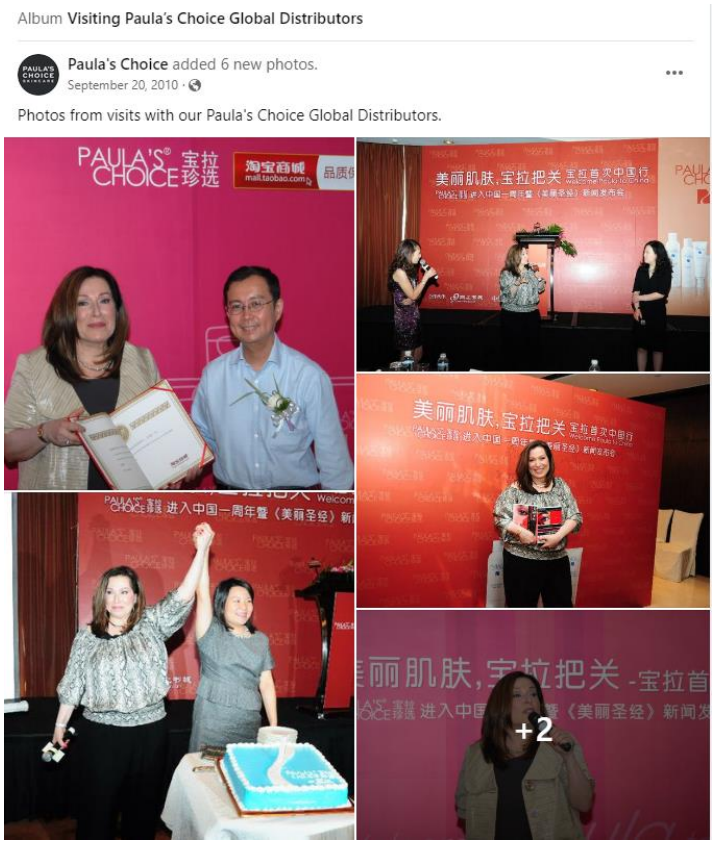

167. In one photo, Begoun is photographed with Yang, with the caption: "Celebrating Paula's Choice in China.[42]

[42] Paula's Choice's Post, FACEBOOK (September 20, 2010), https://www.facebook.com/photo?fbid=439433579003&set=ms.c.eJwzMbY0MTY2MTMxMDDWMwFzTM0tkTgWyDIWSDJmyMrMwMoAXfsPhw~-~-.bps.a.405023569003 (last visited Mar. 12, 2024).

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168. Begoun was also photographed with the CFO of Taobao, Daniel Zhang:[43]

169. Taobao owns Tmall (formerly Taobao Mall), the leading local Chinese E-commerce platform where Paula's Choice products are sold.

170. From 2009 to 2011, Paula's Choice registered five products with the NMPA, each product requiring animal testing. Paula's Choice's NMPA registrations, Ex. 4.

171. In 2012, PETA began exposing multiple cosmetic companies that claimed to be cruelty-free but had been selling products in China and undergoing animal testing.[44]

172. PETA also began removing those companies from its Beauties Without Bunnies certification program if they did not stop selling in China.[45]

173. In 2012, Paula's Choice shut down its Chinese website and instead directed consumers to its Hong Kong website.

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[43] Paula's Choice's Post, FACEBOOK (September 20, 2010), https://www.facebook.com/photo?fbid=439433464003&set=ms.c.eJwzMbY0MTY2MTMxMDDWMwFzTM0tkTgWyDIWSDJmyMrMwMoAXfsPhw~-~-.bps.a.405023569003 (last visited Mar. 12, 2024).

[44] *See* Michelle Reynolds, *Avon, Mary Kay, Estée Lauder Resume Animal Tests*, Feb. 16, 2012, PETA, https://www.peta.org/blog/3-companies-booted-cruelty-free-list/ (last visited Mar. 12, 2024).

[45] *Id.*

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174. The Hong Kong website remained nearly identical to the Chinese website.[46]

175. Upon information and belief, Paula's Choice removed its Chinese website to make it appear that it was no longer doing business in China and only doing business in Hong Kong.

176. On February 20, 2013, PETA reported that Paula's Choice was one of the companies that was no longer going to sell in China.[47]

177. Paula's Choice did not stop selling in China in 2012 or 2013.

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[46] Homepage, PAULA'S CHOICE CHINA, https://web.archive.org/web/20120127102512/http://paulaschoice.com.cn/; Homepage, PAULA'S CHOICE HONG KONG, https://web.archive.org/web/20120430212330/http://www.paulaschoice.hk:80/ (last visited Mar. 12, 2024).

[47] Michelle Reynolds, *Pangea Organics Stops Selling in China to Save Animals*, PETA (February 20, 2013), ("And NYX, Paula's Choice, Yes To Carrots, and Jack Black have all said, "No, thanks!" to the Chinese market until tests on animals are no longer required—and that day is coming closer."), https://www.peta.org/blog/pangea-organics-stops-selling-china/ (last visited Mar. 12, 2024).

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178. In 2012 Paula's Choice registered twelve products with the NMPA in China, each requiring animal testing. Paula's Choice's NMPA registrations, Ex. 4.

179. In 2013, Paula's Choice registered six products with the NMPA in China, each requiring animal testing, and all of them were registered after PETA reported that Paula's Choice was not selling in China. *Id.*

180. If Paula's Choice was only selling in Hong Kong, it would not have needed to obtain the NMPA registrations in China in 2012 and 2013.

181. In 2014, Begoun was interviewed for the blog "In My Bag," which reported: "Paula [Begoun] informed me that if a brand is sold in China, it's compulsory that it has to have been tested on animals . . . That is the reason, according to Paula, that she has taken the (financially challenging) decision not to sell her range there."[48]

182. In 2014, Paula's Choice registered four products with the NMPA in China, each requiring animal testing. Paula's Choice's NMPA registrations, Ex. 4.

183. In 2015, Begoun gave another interview where she was quoted as saying: "We were on the cusp of setting up in China and then we heard about the animal testing and we pulled out. We said 'No'. It's a billion people market. It's a big deal. But 'No.'"[49]

184. From 2015 to 2016, Paula's Choice registered nine products with the NMPA, each requiring animal testing. Paula's Choice's NMPA registrations, Ex. 4.

185. In 2017, Paula's Choice represented on its beautypedia.com website that it did not test on animals and included the following statement about sales in China: "IMPORTANT NOTE ABOUT COSMETICS IMPORTED TO AND SOLD IN CHINA: The Chinese government requires animal testing on all imported cosmetics sold from a physical storefront within mainland China. So, a brand that retails there (in an actual store, not exclusively online) must agree to this

[48] Not Tested on Animals, INMYBAG.CO.ZA (February 28, 2014), https://www.inmybag.co.za/2014/02/28/not-tested-on-animals/ (last visited Mar. 12, 2024).

[49] Meeting Paula Begoun, CAROLINEHIRONS.COM (May 19, 2015), https://www.carolinehirons.com/2015/05/meeting-paula-begoun.html (last visited Mar. 12, 2024).

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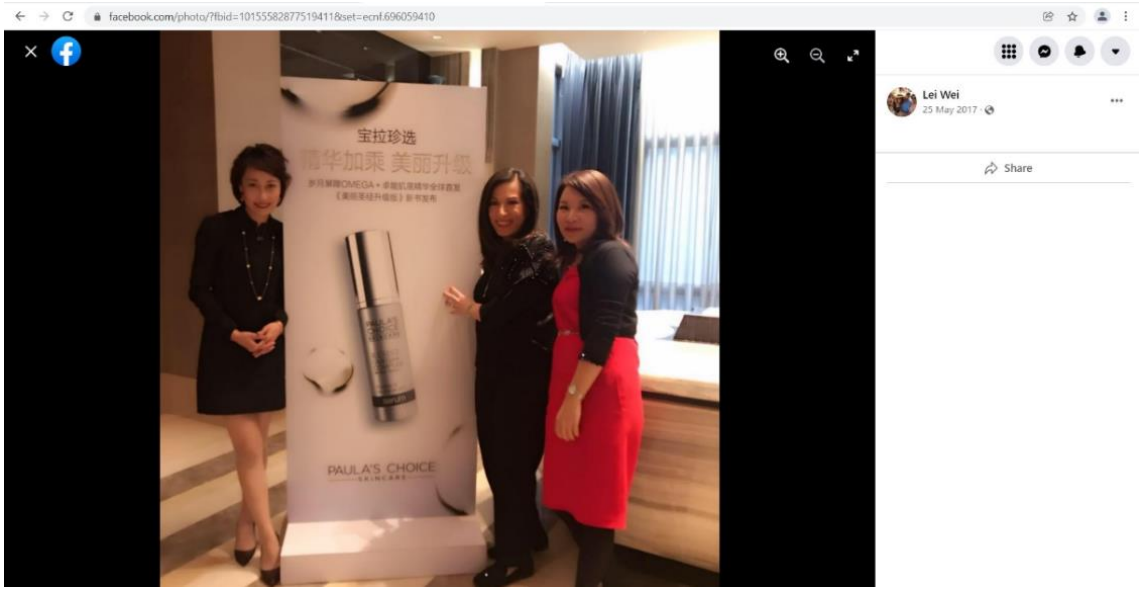
third-party testing even though they may not test on animals themselves or endorse this practice in any other country."[50]

186. But there is no blanket exception for online sales in China. All cosmetics that are imported and sold commercially in China require registration with the NMPA, including the required animal testing, regardless of whether they are sold online or in a physical storefront.

187. On May 25, 2017, Lei Wei, a business partner of Yang, posted a photo of with Begoun and Wang in China, promoting Paula's Choice products.[51]



188. In 2017, Paula's Choice registered nine products with the NMPA in China, each requiring animal testing. Paula's Choice's NMPA registrations, Ex. 4.

189. In 2018, Begoun did an interview for the Chinese website CBO, where she discussed selling Paula's Choice products in China.[52]

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[50] Animal Testing Report Card, BEAUTYPEDIA.COM, https://web.archive.org/web/20170218070715/beautypedia.com/animal-testing/ (last visited Mar. 12, 2024).

[51] Lei Wei Facebook Post, FACEBOOK (May 25, 2017), https://www.facebook.com/photo?fbid=10155582878634411&set=ecnf.696059410 (last visited Mar. 12, 2024).

[52] Wu Sixin, Begoun Interview, CBO (September 15, 2018), https://www.cbo.cn/article/id/46097.html (last visited Mar. 12, 2024).

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190. That article includes photos of Begoun, as well as promotional photos for Paula's Choice China.[53]

191. From 2018 to 2020, Paula's Choice registered fifteen products with the NMPA in China, each requiring animal testing. Paula's Choice's NMPA registrations, Ex. 4.

192. Paula's Choice products sold in China have the required product label and accompanying NMPA registration number. The following are images of a Paula's Choice product sold thorough Amazon China, showing the label and NMPA registration number:

[53] *Id.*

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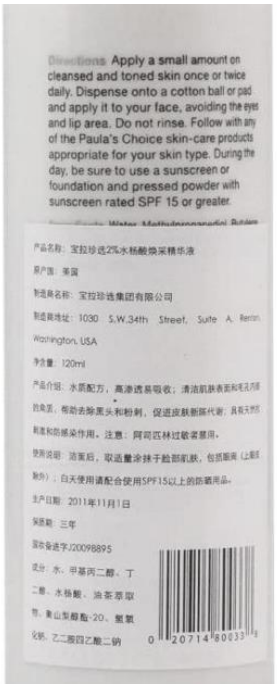

193. To avoid animal testing, Paula's Choice could have availed itself of the 2014 regulations that allowed companies to manufacture non-special use cosmetics in China, rather than import them.

194. Instead, Paula's Choice continued to register and sell imported products in China from 2009 to 2020, an approach that required animal testing for each product.

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195. Although foreign companies have been able to set up domestic manufacturing to avoid animal testing for non-special use cosmetics since 2014, Paula's Choice waited until 2021 to engage domestic manufacturers.

196. In 2021, Paula's Choice began bottling its products in China under the 2014 domestic manufacturing exemption, which allowed Paula's Choice to stop having its non-special use products tested on animals.

197. That same year Paula's Choice stopped registering its products with the NMPA and cancelled several of its NMPA registrations.

198. In total, from 2009 to 2020, Paula's Choice registered 60 products with the NMPA, with multiple registrations each year, all requiring animal testing. Paula's Choice's NMPA registrations, Ex. 4. The distribution of these registrations is as follows:

Number of Paula's Choice Products Registered with the NMPA by Year											
2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
1	3	1	12	6	4	4	5	9	3	3	9

H. Plaintiffs

199. Plaintiff Jesse Vargison purchased Paula's Choice products between 2018–2021.

200. Vargison currently resides in Seattle, Washington.

201. Vargison was first introduced to Paula's Choice through a friend who used to work in their marketing department. Before making his first purchase of Paula's Choice products he investigated and researched whether Paula's Choice products were cruelty-free, as the claim was a "selling point at the time of purchase."

202. Vargison saw the "no animal testing" claims on the Paula's Choice website and marketing and relied on those claims in purchasing Paula's Choice products.

203. Vargison has purchased approximately 30–40 products since 2018.

204. Vargison would not have purchased Paula's Choice products had he known they were tested on animals.

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205. Vargison purchased various products from Paula's Choice, including Skin Recovery Hydrating Treatment Mask, Clear Anti-Redness Exfoliating Solution, Clinical 1% Retinol Treatment, Resist Barrier Repair Moisturizer, Resist Advanced Pore-Refining Treatment 4% BHA, Skin Balancing Pore-Reducing Toner, Resist Anti-Aging Clear Skin Hydrator.

206. The following are photos of the products he currently still has:

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207. Although all Paula's Choice products are represented as being both cruelty-free and made by a cruelty-free company, Paula's Choice obtained NMPA approval for several of Vargison's products in China, meaning those products were tested on animals in a Chinese lab.[54]

208. Vargison generally purchased his Paula's Choice products either directly on the Paula's Choice website or from various retailers and paid the listed retail price.

209. Vargison was not aware that Paula's Choice was testing on animals to sell in China until he contacted undersigned counsel, and no reasonable investigation would have led him to conclude otherwise.

210. Vargison would not have continued to purchase Paula's Choice products through 2021 had he known Paula's Choice tested any of its products on animals, regardless of where that testing occurred.

211. Plaintiff Rachael Forbis (née Laxton) has purchased Paula's Choice products since approximately 2014.

212. Forbis currently resides in Renton, Washington.

213. Forbis was first introduced to Paula's Choice products by her mother who also purchased products and shared them with her.

214. Forbis further investigated and researched the product, including visiting the Paula's Choice website, to verify that the products were cruelty-free.

215. Forbis actively looks for cruelty-free products and companies because she cares about animals and the environment.

216. Forbis does not agree with animal testing and looks for products that are cruelty-free.

217. Forbis relied on Paula's Choice's representations that the products she was purchasing were not tested on animals and that Paula's Choice did not perform animal testing.

[54] One judge has already found that where a company represents that it never tests on animals, it breaks its cruelty-free promise on all products if it tests any products on animals, including those that were not directly tested on animals.

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218. Forbis would not have purchased or continued to purchase Paula's Choice products had she known those products had been tested on animals, regardless of where that animal testing occurred.

219. Forbis has purchased over 90 Paula's Choice products since 2014. She generally purchases her products directly on the Paula's Choice website.

220. The last products Forbis purchased were the Weightless Body Treatment 2% BHA, the Skin-Smoothing Retinol Body Treatment and the Hydrating Gel-to-Cream Cleanser.

221. Forbis purchased the Weightless Body Treatment 2% BHA on July 5, 2023 from the Paula's Choice website for $33.20. This image is a photo of this product:

222. Forbis purchased the Skin-Smoothing Retinol Body Treatment and the Hydrating Gel-to-Cream Cleanser on January 27, 2023 from the Paula's Choice website for $44.92. These images are photos of these products:

223. In making those purchases, Forbis relied on Paula's Choice's representations that its products were cruelty-free, including the representations on the bottles she purchased.

224. Had Forbis known that Paula's Choice products were tested on animals, she would not have purchased these products.

225. Although all Paula's Choice products are represented as being both cruelty-free and made by a cruelty-free company, Paula's Choice obtained NMPA approval for several of Forbis' products in China, meaning those products were tested on animals in a Chinese lab.

226. Forbis was not aware that Paula's Choice was testing on animals to sell in China until she contacted undersigned counsel, and no reasonable investigation would have led her to conclude otherwise.

227. Forbis would not have continued to purchase Paula's Choice products through 2023 had she known Paula's Choice tested any of its products on animals, regardless of where that testing occurred.

I. Terms of Use

228. Paula's Choice has Terms of Use on its website.

229. Those Terms of Use dictate that the "laws of the United States and State of Washington" will govern purchases made on its website.

230. Upon information and belief, up until or around March 14, 2023, the Terms of Use also dictated that website purchases would be subject to the "exclusive jurisdiction of the state and federal courts sitting in the King County in the State of Washington."[55]

231. On or around March 14, 2023, Paula's Choice added an arbitration clause to its Terms of Use, which "will take effect once [Paula's Choice] post[s] them" on the website.[56]

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[55] *Compare* Terms of Use, PAULA'S CHOICE (December 1, 2022), https://web.archive.org/web/20221201074026/https://help.paulaschoice.com/hc/en-us/articles/360035072673-Terms-of-Use (last visited Mar. 12, 2024), *with* Terms of Use, PAULA'S CHOICE (March 14, 2023), https://web.archive.org/web/20230314223729/https://help.paulaschoice.com/hc/en-us/articles/360035072673-Terms-of-Use (last visited Mar. 12, 2024).

[56] Terms of Use, PAULA'S CHOICE (March 14, 2023), https://web.archive.org/web/20230314223729/https://help.paulaschoice.com/hc/en-us/articles/360035072673-Terms-of-Use_ (last visited Mar. 12, 2024).

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V. CLASS ACTION ALLEGATIONS

232. Plaintiffs bring this action under Federal Rule of Civil Procedure 23(b)(2) and (b)(3) individually and for these Classes of similarly situated persons:

> All persons who purchased Paula's Choice products directly from Paula's Choice's U.S. website between December 22, 2009, and the date the arbitration clause was published on Paula's Choice's U.S. website, but no later than March 13, 2023; and
>
> All persons who purchased Paula's Choice products through a third-party retailer on or after December 22, 2009.

233. Excluded from the Class are Paula's Choice and its co-conspirators, their officers, directors, legal representatives, heirs, successors and wholly or partly owned subsidiaries or affiliated companies; class counsel and their employees; and the judicial officers and their immediate family members and associated court staff assigned to this case, and all persons within the third degree of relationship to any such persons. The class is clear and ascertainable using the objective criterion of purchases, which can be proven using Defendant's business records.

234. **Numerosity**. The Class is so numerous that joinder of all members is unfeasible and impracticable. Paula's Choice sells millions of products each year in hundreds of locations. The exact size of the Class is easily ascertainable, as each transaction or purchase can be tracked using Defendant's business records. Any reasonable estimate, based on sales, indicates there are at least hundreds of thousands of Class Members.

235. **Commonality and Predominance**. Questions of law and fact common to all Class Members exist and predominate over questions affecting only individual Class Members, including:

a. Whether Defendant stated or promised on all of the skincare products or packaging associated with those products that its skincare products were cruelty-free or never animal tested anywhere in the world;

b. Whether Defendant advertised its skincare products by stating that Paula's Choice never animal tested anywhere in the world and that it was a cruelty-free company;

c. Whether Defendant performed animal testing, or had animal testing performed on its behalf, on skincare products to sell them in China;

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d. Whether Defendant delivered skincare products to the Class that met its "cruelty-free" promise;

e. Whether Defendant delivered skincare products to the Class that met its promise to never animal test anywhere in the world;

f. Whether Defendant breached its express warranties with the Class Members;

g. Whether Defendant breached its implied warranties of merchantability with the Class Members;

h. Whether Defendant violated the Magnuson-Moss Warranty Act, 15 U.S.C. § 2301, *et seq.*;

i. Whether Defendant violated Washington's Consumer Protection Act, Wash. Rev. Code § 19.86, *et seq.*; and

j. Whether the Class was damaged by paying more for skincare products than they would have if the truth had been disclosed, and, if so, by what amount.

236. **Typicality**. Plaintiffs' claims are typical of the claims of the other members in the Class, as they arise out of conduct of the Defendant that is common to the Class, meaning each Class Member will rely on the same evidence and actions to prove their claims, and such conduct is uniform, standard, and pervasive. Thus, the claims of each Class Member are based on the same legal theories and challenge the same practices of Defendant. Plaintiffs and all Class Members have been subjected to the same falsehoods and practices, hold the same rights, are entitled to the same legal and equitable relief, have suffered the same impact and injury, and sustained similar damage by paying an amount for skincare products that they would not have paid, or greater than they would have paid, had Paula's Choice not affirmatively misrepresented that its skincare products were cruelty-free and never animal tested.

237. **Adequacy**. Plaintiffs and their counsel will fairly and adequately represent the interests of the Class Members. Plaintiffs have no interests antagonistic to, or in conflict with, the interests of the other Class Members, and they will zealously pursue their claims. Plaintiffs' lawyers are highly experienced in the prosecution of consumer class actions and complex commercial litigation, capable of providing the financial resources needed to litigate this matter to conclusion, and have litigated other consumer rights matters in a class context.

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238. **Superiority**. A class action is superior to all other available methods for fairly and efficiently adjudicating the claims of Plaintiffs and the Class Members. Plaintiffs and the Class Members—many of whom are unaware of their rights—have been harmed by Defendant's misrepresentations. Litigating this case as a class action reduces the possibility of repetitious litigation relating to Defendant's wrongful actions and provides an efficient mechanism for adjudication for Class Members, whose claims are too small to warrant individual litigation and Defendant has concealed the truth, thereby preventing Class Members from recognizing and evaluating their rights and claims.

239. **Injunctive and Declaratory Relief**. Paula's Choice has acted or refused to act on grounds that apply generally to the Class and final injunctive relief, or corresponding declaratory relief is appropriate respecting the Class as a whole.

VI. COUNTS

COUNT I

BREACH OF EXPRESS WARRANTY UNDER THE UNIFORM COMMERCIAL CODE

240. Plaintiffs reallege and incorporate by reference the allegations in the preceding paragraphs.

241. Paula's Choice sells skincare products, which qualify as goods under the Uniform Commercial Code. Wash. Rev. Code § 62A.2-105.

242. Plaintiffs purchased various Paula's Choice skincare products during the class period.

243. In connection with the sale of goods, including through representations on the products themselves, Paula's Choice expressly affirmed and warranted that it was a cruelty-free company, and its skincare products were never animal tested anywhere in the world. Wash. Rev. Code § 62A.2-313(1)(a).

244. Paula's Choice's affirmations are part of the basis of the bargain with Plaintiffs. *Id.*

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245. Paula's Choice breached its express warranty that it was a cruelty-free company and its products were never animal tested anywhere in the world by conducting animal tests in China in order to register and sell its products there.

246. Plaintiffs notified Paula's Choice of the nonconformities in the skincare products on December 21, 2023, which was a reasonable time after Plaintiffs discovered the breach. *See* Wash. Rev. Code § 62A.2-607(3)(a).

247. Because of Paula's Choice's breach of its express warranty, Plaintiffs have suffered damages valued at the difference between the value of a skincare product they received and the value of the skincare product they were promised, in an amount to be proven at trial. Wash. Rev. Code § 62A.2-714.

COUNT II

BREACH OF IMPLIED WARRANTY UNDER THE UNIFORM COMMERCIAL CODE

248. Plaintiffs reallege and incorporate by reference the allegations in the preceding paragraphs.

249. Paula's Choice sells skincare products, which qualify as goods under the Uniform Commercial Code. Wash. Rev. Code § 62A.2-105.

250. Plaintiffs purchased various Paula's Choice skincare products during the class period.

251. The implied warranty of merchantability requires a product to conform to the promises or affirmation of fact made on any container or label. Wash. Rev. Code § 62A.2-314.

252. In connection with the sale of goods, including through representations on the containers and labels themselves, Paula's Choice's products have an implied warranty of merchantability that its skincare products were never animal tested anywhere in the world and that it is a cruelty-free company.

253. Paula's Choice breached its implied warranty of merchantability that its products were never animal tested anywhere in the world and it was cruelty-free company by conducting animal tests in China to register and sell its products there.

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254. Plaintiffs notified Paula's Choice of the nonconformities in the skin care products on December 21, 2023, which was a reasonable time after Plaintiffs discovered the breach. Wash. Rev. Code § 62A.2-607(3)(a).

255. Because of Paula's Choice's breach of its implied warranty, Plaintiffs have suffered damages valued at the difference between the value of a skincare product they received and the value of the skincare product they were promised, in an amount to be proven at trial. Wash. Rev. Code § 62A.2-714.

COUNT III

VIOLATION OF THE MAGNUSON-MOSS WARRANTY ACT
15 U.S.C. § 2301, *ET SEQ.*

256. Plaintiffs reallege and incorporate by reference the allegations in the preceding paragraphs.

257. Paula's Choice products are "consumer products" under the Magnuson-Moss Warranty Act, as they are distributed in commerce and used for personal, family, or household purposes. 15 U.S.C. § 2301(1).

258. Plaintiffs are "consumers" under the Magnuson-Moss Warranty Act, as they are buyers of a consumer product. 15 U.S.C. § 2301(3).

259. Paula's Choice is a "supplier" and "warrantor" under the Magnuson-Moss Warranty Act, as it is a supplier who made consumer products and it gave a written warranty on its skincare products, specifically that it is a cruelty-free company and its products are never animal tested anywhere in the world. 15 U.S.C. § 2301(4)–(5).

260. Paula's Choice's representations constitute an "implied warranty" under the Magnuson-Moss Act and Washington law. 15 U.S.C. § 2301(7) ("The term "implied warranty" means an implied warranty arising under State law.").

261. Paula's Choice breached its implied warranty of merchantability that its products were never animal tested anywhere in the world and it was cruelty-free company by conducting animal tests in China to register and sell its products there.

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262. Plaintiffs notified Paula's Choice of the nonconformities in the skincare products on December 21, 2023, which was a reasonable time after Plaintiffs discovered the breach. 15 U.S.C. § 2310.

263. Plaintiffs individually and on behalf of the other Class Members, seek damages or any other equitable and legal relief available under 15 U.S.C. § 2310(d)(1).

264. Plaintiffs, individually and on behalf of the other Class Members, seek costs of court, attorneys' fees under 15 U.S.C. § 2310(d)(2).

COUNT IV

VIOLATION OF THE WASHINGTON CONSUMER PROTECTION ACT ("CPA") WASH. REV. CODE § 19.86, *ET SEQ.*

265. Plaintiffs reallege and incorporate by reference the allegations in the preceding paragraphs.

266. Paula's Choice is engaged in trade or commerce under Wash. Rev. Code § 19.86.10.

267. The CPA declares "unfair methods of competition and unfair or deceptive acts or practices in the conduct of any trade or commerce . . . unlawful." Wash. Rev. Code § 19.86.020.

268. Paula's Choice engaged in unfair or deceptive acts or practices that violated the CPA, as described herein, by representing, certifying, and advertising that its skincare products are cruelty-free and never animal tested anywhere in the world, when Paula's Choice in fact tested its products on animals in order to register and sell such products in China.

269. Paula's Choice's actions occurred in conduct of Paula's Choice trade or commerce, specifically the sale of goods to consumers.

270. Paula's Choice's actions affected the public interest, as it injured Plaintiffs, had the capacity to injure other persons, and continues to have "the capacity to injure other persons." Wash. Rev. Code. § 19.86.093.

271. Paula's Choice's misrepresentations that Paula's Choice's skincare products had never been animal tested anywhere in the world and were cruelty-free, as set forth, were material and had the capacity to deceive a substantial portion of the public.

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272. In purchasing Paula's Choice products, Plaintiffs and the Class relied on the misrepresentations of Paula's Choice regarding its stance on animal testing and that its products were never tested on animals. Paula's Choice's representations turned out not to be true because it tested on animals to sell its products in China.

273. Had Plaintiffs and the other Class Members known they would not receive products that had never been animal tested, they would not have purchased Paula's Choice's skincare products and/or paid as much for them.

274. Plaintiffs suffered ascertainable loss caused by Paula's Choice's misrepresentations and its concealment of and failure to disclose the use of animal testing in Plaintiffs' products.

275. Under Wash. Rev. Code § 19.86.090, Plaintiffs, individually and on behalf of the other Class Members, seek actual damages against Paula's Choice for the harm caused by Paula's Choice's violations of the CPA as alleged.

276. Under Wash. Rev. Code § 19.86.090, Plaintiffs, individually and on behalf of the other Class Members, seek actual damages against Paula's Choice for the harm caused by Paula's Choice's violations of the CPA as alleged. Plaintiffs also seek treble damages because Paula's Choice intentionally and willfully misrepresented material facts that only it knew, specifically that it performed no animal testing and its products were cruelty-free.

277. Plaintiffs seek an order enjoining Defendant's unfair or deceptive acts or practices under Wash. Rev. Code § 19.86.090. Plaintiffs will serve a copy of this Complaint on the attorney general of Washington in compliance with Wash. Rev. Code § 19.86.095.

278. Plaintiffs, individually and on behalf of the other Class Members, seek costs of court, attorneys' fees under Was Rev. Code § 19.86.090, and any other just and proper relief available under the CPA.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs, individually and for members of the Class, respectfully request that the Court enter judgment in their favor and against Defendant, as follows:

A. Certification of the proposed Class, including appointment of Plaintiffs' counsel as Class Counsel and Plaintiffs as Class Representatives;

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B. An order temporarily and permanently enjoining Defendant from continuing the unfair methods of competition and unfair or deceptive acts or practices alleged in this Complaint, including its representation that it has never animal tested;

C. Restitution and/or damages, each in an amount to be determined by the trier of fact;

D. Treble damage award;

E. Pre- and post-judgment interest on any amounts awarded;

F. An award of costs and attorneys' fees where authorized by law; and

G. Such other or further relief as may be appropriate.

DEMAND FOR JURY TRIAL

Plaintiffs demand a trial by jury on all issues so triable.

DATED: March 14, 2024

HAGENS BERMAN SOBOL SHAPIRO LLP

By */s/ Sean R. Matt*

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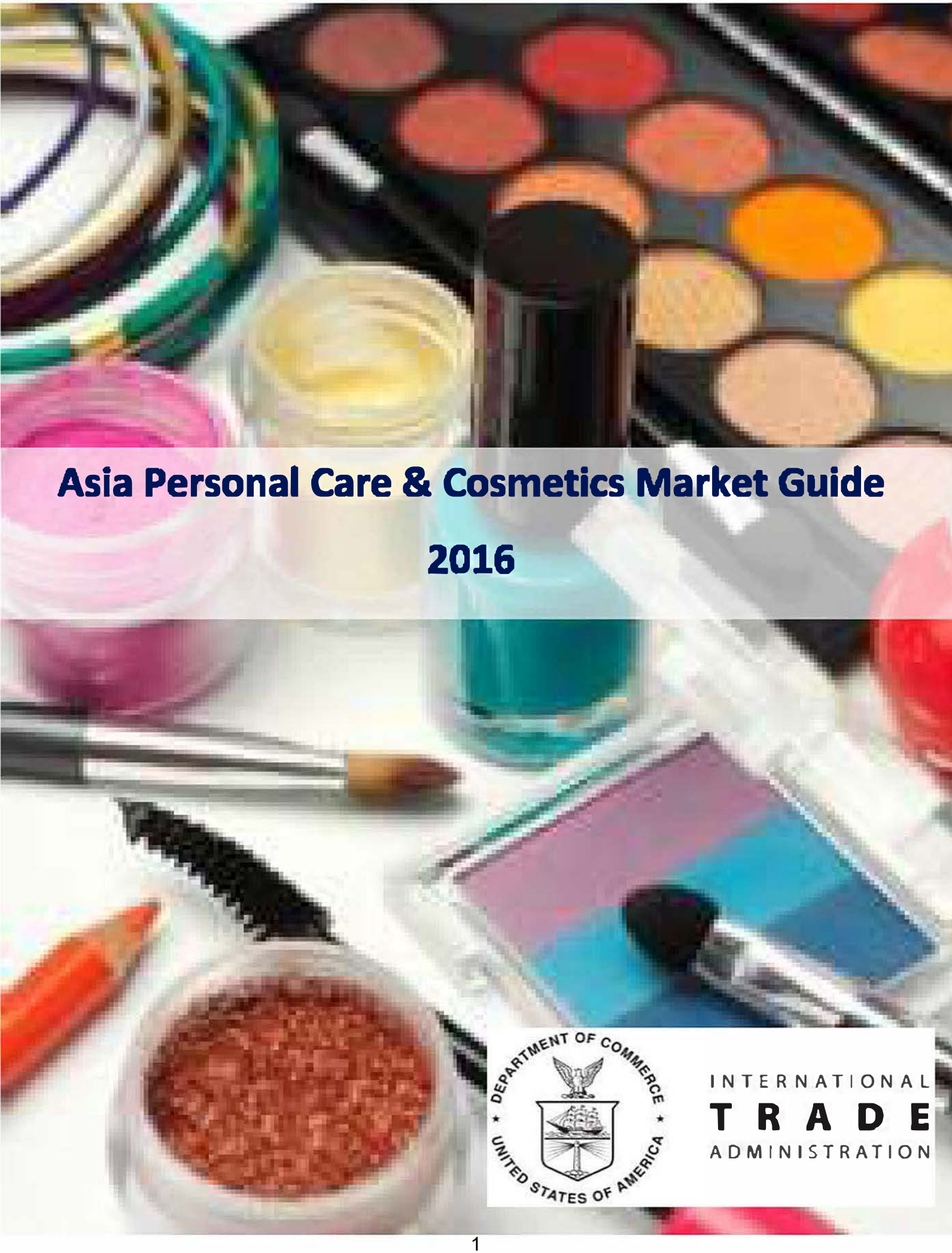
EXHIBIT 1



Asia Personal Care & Cosmetics Market Guide

2016

DEPARTMENT OF COMMERCE
UNITED STATES OF AMERICA

INTERNATIONAL
TRADE
ADMINISTRATION

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INTERNATIONAL TRADE ADMINISTRATION

Tracy Gerstle, International Trade Specialist in the Office of Materials Industries, served as the lead editor of this report. Special thanks to **Swee-keng Cheong** and **Tony Michalski**, for their leadership in prior versions of the Asia Cosmetics Market Guide, as well as in reviewing this report.

Considerable credit is due to ITA's Global Healthcare Team, who authored the country case studies, providing considerable market research and field based insights to this report, including: **Swee-keng Cheong, Janet Coulthart, John Kanawati, Angela Han, Janet Li, Pepsi Maryarini, Chris Ono, Manjushree Pookan, Dey Robles, Yien Rumeral, Smita Sherigar, Shen Yan, Tracy Yeoh, Luanne Theseira,** and **Heesook Baik**.

Comments on this report should be sent to Tracy Gerstle: tracy.gerstle@trade.gov. Contacts for each of the profiled countries are provided in the country profiles.

Partnering with the International Trade Administration

With its network of offices across the United States and in more than 80 countries, the International Trade Administration of the U.S. Department of Commerce utilizes its global presence and international marketing expertise to help U.S. companies sell their products and services worldwide. U.S. companies can work directly with an International Trade Specialist in their local area to receive export assistance in the following areas:

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Locate the U.S. Commercial Service trade specialist in the U.S. nearest you by visiting http://www.export.gov/eac

Comments and Suggestions: We welcome your comments and suggestions regarding this market research. You can e-mail us your comments/suggestions to: Tracy.Gerstle@trade.gov

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Table of Contents

Executive Summary

There Has Never Been a Better Time to Enter Asia's Cosmetics Market Place

The countries in the Asia report represent some of the largest and high potential markets for export-led growth for the U.S. Personal Care and Cosmetics industries. Comprising over 20% of U.S. global exports in the sector, the Asia countries offer the industry over 3 billion potential consumers in the fastest growing global markets. This base includes consumers in well-established markets for U.S. products such as Japan, Australia and South Korea, which together accounted for more than $1 billion in U.S. personal care and cosmetics exports in 2015—to China, which at an estimated $500 to $600 million in U.S. exports that same year, is predicted to become the largest global market for cosmetics in the next two to three years.[1]

In addition, the countries included in the Asia Personal Care and Cosmetics Guide provide access to some of the highest potential future markets—including the growing middle class in countries spanning from Indonesia to Vietnam to Malaysia. These consumers aspire to the quality and sophistication for which U.S. products are known. Currently these markets source most of their basic personal care products such as shampoos, soaps and other grooming products from mass market U.S. companies including Procter and Gamble, Johnson and Johnson and Colgate-Palmolive. Increasingly these consumers are moving beyond the basics to premium skin and hair care as well as color cosmetics opening the door to a wide variety of U.S.-based cosmetics exporters.

Table 1 summarizes three years of U.S. exports to these markets, which have grown across the region by an average annual rate of 6% over the last five years, even during the global downturn of 2011-2012. Stunningly, in some countries U.S. exports have grown by as much as 62% over the past three years in high potential markets such as Indonesia, Vietnam and China and this growth is predicted to continue.

Table 1: Summary of US Exports to the Asia Countries ($USD)

	2013	2014	2015	5 Yr CAGR	3 Year Growth
Hong Kong	$528,831,000	$521,959,000	$516,669,000	NA	-2%
Japan	$568,489,269	$519,891,675	$491,176,604	-1%	-14%
South Korea	$424,921,167	$489,771,533	$487,148,249	4%	15%
China	$324,183,216	$336,578,269	$392,606,007	15%	21%
Singapore	$313,969,980	$324,815,120	$340,553,935	6%	8%
Thailand	$75,207,022	$86,657,330	$80,417,978	2%	7%
Malaysia	$63,298,633	$71,122,679	$78,917,998	9%	25%
Philippines	$52,876,097	$60,813,319	$56,935,354	8%	8%
Vietnam	$40,108,841	$52,840,162	$53,481,224	11%	33%

[1] The estimate of U.S. exports to China is based upon exports to the mainland and an estimated 40% of U.S. exports to Hong Kong, given that these products are then re-exported to China via visitors and other channels.

India	$49,084,035	$55,636,277	$53,771,347	0%	10%
Australia	$19,414,099	$29,362,407	$31,398,239	9%	62%
Total	$2,051,018,946	$2,157,192,939	$2,197,349,243	6%	7%

Source: Trade Policy Information System (TPIS)

Across many of these markets, as illustrated in Table 2, per capita levels of cosmetics spend is still very low, when benchmarked against peer countries such as the U.S. or Japan. China for example, only spends $24 per person annual on personal care and cosmetics products, whereas South Korea and Japan spend $171 and $174 annually.[2]

Table 2: Per Capita Cosmetics Spend in TPP Countries 2014

	Per Capita Cosmetics Spend, 2014
China	$24
U.S.	$139
France	$139
South Korea	$171
Japan	$174
Australia	$30.47
Malaysia	$7 (2013)
New Zealand	$84.92
Singapore	$299 / $90
Vietnam	$5.28 (2013)

Source: US Department of Commerce Estimates

K-Beauty Leads in Product Trends, While U.S. Brands Lead in Trust and Quality

Lead by the global K-Beauty craze, these markets also represent some of the most discerning, sophisticated cosmetics consumers. Vis-s-vis their U.S. counterparts, urban women in many of these countries use five or more products daily. In line with regional cultural values, the most popular products are multifunctional skin care products, protecting skin from harsh sun and other environmental conditions, while promoting skin tone that is even and light. As a result, skin care products represent up to 40% of sales in some markets. However, as Korean and Indian TV and pop-stars dominate popular culture, many young and professional women are increasingly experimenting with color cosmetics. This is reflected in U.S. export statistics in which color cosmetics, including lip, eye and nail polish are among some of the fastest growing categories. The region's men are also increasing consumers of both personal care and grooming products. Similar to women, they value even, clear skin tone and are also willing to experiment with cosmetics. Specialty hair care products, including

[2] Singapore's per capita cosmetics spend is not reflective of domestic demand, as 70% of all cosmetics are re-exported. Therefore, actual per capita spend is likely closer to $90 per person, per annum. (2) Data provided for Malaysia and Vietnam is based on 2013 size of the cosmetics market.

styling products, conditioners and colorants are also growing quickly. These products need to help Asia consumers deal with regional conditions, such as the ability for easy washing due to hard water and stylants that can deal with high humidity, while giving body and UV protection to thicker hair.

To be successful in many Asian markets, companies also need to be sensitive to local trends and competition. For example, Indonesia, representing the largest Muslim population in the world, passed a regulation in 2015 that by 2020 all cosmetics products will have to be labeled as Halal. Whereas in China and India, there is a strong interest in local ingredients and herbal traditions and the properties that these essential oils and other products can bring to personal care products and cosmetics. Asian consumers are also somewhat discerning in terms of packaging. K-Beauty has fueled this trend, with its whimsical and innovative packaging including the air cushion, peel off makeup and lip balms in the shapes of flowers or panda bears.

Smart phone penetration and use for e-commerce is also higher in some Asian countries than even in the U.S., with for example the majority of Chinese consumers having purchased products via e- commerce using their smart phones. Asian consumers also rely on e-commerce to access products, not available in their local market—particularly if they live in second or third tier cities rather than their national capitols. E-Commerce as a result, offers a highly viable entry route for U.S. companies seeking to enter Asian markets. However, companies should be aware that Asia's discerning consumers also expect to be engaged via sophisticated digital marketing and social media campaigns.

Direct Selling is still very popular in some markets, such as the Philippines, where Avon is the largest seller of color cosmetics and Singapore, where direct selling is used as an entry strategy. There is also growing interest in accessing products through specialty salons and doctor-led channels. Private-label brands are also increasingly popular, as the region's retail, hospitality chains and other channels seek to brand these products.

While being mindful of the need to not overly characterize trends across these 11 highly differentiated markets, there are a number of trends of note.

Baby and Child Care Products (Top Export Opportunity)

With Asia's rapidly growing population, baby and child specific products are among the fastest growing product category. While this category is still relatively small—often less than 5% of each country's total domestic market—it is expected to continue to grow, given Asian helicopter parents, who pamper and dote on their children. It also is a highly competitive category for imported products, given consumer sensitivities to the safety of these products, given high levels of adulteration of personal care products. There are still relatively few players in this market outside of Johnson and Johnson, offering a significant niche opportunity for U.S. companies new to the market. Moreover, U.S. branded products also benefit from their strong reputation and track record of safety. However, U.S. companies wishing to enter this product category should be aware that in some Asian markets, there are stricter product approval levels and standards required for these products.

Green or Natural Products (Top Export Opportunity)

Given the number of health scares from adulterated and counterfeit mass market products such as faulty face masks and tainted cosmetics, some Asian consumers are wary of mass products sold via local channels. Green or natural cosmetics, products that contain medicinal, natural or nutritional ingredients such as vitamins, aloe and traditional medicines are very popular. Market data on the trends in these types of products is not readily available, but inputs for these types of products such as essential oils,

are among the fastest growing categories in many products. Note: U.S. companies seeking to market these types of products will need to review their claims, labeling and marketing strategies, as in some Asian markets the use of the terms such as natural and organic are banned.

Color Cosmetics (Top Export Opportunity)

Following skin care, color cosmetics are often the second fastest growing product category, for U.S. exports. Asian consumers expect ABCD, multifunction products as well as creative touches such as cooling face gels.[3] They also expect color pallets adapted to local skin tones and fashion trends. Manicures and pedicures are increasingly popular, with an emphasis on bright colors, color-fast gels, and other attributes that make nails stand out, such as textured, decorative coatings and crystals. There is also increasing interest and growing market for permanent make-up.

Other top prospects, include

1. Dry shampoos, leave-in conditioners and styling treatments
2. Facial skin anti-aging creams, whitening lotions, and masks
3. "Dermo-skincare products" for retail distribution
4. Hair coloring products
5. Body treatments, slimming treatments, massage and bath products for use in spas and professional skincare salons
6. Personal care and cosmetic products using organically-grown and naturally-derived ingredients hypo-allergenic, with low concentrations of fragrance and preservatives. The market is still relatively small. In some markets, local distributors sometimes retail them in smaller bottles than non-organic products for the prices to remain competitive.
7. Use-at-home hand held devices for body and facial skin firming and contouring
8. Skincare products for men
9. Nail colors, nail-care products, artificial nails, soft nail gels
10. Color cosmetics, including long-lasting lip stick, cream-based eye shadows and ABC creams
11. Fake eyelashes & semi-permanent eyelash extensions
12. Private labeling and contract manufacturing of skincare and cosmetic products for the leading retail, hospitality and spa/salon chains

Succeeding in Market Authorization

With the introduction of the ASEAN Cosmetics Directive in 2007 and the variety of regulatory requirements across the Asian markets, which span from ingredient requirements to claims and labeling prescribed lists, it is recommended in most of these markets to partner with a local distributor, who can assist with gaining local or regional market authorization for products as well as marketing. Recognizing the challenges for U.S. firms in initially identifying high potential markets, based both on market research and varying compliance

[3] ABCD stands for product attributes, including anti-aging, beauty balm, correct and cover and disguise and diminish, aka Do All

requirements, in this version of the guide we have included a new Market Readiness Checklist. This new tool, enables a comparison of these requirements across countries. Given that market authorization can cost hundreds or even thousands per product SKU in terms of both government and consulting fees, the labeling, ingredient, and registration requirements can play a large role in determining which markets to enter, particularly for those companies that enter a market with at least ten (10) or more SKUs. The new Market Readiness Checklist, assists companies with an initial scan of compliance requirements that would prohibit entry due to cost or inability to meet the requirements. For example in China, the requirement that imported products be animal tested, makes China inaccessible to many companies who will not animal test their products due to branding or the testing costs. We hope that by proving a high level, comparable country-by-country overview of these requirements, the Market Readiness Checklist will significantly assist companies in identifying potential markets, while reducing the time and cost it takes to gather the requirements. Companies will then be better positioned to work with the International Trade Administration in identifying business partners to facilitate the market authorization process, having identified high potential, feasible targets.

How to Use this Guide

This Guide is comprised of two sections.

1. The 11 Country Overviews, which provide an overview of each of the national markets, highlighting three to five years of market research on trends. Each country guide stratifies the local consumer population, discussing demographic and consumer purchasing trends, as well pricing, distribution and market channels. To assist U.S. companies in understanding their relative positioning in the market, the guides include an overview of the top export countries for each market and the types of products that these countries are successfully marketing. There is also an Annex to each country guide that provides all of the supporting data tables, allowing the reader to drill down more on the data as pertains to their specific product of interest and its performance across the top markets for a given country's imports.

2. The second section is the Market Readiness Checklist, which enables companies to review the market authorization requirements, timelines and costs in each market.

Personal Care & Cosmetics Products

Country Guide: China

Executive Summary

China is 10th largest market for U.S. personal care and cosmetics exports, with U.S. products exports totaling $392.6 million in 2015, representing 3% of the total U.S. export market. Within the next decade, China has the potential to become the largest market for U.S. products, with U.S. exports to China growing 64% in the period between 2010 and 2015. While China's rate of economic growth has slowed over the past few years, it has not impacted the growth of U.S. personal care and cosmetics exports. This is likely due to the current low rates of these products consumption in China, which at $24 per person, per annum in 2014, is still far lower than neighboring countries such as South Korea and Japan, which averaged $223 and $174 respectively.

Overview of the Domestic Market

Market Size, Growth Trends and Consumer Profile

With an estimated $50 billion in domestic sales in 2015 and 7% to 10% annual growth predicted in 2016 and beyond, China is projected to become the largest market for personal care and cosmetics products globally in the next five to ten years. Despite its relatively large market size, merely 10% of the population uses cosmetics regularly. Consumption of cosmetics is most prevalent in tier 1 megacities, with increasing penetration in China's inland tier 2 and tier 3 cities, which are also experiencing the fastest increases in China's growing middle class and consumption of consumer products. The majority of consumers of these products are 20 to 30 year olds, although there is a robust level of consumption among 30 to 39 year olds who also consume higher percentages of premium products. While the market is predicted to grow at 10% or greater over the next five years, U.S. companies considering exporting to China should be aware of the high levels of competition and complex product registration requirements required to enter China. Although a slice of Chinese cosmetics and personal care products consumers are willing to pay for branded or premium products, consumers tend to be less brand-loyal and more price sensitive than U.S. or European counterparts. As a result, product demand, even among well established brands, can be volatile from year to year.

Multinational companies continue to dominate the personal care products market in China, with nine of the top ten largest sellers across categories being foreign companies. The three largest sellers in China currently are Procter and Gamble, L'Oreal and Shiseido. This trend is also reflected in domestic production, with over 80% of products produced via foreign-owned or joint ventures.

Distribution Channels

Market channels for personal care and cosmetics products are changing rapidly, reflecting consumer distrust of mass market channels; the upgrading of Chinese consumer preferences; and the growing middle class in Tier 2 and Tier 3 cities.

Sales of these products via grocery stores is down from 81% in 2011 to 70% in 2014—while sales via non-grocery channels, including beauty specialist, department and pharmacy stores is up approximately

two percent to 18% of all cosmetics sales in the same time period. Direct selling is up by two percent during this period to 13.5% of all sales. Online retailing is by far the fastest growing channel up from 5.3% in 2011 to 15.5% 2014, with estimates that this channel could grow to 30% or more of total sales in the next five to ten years. The increasingly popularity of online channels reflects both the sparser access to retail stores and diversity of brands in Tier 2 and 3 cities. Consumers who shop via this channel are also unique in that they purchase high levels of premium products.

The rise of online sales was also driven in part by the advantageous terms that cross-border, imported products received vis-a-vis domestically produced products and products imported via traditional channels. These cross-border products were not subject to these same product registration requirements, which in China can cost $1,000 or more per SKU and take up to 8 months to complete registration for nonspecial use cosmetics. As a result, many products not registered for consumption in China, were available via the online channel. Similarly, these products were also subject to fewer taxes. In April 2016, China introduced new e-commerce cross-border regulations requiring registration for cosmetics as well as for a number of other consumer goods sold through this channel—putting these products on a more equal footing with products sold via traditional outlets. It is not yet clear how these policy changes will impact online product sales.

Another important consideration for companies marketing to Chinese consumers is the importance that consumers place upon recommendations from their family and friends for purchasing decisions compared to television, internet and other advertising. A 2015 market research report cited that sixty nine percent of Chinese consumers reported that they purchased products based upon the recommendation of a friend. Additionally, Chinese consumers who are online, on average hold six to eight or more social media accounts and tend to use these channels to research and to inform their cosmetics purchases, with women basing their decisions primarily on information gained via this channel.

Product Trends

U.S. companies new to the Chinese market may find the greatest opportunities in premium or niche products, with demand predicted to grow at a faster rate than the demand for traditional products. Chinese consumers grow increasingly suspect of mass market products. In additional, adults born under the one child policy are more inclined to spend on themselves and luxuries. This group travels more frequently abroad than previous generations. The resulting exposure to foreign brands has instilled a cachet and preference for niche products and foreign origins. Chinese consumers are forecasted to buy 44% of the world's luxury goods by 2020, with the average spend on these items growing 11% year over year per a KPMG consumer survey in 2014. This trend is particularly prevalent in cosmetics. For example, via online channels, Chinese consumers ages 30-39 are purchasing 60% of all online cosmetics sales in China, with an average purchase of $275 and a total annual spend of $644 per annum.

Representative retail prices for the following products (US$)

Products	Mass Market		Premium	
	Low	High	Low	High
Perfume	$10	$28	$43	$100
Lipstick	$11	$19	$40	$99
Eye Shadow	$15	$27	$48	$84
Mascara	$9	$22	$37	$61
Sunscreen	$3	$25	$59	$135
Bronzer/Blush	$12	$27	$57	$107

Skin Cream	$4	$20	$87	$382
Shampoo/Conditioner	$3	$21	$61	$132
Hair Styling Product	$1.5	$5	$19	$51
Deodorant	$3	$5.5	$22	$32
Nail Polish	$2	$9	$16	$28
Shaving Prep	$1.5	$7	$25	$40

Exchange Rate: 1USD=6.68CNY

Source: US Department of Commerce, International Trade Administration Field Research

Trends in US Imports and Competing Products

Table 9: Imported Products Share of China's Personal Care and Cosmetics Market (US$) (Thousands)

Top 7 Importers to China	2013	% Market Share	2014	% Market Share	2015	% Market Share
US	$324,183	8%	$336,578	11%	$392,606	9%
France	$481,913	12%	$557,820	18%	$509,255	12%
Japan	$602,059	15%	$901,830	29%	$1,518,563	36%
South Korea	$356,634	9%	$656,422	21%	$1,260,033	30%
Thailand	$70,835	2%	$61,661	2%	$38,182	1%
Brazil	$30,438	1%	$19,817	1%	$33,720	1%
EU 28 (Excluding France)	$2,138,023	53%	$557,820	18%	$509,255	12%
Total Chinese Imports Reported by Exporters	$4,004,084	100%	$3,091,951	100%	$4,261,616	100%
Total Imports Reported by China (1)	$3,068,131	NA	$3,768,144	NA	$6,117,199	NA

Source: Trade Policy Information System (TPIS), if marked with a *, source is Global Trade Atlas

As illustrated in Table 11, demand trends across countries can be volatile from year to year, given the lack of loyalty among Chinese consumers to specific brands. However, there are some prominent trends. The EU has lost significant market share, with exports to China growing at a much slower pace vis-a-vis Japan, South Korea and the U.S. As a result, while China's imports of products have grown exponentially, the EU market share has shrunk with Japan and South Korea now the largest suppliers of personal care and cosmetics products. South Korea's competitive position with China was also significantly strengthening with the signing of a free trade agreement with China in the summer of 2015, which brings in all Korea products with zero tariffs.

US Imports and Competitors

The following section provides a deeper dive on the sales and demand trends in some of the main product categories, as well as identifying categories that may have the highest potential for U.S. companies new to the Chinese market.

Skin products comprise almost half of the domestic sales at 47%. The next largest category is hair care products at 15%; followed by oral care at 9.4% percent; color cosmetics at 7%; and bath and shower products at 6%.

Table 2 in the Appendix provides an overview of trends in U.S. exports across these and other product categories to China, with the top export market prospects for U.S. firms highlighted in this report.

See Appendix Table 2: China Imports of Personal Care and Cosmetics Products from the U.S.Skin and Sun Care (Sun Care: Top Export Opportunity)

Chinese women, similar to their Asian counterparts, use multiple skin care treatments daily, averaging three products. This focus on skin care is also reflected in U.S. exports, with skin care products comprising 34% of all U.S. exports in the sector. Similar to other Asian countries, there is an emphasis on products that whiten or even skin tone. This consumer preference suggests potential for growing sales of sunscreen products, as well as the inclusion of sunscreens in other products in the future; although currently sunscreens comprise only 1% of domestic sales. Cosmeceuticals, products which combine cosmetic and pharmaceutical features such as acne treatment or anti-aging, are also increasingly popular, growing at an estimated 10% to 20% per annum.

The U.S. is currently the fourth largest exporter of products across this category to China, preceded by the EU, South Korea and Japan.

Baby and Child Care Products (Top Export Opportunity)

Baby and child specific products are the fastest growing product category in China's domestic market with 16.3% growth in2014 according to the Fung Business Center. While this category is still relatively small—at only 4% of the total domestic market—it is expected to continue to grow, given both the increasing interest generally of Chinese parents to pamper their children via child-specific products. It also is a highly competitive category for imported products, given consumer sensitivities to the safety of these products, given high levels of adulteration of personal care products. The baby and child care products category is relatively new in China with few players; therefore offering a significant opportunity for U.S. companies new to the Chinese market. Moreover, U.S. branded products also benefit from their strong reputation and track record of safety. However, U.S. companies wishing to enter this product

category should be aware that there are stricter product approval levels and standards required for these products.

Hair Products (Top Export Opportunity)

Niche and premium hair products are the fastest growing segment for U.S. exports to China, up 32.7% over the past three years. Domestically, hair products comprise 15% of the total domestic personal care and cosmetics market, growing at a rate of 3.5% from the prior year. Foreign companies producing locally in China such as Procter and Gamble and L'Oreal dominate the mass market. Imports of U.S. products are growing across all product segments: shampoos, hair preparation and hair spray, with the exception of hair straightening and waving products which are down. Shampoo is the fastest growing segment, followed by other specialty styling products.

Green or Natural Products (Top Export Opportunity)

Given the number of health scares from adulterated and counterfeit mass market products such as faulty face masks and tainted cosmetics, Chinese consumers are increasingly weary of mass products sold via supermarkets and other local channels. Chinese consumers also tend to believe that mass market products, made from synthetic ingredients are less healthy.[8] Green or natural cosmetics, products that contain medicinal, natural or nutritional ingredients such as vitamins, aloe and Chinese traditional medicines are very popular. Market data on the trends in these types of products is not readily available, but inputs for these types of products such as essential oils, grew by an average of 7% over the past three years. Note: U.S. companies seeking to market these types of products in China should seek assistance as to the proper labeling and marketing of these products, as China bans the use of terms such as natural and organic.

Color Cosmetics

Color Cosmetics are the second fastest growing product category, with demand rising over 10% in the past three years.. Hot products continue the focus on evening out skin tone, with color correcting creams and light weight foundations and tinted moisturizers growing in popularity. Color cosmetics comprise approximately 8% of U.S. exports to China in 2015, with lip and eye make-up among the most popular. Manicures and pedicures are increasingly popular especially among Chinese women who prize decorative coatings and artificial nails. There is also increasing interest and growing market for permanent make-up. The U.S. is currently the fourth largest exporter of these products to China, preceded by France, South Korea and Japan.

[8] Masidlover, Nadya. "L'Oréal Pulls Garnier Brand From China; French Cosmetics Firm's Mass-Market Brand Fails to Gain Traction in Key Emerging Market." Wall Street Journal Online. 08 Jan 2014.

Perfumes, Toilet Waters and Fragrances

Finished perfumes and toilet waters are a less promising product category, comprising only 5.2% of the total domestic market. It is a shrinking market for U.S. exports, growing by 15% in 2015. U.S. exports of fragrances inputs, however, are growing. The U.S. is the third largest exporter of fragrance inputs to China and sales of heavily scented personal care products such as personal toilet (bath washes, deodorants and shampoos) are growing rapidly.

See the following tables for more information on the U.S. and competing countries exports to China:

Table 3: China's Imports of Personal Care and Cosmetics Products from Brazil

Table 4: China's Imports of Personal Care and Cosmetics Products from Japan

Table 5: China's Imports of Personal Care and Cosmetics Products from France

Table 6: China's Imports of Personal Care and Cosmetics Products from Thailand

Table 7: China's Imports of Personal Care and Cosmetics Products from South Korea

Table 8: China's Imports of Personal Care and Cosmetics Products from the EU 28

Table 10: HS 34: Make Up Products, US Exports to China

Table 11: HS 35: Hair Care Products, US Exports to China

Table 12: HS 37: Cosmetic & Toilet Preparations, inc. Shaving, Bath Prep and Deodorants, US Exports to China

Top Domestic Trade Fairs

Cosmoprof Asia
November 16 – 18, 2016
Hong Kong Convention & Exhibition Center
Website: http://www.cosmoprof-asia.com/en-us/

Product Categories: Cosmetics & Toiletries, Natural Health, Beauty Salon, Hair Salon, Nail & Accessories, Pack & OEM

2015 Key Figures:

- 63,241 visits from 119 countries and regions
- 84,000 sqm of exhibition area
- 2,504 exhibitors from 46 Countries and Regions
- 22 country and group pavilions: Australia, Belgium, Brazil, California, Chile, China, France, Germany, Hong Kong, Italy, Japan, Korea, Poland, Spain, Switzerland, UK and USA etc.

Shanghai Beauty Expo
May 23 - 25, 2017

Shanghai New International Expo Center
Website: http://www.chinabeautyexpo.com/

Product Categories: Skin care & Cosmetics, Fragrance, Natural, Household detergent, Franchise & Retail Stores, OEM & Private Label, Beauty Salon, Accessories

2016 Key Figures:

- 387,000 visits from more than 100 countries and regions
- 200,000 sqm of exhibition area
- 2,568 exhibitors from 26 countries and regions
- 15 national and group pavilions: Australia, France, Germany, Hong Kong, Italy, Japan, Poland, Singapore, South Korea, Spain, UK, USA etc.

China International Beauty Expo (Guangzhou) Spring 2017
March 9 – 11, 2017
Guangzhou Import & Export Fair Complex
Website: http://www.chinainternationalbeauty.com/gz/

Product Categories: Beauty, Hair, Spa, Packaging, Professional Salon,

2016 Key Figures:

- 183,700 visits
- 260,000 sqm of exhibition area
- 2,000 exhibitors
- Exhibitors from 22 countries and regions, including U.S., Germany, Thailand, South Korea, Japan, Hong Kong etc.

Regulations, Standards, Customs and Tariffs

The Annex to this report provides a table of the requirements to sell cosmetics in China.

Cosmetics in China are categorized as ordinary and special use cosmetics. Perfume, skin care, shampoo and color cosmetics fall into ordinary products and special use products refer to hair dye, hair perm, hair-growing, sunblock, anti-spot, slimming, breast-beautifying, depilatories and deodorant etc.

According to the CFDA (China Food and Drug Administration), all foreign cosmetic product manufacturers must complete a safety and health quality test, and obtain a hygiene permit before they are allowed to sell in the Chinese market. Application for this pre-market approval process can only be carried out by a Chinese legal entity. Overseas cosmetics manufacturers without legal representation in China are thus required to apply for the permit through agent services. The Manufacture needs to sign a "Letter of Authorization" confirming that it authorizes a Chinese company to be the registration responsible party in mainland China for the products.

Safety and Health Quality Test
This test is performed by designated laboratories appointed by the CFDA and are listed on the CFDA website. All these labs have different testing capabilities designated for testing against specific conditions, such as microbiology, hygienic chemistry, toxicology test (which includes animal testing) or conducting safe-for–human-use trials (for special use cosmetics). The test normally takes 2-3 months for

ordinary cosmetics and 3-8 months for special-use cosmetics, while costs vary from $700 to $6,000 depending on the types and complexity of the products.

Following is the information on some of these testing organizations:

China Center for Diseases Control (China CDC)
Institute for Environmental Health and Related Product Safety
Phone: 86-10/6302-2960; Fax: 86-10/6317-0894
www.chinacdc.net.cn

Shanghai Center for Diseases Control (Shanghai CDC)
Environmental Health Section
Phone: 86-21/6275-8710 x 21; Fax: 86-21/6209-6059
www.scdc.sh.cn

Guangdong Center for Diseases Control (Guangdong CDC)
Public Health Research Institute
Phone: 86-20/8419-7952; Fax: 86-20/8446-9324
www.cdcp.org.cn

Hygiene Permit for Imported Cosmetics
Once testing is completed, the designated laboratory will issue a test report which needs to be submitted together with the other required documents for the application of the Hygiene Permit from CFDA. A committee under CFDA convenes to technical review and evaluate of imported cosmetics. The technical review time will be 3 months generally. If one application has been approved, a certificate will be issued by the CFDA. Companies need to submit the following documents (all translated in Chinese and notarized by a Chinese notarization company):

Application form for the cosmetic product to be imported

- Chinese product name and nomenclature;
- Product formula;
- Product quality and safety control file (The product info such as appearance, flavor, batch no and shelf life is required. Other quality control index like heavy metals and microbiology should be provided as well);
- Original product packaging including labelling information and product information sheet;
- Testing report and relevant data from testing organization certified by CFDA;
- Safety assessment report of cosmetics containing potential risk substances;
- Stamped copies of power of attorney and business license of Chinese responsible agent;
- Statement from manufacturer guaranteeing that materials used meet the requirements of BSE free regions.
- Free Sale Certificate at production country (region) or country (region) of origin
- Brief description and diagram of production process
- Technical requirements for cosmetic products in text
- Other relevant information which can support the application

The applicant will be notified by the CFDA within 5 days confirming whether the application is accepted or not. If the application is not accepted, the CFDA will provide explanation of discrepancies or missing documentation allowing the application to be resubmitted.

The Hygiene Certificate is valid for 4 years, and foreign manufacturers are required to renew it at least 4 months before it is expired.

The information of CFDA is as follows:

China Food and Drug Administration
Address: 26 Xuanwumen Xidajie, Beijing, 100053, P.R. China
Fax: 86-010-68310909
Email: inquires@sda.gov.cn
http://eng.sfda.gov.cn/WS03/CL0755/

Free Sales Certificate (FSC)
US companies exporting cosmetics to China needs to obtain a FSC from the FDA in the US indicating that their products are actually being sold and marketed freely in the US market. US FDA Center for Food Safety and Applied Nutrition (CFSAN) is able to issue the certificate at 10 USD in about 3 – 8 weeks.

Details about the FSC issued by FDA can be found at the following webpage:
http://www.fda.gov/Cosmetics/InternationalActivities/Exporters/ucm129593.htm

In addition to FDA, some US trade associations or State or local health department, e.g. California Department of Health also issue this certificate to US companies. Here is the contact information of two such trade associations:

Personal Care Products Council (PCPC)
1101 17th Street, NW, Suite 300, Washington D.C. 20036-4702
Phone: (202) 331-1770
Fax: (202) 331-1969
http://www.personalcarecouncil.org/

Independent Cosmetic Manufacturers & Distributors (ICMD)
1220 W. Northwest Hwy Palatine, Illinois 60067
Phone: 1-800-334-2623
Fax: 847-991-8161
http://www.icmad.org/membership/certificates.asp

Labeling
Cosmetic products imported into China must be labelled according to the mandatory National Standard GB 5296.3-2008 - Instruction for use of consumer products - general labelling for cosmetics. The manufacturer is required to list the following information, in clear simplified Chinese, on a label:

- Product name;
- Name and address of the manufacturer;
- Net content;
- Product ingredients;
- Shelf life;
- The code of hygiene permit or record-keeping certificate;
- Safety statement and guidance on uses (optional);

- For imported cosmetics, country of origin and the name and address of the distributor in China shall also be given on the label because they will shoulder the legal responsibilities in China as foreign manufacturers.

According to the Administrative Measures on the Regulation on the Cosmetics Advertisement (SAIC 1993), the following are prohibited on the label:

- Exaggerating claims in the name, production method, composition, performance or efficacy of the cosmetics
- Use of the name or image of a consumer or other endorser to prove effectiveness;
- Therapeutic claims and medical terms;
- Comparisons that denigrate similar products;
- Using absolute language;
- Data related to performance, function, sales and other similar features

Customs Clearance

After receiving the hygiene permit, foreign manufacturers are qualified to export their cosmetics to China. When goods arrive at Chinese ports, importers need to apply for an inspection from the local branch of the China Inspection and Quarantine Bureau (CIQ). CIQ's inspection is mandatory for cosmetics imported to China and will do a random sample check and conduct Physio-chemical and microbiological tests. CIQ will also review the Chinese label according to GB 5296.3-2008. If the goods pass CIQ's inspections, CIQ will issue a certificate of inspection which is then used by importers for customs clearance.

The following documents are needed to apply for CIQ inspection:

- A self-declaration letter stating that the imported cosmetic product complies with relevant Chinese laws and the normal use of the product will not cause any harm to human health;
- Product formula;
- Hygiene license or record-keeping certificate;
- Sample labels in Chinese, product labels in the original language and the translated text in Chinese;
- Information on the product name, volume/weight, specifications, country of origin, batch number, expiry date (production date and shelf life), target market, and information about packaging company;
- Other documentation required.

Tax & Tariff rate

HS Code	Description	Tariff	VAT	Consumption Tax
33030000* 33041000* 33042000* 33043000* 33049100*	Perfume Lip make-up Eye make-up Manicure or pedicure Powders	10%	17%	15%
33049900*	Skin care	2%	17%	15%

33051000	Shampoo	6.5%	17%	0%

Source: Customs Import and Export Tariff of the People's Republic of China

* Cosmetic products with sale prices of less than 10 yuan per millimeter or 15 yuan a tablet or sheet (exclusive VAT), consumption tax is 0%.

Animal Testing

Animal testing can be waived for China domestic non special use cosmetics from Jun 2014. But for imported cosmetics, all toxicological tests are still performed in animals to assess the safety of non-special-use and special use cosmetics, which are resulting in substantial numbers of U.S. personal care and cosmetics companies choosing to not export to China. Animal testing for cosmetics is a highly controversial practice and companies which conduct animal tests to meet China's requirements risk high-profile criticism from their clients and activists. For foreign companies claiming their products are cruelty free, there is a possible way for them to place their products in China without animal testing. They can export the bulk to China for filling and packing. Then the products manufactured under this way are regarded as domestic cosmetics. Currently, another way to avoid animal testing is selling cosmetics by cross-border e-commerce.

Intellectual Property Rights

Generally speaking, China's trademark registration is fairly inexpensive and straightforward. To show its commitment to WTO pledges, local judges are encouraged to promote rule of law and act against infringements on behalf of the foreign litigants. But, remember that China's system is 'first to register' rather than 'first to market'. So, if you are seriously looking at this market, registering BEFORE you enter China can save a lot of time, money and frustration should you face infringement at a later stage. The worst case scenario is to have a competitor or other local firms register your brand name in the early stages of your market entry, forcing you to fight an uphill battle for your name. To learn more about protecting your trademark in China, please visit: http://beijing.usembassy-china.org.cn/iprtrade.html

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Annex

Annex

I. **Market Readiness Checklist**

II. **Country Data**

COSMETICS MARKET READINESS ASSESSMENT

	ASEAN	China	Japan	EU	South Korea
Local Registration				No	
Legal Representative	Yes By Country	Yes	Yes	Yes	Yes
Product Registration or Notification	Both	Register	Notification	Both	Both
Frequency	Yes	Yes	Yes	Once	Once
By Product or Product Group	Product Group	Yes	Yes	Product	By Product
Product Specification Requirements					
Safety Assessment	Yes	Yes	No	Yes	Yes
Raw Material Registration	No	Yes	No		Yes
Formula: Quantative, Qualitative or Both	Yes	Yes	No	Both	Yes
Material Safety Data Sheet: Raw Materials	No	Yes	No	Yes	
Material Safety Data Sheet: Product	No	Yes	No	Yes	
Preservative Efficacy	Yes	Yes	No		Yes
Nationally Certified Testing Facility	NA	Yes	No	Yes	Yes
Animal Testing	No	Yes	No	No	No
Packaging Assessment	No	Yes	No	Yes	Yes
Product Stability	Yes	Yes	No	Yes	Yes
Good Manufacturing Practice	Yes	Yes	No	Yes	Yes
Proof of Claims	Yes	No	No	Yes	Yes
Trademark Registration	No	No	No	No	No
Product Sample		Yes	No		
Labeling					
Country of Origin	Yes	Yes	Yes	Yes	Yes
Nominal Content: Weight or Volume (?)	Yes	Yes	Yes	Yes	Yes
Registration Number	No	Yes	No	Yes	Yes
Language Requirement	Yes	Yes	Yes	Yes	Yes
Product Function	Yes	Yes	Yes	No	Yes
Formula: Quantative, Qualitative or Both	Yes	Yes	Yes	Yes	Yes
Fragrances	No	No	Yes	Yes	No
Percentage of Ingredients Claimed	No	No	Yes	No	No
Alcohol Content	No	No	No	No	No
Information on Product Use	Yes	Yes	No	Yes	Yes
Warning Statements	Yes	Yes	Yes	Yes	Yes
Manufacturer Name and Address	No	Optional	Yes	No	Yes
Importer Name and Address	Yes	Yes	No	Yes	Yes
Date of Durability	Yes	Yes	Yes	Yes	Yes
Storage Conditions	No	Yes	No	Yes	Yes
Expiration Date	Yes	No	No	Yes	Yes
Claims	Yes	Yes	Yes	Yes	Yes
Environmental Labeling	No	No	Yes	No	Yes
Certificate of Free Sale			Yes		
By Product	Yes	Yes		No	Yes
Post Market Monitoring	Yes	Yes	No		
Consumer Hot Line	No	No		Yes	Yes
Notification of Local Authorities	Yes	Yes	Yes	Yes	Yes
Recall	No	Yes	Yes	Yes	Yes
Government Fee to Notify/Register	See Annex	Yes $1,000 for ordinary	No	No	$5 for first three and $0.80 for additional
Time to Process	See Annex	4-8 months	1 Day	1 Day	3-4 Business Days

Requirements here are for ordinary cosmetics.
ASEAN includes: Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam
The EU is included for ease of comparison, given the overlapping requirements of the Cosmetics Regulation with many Asian countries.

COSMETICS MARKET READINESS ASSESSMENT
CHINA

Cosmetics Definition

China defines cosmetics as products which are applied to any parts of the human body (skin, hair, nails, lips, etc.) by rubbing, spraying, or other similar approaches for the purpose of cleaning, removing bad odors, protecting the skin, beautifying, and making up. Within this definition, China has two classes of cosmetics products: Ordinary and Special, which includes products that have active ingredients that change body functions. Products classified as Ordinary include: Skin Care (lotion, moisturizer, toner, anti-aging, anti-acne, etc); Make-up; Hair Care (shampoo, conditioner, etc); Perfumes and Nail Care. Special cosmetics include skin products to remove spots or whiten the skin; Sun Protection (SPF/PA); hair dyes, relaxers and other chemical treatments; Hair loss; Anti-Perspirants; Depilatory; Body Shaping and Child/Baby products.

	Requirements	Notes
Local Registration		
Legal Representative	Yes	Companies intending to import must assign a legal entity, known as the "responsible person" in China to undertake registration. This entity as well as the manufacturer are responsible for ensuring the product meet China's national standards. For products produced under a contract manufacturing agreement, a copy of the production agreement is required.
Product Registration or Notification	Register	
Frequency	Yes	Every four years, with sample testing not required for renewal, unless there is a change in product specifications or China's test standards.
By Product or Product Group	Yes	China allows by product type, so need to clarify if each individual product requires a registration.
Product Specification Requirements		
Safety Assessment	Yes	For products that contain ingredients that are restricted, a safety assessment is required. The assessment should be performed by a third licensed party, qualified in either medicine, pharmacy, chemistry, toxicology or relevant fields, and have over five years' experience.
Raw Material Registration	Yes	China has lists of prohibited, restricted and allowed ingredients, which includes conditions of use. If an ingredient is not on one of these lists, then it must be registered before use in a product. Letter of commitment stating that the cosmetic ingredients meet therestriction requirements of high-risk substances from regions with high incidence of mad cow disease.
Formula: Quantative, Qualitative or Both	Yes	China requires all imported products to be ingredient tested by local labs. In the product dossier, all ingredients present at 0.01% or greater should be identified by the Chinese INCI name, with special requirements for preservatives, sunscreens, colorants and hair dyes. Ingredients used in special cosmetics that provide function must be identified, with supporting information.
Material Safety Data Sheet: Raw Materials	Yes	
Material Safety Data Sheet: Product	Yes	
Preservative Efficacy	Yes	China provides requirements as to microbiological counts and types of permitted bacteria.
Nationally Certified Testing Facility	Yes	For products sold only in China, products must be tested on Chinese subjects.
Animal Testing	Yes	For both ordinary and special cosmetics.
Packaging Assessment	Yes	Direct contact materials should be toxin free.
Product Stability	Yes	Provide conditions required for safe storage of the product.
Good Manufacturing Practice	Yes	China follows ISO 22716 for Good Manufacturing Practices.
Proof of Claims	No	CFDA provides guidance on product claims, including guidance on product names. Claims of medicinal or therapeutical properities are prohibited.
Trademark Registration	No	
Product Sample	Yes	Samples of the product and packaging required
Labeling		
Country of Origin	Yes	Country of origin on the Chinese label.
Nominal Content: Weight or Volume	Yes	
Registration Number	Yes	Assigned at registration, representing product type, approved date and sequence number. Must be on the product.
Language Requirement	Yes	Chinese label.
Product Function	Yes	On the Chinese label, must include the Chinese product name per the Cosmetic Naming Rule
Formula: Quantative, Qualitative or Both	Yes	All ingredients present at 1% or greater should be identified by the Chinese INCI name. Some special requirements by products, e.g. baby products require nano labeling.
Fragrances	No	

Percentage of Ingredients Claimed	No	This refers to a requirement in some countries that percentages of active ingredients be listed.
Alcohol Content	No	
Information on Product Use	Yes	Safety tatement and guidance on use in Chinese
Warning Statements	Yes	In Chinese, warnings for products that contain restricted ingredients, as well as for special cosmetics.
Manufacturer Name and Address	Optional	Name and address of overseas manfucturer
Importer Name and Address	Yes	Local distribtor or wholesaler information with the company code on the product label.
Date of Durabilty	Yes	Combination of production date and period after manufacturing or batch number of data of minimum durability.
Storage Conditions	Yes	Should be provided if necessary for safety assurance.
Expiration Date	No	See comments on date of durability.
Claims	Yes	Misleading information, including medical and therapeutic claims are prohibited.
Environmental Labeling	No	
Certificate of Free Sale		
By Product	Yes	Accept from FDA, state governments or trade associations in the country of origin.
Post Market Monitoring	Yes	
Consumer Hot Line	No	
Notification of Local Authorities	Yes	The importer should register with local Administration of Industry and Commerce (AIC) and China AQSIQ, with its company name, address, POC, contact information and product information. The importer should also maintain a sales record including recall record. (Effective from March 1, 2017)
Recall	Yes	Importers should have procedures for registration and tracking of product delivery, processing and withdrawal of defective products, and inform AIC and AQSIQ in a timely manner.
Government Fee to Notify/Register	Yes $1,000	For ordinary cosmetics. Special use starts at $3,000 and ingredient registration starts at $12,000 not including testing fees and risk assessment.
Time to Process	4-8 months	4 - 8 months for ordinary cosmetics, 8 - 15 months for special use cosmetics

Product Registration

China has two levels of registration for imported cosmetics. Product classified as ordinary must apply for a registration certificate. Products classified as special must apply for a hygiene liscense. The registration requirements are somewhat similar, with special cosmetics having to justify function of active ingredients and provide a human safety assessment.

Additional Resources

1. Two guides for importing cosmetics to China are as follows:
   - Guidance in a Nutshell: Steps to Exporting Cosmetics Products to China. Reach24 Consulting Group. http://www.in-cosmetics.com/__novadocuments/45611?v=635274255261670000
   - Guidance on Exporting Cosmetics to China. CIRS Consulting Group: http://www.cirs-reach.com/Guidance_on_Exporting_Cosmetics_to_China_2012.pdf
2. CFDA requirements for registration of imported special use cosmetics: http://eng.sfda.gov.cn/WS03/CL0772/98105.html
3. CFDA requirements for registration of ordinary cosmetics: http://eng.sfda.gov.cn/WS03/CL0772/98099.html
4. CFDA requirements for approval of new cosmetics ingredients: http://eng.sfda.gov.cn/WS03/CL0772/98092.html
5. New CFDA safety and technical standard for cosmetics, including test methods and ingredient lists. Goes into effect December 2016: http://www.sda.gov.cn/directory/web/WS01/images/MjAxNcTqtdoyNji6xbmruOa4vbz+LnBkZg==.pdf

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EXHIBIT 2

Hygienic Standard for Cosmetics

Ministry of Health of the People's Republic of China January 2007

Catalogue

Part I General Principle

1 Scope

This specification specifies the hygiene requirements for cosmetic raw materials and their end-products. This specification applies to cosmetics sold in the People's Republic of China.

2 Normative references

欧盟化妆品规程, 76／768／EEC 及其 2005 年 11 月 21 日以前修订内容 (The Cosmetics Directive of the Council European Communities, 76／768／EEC, and amendments until 21 November 2005)。

3 Definition

Cosmetics are applied by rubbing, spraying or other similar methods, dispersed on any part of the human surface (skin, hair, nails, lips, etc.), in order to clean, eliminate bad odours, skin care, beauty and grooming purposes of daily use chemical industrial products.

4 Hygienic requirements for cosmetic products

4.1 General requirements

Under normal and reasonably foreseeable conditions of use, cosmetic products must not be hazardous to human health.

4.2 Raw material requirements

4.2.1 The use of the substances listed in Table 2(1) as cosmetic components is prohibited.

4.2.2 The use of substances listed in Table 2(2) as cosmetic components is prohibited.

4.2.3 Where the substances listed in Table 3 are used as components of cosmetic products, they must comply with the provisions of the table, including the range of use, the maximum permitted concentration, other restrictions and requirements, and the conditions of use and precautions that must be printed on the label.

4.2.4 Preservatives used in cosmetic products must be substances listed in Table 4 and must comply with the provisions of the table, including the maximum permitted concentration, the range and restrictions of use and the conditions of use and precautions that must be printed on the label.

4.2.5 Sunscreens used in cosmetics must be of the substances listed in Table 5 and must comply with the provisions of the table, including the maximum permitted concentration of use and the conditions of use and precautions that must be printed on the label.

4.2.6 Colourants used in cosmetics must be substances listed in Table 6 and must comply with the provisions of the table, including the permissible scope of use and other restrictions and requirements.

4.2.7 Hair colouring agents used in cosmetics must be substances listed in Table 7 and must comply with the provisions of the table, including the maximum permitted concentration, other restrictions and requirements and the conditions of use and precautions that must be printed on the label.

4.3 End product requirements

The ingredients used in cosmetics must comply with the requirements of 4.2 Ingredients above.

Cosmetics must be safe to use, must not cause significant irritation or damage to the application site and must not be infectious.

4.3.1 The microbiological quality of cosmetics should comply with the following regulations.

4.3.1.1 Cosmetics for the eyes and mucous membranes such as lips and mouth, as well as cosmetics for infants and children shall not have a total bacterial count greater than 500 CFU/mL or 500 CFU/g.

4.3.1.2 The total number of colonies for other cosmetics must not be greater than 1000 CFU/mL or 1000 CFU/g.

4.3.1.3 Fecal coliforms, Pseudomonas aeruginosa and Staphylococcus aureus shall not be detected per gram or per millilitre of product.

4.3.1.4 The total number of moulds and yeasts in cosmetics must not be greater than 100 CFU/mL or 100 CFU/g.

4.3.2 Toxic substances in cosmetics must not exceed the limits specified in Table 1.

Table 1 Toxic substance limits in cosmetic products

Common contaminants	Limit (mg/kg)	Remarks
Mercury	1	Except for eye cosmetics containing organic mercury preservatives
Lead	40	
Arsenic	10	
Methanol	2000	

5 Cosmetic packaging requirements

The materials of direct contact containers for cosmetics must be non-toxic and must not contain or release toxic substances that could cause harm to the user Quality.

Table 2(1) Prohibited components of cosmetic products[1][2]

(in alphabetical order)

No.	Chinese Name	English Name
1	α, α, α-三氯甲苯	α, α, α-Trichlorotoluene (CAS No 98-07-7)
2	α, α-二氯甲苯	α, α-Dichlorotoluene (CAS No 98-87-3)
3	α-氯甲苯	α-Chlorotoluene (CAS No100-44-7)
4	1-(1-萘基甲基)喹啉嗡	1-(1-Naphthylmethyl) quinolinium (CAS No 65322-65-8)
5	1-(4-氯苯基)-4,4-二甲基-3-(1,2,4-三唑-1-基甲基)戊-3-醇	1-(4-Chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol(CAS No 107534-96-3)
6	1-(4-甲氧基苯基)-1-戊烯-3-酮	1-(4-Methoxyphenyl)-1-penten-3-one (CAS No 104-27-8)
7	1,1,2-三氯乙烷	1,1,2-Trichloroethane (CAS No 79-00-5)
8	1,1,3,3,5-五甲基-4,6-二硝基茚满(伞花麝香)	1,1,3,3,5-Pentamethyl-4,6-dinitroindane (moskene)
9	硫酸[(1,1'-联苯)-4,4'-二基]二铵	[(1,1'-Biphenyl)-4,4'-diyl]diammonium sulphate (CAS No 531-86-2)
10	苯甲酸[1,1-双(二甲氨基甲基)]丙基酯(戊胺卡因, 阿立平)及其盐类	1,1-Bis(dimethylaminomethyl)propyl benzoate (amydricaine, alypine) and its salts
11	1,2,3,4,5,6-六氯环己烷, 在本附录中别处详细说明的那些除外	1,2,3,4,5,6-Hexachlorcyclohexanes with the exception of those specified elsewhere in this Annex
12	1,2,3-三氯丙烷	1,2,3-Trichloropropane (CAS No 96-18-4)
13	1,2,4-三唑	1,2,4-Triazole (CAS No 288-88-0)
14	1,2-苯基二羧酸支链和直链二C7-11 基酯	1,2-Benzenedicarboxylic acid di-C7-11, branched and linear alkylesters (CAS No 68515-42-4)
15	1,2-苯基二羧酸支链和直链二戊基酯 正戊基异戊基邻苯二甲酸酯 双正戊基邻苯二甲酸酯 双异戊基邻苯二甲酸酯	1,2-Benzenedicarboxylic acid, dipentylester, branched and linear(CAS No 84777-06-0) *n*-Pentyl-isopentylphthalate Di-*n*-pentyl phthalate (CAS No 131-18-0) Diisopentylphthalate (CAS No 605-50-5)
16	1,2-双(2-甲氧乙氧基)乙烷 三乙二醇二甲醚	1,2-Bis(2-methoxyethoxy)ethane triethylene glycol dimethyl ether (CAS No 112-49-2)
17	1,2-二溴-3-氯丙烷	1,2-Dibromo-3-chloropropane (CAS No 96-12-8)
18	1,2-二溴乙烷	1,2-Dibromoethane (CAS No 106-93-4)
19	1,2-环氧-3-苯氧基丙烷	1,2-Epoxy-3-phenoxypropane (CAS No 122-60-1)
20	1,2-环氧丁烷	1,2-Epoxybutane
21	1,3,5-三(环氧乙基甲基)-1,3,5-三嗪-2,4,6(1*H*,3*H*,5*H*)三酮	1,3,5-Tris(oxiranylmethyl)-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione (CAS No 2451-62-9)
22	1,3,5-三-[(2*S* 和 2*R*)-2,3-环氧丙基]-1,3,5 三嗪-2,4,6-(1*H*,3*H*,5*H*)-三酮	1,3,5-Tris-[(2*S* and 2*R*)-2,3-Epoxypropyl]-1,3,5-triazine-2,4,6-(1*H*,3*H*,5*H*)-trione (CAS No 59653-74-6)
23	1,3-双(乙烯基磺酰基乙酰氨基)-丙烷	1,3-Bis(vinylsulfonylacetamido)-propane (CAS No 93629-90-4)

24	1,3-二氯-2-丙醇	1,3-Dichloropropan-2-ol (CAS No 96-23-1)

No.	Chinese Name	English Name
25	1,3-二甲戊胺及其盐类	1,3-Dimethylpentylamine and its salts
26	1,3-二苯胍	1,3-Diphenylguanidine (CAS No 102-06-7)
27	1,3-丙磺酸内酯	1,3-Propanesultone (CAS No 1120-71-4)
28	1,4,5,8-四氨基蒽醌(分散蓝 1)	1,4,5,8-Tetraaminoanthraquinone (Disperse Blue 1) (CAS No 2475-45-8)
29	1,4-二氯苯(对-二氯苯)	1,4-Dichlorobenzene(p-dichlorobenzene) (CAS No 106-46-7)
30	1,4-二氯-2-丁烯	1,4-Dichlorobut-2-ene (CAS No 764-41-0)
31	11-α-羟基孕(甾)-4-烯-3,20-二酮及其酯类,羟基孕甾烯醇酮	11-Alpha-hydroxypregn-4-ene-3,20-dione and its esters
32	1-萘胺和 2-萘胺及它们的盐类	1-and 2-Naphthylamines and their salts
33	1-溴-3,4,5-三氟苯	1-Bromo-3,4,5-trifluorobenzene (CAS No 138526-69-9)
34	1-溴丙烷(正丙基溴化物)	1-Bromopropane(*n*-propyl bromide) (CAS No 106-94-5)
35	1-丁基-3-(*N*-巴豆酰对氨基苯磺酰)脲	1-Butyl-3-(*N*-crotonoylsulphanilyl)urea
36	1-氯-2,3-环氧丙烷	1-Chloro-2,3-epoxypropane (CAS No 106-89-8)
37	1-氯-4-硝基苯	1-Chloro-4-nitrobenzene (CAS No 100-00-5)
38	1-二甲基氨基甲基-1-甲基丙基苯甲酸(阿米卡因)及其盐类	1-Dimethylaminomethyl-1-methylpropyl benzoate (amylocaine) and its salts
39	1-乙基-1-甲基吗啉溴化物	1-Ethyl-1-methylmorpholinium bromide (CAS No 65756-41-4)
40	溴化 1-乙基-1-甲基吡咯烷鎓(盐)	1-Ethyl-1-methylpyrrolidinium bromide (CAS No 69227-51-6)
41	1-甲氧基-2,4-二氨基苯(2,4-二氨基茴香-CI76050)及其盐类	1-Methoxy-2,4-diaminobenzene (2,4-diaminoanisole-CI 76050) and their salts
42	1-甲氧基-2,5-二氨基苯(2,5-二氨基茴香)及其盐类	1-Methoxy-2,5-diaminobenzene (2,5-diaminoanisole) and their salts
43	1-甲基-3-硝基-1-亚硝基胍	1-Methyl-3-nitro-1-nitrosoguanidine (CAS No 70-25-7)
44	斑蝥素(表 3 中所列仅用于头发用品的斑蝥酊中所含斑蝥素除外)	(1*R*,2*S*)-Hexahydro-1,2-dimethyl-3,6-epoxyphthalic anhydride (cantharidin), with the exception of cantharides tincture listed in table 3
45	异艾氏剂	(1*R*,4*S*,5*R*,8*S*)-1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethano- naphthalene (isodrin-ISO)
46	异狄氏剂	(1*R*,4*S*,5*R*,8*S*)-1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4:5,8-dimethano-naphthalene (endrin-ISO)
47	1-乙烯基-2-吡咯烷酮	1-Vinyl-2-pyrrolidone (CAS No 88-12-0)
48	氯鼠酮	2-(2-(4-Chlorophenyl)-2-phenylacetyl) indan 1,3-dione (chlorophacinone-ISO)
49	(+/-)-2-(2,4-二氯苯基)-3-(1*H*-1,2,4-三唑-1-基)丙基-1,1,2,2-四氟乙醚	(+/-)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propyl-1,1,2,2-tetrafluoroethylether (CAS No 112281-77-3)
50	2-(2-甲氧基乙氧基)乙醇	2-(2-Methoxyethoxy)ethanol (CAS No 111-77-3)

No.	Chinese Name	English Name
51	2-(4-烯丙基-2-甲氧苯氧基)-*N*,*N*-二乙基乙酰胺及其盐类	2-(4-Allyl-2-methoxyphenoxy)-*N*,*N*-diethylacetamide and its salts
52	2-(4-叔-丁苯基)乙醇	2-(4-*tert*-Butylphenyl)ethanol (CAS No 5406-86-0)
53	2,2,2-三溴乙醇	2,2,2-Tribromoethanol (tribromoethyl alcohol)
54	2,2,2-三氯乙-1,1-二醇	2,2,2-Trichloroethane-1,1-diol
55	2,2,6-三甲基-4-哌啶基苯甲酸(苯扎明)及其盐类	2,2,6-Trimethyl-4-piperidyl benzoate (benzamine) and its salts
56	2,2'-(亚硝基亚氨基)双乙醇	2,2'-(Nitrosoimino)bisethanol (CAS No 1116-54-7)
57	2,2-二环氧乙烷	2,2'-Bioxirane (CAS No 1464-53-5)
58	2,2'-二羟基-3,3',5,5',6,6'-六氯代二苯基甲烷(六氯酚)	2,2'-Dihydroxy-3,3',5,5',6,6'-hexachlorodiphenylmethane (hexachlorophene)
59	2,2-二溴-2-硝基乙醇	2,2-Dibromo-2-nitroethanol (CAS No 69094-18-4)
60	2,3,4-三氯-1-丁烯	2,3,4-Trichlorobut-1-ene (CAS No 2431-50-7)
61	2,3,7,8-四氯二苯并-对-二口恶 口英	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
62	2,3-二溴-1-丙醇	2,3-Dibromopropan-1-ol (CAS No 96-13-9)
63	2,3-二氯-2-甲基丁烷	2,3-Dichloro-2-methylbutane
64	2,3-二氯丙烯	2,3-Dichloropropene (CAS No 78-88-6)
65	2,3-二硝基甲苯	2,3-Dinitrotoluene (CAS No 602-01-7)
66	2,3-环氧-1-丙醇	2,3-Epoxypropan-1-ol (CAS No 556-52-5)
67	2,3-环氧丙基.邻-甲苯基醚	2,3-Epoxypropyl o-tolyl ether (CAS No 2210-79-9)
68	2,4,5-三甲基苯胺 2,4,5-三甲基苯胺盐酸盐	2,4,5-Trimethylaniline (CAS No 137-17-7) 2,4,5-Trimethylaniline hydrochloride (CAS No 21436-97-5)
69	2,4,6-三氯苯酚	2,4,6-Trichlorophenol (CAS No 88-06-2)
70	2,4-二氨基苯乙醇及其盐类	2,4-Diaminophenylethanol and its salts
71	2,4-二羟基-3-甲基苯甲醛	2,4-Dihydroxy-3-methylbenzaldehyde (CAS No 6248-20-0)
72	2,5-二硝基甲苯	2,5-Dinitrotoluene (CAS No 619-15-8)
73	辛酸 2,6-二溴-4-氰苯酯	2,6-Dibromo-4-cyanophenyl octanoate (CAS No 1689-99-2)
74	(2,6-二甲基-1,3-二恶烷-4-基)乙酸酯	2,6-Dimethyl-1,3-dioxan-4-yl acetate (dimethoxane)
75	2,6-二硝基甲苯	2,6-Dinitrotoluene (CAS No 606-20-2)
76	2-[2-羟基-3-(2-氯苯基)氨基甲酰-1-萘基偶氮]-7-[2-羟基-3-(3-甲基苯基)-2-[2-羟基-3-(3-甲基苯基)-氨基甲酰-1-萘基偶氮]-7-[2-羟基-3-(3-甲基苯基)-氨基甲酰-1-萘基偶氮]芴-9-酮	2-[2-Hydroxy-3-(2-chlorophenyl)carbamoyl-1-naphthylazo]-7-[2-hydroxy-3- (3-methylphenyl) -2-[2-hydroxy-3-(3-methylphenyl)-carbamoyl-1-naphthylazo]-7-[2-hydroxy-3-(3-methylphenyl) -carbamoyl-1-naphthylazo]fluoren-9-one(EC No 420-580-2)

No.	Chinese Name	English Name
77	2-(4-甲氧苄基-*N*-(2-吡啶基)氨基)乙基二甲胺马来酸盐	2-[4-Methoxybenzyl-*N*-(2-pyridyl) amino] ethyldimethylamine maleate
78	2-{4-(2-氨 丙基氨基)-6-[4-羟基-3-(5-甲基-2-甲氧基-4-氨磺酰苯基偶氮)-2-磺化萘-7-基氨基]-1,3,5-三嗪基氨基}-2-氨基丙基甲酸盐	2-{4-(2-Ammonio propylamino)-6-[4-hydroxy-3-(5-methyl-2-methoxy-4-sulfamoylphenylazo)-2-sulfonatonaphth-7-ylamino]-1,3,5-triazin-2-ylamino}-2-aminopropyl formate(EC No 424-260-3)
79	乙酰胆碱及其盐类	2-Acetoxyethyltrimethylammonium hydroxide (acetylcholine)and its salts
80	2-α-环己烷基苯基(*N*,*N*,*N*',*N*'-四乙基)三亚甲基二胺	2-Alpha-cyclohexylbenzyl (*N*,*N*,*N*',*N*'-tetraethyl) trimethylenediamine (phenetamine)
81	2-氨基-1,2-双(4-甲氧苯基)乙醇及其盐类	2-Amino-1,2-bis(4-methoxyphenyl)ethanol and its salts
82	2-氨基-4-硝基苯酚	2-Amino-4-nitrophenol
83	2-氨基-5-硝基苯酚	2-Amino-5-nitrophenol
84	2-溴丙烷	2-Bromopropane (CAS No 75-26-3)
85	2-丁酮肟	2-Butanone oxime (CAS No 96-29-7)
86	2-氯-6-甲基嘧啶-4-基二甲基胺(杀鼠嘧啶)	2-Chloro-6-methylpyrimidin-4-yldimethylamine (crimidine-ISO)
87	3-羟基-4-苯基苯甲酸-2-二乙氨乙基酯及其盐类	2-Diethylaminoethyl 3-hydroxy-4-phenylbenzoate and its salts
88	2-乙氧基乙醇	2-Ethoxyethanol (CAS No110-80-5)
89	乙酸 2-乙氧基乙酯	2-Ethoxyethyl acetate (CAS No 111-15-9)
90	2-乙基己酸	2-Ethylhexanoic acid (CAS No 149-57-5)
91	乙酸 2-乙基己基[[[3,5-双(1,1-二甲基乙基)-4-羟苯基]-甲基]-硫代]酯	2-Ethylhexyl[[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-methyl]thio]acetate (CAS No 80387-97-9)
92	(2-异丙基戊-4-烯酰基)脲	(2-Isopropylpent-4-enoyl) urea (apronalide)
93	2-甲氧基乙醇	2-Methoxyethanol (CAS No109-86-4)
94	乙酸 2-甲氧基乙酯	2-Methoxyethyl acetate (CAS No 110-49-6)
95	乙酸 2-甲氧基丙酯	2-Methoxypronyl aceate (CAS No 70657-70-4)
96	2-甲氧基丙醇	2-Methoxypropanol (CAS No 1589-47-5)
97	2-甲基氮丙啶	2-Methylaziridine (CAS No 75-55-8)
98	2-甲基庚胺及其盐类	2-Methylheptylamine and its salts
99	二异氰酸 2-甲基-间-亚苯酯	2-Methyl-*m*-phenylene diisocyanate (CAS No 91-08-7)
100	2-甲基-间苯二胺	2-Methyl-*m*-phenylenediamine
101	2-萘酚	2-Naphthol
102	2-硝基茴香醚	2-Nitroanisole (CAS No 91-23-6)
103	2-硝基萘	2-Nitronaphthalene (CAS No 581-89-5)

No.	Chinese Name	English Name
104	2-硝基丙烷	2-Nitropropane (CAS No 79-46-9)
105	2-硝基甲苯	2-Nitrotoluene (CAS No 88-72-2)
106	2-亚戊基环己酮	2-Pentylidenecyclohexanone (CAS No 25677-40-1)
107	2-苯基茚满-1,3-二酮(苯茚二酮)	2-Phenylindan-1,3-dione (phenindione)
108	(2*RS*,3*RS*)-3-(2-氯苯基)-2-(4-氟苯基)-[(1*H*-1,2,4-三吡咯-1-基)甲基]环氧乙烷	(2*RS*,3*RS*)-3(2-Chlorophenyl)-2-(4-fluorophenyl)-[1*H*-1,2,4-triazol-1-yl]methyl]oxirane (CAS No 133855-98-8)
109	3-(1-萘基)-4-羟基香豆素	3-(1-Naphthyl)-4-hydroxycoumarin
110	3-(4-氯苯基)-1,1-二甲基尿素三氯乙酸盐; 灭草隆-TCA	3-(4-Chlorophenyl)-1,1-dimethyluronium trichloroacetate;monuron-TCA (CAS No 140-41-0)
111	3-(4-异丙苯基)-1,1-二甲脲	3-(4-Isopropylphenyl)-1,1-dimethylurea (CAS No 34123-59-6)
112	3,3'-二氯联苯胺	3,3'-Dichlorobenzidine (CAS No 91-94-1)
113	3,3'-二氯联苯胺二盐酸盐	3,3'-Dichlorobenzidine dihydrochloride (CAS No 612-83-9)
114	二硫酸二氢 3,3'-二氯联苯胺	3,3'-Dichlorobenzidine dihydrogen bis(sulphate) (CAS No 64969-34-2)
115	3,3'-二氯联苯胺硫酸盐	3,3'-Dichlorobenzidine sulphate (CAS No 74332-73-3)
116	3,3'-二甲氧基联苯胺	3,3'-Dimethoxybenzidine (CAS No119-90-4)
117	二硫酸氢[3,3'-二甲基[1,1'-联苯]-4,4'-二基]二铵	3,3'-Dimethyl [1,1'-biphenyl]-4,4'-diyl]diammonium bis(hydrogen sulphate) (CAS No 64969-36-4)
118	3,3-二(4-羟基苯基)2-苯并[C]呋喃酮(酚酞)	3,3-Bis (4-hydroxyphenyl) phthalide (phenolphthalein)
119	3,4,5-三甲氧苯乙基胺及其盐类	3,4,5-Trimethoxyphenethylamine and its salts
120	3,4',5-三溴水杨酰苯胺(三溴沙仑)	3,4',5-Tribromosalicylanilide (tribromsalan)
121	3,4-二羟基-2-甲氧基-2-甲基-4-苯基-2*H*,5*H* 吡咯(3,2-c)-(1)苯并吡喃-5-酮(环香豆素)	3,4-Dihydro-2-methoxy-2-methyl-4-phenyl-2*H*,5*H*,pyrano [3,2-c]-[1]benzopyran-5-one (cyclocoumarol)
122	3,4-二硝基甲苯	3,4-Dinitrotoluene (CAS No 610-39-9)
123	3,5,5-三甲基环-2-己烯酮	3,5,5-Trimethylcyclohex-2-enone (CAS No 78-59-1)
124	3,5-二溴-4-羟基苄腈	3,5-Dibromo-4-hydroxybenzonitrile (CAS No 1689-84-5)
125	3,5-二硝基甲苯	3,5-Dinitrotoluene (CAS No 618-85-9)
126	3,6,10-三甲基-3,5,9-十一碳三烯-2-酮	3,6,10-Trimethyl-3,5,9-undecatrien-2-one (CAS No 1117-41-5)
127	3,7-二甲基辛烯醇(6,7-二氢拢牛儿醇)	3,7-Dimethyl-2-octen-1-ol (6,7-Dihydrogeraniol) (CAS No 40607-48-5)
128	3'-乙基-5',6',7',8'-四氢-5',5',8',8'-四甲基-2'-乙酰萘(乙酰乙基四甲萘满,AETT)或 7-乙酰基-6-乙基-1,1,4,4-四甲基-1,2,3,4-四羟萘酚	3'-Ethyl-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-acetonaphthone (acetyl ethyl tetra methyl tetralin, AETT) or 7- acetyl-6-ethyl-1,1,4,4-tetramethyl-1,2,3,4- tetrahydronaphtalen
129	(3-氯苯基)-(4-甲氧基-3-硝基苯基)-2-甲基环乙酮	(3-Chlorophenyl)-(4-methoxy-3-nitrophenyl)methanone (CAS No 66938-41-8)

No.	Chinese Name	English Name
130	肉桂酸-3-二乙氨基丙酯	3-Diethylaminopropyl cinnamate
131	3-乙基-2-甲基-2-(3-甲基丁基)-1,3-氧氮杂环戊烷	3-Ethyl-2-methyl-2-(3-methylbutyl)-1,3-oxazolidine (CAS No 143860-04-2)
132	3-咪唑-4-基丙烯酸(尿刊酸)及其乙酯	3-Iimidazol-4-ylacrylic acid and its ethyl ester (urocanic acid)
133	(4-(4-羟基-3-碘苯氧基)-3,5-二碘苯基)乙酸及其盐类	[4-(4-Hydroxy-3-iodophenoxy)-3,5-diiodophenyl] acetic acid and its salts
134	4-(4-甲氧基苯基)-2-丁烯-2-酮	4-(4-Methoxyphenyl)-3-butene-2-one (CAS No 943-88-4)
135	4,4'-(4-亚氨基-2,5-亚环己二烯基亚甲基)双苯胺盐酸盐	4,4'-(4-Iminocyclohexa-2,5-dienylidenemethylene) dianiline hydrochloride (CAS No 569-61-9)
136	4,4'-二邻甲苯胺	4,4'-Bi-*o*-toluidine (CAS No 119-93-7)
137	4,4'-二-邻-甲苯胺二盐酸盐	4,4'-Bi-*o*-toluidine dihydrochloride (CAS No 612-82-8)
138	4,4'-二-邻-甲苯胺硫酸盐	4,4'-Bi-*o*-toluidine sulphate (CAS No74753-18-7)
139	4,4'-双(二甲基氨基)苯甲酮	4,4'-Bis(dimethylamino)benzophenone (Michler's ketone) (CAS No 90-94-8)
140	4,4'-碳亚氨基双(*N*,*N*-二甲基苯胺)	4,4'-Carbonimidoyl bis(*N*,*N*-dimethylaniline) (CAS No 492-80-8)
141	4,4'-二羟基-3,3'-(3-甲基硫代亚丙基) 双香豆素	4,4'-Dihydroxy-3,3'-(3-methylthiopropylidene) dicoumarin
142	4,4'-异丁基亚乙基联苯酚	4,4'-Isobutylethylidenediphenol (CAS No 6807-17-6)
143	4,4'-亚甲基双(2-乙基苯胺)	4,4'-Methylene bis (2-ethylaniline) (CAS No 19900-65-3)
144	4,4'-二苯氨基甲烷	4,4'-Methylenedianiline (CAS No 101-77-9)
145	4,4'-亚甲基-二-邻-甲苯胺	4,4'-Methylenedi-*o*-toluidine (CAS No 838-88-0)
146	4,4'-二氨基二苯醚(对氨基苯基醚)及其盐类	4,4'-Oxydianiline (*p*-aminophenyl ether) and its salts (CAS No 101-80-4)
147	4,4'-二氨基二苯硫醚及其盐类	4,4'-Thiodianiline and its salts (CAS No 139-65-1)
148	4,6-二甲基-8-特丁基香豆素	4,6-Dimethyl-8-*tert*-butylcoumarin (CAS No 17874-34-9)
149	[4-[[4-(二甲基氨基)苯基][4-[乙基(3-磺苯基)氨基]苯基]亚甲基]-2,5-亚环己二烯-1-基](乙基)(3-磺苯基)铵、钠盐	[4-[[4-(Dimethylamino)phenyl][4-ethyl(3-sulphonatobenzyl)amino]phenyl]methylene]cyclohexa-2,5-dien-1-ylidene](ethyl)(3-sulphonatobenzyl)ammonium, sodium salt (CAS No 1694-09-3)
150	4-[4-(1,3-二羟基丙-2-基)苯氨基]-1,8-二羟基-5-硝基蒽醌	4-[4-(1,3-Dihydroxyprop-2-yl)phenylamino]-1,8-dihydroxy-5-nitroanthraquinone (CAS No 114565-66-1)
151	4'-乙氧基-2-苯并咪唑苯胺	4'-Ethoxy-2-benzimidazoleanilide (CAS No 115-96-8)
152	4-氨基-2-硝基酚	4-Amino-2-nitrophenol
153	4-氨基偶氮苯	4-Aminoazobenzene (CAS No 60-09-3)
154	4-氨基水杨酸及其盐类	4-Aminosalicylic acid and its salts
155	4-苄氧基苯酚和 4-乙氧基苯酚	4-Benzyloxyphenol and 4-ethoxyphenol
156	辛酸 4-氰基-2,6-二碘苯酯	4-Cyano-2,6-diiodophenyl octanoate (CAS No 3861-47-0)

No.	Chinese Name	English Name
157	4-乙氧基-间-苯二胺及其盐类	4-Ethoxy-*m*-phenylenediamine and its salts
158	(4-肼基苯基)-*N*-甲基甲烷磺酰胺盐酸盐	4-Hydrazinophenyl)-*N*-methylmethanesulfonamide hydrochloride (CAS No 81881-96-8)
159	二异氰酸 4-甲基-间-亚苯酯	4-Methyl-*m*-phenylene diisocyanate (CAS No 584-84-9)
160	4-甲基-间-苯二胺及其盐类	4-Methyl-*m*-phenylenediamine and its salts
161	4-硝基联苯	4-Nitrobiphenyl (CAS No 92-93-3)
162	4-亚硝基苯酚	4-Nitrosophenol (CAS No 104-91-6)
163	4-邻-甲苯基偶氮-邻-甲苯胺	4-*o*-Tolylazo-*o*-toluidine(CAS No 97-56-3)
164	盐酸柠檬酸柯衣定盐	4-Phenylazophenylene-1, 3-diamine citrate hydrochloride (chrysoidine citrate hydrochloride)
165	4-苯基丁-3-烯-2-酮	4-Phenylbut-3-en-2-one
166	4-叔丁基-3-甲氧基-2,6-二硝基甲苯(葵子麝香)	4-*tert*-Butyl-3-methoxy-2,6-dinitrotoluene (musk ambrette)
167	4-叔丁基苯酚	4-*tert*-Butylphenol
168	4-叔丁基邻苯二酚	4-*tert*-Butylpyrocatechol
169	5-(α,β-二溴苯乙基)-5-甲基乙内酰脲	5-(α,β-Dibromophenethyl)-5-methylhydantoin
170	5-(2,4-二氧代-1,2,3,4-四氢嘧啶)-3-氟-2-羟基甲基四氢呋喃	5-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidine)-3-fluro-2-hydroxymethylterahydrofuran (CAS No 41107-56-6)
171	5-(3-丁酰基-2,4,6-甲基苯基)-2-[1-(乙氧基亚氨基)丙基]-3-羟基环已-2-烯-1-酮	5-(3-Butyryl-2,4,6-trimethylphenyl)-2-[1-(ethoxyimino)propyl]-3-hydroxycyclohex-2-en-1-one (CAS No 138164-12-2)
172	二次亚碘酸 5,5'-二异丙基-2,2'-二甲基联苯-4,4'-二基酯	5,5'-Di-isopropyl-2,2'-dimethylbiphenyl-4,4'-diyl dihypoiodite
173	5,5-二苯基-4-咪唑酮	5,5-Diphenyl-4-imidazolidone
174	5,6,12,13-四氯蒽(2,1,9-d,e,f,:6,5,10-d',e',f')二异喹啉-1,3,8,10(2*H*,9*H*)四酮	5,6,12,13-Tetrachloroanthra(2,1,9-def:6,5,10-d'e'f')diisoquinoline-1,3,8,10(2*H*,9*H*)-tetrone (CAS No 115662-06)
175	5-氯-1,3-二羟基-2H-吲哚-2-酮	5-Chloro-1,3-dihydro-2H-indol-2-one (CAS No 17630-75-0)
176	5-乙氧基-3-三氯甲基-1,2,4-硫代二唑	5-Ethoxy-3-trichloromethyl-1,2,4-thiadiazole (CAS No 2593-15-9)
177	5-甲基-2,3-己二酮	5-Methyl-2,3-hexanedione (CAS No 13706-86-0)
178	5-硝基二氢苊	5-Nitroacenaphthene (CAS No 602-87-9)
179	5-硝基-*o*-甲苯胺 5-硝基-*o*-甲苯胺盐酸盐	5-Nitro-*o*-toluidine (CAS No 99-55-8) 5-Nitro-*o*-toluidine hydrochloride (CAS No 51085-52-0)
180	5-叔丁基-1,2,3-三甲基-4,6-二硝基苯(西藏麝香)	5-*tert*-Butyl-1, 2, 3-trimethyl-4, 6 –dinitrobenzene (musk tibetene)
181	6-(2-氯乙基)-6-(2-甲氧乙氧基)-2, 5, 7, 10-四氧杂-6-硅杂十一烷	6-(2-Chloroethyl)-6-(2-methoxyethoxy)-2,5,7,10-tetraoxa-6-silaundecane (CAS No 37894-46-5)

No.	Chinese Name	English Name
182	甲酸(6-(4-羟基-3-(2-甲氧基苯偶氮基)-2-磺基-7-萘胺基)-1,3,5-三嗪-2,4-基)双[(氨基-1-甲基乙基)铵]	6-(4-Hydroxy-3-(2-methoxyphenylazo)-2-sulfonato-7-naphthylamino)-1,3,5-triazine-2,4-diyl)bis[(amino -1-methylethyl)ammonium]formate (CAS No 108225-03-2)
183	6-(哌嗪基)-2,4-嘧啶二胺-3-氧化物(米诺地尔)及其盐和衍生物	6-(Piperidinyl)-2,4-pyrimidinediamine-3-oxide (minoxidil) and its salts and derivatives
184	6,10-二甲基-3,5,9-十二碳三烯-2-酮	6,10-Dimethyl-3,5,9-undecatrien-2-one (CAS No 141-10-6)
185	6-羟基-1-(3-异丙氧基丙基)-4-甲基-2-氧-5-[4-(苯偶氮基)苯偶氮基]-1,2-二羟-3-吡啶腈	6-Hydroxy-1-(3-isopropoxypropyl)-4-methyl-2-oxo-5-[4-(phenylazo)phenylazo]-1,2-dihydro-3-pyridine carbo-nitrile (CAS No 85136-74-9)
186	6-异丙基-2-十氢萘酚	6-Isopropyl-2-decahydronaphthalenol (CAS No 34131-99-2)
187	2-甲氧基-5-甲基苯胺	6-Methoxy-*m*-toluidine(*p*-cresidine) (CAS No 120-71-8)
188	7,11-二甲基-4,6,10-十二碳三烯-3-酮	7,11-Dimethyl-4,6,10-dodecatrien-3-one (CAS No 26651-96-7)
189	7-[2-羟基-3-(2-羟乙基-*N*-甲氨基)丙基]茶碱	7-[2-Hydroxy-3-(2-hydroxyethyl-*N*-methylamino) propyl] theophylline (xanthinol)
190	7-乙氧基-4-甲基香豆素	7-Ethoxy-4-methylcoumarin(CAS No 87-05-8)
191	7-甲氧基香豆素	7-Methoxycoumarin (CAS No 531-59-9)
192	7-甲基香豆素	7-Methylcoumarin (CAS No 2445-83-2)
193	9-乙烯基咔唑	9-Vinylcarbazole (CAS No 1484-13-5)
194	4-(7-羟基-2,4,4-三甲基-2-苯并二氢吡喃基)间苯二酚-4-基-三(6-重氮基-5,6-二氢化-5-氧代萘-1-磺酸盐)和 4-(7-羟基-2,4,4-三甲基-2-苯并二氢吡喃基)间苯二酚双(6-重氮基-5,6-二氢化-5-氧代萘-1-磺酸盐)的 2:1 混合物	A 2:1 mixture of: 4-(7-hydroxy-2,4,4-trimethyl-2-chromanyl)resorcinol-4-yl-tris(6-diazo-5,6-dihydro-5-oxonaphthalen-1-sulfonate) and 4-(7-hydroxy-2,4,4-trimethyl-2-chromanyl) resorcinolbis(6-diazo-5,6-dihydro-5-oxonaphthalen-1-sulfonate) (CAS No 140698-96-0)
195	1,3,5-三(3-氨基甲基苯基)-1,3,5-(1*H*,3*H*,5*H*)-三嗪-2,4,6-三酮和 3,5-双(3-氨基甲基苯基)-1-聚[3,5-双(3-氨基甲基苯基)-2,4,6-三氧代-1,3,5-(1*H*,3*H*,5*H*)-三嗪-1-基]-1,3,5-(1*H*,3*H*,5*H*)-三嗪-2,4,6-三酮混合低聚物的混合物	A mixture of: 1,3,5-tris(3-aminomethylphenyl)-1,3,5-(1*H*,3*H*,5*H*)-triazine-2,4,6-trione and a mixture of oligomers of 3,5-bis(3-aminomethylphenyl)-1-poly[3,5-bis(3-aminomethylphenyl) -2,4,6-trioxo-1,3,5-(1*H*,3*H*,5*H*)-triazin-1-yl]-1,3,5-(1*H*,3*H*,5*H*)-triazine-2,4,6-trione(EC No 421-550-1)
196	4-[[双-(4-氟苯基)甲基甲硅烷基]甲基]-4H-1,2,4-三唑和 1-[[双-(4-氟苯基)甲基甲硅烷基]甲基]-1H-1,2,4-三唑的混合物	A mixture of: 4-[[bis-(4-fluorophenyl)methylsilyl]methyl]-4H-1,2,4-triazole and 1-[[bis-(4-fluorophenyl)methyl-silyl]methyl]-1H-1,2,4-triazole(EC No 403-250-2)
197	下列化合物的混合物: 4-烯丙基-2,6-双(2,3-环氧丙基)苯酚,4-烯丙基-6-(3-(6-(3-(4-烯丙基-2,6-双(2,3-环氧丙基)-苯氧基)2-羟基丙基)-4-烯丙基-2-(2,3-环氧丙基)-苯氧基-2-羟基丙基)-4-烯丙基-2-(2,3-环氧丙基)-苯氧基-2-羟基丙基-2-(2,3-环氧丙基)苯酚,4-烯丙基-6-(3-(4-烯丙基-2,6-双(2,3-环氧丙基)- 苯氧基-2-羟基丙基)-2-(2,3-环氧丙基)苯氧基)苯酚和 4-烯丙基-6-(3-(6-(3-(4-烯丙基-2,6-双(2,3-环氧丙基)-苯氧基)-2-羟基丙基)-4-烯丙基-2-(2,3-环氧丙基)苯氧基)2-羟基丙基)-2-(2,3-环氧丙基)苯酚	A mixture of: 4-allyl-2,6-bis (2,3-epoxypropyl)phenol, 4-allyl-6-(3-(6-(3-(4-allyl-2,6-bis(2,3-epoxypropyl)-phenoxy)2-hydroxypropyl)-4-allyl-2-(2,3-epoxypropyl)phenoxy)-2-hydroxypropyl)-4-allyl-2-(2,3-epoxypropyl)-phenoxy-2-hydroxypropyl-2-(2,3-epoxypropyl)phenol,4-allyl-6-(3-(4-allyl-2,6-bis(2,3-epoxypropyl)phenoxy)-2-hydroxypropyl)-2-(2,3-epoxypropyl)phenoxy)phenol and 4-allyl-6-(3-(6-(3-(4-allyl-2,6-bis(2,3-epoxypropyl)-phenoxy)-2-hydroxypropyl)-4-allyl-2-(2,3-epoxypropyl)phenoxy)2-hydroxypropyl)-2-(2,3-epoxypropyl)phenol(EC No 417-470-1)
198	5-[(4-[(7-氨基-1-羟基-3-硫代-2-萘基)偶氮]-2,5-二乙氧基苯基)偶氮]-2-[(3-膦酰基苯基)偶氮]苯甲酸和 5-[(4-[(7-氨基-1-羟基-3-硫代-2-萘基)偶氮]-2,5-二乙氧基苯基)偶氮]-3-[(3-膦酰基苯基)偶氮]苯甲酸的混合物	A mixture of: 5-[(4-[(7-amino-1-hydroxy-3-sulfo-2-naphthyl) azo]-2,5-diethoxyphenyl)azo]-2 -[(3-phosphonophenyl)azo]benzoic acid and 5-[(4-[(7-amino-1-hydroxy-3-sulfo-2-naphthyl)azo]-2,5-diethoxyphenyl)azo]-3-[(3-phosphonophenyl) azo]benzoic acid (CAS No 163879-69-4)

No.	Chinese Name	English Name
199	4-(3-乙氧基羰基-4-(5-(3-乙氧基羰基-5-羟基-1-(4-磺酸基苯基)吡唑-4-基)戊-2,4-二烯基)-4,5-二氢化-5-氧代吡唑-1-基)苯磺酸二钠盐和 4-(3-乙氧基羰基-4-(5-(3-乙氧基羰基-5-环氧基-1-(4-磺酸基苯基)吡唑-4-基)戊-2,4-二烯基)-4,5-二氢化-5-氧代吡唑-1-基)苯磺酸三钠盐的混合物	A mixture of: disodium 4-(3-ethoxycarbonyl-4-(5-(3-ethoxycarbonyl-5-hydroxy-1-(4-sulfonatophenyl)pyrazol-4-yl)penta-2,4-dienylidene)-4,5-dihydro-5-oxopyrazol-1-yl) benzenesulfonate and trisodium 4-(3-ethoxycarbonyl-4-(5-(3-ethoxycarbonyl-5-oxido-1-(4-sulfonatophenyl)pyrazol-4-yl)penta-2,4-dienylidene)-4,5-dihydro-5-oxopyrazol-1-yl) benzenesulfonate (EC No 402-660-9)
200	*N*-[3-羟基-2-(2-甲基丙烯酰氨基甲氧基)丙氧基甲基]-2-甲基丙烯酰胺和 N-2,3-双-(2-甲基丙烯酰氨基甲氧基)丙氧基甲基)-2-甲基丙烯酰胺和甲基丙烯酰胺和 2-甲基-N-(2-甲基丙烯酰氨基甲氧基甲基)-丙烯酰胺和 N-(2,3-二羟基丙氧基甲基)-2-甲基丙烯酰胺的混合物(EC No 412-790-8)	A mixture of: *N*-[3-Hydroxy-2-(2-Methylacryloylaminomethoxy)propoxymethyl]-2-methylacrylamide and N-2,3-bis-(2-Methylacryloylaminomethoxy)propoxymethyl]-2-methylacrylamide and methacrylamide and 2-methyl-N-(2-methylacryloylaminomethoxymethyl)-acrylamide and N-(2,3-dihydroxypropoxymethyl)-2-methylacrylamide(EC No 412-790-8)
201	4,4'-亚甲基双[2-(4-羟基苄基)-3,6-二甲基苯酚]和 6-重氮基-5,6-二氢化-5-氧代-萘磺酸盐的 1:2 反应产物及 4,4'-亚甲基双[2-(4-羟基苄基)-3,6-二甲基苯酚]和6-重氮基-5,6-二氢化-5-氧代萘磺酸盐的1:3 反应产物的混合物	A mixture of: reaction product of 4,4'-methylenebis[2-(4-hydroxybenzyl)-3,6-dimethylphenol] and 6-diazo-5,6-dihydro-5-oxo-naphthalenesulfonate(1:2) and reaction product of 4,4'-methylenebis [2-(4-hydroxybenzyl)-3,6-dimethylphenol] and 6-diazo-5,6-dihydro-5-oxonaphthalenesulfonate(1:3)(EC No 417-980-4)
202	苯并[a]芘的含量大于0.005%(w/w)的吸收油，来自双环芳烃和杂环碳氢化合物馏分	Absorption oils, bicyclo arom and heterocylic hydrocarbon fraction (CAS No 101316-45-4), if they contain> 0.005% (w/w) benzo[a]pyrene
203	醋硝香豆素	Acenocoumarol [3-(2-acetyl-1-(p-nitrophenyl) ethyl)-4-hydroxycoumarin]
204	乙酰胺	Acetamide (CAS No 60-35-5)
205	乙腈	Acetonitrile
206	乌头碱(欧乌头主要生物碱)及其盐类	Aconitine (principal alkaloid of *aconitum napellus L.*) and its salts
207	欧乌头属(叶子、根和草药制剂)	*Aconitum napellus L.* (leaves, roots and galenical preparations)
208	丙烯酰胺，在本规范的别处规定的除外	Acrylamide,unless regulated dlsewhere in this Directive (CAS No 79-06-1)
209	丙烯腈	Acrylonitrile (CAS No 107-13-1)
210	侧金盏花及其制剂	*Adonis vernalis L.* and its preparations
211	甲草胺；草不绿	Alachlor (CAS No 15972-60-8)
212	土木香根油	Alanroot oil (*Inula helenium*) (CAS No 97676-35-2)
213	艾氏剂	Aldrin (CAS No 309-00-2)
214	五氰亚硝酰基高铁酸碱金属盐	Alkali pentacyanonitrosylferrate (2-)
215	五氯苯酚的碱金属盐	Alkali salts of pentachlorophenol (CAS No 131-52-2and7778-73-6)
216	丁二烯含量大于0.1%(w/w)的C_{1-2}链烷烃	Alkanes, C_{1-2} (CAS No 68475-57-0), if they contain > 0.1%(w/w) butadiene
217	C_{12-26}支链和直链烷烃，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Alkanes, C_{12-26}-branched and linear (CAS No 90622-53-0), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
218	丁二烯含量大于0.1%(w/w)的富C_3的C_{1-4}烷烃	Alkanes, C_{1-4}, C_3-rich (CAS No 90622-55-2), if they contain > 0.1%(w/w) butadiene

No.	Chinese Name	English Name
219	丁二烯含量大于0.1%(w/w)的C_{2-3}链烷烃	Alkanes, C_{2-3} (CAS No 68475-58-1), if they contain > 0.1%(w/w) butadiene
220	丁二烯含量大于0.1%(w/w)的C_{3-4}链烷烃	Alkanes, C_{3-4} (CAS No 68475-59-2), if they contain > 0.1%(w/w) butadiene
221	丁二烯含量大于0.1%(w/w)的C_{4-5}链烷烃	Alkanes, C_{4-5} (CAS No 68475-60-5), if they contain > 0.1%(w/w) butadiene
222	氯代C_{10-13}烷烃	Alkanes,C_{10-13} chloro (CAS No 85535-84-8)
223	炔醇类以及它们的酯类、醚类、盐类	Alkyne alcohols, their esters、ethers and salts
224	阿洛拉胺及其盐类	Alloclamide and its salts (2-allyloxy-4-choro-*N*- (2-diethylaminoethyl) benzamide)
225	烯丙基氯(3-氯丙烯)	Allyl chloride (3-chloropropene) (CAS No 107-05-1)
226	烯丙缩水甘油醚	Allyl glycidyl ether (CAS No 106-92-3)
227	烯丙基芥子油(异硫氰酸烯丙酯)	Allyl isothiocyanate
228	α-哌嗪-2-基苄基乙酸酯左旋的苏型(左法哌酯)及其盐类	Alpha-piperidin-2-yl benzyl acetate laevorotatory threoform (levophacetoperane) and its salts
229	α-山道年	Alpha-santonin [(3*S*,5a*R*,9b*S*)-3,3a,4,5,5a,9b-hexahydro-3,5a,9-trimethylnaphto [1,2-b] furan-2,8-dione]
230	氨基已酸及其盐类	Aminocaproic acid (6-aminohexanoic acid) and its salts
231	阿米替林及其盐类	Amitriptyline (5- (3-dimethylaminopropylidene)-10, 11-dihydro-5*H*-dibenzo-(a,d) cycloheptene) and its salts
232	杀草强(氨三唑)	Amitrole (CAS No 61-82-5)
233	大阿米芹及其植物制剂	*Ammi majus* and its galenical preparations
234	4-二甲氨基苯甲酸戊酯,混合的异构体(帕地马酯)	Amyl 4-dimethylaminobenzoate, mixed isomers (padimate A (INN))
235	亚硝酸戊酯类	Amyl nitrites
236	印防己(果实)	*Anamirta cocculus L.* (fruit)
237	苯胺及其盐类以及卤化、磺化的衍生物类	Aniline, its salts and its halogenated and sulphonated derivatives
238	蒽油	Anthracene oil
239	甾族结构的抗雄激素物质	Antiandrogens of steroidal structure
240	抗生素类	Antibiotics
241	锑及锑化合物	Antimony and its compounds
242	加拿大大麻(夹竹桃麻,大麻叶罗布麻)及其制剂	*Apocynum cannabinum L.* and its preparations
243	阿扑吗啡及其盐类	Apomorphine (R5,6,6a,7-tetrahydro-6-methyl-4H-dibenzo (de,g)-quinoline-10,11-diol) and its salts
244	槟榔碱	Arecoline (methyl 1,2,5,6-tetrahydro-1-methylnicotinate)
245	马兜铃酸及其酯(盐)；马兜铃属及其制剂	Aristolochic acid and its salts; *Aristolochia* spp and their preparations
246	苯并[a]芘的含量大于0.005%(w/w)的C_{20-28}多环烃芳碳氢化合物,来自煤	Aromatic hydrocarbons, C_{20-28}, polycyclic, mixed coal-tar pitch-polyethylene polypropylene

No.	Chinese Name	English Name
	焦油沥青与聚乙烯聚丙烯混合物的热解衍生物	pyrolysis-derived(CAS No 101794-74-5), if they contain > 0.005% (w/w) benzo[a]pyrene
247	苯并[a]芘的含量大于0.005%(w/w)的C_{20-28}多环芳烃碳氢化合物，来自煤焦油沥青与聚乙烯混合物的热解衍生物	Aromatic hydrocarbons, C_{20-28}, polycyclic, mixed coal-tar pitch-polyethylene pyrolysis-derived (CAS No101794-75-6), if they contain > 0.005% (w/w) benzo[a]pyrene
248	苯并[a]芘的含量大于0.005%(w/w)的C_{20-28}多环芳烃碳氢化合物，来自煤焦油沥青与聚苯乙烯混合物的热解衍生物	Aromatic hydrocarbons, C_{20-28}, polycyclic, mixed coal-tar pitch-polystyrene pyrolysis-derived (CAS No 101794-76-7), if they contain > 0.005% (w/w) benzo[a]pyrene
249	砷及砷化合物	Arsenic and its compounds
250	石棉	Asbestos (CAS No 12001-28-4)
251	颠茄及其制剂	*Atropa belladonna L.* and its preparations
252	阿托品及其盐类和衍生物	Atropine, its salts and derivatives
253	阿扎环醇及其盐类	Azacyclonol (α,α-diphenyl-α-piperid-4-ylmethanol) and its salts
254	唑啶草酮	Azafenidin(CAS No 68049-83-2)
255	吖丙啶; 1-氮杂环丙烷; 环乙亚胺	Aziridine (CAS No 151-56-4)
256	偶氮苯	Azobenzene (CAS No 103-33-3)
257	巴比妥酸盐类	Barbiturates
258	钡盐类(除硫酸钡, 表 3 中的硫化钡及表 6 中着色剂的不溶性钡盐, 色淀和颜料外)	Barium salts, with the exception of barium sulphate, barium sulphide under the conditions laid down in table 3, and lakes, salts and pigments prepared from the colouring agents listed in table 6
259	贝美格及其盐类	Bemegride (ethyl-3-methylglutarimide)and its salts
260	贝那替秦	Benactyzine (2-diethylaminoethyl benzilate)
261	苄氟噻嗪及其衍生物	Bendroflumethiazide (3-benzyl-3,4-dihydro-6-trifluoromethyl-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide) and its derivatives
262	苯菌灵; 苯雷特	Benomyl (CAS No 17804-35-2)
263	苯并[e]醋亚菲	Benz(e)acephenanthrylene (CAS No 205-99-2)
264	苯并[a]蒽	Benz[a]anthracene (CAS No 56-55-3)
265	苯扎托品及其盐类	Benzatropine (tropine benzhydryl ether; 3-(diphenylmethoxy)tropane) and its salts
266	苯并吖庚因及苯并二吖庚因	Benzazepines and benzodiazepines
267	苯	Benzene
268	联苯胺(4,4'-二氨基联苯)	Benzidine (4,4'-diaminobiphenyl)
269	乙酸联苯胺	Benzidine acetate (CAS No 36341-27-2)
270	联苯胺基偶氮染料	Benzidine based azo dyes

No.	Chinese Name	English Name
271	二盐酸联苯胺	Benzidine dihydrochloride (CAS No 531-85-1)
272	硫酸联苯胺	Benzidine sulphate (CAS No 21136-70-9)
273	苯咯溴铵	Benzilonium bromide (1,1-diethyl-3-hydroxypyrrolidinium bromide benzilate)
274	苯并咪唑-2(3*H*)-酮	Benzimidazol-2(3*H*)-one
275	苯并[k]荧蒽	Benzo(k)fluoranthene (CAS No 207-08-9)
276	苯并[a]芘	Benzo[def]chrysene (=benzo[a]pyrene) (CAS No 50-32-8)
277	苯并[e]芘	Benzo[e]pyrene (CAS No 192-97-2)
278	苯并[j]荧蒽	Benzo[j]fluoranthene (CAS No 205-99-2)
279	4-羟基-3-甲氧基肉桂醇的苯甲酸酯(天然精油中的规定含量除外)	Benzoates of 4-hydroxy-3-methoxycinnamyl alcohol except for normal content in natural essences used
280	2,4-二溴-丁酸苄酯	Benzyl 2,4-dibromobutanoate (CAS No 23085-60-1)
281	苯基丁基邻苯二甲酸酯	Benzyl butyl phthalate(CAS No 85-68-7)
282	苄基氰	Benzyl cyanide (CAS No 140-29-4)
283	铍及铍化合物	Beryllium and its compounds
284	贝托卡因及其盐类	Betoxycaine (2-(2-diethylaminoethoxy) ethyl 3-amino-4-butoxybenzoate) and its salts
285	比他维林	Bietamiverine (2-diethylaminoethyl α-phenyl-1-piperidineacetate)
286	乐杀螨	Binapacryl (CAS No 485-31-4)
287	联苯-2-基胺	Biphenyl-2-yl amine (CAS No 90-41-5)
288	4-氨基联苯及其盐	Biphenyl-4-ylamine (CAS No 92-67-1) and its salts
289	邻苯二甲酸双(2-乙基己基)酯	Bis (2-ethylhexyl) phthalate (CAS No 117-81-7)
290	邻苯二甲酸双(2-甲氧乙基)酯	Bis (2-methoxyethyl) phthalate (CAS No 117-82-8)
291	双(2-甲氧基乙基)醚	Bis (2-methyoxyethyl) ether (CAS No 111-96-6)
292	双-(2-氯乙基)醚	Bis(2-chloroethyl) ether (CAS No 111-44-4)
293	双(环戊二烯基)-双(2,6-二氟-3-(吡咯-1-基)-苯基)钛	Bis(cyclopentadienyl)-bis(2,6-difluoro-3-(pyrrol-1-yl)-phenyl)titanium(CAS No125051-32-3)
294	双酚 A(二酚基丙烷)	Bisphenol A(4,4'-isopropylidenediphenol) (CAS No 80-05-7)
295	硫氯酚	Bithionol [2,2'-thiobis (4,6-dichlorophenol)]
296	托西溴苄铵	Bretylium tosilate ((o-bromobenzyl) ethyldimethylammonium p-toluenesulfonate)
297	溴(元素状态)	Bromine, elemental
298	溴米索伐	Bromisoval (1-(2-bromo-3-methylbutyryl) urea)

No.	Chinese Name	English Name
299	溴乙烷	Bromoethane (CAS No 74-96-6)
300	溴乙烯	Bromoethylene (CAS No593-60-2)
301	溴代甲烷	Bromomethane (CAS No 74-83-9)
302	溴苯腈庚酸酯	Bromoxynil heptanoate (ISO)(CAS No 56634-95-8)
303	溴苯那敏及其盐类	Brompheniramine (3-(p-bromophenyl)-N,N-dimethyl-3-pyrid-2-ylpropylamine) and its salts
304	番木鳖碱	Brucine
305	丁二烯	Buta-1,3-diene (CAS No 106-99-0)
306	丁二烯含量大于或等于0.1%(w/w)的丁烷	Butane (CAS No 106-97-8), if it contains ≥0.1%(w/w) butadiene
307	布坦卡因及其盐类	Butanilicaine (2-butylamino-6'-chloro-o-acetotoluidide) and its salts
308	布托哌啉及其盐类	Butopiprine (2-butoxyethyl α-phenyl-1-piperidineacetate) and its salts
309	缩水甘油丁醚	Butyl glycidyl ether (CAS No 2426-08-6)
310	溶剂黄 14	Solvent Yellow 14 (CAS No 842-07-9)
311	镉和镉的化合物	Cadmium and its compounds
312	斑蝥(表 3 中所列仅用于头发用品的斑蝥酊中所含斑蝥素除外)	Cantharides, *cantharis vesicatoria*, with the exception of cantharides tincture listed in table 3
313	敌菌丹	Captafol (2425-06-1)
314	卡普托胺	Captodiame (2-(*p*-butylmercaptobenzhydrylmercapto)-*N*,*N*-dimethylethylamine)
315	卡拉美芬及其盐类	Caramiphen (2-diethylaminoethyl ester of 1-phenylcyclopentanecarboxylic acid) and its salts
316	卡巴多司	Carbadox (CAS No 6804-07-5)
317	甲萘威(甲氨甲酸萘酯)	Carbaryl (CAS No 63-25-2)
318	多菌灵	Carbendazim (CAS No 10605-21-7)
319	二硫化碳	Carbon disulphide
320	一氧化碳	Carbon monoxide (CAS No 630-08-0)
321	四氯化碳	Carbon tetrachloride
322	卡溴脲	Carbromal (1-(2-bromo-2-ethylbutyryl) urea)
323	氨磺丁脲	Carbutamide (*N*'- (butylcarbamoyl) sulfanilamide; 1-butyl-3-sulfanilylurea)
324	卡立普多	Carisoprodol (2-carbamyloxymethyl-2-isopropylcarbamyloxymethylpentane)
325	过氧化氢酶	Catalase
326	儿茶酚	Catechol

No.	Chinese Name	English Name
327	人的细胞、组织或其产品	Cells, tissues or products of human origin
328	吐根酚碱及其盐	Cephaeline and its salts
329	土荆芥(精油)	*Chenopodium ambrosioides* (essential oil)
330	灭螨猛	Chinomethionate (CAS No 2439-01-2)
331	纯氯丹	Chlordane ,pur (CAS No 57-74-9)
332	开蓬；十氯酮	Chlordecone (CAS No 143-50-0)
333	氯苯甲脒	Chlordimeform (CAS No 6164-98-3)
334	氯	Chlorine
335	氮芥及其盐类	Chlormethine (2,2'-dichloro-*N*-methyldiethylamine; bis (2-chloroethyl) methylamine) and its salts
336	氯乙醛	Chloroacetaldehyde (CAS No 107-20-0)
337	氯乙烷	Chloroethane
338	氯仿	Chloroform
339	氯代甲烷	Chloromethane (CAS No 74-87-3)
340	氯气甲基甲基醚	Chloromethyl methyl ether (CAS No 107-30-2)
341	氯美扎酮	Chloromezanone
342	氯丁二烯(2-氯-1,3-丁二烯)	Chloroprene (stabilized) (2-chlorobuta-1,3-diene) (CAS No 126-99-8)
343	四氯二氰苯；百菌清	Chlorothalonil (CAS No 1897-45-6)
344	绿麦隆(*N*'-(3-氯-4-甲苯基)-*N*,*N*-甲基脲)	Chlorotoluron(3-(3-chloro-*p*-tolyl)-1,1-dimethylurea) (CAS No 15545-48-9)
345	氯苯沙明	Chlorphenoxamine (2-[1-(*p*-chlorophenyl)-1-phenylethoxy]-*N*,*N*-dimethylethylamine)
346	氯磺丙脲	Chlorpropamide (1-(*p*-chlorophenylsulfonyl)-3-propylurea)
347	氯普噻吨及其盐类	Chlorprothixene (trans isomer of 3-(2-chlorothioxanthen-9-ylidene) -*N*,*N*-dimethylpropylamine; taractan) and its salts
348	氯噻酮	Chlortalidone [2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl) benzenesulfonamide]
349	氯唑沙宗	Chlorzoxazone (5-chloro-2-benzoxazolinone)
350	乙菌利	Chlozolinate (CAS No 84332-86-5)
351	胆碱盐类及它们的酯类,例如氯化胆碱	Choline salts and their esters, e.g. Choline chloride ((2-hydroxyethyl)- trimethylammonium chloride)
352	铬、铬酸及其盐类	Chromium; chromic acid and its salts
353	艹屈	Chrysene (CAS No 205-99-2)

No.	Chinese Name	English Name
354	辛可卡因及其盐类	Cinchocaine (2-butoxy-*N*-(2-diethylamincethyl) cinchoninamide) and its salts
355	辛可芬及其盐类,衍生物以及衍生物的盐类	Cinchophen (2-phenylcinchoninic acid), its salts, derivatives and salts of these derivatives
356	催化裂解处理的澄清油(石油)	Clarified oils (petroleum),catalytic cracked (CAS No 64741-75-9)
357	加氢脱硫催化裂解的澄清油(石油)	Clarified oils(petroleum), hydrodesulfurised catalytic cracked (CAS No 68333-26-6)
358	麦角菌及其生物碱和草药制剂	*Claviceps purpurea tul.*, its alkaloids and galenical preparations
359	氯非那胺	Clofenamide (4-chloro-1,3-benzenedisulfon-amide)
360	滴滴涕	Clofenotane; DDT (ISO)
361	苯并[a]芘的含量大于0.005%(w/w)的液体溶剂萃取的液态煤	Coal liquids, liq solvent extn (CAS No 94114-48-4), if they contain > 0.005% (w/w) benzo[a]pyrene
362	苯并[a]芘的含量大于0.005%(w/w)的液态煤，来自液体溶剂萃取的煤溶液	Coal liquids, liq solvent extn soln(CAS No 94114-47-3), if they contain > 0.005% (w/w) benzo[a]pyrene
363	苯磺酸钴	Cobalt benzenesulphonate
364	二氯化钴	Cobalt dichloride (CAS No7646-79-9)
365	硫酸钴	Cobalt sulphate (CAS No 10124-43-3)
366	秋水仙碱及其盐类和衍生物	Colchicine, its salts and derivatives
367	秋水仙糖苷及其衍生物	Colchicoside and its derivatives
368	秋水仙及其草药制剂	*Colchicum autumnale L.* And its galenical preparations
369	着色剂 CI 12075 及其色淀、颜料及盐类	Colouring agent CI 12075 and its lakes, pigments and salts
370	着色剂 CI 12140	Colouring agent CI 12140
371	着色剂 CI 13065	Colouring agent CI 13065
372	着色剂 CI 15585	Colouring agent CI 15585
373	着色剂 CI 26105	Colouring agent CI 26105
374	着色剂 CI 42535	Colouring agent CI 42535
375	着色剂 CI 42555 着色剂 CI 42555-1 着色剂 CI 42555-2	Colouring agent CI 42555 Colouring agent CI 42555-1 Colouring agent CI 42555-2
376	着色剂 CI 42640	Colouring agent CI 42640
377	着色剂 CI 45170 和 CI 45170: 1	Colouring agent CI 45170 and CI 45170:1
378	着色剂 CI 61554	Colouring agent CI 61554
379	毒芹碱	Coniine

No.	Chinese Name	English Name
380	毒参(果实、粉末和草药制剂)	*Conium maculatum L.* (fruit, powder, galenical preparations)
381	铃兰毒甙	Convallatoxin
382	木香根油	Costus root oil(Saussurea Lappa Clarke)
383	库美香豆素	Coumetarol (3,3'-(2-methoxyethylidene) bis (4-hydroxycoumarin))
384	苯并[a]芘的含量大于0.005%(w/w)的不含二氢苊的的杂酚油，来自二氢苊馏分	Creosote oil, acenaphthene fraction, acenaphthene-free (CAS No 90640-85-0), if it contains > 0.005% (w/w) benzo[a]pyrene
385	苯并[a]芘的含量大于 0.005%(w/w)的杂酚油，来自洗涤油的二氢苊馏分	Creosote oil, acenaphthene fraction, wash oil, if it contains > 0.005 %(w/w)benzo[a]pyrene (CAS No 90640-84-9)
386	苯并[a]芘的含量大于 0.005%(w/w)的杂酚油，来自洗涤油的高沸点馏分	Creosote oil, high-boiling distillate, wash oil, if it contains > 0.005 %(w/w) benzo[a]pyrene (CAS No 70321-79-8)
387	苯并[a]芘的含量大于 0.005%(w/w)的杂酚油	Creosote oil, if it contains > 0.005 %(w/w) benzo[a]pyrene (CAS No 61789-28-4)
388	苯并[a]芘的含量大于 0.005%(w/w)的杂酚油，来自洗涤油的低沸点馏分	Creosote oil, low-boiling distillate, wash oil, if it contains > 0.005 %(w/w) benzo[a]pyrene (CAS No 70321-80-1)
389	苯并[a]芘的含量大于 0.005%(w/w)的杂酚油	Creosote, if it contains > 0.005 %(w/w) benzo[a]pyrene (CAS No 8001-58-9)
390	巴豆(巴豆油)	*Croton tiglium* (oil)
391	巴豆醛	Crotonaldehyde (CAS No 4170-30-3)
392	粗制和精制煤焦油	Crude and refined coal tars
393	箭毒和箭毒碱	Curare and curarine
394	仙客来醇	Cyclamen alcohol (CAS No 4756-19-8)
395	环拉氨酯	Cyclarbamate (1,1-bis (phenylcarbamoyloxymethyl) cyclopentane)
396	赛克利嗪及其盐类	Cyclizine (1-benzhydryl-4-methylpiperazine) and its salts
397	放线菌酮	Cycloheximide (CAS No 66-81-9)
398	环美酚及其盐类	Cyclomenol (2-cyclohexyl-3,5-xylenol; 2-cyclohexyl-3,5-dimethylphenol) and its salts
399	环磷酰胺及其盐类	Cyclophosphamide (2[bis(2-chloroethyl) amino] tetralydro-2H-1, 3, 2- oxazaphesphorine 2-oxide) and its salts
400	丁酰肼; N-二甲氨基琥珀酰胺酸	Daminozide (CAS No 1596-84-5)
401	曼陀罗及其草药制剂	*Datura stramonium L.* And its galenical preparations
402	醋谷地阿诺	Deanol aceglumate
403	癸亚甲基双(三甲铵)盐类，例如：十烃溴铵	Decamethylenebis (trimethylammonium) salts, e.g.decamethonium bromide
404	右美沙芬及其盐类	Dextromethorphan [(+)-3-methoxy-N-methylmorphinan] and its salts

No.	Chinese Name	English Name
405	右丙氧吩	Dextropropoxyphene (a-(+)-4-dimethylamino-3-methyl-1,2-diphenyl-2-butanol propionate ester)
406	燕麦敌	Di-allate (CAS No 2303-16-4)
407	二氨基甲苯, 工业品－4-甲基-间-苯二胺和 2-甲基-间-苯二胺的混合物 (甲基苯二胺)	Diaminotoluene, technical product -mixture of 4-methyl-m-phenylene diamine and 2-methyl-m-phenylene diamine methyl-phenylenediamine(CAS No 25376-45-8)
408	重氮甲烷	Diazomethane (CAS No 334-88-3)
409	二苯并[a,h]蒽	Dibenz[a,h]anthracene (CAS No 53-70-3)
410	二溴 *N*-水杨酰苯胺类	Dibromosalicylanilides
411	邻苯二甲酸二丁酯	Dibutyl phthalate (CAS No84-74-2)
412	二氯乙烷类(乙烯基氯类)	Dichloroethanes (ethylene chlorides)
413	二氯乙烯类(乙炔基氯类)	Dichloroethylenes (acetylene chlorides)
414	二氯 N-水杨酰苯胺类	Dichlorosalicylanilides
415	双香豆素	Dicoumarol (3,3'-methylenebis (4-hydroxyconmarin))
416	狄氏剂	Dieldrin (CAS No 60-57-1)
417	磷酸-4-硝基苯基二乙基酯	Diethyl 4-nitrophenyl phosphate
418	马来酸二乙酯	Diethyl maleate (CAS No 141-05-9)
419	硫酸二乙酯	Diethyl sulphate (CAS No 64-67-5)
420	二乙基氨基甲酰氯	Diethylcarbamoyl-chloride (CAS No 88-10-8)
421	二苯沙秦	Difencloxazine (4-(2-(*p*-chloro-a-phenylhenzyloxy) ethyl)morpholine)
422	毛地黄苷和洋地黄的各种苷	Digitaline and all heterosides of *digitalis purpurea L.*
423	二氢香豆素	Dihydrocoumarine (CAS No 119-84-6)
424	二氢速甾醇	Dihydrotachysterol (dichystrol)
425	二甲基柠康酸酯	Dimethyl citraconate (CAS No 617-54-9)
426	二甲基亚砜	Dimethyl sulfoxide
427	硫酸二甲酯	Dimethyl sulphate (CAS No 77-78-1)
428	二甲胺	Dimethylamine
429	二甲基氨基甲酰氯	Dimethylcarbamoyl chloride (CAS No 79-44-7)
430	二甲基甲酰胺	Dimethylformamide
431	二甲基亚硝胺	Dimethylnitrosoamine (CAS No 62-75-9)

No.	Chinese Name	English Name
432	二甲基氨磺酰氯化物	Dimethylsulphamoyl-chloride (CAS No 13360-57-1)
433	地美戊胺及其盐类	Dimevamide (4-dimethylamino-2,2-diphenylvaleramide) and its salts
434	三氧化二镍	Dinickel trioxide (CAS No 1314-06-3)
435	二硝基苯酚同分异构体	Dinitrophenol isomers
436	二硝基甲苯，工业级	Dinitrotoluene, technical grade (CAS No 121-14-2)
437	二硝基甲苯	Dinitrotoluene (CAS No 25321-14-6)
438	敌螨普	Dinocap (ISO) (CAS No 39300-45-3)
439	地乐酚[2-(1-甲基正丙基)-4,6-二硝基苯酚]及其盐类和酯类,在本规范的别处规定的除外	Dinoseb (CAS No 88-85-7),its salts and esters with the exception of those specified elsewhere in this list
440	地乐硝酚,它的盐和酯	Dinoterb (CAS No1420-07-1),its salts and esters
441	二恶烷	Dioxane
442	二羟西君及其盐类	Dioxethedrin (1-(3,4-dihydroxyphenyl)-2-ethylamino-1-propanol) and its salts
443	苯海拉明及其盐类	Diphenhydramine (2-diphenylmethoxy-*N*,*N*-dimethylaminc;dimedrol) and its salts
444	地芬诺酯	Diphenoxylate hydrochloride (ethyl ester of 1-(3-cyano-3,3-diphenylpropyl)-4- phenylisonipecotic acid)
445	二苯胺	Diphenylamine (CAS No 122-39-4)
446	二苯醚的八溴衍生物	Diphenylether; octabromo derivate (CAS No 32536-52-0)
447	二苯拉林及其盐类	Diphenylpyraline (4-benzhydryloxy-1-methylpiperidine) and its salts
448	3,3'-[[1,1'-联苯]-4,4'-二基双(偶氮)]双(4-萘胺-1-磺酸)二钠	Disodium 3,3'-[[1,1'-biphenyl]-4,4'-diyl bis(azo)] bis (4-aminonaphthalene-1-sulphonate) (CAS No 573-58-0)
449	4-氨基-3-[[4'-(2,4-二氨基苯)偶氮][1,1'-联苯]-4-基]偶氮]-5-羟基-6-(苯偶氮基)萘-2,7-二磺酸二钠	Disodium 4-amino-3-[[4'-[(2,4-diaminophenyl)azo] [1,1'-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)naphthalene-2,7-disulphonate (CAS No 1937-37-7)
450	[5-[[4'-[[2,6-二羟基-3-[(2-羟基-5-磺苯基)偶氮]苯基][1,1'-联苯]-4-基]偶氮]水杨酰(4-)]铜酸(2-)二钠	Disodium[5-[[4'-[[2,6-dihydroxy-3-[(2-hydroxy-5-sulphophenyl)azo]phenyl][1,1'-biphenyl]-4-yl]azo]salicylato(4-)]cuprate(2-) (CAS No 16071-86-6)
451	分散黄 3	Disperse Yellow 3 (CAS No 2832-40-8)
452	苯并[a]芘的含量大于0.005%(w/w)的含稠环芳烃的煤-石油馏分	Distillates (coal-petroleum), condensed-ring arom(CAS No 68188-48-7), if they contain > 0.005% (w/w) benzo[a]pyrene
453	酸处理的(石油)轻馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), acid-treated light (CAS No 64742-14-9), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
454	酸处理的(石油)中间馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), acid-treated middle (CAS No 64742-13-8), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen

No.	Chinese Name	English Name
455	丁二烯含量大于0.1%(w/w)富戊间二烯的含C_{3-4}的石油馏分	Distillates (petroleum), C_{3-6}, piperylene-rich (CAS No 68477-35-0), if they contain > 0.1%(w/w) butadiene
456	活性炭处理的轻石蜡馏分(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), carbon-treated light paraffinic (CAS No 100683-97-4), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
457	催化重整分馏塔处理的(石油)残液高沸点馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), catalytic reformer fractionator residue, high-boiling (CAS No 68477-29-2), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
458	催化重整分馏塔处理的(石油)残液中沸点馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), catalytic reformer fractionator residue, intermediate-boiling (CAS No 68477-30-5), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
459	催化重整分馏塔处理的(石油)残液低沸点馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), catalytic reformer fractionator residue, low-boiling (CAS No 68477-31-6), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
460	含浓重芳烃的催化重整(石油)馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), catalytic reformer, heavy arom conc CAS No 91995-34-5), except if the full refininghistory is known and it can be shown that the substance from which it is produced is not a carcin ogen
461	化学中和的(石油)中间馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), chemically neutralised middle (CAS No 64742-30-9), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
462	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的重环烷(石油) 馏分	Distillates (petroleum), clay-treated heavy naphthenic (CAS No 64742-44-5), if they contain > 3 % (w/w) DMSO extract
463	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的重石蜡(石油)馏分	Distillates (petroleum), clay-treated heavy paraffinic (CAS No 64742-36-5), if they contain > 3 % (w/w) DMSO extract
464	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的轻环烷(石油) 馏分	Distillates (petroleum), clay-treated light naphthenic (CAS No 64742-45-6), if they contain > 3 % (w/w) DMSO extract
465	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的轻石蜡(石油) 馏分	Distillates (petroleum), clay-treated light paraffinic (CAS No 64742-37-6), if they contain > 3 % (w/w) DMSO extract
466	粘土处理的(石油)中间馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), clay-treated middle (CAS No 64742-38-7), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
467	二甲基亚砜提取物含量大于3%(w/w)的复合脱蜡处理的重石蜡馏分(石油)	Distillates (petroleum), complex dewaxed heavy paraffinic (CAS No 90640-91-8), if they contain > 3 % (w/w) DMSO extract
468	二甲基亚砜提取物含量大于3%(w/w)的复合脱蜡处理的轻石蜡馏分(石油)	Distillates (petroleum), complex dewaxed light paraffinic (CAS No 90640-92-9), if they contain > 3 % (w/w) DMSO extract
469	二甲基亚砜提取物含量大于3%(w/w)的加氢脱蜡的重环烷馏分(石油)	Distillates (petroleum), dewaxed heavy paraffinic, hydrotreated (CAS No 91995-39-0) if they contain > 3 % (w/w) DMSO extract
470	二甲基亚砜提取物含量大于3%(w/w)的加氢脱蜡的轻环烷馏分(石油)	Distillates (petroleum), dewaxed light paraffinic, hydrotreated (CAS No 91995-40-3), if they contain > 3

No.	Chinese Name	English Name
		% (w/w) DMSO extract
471	二甲基亚砜提取物含量大于3%(w/w)的重加氢裂解的(石油)馏分	Distillates (petroleum), heavy hydrocracked (CAS No 64741-76-0), if they contain > 3 % (w/w) DMSO extract
472	深度精练的(石油)中间馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), highly refined middle (CAS No 90640-93-0), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
473	二甲基亚砜提取物含量大于3%(w/w)的加氢裂解溶剂精制的轻馏分(石油)	Distillates (petroleum), hydrocracked solvent-refined light (CAS No 97488-73-8), if they contain > 3 % (w/w) DMSO extract
474	二甲基亚砜提取物含量大于3%(w/w)的脱蜡的加氢裂解溶剂精制馏分(石油)	Distillates (petroleum), hydrocracked solvent-refined, dewaxed (CAS No 91995-45-8), if they contain > 3 % (w/w) DMSO extract
475	加氢脱硫的全程中间馏分(石油)	Distillates (petroleum), hydrodesulfurised full-range middle (CAS No 101316-57-8)
476	加氢脱硫重度催化裂解馏分(石油)	Distillates (petroleum), hydrodesulfurised heavy catalytic cracked (CAS No 68333-28-8)
477	加氢脱硫中度催化裂解馏分(石油)	Distillates (petroleum), hydrodesulfurised intermediate catalytic cracked (CAS No 68333-27-7)
478	加氢脱硫处理的(石油)中间馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), hydrodesulfurised middle (CAS No 64742-80-9), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
479	二甲基亚砜提取物含量大于3%(w/w)的加氢重环烷(石油) 馏分	Distillates (petroleum), hydrotreated heavy naphthenic (CAS No 64742-52-5), if they contain > 3% (w/w) DMSO extract
480	二甲基亚砜提取物含量大于3%(w/w)的加氢重石蜡(石油) 馏分	Distillates (petroleum), hydrotreated heavy paraffinic (CAS No 64742-54-7), if they contain > 3 % (w/w) DMSO extract
481	二甲基亚砜提取物含量大于3%(w/w)的加氢轻环烷(石油) 馏分	Distillates (petroleum), hydrotreated light naphthenic (CAS No 64742-53-6), if they contain > 3 % (w/w) DMSO extract
482	二甲基亚砜提取物含量大于3%(w/w)的加氢轻石蜡(石油) 馏分	Distillates (petroleum), hydrotreated light paraffinic (CAS No 64742-55-8), if they contain > 3 % (w/w) DMSO extract
483	加氢的(石油)中间馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), hydrotreated middle (CAS No 64742-46-7), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
484	活性炭处理的中间馏分石蜡(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), intermediate paraffinic, carbon-treated (CAS No 100683-98-5), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
485	粘土处理的中间馏分石蜡(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), intermediate paraffinic, clay-treated (CAS No 100683-99-6), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
486	轻链烷馏分(石油)	Distillates (petroleum), light paraffinic (CAS No 64741-50-0)
487	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的溶剂脱蜡的重石蜡馏馏分(石油)	Distillates (petroleum), solvent dewaxed heavy paraffinic, clay-treated (CAS No 90640-94-1), if they contain> 3 % (w/w) DMSO extract

No.	Chinese Name	English Name
488	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的溶剂脱蜡轻石蜡馏分(石油)	Distillates (petroleum), solvent dewaxed light paraffinic, clay-treated (CAS No 90640-96-3), if they contain > 3 % (w/w) DMSO extract
489	二甲基亚砜提取物含量大于3%(w/w)的氢化的溶剂脱蜡轻石蜡馏分(石油)	Distillates (petroleum), solvent dewaxed light paraffinic, hydrotreated (CAS No 90640-97-4), if they contain> 3 % (w/w) DMSO extract
490	二甲基亚砜提取物含量大于3%(w/w)的溶剂脱蜡处理的重环烷(石油) 馏分	Distillates (petroleum), solvent-dewaxed heavy naphthenic (CAS No 64742-63-8), if they contain > 3 % (w/w) DMSO extract
491	二甲基亚砜提取物含量大于3%(w/w)的溶剂脱蜡处理的重石蜡(石油) 馏分	Distillates (petroleum), solvent-dewaxed heavy paraffinic (CAS No 64742-65-0), if they contain > 3 % (w/w) DMSO extract
492	二甲基亚砜提取物含量大于3%(w/w)的溶剂脱蜡处理的轻环烷(石油) 馏分	Distillates (petroleum), solvent-dewaxed light naphthenic (CAS No 64742-64-9), if they contain > 3 % (w/w) DMSO extract
493	二甲基亚砜提取物含量大于3%(w/w)的溶剂脱蜡处理的轻石蜡(石油) 馏分	Distillates (petroleum), solvent-dewaxed light paraffinic (CAS No 64742-56-9), if they contain > 3 % (w/w) DMSO extract
494	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制处理的重环烷(石油)馏分	Distillates (petroleum), solvent-refined heavy naphthenic (CAS No 64741-96-4), if they contain > 3 % (w/w) DMSO extract
495	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制处理的重石蜡(石油)馏分	Distillates (petroleum), solvent-refined heavy paraffinic (CAS No 64741-88-4), if they contain > 3 % (w/w) DMSO extract
496	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制的加氢裂解轻馏分(石油)	Distillates (petroleum), solvent-refined hydrocracked light (CAS No 94733-09-2), if they contain > 3 % (w/w) DMSO extract
497	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制的加氢重馏分(石油)	Distillates (petroleum), solvent-refined hydrogenated heavy (CAS No 97488-74-9), if they contain > 3 % (w/w) DMSO extract
498	二甲基亚砜提取物含量大于3%(w/w)的加氢的溶剂精制氢化重馏分(石油)	Distillates (petroleum), solvent-refined hydrotreated heavy, hydrogenated (CAS No 94733-08-1), if they contain> 3 % (w/w) DMSO extract
499	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制处理的轻环烷(石油)馏分	Distillates (petroleum), solvent-refined light naphthenic (CAS No 64741-97-5), if they contain > 3 % (w/w) DMSO extract
500	二甲基亚砜提取物含量大于3%(w/w)的加氢的溶剂精制的轻环烷馏分(石油)	Distillates (petroleum), solvent-refined light naphthenic, hydrotreated (CAS No 91995-54-9), if they contain> 3 % (w/w) DMSO extract
501	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制处理的轻度石蜡(石油)馏分	Distillates (petroleum), solvent-refined light paraffinic (CAS No 64741-89-5), if they contain > 3% (w/w) DMSO extract
502	溶剂精制的(石油)中间馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), solvent-refined middle (CAS No 64741-91-9), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
503	脱硫的(石油)中间馏分，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Distillates (petroleum), sweetened middle (CAS No 64741-86-2), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen

No.	Chinese Name	English Name
504	酸处理的重环烷馏分(石油)	Distillates (petroleum),acid-treated heavy naphthenic (CAS No 64742-18-3)
505	酸处理的重链烷馏分(石油)	Distillates (petroleum),acid-treated heavy paraffinic (CAS No 64742-20-7)
506	酸处理的轻环烷馏分(石油)	Distillates (petroleum),acid-treated light naphthenic (CAS No 64742-19-4)
507	酸处理的轻链烷馏分(石油)	Distillates (petroleum),acid-treated light paraffinic (CAS No 67742-21-8)
508	化学中和的轻环烷馏分(石油)	Distillates (petroleum),chemically neutralized light naphthenic (CAS No 64742-03-6)
509	化学中和的轻链烷馏分(石油)	Distillates (petroleum),chemically neutralized light paraffinic (CAS No 64742-28-5)
510	裂解蒸汽裂解石油馏分(石油)	Distillates (petroleum),cracked steam-cracked petroleum distillates(CAS No 68477-38-3)
511	重环烷馏分(石油)	Distillates (petroleum),heavy naphthenic (CAS No 64741-53-3)
512	重链烷馏分(石油)	Distillates (petroleum),heavy paraffinic (CAS No 64741-51-1)
513	重度热裂解馏分(石油)	Distillates (petroleum),heavy thermal cracked (CAS No 64741-81-7)
514	重度催化裂解馏分(石油)	Distillates (petroleum),heavy,catalytic cracked (CAS No 64741-61-3)
515	重度蒸汽裂解馏分(石油)	Distillates (petroleum),heavy,steam-cracked (CAS No 101631-14-5)
516	加氢脱硫、轻度催化裂解的馏分(石油)	Distillates (petroleum),hydrodesulfurised light catalytic cracked (CAS No 68333-25-5)
517	加氢脱硫中度焦化馏分(石油)	Distillates (petroleum),hydrodesulfurised middle coker (CAS No 101316-59-0)
518	加氢脱硫、热裂解的中间馏分(石油)	Distillates (petroleum),hydrodesulfurised thermal cracked middle (CAS No 85116-53-6)
519	中度催化裂解及热降解的馏分(石油)	Distillates (petroleum),intermediate catalytic cracked,thermally degraded (CAS No 92201-59-7)
520	减压蒸馏的中等沸点馏分(石油)	Distillates (petroleum),intermediate vacuum(CAS No 70592-76-6)
521	轻度催化裂解的馏分(石油)	Distillates (petroleum),light catalytic cracked (CAS No 64741-59-9)
522	轻度催化裂解热降解处理的馏分(石油)	Distillates (petroleum),light catalytic cracked,thermally degraded (CAS No 92201-60-0)
523	轻度加氢裂化处理的石油馏出液	Distillates (petroleum),light hydrocracked (CAS No 64741-77-1)
524	轻环烷馏分(石油)	Distillates (petroleum),light naphthenic (CAS No 64741-52-2)
525	轻度蒸汽裂解石脑油馏分(石油)	Distillates (petroleum),light steam-cracked naphtha (CAS No 68475-80-9)
526	轻度热裂解的馏分(石油)	Distillates (petroleum),light thermal cracked (CAS No 64741-82-8)
527	减压蒸馏的低沸点馏分(石油)	Distillates (petroleum),light vacuum(CAS No 70592-77-7)
528	石油残油减压蒸馏馏分(石油)	Distillates (petroleum),petroleum residues vacuum(CAS No 68955-36-2)
529	减压蒸馏馏分(石油)	Distillates (petroleum),vacuum (CAS No 70592-78-8)
530	化学中和的重环烷馏分(石油)	Distillates(petroleum),chemically neutralized heavy naphthenic (CAS No 64742-34-3)
531	化学中和的重链烷馏分(石油)	Distillates(petroleum),chemically neutralized heavy paraffinic (CAS No 64742-27-4)

No.	Chinese Name	English Name
532	中度催化裂解的馏分(石油)	Distillates(petroleum),intermediate catalytic cracked (CAS No 64741-60-2)
533	双硫仑；塞仑	Disulfiram (tetraethylthiuram disulfide; bis (diethylthiocarbamyl) disulfide); thiram(ISO)
534	二硫代-2,2'-双吡啶-二氧化物 1,1'(添加三水合硫酸镁)(双吡硫酮+硫酸镁)	Dithio-2,2'-bispyridine-dioxide 1,1'(additive with trihydrated magnesium sulphate)-(pyrithione disulphide+magnesium sulphate)
535	敌草隆	Diuron (CAS No 330-54-1)
536	五氧化二钒	Divanadium pentaoxide (CAS No 1314-62-1)
537	4,6-二硝基邻甲酚	DNOC (CAS No534-52-1)
538	十二氯五环[5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$]癸烷	Dodecachloropentacyclo[5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$]decane (CAS No 2385-85-5)
539	多西拉敏及其盐类	Doxylamine (2-[α-(2-dimethylaminoethoxy)-α-methylbenzyl] pyridine; histadoxylamine) and its salts
540	依米丁及其盐类和衍生物	Emetine, its salts and derivatives
541	麻黄碱及其盐类	Ephedrine and its salts
542	肾上腺素	Epinephrine (3,4-dihydroxy-α-methylaminomethylbenzyl alcohol; adrenaline)
543	氟环唑	Epoxiconazole (CAS No 133855-98-8)
544	(环氧乙基)苯	(Epoxyethyl)benzene (CAS No 96-09-3)
545	骨化醇和胆骨化醇(维生素D_2和D_3)	Ergocalciferol and cholecalciferol (vitamins D_2 and D_3)
546	毛沸石	Erionite (CAS No 12510-42-8)
547	毒扁豆碱(依色林)及其盐类	Eserine or physostigmine and its salts
548	带游离氨基的 4-氨基苯甲酸酯类(表 5 中允许使用的除外)	Esters of 4-aminobenzoic acid, with the free amino group, with the exception of that given in table 5
549	乙硫异烟胺	Ethionamide (2-ethylisonicotinthioamide; α -ethylisonicotinic thioamide; 2-ethyl-4-thiocarbamoylpyridine)
550	依索庚嗪及其盐类	Ethoheptazine (4-carbethoxy-1-methyl-4-phenylhexamethylenimine) and its salts
551	丙烯酸乙酯	Ethyl acrylate (CAS No 140-88-5)
552	双(4-羟基-2-氧代-1-苯并吡喃-3-基)乙酸乙酯及酸的盐类	Ethyl bis (4-hydroxy-2-oxo-1-benzopyran-3-yl) acetate and salts of the acid
553	乙二醇二甲醚	Ethylene glycol dimethyl ether (CAS No 110-71-4)
554	环氧乙烷	Ethylene oxide
555	苯丁酰脲	Ethylphenacemide (1-(2-phenylbutyryl) urea)
556	苯并[a]芘的含量大于0.005%(w/w)的褐煤提取残渣	Extract residues (coal), brown (CAS No 91697-23-3), if they contain > 0.005% (w/w) benzo[a]pyrene
557	苯并[a]芘的含量大于 0.005%(w/w)的煤提取残渣, 来自洗涤油提取残渣的酸化杂酚油	Extract residues (coal), creosote oil acid, wash oil extract residue, if it contains > 0.005 %(w/w) benzo[a]pyrene (CAS No 122384-77-4)

No.	Chinese Name	English Name
558	二甲基亚砜提取物含量大于3%(w/w)的含高浓度芳烃的重环烷馏分溶剂提取液(石油)	Extracts (petroleum), heavy naphthenic distillate solvent, arom conc (CAS No 68783-00-6), if they contain> 3 % (w/w) DMSO extract
559	二甲基亚砜提取物含量大于3%(w/w)的加氢脱硫重环烷馏分溶剂提取(石油)	Extracts (petroleum), heavy naphthenic distillate solvent, hydrodesulfurised (CAS No 93763-10-1), if they contain > 3 % (w/w) DMSO extract
560	二甲基亚砜提取物含量大于3%(w/w)的加氢重环烷馏分溶剂提取物(石油)	Extracts (petroleum), heavy naphthenic distillate solvent, hydrotreated (CAS No 90641-07-9), if they contain> 3 % (w/w) DMSO extract
561	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的重石蜡馏分的溶剂提取物	Extracts (petroleum), heavy paraffinic distillate solvent, clay-treated (CAS No 92704- 08-0), if they contain > 3 % (w/w) DMSO extract
562	二甲基亚砜提取物含量大于3%(w/w)的加氢重石蜡馏分溶剂提取物(石油)	Extracts (petroleum), heavy paraffinic distillate solvent, hydrotreated (CAS No 90641-08-0), if they contain> 3 % (w/w) DMSO extract
563	二甲基亚砜提取物含量大于3%(w/w)的重石蜡馏分溶剂脱沥青提取液(石油)	Extracts (petroleum), heavy paraffinic distillates, solvent-deasphalted (CAS No 68814-89-1), if they contain> 3 % (w/w) DMSO extract
564	二甲基亚砜提取物含量大于3%(w/w)的加氢轻石蜡馏分溶剂提取物(石油)	Extracts (petroleum), hydrotreated light paraffinic distillate solvent (CAS No 91995- 73-2), if they contain > 3 % (w/w) DMSO extract
565	二甲基亚砜提取物含量大于3%(w/w)的加氢脱硫轻环烷馏分溶剂提取物(石油)	Extracts (petroleum), light naphthenic distillate solvent, hydrodesulfurised (CAS No 91995-75-4), if they contain> 3 % (w/w) DMSO extract
566	二甲基亚砜提取物含量大于3%(w/w)的酸处理的轻石蜡馏出液溶剂提取物(石油)	Extracts (petroleum), light paraffinic distillate solvent, acid-treated (CAS No 91995-76-5), if they contain > 3 % (w/w) DMSO extract
567	二甲基亚砜提取物含量大于3%(w/w)的活性炭处理的轻石蜡馏分的溶剂提取物(石油)	Extracts (petroleum), light paraffinic distillate solvent, carbon-treated (CAS No 100684-02-4), if they contain> 3 % (w/w) DMSO extract
568	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的轻石蜡馏分的溶剂提取物(石油)	Extracts (petroleum), light paraffinic distillate solvent, clay-treated (CAS No 100684- 03-5), if they contain > 3 %(w/w) DMSO extract
569	二甲基亚砜提取物含量大于3%(w/w)的加氢脱硫的轻石蜡馏出液溶剂提取物(石油)	Extracts (petroleum), light paraffinic distillate solvent, hydrodesulfurised (CAS No 91995-77-6), if they contain> 3 % (w/w) DMSO extract
570	二甲基亚砜提取物含量大于3%(w/w)的加氢轻石蜡馏分溶剂提取物(石油)	Extracts (petroleum), light paraffinic distillate solvent, hydrotreated (CAS No 90641-09-1), if they contain > 3 % (w/w) DMSO extract
571	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的轻减压柴油溶剂提取物(石油)	Extracts (petroleum), light vacuum gas oil solvent, clay-treated (CAS No 100684-05-7), if they contain > 3 % (w/w) DMSO extract
572	二甲基亚砜提取物含量大于3%(w/w)的加氢的轻减压瓦斯油溶剂提取物(石油)	Extracts (petroleum), light vacuum gas oil solvent, hydrotreated (CAS No 91995-79-8), if they contain > 3 % (w/w) DMSO extract
573	二甲基亚砜提取物含量大于3%(w/w)的活性炭处理的轻减压柴油溶剂提取物(石油)	Extracts (petroleum), light vacuum, gas oil solvent, carbon-treated (CAS No 100684-04-6), if they contain > 3 % (w/w) DMSO extract

No.	Chinese Name	English Name
574	二甲基亚砜提取物含量大于3%(w/w)的加氢脱硫的溶剂脱蜡重石蜡馏分溶剂提取物	Extracts (petroleum), solvent-dewaxed heavy paraffinic distillate solvent, hydrodesulfurised (CAS No 93763-11-2), if they contain > 3 % (w/w) DMSO extract
575	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制处理的重石蜡馏分溶剂提取液(石油)	Extracts (petroleum), solvent-refined heavy paraffinic distillate solvent (CAS No 68783-04-0), if they contain> 3 % (w/w) DMSO extract
576	重环烷馏分的溶剂提取物(石油)	Extracts (petroleum),heavy naphthenic distillate solvent (CAS No 64742-11-6)
577	重链烷馏分的溶剂提取物(石油)	Extracts (petroleum),heavy paraffinic distillate solvent (CAS No 64742-04-7)
578	轻环烷馏分的溶剂提取物(石油)	Extracts (petroleum),light naphthenic distillate solvent (CAS No 64742-03-6)
579	轻链烷馏分的溶剂提取物(石油)	Extracts (petroleum),light paraffinic distillate solvent (CAS No 64742-05-8)
580	轻减压瓦斯油的溶剂提取物(石油)	Extracts (petroleum),light vacuum gas oil solvent (CAS No 91995-78-7)
581	酚二唑	Fenadiazole (*o*-(1,3,4-oxadiazol-2-yl) phenol)
582	异嘧菌醇	Fenarimol (CAS No 60168-88-9)
583	非诺唑酮	Fenozolone (2-ethylamino-5-phenyl-2-cxazolin-4-one)
584	丁苯吗啉	Fenpropimorph (CAS No 67564-91-4)
585	倍硫磷	Fenthion (CAS No 55-38-9)
586	薯瘟锡	Fentin acetate (CAS No 900-95-8)
587	毒菌锡	Fentin hydroxide (CAS No 76-87-9)
588	非尼拉朵	Fenyramidol [α -(2-pyridylaminomethyl) benzyl alcohol]
589	无花果叶的纯净萃	Fig leaf absolute (*Ficus carica*) (CAS No 68916-52-9)
590	氟阿尼酮	Fluanisone (4'-fluoro-4-[4-(*o*-methoxyphenyl) piperazin-1-yl] butyrophenone)
591	氟甲吡啶氧酚丙酸丁酯	Fluazifop-buty1(CAS No 69806-50-4)
592	氟甲吡啶氧酚丙酸丁酯(稳杀得; 吡氟乐草灵; 氟草除)	Fluazifo-P-butyl (CAS No 79241-46-6)
593	氟噁嗪酮	Flumioxazin (CAS No 103361-09-07)
594	氟苯乙砜	Fluoresone (ethyl *p*-fluorophenyl sulfone)
595	氟尿嘧啶	Fluorouracil (5-fluorouracil)
596	氟硅唑	Flusilazole (CAS No 85509-19-9)
597	二甲基亚砜提取物含量大于3%(w/w)的脚子油(石油)	Foots oil (petroleum) (CAS No 64742-67-2), if it contains > 3 % (w/w) DMSO extract
598	二甲基亚砜提取物含量大于3%(w/w)的酸处理的脚子油(石油)	Foots oil (petroleum), acid-treated (CAS No 93924-31-3), if it contains > 3 % (w/w) DMSO extract
599	二甲基亚砜提取物含量大于3%(w/w)的活性炭处理的脚子油(石油)	Foots oil (petroleum), carbon-treated (CAS No 97862-76-5), if it contains > 3 % (w/w) DMSO extract
600	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的脚子油(石油)	Foots oil (petroleum), clay-treated (CAS No 93924-32-4), if it contains > 3 % (w/w) DMSO extract

No.	Chinese Name	English Name
601	二甲基亚砜提取物含量大于3%(w/w)的加氢脚子油(石油)	Foots oil (petroleum), hydrotreated (CAS No 92045-12-0), if it contains > 3 % (w/w) DMSO extract
602	二甲基亚砜提取物含量大于3%(w/w)的硅酸处理的脚子油(石油)	Foots oil (petroleum), silicic acid-treated (CAS No 97862-77-6), if it contains > 3 % (w/w) DMSO extract
603	甲酰胺	Formamide (CAS No 75-12-7)
604	丁二烯含量大于0.1%(w/w)的燃料油，来自原油馏分	Fuel gases, crude oil distillates (CAS No 68476-29-9), if they contain > 0.1%(w/w) butadiene
605	6 号燃料油	Fuel oil, No 6(CAS No 68553-00-4)
606	4 号燃料油	Fuel oil, No.4 (CAS No 68476-31-3)
607	燃料油残液	Fuel oil, residual (CAS No 68476-33-5)
608	高硫燃料油，来自直馏柴油残液	Fuel oil, residues-straight-run gas oils, high-sulfur (CAS No 68476-32-4)
609	丁二烯含量大于0.1%(w/w)的燃料油	Fuel-gases (CAS No 68476-26-6), if they contain > 0.1%(w/w) butadiene
610	柴油机燃料，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Fuels, diesel (CAS No 68334-30-5), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
611	柴油机燃料，来自加氢裂解氢化煤的溶剂提取液	Fuels, diesel, coal solvent extn., hydrocracked hydrogenated (CAS No 94114-59-7)
612	2 号柴油机燃料	Fuels, diesel, No.2 (CAS No 68476-34-6)
613	喷气飞机燃料，来自加氢裂解氢化煤的溶剂提取液	Fuels,jet aircraft,coal solvent extn., hydrocracked hydrogenated (CAS No 94114-58-6)
614	高硫高沸点燃料油	Fues oil, heavy, high-sulfur (CAS No 92045-14-2)
615	2 号燃料油	Fues oil, No.2 (CAS No 68476-30-2)
616	呋喃	Furan (CAS No 110-00-9)
617	呋喃唑酮	Furazolidone (3-(5-nitro-2-furfurylideneamino)-2-oxazolidinone)
618	糠基三甲基铵盐类,例如：呋嗦碘铵	Furfuryltrimethylammonium salts, e.g.furtrethonium iodide
619	呋喃香豆素类(如: 三甲沙林, 8-甲氧基补骨脂素(花椒毒素), 5-甲氧基补骨脂素(佛手柑内酯)等)，天然香精中存在的正常含量除外。在防晒和晒黑产品中，呋喃香豆素的含量应小于 1mg/kg.	Furocoumarines (e.g. Trioxysalan , 8-methoxypsoralen, 5-methoxypsoralen) except for normal content in natural essences used. In sun protection and in bronzing products, furocoumarines shall be below 1 mg/kg.
620	加兰他敏	Galantamine (1, 2, 3, 4, 6, 7, 7a, 11c-octahydro-9-methoxy-2-methylbenzofuro- (4, 3, 2- e, f, g) (2) benzazocin-2-ol)
621	戈拉碘铵	Gallamine triethiodide (1,2,3-tris(2-diethylaminoethoxy) benzene trethiodide)
622	酸处理的柴油(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Gas oils (petroleum), acid-treated (CAS No 64742-12-7), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
623	化学中和的柴油(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Gas oils (petroleum), chemically neutralised (CAS No 64742-29-6), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen

No.	Chinese Name	English Name
624	常压蒸馏的高沸点柴油(石油)	Gas oils (petroleum), heavy atmospheric (CAS No 68783-08-4)
625	加氢脱硫的柴油(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Gas oils (petroleum), hydrodesulfurised (CAS No 64742-79-6), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
626	溶剂精制的柴油(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Gas oils (petroleum), solvent-refined (CAS No 64741-90-8), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
627	重度减压处理的柴油(石油)	Gas oils (petroleum),heavy,vacuum (CAS No 64741-57-7)
628	加氢脱硫焦化减压蒸馏高沸点柴油(石油)	Gas oils (petroleum),hydrodesulfurised coker heavy vacuum (CAS No 85117-03-9)
629	加氢脱硫减压蒸馏高沸点柴油(石油)	Gas oils (petroleum),hydrodesulfurised heavy vacuum (CAS No 64742-086-5)
630	加氢减压蒸馏的柴油(石油)	Gas oils (petroleum),hydrotreated vacuum (CAS No 64742-59-2)
631	轻度减压热裂解加氢脱硫的柴油(石油)	Gas oils (petroleum),light vacuum,thermal-cracked hydrodesulfurised (CAS No 97926-59-5)
632	蒸汽裂解的柴油(石油)	Gas oils (petroleum),steam-cracded (CAS No 68527-18-4)
633	热裂解加氢脱硫处理的柴油(石油)	Gas oils (petroleum),thermal-cracked,hydrodesulfurised (CAS No 92045-29-9)
634	加氢柴油, 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Gas oils, hydrotreated (CAS No 97862-78-7), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
635	石蜡柴油, 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Gas oils, paraffinic (CAS No 93924-33-5), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
636	丁二烯含量大于0.1%(w/w)的采用烷基化进料的汽油(石油)	Gases (petroleum), alkylation feed (CAS No 68606-27-9), if they contain > 0.1%(w/w) butadiene
637	丁二烯含量大于0.1%(w/w)的氨系统进料汽油(石油)	Gases (petroleum), amine system feed (CAS No 68477-65-6), if they contain > 0.1%(w/w) butadiene
638	丁二烯含量大于0.1%(w/w)的苯单元产生的加氢脱硫的汽油(石油)尾气	Gases (petroleum), benzene unit hydrodesulferised off (CAS No 68477-66-7), if they contain > 0.1%(w/w) butadiene
639	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自苯单元加氢脱戊烷塔塔顶馏馏分	Gases (petroleum), benzene unit hydrotreater depentaniser overheads (CAS No 68602-82-4), if they contain> 0.1%(w/w)butadiene
640	丁二烯含量大于0.1%(w/w)富氢的苯系统循环的汽油(石油)	Gases (petroleum), benzene unit recycle, hydrogen-rich (CAS No 68477-67-8), if they contain > 0.1%(w/w) butadiene
641	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自富氢氮的调合油	Gases (petroleum), blend oil, hydrogen-nitrogen-rich (CAS No 68477-68-9), if they contain > 0.1%(w/w) butadiene
642	丁二烯含量大于0.1%(w/w)的汽油(石油),丁烷分离塔塔顶馏分	Gases (petroleum), butane splitter overheads (CAS No 68477-69-0), if they contain > 0.1%(w/w) butadiene
643	丁二烯含量大于0.1%(w/w)的含C_{1-5}湿汽油(石油)	Gases (petroleum), C_{1-5}, wet (CAS No 68602-83-5), if they contain > 0.1%(w/w) Butadiene
644	丁二烯含量大于0.1%(w/w)的含C_{2-3}汽油(石油)	Gases (petroleum), C_{2-3} (CAS No 68477-70-3), if they contain > 0.1%(w/w) butadiene
645	丁二烯含量大于0.1%(w/w)的脱硫的C_{2-4}汽油(石油)	Gases (petroleum), C_{2-4}, sweetened (CAS No 68783-65-3), if they contain > 0.1%(w/w) butadiene

No.	Chinese Name	English Name
646	丁二烯含量大于0.1%(w/w)的C_2溢流汽油(石油)	Gases (petroleum), C_2-return stream (CAS No 68477-84-9), if they contain > 0.1%(w/w) butadiene
647	丁二烯含量大于0.1%(w/w)的含C_{3-4}汽油(石油)	Gases (petroleum), C_{3-4} (CAS No 68131-75-9), if they contain > 0.1%(w/w) butadiene
648	丁二烯含量大于0.1%(w/w)富异丁烷的含C_{3-4}的汽油(石油)	Gases (petroleum), C_{3-4}, isobutane-rich (CAS No 68477-33-8), if they contain > 0.1%(w/w) butadiene
649	丁二烯含量大于0.1%(w/w)的烯烃-烷烃烷基化进料的C_{3-5}汽油(石油)	Gases (petroleum), C_{3-5} olefinic-paraffinic alkylation feed (CAS No 68477-83-8), if they contain > 0.1%(w/w) butadiene
650	丁二烯含量大于0.1%(w/w)的富C_4汽油(石油)	Gases (petroleum), C_4-rich (CAS No 68477-85-0), if they contain > 0.1%(w/w) butadiene
651	丁二烯含量大于0.1%(w/w)的C_{6-8}催化重整的汽油(石油)	Gases (petroleum), C_{6-8} catalytic reformer (CAS No 68477-81-6), if they contain > 0.1%(w/w) butadiene
652	丁二烯含量大于0.1%(w/w)的C_{6-8}催化重整循环的汽油(石油)	Gases (petroleum), C_{6-8} catalytic reformer recycle (CAS No 68477-80-5), if they contain > 0.1%(w/w) butadiene
653	丁二烯含量大于0.1%(w/w)的催化重整循环的富氢C_{6-8}汽油(石油)	Gases (petroleum), C_{6-8} catalytic reformer recycle, hydrogen-rich (CAS No 68477-82-7), if they contain > 0.1%(w/w)butadiene
654	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自催化裂解石脑油脱丁烷塔	Gases (petroleum), catalytic cracked naphtha debutanizer (CAS No 68952-76-1), if they contain > 0.1%(w/w) butadiene
655	丁二烯含量大于0.1%(w/w)的富C_3无酸汽油(石油), 来自催化裂解石脑油脱丙烷塔塔顶馏分	Gases (petroleum), catalytic cracked naphtha depropaniser overhead, C_3-rich acid-free (CAS No 68477-73-6), if they contain > 0.1%(w/w) butadiene
656	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自催化裂解塔顶馏分	Gases (petroleum), catalytic cracked overheads (CAS No 68409-99-4), if they contain > 0.1%(w/w) butadiene
657	丁二烯含量大于0.1%(w/w)的催化裂解汽油(石油)	Gases (petroleum), catalytic cracker (CAS No 68477-74-7), if they contain > 0.1%(w/w) butadiene
658	丁二烯含量大于0.1%(w/w)的富C_{1-5}催化裂解汽油(石油)	Gases (petroleum), catalytic cracker, C_{1-5}-rich (CAS No 68477-75-8), if they contain > 0.1%(w/w) butadiene
659	丁二烯含量大于0.1%(w/w)的催化裂解汽油(石油)	Gases (petroleum), catalytic cracking (CAS No 68783-64-2), if they contain > 0.1%(w/w) butadiene
660	丁二烯含量大于0.1%(w/w)的富C_{2-4}汽油(石油), 来自催化聚合石脑油稳定塔塔顶馏分	Gases (petroleum), catalytic polymd naphtha stabiliser overhead, C_{2-4}-rich (CAS No 68477-76-9), if they contain > 0.1%(w/w) butadiene
661	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自催化重整石脑油汽提塔塔顶馏分	Gases (petroleum), catalytic reformed naphtha stripper overheads (CAS No 68477-77-0), if they contain > 0.1%(w/w) butadiene
662	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自催化重整直馏石脑油稳定塔塔顶馏分	Gases (petroleum), catalytic reformed straight-run naphtha stabiliser overheads (CAS No 68513-14-4), if they contain > 0.1%(w/w) butadiene
663	丁二烯含量大于0.1%(w/w)的催化重整的富C_{1-4}汽油(石油)	Gases (petroleum), catalytic reformer, C_{1-4}-rich (CAS No 68477-79-2), if they contain > 0.1%(w/w)butadiene
664	丁二烯含量大于0.1%(w/w)的富C_4无酸汽油(石油), 来自催化裂解柴油脱丙烷塔塔底物	Gases (petroleum), catalytic-cracked gas oil depropaniser bottoms, C_4-rich acid-free (CAS No 68477-71-4), if they contain > 0.1%(w/w) butadiene
665	丁二烯含量大于0.1%(w/w)的富C_{3-5}汽油(石油), 来自催化裂解石脑油脱丁烷塔塔底物	Gases (petroleum), catalytic-cracked naphtha debutaniser bottoms, C_{3-5}-rich (CAS No 68477-72-5), if they contain > 0.1%(w/w) butadiene

No.	Chinese Name	English Name
666	丁二烯含量大于0.1%(w/w)的原油蒸馏及催化裂解的汽油(石油)	Gases (petroleum), crude distn and catalytic cracking (CAS No 68989-88-8), if they contain > 0.1%(w/w) butadiene
667	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自原油分馏尾气	Gases (petroleum), crude oil fractionation off (CAS No 68918-99-0), if they contain > 0.1%(w/w) butadiene
668	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自脱乙烷塔塔顶馏分	Gases (petroleum), deethaniser overheads (CAS No 68477-86-1), if they contain > 0.1%(w/w) butadiene
669	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自脱己烷尾气	Gases (petroleum), dehexaniser off (CAS No 68919-00-6), if they contain > 0.1%(w/w)butadiene
670	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自脱异丁烷塔塔顶馏分	Gases (petroleum), deisobutaniser tower overheads (CAS No 68477-87-2), if they contain > 0.1%(w/w)butadiene
671	丁二烯含量大于0.1%(w/w)的汽油，来自脱丙烷油脚分馏塔尾气	Gases (petroleum), depropaniser bottoms fractionation off (CAS No 68606-34-8), if they contain > 0.1%(w/w) butadiene
672	丁二烯含量大于0.1%(w/w)的富丙烯汽油(石油)，来自脱丙烷干塔	Gases (petroleum), depropaniser dry, propene-rich (CAS No 68477-90-7), if they contain > 0.1%(w/w)butadiene
673	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自脱丙烷塔塔顶馏分	Gases (petroleum), depropaniser overheads (CAS No 68477-91-8), if they contain > 0.1%(w/w) butadiene
674	丁二烯含量大于0.1%(w/w)的汽油，来自加氢精制脱硫汽提塔馏分尾气	Gases (petroleum), distillate unifiner desulfurisation stripper off (CAS No 68919-01-7), if they contain > 0.1%(w/w) butadiene
675	丁二烯含量大于0.1%(w/w)的干酸汽油(石油)尾气，来自汽油浓缩单元	Gases (petroleum), dry sour, gas-concn- unit-off (CAS No 68477-92-9), if they contain > 0.1%(w/w) butadiene
676	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自流化催化裂解分馏塔尾气	Gases (petroleum), fluidised catalytic cracker fractionation off (CAS No 68919-02-8) if they contain > 0.1%(w/w) butadiene
677	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自流化催化裂解洗气二级吸收塔尾气	Gases (petroleum), fluidised catalytic cracker scrubbing secondary absorber off (CAS No 68919-03-9), if they contain > 0.1%(w/w) butadiene
678	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自流化催化裂解分流塔塔顶馏分	Gases (petroleum), fluidised catalytic cracker splitter overheads (CAS No 68919-20-0), if they contain > 0.1%(w/w)butadiene
679	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自全程馏分的直馏石脑油脱己烷塔尾气	Gases (petroleum), full-range straight-run naphtha dehexaniser off (CAS No 68513-15-5), if they contain> 0.1%(w/w) butadiene
680	丁二烯含量大于0.1%(w/w)的经汽油浓缩再吸收塔蒸馏的汽油(石油)	Gases (petroleum), gas concn reabsorber distn(CAS No 68477-93-0), if they contain > 0.1%(w/w) butadiene
681	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自二乙醇胺洗涤塔尾气的柴油	Gases (petroleum), gas oil diethanolamine scrubber off (CAS No 92045-15-3), if they contain > 0.1%(w/w) butadiene
682	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自加氢脱硫的柴油流出液	Gases (petroleum), gas oil hydrodesulfurisation effluent (CAS No 92045-16-4), if they contain > 0.1%(w/w) butadiene
683	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自加氢脱硫清洗的柴油	Gases (petroleum), gas oil hydrodesulfurisation purge (CAS No 92045-17-5), if they contain > 0.1%(w/w) butadiene

No.	Chinese Name	English Name
684	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自汽油回收工厂脱丙烷塔塔顶馏分	Gases (petroleum), gas recovery plant depropaniser overheads (CAS No 68477-94-1), if they contain > 0.1%(w/w) butadiene
685	丁二烯含量大于0.1%(w/w)的经Girbatol单元进料处理的汽油(石油)	Gases (petroleum), Girbatol unit feed (CAS No 68477-95-2), if they contain > 0.1%(w/w) butadiene
686	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自加氢脱硫汽提塔重馏分尾气	Gases (petroleum), heavy distillate hydrotreater desulfurisation stripper off (CAS No 68919-04-0), if they contain > 0.1%(w/w) butadiene
687	丁二烯含量大于0.1%(w/w)的富碳氢汽油(石油)，来自加氢裂解脱丙烷塔尾气	Gases (petroleum), hydrocracking depropaniser off, hydrocarbon-rich (CAS No 68513-16-6), if they contain> 0.1%(w/w) butadiene
688	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自加氢裂解低压分离塔	Gases (petroleum), hydrocracking low-pressure separator (CAS No 68783-06-2), if they contain > 0.1%(w/w)butadiene
689	丁二烯含量大于0.1%(w/w)的汽油(石油)尾气，来自氢吸收塔	Gases (petroleum), hydrogen absorber off (CAS No 68477-96-3), if they contain > 0.1%(w/w) butadiene
690	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自加氢流出液闪蒸槽尾气	Gases (petroleum), hydrogenator effluent flash drum off (CAS No 92045-18-6), if they contain > 0.1%(w/w) butadiene
691	丁二烯含量大于0.1%(w/w)的富氢汽油(石油)	Gases (petroleum), hydrogen-rich (CAS No 68477-97-4), if they contain > 0.1%(w/w) butadiene
692	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自加氢酸化煤油脱戊烷稳定塔的尾气	Gases (petroleum), hydrotreated sour kerosine depentaniser stabiliser off (CAS No 68911-58-0), if they contain> 0.1%(w/w) butadiene
693	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自加氢酸化煤油闪蒸槽	Gases (petroleum), hydrotreated sour kerosine flash drum (CAS No 68911-59-1), if they contain > 0.1%(w/w) butadiene
694	丁二烯含量大于0.1%(w/w)的富氢-氮汽油(石油), 来自循环加氢调和油	Gases (petroleum), hydrotreater blend oil recycle, hydrogen-nitrogen-rich (CAS No 68477-98-5), if they contain> 0.1%(w/w) butadiene
695	丁二烯含量大于0.1%(w/w)的无硫化氢富C_4汽油(石油)，来自异构化石脑油分馏塔	Gases (petroleum), isomerised naphtha fractionator, C_4-rich, hydrogen sulfide-free (CAS No 68477-99-6), if they contain > 0.1%(w/w) butadiene
696	丁二烯含量大于0.1%(w/w)的轻蒸汽裂浓丁二烯的汽油(石油)	Gases (petroleum), light steam-cracked, butadiene conc(CAS No 68955-28-2), if they contain > 0.1%(w/w) butadiene
697	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自轻直馏汽油分馏稳定塔尾气	Gases (petroleum), light straight run gasoline fractionation stabiliser off (CAS No 68919-05-1), if they contain> 0.1%(w/w)butadiene
698	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自轻直馏石脑油稳定塔尾气	Gases (petroleum), light straight-run naphtha stabiliser off (CAS No 68513-17-7), if they contain > 0.1%(w/w) butadiene
699	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自石脑油蒸汽裂解的高压残液	Gases (petroleum), naphtha steam cracking high-pressure residual (CAS No 92045-19-7), if they contain > 0.1%(w/w)butadiene
700	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自石脑油精制加氢脱硫汽提塔尾气	Gases (petroleum), naphtha unifiner desulfurisation stripper off (CAS No 68919-06-2), if they contain > 0.1%(w/w) butadiene
701	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自炼油厂汽油蒸馏尾气	Gases (petroleum), oil refinery gas distn off (CAS No 68527-15-1), if they contain > 0.1%(w/w) butadiene

No.	Chinese Name	English Name
702	丁二烯含量大于0.1%(w/w)的汽油(石油)， 来自铂重整产品分离塔尾气	Gases (petroleum), platformer products separator off (CAS No 68814-90-4), if they contain > 0.1%(w/w) butadiene
703	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自轻馏分分馏的铂重整稳定塔尾气	Gases (petroleum), platformer stabiliser off, light ends fractionation (CAS No 68919-07-3), if they contain> 0.1%(w/w) butadiene
704	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自原油蒸馏的预闪蒸塔尾气	Gases (petroleum), preflash tower off, crude distn(CAS No 68919-08-4), if they contain > 0.1%(w/w)butadiene
705	丁二烯含量大于0.1%(w/w)的循环处理的富氢汽油(石油)	Gases (petroleum), recycle, hydrogen-rich (CAS No 68478-00-2), if they contain > 0.1%(w/w) butadiene
706	丁二烯含量大于0.1%(w/w)的炼油厂汽油(石油)	Gases (petroleum), refinery (CAS No 68814-67-5), if they contain > 0.1%(w/w) butadiene
707	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自精炼厂的调合油	Gases (petroleum), refinery blend (CAS No 68783-07-3), if they contain > 0.1%(w/w) butadiene
708	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自重整流出液高压闪蒸槽尾气	Gases (petroleum), reformer effluent high-pressure flash drum off (CAS No 68513-18-8), if they contain > 0.1%(w/w) butadiene
709	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自重整流出液低压闪蒸槽尾气	Gases (petroleum), reformer effluent low-pressure flash drum off (CAS No 68513-19-9), if they contain > 0.1%(w/w) butadiene
710	丁二烯含量大于0.1%(w/w)的重整补偿的富氢汽油(石油)	Gases (petroleum), reformer make-up, hydrogen-rich (CAS No 68478-01-3), if they contain > 0.1%(w/w) butadiene
711	丁二烯含量大于0.1%(w/w)的重整加氢汽油(石油)	Gases (petroleum), reforming hydrotreater (CAS No 68478-02-4), if they contain > 0.1%(w/w) butadiene
712	丁二烯含量大于0.1%(w/w)的富氢汽油(石油)，来自补偿重整加氢塔	Gases (petroleum), reforming hydrotreater make-up, hydrogen-rich (CAS No 68478-04-6), if they contain> 0.1%(w/w) butadiene
713	丁二烯含量大于0.1%(w/w)的富氢-甲烷汽油(石油)，来自重整加氢塔	Gases (petroleum), reforming hydrotreater, hydrogen-methane-rich (CAS No 68478-03-5), if they contain> 0.1%(w/w) butadiene
714	丁二烯含量大于0.1%(w/w)的汽油(石油)，来自残渣减粘轻度裂解尾气	Gases (petroleum), residue visbreaking off (CAS No 92045-20-0), if they contain > 0.1%(w/w) butadiene
715	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自流化催化裂解塔顶馏出物分馏塔的二级吸收塔尾气	Gases (petroleum), secondary absorber off, fluidised catalytic cracker overheads fractionator (CAS No 68602-84-6), if they contain > 0.1%(w/w) butadiene
716	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自流化催化裂解及柴油脱硫塔顶馏分分馏的海绵吸收塔尾气	Gases (petroleum), sponge absorber off, fluidised catalytic cracker and gas oil desulfuriser overhead fractionation(CAS No 68955-33-9), if they contain > 0.1%(w/w) butadiene
717	丁二烯含量大于0.1%(w/w)的蒸汽裂解富C_3汽油(石油)	Gases (petroleum), steam-cracker C_3-rich (CAS No 92045-22-2), if they contain > 0.1%(w/w) butadiene
718	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自直馏石脑油催化重整稳定塔塔顶馏分	Gases (petroleum), straight-run naphtha catalytic reformer stabiliser overhead (CAS No 68955-34-0), if they contain > 0.1%(w/w) butadiene
719	丁二烯含量大于0.1%(w/w)的汽油(石油), 来自直馏石脑油催化重整尾气	Gases (petroleum), straight-run naphtha catalytic reforming off (CAS No 68919-09-5), if they contain > 0.1%(w/w) butadiene
720	丁二烯含量大于0.1%(w/w)的汽油(石油)，直馏稳定塔尾气	Gases (petroleum), straight-run stabiliser off (CAS No 68919-10-8), if they contain > 0.1%(w/w)

No.	Chinese Name	English Name
		butadiene
721	丁二烯含量大于0.1%(w/w)的来自焦油汽提塔尾气的汽油(石油)	Gases (petroleum), tar stripper off (CAS No 68919-11-9), if they contain > 0.1%(w/w)butadiene
722	丁二烯含量大于0.1%(w/w)的热裂解蒸馏汽油(石油)	Gases (petroleum), thermal cracking distn (CAS No 68478-05-7), if they contain > 0.1%(w/w) butadiene
723	丁二烯含量大于0.1%(w/w)的来自加氢精制汽提塔尾气的汽油(石油)	Gases (petroleum), unifiner stripper off (CAS No 68919-12-0), if they contain > 0.1%(w/w) butadiene
724	糖皮质激素类	Glucocorticoids
725	格鲁米特及盐类	Glutethimide (2-ethyl-2-phenylglutarimide) and its salts
726	格列环脲	Glycyclamide (1-cyclohexyl-3-(p-toluenesulfonyl) urea)
727	金盐类	Gold salts
728	愈创甘油醚	Guaifenesin [3-(0-methoxyphenoxy)-1,2-propanediol; glyceryl guaiacolate]
729	胍乙啶及其盐类	Guanethidine (1-[2-(1-azacyclooctyl)ethyl] guanidine) and its salts
730	氟哌啶醇	Haloperidol (4-[4-(p-chlorophenyl) -4-hydroxypiperidino]-4'-fluorobutyrophenone)
731	七氯	Heptachlor (CAS No 76-44-8)
732	七氯一环氧化物	Heptachlor-epoxide (CAS No 1024-57-3)
733	六氯苯	Hexachlorobenzene (CAS No 118-74-1)
734	六氯乙烷	Hexachloroethane
735	四磷酸六乙基酯	Hexaethyl tetraphosphate
736	六氢化香豆素	Hexahydrocoumarin(CAS No 700-82-3)
737	六氢化环戊(c)吡咯-1-(1*H*)-铵　　*N*-乙氧基羰基-*N*-(聚砜基)氮烷化物	Hexahydrocyclopenta(c)pyrrole-(1*H*)-ammorium *N*-ethoxycarbonyl-*N*-(polylsulfonyl)azanide (EC No418-350-1)
738	六甲基磷酸-三酰胺	Hexamethylphosphoric-triamide (CAS No 680-31-9)
739	2-己酮	Hexan-2-one (CAS No 591-78-6)
740	己烷	Hexane (CAS No 110-54-3)
741	己丙氨酯	Hexapropymate (1-(2-propynyl) cyclohexanol carbamate)
742	北美黄连碱和北美黄连次碱以及它们的盐类	Hydrastine, hydrastinine and their salts
743	酰肼类及其盐类	Hydrazides and their salts
744	肼，肼的衍生物以及它们的盐类	Hydrazine, its derivatives and their salts
745	氢化松香基醇	Hydroabietyl alcohol (CAS No 13393-93-6)
746	富含芳烃的C_{26-55}碳氢化合物	Hydrocarbons C_{26-55},arom.Rich (CAS No 97722-04-8)
747	来自溶剂萃取的轻环烷烃C_{11-17}碳氢化合物，除非清楚全部精炼过程并且	Hydrocarbons, C_{11-17}, solvent-extd light naphthenic (CAS No 97722-08-2), except if the full refining

No.	Chinese Name	English Name
	能够证明所获得的物质不是致癌物	history is known and it can be shown that the substance from which it is produced is not a carcinogen
748	来自加氢石蜡轻馏分的C_{12-20}碳氢化合物, 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Hydrocarbons, C_{12-20}, hydrotreated paraffinic, distn lights (CAS No 97675-86-0), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
749	丁二烯含量大于0.1%(w/w)的C_{1-3}碳氢化合物	Hydrocarbons, C_{1-3} (CAS No 68527-16-2), if they contain > 0.1%(w/w) butadiene
750	二甲基亚砜提取物含量大于3%(w/w)的C_{13-27}碳氢化合物, 来自溶剂提取的轻环烷	Hydrocarbons, C_{13-27}, solvent-extd light naphthenic (CAS No 97722-09-3), if they contain > 3 % (w/w) DMSO extract
751	二甲基亚砜提取物含量大于3%(w/w)的C_{13-30}碳氢化合物, 来自富芳烃的溶剂提取的环烷馏分	Hydrocarbons, C_{13-30}, arom-rich, solvent-extd naphthenic distillate (CAS No 95371-04-3), if they contain> 3 % (w/w) DMSO extract
752	丁二烯含量大于0.1%(w/w)的C_{1-4}碳氢化合物	Hydrocarbons, C_{1-4} (CAS No 68514-31-8), if they contain > 0.1%(w/w) butadiene
753	丁二烯含量大于0.1%(w/w)的脱丁烷馏分C_{1-4}碳氢化合物	Hydrocarbons, C_{1-4}, debutanizer fraction (CAS No 68527-19-5), if they contain > 0.1%(w/w) butadiene
754	丁二烯含量大于0.1%(w/w)的脱硫C_{1-4}碳氢化合物	Hydrocarbons, C_{1-4}, sweetened (CAS No 68514-36-3), if they contain > 0.1%(w/w) butadiene
755	二甲基亚砜提取物含量大于3%(w/w)的C_{14-29}碳氢化合物, 来自溶剂提取的轻环烷	Hydrocarbons, C_{14-29}, solvent-extd light naphthenic (CAS No 97722-10-6), if they contain > 3 % (w/w) DMSO extract
756	来自加氢中间馏分的轻C_{16-20}碳氢化合物, 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Hydrocarbons, C_{16-20}, hydrotreated middle distillate, distn Lights (CAS No 97675- 85-9), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
757	二甲基亚砜提取物含量大于3%(w/w)的C_{16-32}碳氢化合物, 来自富芳烃的溶剂提取的环烷馏分	Hydrocarbons, C_{16-32}, arom rich, solvent-extd naphthenic distillate (CAS No 95371-05-4), if they contain> 3 % (w/w) DMSO extract
758	二甲基亚砜提取物含量大于3%(w/w)的C_{17-30}碳氢化合物, 来自加氢蒸馏的轻馏分	Hydrocarbons, C_{17-30}, hydrotreated distillates, distn Lights (CAS No 97862-82-3), if they contain > 3 % (w/w) DMSO extract
759	二甲基亚砜提取物含量大于3%(w/w)的C_{17-30}碳氢化合物, 来自加氢溶剂脱沥青常压蒸馏的残液的轻馏分	Hydrocarbons, C_{17-30}, hydrotreated solvent-deasphalted atm distn residue, distn lights (CAS No 97675-87-1), if they contain > 3 % (w/w) DMSO extract
760	二甲基亚砜提取物含量大于3%(w/w)的C_{17-40}碳氢化合物, 来自加氢溶剂脱沥青蒸馏残液的减压蒸馏轻馏分	Hydrocarbons, C_{17-40}, hydrotreated solvent-deasphalted distn residue, vacuum distn lights (CAS No 97722-06-0), if they contain > 3 % (w/w) DMSO extract
761	二甲基亚砜提取物含量大于3%(w/w)的C_{20-50}碳氢化合物, 来自残油的氢化减压馏分	Hydrocarbons, C_{20-50}, residual oil hydrogenation vacuum distillate (CAS No 93924- 61-9), if they contain > 3 % (w/w) DMSO extract
762	二甲基亚砜提取物含量大于3%(w/w)的氢化的溶剂脱蜡重石蜡C_{20-50}碳氢化合物	Hydrocarbons, C_{20-50}, solvent dewaxed heavy paraffinic, hydrotreated (CAS No 90640-95-2), if they contain> 3 % (w/w) DMSO extract
763	二甲基亚砜提取物含量大于3%(w/w)的加氢C_{20-58}碳氢化合物	Hydrocarbons, C_{20-58}, hydrotreated (CAS No 97926-70-0), if they contain > 3 % (w/w) DMSO extract
764	丁二烯含量大于0.1%(w/w)的C_{2-4}碳氢化合物	Hydrocarbons, C_{2-4} (CAS No 68606-25-7), if they contain > 0.1%(w/w) butadiene
765	丁二烯含量大于0.1%(w/w)富C_3的C_{2-4}碳氢化合物	Hydrocarbons, C_{2-4}, C_3-rich (CAS No 68476-49-3), if they contain > 0.1%(w/w) butadiene

No.	Chinese Name	English Name
766	二甲基亚砜提取物含量大于3%(w/w)的脱芳构化C_{27-42}碳氢化合物	Hydrocarbons, C_{27-42}, dearomatised (CAS No 97862-81-2), if they contain > 3 % (w/w) DMSO extract
767	二甲基亚砜提取物含量大于3%(w/w)的C_{27-42}环烷烃碳氢化合物	Hydrocarbons, C_{27-42}, naphthenic (CAS No 97926-71-1), if they contain > 3 % (w/w) DMSO extract
768	二甲基亚砜提取物含量大于3%(w/w)的脱芳构化C_{27-45}碳氢化合物	Hydrocarbons, C_{27-45}, dearomatised (CAS No 97926-68-6), if they contain > 3 % (w/w) DMSO extract
769	二甲基亚砜提取物含量大于3%(w/w)的C_{27-45}碳氢化合物，来自环烷减压蒸馏	Hydrocarbons, C_{27-45}, naphthenic vacuum distn(CAS No 97862-83-4), if they contain > 3 % (w/w) DMSO extract
770	丁二烯含量大于0.1%(w/w)的C_3碳氢化合物	Hydrocarbons, C_3 (CAS No 68606-26-8), if they contain > 0.1%(w/w) butadiene
771	丁二烯含量大于0.1%(w/w)的C_{3-4}碳氢化合物	Hydrocarbons, C_{3-4} (CAS No 68476-40-4), if they contain > 0.1%(w/w) butadiene
772	丁二烯含量大于0.1%(w/w)的碳氢化合物，来自富C_{3-4}的石油馏分	Hydrocarbons, C_{3-4}-rich, petroleum distillate (CAS No 68512-91-4), if they contain > 0.1%(w/w) butadiene
773	二甲基亚砜提取物含量大于3%(w/w)的C_{37-65}碳氢化合物，来自加氢脱沥青的减压蒸馏的残液	Hydrocarbons, C_{37-65}, hydrotreated deasphalted vacuum distn Residues (CAS No 95371-08-7), if they contain> 3 % (w/w) DMSO extract
774	二甲基亚砜提取物含量大于3%(w/w)的C_{37-68}碳氢化合物，来自脱蜡脱沥青加氢的减压蒸馏的残液	Hydrocarbons, C_{37-68}, dewaxed deasphalted hydrotreated vacuum distn Residues (CAS No 95371-07-6), if they contain > 3 % (w/w) DMSO extract
775	丁二烯含量大于0.1%(w/w)的C_4碳氢化合物	Hydrocarbons, C_4 (CAS No 87741-01-3), if they contain > 0.1%(w/w) butadiene
776	丁二烯含量大于0.1%(w/w)的无1,3-丁二烯和异丁烯的C_4碳氢化合物	Hydrocarbons, $C_{4,\ 1,3}$-butadiene- and isobutene-free (CAS No 95465-89-7), if they contain > 0.1%(w/w) butadiene
777	丁二烯含量大于0.1%(w/w)的蒸汽裂解C_4馏分的碳氢化合物	Hydrocarbons, C_4, steam-cracker distillate (CAS No 92045-23-3), if they contain > 0.1%(w/w) butadiene
778	丁二烯含量大于0.1%(w/w)的C_{4-5}碳氢化合物	Hydrocarbons, C_{4-5} (CAS No 68476-42-6), if they contain > 0.1%(w/w) butadiene
779	二甲基亚砜提取物含量大于3%(w/w)的碳氢化合物，来自溶剂脱蜡的加氢裂的石蜡蒸馏残液	Hydrocarbons, hydrocracked paraffinic distn residues, solvent-dewaxed (CAS No 93763-38-3), if they contain> 3 % (w/w) DMSO extract
780	C_{16-20}碳氢化合物，来自溶剂脱蜡、加氢裂解的烷烃蒸馏残液	Hydrocarbons,C_{16-20}, solvent-dewaxed hydrocracked paraffinic distn.Residue (CAS No 97675-88-2)
781	氢氟酸及其正盐,配合物以及氢氟化物(表 3 中的氟化合物除外)	Hydrofluoric acid, its normal salts, its complexes and hydrofluorides with the exception of those given in table 3
782	氰化氢及其盐类	Hydrogen cyanide and its salts
783	8-羟喹啉及其硫酸盐(表 3 中的 8-羟喹啉及其硫酸盐除外)	Hydroxy-8-quinoline and its sulphate, except for the uses provided in table 3
784	羟嗪	Hydroxyzine [2-(2-(4-(p-chlore- α -phenylbenzyl)-1-piperazinyl) ethoxy)ethanol]
785	东莨菪碱及其盐类和衍生物	Hyoscine, its salts and derivatives
786	莨菪碱及其盐类和衍生物	Hyoscyamine, its salts and derivatives
787	莨菪(叶、果实、粉和草药制剂)	*Hyoscyamus niger L.* (leaves, seeds, powder and galenical preparations)
788	咪唑啉-2-硫酮	Imidazolidine-thione (CAS No 96-45-7)

No.	Chinese Name	English Name
789	欧前胡内酯	Imperatorin (9-(3-methoxylbut-2-enyloxy)furo(3, 2-g) chromen-7-one)
790	无机亚硝酸盐类(亚硝酸钠除外)	Inorganic nitrites, with the exception of sodium nitrite
791	2,5-双(1-氮杂环丙烯基)-3,6-二丙氧基-1,4-苯醌	Inproquone (2,5-bis (1-aziridinyl)-3,6-dipropoxy-1,4-benzoquinone)
792	碘	Iodine
793	碘代甲烷	Iodomethane (CAS No 74-88-4)
794	碘苯腈; 4-羟基-3,5-二碘苯甲腈	Ioxynil (CAS No 1689-83-4)
795	吐根(根、粉末及草药制剂)	Ipecacuanha (*cephaelis ipecacuanha brot.* And related species) (roots, powder and galenical preparations)
796	异丙二酮	Iprodione (CAS No 36734-19-7)
797	丁二烯含量大于或等于0.1%(w/w)的异丁烷	Isobutane (CAS No 75-28-5), if it contains ≥0.1%(w/w) butadiene
798	亚硝酸异丁酯	Isobutyl nitrite(CAS No 542-56-3)
799	异卡波肼	Isocarboxazide (1-benzyl-2-(6-methylisoxazol-3-ylcarbonyl) hydrazine)
800	异美汀及其盐类	Isometheptene (6-methyl-2-methylaminohept-5-ene) and its salts
801	异丙肾上腺素	Isoprenaline (3,4-dihydroxy-α-(isopropylaminomethyl) benzyl alcohol)
802	稳定的橡胶基质(2-甲基-1,3-丁二烯)	Isoprene (stabilized) (2-methyl-1,3-butadiene) (CAS No 78-79-5)
803	硝酸异山梨酯	Isosorbide dinitrate (1,4:3,6-dianhydrosorbitol 2,5-dinitrate)
804	异噁氟草	Isoxaflutole (CAS No 141112-29-0)
805	叉子园柏的叶子, 精油及其草药制剂	*Juniperus sabina L.* (leaves, essential oil and galenical preparations)
806	酮康唑	Ketoconazole
807	亚胺菌	Kresoxim-methyl (CAS No 143390-89-0)
808	铅和铅化合物	Lead and its compounds
809	利多卡因	Lidocaine
810	利农伦	Linuron (CAS No 330-55-2)
811	北美山梗菜及其草药制剂	*Lobelia inflata L.* And its galenical preparations
812	洛贝林及其盐类	Lobeline (2-(β-hydroxyphenethyl)-1-methyl-6-phenacylpiperidine) and its salts
813	润滑脂, 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Lubricating greases (CAS No 74869-21-9), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
814	二甲基亚砜提取物含量大于3%(w/w)的润滑油	Lubricating oils (CAS No 74869-22-0), if they contain > 3 % (w/w) DMSO extract

No.	Chinese Name	English Name
815	二甲基亚砜提取物含量大于3%(w/w)的来自原油的石蜡润滑油(石油)	Lubricating oils (petroleum), base oils, paraffinic (CAS No 93572-43-1), if they contain > 3 % (w/w) DMSO extract
816	二甲基亚砜提取物含量大于3%(w/w)的溶剂萃取、脱沥青、脱蜡加氢处理的碳原子数大于25的润滑油(石油)	Lubricating oils (petroleum), C>25, solvent-extd, deasphalted, dewaxed, hydrogenated (CAS No 101316-69-2), if they contain > 3 % (w/w) DMSO extract
817	二甲基亚砜提取物含量大于3%(w/w) 的加氢中性油基高粘C_{15-30}润滑油(石油)	Lubricating oils (petroleum), C_{15-30}, hydrotreated neutral oil-based (CAS No 72623- 86-0), if they contain > 3 % (w/w) DMSO extract
818	二甲基亚砜提取物含量大于3%(w/w)的溶剂萃取、脱蜡加氢的C_{17-32}润滑油(石油)	Lubricating oils (petroleum), C_{17-32}, solvent-extd, dewaxed, hydrogenated (CAS No 101316-70-5), if they contain > 3 % (w/w) DMSO extract
819	二甲基亚砜提取物含量大于3%(w/w)的加氢的溶剂萃取及脱蜡的C_{17-35}润滑油(石油)	Lubricating oils (petroleum), C_{17-35}, solvent-extd, dewaxed, hydrotreated (CAS No 92045-42-6), if they contain> 3 % (w/w) DMSO extract
820	二甲基亚砜提取物含量大于3%(w/w)的加氢裂解溶剂脱蜡的润滑油(石油)	Lubricating oils (petroleum), C_{18-27}, hydrocracked solvent-dewaxed (CAS No 97488-95-4), if they contain > 3 % (w/w) DMSO extract
821	二甲基亚砜提取物含量大于3%(w/w)的C_{18-40}润滑油，以溶剂脱蜡的加氢裂解轻馏分为基础	Lubricating oils (petroleum), C_{18-40}, solvent-dewaxed hydrocracked distillate-based (CAS No 94733-15-0), if they contain > 3 % (w/w) DMSO extract
822	二甲基亚砜提取物含量大于3%(w/w)的C_{18-40}润滑油，以溶剂脱蜡的加氢残油为基础	Lubricating oils (petroleum), C_{18-40}, solvent-dewaxed hydrogenated raffinate-based (CAS No 94733-16-1), if they contain > 3 % (w/w) DMSO extract
823	二甲基亚砜提取物含量大于3%(w/w)的溶剂萃取、脱蜡加氢的C_{20-35}润滑油(石油)	Lubricating oils (petroleum), C_{20-35}, solvent-extd, dewaxed, hydrogenated (CAS No 101316-71-6), if they contain > 3 % (w/w) DMSO extract
824	二甲基亚砜提取物含量大于3%(w/w)的加氢中性油基高粘C_{20-50}润滑油(石油)	Lubricating oils (petroleum), C_{20-50}, hydrotreated neutral oil-based, high-viscosity (CAS No 72623-85-9), if they contain > 3 % (w/w) DMSO extract
825	二甲基亚砜提取物含量大于3%(w/w) 的加氢中性油基高粘C_{20-50}润滑油(石油)	Lubricating oils (petroleum), C_{20-50}, hydrotreated neutral oil-based (CAS No 72623- 87-1), if they contain > 3 % (w/w) DMSO extract
826	二甲基亚砜提取物含量大于3%(w/w)的溶剂萃取、脱蜡加氢的C_{24-50}润滑油(石油)	Lubricating oils (petroleum), C_{24-50}, solvent-extd, dewaxed, hydrogenated (CAS No 101316-72-7), if they contain > 3 % (w/w) DMSO extract
827	二甲基亚砜提取物含量大于3%(w/w)的加氢裂解非芳香性的溶剂脱石蜡处理的润滑油(石油)	Lubricating oils (petroleum), hydrocracked nonarom solvent-deparaffined (CAS No 92045-43-7), if they contain> 3 % (w/w) DMSO extract
828	麦角二乙胺及其盐类	Lysergide (*N*,*N*-diethyllysergamide; lysergic acid diethylamide) and its salts
829	孔雀石绿的盐酸盐和草酸盐	Malachite green hydrochloride (CAS No 569-64-2) malachite green oxalate (CAS No 18015-76-4)
830	丙二腈	Malononitrile
831	甘露莫司汀及其盐类	Mannomustine (1,6-bis (2-chloroethylamino)-1,6-dideoxy-D-mannitol) and its salts
832	美卡拉明(3-甲氨基异莰烷)	Mecamylamine (3-methylaminoisobornane)

No.	Chinese Name	English Name
833	美非氯嗪及其盐类	Mefeclorazine (1-(*o*-chlorophenyl)-4-(3,4-dimethoxyphenethyl) piperazine) and its salts
834	美芬新及其酯类	Mephenesin (*o*-cresyl glyceryl ether; 3-(*o*-methylphenoxy)-1,2-propanediol) and its esters
835	甲丙氨酯	Meprobamate (2-methyl-2-propyl-1,3-propanediol dicarbamate)
836	汞和汞化合物(表 4 中的汞化合物除外)	Mercury and its compounds, except those special cases included in table 4
837	聚乙醛	Metaldehyde
838	甲胺苯丙酮及其盐类	Metamfepramone (2-dimethylaminopropiophenone) and its salts
839	美索庚嗪及其盐类	Metethoheptazine (4-ethoxycarbonyl-1,3-dimethyl-4-phenylhexamethylenimine) and its salts
840	二甲双胍及其盐类	Metformin (1,1-dimethylbiguanide; *N*,*N*-dimethylguanylguanidine) and its salts
841	甲醇	Methanol
842	美沙吡林及其盐类	Methapyrilene (*N*,*N*-dimethyl-*N*'-(2-pyridyl)-*N*'-(2-thenyl) ethylenediamine) and its salts
843	美庚嗪及其盐类	Metheptazine (4-carbomethoxy-1,2-dimethyl-4-phenylhexamethylenimine) and its salts
844	美索巴莫	Methocarbamol
845	甲氨嘌呤	Methotrexate (*N*-[*p*-[(2, 4-diamino-6-pteridylmethyl) methylamino] benzoyl]-L-(+)-glutamic acid)
846	甲氧基乙酸	Methoxyacetic acid (CAS No 625-45-6)
847	异氰酸甲酯	Methyl isocyanate (CAS No 624-83-9)
848	反式-2-丁烯酸甲基酯	Methyl *trans*-2-butenoate (CAS No 623-43-8)
849	(亚甲基双(4,1-亚苯基偶氮(1-(3-(二甲基氨基)丙基)-1,2-二氢化-6-羟基-4-甲基-2-氧代嘧啶-5,3-二基)))-1,1'-二吡啶盐的二氯化物二盐酸化物	(Methylenebis(4,1-phenylenazo(1-(3-(dimethylamino)propyl)-1,2-dihydro-6-hydroxy-4-methyl-2-oxopyridine-5,3-diyl)))-1,1'-dipyridinium dichloride dihydrochloride(EC No 401-500-5)
850	甲基丁香酚，除天然香料含有并在产品中含量不大于以下浓度外:(a) 0.01%香精中含量; (b) 0.004%古龙水中含量; (c) 0.002%香脂中含量; (d) 0.001%淋洗类产品; (e) 0.0002% 其它驻留类产品和口腔卫生产品	Methyleugenol (CAS No 93-15-2) except for normal content in the natural essences used and provided that the concentration does not exceed: (a) 0.01% in fine fragrance; (b) 0.004% in eau de toilette; (c) 0.002% in fragrance cream; (d) 0.001% in rinse-off products; (e) 0.0002% in other leave-on products and oral hygiene products
851	乙酸(甲基-*ONN*-氧化偶氮基)甲酯	(Methyl-*ONN*-azoxy)methyl acetate (CAS No 592-62-1)
852	甲基环氧乙烷	Methyloxirane (CAS No 75-56-9)
853	哌甲酯及其盐类	Methylphenidate (methyl α-phenyl-2-piperid-2-ylacetate) and its salts
854	甲乙哌酮及其盐类	Methyprylon (3,3-diethyl-5-methyl-2,4-piperidinedione) and its salts
855	甲硝唑	Metronidazole
856	美替拉酮	Metyrapone (2-methyl-1,2-dipyrid-3-yl-1-propanone)
857	矿石棉，[不规则晶体排列，且碱金属氧化物和碱土金属氧化物(Na_2O +	Mineral wool,with the exception of those specified elsewhere in this Annex;[Man-made vitreous

No.	Chinese Name	English Name
	K_2O + CaO + MgO + BaO)含量大于 18%(以重量计)的人造玻璃质(硅酸盐)纤维], 在本附录中别处详细说明的那些除外	(silicate) fibres with random orientation with alkaline oxide and alkali earth oxide (Na_2O+K_2O+CaO+MgO+BaO)content greater than 18% by weight]
858	莫非布宗	Mofebutazone (4-butyl-1-phenyl-3,5-pyrazolidinedione)
859	禾草敌	Molinate (ISO) (CAS No 2212-67-1)
860	久效磷	Monocrotophos (CAS No 6923-22-4)
861	灭草隆	Monuron (CAS No 150-68-5)
862	吗啉及其盐类	Morpholine (diethyleneimideoxide) and its salts
863	吗啉-4-碳酰氯	Morpholine-4-carbonyl chloride (CAS No 15159-40-7)
864	间苯二胺及其盐类	*m*-Phenylenediamine and its salts (CAS No 108-45-2)
865	二异氰酸间-甲苯亚基酯	*m*-Tolylidene diisocyanate (CAS No 26471-62-5)
866	[(间-甲苯氧基)甲基]环氧乙烷	[(*m*-Tolyloxy)methyl]oxirane (CAS No 2186-25-6)
867	腈菌唑, 2-(4-氯苯基)-2-(1*H*-1,2,4-三唑-1-基甲基)己腈	Myclobutanil, 2-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl methyl)hexanenitrile (CAS No 88671-89-0)
868	*N*-(3-氨甲酰基-3,3-二苯丙基)-*N*,*N*-二异丙基甲基铵盐类。例如: 异丙碘铵	*N*-(3-carbamoyl-3,3-diphenylpropyl)-*N*,*N*-diisopropylmethylammonium salts, e.g. Isopropamide iodide
869	*N*-(三氯甲基硫代)-4-环己烯-1,2-联羧酰胺(克霉丹)	*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide (captan)
870	*N*-(三氯甲硫基)邻苯二甲酰亚胺	*N*-(trichloromethylthio)phthalimide (CAS No 133-07-3)
871	*N*,*N*,*N*',*N*'-四缩水甘油基-4,4'-二氨基-3,3'-二乙基二苯基甲烷	*N*,*N*,*N*',*N*'-tetraglycidyl-4,4'-diamino-3,3'-diethyldiphenylmethane (CAS No 130728-76-6)
872	*N*,*N*,*N*',*N*'-四甲基-4,4'-二苯氨基甲烷	*N*,*N*,*N*',*N*'-tetramethyl-4,4'-methylendianiline(CAS No 101-61-1)
873	*N*,*N*'-((甲基亚氨基)二乙烯)双(乙基二甲基氨)盐,如: 阿扎溴铵	*N*,*N*'-[(methylimino) diethylene] bis (ethyldimethylammonium) salts, e.g. Azamethonium bromide
874	*N*,*N*'-五甲亚基双(三甲基铵)盐,如: 五甲溴铵	*N*,*N*'-pentamethylenebis (trimethylammonium) salts, e.g. Pentamethonium bromide
875	*N*,*N*-双(2-氯乙基)甲胺-*N*-氧化物及其盐类	*N*,*N*-bis(2-chloroethyl)methylamine *N*-oxide and its salts
876	*N*,*N*-二甲基乙酰胺	*N*,*N*-dimethylacetamide (CAS No 127-19-5)
877	*N*,*N*-二甲基苯胺	*N*,*N*-dimethylaniline (CAS No 121-69-7)
878	*N*,*N*-二甲基苯胺四(戊氟化苯基)硼酸盐	*N*,N-dimethylanilinium tetrakis (pentafluorophenyl)borate (CAS No 118612-00-3)
879	*N*,*N*-二甲基甲酰胺	*N*,*N*-dimethylformamide (CAS No 68-12-2)
880	*N*,*N*'-六甲亚基双(三甲基铵)盐, 如: 六甲溴铵	*N*,*N*'-hexamethylenebis(trimethylammonium) salts, e g hexamethonium bromide
881	*N*-[2-(3-乙酰基-5-硝基噻吩-2-基偶氮)-5-二乙基氨基苯基]乙酰胺	*N*-[2-(3-acetyl-5-nitrothiophen-2-ylazo)-5-diethylaminophenyl] acetamide (EC No 416-860-9)
882	*N*'-(4-氯-邻-甲苯基)N,N-二甲基甲脒-氢氯化物	*N*'-(4-chloro-o-tolyl)-*N*,N-dimethylformamidine monohydrochloride (CAS No 19750-95-9)
883	*N*-2-萘基苯胺	*N*-2-naphthylaniline (CAS No 135-88-6)

No.	Chinese Name	English Name
884	*N*-5-氯苯唑唑-2-基乙酰胺	*N*-5-chlorobenzoxazol-2-ylacetamide
885	烯丙吗啡及其盐类和醚类	Nalorphine (*N*-allylnormorphine; *N*-allyl-*N*-desmethylmorphine), its salts and ethers
886	萘甲唑啉及其盐类	Naphazoline [2-(1-naphthylmethyl)-2-imidazoline] and its salts
887	溶剂精制、加氢脱硫的重石脑油(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Naphtha (petroleum), solvent-refined hydrodesulfurised heavy (CAS No 97488-96-5), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
888	萘	Naphthalene (CAS No 91-20-3)
889	二甲基亚砜提取物含量大于3%(w/w)的催化脱蜡处理的重环烷油(石油)	Naphthenic oils (petroleum), catalytic dewaxed heavy (CAS No 64742-68-3), if they contain > 3 % (w/w) DMSO extract
890	二甲基亚砜提取物含量大于3%(w/w)的催化脱蜡处理的轻环烷油(石油)	Naphthenic oils (petroleum), catalytic dewaxed light (CAS No 64742-69-4), if they contain > 3 % (w/w) DMSO extract
891	二甲基亚砜提取物含量大于3%(w/w)的复合脱蜡处理的重环烷油(石油)	Naphthenic oils (petroleum), complex dewaxed heavy (CAS No 64742-75-2), if they contain > 3 % (w/w) DMSO extract
892	二甲基亚砜提取物含量大于3%(w/w)的复合脱蜡处理的轻环烷油(石油)	Naphthenic oils (petroleum), complex dewaxed light (CAS No 64742-76-3), if they contain > 3 % (w/w) DMSO extract
893	麻醉药类(凡是中华人民共和国药政法规定管制的麻醉药品品种)	Narcotics, natural and synthetic controlled by the Drug Administration Law of the People's Republic of China
894	*N*-环己基-*N*-甲氧基-2,5-二甲基-3-糠酰胺	*N*-cyclohexyl-*N*-methoxy-2,5-dimethyl-3-furamide (CAS No 60568-05-0)
895	钕和钕盐类	Neodymium and its salts
896	新斯的明及其盐类, 如溴新斯的明	Neostigmine and its salts (e.g.neostigmine bromide)
897	镍	Nickel (CAS No 7440-02-0)
898	碳酸镍	Nickel carbonate (CAS No 3333-67-3)
899	二氢氧化镍	Nickel dihydroxide (CAS No 12054-48-7)
900	二氧化镍	Nickel dioxide (CAS No 12035-36-8)
901	一氧化镍	Nickel monoxide (CAS No 1313-99-1)
902	硫酸镍	Nickel sulphate (CAS No 7786-81-4)
903	硫化镍	Nickel sulphide (CAS No 16812-54-7)
904	尼古丁及其盐类	Nicotine [3- (1-methyl-2-pyrrolidyl) pyridine] and its salts
905	硝基苯	Nitrobenzene
906	硝基甲酚类及其碱金属盐	Nitrocresols and their alkali metal salts
907	咔唑的硝基衍生类	Nitroderivatives of carbazole

No.	Chinese Name	English Name
908	除草醚	Nitrofen (CAS No 1836-75-5)
909	呋喃妥因	Nitrofurantoin (1-(5-nitro-2-furfurylideneamino)-hydantoin)
910	亚硝胺	Nitrosamines
911	亚硝基二丙胺	Nitrosodipropylamine (CAS No 621-64-7)
912	硝基芪(硝基 1,2 二苯乙烯)类，它们的同系物和衍生物	Nitrostilbenes, their homologues and their derivatives
913	硝羟喹啉及其盐类	Nitroxoline (5-nitro-8-quinolinol) and its salts
914	*N*-甲基乙酰胺	*N*-Methylacetamide (CAS No 79-16-3)
915	*N*-甲基甲酰胺	*N*-Methylformamide (CAS No 123-39-7)
916	壬基苯酚 支链 4-壬基苯酚	Nonylphenol (CAS No 25154-52-3) 4-nonylphenol, branched (CAS No 84852-15-3)
917	去甲肾上腺素及其盐类	Noradrenaline (norepinephrine) and its salts
918	那可丁及其盐类	Noscapine [(-)-1-(6, 7-dimethoxy-3-phthalidyl) -8-methoxy-2-methyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline] and its salts
919	*O,O*'-(乙烯基甲基硅烯)二[(4-甲基-2-酮)]肟	*O,O*'-(ethenylmethylsilylene) di[(4-methylpentan-2-one) oxime](EC No 421-870-1)
920	*O,O*'-二乙酰基-*N*-烯丙基-*N*-去甲基吗啡	*O,O*'-diacetyl-*N*-allyl-*N*-normorphine
921	*O,O*'-二乙基-*O*-(4-硝基苯基)硫代磷酸酯(对硫磷)	*O,O*'-diethyl *O*-4-nitrophenyl phosphorothioate (parathion-ISO)
922	邻-茴香胺(甲氧基苯胺；氨基苯甲醚)	*o*-Anisidine (CAS No 90-04-0)
923	奥他莫辛	Octamoxin (1-(1-methylheptyl)-hydrazine) and its salts
924	辛戊胺	Octamylamine (2-isoamylamino-6-methylheptane) and its salts
925	奥托君及其盐类	Octodrine (1,5-dimethylhexylamine; 2-amino-6-methylheptane) and its salts
926	邻-联(二)茴香胺基偶氮染料	*o*-Dianisidine based azo dyes
927	雌激素类	Oestrogens
928	月桂树籽油	Oil from the seeds of *Laurus nobilis L.*
929	欧夹竹桃苷	Oleandrin
930	邻苯二胺及其盐类	*o*-Phenylenediamine and its salts
931	邻-联甲苯胺基染料	*o*-Tolidine based dyes
932	稻思达	Oxadiargyl (ISO) (CAS No 39807-15-3)
933	(乙二酰双亚氨乙烯)双[(邻-氯苯基)二乙基铵]盐，如：安贝氯铵	(Oxalylbisiminoethylene) bis [(*o*-chlorobenzyl) diethylammonium]salts, e.g. ambenomium chloride
934	奥沙那胺及其衍生物	Oxanamide (2,3-epoxy-2-ethylhexanamide) and its derivatives

No.	Chinese Name	English Name
935	环氧乙烷甲醇, 4-甲苯磺酸盐(S)-	Oxiranemethanol, 4-methylbenzene-sulfonate, (S)- (CAS No 70987-78-9)
936	羟芬利定及其盐类	Oxpheneridine (ethyl ester of 1-(β-hydroxyphenethyl)-4-phenylpiperidine-4- carboxylic acid) and its salts
937	氧代双(氯甲烷),双(氯甲基)醚	Oxybis[chloromethane],bis (Chloromethyl) ether (CAS No 542-88-1)
938	二甲基亚砜提取物含量大于3%(w/w)的催化脱蜡处理的重石蜡油(石油)	Paraffin oils (petroleum), catalytic dewaxed heavy (CAS No 64742-70-7), if they contain > 3 % (w/w) DMSO extract
939	二甲基亚砜提取物含量大于3%(w/w)的催化脱蜡处理的轻石蜡油(石油)	Paraffin oils (petroleum), catalytic dewaxed light (CAS No 64742-71-8), if they contain > 3 % (w/w) DMSO extract
940	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制的脱蜡重石蜡油(石油)	Paraffin oils (petroleum), solvent-refined dewaxed heavy (CAS No 92129-09-4), if they contain > 3 % (w/w) DMSO extract
941	苯并[a]芘的含量大于0.005%(w/w)的固体石蜡，来自褐煤高温煤焦油	Paraffin waxes (coal), brown-coal high-temp tar (CAS No 92045-71-1), if they contain > 0.005% (w/w) benzo[a]pyrene
942	苯并[a]芘的含量大于0.005%(w/w)的固体石蜡，来自活性炭处理的褐煤高温煤焦油	Paraffin waxes (coal), brown-coal high-temp tar, carbon-treated (CAS No 97926-76-6), if they contain > 0.005% (w/w) benzo[a]pyrene
943	苯并[a]芘的含量大于0.005%(w/w)的固体石蜡，来自粘土处理的褐煤高温煤焦油	Paraffin waxes (coal), brown-coal high-temp tar, clay-treated (CAS No 97926-77-7), if they contain > 0.005% (w/w) benzo[a]pyrene
944	苯并[a]芘的含量大于0.005%(w/w)的固体石蜡，来自加氢处理的褐煤高温煤焦油	Paraffin waxes (coal), brown-coal high-temp tar, hydrotreated (CAS No 92045-72-2), if they contain > 0.005% (w/w) benzo[a]pyrene
945	苯并[a]芘的含量大于0.005%(w/w)的固体石蜡，来自硅酸处理的褐煤高温煤焦油	Paraffin waxes (coal), brown-coal high-temp tar, silicic acid-treated (CAS No 97926-78-8), if they contain> 0.005% (w/w) benzo[a]pyrene
946	帕拉米松	Paramethasone (6α-fluoro-16α-methylpregna-1,4-diene-11β,17,21-triol-3,20-dione)
947	对乙氧卡因及其盐类	Parethoxycaine (2-diethylaminoethyl ester of pethoxybenzoic acid) and its salts
948	*p*-氯三氯甲基苯	*p*-Chlorobenzotrichloride (CAS No 5216-25-1)
949	石榴皮碱(异石榴皮碱)及其盐类	Pelletierine (isopelletierine) and its salts
950	匹莫林及其盐类	Pemoline (2-amino-5-phenyl-2-oxazolin-4-one) and its salts
951	五氯乙烷	Pentachloroethane
952	五氯苯酚	Pentachlorophenol (CAS No 87-86-5)
953	戊四硝酯	Pentaerithrityl tetranitrate (pentaerythritol tetranitrate)
954	秘鲁香酯	Peru balsam(INCI name: Myroxylon pereirae; CAS No 8007-00-9)
955	陪曲氯醛	Petrichloral (1,1',1",1'"-(neopentanetetryltetraoxy) tetrakis (2,2,2-trichloroethanol))
956	矿脂，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Petrolatum (CAS No 8009-03-8), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen

No.	Chinese Name	English Name
957	氧化铝处理的矿脂(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Petrolatum (petroleum), alumina-treated (CAS No 85029-74-9), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
958	活性炭处理的矿脂(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Petrolatum (petroleum), carbon-treated (CAS No 97862-97-0), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
959	粘土处理的矿脂(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Petrolatum (petroleum), clay-treated (CAS No 100684-33-1), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
960	加氢的矿脂(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Petrolatum (petroleum), hydrotreated (CAS No 92045-77-7), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
961	氧化处理的矿脂(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Petrolatum (petroleum), oxidised (CAS No 64743-01-7), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
962	硅酸处理的矿脂(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Petrolatum (petroleum), silicic acid-treated (CAS No 97862-98-1), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
963	石油	Petroleum (CAS No 8002-05-9)
964	丁二烯含量大于0.1%(w/w)的液化石油气	Petroleum gases, liquefied (CAS No 68476-85-7), if they contain > 0.1%(w/w) butadiene
965	丁二烯含量大于0.1%(w/w)的脱硫液化石油气	Petroleum gases, liquefied, sweetened (CAS No 68476-86-8), if they contain > 0.1%(w/w) butadiene
966	丁二烯含量大于0.1%(w/w)的脱硫C_4馏分液化石油气	Petroleum gases, liquefied, sweetened, C_4 fraction (CAS No 92045-80-2), if they contain > 0.1%(w/w) butadiene
967	丁二烯含量大于0.1%(w/w)的石油产品, 来自炼油厂气油	Petroleum products, refinery gases (CAS No 68607-11-4), if they contain > 0.1%(w/w) butadiene
968	醋醢尿素苯	Phenacemide
969	非那二醇	Phenaglycodol
970	芬美曲秦及其衍生物和盐类	Phenmetrazine (3-methyl-2-phenylmorpheline) its derivatives and salts
971	苯酚	Phenol (CAS No 108-95-2)
972	吩噻秦及其化合物	Phenothiazine (dibenzoparathiazine; thiodiphenylamine) and its compounds
973	苯丙氨酯	Phenprobamate (3-phenylpropyl carbamate)
974	苯丙香豆素	Phenprocoumon (4-hydroxy-3-(1-phenylpropyl) coumarin)
975	保泰松	Phenylbutazone (4-butyl-2,2-diphenyl-3,5-pyrazolidinedione)
976	磷胺; 大灭虫	Phosphamidon (CAS No 13171-21-6)
977	磷及金属磷化物	Phosphorus and metal phosphides
978	毒扁豆	*Physostigma venenosum balf*
979	商陆及其制剂	*Phytolacca spp*. and their preparations

No.	Chinese Name	English Name
980	苦味酸(2,4,6-三硝基苯酚)	Picric acid (2,4,6-trinitrophenol)
981	印防己毒素	Picrotoxin
982	毛果云香碱及其盐类	Pilocarpine (5-((4-ethyl-2,3,4,5-tetrahydrofuran-5-on-3-yl) methyl)-1-methylimidazole) and its salts
983	毛果芸香及其草药制剂	*Pilocarpus jaborandi holmes* and its galenical preparations
984	匹哌氮酯及其盐类	Pipazetate (2-(2-piperid-1-ylethoxy) ethyl ester of 10*H*-pyrido (3,2-b)(1,4) benzothiazine 10-carboxylic acid) and its salts
985	哌苯甲醇及其盐类	Pipradrol (α-piperid-2-ylbenzhydrol) and its salts
986	哌库碘铵	Piprocurarium
987	苯并[a]芘的含量大于 0.005%(w/w)的沥青	Pitch (CAS No 61789-60-4) if it contains >0.005%((w/w))benzo[a]pyrene
988	苯并[a]芘的含量大于0.005%(w/w)的沥青，来自热处理的高温煤焦油	Pitch, coal tar, high-temp, heat-treated (CAS No 121575-60-8), if it contains > 0.005% (w/w) benzo[a]pyrene
989	苯并[a]芘的含量大于0.005%(w/w)的沥青，来自高温煤焦油次级馏分	Pitch, coal tar, high-temp, secondary (CAS No 94114-13-3), if it contains > 0.005% (w/w) benzo[a]pyrene
990	苯并[a]芘的含量大于0.005%(w/w)的沥青，来自低温煤焦油	Pitch, coal tar, low-temp(CAS No 90669-57-1), if it contains > 0.005% (w/w) benzo[a]pyrene
991	苯并[a]芘的含量大于0.005%(w/w)的沥青，来自热处理的低温煤焦油	Pitch, coal tar, low-temp, heat-treated (CAS No 90669-58-2), if it contains > 0.005% (w/w) benzo[a]pyrene
992	苯并[a]芘的含量大于0.005%(w/w)的沥青，来自氧化的低温煤焦油	Pitch, coal tar, low-temp, oxidised (CAS No 90669-59-3), if it contains > 0.005% (w/w) benzo[a]pyrene
993	苯并[a]芘的含量大于0.005%(w/w)的沥青，来自煤焦油-石油	Pitch, coal tar-petroleum (CAS No 68187-57-5), if it contains > 0.005% (w/w) benzo[a]pyrene
994	甲硫泊尔定	Poldine methylsulfate (2-benzilyloxymethyl-1,1-dimethylpyrrolidinium methosulfate)
995	溴酸钾	Potassium bromate (CAS No 7758-01-2)
996	对氨基苯乙醚(4-乙氧基苯胺)	*p*-Phenetidine (4-ethoxyaniline)(CAS No 156-43-4)
997	普莫卡因	Pramocaine
998	丙磺舒	Probenecid (*p*-(dipropylsulfamoyl) benzoic acid)
999	普鲁卡因胺及其盐类和衍生物	Procainamide (*p*-amino-*N*-(2-diethylaminoethyl) benzamide), its salts and derivatives
1000	孕激素类	Progestogens
1001	硝酸甘油(丙三醇三硝酸酯)	Propane-1,2,3-triyl trinitrate
1002	克螨特	Propargite (ISO) (CAS No 2312-35-8)
1003	丙帕硝酯	Propatylnitrate (2-ethyl-2-(hydroxymethyl)-1,3-propanediol trinitrate 1,1,1-trisnitrato methylpropane)
1004	丙唑嗪	Propazine (CAS No 139-40-2)
1005	丙醇酸内酯	Propiolactone (CAS No 57-57-8)

No.	Chinese Name	English Name
1006	异丙安替比林	Propyphenazone (4-isopropyl-2, 3-dimethyl-1-phenyl-3-pyrazolin-5-one)
1007	氯甲丙炔基苯甲酰胺	Propyzamide (CAS No 23950-58-5)
1008	桂樱(樱桂水)	*Prunus laurocerasus L.* ('cherry laurel water')
1009	赛洛西宾	Psilocybine [3-(2-dimethylaminoethyl) indol-4-yl dihydrogen phosphate]
1010	[(对-甲苯氧基)甲基]环氧乙烷	[(*p*-Tolyloxy)methyl]oxirane (CAS No 2186-24-5)
1011	吡蚜酮	Pymetrozine (ISO) (CAS No 123312-89-0)
1012	除虫菊及其草药制剂	*Pyrethrum album L.* And its galenical preparations
1013	吡硫鎓钠	Pyrithione sodium (INNM)
1014	焦棓酚	Pyrogallol
1015	一水化膦酸(*R*)-α-苯乙铵(-)-(1*R*,2*S*)-(1,2-环丙)酯	(*R*)-α-phenylethylammonium (-)-(1*R*,2*S*)-(1,2-epoxypropyl)phosphonate monohydrate (CAS No 25383-07-7)
1016	(*R*)-5-溴-3-(1-甲基-2-吡咯)	(*R*)-5-bromo-3-(1-methyl-2-pyrrolidinyl methyl)-1*H*-indole (CAS No 143322-57-0)
1017	*R*-1-氯-2,3-环氧丙烷	*R*-1-Chloro-2,3-epoxypropane (CAS No 51594-55-9)
1018	*R*-2,3-环氧-1-丙醇	*R*-2,3-Epoxy-1-propanol (CAS No 57044-25-4)
1019	放射性物质 (1)	Radioactive substances (1)
1020	含饱和及不饱和C_{3-5}但不含丁二烯的残油(石油), 来自蒸汽裂解C_4馏分的乙酸亚铜铵萃取物	Raffinates (petroleum), steam-cracked C_4 fraction cuprous ammonium acetate extn, C_{3-5} and C_{3-5} unsatd, butadiene-free (CAS No 97722-19-5)
1021	萝芙木生物碱类及其盐类	*Rauwolfia serpentina* alkaloids and their salts
1022	苯乙酮, 甲醛, 环己胺, 甲醇和乙酸的反应产物	Reaction product of acetophenone, formaldehyde, cyclohexylamine, methanol and acetic acid(EC No 406-230-1)
1023	石油残油	Residual oils (petroleum) (CAS No 93821-66-0)
1024	二甲基亚砜提取物含量大于3%(w/w)的活性炭处理的溶剂脱蜡的残油(石油)	Residual oils (petroleum), carbon-treated solvent-dewaxed (CAS No 100684-37-5), if they contain > 3 % (w/w) DMSO extract
1025	二甲基亚砜提取物含量大于3%(w/w)的催化脱蜡的石油残油	Residual oils (petroleum), catalytic dewaxed (CAS No 91770-57-9), if they contain > 3 % (w/w) DMSO extract
1026	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的(石油)残油	Residual oils (petroleum), clay-treated (CAS No 64742-41-2), if they contain > 3 % (w/w) DMSO extract
1027	二甲基亚砜提取物含量大于3%(w/w)的粘土处理的溶剂脱蜡的残油(石油)	Residual oils (petroleum), clay-treated solvent-dewaxed (CAS No 100684-38-6), if they contain > 3 % (w/w) DMSO extract
1028	二甲基亚砜提取物含量大于3%(w/w)的加氢解酸处理及溶剂脱蜡处理的残油(石油)	Residual oils (petroleum), hydrocracked acid-treated solvent-dewaxed (CAS No 92061-86-4), if they contain> 3 % (w/w) DMSO extract

No.	Chinese Name	English Name
1029	二甲基亚砜提取物含量大于3%(w/w)的加氢(石油)残油	Residual oils (petroleum), hydrotreated (CAS No 64742-57-0), if they contain > 3 % (w/w) DMSO extract
1030	二甲基亚砜提取物含量大于3%(w/w)的加氢溶剂脱蜡的(石油)残油	Residual oils (petroleum), hydrotreated solvent dewaxed (CAS No 90669-74-2), if they contain > 3 % (w/w) DMSO extract
1031	二甲基亚砜提取物含量大于3%(w/w)的溶剂脱沥青处理的(石油)残油	Residual oils (petroleum), solvent deasphalted (CAS No 64741-95-3), if they contain > 3 % (w/w) DMSO extract
1032	二甲基亚砜提取物含量大于3%(w/w)的溶剂脱蜡处理的(石油)残油	Residual oils (petroleum), solvent-dewaxed (CAS No 64742-62-7), if they contain > 3 % (w/w) DMSO extract
1033	二甲基亚砜提取物含量大于3%(w/w)的溶剂精制处理的(石油)残油	Residual oils (petroleum), solvent-refined (CAS No 64742-01-4), if they contain > 3 % (w/w) DMSO extract
1034	苯并[a]芘的含量大于 0.005%(w/w)的煤焦油残渣，来自杂酚油蒸馏	Residues (coal tar), creosote oil distn., if it contains > 0.005 %(w/w) benzo[a]pyrene (CAS No 92061-93-3)
1035	苯并[a]芘的含量大于0.005%(w/w)的液体溶剂萃取的煤残留物	Residues (coal), liq solvent extn(CAS No 94114-46-2), if they contain > 0.005% (w/w) benzo[a]pyrene
1036	丁二烯含量大于0.1%(w/w)的来自烷基化分流塔的富C_4石油残渣	Residues (petroleum), alkylation splitter, C_4-rich (CAS No 68513-66-6), if they contain > 0.1%(w/w) butadiene
1037	催化重整分馏塔残渣蒸馏的残液(石油)	Residues (petroleum), catalytic reformer fractionator residue distn. (CAS No 68478-13-7)
1038	含稠环芳烃的焦化洗涤塔处理物的蒸馏残液(石油)	Residues (petroleum), coker scrubber,condensed-ring-arom.-contg (CAS No 68783-13-1)
1039	重焦化减压蒸馏的低沸点残液(石油)	Residues (petroleum), heavy coker and light vacuum (CAS No 68512-61-8)
1040	重焦化柴油及减压蒸馏柴油的残液(石油)	Residues (petroleum), heavy coker gas oil and vacuum gas oil (CAS No 68478-17-1)
1041	减压蒸馏的低沸点残液(石油)	Residues (petroleum), light vacuum (CAS No 68512-62-9)
1042	蒸汽裂解低沸点残液(石油)	Residues (petroleum), steam-cracked light (CAS No68513-69-9)
1043	初馏低硫残液(石油)	Residues (petroleum), topping plant ,low-sulfur(CAS No 68607-30-7)
1044	常压塔处理的残液(石油)	Residues (petroleum),atm.tower (CAS No 64741-57-7)
1045	常压蒸馏残液(石油)	Residues (petroleum),atmospheric (CAS No 68333-22-2)
1046	催化裂解残液(石油)	Residues (petroleum),catalytic cracking (CAS No 92061-97-7)
1047	催化重整分馏塔处理的残液(石油)	Residues (petroleum),catalytic reformer fractionator (CAS No 64741-67-9)
1048	加氢裂解残液(石油)	Residues (petroleum),hydrocracked (CAS No 64741-75-9)
1049	加氢脱硫常压塔蒸馏残液(石油)	Residues (petroleum),hydrodesulfurised atmospheric tower (CAS No 64742-78-5)
1050	加氢蒸汽裂解石脑油残液(石油)	Residues (petroleum),hydrogenated steam-cracked naphtha (CAS No 92062-00-5)
1051	蒸汽裂解残液(石油)	Residues (petroleum),steam-cracked (CAS No 64742-90-1)
1052	蒸汽裂解热裂解石脑油残液(石油)	Residues (petroleum),steam-cracked heat-soaked naphtha (CAS No 93763-85-0)

No.	Chinese Name	English Name
1053	蒸汽裂解石脑油蒸馏残液(石油)	Residues (petroleum),steam-cracked naphtha distn. (CAS No 92062-04-9)
1054	蒸汽裂解蒸馏残液(石油)	Residues (petroleum),steam-cracked,distillates (CAS No 90669-75-3)
1055	蒸汽裂解的树脂状塔底残液(石油)	Residues (petroleum),steam-cracked,resinous (CAS No 68955-36-2)
1056	热裂解残液(石油)	Residues (petroleum),thermal cracked (CAS No64741-80-6)
1057	减压蒸馏的低沸点残液(石油)	Residues (petroleum),vacuum,light (CAS No 90669-76-4)
1058	蒸汽裂解及热处理的残液(石油)	Residues, steam cracked, thermally treated (CAS No 98219-64-8)
1059	间苯二酚二缩水甘油醚	Resorcinol diglycidyl ether (CAS No 101-90-6)
1060	(S)-2,3-二氢-1*H*-吲哚-羧酸	(*S*)-2,3-Dihydro-1*H*-indole-carboxylic acid (CAS No 79815-20-6)
1061	黄樟素(黄樟脑), [当加入化妆品中的天然成分中含有，且不超过如下浓度时除外: 化妆品成品中 100mg/kg; 牙齿及口腔卫生用品中 50mg/kg(专供儿童使用的牙膏中禁止使用)]	Safrole except for normal content in the natural essences used and provided the concentration does not exceed: 100mg/kg in the finished product; 50mg/kg in products for dental and oral hygiene, and provided that safrole is not present in toothpastes intended specifically for children
1062	4,4'-碳亚氨基双(*N*,*N*-二甲基苯胺)的盐	Salts of 4,4'-carbonimidoyl bis(*N*,*N*-dimethylaniline)
1063	*O*-烷基二硫代碳酸的盐类	Salts of *O*-alkyldithiocarbonic acids
1064	邻-联(二)茴香胺的盐	Salts of *o*-dianisidine
1065	种子藜芦(沙巴草)(种子和草药制剂)	Schoenocaulon officinale Lind. (seeds and galenical preparations)
1066	仲链烷胺和仲链烷醇胺类和它们的盐类	Secondary alkyl and alkanolamine and their salts
1067	硒及其化合物(表 3 中在限定条件下使用的二硫化硒除外)	Selenium and its compounds with the exception of selenium disulphide under the conditions set out under the reference in table 3
1068	西玛津	Simazine (CAS No 122-34-9)
1069	软蜡(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum) (CAS No 64742-61-6), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1070	酸处理的软蜡(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum), acid-treated (CAS No 90669-77-5), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1071	活性炭处理的软蜡(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum), carbon-treated (CAS No 100684-49-9), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1072	粘土处理的软蜡(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum), clay-treated (CAS No 90669-78-6), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1073	加氢的软蜡(石油), 除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum), hydrotreated (CAS No 92062-09-4), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1074	低熔点软蜡(石油)， 除非清楚全部精炼过程并且能够证明所获得的物质	Slack wax (petroleum), low-melting (CAS No 92062-10-7), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen

No.	Chinese Name	English Name
	不是致癌物	
1075	活性炭处理的低熔点软蜡(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum), low-melting, carbon-treated (CAS No 97863-04-2), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1076	粘土处理的低熔点软蜡(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum), low-melting, clay-treated (CAS No 97863-05-3), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1077	加氢的低溶点软蜡(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum), low-melting, hydrotreated (CAS No 92062-11-8), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1078	硅酸处理的低熔点软蜡(石油)，除非清楚全部精炼过程并且能够证明所获得的物质不是致癌物	Slack wax (petroleum), low-melting, silicic acid-treated (CAS No 97863-06-4), except if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen
1079	2-(1-羟甲基环己基)乙酸钠	Sodium hexacyclonate (sodium 2-(1-hydroxymethylcyclohexyl)acetate)
1080	龙葵及其草药制剂	*Solanum nigrum L.* And its galenical preparations
1081	司巴丁及其盐类	Sparteine and its salts
1082	螺内酯	Spironolactone (17-hydroxy-7-mercapto-3-oxo-17 α -pregn-4-eno-21-carboxylic acid r-tactone 7-acetate)
1083	乳酸锶	Strontium lactate
1084	硝酸锶	Strontium nitrate
1085	多羧酸锶	Strontium polycarboxylate
1086	羊角拗质素及其糖苷配基以及相应的衍生物	Strophantines, their aglucones and their respective derivatives
1087	羊角拗及其草药制剂	*Strophantus* species and their galenical preparations
1088	士的宁及其盐类	Strychnine and its salts
1089	马钱子和它的草药制剂	*Strychnos* species and their galenical preparations
1090	具有雄激素效应的物质	Substances with androgenic effect
1091	丁二腈(琥珀腈)	Succinonitrile
1092	草克死	Sulfallate (CAS No 95-06-7)
1093	磺砒酮	Sulfinpyrazone (1,2-diphenyl-4-(2-phenylsulfinylethyl)-3,5-pyrazolidinedione)
1094	磺胺类药物(磺胺和其氨基的一个或多个氢原子被取代的衍物)及其盐类	Sulphonamides (sulphanilamide and its derivatives obtained by substitution of one or more H-atoms of the -NH_2 groups) and their salts
1095	舒噻美	Sultiame (sulthiane; [2-(p-sulfamoylphenyl) tetrahydro-1,2-thiazine 1,1-dioxide])
1096	对中枢神经系统起作用的拟交感胺类和中国卫生部发布的管制精神类药品(咖啡因除外)	Sympathicomimetic amines acting on the central nervous system and the medicins, natural and synthetic, controlled by the Drug Administration Law of the People's Republic of China (except caffien)

No.	Chinese Name	English Name
1097	合成箭毒类	Synthetic curarizants
1098	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化裂解澄清油及热裂解分馏回流接收器的减压渣油	Tail gas (petroleum), catalytic cracked clarified oil and thermal cracked vacuum residue fractionation reflux drum (CAS No 68478-21-7), if it contains > 0.1%(w/w) butadiene
1099	丁二烯含量大于0.1%(w/w)的石油尾气，来自石油催化裂解的馏分及·催化裂解石脑油馏分吸收塔	Tail gas (petroleum), catalytic cracked distillate and catalytic cracked naphtha fractionation absorber (CAS No68307-98-2), if it contains > 0.1%(w/w) butadiene
1100	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化裂解馏分及石脑油的稳定塔	Tail gas (petroleum), catalytic cracked distillate and naphtha stabiliser (CAS No 68952-77-2), if it contains> 0.1%(w/w) butadiene
1101	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化裂解石脑油稳定吸收塔	Tail gas (petroleum), catalytic cracked naphtha stabilisation absorber (CAS No 68478-22-8), if it contains> 0.1%(w/w) butadiene
1102	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化裂解分馏吸收塔	Tail gas (petroleum), catalytic cracker refractionation absorber (CAS No 68478-25-1), if it contains > 0.1%(w/w)butadiene
1103	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化裂解，催化重整及加氢脱硫联合分馏塔	Tail gas (petroleum), catalytic cracker, catalytic reformer and hydrodesulferised combined fractionater (CAS No68478-24-0), if it contains > 0.1%(w/w) butadiene
1104	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化加氢脱硫石脑油分离塔	Tail gas (petroleum), catalytic hydrodesulfurised naphtha separator (CAS No 68952-79-4), if it contains > 0.1%(w/w)butadiene
1105	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化聚合石脑油分馏稳定塔	Tail gas (petroleum), catalytic polymn. naphtha fractionation stabiliser (CAS No68307-99-2), if it contains > 0.1%(w/w) butadiene
1106	丁二烯含量大于0.1%(w/w)的无硫化氢石油尾气，来自催化重整石脑油分馏稳定塔	Tail gas (petroleum), catalytic reformed naphtha fractionation stabiliser, hydrogen sulfide-free (CAS No 68308-00-9), if it contains > 0.1%(w/w) butadiene
1107	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化重整石脑油分馏稳定塔	Tail gas (petroleum), catalytic reformed naphtha fractionation stabiliser (CAS No 68478-26-2), if it contains > 0.1%(w/w) butadiene
1108	丁二烯含量大于0.1%(w/w)的石油尾气，来自经催化重整石脑油分离器	Tail gas (petroleum), catalytic reformed naphtha separator (CAS No 68478-27-3), if it contains > 0.1%(w/w) butadiene
1109	丁二烯含量大于0.1%(w/w)的石油尾气，来自催化重整石脑油稳定塔	Tail gas (petroleum), catalytic reformed naphtha stabiliser (CAS No 68478-28-4), if it contains > 0.1%(w/w) butadiene
1110	丁二烯含量大于0.1%(w/w)的石油尾气，来自加氢分离塔的裂解馏分	Tail gas (petroleum), cracked distillate hydrotreater separator (CAS No 68478-29-5), if it contains > 0.1%(w/w) butadiene
1111	丁二烯含量大于0.1%(w/w)的石油尾气，来自石油裂解馏分催化加氢汽提塔	Tail gas (petroleum), cracked distillate hydrotreater stripper (CAS No 68308-01-0), if it contains > 0.1%(w/w) butadiene
1112	丁二烯含量大于0.1%(w/w)的石油尾气，来自柴油催化裂解吸收塔	Tail gas (petroleum), gas oil catalytic cracking absorber (CAS No 68308-03-2), if it contains > 0.1%(w/w) butadiene
1113	丁二烯含量大于0.1%(w/w)的石油尾气，来自汽油回收工厂	Tail gas (petroleum), gas recovery plant (CAS No 68308-04-3), if it contains > 0.1%(w/w) butadiene
1114	丁二烯含量大于0.1%(w/w)的石油尾气，来自汽油回收工厂脱乙烷塔	Tail gas (petroleum), gas recovery plant deethaniser (CAS No 68308-05-4), if it contains > 0.1%(w/w)

No.	Chinese Name	English Name
		butadiene
1115	丁二烯含量大于0.1%(w/w)的无酸石油尾气，来自加氢脱硫馏分及加氢脱硫石脑油分馏塔	Tail gas (petroleum), hydrodesulfurised distillate and hydrodesulfurised naphtha fractionator, acid-free (CAS No68308-06-5), if it contains > 0.1%(w/w) butadiene
1116	丁二烯含量大于0.1%(w/w)的石油尾气，来自加氢脱硫直馏石脑油分离塔	Tail gas (petroleum), hydrodesulfurised straight-run naphtha separator (CAS No 68478-30-8), if it contains> 0.1%(w/w) butadiene
1117	丁二烯含量大于0.1%(w/w)的无硫化氢石油尾气，来自加氢脱硫真空柴油汽提塔	Tail gas (petroleum), hydrodesulfurised vacuum gas oil stripper, hydrogen sulfide-free (CAS No 68308-07-6), if it contains > 0.1%(w/w) butadiene
1118	丁二烯含量大于0.1%(w/w)的石油尾气，来自异构化石脑油分馏稳定塔	Tail gas (petroleum), isomerised naphtha fractionation stabiliser (CAS No 68308-08-7), if it contains > 0.1%(w/w) butadiene
1119	丁二烯含量大于0.1%(w/w)的无硫化氢石油尾气，来自直馏石脑油分馏稳定塔的轻馏分	Tail gas (petroleum), light straight-run naphtha stabiliser, hydrogen sulfide-free (CAS No 68308-09-8), if it contains > 0.1%(w/w) butadiene
1120	丁二烯含量大于0.1%(w/w)的石油尾气，来自丙烷-丙烯烷基化进料预处理脱乙烷塔	Tail gas (petroleum), propane-propylene alkylation feed prep deethaniser (CAS No 68308-11-2), if it contains> 0.1%(w/w) butadiene
1121	丁二烯含量大于0.1%(w/w)的石油尾气，来自饱和汽油工厂的富C_4混流	Tail gas (petroleum), saturate gas plant mixed stream, C_4-rich (CAS No 68478-32-0), if it contains > 0.1%(w/w) butadiene
1122	丁二烯含量大于0.1%(w/w)的富C_{1-2}石油尾气，来自饱和汽油回收工厂	Tail gas (petroleum), saturate gas recovery plant, C_{1-2}-rich (CAS No 68478-33-1), if it contains > 0.1%(w/w) butadiene
1123	丁二烯含量大于0.1%(w/w)的无硫化氢石油尾气，来自加氢脱硫处理的直馏馏分	Tail gas (petroleum), straight-run distillate hydrodesulferised, hydrogen sulfide-free (CAS No 68308-10-1), if it contains > 0.1%(w/w) butadiene
1124	丁二烯含量大于0.1%(w/w)的石油尾气，来自加氢脱硫的直馏石脑油	Tail gas (petroleum), straight-run naphtha hydrodesulferised (CAS No 68952-80-7), if it contains > 0.1%(w/w) butadiene
1125	丁二烯含量大于0.1%(w/w)的石油尾气，来自热裂解碳氢化合物分馏稳定塔的石油焦化产物	Tail gas (petroleum), thermal cracked hydrocarbon fractionation stabiliser, petroleum coking (CAS No 68952-82-9), if it contains > 0.1%(w/w) butadiene
1126	丁二烯含量大于0.1%(w/w)的石油尾气，来自热裂解馏分、柴油及石脑油吸收塔	Tail gas (petroleum), thermal-cracked distillate, gas oil and naphtha absorber (CAS No 68952-81-8), if it contains > 0.1%(w/w) butadiene
1127	丁二烯含量大于0.1%(w/w)的无硫化氢石油尾气，来自加氢脱硫的真空瓦斯油	Tail gas (petroleum), vacuum gas oil hydrodesulferised, hydrogen sulfide-free (CAS No 68308-12-3), if it contains > 0.1%(w/w) butadiene
1128	丁二烯含量大于0.1%(w/w)的石油尾气，来自热裂解真空渣油	Tail gas (petroleum), vacuum residues thermal cracker (CAS No 68478-34-2), if it contains > 0.1%(w/w) butadiene
1129	替法唑啉及其盐类	Tefazoline (2-(5,6,7,8-tetrahydronaphth-1-ylmethyl)-2-imidazoline) and its salts
1130	碲及碲化合物	Tellurium and its compounds
1131	丁苯那嗪及其盐类	Tetrabenazine(1,3,4,6,7,llb-hexahydro-3-isobutyl-9,10-dimethoxy-2*H*-berzo(a)quinolizin-2-one) and its salts

No.	Chinese Name	English Name
1132	四溴 *N*-水杨酰苯胺	Tetrabromosalicylanilides
1133	丁卡因及其盐类	Tetracaine (deanol *p*-butylaminobenzoate) and its salts
1134	四羰基镍	Tetracarbonyl nickel (CAS No 13463-39-3)
1135	四氯乙烯	Tetrachloroethylene
1136	四氯 *N*-水杨酰苯胺	Tetrachlorosalicylanilides
1137	焦磷酸四乙酯	Tetraethyl pyrophosphate; TEPP (ISO)
1138	丙酸(+/-)-四羟糠基-(*R*)-2-[4-(6-氯-2-喹喔啉氧基)苯氧基]酯	(+/-)-Tetrahydrofurfuryl-(*R*)-2-[4-(6-chloroquinoxalin-2-yloxy)phenyloxy]propionate(CAS No 119738-06-6)
1139	四氢化噻喃-3-甲醛	Tetrahydrothiopyran-3-carboxaldehyde (CAS No 61571-06-0)
1140	四氢咪唑啉及其盐类	Tetrahydrozoline and its salts
1141	3,3'-[[1,1'-联苯]-4,4'-二基-双(偶氮)]双[5-氨基-4-羟基萘-2,7-二磺酸四钠	Tetrasodium 3,3'-[[1,1'-biphenyl]-4,4'-diyl bis(azo)]bis [5-amino-4-hydroxynaphthalene-2,7-disulphonate] (CAS No 2602-46-2)
1142	四乙溴铵	Tetrylammonium bromide (tetraethylammonium bromide)
1143	沙立度胺及其盐类	Thalidomide [*N*-(2,6-dicxopiperid-3-yl) phthalimide] and its salts
1144	铊和铊的化合物	Thallium and its compounds
1145	(1) 头颅骨, 包括脑以及眼、扁桃体和脊髓: ——达到 12 月龄的牛科动物 ——12 月龄以上或从牙龈已萌出一个永久性门齿的羊和山羊科动物 (2) 羊和山羊科动物的脾脏以及由此获得的原料。 (3) 卫生部 2002 年第 3 号公告中Ⅰ类牛、羊动物源性原料成分。 但是, 牛羊脂衍生物(含在卫生部发布的 2002 年第 3 号公告中Ⅱ类牛、羊动物源性原料成分)可以使用, 如果生产者使用下述方法, 并且是严格保证的: ——酯基转移作用或水解作用至少是在 200℃,以及适宜的相应压力下 20 分钟(甘油和脂肪酸及酯)的条件下进行 ——与 NaOH(12mol/L)皂化作用(甘油和肥皂)是在下述条件下进行: 分批法: 95℃ 3h 连续法: 140℃, 2bars (2000h Pa) 8 分钟或相等条件	(1) The skull , including the brain and eyes, tonsils and spinal cord of: ——bovine animals aged 12 months ——ovine and caprine animals which are aged over 12 months or have a permanent incissor tooth erupted through the gum; (2) The spleens of ovine and caprine animals and ingredients derived therefrom Ingredients list as class 1 substances in the bullten No.3, 2002 promulgated by ministry of public health and it's salts. However, tallow derivatives (including the substances list as class 2 ingredients in the bullten No.3, 2002 promulgated by ministry of public health) may be used provided that the following methods have been used and strictly certified by the producer: ——transesterification or hydrolysis at at least 200℃ and at an appropriate corresponding pressure, for 20 minutes (glycerol, fatty acids and fatty acid esters) ——saponification with NaOH 12mol/L (glycerol and soap): batch process: at 95℃ for 3 hours; or continuous process : at 140℃, 2 bars (2000 h pa) for 8 minutes or equivalent conditions.
1146	黄花夹竹桃苷提取物	*Thevetia neriifolia juss.* Glycoside extract
1147	甲巯咪唑	Thiamazole (1-methyl-2-imidazolethiol)
1148	硫代乙酰胺	Thioacetamide (CAS No 62-55-5)

No.	Chinese Name	English Name
1149	噻吩甲酸甲酯	Thiophanate-methyl (CAS No 23564-05-8)
1150	噻替派	Thiotepa [tris(1-aziridinyl) phosphine sulfide]
1151	硫脲及其衍生物(表 3 中限用的除外)	Thiourea and its derivatives, with the exception of the one listed in table 3.
1152	秋兰姆二硫化物类	Thiuram disulphides
1153	秋兰姆单硫化物类	Thiuram monosulphides
1154	甲状丙酸及其盐类	Thyropropic acid (4-(4-hydroxy-3-iodophenoxy)-3,5-diiodohydrocinnamie acid) and its salts
1155	短杆菌素	Thyrothricine
1156	托硼生	Tolboxane (5-methyl-5-propyl-2-p-tolyl-1,3,2-dioxaborinane)
1157	甲苯磺丁脲	Tolbutamide (1-butyl-3-(p-toluenesulfonyl) urea; 1-butyl-3-tosylurea)
1158	硫酸甲苯胺(1:1)	Toluidene sulphate(1:1) (CAS No540-25-0)
1159	甲苯胺类及其同分异构体,盐类以及卤化和磺化衍生物	Toluidines, their isomers, salts and halogenated and sulphonated derivatives
1160	4-甲苯胺盐酸盐	Toluidium chloride (CAS No 540-23-8)
1161	[(甲苯氧基)甲基]环氧乙烷, 羟甲苯基缩水甘油醚	[(Tolyloxy)methyl]oxirane,cresyl glycidyl ether (CAS No 26447-14-3)
1162	毒杀芬	Toxaphene (CAS No 8001-35-2)
1163	反式-2-庚烯醛	*Trans*-2-heptenal (CAS No 18829-55-5)
1164	反式-2-己烯醛二乙基乙缩醛	*Trans*-2-hexenal diethyl acetal (CAS No 67746-30-9)
1165	反式-2-己烯醛二甲基乙缩醛	*Trans*-2-hexenal dimethyl acetal (CAS No 18318-83-7)
1166	反式-4-环己基-L-脯氨酸-盐酸盐	*Trans*-4-cyclohexyl-L-proline monohydro-chloride (CAS No 90657-55-9)
1167	反式-4-苯基-L-脯氨酸	*Trans*-4-phenyl-L-proline(CAS No 96314-26-0)
1168	反苯环丙胺及其盐类	Tranylcypromine (DL-trans-2-phenylcyclopropylamine) and its salts
1169	曲他胺	Tretamine (2,4,6-tris (1-aziridinyl)-s-triazine; triethylenemelamine)
1170	维甲酸(视黄酸)及其盐类	Tretinoin (retinoic acid) and its salts
1171	氨苯喋啶及其盐类	Triamterene (2,4,7-triamino-6-phenylpteridine) and its salts
1172	磷酸三丁酯	Tributyl phosphate (CAS No 126-73-8)
1173	三氯氮芥及其盐类	Trichlormethine (tris (2-chloroethyl) amine; 2,2',2"-trichlorotriethylamine) and its salts
1174	三氯乙酸	Trichloroacetic acid
1175	三氯乙烯	Trichloroethylene (CAS No 79-01-6)
1176	三氯硝基甲烷(氯化苦)	Trichloronitromethane (chloropicrine)

No.	Chinese Name	English Name
1177	克啉菌; 十三吗啉	Tridemorph (CAS No 24602-86-6)
1178	三氟碘甲烷	Trifluoroiodomethane (CAS No 2314-97-8)
1179	三氟哌多	Trifluperidol (1-[3-(p-fluorobenzoyl) propyl]-4-(m-trifluoromethylpheryl)-4- piperidinol)
1180	二硫化三镍	Trinickel disulphide (CAS No 12035-72-2)
1181	三聚甲醛(1,3,5-三恶烷)	Trioxymethylene(1,3,5-trioxan) (CAS No 110-88-3)
1182	曲帕拉醇	Triparanol (2-(*p*-chlorophenyl)-1-[*p*-(2-diethylaminoethoxy)phenyl-1-(*p*-tolyl)]ethanol)
1183	曲吡那敏	Tripelennamine [*N*-benzyl-*N'*,*N'*-dimethyl-*N*-(2-pyridyl) ethylenediamine]
1184	磷酸三(2-氯乙)酯	Tris(2-chloroethyl) phosphate (CAS No 115-96-8)
1185	双(7-乙酰氨基-2-(4-硝基-2-氧苯偶氮基)-3-磺基-1-萘酚基)-1-铬酸三钠	Trisodium bis (7-acetamido-2-(4-nitro-2-oxidophenylazo)-3-sulfonato-1-naphtholato)chromate (1-)(EC No400-810-8)
1186	三钠[4'-(8-乙酰氨基-3,6-二磺基-2-萘偶氮基)-4''-(6-苯甲酰氨基-3-磺基-2-萘偶氮基)-联苯-1,3',3'',1'''-四羟连-O,O',O'',O'''] 铜(II) (EC No 413-590-3)	Trisodium[4'-(8-acetylamino-3,6-disulfonato-2-naphthylazo)-4''-(6-benzoylamino-3-sulfonato-2-naphthylazo)-biphenyl-1,3',3'',1'''-tetraolato-O,O',O'',O''']copper(II)(EC No 413-590-3)
1187	磷酸三甲酚酯	Tritolyl phosphate
1188	异庚胺及其同分异构体和盐类	Tuaminoheptane (2-aminoheptane; 2-heptylamine), its isomers and salts
1189	尿烷; 氨基甲酸乙酯	Urethane (CAS No 51-79-6)
1190	(白)海葱及其草药制剂	*Urginea scilla stern.* and its galenical preparations
1191	以下化合物的 UVCB 缩合产物: 四倍-氯化羟基甲基膦, 尿素和蒸馏的氢化 C_{16-18} 牛油烷基胺	UVCB condensation product of: tetrakis-hydroxymethylphosphonium chloride, urea and distilled hydrogenated C_{16-18} tallow alkylamine (CAS No 166242-53-1)
1192	疫苗、毒素或血清	Vaccines, toxins or serums
1193	α-氨基异戊酰胺	Valinamide (CAS No 20108-78-5)
1194	戊诺酰胺	Valnoctamide (2-ethyl-3-methylvaleramide)
1195	藜芦碱,其盐类及其草药制剂	Veratrine, its salts and galenical preparations
1196	藜芦的根及草药制剂	*Veratrum spp.* And their preparations
1197	马鞭草油	Verbena oil (*Lippia citriodora* Kunth) (CAS No 8024-12-2)
1198	烯菌酮	Vinclozolin (CAS No 50471-44-8)
1199	氯乙烯单体	Vinyl chloride monomer
1200	偏氯乙烯(1,1-二氯乙烯)	Vinylidene chloride(1,1-dichloroethylene) (CAS No 75-35-4)
1201	华法林及其盐类	Warfarin (3- (α-acetonylbenzyl) -4-hydroxycaumarin) and its salts

No.	Chinese Name	English Name
1202	苯并[a]芘的含量大于0.005%(w/w)的固体废弃物，来自煤焦油的沥青炼焦过程	Waste solids, coal-tar pitch coking (CAS No 92062-34-5), if they contain > 0.005% (w/w) benzo[a]pyrene
1203	二甲苯胺类及它们的同分导构体，盐类以及卤化的和磺化的衍生物	Xylidines, their isomers, salts and halogenated and sulphonated derivatives
1204	赛洛唑啉及其盐类	Xylometazoline [2-(4-*tert*-butyl-2,6-dimethylbenzyl)-2-imidazoline] and its salts
1205	育亨宾及其盐类	Yohimbine (16α-carbomethoxyyohimban-17α-ol; ester of yohimbic acid) and its salts
1206	二甲基二硫代氨基甲酸锌；福美锌	Ziram (CAS No 137-30-4)
1207	锆和它的化合物(表 3 中锆的配合物类以及表 6 中着色剂的锆色淀，盐和颜料除外)	Zirconium and its compounds,with the exception of the complexes listed in table 3 and of zirconium lakes, salts and pigments of colouring agents listed in table 6
1208	氯苯唑胺	Zoxazolamine (2-amino-5-chlorobenzoxazole)

(1) Naturally occurring radioactive substances and radioactive substances resulting from man-made environmental pollution are not included in the limits. However, the amount of these radioactive substances must not be increased during the production of cosmetics and must not exceed the basic limits set for the protection of workers' health and the protection of the public from radiation damage.

(2) Strontium and its compounds other than strontium lactate, strontium nitrate and strontium polycarboxylate listed in this table are not included in this provision.

Table 2(2) Prohibited Components for Cosmetics[(1)]

(in Latin alphabetical order)

No.	Chinese Name	Protoplankton Latin name
1	毛茛科乌头属植物	*Aconitum L, (Ranunculaceae).*
2	毛茛科侧金盏花属植物	*Adonis L, (Ranunculaceae).*
3	卜芥	*Alocasia cucullata (Lour.) Schott*
4	海芋	*Alocasia odora (Roxb.) K.Koch*
5	蒟蒻	*Amorphophallus rivieri Durieu; Amorphophallus sinensis Belval*
6	打破碗花花	*Anemone hupehensis Lemoine*
7	白芷	*Angelica dahurica(Fisch. ex Hoffm.) Benth. et Hook. f.*
8	杭白芷	*Angelica dahurica(Fisch. ex Hoffm.)Benth. et Hook. f. var. formosana(Boiss.) Shan et Yuan*
9	茄科山莨菪属植物	*Anisodus Link et Otto, (Solanaceae).*
10	槟榔	*Areca catechu L.*
11	青木香	*Aristolochia delilis siel et Zuuo.*
12	广防己	*Aristolochia fangchi Y. C. Wu ex L. D. Chou et S. M. Hwang*
13	关木通	*Aristolochia manshuriensis Kom.*
14	马兜铃科细辛属植物	*Asarum L, (Aristolochiaceae).*
15	芥子	*Brassica juncea (L.) Czern. et Coss.; Sinapis alba L.*
16	鸦胆子	*Brucea javanica (L.) Merr.*
17	蟾酥	*Bufo bufo gargarizans Cantor ; Bufo melanostictus Schneider*
18	长春花	*Catharanthus roseus (L.) G.Don*
19	牛心茄子(海杧果)	*Cerbera manghas L.*
20	白屈菜	*Chelidonium majus L.*
21	藜	*Chenopodium album L.*
22	威灵仙	*Clematis chinensis Osbeck; Clematis hexapetala Pall.; Clematis manshurica Rupr.*
23	铃兰	*Convallaria keiskei Miq.*
24	马桑	*Coriaria sinica Maxim.*
25	紫堇	*Corydalis incisa (Thunb.) Pers.*
26	文殊兰	*Crinum asiaticum L. var. sinicum Bak.*

No.	Chinese Name	Protoplankton Latin name
27	野百合(农吉利)	*Crotalaria sessiliflora L.*
28	大戟科巴豆属植物	*Croton L, (Euphorbiaceae).*
29	芫花(根、全草)	*Daphne genkwa Sieb. et Zucc.*
30	茄科蔓陀罗属植物	*Datura L., (Solanaceae).*
31	鱼藤	*Derris trifoliata Lour.*
32	玄参科毛地黄属植物	*Digitalis L, (Scrophulariaceae).*
33	白薯莨	*Dioscorea hispida Dennst.*
34	茅膏菜	*Drosera peltata Sm.var.lunata (Buch.-Ham.)C.B.Clarke*
35	绵马贯众	*Dryopteris crassirhizoma Nakai*
36	麻黄科麻黄属植物	*Ephedra Tourn. ex L, (Ephedraceae).*
37	葛上亭长	*Epicauta gorhami Mars.*
38	大戟科大戟属植物	*Euphorbia L, (Euphorbiaceae).*
39	藤黄	*Garcinia morella Desv.*
40	钩吻	*Gelsemium elegans Benth.*
41	红娘子	*Huechys sanguinea De Geer.*
42	大风子	*Hydnocarpus anthelmintica Pierre ; Hydnocarpus hainanensis (Merr.)Sleum.*
43	天仙子	*Hyoscyamus niger L. (Leaves Seeds)*
44	莽草	*Illicium lanceolatum A.C.Smith*
45	丽江山慈姑	*Iphigenia indica Kunth et Benth.*
46	桔梗科半边莲属植物	*Lobelia L, (Campanulaceae).*
47	石蒜	*Lycoris radiata Herb.*
48	青娘子	*Lytta caraganae Pallas*
49	博落回	*Macleaya cordata (Willd.) R. Br.*
50	地胆	*Meloe coarctatus Motsch.*
51	含羞草	*Mimosa pudica L.*
52	夹竹桃	*Nerium indicum Mill.*
53	臭常山	*Orixa japonica Thunb.*
54	北五加皮(香加皮)	*Periploca sepium Bge.*

No.	Chinese Name	Protoplankton Latin name
55	牵牛子	*Pharbitis nil(L.) Choisy. ; Pharbitis purpurea (L.) Voigt*
56	商陆	*Phytolacca acinosa Roxb; Phytolacca americana L.*
57	半夏	*Pinellia ternata (Thunb.) Breit.*
58	紫雪花	*Plumbago indica L.*
59	白花丹	*Plumbago zeylanica L.*
60	补骨脂	*Psoralea corylifolia L.*
61	毛茛科毛茛属植物	*Ranunculus L, (Ranunculaceae).*
62	罗芙木	*Rauvolfia verticillata (Lour.)Baill.*
63	闹羊花	*Rhododendron molle G. Don*
64	万年青	*Rohdea japonica Roth*
65	乌桕	*Sapium sebiferum (L.) Roxb.*
66	一叶萩	*Securinega suffruticosa (Pall.) Rehd.*
67	苦参实	*Sophora flavescens Ait.*
68	羊角拗子	*Strophanthus divaricatus (Lour.) Hook. et Arn.*
69	菊科千里光属植物	*Senecio L, (Compositae).*
70	茵芋	*Skimmia reevesiana Fortune*
71	狼毒	*Stellera chamaejasme L.*
72	马钱科马钱属植物	*Strychnos L, (Loganiaceae).*
73	黄花夹竹桃	*Thevetia peruviana (Pers.) K. Schum.*
74	昆明山海棠	*Tripterygium hypoglaucum (LéVL.) Hutch.*
75	雷公藤	*Tripterygium wilfordii Hook.f.*
76	白附子	*Typhonium giganteum Engl.*
77	百合科藜芦属植物	*Veratrum L, (Liliaceae).*
78	了哥王	*Wikstroemia indica (L.) C.A.Mey.*

(1) Prohibited substances in this list include their extracts and products

Table 3 Restricted substances in cosmetic components

(in alphabetical order by INCI name)

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
1	α-羟基酸及其盐类和酯类(1)	-Hydroxy acids and their salts, esters	None		Total 6% (as acid)	pH ≥ 3.5 (except for drenching hair products)	If used in non-sunscreen skin care cosmetics containing ≥ 3% -hydroxy acids or if -hydroxy acids are claimed on the label, it should be stated "with sunscreen Cosmetics used simultaneously"
2	6-甲基香豆素	6-Methyl coumarin	6-Methyl coumarin	Oral hygiene products	0.003%		
3	(1) 碱金属的硫化物类 (2) 碱土金属的硫化物类	(1) Alkali sulphides (2) Alkaline earth sulphides	(1) Alkali sulfides (2) Alkaline earth sulfides	Depilatories Depilatories	2% (in sulphur) 6% (in sulphur)	pH ≤ 12.7 pH ≤ 12.7	Prevent children from grasping; avoid contact with eyes Prevent children from grasping; avoid contact with eyes
4	烷基(C_{12}-C_{22})三甲基铵溴化物或氯化物(2)	Alkyl(C12-C22) trimethyl ammonium, bromide and chloride	Alkyl(C12-C22) trimonium bromide and chloride	(a) Residency products (b) Drenching products	(a) 0.25%		
5	氟化铝	Aluminium fluoride	Aluminium fluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Contains aluminium fluoride

6	氯化羟锆铝配合物($Al_xZr(OH)_yCl_z$)和氯化羟锆铝甘氨酸配合物	Aluminium zirconium chloride hydroxide complexes; $AlxZr(OH)_{y\ Clz}$ and the aluminium zirconium chloride hydroxide glycine complexes	Aluminium zirconium chloride hydroxide complexes $AlxZr(OH)_{yClz}$ and the aluminium zirconium chloride hydroxide glycine complexes	Antiperspirant	20% (as anhydrous aluminium hydroxyzirconium chloride) 5.4% (in zirconium)	The ratio of the number of aluminium atoms to the number of zirconium atoms should be between 2 and 10; the ratio of the number of atoms of (Al+Zr) to the number of chlorine atoms should be between 0.9 and Between 2.1; not for use in aerosol products	Not to be used on irritated or damaged skin
7	氨	Ammonia	Ammonia		6% (as NH_3)		If containing more than 2% ammonia, specify "contains ammonia".
8	氟化铵	Ammonium fluoride	Ammonium fluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Contains ammonium fluoride
9	氟硅酸铵	Ammonium fluorosilicate	Ammonium fluorosilicate	Oral hygiene products	0.15% (in F), when compared to the permitted When other fluorides are mixed, the total F concentration not more than 0.15%		Ammonium fluorosilicate containing

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
10	单氟磷酸铵	Ammonium monofluorophosphate	Ammonium monofluorophosphate	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Contains ammonium monofluorophosphate
11	苯扎氯铵，苯扎溴铵，苯扎糖精铵 (2)	Benzalkonium chloride, bromide and saccharinate	Benzalkonium chloride, bromide and saccharinate	(a) Drenching hair products (b) Other products	(a) 3% (as benzalkonium chloride) (b) 0.1% (as benzalkonium chloride)	(a) If the alkyl chain of benzalkonium chloride, benzalkonium bromide, benzalkonium saccharin used in the finished product equal to or less than C_{14}, the amount shall not be greater than 0.5% (to Benzalkonium chloride)	(a) Avoid contact with eyes (b) Avoid contact with eyes
12	苯甲酸及其钠盐 (2)	Benzoic acid Sodium benzoate	Benzoic acid Sodium benzoate	(a) Drenching products (b) Oral Care Products	(a) 2.5% (as acid) (b) 1.7% (as acid)		
13	过氧苯甲酰	Benzoyl peroxide	Benzoyl peroxide	Artificial nail system	0.7% (concentration at the time of use)	For professional use only	For professional use only; avoid contact with skin; read instructions carefully
14	苯甲醇（2）	Benzyl alcohol	Benzyl alcohol	Solvents, perfumes and fragrances			

15	(1) 硼酸, 硼酸盐和四硼酸盐(禁用物质表 2(1) 所列成分除外)	(1) Boric acid, borates and tetraborates with the exception of substances in table 2(1)	(1) Boric acid, borates and tetraborates with the exception of substances in table 2(1)	(a) talcum powder (b) Oral hygiene products (c) Other products (Bathing and perm products excluded)	(a) 5% (as boric acid) (b) 0.1% (as boric acid) (c) 3% (as boric acid)	(a) Not for use on children under 3 years of age; not for use on flaking or irritated skin when the concentration of free soluble borate in the product exceeds 1.5% (as boric acid) (b) shall not be used for persons under three years of age Products for children (c) shall not be used for persons under three years of age Products for children; Products Free soluble borates in Concentrations above 1.5% (as boric acid) not for stripping (in the case of a single count) of or irritated skin	(a) Do not use on children under 3 years of age; do not use if skin is flaking or irritated (b) Do not swallow; under 3 years of age Do not use on children (c) Children under three years of age Do not use; skin peeling or Do not use when irritated

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
	(2) 四硼酸盐	(2) Tetraborates	(2) Tetraborates	(a) Bath products (b) Perm products	(a) 18% (as boric acid) (b) 8% (as boric acid)	(a) products that must not be used on children under three years of age	(a) Do not use on children under 3 years of age (b) Rinse well
16	氟化钙	Calcium fluoride	Calcium fluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Contains calcium fluoride
17	氢氧化钙	Calcium hydroxide	Calcium hydroxide	(a) Hair straighteners containing calcium hydroxide and guanidine salts (b) pH regulator for hair removal agents (c) Other applications, such as pH Conditioners, processing aids	(a) 7% (by weight of calcium hydroxide)	(b) pH ≤ 12.7 (c) pH ≤ 11	(a) Contains strong alkali; avoid contact with eyes; may cause blindness; prevent children from grasping (b) Contains strong alkali; avoid contact with eyes; prevent children from grasping
18	单氟磷酸钙	Calcium monofluorophosphate	Calcium monofluorophosphate	Oral hygiene products	0.15% (in F), when mixed with other fluorides permitted in this table, total F concentration		Contains calcium monofluorophosphate

					not more than 0.15%		
19	斑蝥素	Cantharides tincture	None	For use in hair regrowth agents only	1%	Banned in children's products	Contains Bacitracin; prevent children from grasping; do not use on children; Avoid contact with eyes
20	鯨蜡基胺氢氟酸盐	Hexadecyl ammonium fluoride	Cetylamine hydrofluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Cetylamine containing hydrofluoric acid salt
21	氯胺T	Tosylchloramide sodium	Chloramine T		0.2%		
22	碱金属的氯酸盐类	Chlorates of alkali metals	Chlorates of alkali metals	(a) Toothpaste (b) Other uses	(a) 5% (b) 3%		
23	二氨基嘧啶氧化物	2,4-Diamino-pyrimidine 3-oxide	Diaminopyrimidine oxide	Hair care products	1.5%		

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
24	二氯甲烷	Dichloromethane	Dichloromethane		35% (total concentration must not exceed 35% when mixed with 1,1,1-trichloroethane)	The maximum impurity content must not exceed 0.2%.	
25	双氯酚	Dichlorophen	Dichlorophen		0.5%		Contains diclofenac
26	二(羟甲基)亚乙基硫脲	1,3-Bis(hydroxymethyl)imidazolidine-2-thione	Dimethylol ethylene thiourea	(a) Hair care products (b) Nail Care Products	(a) 2% (b) 2%	(a) Not for use in aerosol products (b) pH of the product at the time of use The value must be below 4	Bis(hydroxymethyl)ethylene thiourea containing
27	羟乙磷酸及其盐类	Etidronic acid and its salts(1-hydroxyethylidene-di-phosphonic acid and its salts)	Etidronic acid and its salts	(a) Hair care products (b) Soap, Soap	(a) 1.5% (as hydroxyethyl phosphate) (b) 2% (as hydroxyethyl phosphate)		
28	脂肪酸双链烷酰胺及脂肪酸双链烷醇酰胺	Fatty acid dialkylamides and dialkanolamides	Fatty acid dialkylamides and dialkanolamides		Secondary chain alkylamine max 0.5%	Not for use with nitrosating systems; sec-alkylamine max 5% (for raw materials); nitrosamines max 50g/kg; store in nitrite free containers	
29	甲醛[2]	Formaldehyde	Formaldehyde	Nail hardener	5% (in formaldehyde)	Formaldehyde is required for concentrations above 0.05%.	Contains formaldehyde[3]; Protects the skin with oil

30	过氧化氢和其它释放过氧化氢的化合物或混合物, 如过氧化脲和过氧化锌	Hydrogen peroxide, and other compounds or mixtures that release hydrogen peroxide, including carbamide peroxide and zinc peroxide	Hydrogen peroxide and other compounds or mixtures that release hydrogen peroxide, including urea peroxide and zinc peroxide	(a) Hair care products (b) Skin care products (c) Finger (Toe) nail hardening products	(a) 12% (40 v/v of oxygen, in terms of H_2O_2 present or released) (b) 4% (in terms of the presence or release of H_2O_2 count) (c) 2% (based on the presence or release of H_2O_2 count)		(a) Wear suitable gloves; contains hydrogen peroxide; avoid contact with eyes; rinse immediately if product accidentally gets into eyes (b) Contains hydrogen peroxide; avoid contact with eyes; rinse immediately if product accidentally gets into eyes (c) Contains hydrogen peroxide; avoid contact with eyes; rinse immediately if product accidentally gets into eyes

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
				(d) Oral hygiene products	(d) 0.1% (based on the presence or release of H_2O_2 count)		
31	氢醌	Hydroquinone	Hydroquinone	Artificial nail system	0.02% (concentration at the time of use)	For professional use only	For professional use only; Avoid contact with skin; Carefully Read usage notes
32	氢醌二甲基醚	Hydroquinone methylether	None	Artificial nail system	0.02% (concentration at the time of use)	For professional use only	For professional use only; avoid contact with skin; read instructions carefully
33	无机亚硫酸盐类和亚硫酸氢盐类 (2)	Inorganic sulphites and hydrogen sulphites	Inorganic sulfites and hydrogen sulfites	(a) Oxidative hair dyes (b) Perm and straightening products (c) Automatic tanning products for the face (d) Automatic tanning for the body Products	(a) 0.67 % (as free SO_2) (b) 6.7 % (as free SO_2) (c) 0.45% (as free SO_2) (d) 0.40% (in terms of free SO_2)		

34	氢氧化锂	Lithium hydroxide	Lithium hydroxide	(a) Hair straightener 1. Generally used 2. Professional use (b) pH regulator for hair removal agents (c) Other applications, e.g. pH regulator (for drench products only)	(a) 1. 2% by weight [4] 2. 4.5% by weight [4]	(b) pH ≤ 12.7 (c) pH ≤ 11	(a) 1. Contains strong alkali; avoid contact with eyes; may cause blindness; prevent children from grasping 2. For professional use only; avoid contact with eyes; may cause blindness (b) Contains strong alkalis; avoid contact with eyes; prevent children from grasping
35	氟化镁	Magnesium fluoride	Magnesium fluoride	Oral hygiene products	0.15% (in F), when mixed with other fluorides permitted in this table, total F concentration not more than 0.15%		Contains magnesium fluoride

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
36	氟硅酸镁	Magnesium fluorosilicate	Magnesium fluorosilicate	Oral hygiene products	0.15% (in F), when mixed with other fluorides permitted in this table, total F concentration not more than 0.15%		Magnesium fluorosilicate containing
37	单链烷胺, 单链烷醇胺及它们的盐类	Monoalkylamines, monoalkanolamines and their salts	Monoalkylamines, monoalkanolamines and their salts			Not for use with nitrosating systems; minimum purity: 99%; maximum seco-alkylamine content 0.5% (for raw materials); Nitrosamines max 50g/kg; Store in a nitrite free room. Inside the container of nitrates	
38	麝香酮	Musk ketone	Musk ketone	All cosmetics (except oral hygiene products)	(a) Flavouring 1.4% (b) Floral water 0.56% (c) Other products 0.042%		
39	麝香二甲苯	Musk xylene	None	All cosmetics (except oral hygiene products)	(a) Flavouring 1.0% (b) Floral water 0.4% (c) Other products 0.03%		
40	尼克(甲)醇氢氟酸盐	Nicomethanol hydrofluoride	Nicomethanol hydrofluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration		Hydrofluoric acid salt containing nikonomers

					shall not exceed 0.15%		
41	硝甲烷	Nitromethane	Nitromethane	Rust inhibitor	0.3%		
42	氟化十八烯基铵	Octadecenyl-ammonium fluoride	Octadecenyl ammonium fluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Octadecylammonium fluoride
43	奥拉氟	3-(N-Hexadecyl-N-2-hydroxyethylammonio)propylbis(2-hydroxyethyl)ammonium difluoride	Olaflur	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Contains Olafur
44	草酸及其酯类和碱金属盐类	Oxalic acid, its esters and alkaline salts	Oxalic acid, its esters and alkaline salts	Hair care products	5%		For professional use only

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
45	羟基喹啉, 羟基喹啉硫酸盐	Quinolin-8-ol and bis(8-hydroxyquinolinium) sulphate	Oxyquinoline, oxyquinoline sulfate	(a) Stabilizer for hydrogen peroxide in drench hair care products (b) Used as peroxide in non-rinse hair care products Hydrogen stabilizer	(a) 0.3% (in bases) (b) 0.03% (in bases)		
46	二氢氟酸棕榈酰基三羟乙基丙烯二胺	*NN'N'*-Tris (polyoxyethylene)-N-hexadecylpropylenediamine dihydrofluoride	Palmityl trihydroxyethyl propylenediamine dihydrofluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Palmitoyl tris(hydroxyethyl)propylene diamine containing dihydrofluoric acid
47	苯氧异丙醇 (2)	l-Phenoxy-propan-2-ol	Phenoxyisopropanol	(a) For drenching products only (b) Forbidden for oral hygiene products	2%		
48	聚丙烯酰胺	Polyacrylamides	Polyacrylamides	(a) Residual Skin Care Products (b) Other products		(a) Maximum residue of acrylamide monomer 0.1mg/kg (b) Maximum residue of acrylamide monomer 0.5mg/kg	

49	氟化钾	Potassium fluoride	Potassium fluoride	Oral hygiene products	0.15% (in F), when mixed with other fluorides permitted in this table, total F concentration not more than 0.15%		Contains potassium fluoride
50	氟硅酸钾	Potassium fluorosilicate	Potassium fluorosilicate	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Potassium fluorosilicate containing
51	氢氧化钾(或氢氧化钠)	Potassium or sodium hydroxide	Potassium hydroxide, sodium hydroxide	(a) Finger (toe) nail care fluids	(a) 5% (by weight)[4]		(a) Contains strong alkali; avoid contact with eyes; may cause blindness; prevent children from grasping

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
				(b) Hair straighteners 1. Generally used 2. Professional use (c) pH regulator for hair removal agents (d) Other applications, such as pH Conditioners	(b) 1. 2% (by weight)(4) 2. 4.5% (by weight)(4)	(c) pH ≤ 12.7 (d) pH ≤ 11	(b) 1. Contains strong alkali; avoid contact with eyes; may cause blindness; prevent children from grasping 2. For professional use only; avoid contact with eyes; may cause blindness (c) Avoid contact with eyes; prevent children from grasping it
52	单氟磷酸钾	Potassium monofluorophosphate	Potassium monofluorophosphate	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Contains potassium monofluorophosphate
53	奎宁及其盐类	Quinine and its salts	Quinine and its salts	(a) Shampoo (drenching type) (b) Hairspray (leave-in)	(a) 0.5% (as quinine) (b) 0.2% (as quinine)		

54	间苯二酚	Resorcinol	Resorcinol	Hair lotions and shampoos	0.5%		Contains resorcinol
55	水杨酸 (2)	Salicylic acid	Salicylic acid	(a) Leave-in and shower skin care products (b) Drenching hair products	(a) 2.0% (b) 3.0%	Not to be used in products for children under 3 years of age, except for shampoos	Contains salicylic acid
56	硫化硒	Selenium disulphide	Selenium disulfide	Anti-dandruff shampoo	1%		Contains selenium sulphide; avoid contact with eyes or damaged skin
57	硝酸银	Silver nitrate	Silver nitrate	Products that can only be used to colour eyelashes and eyebrows exclusively	4%		Contains silver nitrate; if product is accidentally introduced into the eyes, rinse immediately
58	氟化钠	Sodium fluoride	Sodium fluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Contains sodium fluoride

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
59	氟硅酸钠	Sodium fluorosilicate	Sodium fluorosilicate	Oral hygiene products	0.15% (in F), when mixed with other fluorides permitted in this table, total F concentration not more than 0.15%		Sodium fluorosilicate containing
60	单氟磷酸钠	Sodium monofluorophosphate	Sodium monofluorophosphate	Oral hygiene products	0.15% (in F), when mixed with other fluorides permitted in this table, total F concentration not more than 0.15%		Contains sodium monofluorophosphate
61	亚硝酸钠	Sodium nitrite	Sodium nitrite	Rust inhibitor	0.2%	Not to be mixed with secondary and/or tertiary chain alkylamines or other substances that can form nitrosamines	
62	氟化亚锡	Stannous fluoride	Stannous fluoride	Oral hygiene products	0.15% (as F), when mixed with other fluorides permitted in this table, the total F concentration shall not exceed 0.15%		Contains stannous fluoride
63	乙酸锶半水合物(5)	Strontium acetate hemihydrate	Strontium acetate hemihydrate	Toothpaste	3.5% (in strontium), when mixed with other permitted strontium products, the total strontium content shall not exceed 3.5%		Contains strontium acetate; not suitable for children

64	氯化锶六水合物[5]	Strontium chloride hexahydrate	Strontium chloride hexahydrate	(a) Toothpaste (b) Shampoos and face care products	(a) 3.5% (in strontium), when mixed with other permitted strontium products, the total strontium content shall not exceed 3.5% (b) 2.1% (in strontium), when mixed with other permitted strontium products, the total strontium content shall not exceed 2.1%		Contains strontium chloride; not recommended for children
65	氢氧化锶[5]	Strontium hydroxide	Strontium hydroxide	pH regulators in hair removal products	3.5% (in strontium)	pH ≤ 12.7	Prevent children from grasping; avoid contact with eyes
66	过氧化锶[5]	Strontium peroxide	Strontium peroxide	Professional hair care products for drenching	4.5% (based on strontium in ready-to-use products)	All products must comply with the requirements for the release of hydrogen peroxide	Avoid contact with eyes; rinse immediately if product accidentally enters eyes; for professional use only; suitable for wearing of gloves
67	滑石：水合硅酸镁	Talc: hydrated magnesium silicate	Talc: hydrated magnesium silicate	(a) Powdered products for children under 3 years (b) Other products			(a) Keep powders away from the nose and mouth of children

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
68	(1) 巯基乙酸及其盐类 (1) 巯基乙酸酯类	(1) Thioglycollic acid and its salts (2) Thioglycollic acid esters	(1) Thioglycolic acid and its salts (2) Thioglycolic acid esters	(a) Hair curlers or straighteners 1. Generally used 2. Professional use (b) Depilatories (c) Other hair care products that remove after use Hair curlers or straighteners 1. Generally used 2. Professional use	(a) 1. 8% ready to use, pH 7-9.5 2. 11% ready to use, pH 7-9.5 (b) 5% ready to use, pH 7-12.7 (c) 2% ready to use, pH 7-9.5 The above percentages are based on thioglycolic acid	(a) The following notes are required: Avoid contact with eyes; if product gets into eyes, rinse immediately with plenty of water and seek medical attention; wear suitable gloves (b) The following notes are required: Avoid contact with eyes; if product is accidentally introduced into the eyes, flush with plenty of water and seek medical attention immediately (c) The following notes are required: Avoid contact with eyes; if product gets into eyes, rinse immediately with plenty of water and seek medical attention; wear suitable gloves The following notes are required: May cause allergic	(a) Contains thioglycolic acid; Use as directed; Prevent children from grasping; For professional use only (b) Contains thioglycolic acid; Use as directed; Prevent children from grasping (c) Contains thioglycolic acid salt; Use as directed; Prevent children from grasping Contains thioglycolate; Use as

					1. 8% ready to use, pH 6-9.5 2. 11% ready to use, pH 6-9.5 The above percentages are based on thioglycolic acid	reactions in contact with skin; avoid contact with eyes; if product gets into eyes accidentally, flush with plenty of water and seek medical attention immediately; wear suitable gloves	directed; Protect from children; For professional use only
69	三链烷胺, 三链烷醇胺及它们的盐类	Trialkylamines, trialkanolamines and their salts	Trialkylamines, trialkanolamines and their salts	(a) Non-showering products (b) Other products	(a) 2.5%	Not for use with Nitrosating system; Minimum purity: 99%; Maximum seco-alkylamine content 0.5% (for raw materials); Nitrosamines max 50g/kg; Store in a nitrite free room. Inside the container of nitrates	
70	三氯卡班 (2)	Triclocarban (INN)	Triclocarban	Drenching skin care products	1.5%	Purity standard: 3,3',4,4'-Tetrachloroazobenzene less than 1mg/kg;	

No.	Name of substance			Restrictions			The conditions of use that must be printed on the cosmetic label and Cautions
	Chinese Name	English Name	INCI Name	Application and/or scope of use	Maximum permissible concentration in cosmetic products	Other restrictions and requirements	
						3,3',4,4'-Tetrachloroazo Benzene less than 1mg/kg	
71	水溶性锌盐(苯酚磺酸锌和吡啶翁锌除外)	Water-soluble zinc salts with the exception of zinc 4-Hydroxybenzenesulphonate and zinc pyrithione	Water-soluble zinc salts with the exception of zinc phenolsulphonate and zinc pyrithione		1% (in zinc)		
72	苯酚磺酸锌	Zinc 4-Hydroxybenzene sulphonate	Zinc phenolsulfonate	Deodorants, antiperspirants and astringents	6% (as anhydrous matter)		Avoid contact with eyes
73	吡硫翁锌 (2)	Pyrithione zinc (INN)	Zinc pyrithione	Anti-dandruff drenching hair products	1.5%		

(1) Alpha-hydroxy acids are carboxylic acids in which the alpha-carbon hydrogen is replaced by a hydroxyl group, including tartaric, glycolic, malic, lactic, citric and other acids. "Salts" refers to their sodium, potassium, calcium, magnesium, ammonium and alcoholamine salts; "Esters" refers to methyl, ethyl, propyl, isopropyl, butyl, isobutyl and phenyl esters, etc.

(2) When these restricted substances are used as preservatives, the specific requirements are listed in Table 4 of the restricted preservatives.

(3) Only when the concentration exceeds 0.05% should it be labelled.

(4) The content of NaOH, LiOH or KOH is given by weight of NaOH. In the case of mixtures, the total amount must not exceed the requirements in the column "Maximum permitted concentration in cosmetics".

(5) Strontium and its compounds other than those listed in this table are not included in this provision.

Table 4 Restricted preservatives in cosmetic components [1]

(in alphabetical order by INCI name)

No.	Name of substance			Maximum permissible concentration in cosmetic products	Scope of use and restrictions	Conditions of use and precautions that must be printed on the label
	Chinese Name	English Name	INCI Name			
1	2-溴-2-硝基丙烷-1,3 二醇	Bronopol (INN)	2-Bromo-2-nitropropane-1,3-diol	0.1%	Avoiding the formation of nitrosamines	
2	5-溴-5-硝基-1,3-二噁烷	5-Bromo-5-nitro-1,3-dioxane	5-Bromo-5-nitro-1,3-dioxane	0.1%	For drench products only; to avoid the formation of nitrosamines	
3	7-乙基二环噁唑啉	5-Ethyl-3,7-dioxa-1-azabicyclo[3.3.0]octane	7-Ethylbicyclooxazolidine	0.3%	Forbidden in oral hygiene products and products that come into contact with mucous membranes	
4	烷基(C_{12}-C_{22})三甲基铵溴化物或氯化物 [2]	Alkyl($_{C12-C22}$) trimethyl ammonium, bromide and chloride	Alkyl($_{C12-C22}$) trimonium bromide and chloride	0.1%		
5	苯扎氯铵, 苯扎溴铵, 苯扎糖精铵 [2]	Benzalkonium chloride, bromide and saccharinate	Benzalkonium chloride, bromide and saccharinate	0.1% (as benzalkonium chloride)		Avoid contact with eyes
6	苄索氯铵	Benzethonium chloride	Benzethonium chloride	0.1%	(1) Drenching products (2) Residual products other than oral hygiene products	
7	苯甲酸及其盐类和酯类 [2]	Benzoic acid, its salts and esters	Benzoic acid, its salts and esters	0.5% (as acid)		
8	苯甲醇 [2]	Benzyl alcohol	Benzyl alcohol	1.0%		
9	甲醛苄醇半缩醛	Benzylhemiformal	Benzylhemiformal	0.15%	For drenching products only	

10	溴氯芬	6,6-Dibromo-4,4-dichloro-2,2'-methylene-diphenol	Bromochlorophen	0.1%		
11	氯己定及其二葡萄糖酸盐, 二醋酸盐和二盐酸盐	Chlorhexidine (INN) and its digluconate,diacetate and dihydrochloride	Chlorhexidine and its digluconate, diacetate and dihydrochloride	0.3% (expressed as chlorhexidine)		
12	氯乙酰胺	2-Chloroacetamide	Chloroacetamide	0.3%		Chloroacetamide
13	三氯叔丁醇	Chlorobutanol (INN)	Chlorobutanol	0.5%	Not for use in aerosol products	Contains trichloro-tert-butanol
14	苄氯酚	2-Benzyl-4-chlorophenol	Chlorophene	0.2%		
15	氯二甲酚	4-Chloro-3,5-xylenol	Chloroxylenol	0.5%		
16	氯苯甘醚	3-(p-chlorophenoxy)-propane-1,2-diol	Chlorphenesin	0.3%		

No.	Name of substance			Maximum permissible concentration in cosmetic products	Scope of use and restrictions	Conditions of use and precautions that must be printed on the label
	Chinese Name	English Name	INCI Name			
17	氯咪巴唑	1-(4-chlorophenoxy)-1-(imidazol-1-yl)-3,3-dimethylbutan-2-one	Climbazole	0.5%		
18	脱氢醋酸及其盐类	3-Acetyl-6-methylpyran-2,4 (3H)-dione and its salts	Dehydroacetic acid	0.6% (as acid)	Not for use in aerosol products	
19	双(羟甲基)咪唑烷基脲	*N*-(Hydroxymethyl)-*N*-(dihydroxymethyl-1,3-dioxo-2,5-imidazolinidyl-4)-*N'*-(hydroxymethyl) urea	Diazolidinyl urea	0.5%		
20	二溴己脒及其盐类，包括二溴己脒羟乙磺酸盐	3,3'-Dibromo-4,4'-hexamethylene dioxydibenzamidine and its salts (including isethionate)	Dibromohexamidine and its salts, including dibromohexamidine isethionate	0.1%		
21	二氯苯甲醇	2,4-Dichlorobenzyl alcohol	Dichlorobenzyl alcohol	0.15%		
22	二甲基噁唑烷	4,4-Dimethyl-1,3-oxazolidine	Dimethyl oxazolidine	0.1%	The pH of the final product must not be less than 6	
23	DMDM 乙内酰脲	1,3-Bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione	DMDM hydantoin	0.6%		
24	甲醛和多聚甲醛 (2)	Formaldehyde and paraformaldehyde	Formaldehyde and paraformaldehyde	0.2% (except oral hygiene products) 0.1% (Oral hygiene products) (based on free formaldehyde)	Not for use in aerosol products	
25	甲酸及其钠盐	Formic acid and its sodium salt	Formic acid and its sodium salt	0.5% (as acid)		
26	戊二醛	Glutaraldehyde (Pentane-1,5-dial)	Glutaral	0.1%	Not for use in aerosol products	Contains glutaraldehyde (when the concentration of

						glutaraldehyde in the finished product exceeds 0.05%)
27	己脒定及其盐, 包括己脒定二个羟乙基磺酸盐和己脒定对羟基苯甲酸盐	1,6-Di(4-amidinophenoxy)-n-hexane and its salts (including isethionate and p-hydroxybenzoate)	Hexamidine and its salts, including hexamidine diisethionate and hexamidine paraben	0.1%		
28	海克替啶	Hexetidine (INN)	Hexetidine	0.1%		
29	咪唑烷基脲	3,3'-Bis(1-hydroxymethyl-2,5-dioxoimidazolidin-4-yl)-1,1'-methylenediurea	Imidazolidinyl urea	0.6%		
30	无机亚硫酸盐类和亚硫酸氢盐类 (2)	Inorganic sulphites and hydrogensulphites	Inorganic sulfites and hydrogen sulfites	0.2% (as free SO_2)		

No.	Name of substance			Maximum permissible concentration in cosmetic products	Scope of use and restrictions	Conditions of use and precautions that must be printed on the label
	Chinese Name	English Name	INCI Name			
31	碘丙炔醇丁基氨甲酸酯	3-Iodo-2-propynylbutylcarbamate	Iodopropynyl butylcarbamate	0.05%	Not for use in oral hygiene and lip products	For products left on the skin after use, when the concentration exceeds 0.02%, the following warning should be stated: Contains iodine
32	乌洛托品	Hexamethylenetetramine (INN)	Methenamine	0.15%		
33	甲基二溴戊二腈	1,2-Dibromo-2,4-dicyanobutane	Methyldibromo glutaronitrile	0.1%	For drenching products only	
34	甲基异噻唑啉酮	2-Methylisothiazol-3(*2H*)-one	Methylisothiazolinone	0.01%		
35	甲基氯异噻唑啉酮和甲基异噻唑啉酮与氯化镁及硝酸镁的混合物	Mixture of 5-chloro-2-methylisothiazol-3(*2H*)-one and 2-Methylisothiazol-3(*2H*)-one with magnesium chloride and magnesium nitrate	Mixture of methylchloroisothiazolinone and methylisothiazolinone with magnesium chloride and magnesium nitrate	0.0015% (based on a 3:1 mixture of methylchloroisothiazolinone and methylisothiazolinone)		
36	*o*-伞花烃-5-醇	4-Isopropyl-m-cresol	o-Cymen-5-ol	0.1%		
37	*o*-苯基苯酚	Biphenyl-2-ol and its salts	o-Phenylphenol	0.2% (as phenol)		
38	4-羟基苯甲酸及其盐类和酯类	4-Hydroxybenzoic acid and its salts and esters	4-Hydroxybenzoic acid and its salts and esters	Monoester: 0.4% (as acid) Mixed esters: 0.8% (as acid)		
39	*p*-氯-*m*-甲酚	4-Chloro-m-cresol	p-Chloro-m-cresol	0.2%	Forbidden to use on products that come into	

					contact with mucous membranes	
40	苯氧乙醇	2-Phenoxyethanol	Phenoxyethanol	1.0%		
41	苯氧异丙醇 (2)	1-Phenoxypropan-2-ol	Phenoxyisopropanol	1.0%	For drenching products only	
42	吡罗克酮乙醇胺盐	1-Hydroxy-4-methyl-6(2,4,4-trimethylpentyl)2-pyridon and its monoethanolamine salt	Piroctone olamine	(a) 1.0% (b) 0.5%	(a) Drenching products (b) Other products	
43	盐酸聚氨丙基双胍	Poly(1-hexamethylenebiguanide) hydrochloride	Polyaminopropyl biguanide hydrochloride	0.3%		
44	丙酸及其盐类	Propionic acid and its salts	Propionic acid and its salts	2% (as acid)		
45	聚季铵盐-15	Methenamine 3-chloroallylochloride (INNM)	Quaternium-15	0.2%		
46	水杨酸及其盐类 (2)	Salicylic acid and its salts	Salicylic acid and its salts	0.5% (as acid)	Not to be used in products for children under 3 years of age, except for shampoos	Do not use on children under three years old (3)

No.	Name of substance			Maximum permissible concentration in cosmetic products	Scope of use and restrictions	Conditions of use and precautions that must be printed on the label
	Chinese Name	English Name	INCI Name			
47	苯汞的盐类, 包括硼酸苯汞	Phenylmercuric salts (including borate)	Salts of phenylmercury, including borate	0.007% (in Hg), if mixed with other mercury compounds in this specification, the maximum concentration of Hg remains 0.007%	For use with eye make-up and eye make-up removers only	Phenylmercury containing compounds
48	沉积在二氧化钛上的氯化银	Silver chloride deposited on titanium dioxide	Silver chloride deposited on titanium dioxide	0.004% (as AgCl)	20% ((w/w)) AgCl deposited on $_{TiO2}$, prohibited for use in products for children under 3 years of age, oral hygiene products and and products for the eye and lip area	
49	羟甲基甘氨酸钠	Sodium hydroxymethylamino acetate	Sodium hydroxymethylglycinate	0.5%		
50	碘酸钠	Sodium iodate	Sodium iodate	0.1%	For drenching products only	
51	山梨酸及其盐类	Sorbic acid (hexa-2,4-dienoic acid) and its salts	Sorbic acid and its salts	0.6% (as acid)		
52	硫柳汞	Thiomersal (INN)	Thimerosal	0.007% (in Hg), if mixed with other mercury compounds in this specification, the maximum concentration of Hg remains 0.007%	For use with eye make-up and eye make-up removers only	Thimerosal containing

53	三氯卡班 (2)	Triclocarban (INN)	Triclocarban	0.2%	Purity standard: 3,3',4,4'-Tetrachloroazobenzene less than 1mg/kg; 3,3',4,4'-Tetrachloroazobenzene oxide less than 1mg/kg	
54	三氯生	Triclosan (INN)	Triclosan	0.3%		
55	十一烯酸及其盐类	Undec-10-enoic acid and salts	Undecylenic acid and salts	0.2% (as acid)		
56	吡硫翁锌 (2)	Pyrithione zinc (INN)	Zinc pyrithione	0.5%	For use in drench products, not for use in oral hygiene products	

(1) a The preservatives listed in the table are substances added to cosmetics with the aim of inhibiting the growth of micro-organisms in that cosmetic.

b Other substances in cosmetic products that have an anti-microbial effect, such as many essential oils and certain alcohols, are not included in this table.

c In the table, "salts" means salts of a preservative with cations of sodium, potassium, calcium, magnesium, ammonium and ammonium alcohol; or salts of a preservative with anions of chloride, bromide, sulphate and acetate. In the table, "esters" means methyl, ethyl, propyl, isopropyl, butyl, isobutyl and phenyl esters.

d All cosmetic products containing formaldehyde or any of the formaldehyde-releasing substances listed in this table must be labelled "Contains formaldehyde" when the concentration of formaldehyde in the finished product exceeds 0.05% (in terms of free formaldehyde).

(2) When these preservatives are restricted substances, the requirements are listed in Table 3 of the restricted substances.

(3) Only if the product is likely to be used by children under 3 years of age and in prolonged contact with the skin.

Table 5 Restricted sunscreens in cosmetic components [1]

(in alphabetical order by INCI name)

No.	Name of substance			Maximum permissible concentration in cosmetic products	Other restrictions and requirements	Conditions of use and precautions that must be printed on the label
	Chinese Name	English Name	INCI Name			
1	3-亚苄基樟脑	3-Benzylidene camphor	3-Benzylidene camphor	2%		
2	4-甲基苄亚基樟脑	3-(4'-Methylbenzylidene)-d-l camphor	4-Methylbenzylidene camphor	4%		
3	二苯酮-3	Oxybenzone (INN)	Benzophenone-3	10%		Diphenyl acetone-3[2]-containing
4	二苯酮-4二苯酮-5	2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid and its sodium salt	Benzophenone-4 Benzophenone-5	5% (as acid)		
5	亚苄基樟脑磺酸	Alpha-(2-oxoborn-3-ylidene)-toluene-4-sulphonic acid and its salts	Benzylidene camphor sulfonic acid	6% (as acid)		
6	双-乙基己氧苯酚甲氧苯基三嗪	(1,3,5)-Triazine-2,4-bis((4-(2-ethyl-hexyloxy)-2-hydroxy)-phenyl)-6-(4-methoxyphenyl)	Bis-ethylhexyloxyphenol methoxyphenyl triazine	10%		
7	丁基甲氧基二苯甲酰基甲烷	1-(4-Tert-butylphenyl)-3-(4-methoxyphenyl) propane-1,3-dione	Butyl methoxydibenzoylmethane	5%		
8	樟脑苯扎铵甲基硫酸盐	*N*,*N*,N-trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulphate	Camphor benzalkonium methosulfate	6%		
9	二乙氨基羟苯甲酰基苯甲酸己酯	Benzoic acid, 2-(4-(diethylamino) -2-hydyoxybenzoyl) -,hexyl ester	Diethylamino hydyoxybenzoyl hexyl benzoate	10%		
10	二乙基己基丁酰胺基三嗪酮	Benzoic acid, 4,4'-((6-(((((1,1-dimethylethyl) amino) carbonyl)phenyl)amino) 1,3,5-triazine-2,4-diyl)diimino)bis-, bis-(2-ethylhexyl) ester	Diethylhexyl butamido triazone	10%		

11	2,2'-双-(1,4-亚苯基)1*H*-苯并咪唑-4,6-二磺酸)的二钠盐	Disodium salt of 2,2'-bis-(1,4-phenylene)1H-benzimidazole-4,6-disulphonic acid	Disodium phenyl dibenzimidazole tetrasulfonate	10% (as acid)		
12	甲酚曲唑三硅氧烷	Phenol, 2-(2H-benzotriazol-2-yl)-4-methyl-6-(2-Methyl-3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)-disiloxanyl)propyl	Drometrizole trisiloxane	15%		
13	PABA 乙基己酯	4-Dimethyl amino benzoate of ethyl-2-hexyl	Ethylhexyl dimethyl PABA	8%		
14	甲氧基肉桂酸乙基己酯	2-Ethylhexyl 4-methoxycinnamate	Ethylhexyl methoxycinnamate	10%		
15	水杨酸乙基己酯	2-Ethylhexyl salicylate	Ethylhexyl salicylate	5%		

16	乙基己基三嗪酮	2,4,6-Trianilino-(p-carbo-2'-ethylhexyl-l'-oxy)-1,3,5-triazine	Ethylhexyl triazone	5%		
17	胡莫柳酯	Homosalate (INN)	Homosalate	10%		
18	p-甲氧基肉桂酸异戊酯	Isopentyl-4-methoxycinnamate	Isoamyl p-methoxycinnamate	10%		
19	亚甲基双-苯并三唑基四甲基丁基酚	2,2'-Methylene bis-6-(2H-benzotriazol-2yl)-4-(tetramethyl-butyl)-1,1,3,3-phenol	Methylene bis-benzotriazolyl tetramethylbutylphenol	10%		
20	奥克立林	2-Cyano-3,3-diphenyl acrylic acid, 2-ethylhexyl ester	Octocrylene	10% (as acid)		
21	对氨基苯甲酸	4-Aminobenzoic acid	PABA	5%		
22	PEG-25 对氨基苯甲酸	Ethoxylated ethyl-4-aminobenzoate	PEG-25 PABA	10%		
23	苯基苯并咪唑磺酸及其钾、钠和三乙醇胺盐	2-Phenylbenzimidazole-5-sulphonic acid and its potassium, sodium, and triethanolamine salts	Phenylbenzimidazole sulfonic acid and its potassium, sodium, and triethanolamine salts	8% (as acid)		
24	聚丙烯酰胺甲基亚苄基樟脑	Polymer of N-{(2 and 4)-[(2-oxoborn-3-ylidene) methyl]benzyl} acrylamide	Polyacrylamidomethyl benzylidene camphor	6%		
25	聚硅氧烷-15	Dimethicodiethylbenzalmalonate	Polysilicon-15	10%		
26	对苯二亚甲基二樟脑磺酸	3,3'-(1,4-Phenylenedimethylene)bis(7,7-dimethyl)-2-oxobicyclo-[2.2.1]hept-1-yl-methanesulphonic acid) and its salts	Terephthalylidene dicamphor sulfonic acid and its salts	10% (as acid)		
27	二氧化钛(3)	Titantum dioxide	Titantum dioxide	25%		
28	氧化锌(3)	Zinc oxide	Zinc oxide	25%		

(1) For the purposes of this specification, sunscreens are substances added to sunscreen cosmetics to filter out certain ultraviolet rays in order to protect the skin from certain harmful effects of radiation. These sunscreens may be added to other cosmetic products under the limits and conditions of use specified in this Code. Other sunscreens added to cosmetic products solely for the

purpose of protecting the product from UV damage are not included in this list, but are used in amounts proven safe by safety assessment.

(2) This is not required on the label if the concentration is 0.5% or less and the purpose of use is only to protect the product.

(3) When these sunscreens are used as colourants, the specific requirements are shown in Colourants Table 6.

Table 6 Restricted colourants in cosmetic components [1]

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
1	CI 10006	PIGMENT GREEN 8	Green	颜料绿 8				+	
2	CI 10020	ACID GREEN 1	Green	酸性绿 1			+		
3	CI 10316 [2]	ACID YELLOW 1	Yellow	酸性黄 1		+			1-Naphthol Not more than 0.2%; 2,4-Dinitro-1-naphthol (2,4-Dinitro-1-naphthol) not more than 0.03%
4	CI 11680	FOOD YELLOW 1	Yellow	食品黄 1			+		
5	CI 11710	PIGMENT YELLOW 3	Yellow	颜料黄 3			+		
6	CI 11725	PIGMENT ORANGE 1	Orange	颜料橙 1				+	
7	CI 11920	FOOD ORANGE 3	Orange	食品橙 3	+				
8	CI 12010	SOLVENT RED 3	Red	溶剂红 3			+		

9	CI 12085 (2)	PIGMENT RED 4	Red	颜料红 4	+				2-Chloro-4-nitroaniline (2-Chloro-4-nitrobenzenamine) not more than 0.3%; 2-Naphthol (2-Naphthalenol) not more than 1%; 2,4-Dinitrophenylamine (2,4-Dinitrobenzenamine) not more than 0.02%; 1-[(2,4-Dinitrophenyl)]-2-naphthol (1-[(2,4-Dinitrophenyl)azo]-) 2-naphthalenol) not more than 0.5%; 4-[(2-chloro-4-nitrophenyl)azo]-1-naphthalenol (4-[(2-Chloro-4-nitrophenyl)azo]-1-naphthalenol) not more than 0.5%; 1-[(4-nitrophenyl)azo]-2- naphthalenol (1-[(4- Nitrophenyl)azo]-2-naphthalenol) not more than 0.3%; 1-[(4-chloro-2-nitrophenyl)azo]-2-naphthalenol (1-[(4-Chloro-2-nitrophenyl)azo]-2-naphthalenol) not more than 0.3%
10	CI 12120	PIGMENT RED 3	Red	颜料红 3				+	
11	CI 12370	PIGMENT RED 112	Red	颜料红 112				+	
12	CI 12420	PIGMENT RED 7	Red	颜料红 7				+	Maximum concentration of 4-Chloro-o-toluidine in this colourant: 5mg/kg
13	CI 12480	PIGMENT BROWN 1	Brown	颜料棕 1				+	

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
14	CI 12490	PIGMENT RED 5	Red	颜料红 5	+				
15	CI 12700	DISPERSE YELLOW 16	Yellow	分散黄 16				+	
16	CI 13015	FOOD YELLOW 2	Yellow	食品黄 2	+				
17	CI 14270	ACID ORANGE 6	Orange	酸性橙 6	+				
18	CI 14700	FOOD RED 1	Red	食品红 1	+				5- 氨基 -2,4- 二甲基 -1- 苯磺酸及其钠盐(5-Amino-2,4-dimethyl-1-benzenesulfonic acid and its sodium salt) 不超过 0.2%; 4- 羟基-1- 萘磺酸及其钠盐(4-Hydroxy-1-naphthalenesulfonic acid and its sodium salt) 不超过 0.2%
19	CI 14720	FOOD RED 3	Red	食品红 3	+				4-Aminonaphthalene-1-sulfonic acid and 4-Hydroxynaphthalene-1-sulfonic acid not exceeding 0.5% in total; unsulphonated aromatic primary amines not exceeding 0.01% (as aniline))
20	CI 14815	FOOD RED 2	Red	食品红 2	+				
21	CI 15510 (2)	ACID ORANGE 7	Orange	酸性橙 7		+			2-Naphthol not more than 0.4%; Sulfanilic acid, sodium salt not more than 0.2%; 4,4'-(Diazoamino)-dibenzenesulfonic acid not more than 0.1%; 4,4'-(Diazoamino)-dibenzenesulfonic acid dibenzenesulfonic acid) not more than 0.1%

22	CI 15525	PIGMENT RED 68	Red	颜料红 68	+				
23	CI 15580	PIGMENT RED 51	Red	颜料红 51	+				
24	CI 15620	ACID RED 88	Red	酸性红 88				+	
25	CI 15630 (2)	PIGMENT RED 49	Red	颜料红 49	+				Maximum concentration in cosmetic products 3%
26	CI 15800	PIGMENT RED 64	Red	颜料红 64			+		Aniline not more than 0.2%; Calcium 3-hydroxy-2-naphthoate (3-Hydroxy-2-naphthoic acid, calcium salt) not more than 0.4%
27	CI 15850 (2)	PIGMENT RED 57	Red	颜料红 57	+				2- 氨 基 -5- 甲 基 苯 磺 酸 钙 盐 (2-Amino-5-methylbenzensulfonic acid, calcium salt)不超过 0.2%; 3-羟基-2- 萘基羰酸钙盐(3-Hydroxy-2-naphthalene carboxylic acid, calcium salt)不超过 0.4%; 未磺化芳香伯胺不超过 0.01%(以苯胺计)

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
28	CI 15865 (2)	PIGMENT RED 48	Red	颜料红 48	+				
29	CI 15880	PIGMENT RED 63	Red	颜料红 63	+				2-氨基-1-萘磺酸钙(2-Amino-1- naphthalenesulfonic acid, calcium salt)不超过 0.2%; 3-羟基-2-萘甲酸(3-Hydroxy-2-naphthoic acid)不超过 0.4%
30	CI 15980	FOOD ORANGE 2	Orange	食品橙 2	+				
31	CI 15985 (2)	FOOD YELLOW 3	Yellow	食品黄 3	+				4-氨基苯-1-磺酸(4-Aminobenzene-1-sulfonic acid)、3-羟基萘-2,7-二磺酸(3-Hydroxynaphthalene- 2,7-disulfonic acid)、6-羟基萘-2-磺酸(6-Hydroxynaphthalene-2-sulfonic acid)、7- 羟 基 萘 -1,3- 二 磺 酸 (7-Hydroxynaphthalene 1,3-disulfonic acid) and 4,4'-diazoaminodi(benezene sulfonic acid) in a total amount not exceeding 0.5%; 6,6 6,6'-Oxydi(2-naphthalene sulfonic acid) disodium salt not more than 1.0%; unsulphonated aromatic primary amine not more than 0.01 % (as aniline)
32	CI 16035	FOOD RED 17	Red	食品红 17	+				6-Hydroxy-2-naphthalene sulfonic acid, sodium salt, not more than 0.3%; 4-Amino-5-methoxy-2-methylbenezene 6,6'-Oxydi(2-naphthalene sulfonic acid) disodium salt (not more than 1.0%); 6,6'-Oxydi(2-naphthalene sulfonic acid) disodium salt Not more than 1.0%; Unsulphonated aromatic primary amines not exceeding 0.01% (as aniline)

33	CI 16185	FOOD RED 9	Red	食品红 9	+				4-氨基萘-1-磺酸(4-Aminonaphthalene-1-sulfonic acid)、3-羟基萘-2,7- 二磺酸(3-Hydroxynaphthalene-2,7-disulfonic acid)、6-羟基萘-2-磺酸(6-Hydroxynaphthalene-2-sulfonic acid) 、 7- 羟 基 萘 -1,3- 二 磺 酸 (7- Hydroxynaphthalene-1,3-disulfonic acid)和 7-羟基萘-1,3,6- 三磺酸(7-Hydroxy naphthalene-1,3,6- trisulfonic acid)总量不超过 0.5%; 未磺化芳香伯胺不超过 0.01%(以苯胺计)
34	CI 16230	ACID ORANGE 10	Orange	酸性橙 10			+		

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
35	CI 16255 (2)	FOOD RED 7	Red	食品红 7	+				4-氨基萘-1-磺酸(4-Aminonaphthalene-1-sulfonic acid)、3-羟基萘-2,7-二磺酸(3- Hydroxynaphthalene-2,7-disulfonic acid)、6-羟基萘-2-磺酸(6-Hydroxynaphthalene-2-sulfonic acid) 、 7- 羟基萘 -1,3- 二磺酸 (7-Hydroxynaphthalene 1,3-disulfonic acid) and 7-Hydroxy naphthalene-1,3,6-trisulfonic acid (not more than 0.5% in total); unsulphonated aromatic primary amines not more than 0.01% (as aniline)
36	CI 16290	FOOD RED 8	Red	食品红 8	+				
37	CI 17200 (2)	FOOD RED 12	Red	食品红 12	+				4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid, disodium salt; 4,5-dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid, disodium salt; 4,5-dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid, disodium salt; 4,5-dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid, disodium salt; 4,5-dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid, disodium salt 4,5-Dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid, disodium salt Not more than 3%; Aniline Not more than 25mg/kg; 4-aminoazobenzene Not more than 0.3%; 4,5-Dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid, disodium salt Not more than 0.3%; 4,5-Dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid, disodium salt Aminoazobenzene not more than 100g/kg; 1,3-Diphenyltriazene not more than 125g/kg; 4-Aminobiphenyl not more than 275 g/kg;

									Azobenzene not more than 1mg/kg; Benzidine not more than 20g/kg
38	CI 18050	FOOD RED 10	Red	食品红 10			+		5-Acetamido-4-hydroxynaphthalene-2,7-disulfonic acid and 5-Amino-4-hydroxynaphthalene-2,7-disulfonic acid in a total quantity of not more than 0.5%; unsulphonated aromatic primary amines not more than 0.01% (as aniline). not more than 0.5% in total; not more than 0.01% (as aniline) of unsulphonated aromatic primary amines)
39	CI 18130	ACID RED 155	Red	酸性红 155				+	
40	CI 18690	ACID YELLOW 121	Yellow	酸性黄 121				+	
41	CI 18736	ACID RED 180	Red	酸性红 180				+	
42	CI 18820	ACID YELLOW 11	Yellow	酸性黄 11				+	
43	CI 18965	FOOD YELLOW 5	Yellow	食品黄 5	+				

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
44	CI 19140 (2)	FOOD YELLOW 4	Yellow	食品黄 4	+				4-Hydrazinobenzene sulfonic acid; 4-Aminobenzene; 4-Hydrazinobenzene sulfonic acid 1-sulfonic acid (4-Aminobenzene-1-sulfonic acid), 5-Oxo-1-(4-sulfophenyl)-2-pyrazoline-3-carboxylic acid (5-Oxo-1-(4-sulfophenyl)-2-pyrazoline-3-carboxylic acid), 4,4 4,4'-Diazoaminodi(benzene sulfonic acid) and Tetrahydroxy succinic acid in a total amount not exceeding 0.5%; unsulphonated aromatic Not more than 0.01% by weight of aniline
45	CI 20040	PIGMENT YELLOW 16	Yellow	颜料黄 16				+	Maximum concentration of 3,3'-dimethylbenzidine in this colourant: 5mg/kg
46	CI 20470	ACID BLACK 1	Black	酸性黑 1				+	
47	CI 21100	PIGMENT YELLOW 13	Yellow	颜料黄 13				+	Maximum concentration of 3,3'-dimethylbenzidine in this colourant: 5mg/kg
48	CI 21108	PIGMENT YELLOW 83	Yellow	颜料黄 83				+	Maximum concentration of 3,3'-dimethylbenzidine in this colourant: 5mg/kg
49	CI 21230	SOLVEN TYELLOW 29	Yellow	溶剂黄 29			+		
50	CI 24790	ACID RED 163	Red	酸性红 163				+	

51	CI 27755	FOOD BLACK 2	Black	食品黑 2	+				
52	CI 28440	FOOD BLACK 1	Black	食品黑 1	+				4-Acetamido-5-hydroxy naphthalene-1,7-disulfonic acid, 4-Amino-5-hydroxy naphthalene-1,7-disulfonic acid, 8-Aminonaphthalene-2-sulfonic acid and 4,4'-bis-azoamines. disulfonic acid, 8-Aminonaphthalene-2-sulfonic acid and 4,4'-diazoaminodiphenyl sulfonic acid. diazoaminodi-(benzenesulfonic acid) not more than 0.8% in total; unsulphonated aromatic primary amines not more than 0.01% (as aniline)
53	CI 40215	DIRECT ORANGE 39	Orange	直接橙 39				+	
54	CI 40800	FOOD ORANGE 5	Orange	食品橙 5	+				
55	CI 40820	FOOD ORANGE 6	Orange	食品橙 6	+				

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
56	CI 40825	FOOD ORANGE 7	Orange	食品橙 7	+				
57	CI 40850	FOOD ORANGE 8	Orange	食品橙 8	+				
58	CI 42045	ACID BLUE 1	Blue	酸性蓝 1			+		
59	CI 42051 (2)	FOOD BLUE 5	Blue	食品蓝 5	+				3-Hydroxy benzaldehyde, 3-Hydroxy benzoic acid, 3-Hydroxy-4-sulfobenzoic acid and N,N Not more than 0.5% total diethylamino benzenesulfonic acid; not more than 4.0% colourless parent (Leuco base); not more than 0.01% unsulphonated aromatic primary amine (as aniline)
60	CI 42053	FOOD GREEN 3	Green	食品绿 3	+				无色母体(Leuco base)不超过 5%; 2-,3-,4-甲酰基苯磺酸及其钠盐 (2-,3-,4-Formylbenzenesulfonic acids and their sodium salts)总量不超过 0.5%; 3-和 4-[乙基(4-磺苯基)氨基]甲基苯磺酸及其二钠盐(3- and 4-[(Ethyl(4-sulfophenyl) amino)methyl]benzenesulfonic acid and its disodium salts) 总量不超过 0.3%; 2- 甲酸基-5- 羟基苯磺酸及其钠盐(2-Formyl-5- hydroxybenzenesulfonic acid and its sodium salt) 不超过 0.5%
61	CI 42080	ACID BLUE 7	Blue	酸性蓝 7				+	

62	CI 42090	FOOD BLUE 2	Blue	食品蓝 2	+				2-,3- and 4-Formyl benzene sulfonic acids, not more than 1.5% total; 3-(Ethyl(4-sulfophenyl)amino)methyl benzene sulfonic acid) not more than 0.3%; colourless parent (Leuco base) not more than 5.0%; unsulphonated aromatic primary amines not more than 0.01% (as aniline)
63	CI 42100	ACID GREEN 9	Green	酸性绿 9				+	
64	CI 42170	ACID GREEN 22	Green	酸性绿 22				+	
65	CI 42510	BASIC VIOLET 14	Purple	碱性紫 14			+		
66	CI 42520	BASIC VIOLET 2	Purple	碱性紫 2				+	Maximum concentration in cosmetic products 5mg/kg
67	CI 42735	ACID BLUE 104	Blue	酸性蓝 104			+		

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
68	CI 44045	BASIC BLUE 26	Blue	碱性蓝 26			+		
69	CI 44090	FOOD GREEN 4	Green	食品绿 4	+				4,4'-Bis(dimethylamino) benzhydryl alcohol not more than 0.1%; 4,4'-bis(dimethylamino) benzophenone 4,4'-Bis(dimethylamino) benzophenone) not more than 0.1%; 3-Hydroxynaphthalene-2,7- disulfonic acid not more than 0.2%; colourless parent (Leuco base) not more than 5.0%; Unsulphonated aromatic primary amine not more than 0.01% (as aniline)
70	CI 45100	ACID RED 52	Red	酸性红 52				+	
71	CI 45190	ACID VIOLET 9	Purple	酸性紫 9				+	
72	CI 45220	ACID RED 50	Red	酸性红 50				+	
73	CI 45350	ACID YELLOW 73	Yellow	酸性黄 73	+				Maximum concentration in cosmetics 6%; Resorcinol not more than 0.5%; Phthalic acid not more than 1%; 2-(2,4-Dihydroxybenzoyl) benzoic acid not more than 0.5%
74	CI 45370 (2)	ACID ORANGE 11	Orange	酸性橙 11	+				2-(6-Hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid, not more than 1%; 2-(Bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid, not more than 2%. (Bromo-6- hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid) not more than 2%

75	CI 45380 (2)	ACID RED 87	Red	酸性红 87	+				2-(6-Hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid, not more than 1%; 2-(Bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (Bromo-6- hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid) not more than 2%; 2-(Bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid
76	CI 45396	SOLVEN TORANGE 16	Orange	溶剂橙 16	+				For lipstick, only the free (acidic) form of the colourant is permitted, and the maximum concentration is 1%.
77	CI 45405	ACID RED 98	Red	酸性红 98		+			2-(6-Hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid, not more than 1%; 2-(Bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (Bromo-6- hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid) not more than 2%; 2-(Bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid
78	CI 45410 (2)	ACID RED 92	Red	酸性红 92	+				2-(6-Hydroxy-3-oxo-3H-occupant-9-yl) benzoic acid

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
									(2-(6-Hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid) not more than 1%; 2-(Bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (2-(Bromo-6- hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid) not more than 2%
79	CI 45425	ACID RED 95	Red	酸性红 95	+				Triiodoresorcinol not more than 0.2%; 2-(2,4-dihydroxy-3,5-dicarbonylbenzoyl) benzoic acid (2-(2,4-dihydroxy-3,5-carbonyl)benzoic acid) -dioxobenzoyl) benzoic acid) not more than 0.2%
80	CI 45430 (2)	FOOD RED 14	Red	食品红 14	+				Triiodoresorcinol not more than 0.2%; 2-(2,4-dihydroxy-3,5-dicarbonylbenzoyl) benzoic acid not more than 0.2%; 2-(2,4-dihydroxy-3,5-dioxobenzoyl) benzoic acid
81	CI 47000	SOLVENTYELLOW 33	Yellow	溶剂黄 33			+		Phthalic acid not more than 0.3%; 2-Methylquinoline (Quinaldine) not more than 0.2%
82	CI 47005	FOOD YELLOW 13	Yellow	食品黄 13	+				2-methylquinoline, 2-methylquinoline sulfonic acid, phthalic acid, 2,6-dimethyl quinoline and 2,6-dimethyl quinoline 2-(2-quinolinyl)2,3-dihydro-1,3-indane-1,3-dione 4mg/kg; Unsulphonated aromatic primary amine not more than 0.01% (as aniline)
83	CI 50325	ACID VIOLET 50	Purple	酸性紫 50				+	

84	CI 50420	ACID BLACK 2	Black	酸性黑 2			+		
85	CI 51319	PIGMENT VIOLET 23	Purple	颜料紫 23				+	
86	CI 58000	PIGMENT RED 83	Red	颜料红 83	+				
87	CI 59040	SOLVENT GREEN 7	Green	溶剂绿 7			+		1,3,6- 芘三磺 酸三钠 (Trisodium salt of 1,3,6-pyrene trisulfonic acid) 不超过 6%; 1,3,6,8- 芘四磺酸四钠 (Tetrasodium salt of 1,3,6,8-pyrene tetrasulfonic acid) 不 超过 1%; 芘(Pyrene)不超过 0.2%
88	CI 60724	DISPERSE VIOLET 27	Purple	分散紫 27				+	
89	CI 60725	SOLVENT VIOLET 13	Purple	溶剂紫 13	+				p-Toluidine not more than 0.2%; 1-Hydroxy-9,10-anthracenedione not more than 0.5%; 1,4-dihydroxy

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
									1,4-Dihydroxy-9,10-anthracenedione (1,4-Dihydroxy-9,10-anthracenedione) not more than 0.5%
90	CI 60730	ACID VIOLET 43	Purple	酸性紫 43			+		1-Hydroxy-9,10-anthracenedione not more than 0.2%; 1,4-dihydroxy-9,10-anthracenedione anthracenedione) not more than 0.2%; *p-Toluidine* (p-Toluidine) not more than 0.1%; p-Toluidine sulfonic acids (sodium salts) not more than 0.2%
91	CI 61565	SOLVENT GREEN 3	Green	溶剂绿 3	+				p-Toluidine Not more than 0.1%; 1,4-Dihydroxyanthraquinone Not more than 0.2%; 1-Hydroxy-4-[(4-methyl phenyl)amino]-9,10-anthracenedione Not more than 5%; 1-Hydroxy-4-[(4-methyl phenyl)amino]-9,10-anthracenedione 1-Hydroxy-4-[(4-methyl phenyl)amino]-9,10-anthracenedione (not more than 5%)
92	CI 61570	ACID GREEN 25	Green	酸性绿 25	+				1,4-Dihydroxy anthraquinone Not to exceed 2-Amino-m-toluene sulfonic acid; 0.2%; 2-Amino-m-toluene sulfonic acid acid) not more than 0.2%
93	CI 61585	ACID BLUE 80	Blue	酸性蓝 80				+	
94	CI 62045	ACID BLUE 62	Blue	酸性蓝 62				+	
95	CI 69800	FOOD BLUE 4	Blue	食品蓝 4	+				

96	CI 69825	VAT BLUE 6	Blue	还原蓝 6	+				
97	CI 71105	VAT ORANGE 7	Orange	还原橙 7			+		
98	CI 73000	VAT BLUE 1	Blue	还原蓝 1	+				
99	CI 73015	FOOD BLUE 1	Blue	食品蓝 1	+				Isatin-5-sulfonic acid, 5-Sulfoanthranilic acid and Anthranilic acid in a total amount not exceeding 0.5%; unsulphonated aromatic primary amines not exceeding 0.01% (as aniline)
100	CI 73360	VAT RED 1	Red	还原红 1	+				
101	CI 73385	VAT VIOLET 2	Purple	还原紫 2	+				
102	CI 73900	PIGMENT VIOLET 19	Purple	颜料紫 19				+	

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
103	CI 73915	PIGMENT RED 122	Red	颜料红 122				+	
104	CI 74100	PIGMENT BLUE 16	Blue	颜料蓝 16				+	
105	CI 74160	PIGMENT BLUE 15	Blue	颜料蓝 15	+				
106	CI 74180	DIRECT BLUE 86	Blue	直接蓝 86				+	
107	CI 74260	PIGMENT GREEN 7	Green	颜料绿 7		+			
108	CI 75100	NATURAL YELLOW 6	Yellow	天然黄 6	+				
109	CI 75120	NATURAL ORANGE 4	Orange	天然橙 4	+				
110	CI 75125	NATURAL YELLOW 27	Yellow	天然黄 27	+				
111	CI 75130	NATURAL YELLOW 26	Orange	天然黄 26	+				
112	CI 75135	RUBIXANTHIN	Yellow	玉红黄	+				
113	CI 75170	NATURAL WHITE 1	White	天然白 1	+				
114	CI 75300	NATURAL YELLOW 3	Yellow	天然黄 3	+				
115	CI 75470	NATURAL RED 4	Red	天然红 4	+				

116	CI 75810	NATURAL GREEN 3	Green	天然绿 3	+				
117	CI 77000	PIGMENT METAL 1	White	颜料金属 1 (铝, Al)	+				
118	CI 77002	PIGMENT WHITE 24	White	颜料白 24	+				
119	CI 77004	PIGMENT WHITE 19	White	颜料白 19	+				
120	CI 77007	PIGMENT BLUE 29	Blue	颜料蓝 29	+				
121	CI 77015	PIGMENT RED 101, 102	Red	颜料红 101, 102 (氧化铁着色的硅酸镁)	+				
122	CI 77019	PIGMENT WHITE 20	White	颜料白 20(云母)	+				

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
123	CI 77120	PIGMENT WHITE 21, 22	White	颜料白 21, 22 (硫酸钡, $BaSO_4$)	+				
124	CI 77163	PIGMENT WHITE 14	White	颜料白 14 (氯氧化铋, BiOCl)	+				
125	CI 77220	PIGMENT WHITE 18	White	颜料白 18 (碳酸钙, $CaCO_3$)	+				
126	CI 77231	PIGMENT WHITE 25	White	颜料白 25 (硫酸钙, $CaSO_4$)	+				
127	CI 77266	PIGMENT BLACK 6,7	Black	颜料黑 6, 7	+				Polycyclic aromatic hydrocarbon limit: 1g of colourant sample plus 10g of cyclohexane, the extract should be colourless and the fluorescence intensity under UV light should not exceed that of a control solution of quinine sulfate (0.1g of quinine sulfate dissolved in 1000mL of 0.01mol/L sulphuric acid)
128	CI 77267	PIGMENT BLACK 9	Black	颜料黑 9	+				
129	CI 77268:1	FOOD BLACK 3	Black	食品黑 3	+				
130	CI 77288	PIGMENT GREEN 17	Green	颜料绿 17 (三氧化二铬, Cr_2O_3)	+				No free chromate ions
131	CI 77289	PIGMENT GREEN 18	Green	颜料绿 18	+				No free chromate ions

				($Cr_2O(OH)_4$)					
132	CI 77346	PIGMENT BLUE 28	Blue	颜料蓝 28	+				
133	CI 77400	PIGMENT METAL 2	Brown	颜料金属 2 (铜, Cu)	+				
134	CI 77480	PIGMENT METAL 3	Brown	颜料金属 3 (金, Au)	+				
135	CI 77489	FERROUS OXIDE	Orange	氧化亚铁, FeO	+				
136	CI 77491	PIGMENT RED 101, 102	Red	颜料红 101, 102 (氧化铁, Fe_2O_3)	+				
137	CI 77492	PIGMRNT YELLOW 42, 43	Yellow	颜料黄 42, 43 ($FeO(OH).nH_2O$)	+				

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
138	CI 77499	PIGMENT BLACK 11	Black	颜料黑 11 ($FeO+Fe_2O_3$)	+				
139	CI 77510	PIGMENT BLUE 27	Blue	颜料蓝 27 ($Fe_4(Fe(CN)_6)_3+FeNH_4Fe(CN)_6$)	+				Cyanide ion free
140	CI 77713	PIGMENT WHITE 18	White	颜料白 18 (碳酸锰, $MnCO_3$)	+				
141	CI 77718	PIGMENT WHITE 26	White	颜料白 26(滑石)	+				
142	CI 77742	PIGMENT VIOLET 16	Purple	颜料紫 16 ($(NH_4)_2MnP_2O_7$)	+				
143	CI 77745	MANGANESE PHOSPHATE	Red	磷酸锰, $Mn_3(PO_4)_2.7H_2O$	+				
144	CI 77820	SILVER	White	银, Ag	+				
145	CI 77891[3]	PIGMENT WHITE 6	White	颜料白 6 (二氧化钛, TiO_2)	+				
146	CI 77947[3]	PIGMENT WHITE 4	White	颜料白 4 (氧化锌, ZnO)	+				
147		ACID RED 195	Red	酸性红 195			+		

148		ALUMINUM, ZINC, MAGNESINM and CALCIUM STEARATE	White	硬脂酸铝、锌、镁、钙盐	+				
149		ANTHOCYANINS	Red	花色素苷	+				
150		BEET ROOT RED	Red	甜菜根红	+				
151		BROMOCRESOL GREEN	Green	溴甲酚绿				+	
152		BROMOTHYMOL BLUE	Blue	溴百里酚蓝				+	
153		CAPSANTHIN, CAPSORUBIN	Orange	辣椒红	+				

No.	Colourant index number (Color Index)	Colorant Index Generic Name (C.I. generic name)	Colour	Colorant Index Common Chinese Name	Permissible range of use				Other restrictions and requirements
					1	2	3	4	
					Various cosmetics	Cosmetics other than those for the eyes	Specifically for cosmetics that do not come into contact with mucous membranes	Cosmetics designed for temporary contact with the skin only	
154		CARAMEL	Brown	焦糖	+				
155		LACTOFLAVIN	Yellow	乳黄素	+				
156		SORGHUM RED	Coffee	高粱红		+			

(1) a Salts and colour deposits formed by the colouring agents listed in a are also permitted with substances not included in Schedule 2 of the Prohibited Substances.

b Colourants with more than one salt are indicated by a number after a colon, e.g. 15850:1, 15850:2. If not specified, the common Chinese Name is taken as the main name without the colon. If there is more than one common Chinese Name, the name with "food" is used.

(2) Insoluble barium, strontium, zirconium precipitates, salts and pigments are also permitted for these colourants, which must pass an insolubility test.

(3) When these colourants are used as sunscreens, the specific requirements are listed in Table 5 of the sunscreens.

Table 7 Temporary permission to use hair dyes in cosmetic components [1]

(in alphabetical order by INCI name)

No.	Name of substance		Maximum permissible concentration in cosmetic products	Other restrictions and requirements	Conditions of use and precautions that must be printed on the label
	Chinese Name	INCI Name			
1	1,3-双-(2,4-二氨基苯氧基)丙烷 HCl	1,3-Bis-(2,4-diaminophenoxy) propane HCl	2.0 (in free base)	When used in combination with oxidised milk, the maximum concentration should be 1.0%.	
2	1,3-双-(2,4-二氨基苯氧基)丙烷	1,3-Bis-(2,4-diaminophenoxy) propane	2.0	When used in combination with oxidised milk, the maximum concentration should be 1.0%.	
3	1,5-萘二酚(CI76625)	1,5-Naphthalenediol	1.0	When mixed with oxidised milk, the maximum concentration should be 0.5%.	
4	1-羟乙基 4,5-二氨基吡唑硫酸盐	1-Hydroxyethyl 4,5-Diaminopyrazole sulfate	2.25	When mixed with Milk Oxide, the maximum concentration should be 1.125%.	
5	1-萘酚(CI76605)	1-Naphthol	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	Contains 1-naphthol
6	2,4-二氨基苯酚 [2]	2,4-Diaminophenol	10.0		Diaminophenol containing
7	2,4-二氨基苯酚 HCl [2]	2,4-Diaminophenol HCl	10.0 (in free base)		Diaminophenol containing
8	2,4-二氨基苯氧基乙醇HCl	2,4-Diaminophenoxyethanol HCl	4.0 (in free base)	When mixed with Milk Oxide, the maximum concentration should be 2.0%.	
9	2,4-二氨基苯氧基乙醇硫酸盐	2,4-Diaminophenoxyethanol sulfate	4.0 (in free base)	When mixed with Milk Oxide, the maximum concentration should be 2.0%.	
10	2,6-二氨基吡啶	2,6-Diaminopyridine	0.004	When mixed with milk oxide, the maximum concentration should be 0.002%.	
11	2,6-二氨基吡啶硫酸盐	2,6-Diaminopyridine sulfate	0.004 (in free base)	When mixed with milk oxide, the maximum concentration should be 0.002%.	

12	2,6-二羟乙基氨甲苯	2,6-Dihydroxyethylaminotoluene	2.0	When mixed with Milk Oxide, the maximum concentration should be 1.0%.	
13	2,6-二甲氧基-3,5-吡啶二胺 HCl	2,6-Dimethoxy-3,5-pyridinediamine HCl	0.5	When mixed with oxidised milk, the maximum concentration should be 0.25%.	
14	2,7-萘二酚(CI76645)	2,7-Naphthalenediol	1.0	When mixed with oxidised milk, the maximum concentration should be 0.5%.	
15	2-氨基-3-羟基吡啶	2-Amino-3-hydroxypyridine	0.6	When mixed with oxidised milk, the maximum concentration should be 0.3%.	
16	2-氨基-4-羟乙氨基茴香醚	2-Amino-4-hydroxyethylaminoanisole	3.0	When mixed with oxidised milk, the maximum concentration should be 1.5%.	
17	2-氨基-4-羟乙氨基茴香醚硫酸盐	2-Amino-4-hydroxyethylaminoanisole sulfate	3.0 (in free base)	When mixed with oxidised milk, the maximum concentration should be 1.5%.	
18	2-氨基-6-氯-4-硝基苯酚	2-Amino-6-chloro-4-nitrophenol	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	
19	2-氨基-6-氯-4-硝基苯酚HCl	2-Amino-6-chloro-4-nitrophenol HCL	2.0 (in free base)	When mixed with milk oxide, the maximum concentration should be 1.0%.	
20	2-氯-*p*-苯二胺	2-Chloro-p-phenylenediamine	0.1	When mixed with oxidised milk, the maximum concentration should be 0.05%.	
21	2-氯-*p*-苯二胺硫酸盐	2-Chloro-p-phenylenediamine sulfate	1.0	When mixed with milk oxide, the maximum concentration should be 0.5%.	

No.	Name of substance		Maximum permissible concentration in cosmetic products	Other restrictions and requirements	Conditions of use and precautions that must be printed on the label
	Chinese Name	INCI Name			
22	2-羟乙基苦氨酸	2-Hydroxyethyl picramic acid	(a) 3.0 (b) 2.0(3)	When mixed with oxidised milk, the maximum concentration should be 1.5%.	
23	2-甲基-5-羟乙氨基苯酚	2-Methyl-5-hydroxyethylaminophenol	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	
24	2-甲基雷琐辛	2-Methylresorcinol	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	Containing 2-Methylresorcin
25	2-硝基-*p*-苯二胺	2-Nitro-p-phenylenediamine	0.3	When mixed with oxidised milk, the maximum concentration should be 0.15%.	
26	2-硝基-*p*-苯二胺 2HCl	2-Nitro-p-phenylenediamine dihydrochloride	0.3 (in free base)	When mixed with oxidised milk, the maximum concentration should be 0.15%.	
27	2-硝基-*p*-苯二胺硫酸盐	2-Nitro-p-phenylenediamine sulfate	0.3 (in free base)	When mixed with oxidised milk, the maximum concentration should be 0.15%.	
28	3-硝基-*p*-羟乙氨基酚	3-Nitro-p-hydroxyethylaminophenol	6.0	When mixed with Milk Oxide, the maximum concentration should be 3.0%.	
29	4,4'-二氨基二苯胺(2)	4,4'-Diaminodiphenylamine	6.0		Phenylenediamine containing
30	4,4'-二氨基二苯胺硫酸盐(2)	4,4'-Diaminodiphenylamine sulfate	6.0 (in free base)		Phenylenediamine containing
31	4-氨基-2-羟基甲苯	4-Amino-2-hydroxytoluene	3.0	When mixed with milk oxide, the maximum concentration should be 1.5%.	
32	4-氨基-3-硝基苯酚	4-Amino-3-nitrophenol	3.0	When mixed with oxidised milk, the maximum concentration should be 1.5%.	
33	4-氨基-*m*-甲酚	4-Amino-m-cresol	3.0	When mixed with milk oxide, the maximum concentration should be 1.5%.	

34	4-氯雷琐辛	4-Chlororesorcinol	1.0	When mixed with milk oxide, the maximum concentration should be 0.5%.	
35	4-羟丙氨基-3-硝基苯酚	4-Hydroxypropylamino-3-nitrophenol	(a) 5.2 (b) 2.6[3]	When mixed with oxidised milk, the maximum concentration should be 2.6%.	
36	4-硝基-*o*-苯二胺	4-Nitro-o-phenylenediamine	1.0	When mixed with milk oxide, the maximum concentration should be 0.5%.	
37	4-硝基-*o*-苯二胺硫酸盐	4-Nitro-o-phenylenediamine sulfate	1.0 (in free base)	When mixed with milk oxide, the maximum concentration should be 0.5%.	
38	5-氨基-4-氯-*o*-甲酚	5-Amino-4-chloro-o-cresol	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	
39	5-氨基-6-氯-*o*-甲酚	5-Amino-6-chloro-o-cresol	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	
40	6-氨基-*m*-甲酚	6-Amino-m-cresol	2.4	When mixed with milk oxide, the maximum concentration should be 1.2%.	
41	6-氨基-*o*-甲酚	6-Amino-o-cresol	3.0	When mixed with milk oxide, the maximum concentration should be 1.5%.	
42	6-羟基吲哚	6-Hydroxyindole	1.0	When mixed with oxidised milk, the maximum concentration should be 0.5%.	
43	6-甲氧基-2-甲氨基-3-氨基吡啶 HCl (HC 蓝 7 号)	6-Methoxy-2-methylamino-3-aminopyridine HCl	2.0	When mixed with Milk Oxide, the maximum concentration should be 1.0%.	
44	酸性橙3号(CI10385)	Acid Orange 3	0.2		

No.	Name of substance		Maximum permissible concentration in cosmetic products	Other restrictions and requirements	Conditions of use and precautions that must be printed on the label
	Chinese Name	INCI Name			
45	酸性紫43号(CI60730)	Acid Violet 43	1.0	The purity of the dyestuff used must not be <80%, and the impurity content must meet the following requirements: volatile components (135°C) and chlorides and sulphates (as sodium salts) <18%, water insoluble matter must not be <0.4%, 1-hydroxy -9,10-anthracenedione (1-hydroxy-9,10-anthracenedione) Less than 0.2%, p-甲苯胺(p-toluidine)小于0.1%, p-甲苯胺磺酸钠(p-tolluidine sulfonic acids, sodium salts)小于0.2%, 其它染 料 (subsidiary colors) 小于 1%, 铅小于 20mg/kg, 砷小于3mg/kg, 汞小于1mg/kg	
46	碱性蓝26号(CI44045)	Basic Blue 26	0.5	When mixed with milk oxide, the maximum concentration should be 0.25%.	
47	碱性橙 31 号	Basic orange 31	0.2	When mixed with oxidised milk, the maximum concentration should be 0.1%.	
48	碱性红 51 号	Basic red 51	0.2	When mixed with oxidised milk, the maximum concentration should be 0.1%.	
49	碱性红 76 号(CI12245)	Basic red 76	2.0		
50	碱性紫14号(CI42510)	Basic Violet 14	0.3	When mixed with milk oxide, the maximum concentration should be 0.15%.	
51	碱性黄 87 号	Basic yellow 87	0.2	When mixed with oxidised milk, the maximum concentration should be 0.1%.	
52	分散黑 9 号	Disperse Black 9	0.4		
53	分散紫1号	Disperse Violet 1	1.0	When mixed with milk oxide, the maximum	

				concentration should be 0.5%.	
54	分散紫4号(CI61105)	Disperse violet 4	0.08	When mixed with oxidised milk, the maximum concentration should be 0.04%.	
55	HC橙1号	HC Orange No.1	3.0		
56	HC红1号	HC Red No.1	0.5		
57	HC红3号	HC Red No.3	0.5	The free diethanolamine content in the raw material is ≤ 0.5% and must not be combined with nitrosated substances.	
58	HC黄2号	HC Yellow No.2	3.0	When mixed with milk oxide, the maximum concentration should be 1.5%.	
59	HC 黄 4 号	HC Yellow No.4	3.0		
60	HC 黄 6 号	HC Yellow No.6	(a) 2.0 (b) 1.0(3)	When mixed with Milk Oxide, the maximum concentration should be 1.0%.	
61	氢醌(4)	Hydroquinone	0.3		Contains hydroquinone
62	羟苯并吗啉	Hydroxybenzomorpholine	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	
63	羟乙基-2-硝基-*p*-甲苯胺	Hydroxyethyl-2-nitro-p-toluidine	(a) 2.0 (b) 1.0(3)	When mixed with Milk Oxide, the maximum concentration should be 1.0%.	

No.	Name of substance		Maximum permissible concentration in cosmetic products	Other restrictions and requirements	Conditions of use and precautions that must be printed on the label
	Chinese Name	INCI Name			
64	羟乙基-3,4-亚甲二氧基苯胺HCl	Hydroxyethyl-3,4-methylenedioxyaniline HCl	3.0	When mixed with milk oxide, the maximum concentration should be 1.5%.	
65	羟乙基-*p*-苯二胺硫酸盐	Hydroxyethyl-p-phenylenediamine sulfate	3.0	When mixed with oxidised milk, the maximum concentration should be 1.5%.	
66	羟丙基双(*N*-羟乙基-*p*-苯二胺)HCl	Hydroxypropylbis(N-hydroxyethyl-p-phenylenediamine) HCl	3.0	When mixed with oxidised milk, the maximum concentration should be 1.5%.	
67	*m*-氨基苯酚	m-Aminophenol	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	
68	*m*-氨基苯酚HCl	m-Aminophenol HCl	2.0 (in free base)	When mixed with milk oxide, the maximum concentration should be 1.0%.	
69	*m*-氨基苯酚硫酸盐	m-Aminophenol sulfate	2.0 (in free base)	When mixed with milk oxide, the maximum concentration should be 1.0%.	
70	*N*,*N*-双(2-羟乙基)-*p*-苯二胺硫酸盐 (2)	*N*,N-bis(2-hydroxyethyl)-p-phenylenediamine sulfate	6.0 (in free base)		Phenylenediamine containing
71	*N*,*N*-二乙基-*p*-苯二胺硫酸盐 (2)	*N*,N-diethyl-p-phenylenediamine sulfate	6.0 (in free base)		Phenylenediamine containing
72	*N*,*N*-二乙基甲苯-2,5-二胺HCl (2)	*N*,N-diethyltoluene-2,5-diamine HCl	10.0 (in free base)		Phenylenediamine containing
73	*N*,*N*-二甲基-*p*-苯二胺 (2)	*N*,N-dimethyl-p-phenylene diamine	6.0		Phenylenediamine containing
74	*N*,*N*-二甲基-*p*-苯二胺硫酸盐 (2)	*N*,N-dimethyl-p-phenylenediamine sulfate	6.0 (in free base)		Phenylenediamine containing
75	*N*-苯基-*p*-苯二胺(CI76085) (2)	N-phenyl-p-phenylenediamine	6.0		Phenylenediamine containing

76	*N*-苯基-*p*-苯二胺HCl(CI76086)[2]	N-phenyl-p-phenylenediamine HCl	6.0 (in free base)		Phenylenediamine containing
77	*N*-苯基-*p*-苯二胺硫酸盐[2]	N-phenyl-p-phenylenediamine sulfate	6.0 (in free base)		Phenylenediamine containing
78	*o*-氨基苯酚	o-Aminophenol	2.0	When mixed with milk oxide, the maximum concentration should be 1.0%.	
79	*o*-氨基苯酚硫酸盐	o-Aminophenol sulfate	2.0 (in free base)	When mixed with milk oxide, the maximum concentration should be 1.0%.	
80	*p*-氨基苯酚	p-Aminophenol	1.0	When mixed with milk oxide, the maximum concentration should be 0.5%.	
81	*p*-氨基苯酚硫酸盐	p-Aminophenol sulfate	1.0 (in free base)	When mixed with milk oxide, the maximum concentration should be 0.5%.	
82	苯基甲基吡唑啉酮	Phenyl methyl pyrazolone	0.5	When mixed with milk oxide, the maximum concentration should be 0.25%.	
83	*p*-甲基氨基苯酚	p-Methylaminophenol	3.0	When mixed with milk oxide, the maximum concentration should be 1.5%.	
84	*p*-甲基氨基苯酚硫酸盐	p-Methylaminophenol sulfate	3.0 (in free base)	When mixed with milk oxide, the maximum concentration should be 1.5%.	
85	*p*-苯二胺[2]	p-Phenylenediamine	6.0		Phenylenediamine containing

No.	Name of substance		Maximum permissible concentration in cosmetic products	Other restrictions and requirements	Conditions of use and precautions that must be printed on the label
	Chinese Name	INCI Name			
86	*p*-苯二胺HCl (2)	p-Phenylenediamine HCl	6.0 (in free base)		Phenylenediamine containing
87	*p*-苯二胺硫酸盐 (2)	p-Phenylenediamine sulfate	6.0 (in free base)		Phenylenediamine containing
88	间苯二酚(4)	Resorcinol	5.0		Contains resorcinol
89	苦氨酸钠	Sodium picramate	0.1	When mixed with oxidised milk, the maximum concentration should be 0.05%.	
90	四氨基嘧啶硫酸盐	Tetraaminopyrimidine sulfate	5.0	When mixed with oxidised milk, the maximum concentration should be 2.5%.	
91	甲苯-2,5-二胺 (2)	Toluene-2,5-diamine	10.0		Phenylenediamine containing
92	甲苯-2,5-二胺硫酸盐 (2)	Toluene-2,5-diamine sulfate	10.0 (in free base)		Phenylenediamine containing
93	甲苯-3,4-二胺 (2)	Toluene-3,4-diamine	10.0		Phenylenediamine containing

(1) The following warnings are required on all product labels: may cause allergic reactions in some individuals, skin testing should be carried out according to the instructions; should not be used to colour eyebrows or eyelashes and should be rinsed immediately if accidentally inserted into the eyes; suitable gloves should be worn for professional use.

(2) These substances may be used individually or in combination, the sum of the ratio of the concentration of each ingredient in the cosmetic product to the maximum limit concentration specified in the table must not be greater than 1.

(3) Maximum concentration for use as an ingredient in semi-permanent hair dyes.

(4) These substances may be used individually or in combination, the sum of the ratio of the concentration of each ingredient in the cosmetic product to the maximum limit concentration specified in the table must not be greater than 2.

Part II Methods of Toxicological Test

I. General Provisions

1 Scope

This specification specifies the toxicological testing requirements for the safety evaluation of cosmetic ingredients and their products.

2 Testing of cosmetic ingredients

New raw materials for cosmetic products, generally requiring the following toxicological tests.

（1） Acute oral and acute percutaneous toxicity tests.

（2） Skin and acute eye irritation/corrosion tests.

（3） Skin metaplasia test.

（4） Skin phototoxicity and photosensitivity test* (this test is required for raw materials with UV absorption properties).

（5） Mutagenicity tests (at least one mutation test and one chromosomal aberration test should be included).

（6） Subchronic oral and percutaneous toxicity tests.

（7） Teratogenicity tests.

（8） Chronic toxicity/carcinogenicity combination test.

（9） Toxic metabolism and kinetic tests*.

（10） Depending on the characteristics and use of the raw material, other necessary tests may also be considered.

If the new ingredient has a similar chemical structure and properties to those already used in cosmetics, some tests may be considered for reduction.

*Test methods refer to GB7919-87 Procedures and methods for evaluating the safety of cosmetic products:

OECD Guidelines for Testing of Chemicals.

3 Testing of cosmetic products

3.1 Test items

In general, before a newly developed cosmetic product is placed on the market, tests should be carried out to evaluate its safety, depending on the use and type of product.

3.2 Principles for the selection of test items

3.2.1 Due to the wide range of cosmetic products, the choice of test items should be determined on a case-by-case basis.

3.2.2 Cosmetics used daily are subjected to multiple skin irritation tests, those subjected to multiple skin

irritation tests are no longer subjected to acute skin irritation tests, those used several days apart and those rinsed after use are subjected to acute skin irritation tests.

3.2.3 Acute eye irritation tests are not required for products with low potential for eye contact.

II. Acute oral toxicity test

1 Scope

This specification sets out the basic principles, requirements and methods for acute oral toxicity testing in animals. This specification applies to the toxicological testing of cosmetic raw material safety.

2 Normative references

OECD Guidelines for Testing of Chemicals（No. 401, Feb. 1987） USEPA OPPTS Harmonized Test Guidelines（Series 870.1100, Aug. 1998）

3 Purpose of the test

The acute oral toxicity test is the first step in assessing the toxicity properties of cosmetic ingredients and provides information on health hazards through short oral exposure. The results of the test can be used as a basis for classification and labelling of cosmetic ingredients and for determining doses for sub-chronic toxicity tests and other toxicological tests.

4 Definition

4.1 Acute oral toxicity: The short-term detrimental effect on the health of an animal following a single oral administration or multiple oral administrations of a test substance over a 24h period.

4.2 Oral LD50 (Medium lethal dose): The statistical dose of a toxicant that causes the death of half of the total number of experimental animals after a single oral administration of the test substance. It is expressed as the weight per unit body weight of the test substance received (mg/kg or g/kg).

5 Basic principles of the test

The animals in each test group are given different doses of the test substance orally by tube feeding, one dose per group, the choice of the dose to be contaminated can be determined by pre-testing. The animals are observed for toxicity and mortality. Animals that die during the test are subjected to post-mortem examination and those that are still alive at the end of the test are executed and subjected to post-mortem examination. This method is primarily applicable to rodent studies, but can also be used for non-rodent studies.

6 Test method

6.1 Subjects

The test substance should be dissolved or suspended in a suitable medium, water is recommended as a first choice, followed by vegetable oil (e.g. corn oil) or other media (e.g. carboxymethyl cellulose, gelatine, starch, etc.) should be considered. For non-water soluble media, the toxicological properties should be known, otherwise the toxicity should be determined prior to testing. The maximum volume of liquid to be administered orally at any one time depends on the size of the animal, and is generally 1mL/100g for

rodents, up to 2mL/100g for aqueous solutions.

6.2 Laboratory animals and housing environment

Healthy adult rats and mice are preferred, but other sensitive animals may also be used. The female animals used should be non-pregnant and have not given birth. The difference in body weight between animals should not exceed 20% of the average body weight. The animals should be acclimatised in the animal house environment for at least 3-5 d prior to testing.

Laboratory animals and laboratory animal houses should comply with the corresponding national regulations. Conventional feed is selected and water is not restricted.

6.3 Dose level

Depending on the requirements of the chosen method, there should in principle be 4 to 6 dose groups of generally 10 animals each, 50/50 male and female. The spacing of the dose groups should be such as to produce a balance between toxicity and mortality, usually with a larger group spacing and a smaller number of animals for pre-testing. If the toxicity of the test substance is very low, a single limit method can be used, i.e. 10 animals (half male and half female) given an oral dose of 5000 mg/kg bw, and when no mortality is caused, multiple doses of acute oral toxicity tests can be considered.

6.4 Test procedure

6.4.1 Prior to the test, experimental animals are fasted overnight with no restriction on water intake. If other animals with high metabolic rates are used, the fasting period can be shortened.

6.4.2 For formal testing, animals are weighed and randomly grouped, then each group is given a single dose by tube feeding, or if the toxicity is estimated to be low and a single dose is too large, the animals may be given two to three doses over a 24h period, but combined as one dose. If multiple doses are administered in batches, the animals may be given a certain amount of food and water if necessary, depending on the length of the dose interval.

6.4.3 Each animal should be individually and thoroughly recorded after poisoning, with regular observation of signs of poisoning and death during the first day of poisoning, followed by a careful examination at least once a day. Keep detailed records of changes in coat and skin, eyes and mucous membranes, respiration, circulation, autonomic and central nervous system, limb movements and behaviour. Pay particular attention to the presence of tremors, convulsions, salivation, diarrhoea, lethargy and coma. The time of appearance and disappearance of signs of toxic effects and the time of death should be recorded.

6.4.4 The duration of observation is usually no more than 14 d, but is not constant and depends on the severity of the reaction, the speed of onset of symptoms and the length of the recovery period. If there are signs of delayed death, the observation period may be extended.

Surviving animals should be weighed weekly during the observation period and at the end of the observation period surviving animals should be weighed and executed for post-mortem examination.

6.4.5 Gross anatomical examination of the animals was carried out and all gross pathological changes were recorded. The animals were examined for death and survival for 24h and Orgaos that are more than 24h old and have gross pathological changes should be subjected to pathological histological examination.

6.4.6 A variety of methods can be used to determine $LD_{50;}$ Horn's method, the top-down method, the probability unit-log plot method and the Kou's method are recommended.

6.5 Evaluation of test results

When evaluating the results of a test, the $LD_{50\ should\ be}$ considered in conjunction with the observed toxic effects and the post-mortem findings. The LD_{50} value is the basis for classification and labelling of the toxicity of the test substance and for determining the likelihood of death in animals following ingestion through the gastrointestinal tract. The $LD_{50\ value\ should}$ always be quoted with reference to the species, sex, route of exposure and duration of observation of the experimental animal used. The evaluation should include the relationship between exposure and the incidence and severity of abnormalities (including behavioural and clinical changes, gross lesions, weight changes, lethal effects and other toxic effects) in the animal.

See Table 1 for toxicity classification.

7 Test reports

The test report shall include the following.

（1） Name of the test substance, physicochemical properties, method of preparation, concentration used.

（2） The species, strain and origin of the experimental animal (indicating the certificate of conformity number and animal class).

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal house certificate number.

（4） The dose and grouping of animals used, the sex, number and weight range of animals used in each group.

（5） Manifestations of poisoning and death of the animal after contamination and the time of appearance, gross anatomical and pathological findings.

（6） The method for calculating LD_{50}.

（7） Tabulate the results and report the calculated LD_{50} with its 95% confidence interval (see Table 2 for suggested tabular format).

（8） Conclusion.

8 Interpretation of test results

The toxicity of the subjects can be evaluated by acute oral toxicity tests and LD_{50} measurements. The results are of limited validity for extrapolation to humans.

Table 1 Oral toxicity classification

LD50 (mg/kg)	Toxicity classification
≤ 50	Highly toxic
＞ 50-500	Moderately toxic
＞ 500 to 5000	Low toxicity
＞ 5000	Practically non-toxic

Table 2 Results of the acute oral toxicity test in mice

Animal sex	Dose (mg/kg)	Number of Animals	Body weight (x±SD) (g)			Number of animals killed	Mortality (%)
			0 day	7 days	14 days		
LD50 and 95% confidence interval.							
Males:							
Females:							

III. Acute percutaneous toxicity test

1 Scope

This specification sets out the basic principles, requirements and methods for acute dermal toxicity testing in animals. This specification applies to the toxicological testing of cosmetic raw material safety.

2 Normative references

OECD Guidelines for Testing of Chemicals（No.402，Feb. 1987）

USEPA OPPTS Harmonized Test Guidelines (Series 870.1200, Aug. 1998)

3 Purpose of the test

The acute dermal toxicity test determines whether the test substance can be absorbed through the skin and the short-term effects of toxic reactions, which can provide a basis for the classification and labelling of cosmetic ingredients and for determining the dose of sub-chronic toxicity tests and other toxicological tests.

4 Definition

4.1 Acute dermal toxicity: The effect of health damage in animals within a short period of time following a single transdermal application of the test substance.

4.2 Transdermal $_{LD50}$ (Medium lethal dose): The statistical dose of a toxicant that causes half of the total mortality of an experimental animal after a single transdermal application of the test substance. Expressed as mg/kg or g/kg per unit body weight of test substance applied.

5 Basic principles of the test

The subjects were given transdermally at different doses to each group of experimental animals, with one dose used for each group. Animals are observed for toxic reactions and mortality after contamination. Animals that die during the test are subjected to post-mortem examination and those that are still alive at the end of the test are executed and subjected to post-mortem examination. Acute percutaneous toxicity tests may not be performed if the test substance is known to be corrosive or highly irritating.

6 Test method

6.1 Subjects

Liquid subjects do not normally require dilution. If the test substance is solid, it should be ground to a fine powder and mixed with an appropriate amount of water or a medium that is non-toxic, non-irritating, does not interfere with the penetration of the test substance into the skin and does not react with the test substance to ensure good contact between the test substance and the skin. Commonly used media are olive oil, lanolin, petroleum jelly, etc.

6.2 Laboratory animals and housing environment

Healthy adult rats, rabbits or guinea pigs may be used as experimental animals, but other species of animals may also be used for testing. Female animals should be non-pregnant and have not given birth. The recommended weight range is 200g-300g for rats, 2kg-3kg for rabbits and 350g-450g for guinea pigs, and the skin should be healthy and unbroken. The animals should be acclimatised in the animal house environment for at least 3-5 days prior to testing.

Laboratory animals and laboratory animal houses should comply with the corresponding national regulations. Conventional feed is selected and water is not restricted.

6.3 Dose level

Depending on the requirements of the method chosen, there should in principle be 4 to 6 dose groups of generally 10 animals each, 50/50 male and female. The spacing of the dose groups should be such as to produce a balance between toxicity and mortality, usually with a larger group spacing and a smaller number of animals for pre-testing. If the toxicity of the test substance is very low, a single limit of 10 animals (half male and half female) may be used, i.e. 2000 mg/kg applied to the skin.

Body weight doses, when they do not cause animal mortality, may be considered without further acute percutaneous toxicity tests at multiple doses.

6.4 Test procedure

6.4.1 24h before the start of the test, cut or shave the hair on the back of the animal's trunk in the area to be poisoned. The area to be skinned should be approximately 10% of the animal's body surface area and should be determined according to the animal's body weight. For rats weighing 200g to 300g, the area should be approximately 30cm2 to 40cm2, for rabbits weighing 2kg to 3kg, 160cm2 to 210cm2 and for guinea pigs weighing 350g to 450g, 46cm2 to 54cm2.

6.4.2 The test substance is applied evenly to the dorsal skin of the animal and then covered with a thin layer of film, secured with non-irritating tape to prevent licking by the animal. If the test substance is highly toxic, the area of application may be reduced, but the application should be as thin and uniform as possible. Exposure is normally closed for 24h.

6.4.3 At the end of the contamination, the residual test substance should be removed using water or other suitable solution.

6.4.4 The period of observation should normally not exceed 14 d, but will depend on the severity of the animal's reaction, the speed of onset of symptoms and the length of the recovery period. If there are signs of delayed mortality, longer observation periods may be considered.

6.4.5 Each animal should be individually and thoroughly documented, with regular observation of signs of intoxication and death during the first day of poisoning, followed by a careful examination at least once a day. This should include changes in coat and skin, eyes and mucous membranes, as well as respiratory, circulatory, autonomic and central nervous system, limb movements and behavioural activity. Pay particular attention to the presence of tremors, convulsions, salivation, diarrhoea, lethargy, and coma. The time of death should be recorded as accurately as possible.

Surviving animals should be weighed weekly during the observation period and at the end of the observation period, and post-mortem examination should be carried out after execution.

6.4.6 Gross anatomical examination of the animals was carried out and all gross pathological changes were recorded. The animals were examined for death and survival for 24h and organs that are more than 24h old and have gross pathological changes should be subjected to pathological histological examination.

6.4.7 A variety of methods can be used to determine $LD_{50;}$ Horn's method, the top-down method, the

probability unit-log plot method and the Kou's method are recommended.

6.5 Evaluation of test results

When evaluating test results, the transdermal $LD50$ should be considered in conjunction with the observed toxic effects and post-mortem findings. The $LD50$ value is the basis for classification and labelling of the toxicity of the test substance and for determining the likelihood of death in animals following dermal absorption. The $LD50$ value should always be quoted with reference to the species, sex, route of exposure and duration of observation of the experimental animal used. The evaluation should include the relationship between exposure and the incidence and severity of abnormalities (including behavioural and clinical changes, gross lesions, weight changes, lethal effects and other toxic effects) in the animal.

See Table 1 for toxicity classification.

Table 1 Dermal toxicity classification

LD50 (mg/kg)	Toxicity classification
< 5	Extremly Highly toxic
5 to 44	Highly toxic
44 to 350	Moderately toxic
350 to 2180	Low toxic
＞ 2180	Slightly toxic

7 Test reports

The test report shall include the following.

（1） Name of the test substance, physicochemical properties, method of preparation, concentration used.

（2） The species, strain and origin of the experimental animal (indicating the certificate of conformity number and animal class).

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal house certificate number.

（4） The dose and grouping of animals used, the sex, number and weight range of animals used in each group.

（5） Manifestations of poisoning and death of the animal after contamination and the time of appearance, gross anatomical and pathological findings.

（6） The method for calculating LD50.

（7） Tabular reporting of results and calculation of $_{LD50}$ and its 95% confidence interval (see Table 2 for suggested tabular format).

（8） Conclusion.

Table 2 Results of the acute oral toxicity test in mice

Animal sex	Dose (mg/kg)	Number of Animals	Body weight (x±SD) (g)			Number of animals killed	Mortality (%)
			0 day	7 days	14 days		
LD50 and 95% confidence interval.							
Males:							
Females:							

8 Interpretation of test results

Acute percutaneous toxicity test studies and percutaneous $_{LD50}$ determinations provide information on the toxicity of the test substance percutaneously. The results have limited validity for extrapolation to humans. The results of acute percutaneous toxicity tests should be evaluated in conjunction with the results of acute toxicity tests for other routes of exposure.

IV. Skin irritation/corrosion test

Dermal Irritation/Corrosion Test

1 Scope

This specification specifies the basic principles, requirements and methods for testing skin irritation or corrosiveness in animals. This specification applies to toxicological tests for the safety of cosmetic raw materials and their products.

2 Normative references

OECD Guidelines for Testing of Chemicals（No.404，April 2002）

USEPA OPPTS Harmonized Test Guidelines (Series 870. 2500, Aug. 1998)

3 Purpose of the test

To determine and evaluate whether and to what extent cosmetic ingredients and their products have a local irritant or corrosive effect on mammalian skin.

4 Definition

4.1 Dermal irritation: Local reversible inflammatory changes following the application of a test substance to the skin.

4.2 Dermal corrosion: Irreversible tissue damage caused locally by the application of a test substance to the skin.

5 Basic principles of the test

The test substance is applied once (or more) to the skin of the test animal and the degree of local irritation of the animal's skin is observed and scored at defined time intervals. Self-control is used to evaluate the irritating effect of the test substance on the skin. The duration of observation of the acute skin irritation test should be sufficient to evaluate the reversibility or irreversibility of the effect.

Animals should be humanely executed if they show signs of severe depression and distress at any stage of the test. Subjects should be evaluated appropriately in the light of the test.

6 Test method

6.1 Subjects

Liquid subjects generally do not require dilution and can be used directly as a stock solution. If the test substance is solid, it should be ground to a fine powder and well moistened with water or other non-irritating solvent to ensure good contact with the skin. If other solvents are used, the effect of the solvent on skin irritation should be taken into account. For products to be used diluted, a skin irritation/corrosion test of

the prototype should be carried out first and if the test results indicate moderate irritation or more, a further skin irritation/corrosion test may be carried out on the subject at the concentration used.

If the test substance is a strong acid or base (pH ≤ 2 or ≥ 11.5), the skin irritation test may not be performed. In addition, acute skin irritation tests are not required if the test substance is known to be highly toxic by percutaneous absorption, if the percutaneous $_{LD50\ is}$ less than 200 mg/kg bw or if no skin irritation occurs at a dose of 2000 mg/kg bw in an acute percutaneous toxicity test.

6.2 Laboratory animals and housing environment

A wide range of mammals can be selected as experimental animals, with white rabbits being preferred. Adult, healthy animals with undamaged skin should be used, both females and males, but females should be non-pregnant and have not given birth. A minimum of four animals should be used, with additional animals required to clarify certain suspected reactions. Animals should be housed in a single cage and acclimatised in a laboratory environment for at least 3 days prior to testing.

Laboratory animals and laboratory animal houses should comply with the corresponding national regulations. Conventional feed is selected and water is not restricted.

6.3 Acute skin irritation test procedure

6.3.1 About 24 h before the test, the hair on both sides of the back of the animal was cut off without damaging the epidermis, to the left and right of the animal.

6.3.2 Approximately 0.5mL (g) of the test substance is applied directly to the skin and then covered with two layers of gauze (2.5cm x 2.5cm) and a layer of cellophane or similar, and secured with non-irritating tape and bandages. The other side of the skin was used as a control. For cosmetic products, the duration of application can be extended or shortened depending on the actual use and type of product. For cosmetic products that are rinsed after use, only the 2h application test is used. At the end of the test the residue is removed with warm water or a non-irritating solvent.

If it is suspected that the test substance may cause serious irritation or corrosion, the test can be carried out in stages by applying three pieces of gauze coated with the test substance to the skin of a rabbit at the same time or in sequence, and removing one piece of gauze at 3min, 60min and 4h after application.

6.3.3 Skin reactions were observed at 1, 24, 48 and 72 h after removal of the test substance, and the skin reactions were scored according to Table 1.

6.3.4 The duration of observation should be sufficient to observe the full course of reversible or irreversible stimulus action, usually no more than 14 d.

6.4 Multiple skin irritation test procedure

6.4.1 Before the test, the hair on both sides of the spine was cut off and the area of hair removal was 3cm x 3cm each, and the area of application was 2.5cm x 2.5cm.

6.4.2 Approximately 0.5mL (g) of the test substance is applied to one side of the skin, and when the test substance is prepared with a non-irritating solvent, the other side is applied as a control, once daily for 14 d. From the second day onwards, the hair should be cut before each application and the residual test substance removed with water or a non-irritating solvent. Observe the results after one hour and score according to Table 1, treating the control and test areas equally.

6.4.3 Evaluation of results: The mean score per animal per day was calculated according to the following formula to determine the intensity of skin irritation using Table 2.

$$\text{Average points per animal per day} = \frac{\text{Erythema and oedema points}}{14}$$

Table 1 Skin irritation response scores

Skin Reaction	Points
Erythema and scab formation	
No erythema	0
Slight erythema (barely visible)	1
Visible erythema	2
Moderate to severe erythema	3
Severe erythema (purplish red) to slight scab formation	4
Edema formation	
No edema	0
Slight oedema (barely visible)	1

Mild oedema (well-defined skin elevation)	2
Moderate oedema (skin elevation of about 1mm)	3
Severe oedema (skin bulge of more than 1mm, widening)	4
Maximum points	8

7 Test reports

The test report should be summarised in tabular form and include the following.

（1） Name of test substance, physical and chemical properties, method of preparation and dosage. If necessary, the pH of the test substance.

（2） The species, strain, sex, weight and origin of the experimental animal (indicating the certificate of conformity number and animal class).

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal house certificate number.

（4） The skin irritation response score for each animal at each observation time point.

（5） A specific description of toxic effects other than irritation.

（6） Conclusion.

8 Interpretation of test results

Acute skin irritation test results have limited reliability for extrapolation from animals to humans. White rabbits are in most cases more sensitive to irritating or corrosive substances than humans. The reliability of extrapolation from animals to humans would be increased if similar results were obtained when testing with other strains of animals. The use of closed exposure in the tests is an extraordinary laboratory condition, which is rarely present in the actual use of cosmetics in humans.

Table 2 Classification of skin irritation intensity

Average points value	Strength
0~<0.5	Non-irritating
0.5~<2.0	Lightly irritating
2.0~<6.0	Medium irritant
6.0~8.0	Strong irritant

Table 3 Results of the ××× test for acute skin irritation in rabbits

Serial Number of Animals	Sex	Weight	1h						24h						48h						72h					
			Sample			Control Group			Sample			Control Group			Sample			Control Group			Sample			Control Group		
			erythema	edema	Total score	erythema	edema	Total score	erythema	edema	Total score	erythema	edema	Total score	erythema	edema	Total score	erythema	edema	Total score	erythema	edema	Total score	erythema	edema	Total score
1																										
2																										
3																										
4																										
Average of total score																										

Average stimulus intensity

Table 4 Results of multiple skin irritation tests on rabbits

Number of days	Number of animals	Score of reaction to stimulus					
		Sample			Control Group		
		erythema	edema	Total score	erythema	edema	Total score
1.	4						
2.	4						
3.	4						
4.	4						
5.	4						
6.	4						
7.	4						
8.	4						
9.	4						
10.	4						
11.	4						
12.	4						
13.	4						
14.	4						
Average points per animal in 14 days							
Average points per animal each day							

V. Acute eye irritation/corrosion test

1 Scope

This specification specifies the basic principles, requirements and methods for acute eye irritation or corrosiveness tests in animals. This specification applies to toxicological tests for the safety of cosmetic raw materials and their products.

2 Normative references

OECD Guidelines for Testing of Chemicals（No 405，April 2002）

USEPA OPPTS Harmonized Test Guidelines (Series 870.2400, Aug. 1998)

3 Purpose of the test

To determine and evaluate whether and to what extent cosmetic ingredients and their products have an irritating or corrosive effect on the eyes of mammals.

4 Definition

4.1 Eye irritation: A reversible inflammatory change in the surface of the eye following contact with a test substance.

4.2 Eye corrosion: Irreversible tissue damage caused by contact with a test substance on the surface of the eye.

5 Basic principles of the test

A single dose of the test substance is placed in the conjunctival sac of one eye of each animal and the untreated eye is used as its own control. The irritating effect of the test substance on the eyes of the animals is evaluated by observing and scoring the extent of the irritating and corrosive effect on the eyes of the animals at specified time intervals. The observation period should be sufficient to evaluate the reversibility or irreversibility of the irritant effect.

Animals that show signs of severe depression and distress at any stage of the test should be humanely put to death and the subject evaluated appropriately in the light of the test. Animals that show corneal perforation, corneal ulceration, corneal 4 points for more than 48h, lack of light reflex for more than 72h, conjunctival ulceration, gangrene and decay, which are usually signs of irreversible damage, should also be humanely executed.

6 Test method

6.1 Subjects

The liquid test substance is normally used without dilution and can be used directly as a stock solution in an amount of 0.1 mL. If the test substance is solid or granular, it should be ground to a fine powder in an

amount of 0.1 mL by volume or not more than 100 mg by weight (the amount of toxicity should be recorded).

The eye irritation test may be dispensed with if the test substance is a strong acid or base (pH ≤ 2 or ≥ 11.5) or if it has been shown to be corrosive or highly irritating to the skin.

The aerosol product needs to be sprayed into a container and its liquid collected before use.

6.2 Laboratory animals and housing environment

Healthy adult white rabbits are preferred. A minimum of 3 rabbits should be used. The animals will be acclimatised in the experimental animal house environment for at least 3 d before the test. Both eyes of the animals should be examined (including with sodium fluorescein) within 24h prior to the start of the test. Animals with signs of eye irritation, corneal defects and conjunctival damage should not be used for the test.

Laboratory animals and laboratory animal houses should comply with the corresponding national regulations. Conventional feed is selected and water is not restricted.

6.3 Test procedure

6.3.1 The lower eyelid of one eye of the rabbit is gently pulled open and 0.1 mL (100 mg) of the test substance is dropped (or applied) into the conjunctival sac, allowing the upper and lower eyelids to close passively for 1 s to prevent loss of the test substance. The other eye was left untreated as its own control. The eye is not flushed for 24h after the drop is applied. If deemed necessary, irrigation may be performed at 24h.

6.3.2 If the results of the above test indicate that the test substance is irritating, three additional rabbits should be used for the flushing effect test, i.e. 30 s after the test substance has been administered to the rabbit's eye, a sufficient amount of water should be flushed for at least 30 s with a fast flow of water that does not cause damage to the animal's eye.

6.3.3 Clinical examination and scoring: The eyes of the animals are examined at 1, 24, 48, 72h and at 4d and 7d after the drop has been administered. If no irritation occurs at 72h, the test is terminated. If corneal involvement or other ocular irritation is found and does not recover within 7d, the observation period should be extended to determine the reversibility or irreversibility of the damage, usually for no more than 21d, and a report of the observations made at 7d, 14d and 21d should be provided. All effects of damage should be recorded and reported, except for observation of the cornea, iris and conjunctiva. The score of the ocular irritation response should be recorded at each examination according to the scoring scale for ocular damage in Table 1.

Eye irritation responses can be examined using a magnifying glass, hand-held slit lamp, biomicroscope or other applicable instrumentation. After 24h of observation and recording, the eyes of all animals are further examined by applying sodium fluorescein.

6.3.4 For post-rinsing products (e.g. facial cleansers, hair products, hair care rinses, etc.), the 30-s rinse test is performed only, i.e. the eye is closed for 1 s after the drop has been applied to the subject, and then rinsed for 30 s at the 30th s with a sufficient amount of water flowing at a fast rate that does not cause damage to the animal's eye, and then examined and scored according to 6.3.3.

6.3.5 For hair dye products, only a 4s rinse test is performed, i.e. the eye is closed for 1s after the drop has been applied and then rinsed for 30s at 4s with a sufficient amount of water flowing fast enough not to cause damage to the animal's eye, and then examined and scored according to 6.3.3.

Table 1 Scoring criteria for eye damage

Eye damage	Points
Cornea: cloudy (whichever is the densest part)	
No ulcer formation or cloudiness	0
Diffuse or diffuse clouding with a clearly visible iris	1
Translucent areas easily distinguished, iris blurred	2
Appearance of greyish translucent areas with poor iris detail and barely visible pupil size	3
Cloudy corneas and unrecognisable irises	4
Iris: normal	0
marked deepening of the folds, congestion, swelling, moderate pericorneal congestion, pupil to Light still responsive	1
Bleeding, visible destruction to the naked eye, no reaction to light (or one of these reactions occurs)	2

Conjunctiva: congested (refers to lid conjunctiva, bulbar conjunctiva area)	
Normal blood vessels	0
Blood vessels are congested and bright red	1
Vascular congestion of a deep red colour and diffuse congestion of a purplish colour with vessels not easily distinguishable	2
Edema	3
None	
Slight oedema (including transepithelium)	0
Significant oedema with partial eyelid ectropion oedema to near half-closed eyelids	1
Edema to eyelid mostly closed	2
	3
	4

7 Evaluation of results

Cosmetic ingredients - The intensity of irritation to the eye was assessed by the mean value of the irritation response score and the recovery time at the 24, 48 and 72 h observation points for the cornea, iris or conjunctiva of the animal after administration of the test substance, and was graded according to Table 2.

Table 2 Grading of eye irritation reactions

Reversible eye damage	Class 2A (mildly irritating)	Mean stimulus response scores for 2/3 animals: corneal clouding ≥ 1; iris ≥ 1; conjunctival congestion ≥ 2; conjunctival oedema ≥ 2 and full recovery of the above stimulus response scores at ≤ 7 days Repeat
	Level 2B (irritating)	Mean stimulus response scores for 2/3 animals: corneal clouding ≥ 1; iris ≥ 1; conjunctival congestion ≥ 2; conjunctival oedema ≥ 2 and full recovery of the above stimulus response scores at <21 days Repeat
Irreversible eye damage		(i) the corneal, iris and/or conjunctival irritation points of either animal did not fully recover during the 21-day observation period. ②Mean stimulus response score for 2/3 animals: corneal clouding ≥3 and/or iris >1.5

Cosmetic products - The intensity of irritation to the eye of the test substance was assessed by the maximum integral and recovery time of the irritation response of the animal's cornea, iris or conjunctiva at the 24, 48 or 72h observation time point after administration of the test substance, as determined by the eye irritation response classification in Table 3.

Table 3 Grading of eye irritation reactions

Reversible eye damage	Slightly irritating	Animal's corneal and iris score = 0; conjunctival congestion and/or conjunctival oedema score ≤ 2 and score falls to 0 within <7 days
	Light irritation	Corneal, iris and conjunctival scores of animals fall to 0 in ≤7 days
	Irritability	Corneal, iris and conjunctival integrals of animals drop to 0 within 8-21 days
Irreversible eye damage	Corrosive	(i) The animal's corneal, iris and/or conjunctival score is >0 at day 21. (②2/3 animals with eye irritation response score: corneal clouding ≥3 and/or iris = 2

Note: When the corneal, iris and conjunctival scores are 0, it is judged to be non-irritating.

8 Test reports

The test report shall include the following.

（1） The name of the test substance, its physicochemical properties, method of preparation and dosage, and, if necessary, the pH value of the test substance.

（2） The species, strain and origin of the experimental animal (indicating the certificate of conformity

number and animal class).

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal house certificate number.

（4） Tabulate the stimulus response for each animal at each observation time point (e.g. 1, 24, 48 and 72h) (see Table 4 for a suggested tabular format), tabulating the results of the experimental conditions without and 30-second rinses or 4-second rinses, respectively.

（5） A specific description of roles other than eye.

（6） Describe the method of examination (e.g. hand-held slit lamp, with sodium fluorescein) when integrated at each observation time point.

（7） Conclusion.

Table 4 XXX results of rabbit eye irritation test results

Experimental conditions: don't wash

30 (or 4) seconds

No. Of animalss	section	Eye irritation response score											
		1h		24h		48h		72h		4d		7d	
		Sample	Control group	Sample	Control group	Sample	Control group	Sample	Control group	Sample	Control group	Sample	Control group
1	conjunctiva												
	iris												
	cornea												
2	conjunctiva												
	iris												
	cornea												
3	conjunctiva												
	iris												
	cornea												
Level of stimulus													

9 Interpretation of test results

There is limited reliability in extrapolating the results of the acute eye irritation test from animals to humans. White rabbits are in most cases more sensitive to irritating or corrosive substances than humans. The reliability of extrapolation from animals to humans would be increased if similar results were obtained when testing with other strains of animals.

VI. Skin metaplasia test

Skin Sensitisation Test

1 Scope

This specification specifies the basic principles, requirements and methods for skin metabolic tests on animals. This specification applies to toxicological tests for the safety of cosmetic raw materials and their products.

2 Normative references

OECD Guidelines for Testing of Chemicals（No 406，July 1992）

USEPA OPPTS Harmonized Test Guidelines (Series 870.2600, Aug. 1998)

3 Purpose of the test

To determine whether and to what extent repeated exposure to cosmetics and their raw materials can cause metabolic reactions in mammals.

4 Definition

4.1 Skin sensitization (allergic contact dermatitis)

It is an immunogenic cutaneous reaction of the skin to a substance. In humans this reaction may be characterised by pruritus, erythema, papules, blisters and fused blisters. In animals the reaction is different and may be seen only as erythema and oedema of the skin.

4.2 Induction exposure

Refers to an experimental exposure in which the body induces an allergic state through exposure to a test substance.

4.3 Induction period

The time required for the body to induce an allergic state through exposure to the test substance, usually at least one week.

4.4 Challenge exposure

The organism receives an induction exposure followed by a test exposure to the test substance again to determine whether an allergic reaction to the skin will occur.

5 Basic principles of the test

The animals were dermatologically applied (induction of exposure) or injected intradermally for 10 to 14 d (induction phase), then given a stimulated dose of the test substance and observed and compared with control animals for the intensity of the dermal response to the stimulated exposure.

5.1 Laboratory animals and housing environment

Healthy, adult male or female guinea pigs are generally used, and females should be used if they are not pregnant or have not given birth.

The experimental animals and the experimental animal house should comply with the corresponding national regulations. The animals should be fed a conventional diet with unrestricted water intake and should be provided with appropriate amounts of Vc.

5.2 Preparation for animal testing

The animals should be acclimatised in the experimental animal house environment for at least 3 d to 5 d before the test. The animals should be randomly divided into subject and control groups and the skin should be prepared (debrided) in the appropriate area according to the test method chosen to avoid damage to the skin. Animal weights should be recorded at the beginning and end of the test.

5.3 Complete observation of the animal, including systemic and local responses, should be carried out during both the induction and stimulation phases and fully documented.

5.4 Checking the reliability of test methods

Check every 6 months using a positive known to cause mild/moderate sensitisation. Topical closed skin coating method with at least

Allergic skin reactions occur in 30% of animals; at least 60% of animals have skin reactions by intradermal injection. Positives are generally taken

Use 2,4-dinitrochlorobenzene, cinnamaldehyde, 2-mercaptobenzothiazole or ethyl p-aminobenzoate.

6 Test method

6.1 Buehler Test (BT) for local closure skin coating

6.1.1 Number of animals

At least 20 in the test group and 10 in the control group.

6.1.2 Dose level

The concentration of induced exposure is the highest concentration that causes a mild skin irritation and the concentration of stimulated exposure is the highest concentration that does not cause a skin irritation. The test concentration level can be obtained by pre-testing a small number of animals (2 to 3).

Water soluble subjects can be excipients with water or non-irritating surfactants, other subjects can be excipients with 80% ethanol

(induced exposure) or acetone (excited exposure) as excipient.

6.1.3 Test procedure

6.1.3.1 About 24h before the test, the left side of the guinea pig's back was deboned to the extent of 4cm2~6cm2.

6.1.3.2 Induction of exposure: approximately 0.2 mL (g) of the test substance was applied to the skin of the de-haired area of the experimental animal, covered with two layers of gauze and one layer of cellophane, and then closed and fixed with non-irritating adhesive tape for 6 h. The procedure was repeated once on d 7 and once on d 14 in the same way.

6.1.3.3 Stimulated exposure: 14d-28d after the last induction, approximately 0.2mL of the test substance was applied to the right side of the guinea pig's back 2cm x 2cm

The debrided area (24h hair removal before contact) is then covered with two layers of gauze and a layer of cellophane and then fixed with non-irritating tape for 6h.

6.1.3.4 Skin reactions were observed at 24h and 48h after stimulated exposure and scored according to Table 1.

6.1.3.5 A negative control group is required for the test, using methods 6.1.3.2 and 6.1.3.3, with only the solvent applied as a control at the time of induction of exposure and the test substance at the time of provocation of exposure. The animals in the control group must be the same as those in the subject group. A positive control group is required at the beginning of a laboratory test for metabolic reactions or when a new animal species or strain is used.

Table 1 Skin reaction scores for allergic reaction tests

Skin Reaction	Points
Erythema and scab formation	
No erythema	0
Slight erythema (barely visible)	1
Visible erythema (scattered or small patches of erythema)	2
Moderate to severe erythema	3
Severe erythema (purplish red) to slight scab formation	4

Edema formation	
No edema	0
Slight oedema (barely visible)	1
Moderate oedema (well-defined skin elevation)	2
Severe oedema (skin elevation of about 1mm or more)	3
Maximum points	7

6.1.4 Evaluation of results

6.1.4.1 When a skin reaction score of ≥2 is observed in the test group, the animal is considered to have a positive skin reaction and the sensitizing strength of the test is determined according to Table 3.

6.1.4.2 If the results of the excitation exposure are still inconclusive, a second excitation should be given one week after the first excitation and the control groups were treated synchronously or evaluated according to 6.2.

6.2 Guinea Pig Maximinatim Test (GPMT)

The possibility of sensitisation was tested by intradermal injection of Freund Complete Adjvant (FCA)

6.2.1 Number of animals

A minimum of 10 animals should be used for the test group and a minimum of 5 for the control group. If the test results make it difficult to determine the sensitisation of the test substance, the number of animals should be increased to 20 in the test group and 10 in the control group.

6.2.2 Dose level

The concentration of induced exposure is the highest concentration that causes a mild skin irritation and the concentration of stimulated exposure is the highest concentration that does not cause a skin irritation. The test concentration level can be obtained by pre-testing a small number of animals (2 to 3).

6.2.3 Test procedure

6.2.3.1 Induced exposure (d 0)

Subjects: Three symmetrical points were designated on either side of the midline of the de-haired area on the back of the neck (2cm x 4cm) and each point was injected intradermally with 0.1mL of the solution described below.

Point 1 1:1 (v/v) mixture of FCA/water or saline. Point 2 Tolerated concentration of the subject.

Point 3 Subjects prepared with 1:1 (v/v) FCA/water or saline at the same concentration as point 2. Control group: same injection site as the test substance group.

Point 1 1:1 (v/v) mixture of FCA/water or saline. Point 2 Undiluted solvent.

Point 3 A solvent at a concentration of 50% (w/v) prepared with 1:1 (v/v) FCA/water or saline.

6.2.3.2 Induced exposure (d7).

A 2cm x 4cm filter paper coated with 0.5g (mL) of the subject was applied to the injection site for the above re-depilation, then covered with two layers of gauze and one layer of cellophane and fixed with non-irritating tape for 48 h. For subjects without skin irritation, sensitisation was added and 10% sodium dodecyl sulphate (SLS) 0.5mL was applied to the injection site 24h before the second induction exposure. control group Induction treatment with solvent only.

6.2.3.3 Excitation of exposure (21st d)

The guinea pig carcass is debrided and a 2 cm x 2 cm filter paper sheet coated with 0.5 g (mL) of the

test substance is applied to the debrided area, then covered with two layers of gauze and a layer of cellophane and fixed in place with non-stimulating tape for 24 h. The control animals are treated similarly. If the results of the stimulated contact are uncertain, a second stimulated contact can be made one week after the first stimulated contact. The control group is treated simultaneously.

6.2.4 Observation and evaluation of results

At the end of the stimulated exposure, 24, 48 and 72 h after removal of the filter paper coated with the test substance, the skin reaction should be observed (if it is necessary to remove the test residue, use water or a solvent that does not alter the existing skin reaction and does not damage the skin) and scored according to Table 2. When the skin reaction score is ≥1, the animals in the test group should be classified as positive for allergic reactions and the test should be graded for sensitization intensity according to Table 3.

Table 2 Skin reaction score for allergic reaction test

Rating	Skin reactions
0	No skin reaction seen
1	Scattered or small patches of erythema
2	Moderate erythema and fused erythema
3	Severe erythema and oedema

7 Test reports

The report should include the following.

（1） Name of the test substance, physicochemical properties, method of preparation, concentration used.

（2） The species, strain, source (indicating the certificate number and class of animal), sex and number of laboratory animals.

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal house certificate number.

（4） Test methods.

（5） Animal weights at the beginning and end of the test.

（6） Results: Report skin reactions and sensitisation rates etc. for each group of animals in tabular form (see Table 4 for suggested tabular form)

(and Table 5).

（7） Conclusion.

Table 3 Allergenic intensity

Sensitization rate (%)		Sensitising intensity
0 to	8	weak
9~ 28		Light
29~ 64		Medium

65~ 80	Strong
81~100	Extremely strong

Note: When the sensitization rate is 0, no skin metaplasia is seen.

Positive control test date.

Note: The number of animals that reacted as a proportion of the number of animals tested when the skin reaction score was 0, 1, 2, 3... should be entered in the skin reaction intensity column

8 Interpretation of test results

The results of the test should yield the sensitizing capacity and strength of the test substance. These results can only be extrapolated to humans to a very limited extent. Substances that cause strong reactions in guinea pigs may also cause some degree of metamorphosis in the population, while substances that cause weaker reactions in guinea pigs may not cause metamorphosis in the population.

VII. Skin phototoxicity test

Skin Phototoxicity Test

1 Scope

This specification sets out the basic principles, requirements and methods for skin phototoxicity testing. This specification applies to the toxicological testing of cosmetic raw materials and their product safety.

2 Purpose of the test

Evaluates the potential for cosmetic ingredients and their products to cause skin phototoxicity.

3 Definition

Phototoxicity: A dermal toxic reaction caused by a single exposure of the skin to a chemical followed by exposure to ultraviolet light, or a similar reaction occurring after systemic application of a chemical followed by exposure to ultraviolet light.

4 Basic principles of the test

A quantity of the test substance was applied to the de-haired skin of the animal's back and exposed to UVA light at regular intervals to observe the skin reaction and to determine whether the test substance was phototoxic.

5 Test method

5.1 Subjects

Liquid subjects generally do not need to be diluted and can be used directly as a stock solution. If the test substance is solid, it should be ground to a fine powder and well moistened with water or other solvents, taking into account the effect of the solvent on the skin irritation of the test animal. For cosmetic products, the original cream or liquid is generally used. The positive control is 8- methoxypsoralen

(8-methoxypsoralen, 8-Mop).

5.2 Experimental animals and housing conditions

Adult white rabbits or albino guinea pigs, half male and half female where possible, were used. Six animals are selected for the official test. The animals should be acclimatised in the experimental animal house environment for at least 3-5 d prior to testing.

The experimental animals and the experimental animal house should comply with the corresponding national regulations. The animals should be fed a conventional diet with unrestricted water intake and should be provided with appropriate amounts of Vc.

5.3 UV light source

5.3.1 UV light source: UVA with a wavelength of 320nm~400nm, if it contains UVB, the dose should not exceed 0.$^{1J/cm2.}$

5.3.2 Determination of intensity: 6 points on the back of the animal should be set up with a radiometer before use to determine the intensity of light

($^{mW/cm2}$), in mean values.

5.3.3 Calculation of exposure time: For an exposure dose of 10 $^{J/cm2}$, calculate the exposure time according to the formula below.

$$\text{Illumination time (sec)} = \frac{\text{Irradiation dose (10000mJ / } ^{cm2}\text{)}}{\text{Light intensity (mJ / } ^{cm2}\text{ /sec)}}$$

Note: $^{1mW/cm2}$ = 1mJ/cm2/sec

5.4 Test procedure

5.4.1 Between 18h and 24h prior to the formal phototoxicity test, the skin on both sides of the spine is debrided and the skin at the test site must be intact and free from damage and abnormalities. Four de-hairing areas are prepared (see Figure 1), each measuring approximately 2cm x 2cm.

5.4.2 The animals were fixed and 0.2mL (g) of test material was applied to debridement areas 1 and 2 as shown in Table 1. Subjects used

After 30 min, the left side (depilation zones 1 and 3) was covered with aluminium foil and taped in place, while the right side was irradiated with UVA.

5.4.3 Skin reactions were observed at 1, 24, 48 and 72h after completion and each animal's skin reaction score was determined according to Table 2.

5.4.4 To ensure the reliability of the test method, check with a positive control at least every six months. i.e. in depilation zones 1 and 2

Apply the positive control as in 5.4.2.

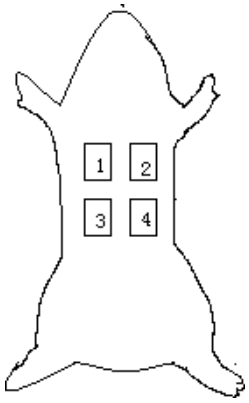


Figure 1 Schematic diagram of the location of the skin debridement area of the animal

Table 1 Experimental arrangements for animal de-hairing areas

De-hairing zone number	Test treatment
1	Coating of the subject, without irradiation
2	Coating the subject, irradiation
3	No subject applied, no irradiation
4	No subject applied, irradiated

Table 2 Skin irritation response scores

Skin Reaction	Points
Erythema and scab formation	
No erythema	0
Slight erythema (barely visible)	1
Visible erythema	2
Moderate to severe erythema	3

Severe erythema (purplish red) to slight scab formation	4
Edema formation	
No edema	0
Slight oedema (barely visible)	1
Mild oedema (well-defined skin elevation)	2
Moderate oedema (skin elevation of about 1mm)	3
Severe oedema (skin bulge of more than 1mm, widening)	4
Maximum points	8

6 Evaluation of results

If the number of animals with a sum of 2 or more skin reaction scores in the irradiated area after application of the test substance is 1 or more, the test substance is considered to be phototoxic if no skin reaction occurs in the unirradiated area alone.

7 Test reports

The report should include the following.

（1） Name of the test substance, physicochemical properties, method of preparation, concentration used.

（2） Animal species, strain, sex, weight, source (specify certificate of conformity number and animal class).

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal house certificate number.

（4） The production plant, specification of the light source.

（5） Light intensity and duration of exposure and test methods.

（6） RESULTS: The scores of animals showing skin reactions are reported in tabular form (see Tables 3 and 4).

（7） Conclusion.

Table 3 Results of the ××× dermal phototoxicity test on guinea pigs

Animal number	Sex	Weight (g)	Skin reaction score															
			1h				24h				48h				72h			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1																		
2																		
3																		
4																		
5																		
6																		

Note: 1, 2, 3 and 4 are the test areas shown in Table 1.

Table 4 Results of positive controls for dermal phototoxicity in guinea pigs

Skin reaction score

Animal number	Gender	Body weight (g)	1h				24h				48h				72h			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1																		
2																		
3																		
4																		
5																		
6																		

Note: 1, 2, 3 and 4 are the experimental areas shown in Table 1. Date of experiment.

VIII. Salmonella typhimurium/reversion mutation test

1 Scope

This specification defines the basic principles, requirements and methods of the Salmonella typhimurium / revert mutation test. This specification applies to the testing of cosmetic ingredients and their products for mutations.

2 Normative references

OECD Guidelines for Testing of Chemicals（No.471，Adopted：21，July 1997）

3 Definition

3.1 Reverse mutation (Reverse mutation)

Bacteria change back from nutrient-deficient to prototrophic in response to chemical mutagens.

3.2 Gene mutation (Gene mutation)

Changes in the order of base pairs in cellular DNA in response to chemical mutagens.

3.3 Base substitution mutation A substitution of one or more base pairs in the DNA strand.

There are two forms of base substitution: transition and conversion.

A switch is the replacement of one pyrimidine on a DNA strand by another pyrimidine, or the substitution of one purine by another purine. A switch is the replacement of one pyrimidine on the DNA strand by another purine, or the substitution of one purine by another pyrimidine.

3.4 Frameshift mutation

Causes the addition or deletion of one or more base pairs to the DNA strand.

3.5 Salmonella typhimurium/reverse mutation assay

A test method for determining the histidine-deficient (his-) → protochthonous (his+) reversion mutation induced by chemicals causing base substitution or code shift mutations in Salmonella using a group of Salmonella typhimurium histidine-deficient test strains.

3.6 $_{S9}$

Liver homogenates were prepared from rats induced by the combination of polychlorinated biphenyls (PCB mixture) or sodium phenobarbital and β-naphthoflavone in

The supernatant of the liver homogenate after centrifugation at 9000g for 10min.

4 Principle

Salmonella typhimurium histidine nutrient-deficient strains cannot synthesise histidine, so only a few spontaneously revert to mutant growth on histidine-deficient media. If a mutagen is present, the nutrient-deficient bacteria revert to the protoplastic form and therefore grow to form colonies, which determines whether the test is mutagenic.

Some mutagens require metabolic activation to cause revertant mutations, so a mixture of $_{S9}$ prepared from rat liver induced by an inducer is added.

5 Instruments and equipment

Incubators, constant temperature water baths, oscillating water bath shakers, pressure steam sterilisers, dry heat ovens, cryogenic refrigerators (-80°C) or liquid nitrogen biocontainers, ordinary refrigerators, balances (precision 0.1g and 0.0001g), mixer shakers, homogenisers, colony counters, cryogenic high-speed centrifuges, glassware, etc.

6 Culture media and reagents

6.1 0.5 mmol/L histidine-0.5 mmol/L biotin solution

Ingredient: L-Histidine (MW155)	78mg
D-Biotin (MW244)	122mg
Add distilled water to	1000mL

Preparation: Heat the above ingredients to dissolve the biotin, then autoclave for 20min at 0.068MPa and store in a 4°C refrigerator.

6.2 Top agar medium

Ingredients: Agar powder	1.2g
Sodium chloride	1.0g
Add distilled water to	200mL

Preparation: The above ingredients were mixed and autoclaved at 0.103 MPa for 30 min. 20 mL of 0.5 mmol/L histidine-0.5 mmol/L biotin solution was added for the experiment.

6.3 Vogel-Bonner (V-B) medium E

Ingredients: raffinate (C6H8O7-H2O)	100g
Dipotassium hydrogen phosphate ($_{K2HPO4}$)	500g
Sodium ammonium hydrogen phosphate (NaNH4HPO4-4H2O)	175g
Magnesium Sulphate (MgSO4-7H2O)	10g
Add distilled water to	1000mL

Preparation: first dissolve the first three ingredients by heating, then slowly pour the dissolved magnesium sulfate into a volumetric flask and add distilled water to

1000mL, autoclaved at 0.103MPa for 30min and stored in a 4°C refrigerator.

6.4 20% dextrose solution

Ingredients: Glucose	200g
Add distilled water to	1000mL

Preparation: Dissolve the glucose in a small amount of distilled water, add distilled water to 1000 mL, autoclave for 20 min at 0.068 MPa and store in a refrigerator at 4°C.

6.5 Bottom agar medium

Ingredients: Agar powder	7.5g
Distilled water	480mL
V-B medium	E10mL
20% dextrose solution	10mL

Preparation: The first two components were autoclaved at 0.103 MPa for 30 min, then the second two components were added and mixed thoroughly and poured onto the bottom plate. The plates were prepared at 25mL per dish, cold cured and placed in an incubator at 37°C for 24h and set aside.

6.6 Nutrient Broth Medium

Ingredients: Beef paste	2.5g
Tryptone	5.0g
Dipotassium hydrogen phosphate ($_{K2HPO4}$)	1.0g
Add distilled water to	500mL

Preparation: Mix the above ingredients, autoclave at 0.103MPa for 30min and store at 4°C in a refrigerator.

6.7 Salt solution (1.65 mol/L KCl + 0.4 mol/L $_{MgCl2}$)

Ingredients: Potassium chloride (KCl)	61.5g
Magnesium chloride (MgCl2-6H2O)	40.7g
Add distilled water to	500mL

Preparation: Dissolve the above ingredients in water, autoclave for 30min at 0.103MPa and store at 4°C in a refrigerator.

6.8 0.2 mol/L phosphate buffer (pH 7.4)

Ingredients: Sodium dihydrogen phosphate (NaH2PO4-2H2O)	2.965g
Disodium hydrogen phosphate (Na2HPO4-12H2O)	29.015g
Add distilled water to	500mL

Preparation: Dissolve the above ingredients, autoclave at 0.103MPa for 30min and store at 4°C in the refrigerator.

6.9 $_{S9}$ Mixture

Ingredients.	Per ml $_{S9}$ mix
Liver S9100L	
Salt solution	20L
Sterilised distilled water	380L
0.2 mol/L phosphate buffer	500L
Coenzyme II (NADP)	4mol
Glucose 6-phosphate (G-6-P)	5mol

Preparation: Coenzyme II and Glucose 6-Phosphate are weighed in a sterilised triangular flask and the components are added in the reverse order described above, so that Liver $_{S9\ is}$ added to the existing buffer solution. The mixture must be prepared as needed and stored in an ice water bath. At the end of the experiment, the remaining $_{S9}$ mixture should be discarded.

6.10 Reagents for strain identification and special applications

6.10.1 Histidine-biotin plates

Ingredients: Agar powder	15g
Distilled water	944mL
V-B Medium	E20mL
20% Dextrose	20mL
Sterilized aqueous histidine hydrochloride solution (0.5g/100mL)	10mL
Sterilised 0.5 mmol/L biotin solution	6mL

Preparation: After autoclaving the agar and water, add the sterilised 20% glucose, V-B medium and histidine solution to the hot agar solution. After the solution has cooled slightly, sterilised biotin is added, mixed and the plates are poured.

6.10.2 Ampicillin plates and ampicillin/tetracycline plates

Ingredients: agar powder	15g
Distilled water	940mL
V-B Salt solution	20mL
20% dextrose	20mL
Sterilized histidine hydrochloride solution (0.5g/100mL)	10mL

Sterilisation 0.5 mmol/L biotin solution	6mL
Ampicillin solution (8 mg/mL in 0.02 mol/L NaOH)	3.15mL
Tetracycline solution (8mg/mL in 0.02mol/L HCl)	0.25mL

Preparation: Autoclave the agar and water for 20 min. Add sterile glucose, VB salt solution and histidine-biotin solution to the hot solution and mix well. Cool to approx. 50°C and add tetracycline solution and/or ampicillin solution under aseptic conditions.

The master plate should be prepared within a few days of pouring the agar plate.

6.10.3 Nutrient agar plates

Ingredients: Agar powder	7.5g
Nutrient Broth Medium	500mL

Preparation: Autoclave at 0.103MPa for 30min and pour onto the plate.

7 Identification of test strains and their biological characteristics

7.1 Test strains

A group of standard test strains TA97, TA98, TA100 and TA102 were used.

7.2 Biological characterisation

Newly acquired or long preserved strains must be biologically characterised prior to testing. The criteria for strain identification are shown in Table 1.

Table 1 Judgement criteria for the identification of test strains

Strain	Histidine Defects	Lipopolysaccharide Barrier Defects	Ampicillin Vegetation resistance	Excision Repair of defects	Tetracycline Resistance	Spontaneous return to change Number of colonies*
TA 97	+	+	+	+	-	90-180
TA 98	+	+	+	+	-	30- 50
TA100	+	+	+	+	-	100-200
TA102	+	+	+	-	+	240-320
Note	"+" indicates the need for histidine	"+" indicates an rfa mutation	"+" indicates a factor of R	"+" indicates that it has ΔuvrB mutation	"+" indicates the presence of the pAQ1 plasmid	*Metabolically active in vitro Slight increase in the number of spontaneous revertant colonies under chemosynthetic conditions

7.2.1 Histidine Deficiency

Principle: Histidine-deficient test strains cannot synthesise histidine themselves and can only grow on histidine-supplemented media, but not on histidine-deficient media.

Identification method: The test strain enrichment solution was scribed on histidine-containing medium plates and histidine-free plates on

The results were observed after 24h incubation at 37°C.

The results showed that the histidine-deficient strains grew on histidine-containing plates, but not on histidine-free plates.

7.2.2 Lipopolysaccharide barrier deficiency

Principle: Strains with deep roughened (rfa) are missing a lipopolysaccharide barrier on their surface, so some large molecules such as crystalline violet can penetrate the membrane and inhibit their growth, whereas wild-type strains are unaffected by this.

Identification: aspirate 0.1mL of the bacterium to be tested onto a nutrient agar plate and place a strip of filter paper moistened with 0.1% crystalline violet solution across the line. incubate at 37°C for 24h and observe the results.

Judgement: If the bacterium to be tested appears as a transparent band at the intersection of the filter paper strip and the scribe line, it means that the strain to be tested has rfa

Mutation.

7.2.3 Ampicillin resistance

Principle: The test strain containing the R factor is resistant to ampicillin. The presence or absence of the plasmid was determined using ampicillin because the R factor is not very stable and can be easily lost.

Identification method: aspirate 0.1mL of the strain to be tested, scribe on a benzylpenicillin plate and incubate at 37°C for 24h before observing the results.

Judgement of results: If the test organism grows on ampicillin plates, the test organism is resistant to ampicillin and contains the R factor; otherwise, the test organism does not contain the R factor or the R factor is missing.

7.2.4 UV sensitivity

Principle: Strains with the ΔuvrB mutation are sensitive to UV light and cannot grow when exposed to UV light, while strains with the wild-type excision repair enzyme can grow as usual.

Identification method: 0.1mL of the bacterium to be tested was drawn onto a nutrient agar plate, half of the plate was covered with black paper and placed under a UV lamp (15W, distance 33cm) for 8 seconds. Incubate at 37°C for 24h and observe the results.

The results showed that the strains with the ΔuvrB mutation were sensitive to UV light and did not grow after radiation, while the strains with intact

The strains of the excision repair system are grown as usual.

7.2.5 Tetracycline resistance

Principle: strains with pAQI are resistant to tetracycline.

Identification method: 0.1mL of the strain to be tested was drawn onto a benzylpenicillin/tetracycline plate, incubated at 37°C for 24h and then observed.

Judgement: If the test organism grows on the ampicillin/tetracycline plate as usual, the test strain is resistant to both ampicillin and tetracycline and has the pAQI plasmid; otherwise, the test strain does not contain the pAQI plasmid.

7.2.6 Spontaneous return to change

Principle: Each of the test strains produces a spontaneous gyration at a certain frequency, called spontaneous gyration. This spontaneous gyration is a characteristic of each test strain.

Identification method: Add 0.1mL of the strain to be tested to 2mL of top agar medium containing histidine-biotin, mix well and spread onto the bottom agar plate, allow the agar to solidify, incubate at 37°C for 48h and then count the number of colonies per dish.

Judgement of results: The number of spontaneous revertant colonies for each standard test strain should meet the requirements of Table 1. The number of spontaneous revertant colonies after in vitro metabolic activation should be slightly higher than under direct action.

7.2.7 Return variation characteristics - diagnostic test

Principle: The nature of the mutagenic effect of each test strain and the effect of the $_{S9}$ mixture vary for each diagnostic mutagen. Identification method: Follow the procedure for the plate admixture test. The subjects are replaced with the diagnostic mutagen. Judgement of results: See Table 2 for the results of the standard strains of mutagenicity specific to certain diagnostic mutagens.

Table 2 Testing strains for mutagenicity

Mutagens	Dose (g)	$_{S9}$	TA97	TA98	TA100	TA102
Zolomycin	6.0		-124	3123	47	592
Sodium azide	1.5		-76	3	3000	188
ICR-191	1.0		-1640	63	185	0
Streptavidin	0.25		-inh	inh	inh	2230
Mitomycin C	0.5		-inh	inh	inh	2772
2,4,7-Trinitro-9-fluorenone	0.20		-8377	8244	400	16
4-Nitro-O-phenylenediamine	20	-Such as	2160	1599	798	0
4-Nitroquinoline-N-oxide	0.5		-528	292	4220	287
Methyl	1.0		-174	23	2730	6586

methanesulfonate					
2-Aminofluorene	10	+1742	6194	3026	261
Benzo(a)pyrene	1.0	+337	143	937	255

Note: inh indicates inhibition. Values in the table are net of solvent control back-variant colonies.

8 Induction of rat liver microsomal enzymes and preparation of S_9

8.1 Induction

The most widely used inducer of liver microsomal enzymes in rats is polychlorinated biphenyls (PCB mixture), which is administered intraperitoneally at a dose of 500 mg/kg body weight to healthy male rats weighing approximately 200 g. The inducer is dissolved in corn oil at a concentration of 200 mg/mL. The animals were decapitated on the fifth day after PCB induction and stopped eating and drinking for 12 h prior to execution.

The combination of sodium phenobarbital and β-naphthoflavone can also be used as an induction agent. Healthy male rats weighing approximately 200 g are injected orally or intraperitoneally with 80 mg/kg sodium phenobarbital and 80 mg/kg β-naphthoflavone for 3 days. Diet was stopped 16h before execution, but water was freely available. As the chemicals are injected intraperitoneally, they tend to cause the liver to form an epithelium that cannot be easily peeled off, so the use of transoral instillation is recommended.

The way to the stomach.

8.2 $_{S9}$ Preparation

First, the animal was disinfected with 75% alcohol and the abdomen was dissected. The liver was removed under sterile conditions, the connective tissue was removed and the liver was washed in an ice bath with 0.15 mol/L potassium chloride solution and placed in a beaker containing 0.15 mol/L potassium chloride solution. The liver was then centrifuged at 9000g for 10 min at 4°C on a low temperature high speed centrifuge and the supernatant ($_{S9)\ was}$ dispensed into plastic tubes. Store in a liquid nitrogen biocontainer or in a -80°C refrigerator.

All procedures are carried out in an ice-water bath and under sterile conditions. All surgical instruments and utensils used for the preparation of liver $_{S9}$ are sterilised and the viability of $_{S9\ is}$ determined by diagnostic mutagens after preparation.

9 Selection of solvents

If the test substance is water-soluble, sterilised distilled water can be used as the solvent; if it is fat-soluble, organic solvents with low toxicity to the test strain and no mutagenicity should be chosen. In general operation, in order to reduce the error and the influence of the solvent, the same solvent is often used in different concentrations for each dish, with a fixed addition of 100L.

10 Design of the dose

The criteria for determining the highest dose of a test substance are the toxicity to bacteria and their solubility. A reduction in the number of spontaneous gyrations, a clearing of the background bacteria or a reduction in the number of viable bacteria in the treated culture are all signs of toxicity.

For raw materials, the maximum dose group is generally 5mg/dish. For products, the highest dose may be the lowest inhibitory concentration for subjects with a bactericidal effect and the highest dose may be the stock solution for subjects without a bactericidal effect. There should be a minimum of four dose groups. Three parallel plates should be made for each dose.

11 Test procedure

11.1 Bacterial growth cultures

The strain should be incubated for 10 h at 37°C with 100 shaking cycles/min. The strain should be incubated at least 1-2 times per ml.

$\times\ ^{109}$ viable bacteria count.

11.2 Flat Blending Method

For the experiment, dispense 2.0mL of top agar medium containing 0.5mmol/L histidine-0.5mmol/L biotin solution into test tubes and keep warm in a water bath at 45°C. Then add 0.1mL of test strain enrichment solution, 0.1mL of subject solution and 0.5mL of $_{S9}$ mixture (when metabolic activation is required) to each tube in turn, mix thoroughly. Pour rapidly onto the bottom agar plate and rotate the plate to distribute evenly. Incubate horizontally for 48 h in a 37°C incubator and count the number of colonies per dish.

In addition to the dose groups of subjects, there should also be a blank control, a solvent control, a positive mutagen control and a sterile control.

12 Data processing and judgement of results

The number of colonies per dish for each dose group, blank control (spontaneous regression), solvent control and positive mutagen control were recorded and the mean and standard deviation were calculated.

If the number of revertant colonies is twice or more than the number of solvent control colonies and there is a dose-response relationship, the test is judged to be positive for mutagenicity.

A subject is reported as mutagenic for Salmonella typhimurium if it is positive for one of the four test strains, either with or without the addition of S9. If the test is negative for all four test strains, both with and without S9, the test will be reported as mutagenic negative.

13 Test reports

The test report shall include the following.

（1） Name of the test substance, physical and chemical properties, method of preparation, solvent used.

（2） Test strain: the test strain used.

（3） Metabolic activation systems: inducers used.

（4） Test method: a brief description of the procedure and, in addition to the dose grouping of the subjects, a description of the blank control, solvent control and positive control, and the criteria for determining a positive result.

（5） Results: The results of the Ames assay are reported in tabular form for the subjects (see Table 1).

（6） Conclusion.

Table 1 Results of the back-variation of the Ames test strains (mean ± standard deviation)

Group	Dose mg/pad	TA97		TA98		TA100		TA102	
		-(S9)	+(S9)	-(S9)	+(S9)	-(S9)	+(S9)	-(S9)	+(S9)
testent									
Spontaneous inversion									
Solution control group									
Positive control group									

IX. In vitro mammalian cell chromosome aberration assay

1 Scope

This specification specifies the basic principles, requirements and methods for in vitro mammalian cell chromosome aberration tests. This specification applies to the detection of mutagenicity in cosmetic raw materials and their products.

2 Normative references

OECD Guidelines for Testing of Chemicals（No.473，July 1997）

3 Purpose of the test

This test is used to detect chromosomal aberrations in cultured mammalian cells to evaluate the mutagenic potential of the subject.

4 Definition

4.1 Structural aberration: A change in the structure of a chromosome that is detected microscopically at the mid-phase stage of cell division, manifesting itself as a deletion, fragmentation or interchange. Structural aberrations can be divided into the following two categories.

4.1.1 Chromosome-type aberration: Structural damage to a chromosome, manifested by a break or breakage at the same site on both chromosomes.

4.1.2 Chromatid-type aberration: Structural damage to chromosomes, manifested by damage to chromosome breaks or recombination of chromosome breaks.

4.2 Mitotic index: the ratio of the number of cells in the intermediate phase to the total number of cells observed; an indicator of the degree of cell proliferation.

5 Basic principles of testing

Cultured mammalian cells are exposed to the test subjects with and without the addition of a metabolic activation system. Cells are treated with a mid-phase division blocker (e.g. colchicine or colchicine) to stop the cells in mid-phase division and subsequently harvested, sectioned, stained and analysed for chromosomal aberrations.

Most mutagenic agents result in chromosomal haplotype aberrations, with occasional chromosomal type aberrations occurring. Although an increase in polyploidy may indicate the possibility of chromosome number aberrations, this method is not suitable for determining chromosome number aberrations.

6 Test method

6.1 Reagent and subject preparation

6.1.1 Positive controls: Suitable positive controls may be selected according to the nature and structure of the subject. Positive controls should be known breakers that elicit detectable and reproducible positive results. When exogenous activation systems are not present, methyl methanesulphonate (MMS), ethyl methanesulphonate (MMS), ethyl methanesulphonate (MMS), ethyl methanesulphonate (MMS) and ethyl methanesulphonate (MMS) may be used.

(EMS), ethyl nitrosourea, mitomycin C, 4-nitroquinoline-N

Oxide (4-nitroquinoline-N-oxide). When exogenous activation systems are present, benzo(a)pyrene (benzo

(a) pyrene), cyclophosphamide (cyclophosphamide).

6.1.2 Negative controls: There should be a negative control, i.e. containing only the same solvent as the subject group, without the subject, and otherwise treated exactly the same as the subject group. In addition, there should be a blank control if it is not proven that the selected solvent is not mutagenic and the solvent control differs significantly from the background information of the blank control in this laboratory.

6.1.3 Subjects

6.1.3.1 Preparation of the test substance: solid test substances should be dissolved or suspended in a solvent and diluted to a suitable concentration prior to use; liquid test substances can be added directly to the test system and/or diluted to a suitable concentration prior to use. The test substance should be freshly prepared prior to use, otherwise it must be verified that storage does not affect its stability.

6.1.3.2 Choice of solvent: The solvent must be non-mutagenic, not chemically reactive with the subject and not affect cell survival or $_{S9}$ activity. Preferred solvents are culture medium (without serum) or water. Dimethyl sulfoxide (DMSO) is also a common solvent and should be used at a concentration of no more than 0.5%.

6.1.3.3 Subject concentration setting

（1） Selection of maximum concentration.

The factors that determine the maximum concentration are cytotoxicity, solubility of the test substance in the test system and changes in pH or osmolality.

（2） Determination of cytotoxicity.

Cytotoxicity should be determined in the presence or absence of the activation system using indicators indicative of cell integrity and growth, such as degree of confluency, viable cell counts or mitotic index. Cytotoxicity and solubility should be determined in a pre-test.

（3） Dose setting.

①At least 3 concentrations should be available for analysis. When cytotoxic, the concentration range should include a range from maximum toxicity to

Virtually non-toxic; usually concentration interval factor not greater than 2 to $\sqrt{10}$.

(ii) When harvesting cells, the highest concentration should significantly reduce the degree of cell coverage, cell count or mitotic index (all should be greater than 50%).

(iii) For those compounds that are relatively non-cytotoxic, the maximum concentration should be 5 L/mL, 5 mg/mL or 0.01 mol/L.

④ For relatively insoluble substances that remain non-toxic at concentrations below the insoluble concentration, the highest dose should be, when the treatment period is over, one concentration above the solubility limit in the final culture solution. In some cases (i.e. where cytotoxicity occurs only above the lowest insoluble concentration), more than one concentration at which precipitation can be seen should be used. It is advisable to evaluate solubility both at the beginning and at the end of the test treatment, as solubility may change during exposure within the test system due to the presence of cells, $_{S9,}$ etc. Insolubility can be identified visually, but precipitation should not interfere with observation.

6.1.4 Culture medium: MEM (Eagle) with non-essential amino acids and antimicrobials (penicillin and streptomycin at 100 IU/mL) and fetal calf serum or calf serum at 10%. Other suitable cultures can also be used.

6.1.5 Revitalisation systems

S9 mix is usually used and is obtained from rodent livers treated with an enzyme inducer (Aroclor 1254 or a combination of sodium phenobarbital and β-naphthoflavone). The amount of cofactor added to the S9 mix is left to the discretion of each laboratory, but the activity of S9mix must be identified and the positive control must be significantly activated. The following can also be used

	S90.125mL
MgC12 (0.4mol/L)	0.02mL
KC1 (1.65 mol/L)	0.02mL
Glucose-6-phosphate	1.791mg
Coenzyme II (oxidative, NADP)	3.0615mg

Top up to 1mL with serum-free MEM culture.

6.2 Test procedure

6.2.1 Cells: Established cell lines or cell lines may be used, or primary cultured cells may be used. The cells used should be stable in terms of growth performance, chromosome number and karyotype, and rate of spontaneous chromosomal aberrations. The Chinese gopher ovary (CHO) cell line or Chinese gopher lung (CHL) cell line is recommended.

6.2.2 The test should be accompanied by a positive control, a negative control and at least 3 concentration groups of subjects available for analysis.

6.2.3 The day before the test, a certain number of cells were inoculated into culture dishes (bottles) and incubated in a $_{CO2}$ incubator.

6.2.4 The test is carried out with and without the addition of $_{S9}$ mix. At the end of the test, the culture medium containing the test substance is aspirated, the cells are washed three times with Hanks' solution, a culture medium containing 10% fetal bovine serum is added and the cells are returned to the incubator. The cells were then returned to the incubator and harvested within 24h. A mid-cell division blocker (e.g. colchicine, 4h, final concentration 1g/mL) was added 2h-4h before harvesting.

If negative results are obtained with and without the addition of $_{S9}$ mix as described above, an additional test should be added, i.e. the contact time between the subject and the test system should be extended to 24 h without the addition of $_{S9}$ mix.

Repeat test for touch time etc.

6.2.5 When the cells were harvested, they were digested with 0.25% trypsin solution. After the cells were dislodged, the trypsin action was terminated by adding a culture solution containing 10% fetal calf or calf serum, mixed well, centrifuged at 1000rpm~1200rpm for 5min~7min in a centrifuge tube, the supernatant was discarded and treated with 0.075mol/L KC1 solution at hypotonicity The supernatant was discarded and treated with 0.075 mol/L KC1 solution, followed by fixation with freshly prepared methanol and glacial acetic acid solution (3:1 volume ratio). The films were routinely produced by air-drying or flame-drying methods and stained with Kimsa stain.

6.2.6 For chromosome analysis, 100 well-dispersed mid-phase schizograms (2n±2 chromosomes) per treatment group are selected for chromosome aberration analysis for cosmetic end-products. For cosmetic raw materials, 200 (100 for positive controls) well-dispersed mid-phase divisions (2n±2 chromosomes) per treatment group are selected for chromosomal aberration analysis. The number of chromosomes in each observed cell should be recorded during the analysis and, in the case of aberrant cells, the position of the coordinates of the microscopic field and the type of aberration should also be recorded.

6.3 Statistical treatment: The rate of chromosomally aberrant cells was tested with the ^{X2} test to evaluate the mutagenicity of the subjects.

6.4 Evaluation of results: A subject is judged to be mutagenic in this test system if either.

（1） The number of chromosomal structural aberrations caused by the subjects was statistically significant and dose-related.

（2） The subject causes a statistically significant and reproducible increase at any one dose condition.

A combination of biological and statistical significance should be considered in the evaluation.

7 Test reports

The test report shall include the following.

（1） The name of the test substance, the relevant physicochemical properties, the solvent used and its preparation, the choice of dose (the method of determination of the cytotoxicity of the test substance, its dissolution, etc. should be indicated).

（2） The name of the cell line.

（3） Experimental conditions and methods

(i) Metabolic activation system: inducer of the enzyme used in the preparation of $_{S9}$, the animal species

and source chosen, formulation of $_{S9}$ mix.

②Controls: name of positive control and concentration chosen; name of negative (solvent) control and concentration used.

(iii) Culture fluid: name of culture fluid used, type of serum and concentration used.

④ The cell density at the time of inoculation and the size of the culture dish (flask) used.

⑤ Mid-term division blockers: name, concentration used, duration of action.

(vi) Handling time: the contact time of the test subject with the test system.

(vii) Briefly describe the filming method, the number of intermediate split phases analysed, and the method of evaluating the results.

（4） Results

① Determination of the maximum dose of the test substance and the results of the test: determination of cytotoxicity (see Table 1 for suggested tables); dissolution (see Table 2 for suggested tables); effect on pH and osmolality (osmolality) concentration, if any.

(ii) The rate of chromosomal aberrations in each treatment and control group (see Table 3 for a suggested table).

(iii) Positive controls and negative controls (commonly used solvents, e.g. DMSO) in this laboratory have historically been

Range, mean and standard deviation of chromosome aberration rates (indicate number of samples).

（5） Conclusion.

Table 1 Toxicity of the test substance to cells

Live Cell Counting Method				Splitting Index Method		
Subject concentration Degree (μg/mL)	Number of inoculated cells Number of live cells /mL/mL	Survival rate (%)	Counting Cells Number	Mid-term Number of splitting phases	Splitting Index	Cell Coat Level of cover

Table 2 Record of dissolution of the test substance in the selected solvent

Concentration of the test substance (μg/mL)	Name of solvent	Presence of precipitation (slightly visible)

Table 3 test results of the subject

8 Interpretation of results

Positive results indicate that the test substance caused structural aberrations in the chromosomes of cultured mammalian somatic cells.

The negative results indicate that the subjects did not cause structural aberrations in the chromosomes of cultured mammalian somatic cells under the conditions of this test.

X. In vitro mammalian cell gene mutation assay

In Vitro Mammalian Cell Gene Mutation Test

1 Scope

This specification specifies the basic principles, requirements and methods for in vitro mammalian cell mutagenicity testing. This specification applies to the detection of mutagenicity in cosmetic raw materials and their products.

2 Normative references

GB15193 Procedures and Methods for the Toxicological Evaluation of Food Safety 15193.12; 202003 OECD Guidelines for Testing of Chemicals (No. 476, 1997)

3 Purpose of the test

The test system is used to detect mutations caused by cosmetic ingredients and their products, including base pair mutations, shift mutations and deletions, in order to evaluate the likelihood of mutations caused by the subject.

4 Definition

Forward mutation: A mutation in a gene from the prototype to a mutant subtype that causes changes in enzymes and functional proteins.

Mutant frequency: The ratio of the number of mutant cells observed to the number of surviving cells.

5 Test principle

Cells were exposed to the test substance for a certain period of time with and without the addition of a metabolic activation system, and then the cells were re-passaged and cultured. Cells with normal levels of thymidine kinase are sensitive to, for example, trifluorothymidine (TFT) and therefore fail to grow and divide in culture, whereas mutant cells are not and continue to divide and form colonies in selective cultures containing 6-thioguanine (6-TG), 8-azaguanine (AG) or TFT. The mutant colonies were divided and formed. Based on the number of mutant colonies, the mutation frequency was calculated to evaluate the mutagenicity of the subject.

6 Test method

6.1 Reagent and subject preparation

6.1.1 Subjects

6.1.1.1 Preparation of subjects: solid subjects should be dissolved or suspended in a solvent and diluted to a suitable concentration prior to use; liquid subjects can be added directly to the test system/or diluted to a

suitable concentration prior to use. Subjects should be freshly prepared prior to use, otherwise it must be verified that storage does not affect their stability.

6.1.1.2 Choice of solvent: The solvent must be non-mutagenic, not chemically reactive with the subject and not affect cell survival or $_{S9}$ activity. The preferred solvent is water or an aqueous solvent. Dimethyl sulfoxide (DMSO) is also commonly used, but should not be used at concentrations greater than 0.5%.

6.1.1.3 Subject concentration setting

6.1.1.3.1 Choice of maximum concentration: The factors that determine the maximum concentration are cytotoxicity, solubility of the test substance in the test system and changes in pH or osmolality.

6.1.1.3.2 Determination of cytotoxicity: Cytotoxicity should be determined in the presence or absence of the activation system using indicators indicative of cell integrity and growth, such as relative colony formation rate or relative total cell growth (total growth). Cytotoxicity and solubility should be determined in a pre-test.

6.1.1.3.3 Dose setting

A minimum of 4 concentrations should be available for analysis. When cytotoxic, the range of concentrations should include from maximum toxicity to several

Virtually non-toxic. Usually the concentration interval factor is between 2 and $\sqrt{10}$ Between.

If the highest concentration is based on cytotoxicity, then the relative cell survival (relative colony formation rate) or relative total cell growth should be 10% to 20% (not less than 10%) for this concentration group.

For those compounds with very low cytotoxicity, the maximum concentration should be 5μL/mL, 5mg/mL or 0.0lmol/L.

For relatively insoluble substances, the maximum concentration should be at or above the solubility limit in the cell culture state. It is advisable to evaluate solubility both at the beginning and at the end of the test treatment, as solubility may change during exposure within the test system due to the presence of $_{S9}$ etc. Insolubility can be identified visually, but precipitation should not interfere with observation.

6.1.2 Controls: In each test, there should be a positive control and a negative (solvent) control in the presence and absence of the metabolic activation system.

6.1.2.1 Positive control: When using a metabolic activation system, the positive control must be a substance that requires metabolic activation and is capable of causing a mutation. In the absence of a metabolic activation system, positive controls may be ethyl methanesulfonate-EMS (HPRT), methyl methanesulphonate (MMS, TK), ethyl nitrosourea (ENU, HPRT), etc. nitrosourea-ENU (HPRT test), etc. In the presence of a metabolic activation system, 3-methylcholanthrene (HPRT test; TK test), cyclophosphamide

N-nitroso-dimethylamine (HPRT), 7,12-dimethylbenzanthracene (HPRT), etc. Other suitable positive controls may also be used.

6.1.2.2 Negative controls: Negative controls (including solvent controls) should be treated the same as the test except that they do not contain the test substance. In addition, a blank control should be provided when no laboratory history is available to confirm that the solvent used is not mutagenic and has no other deleterious effects.

6.1.3 Cells: Chinese hamster lung cell line (V-79) and Chinese hamster ovary cell line commonly used for HPRT locus mutation analysis

The mouse lymphoma cell line (L5178Y) and the human lymphoblastoid cell line (TK6) are commonly used for TK locus mutation analysis. Cells should be checked for mycoplasma contamination prior to use.

6.1.4 Culture medium: The appropriate medium should be selected according to the system and cell type used for the experiment. For V-79 or CHO cells, MEM (Eagle) medium with 10% fetal bovine serum and an appropriate amount of antimicrobial agent is commonly used. For L5178Y or TK6 cells, RPMI 1640 medium with 10% horse serum and an appropriate amount of antimicrobial agent is commonly used.

6.1.5 Activation system: same as in vitro mammalian cell chromosome aberration assay.

6.1.6 Agent of choice: 6-Thioguanine (6-TG): recommended final concentration 5g/mL to 10g/mL. trifluorothymidine

(TFT): recommended final concentration of 3 g/mL.

6.1.7 Pre-treatment culture solution: THMG/THG

To reduce the rate of spontaneous mutations, cells were incubated in THMG-containing medium for 24h before the test to kill spontaneous mutated cells, and then the cells were inoculated in THG (THMG medium without methotrexate) for 1d to 3d.

THMG contains the following final concentrations of each substance other than the culture composition.

Thymidine	$^{510\text{-}6}$ mol/L
Hypoxanthine	$^{510\text{-}5}$ mol/L
Aminoglutethimide	$^{410\text{-}7}$ mol/L
Glycine	$^{110\text{-}4}$ mol/L

6.2 Test procedure

6.2.1 HPRT locus mutation analysis

6.2.1.1 The cells were inoculated in culture flasks 1 d before the test and incubated at 37°C.

6.2.1.2 The test was performed by aspirating the culture solution from the flask and adding a certain concentration of the subject, S9-mix (without S9-mix)

After incubation for 3h-6h, the cells were washed three times with Hank's solution and culture medium containing fetal bovine serum was added.

6.2.1.3 Cells were seeded at low density on the same day and at day 3 and inoculated at day 7 with 3 vials of each dose, and stained after 7 d to determine cell viability. An additional number of cells were inoculated in each culture flask, 8 flasks per dose, and 6-TG (final concentration 5 g/mL) was added after 3 h. After 10 d, the cells were stained and the mutant cell colonies counted.

6.2.1.4 The test results were statistically analysed using the χ^2 test.

6.2.2 TK locus mutation analysis (L5178Y cells, 96-well plate assay)

6.2.2.1 Treatment: Take well-grown cells, adjust the density to 5 x 105/mL, add the test material at 1% volume and shake at 37°C for 3 hours. Centrifuge, discard the supernatant, wash the cells twice with PBS or serum-free medium, resuspend the cells in RPMI 1640 medium containing 10% horse serum and adjust the cell density to 2×105/mL.

6.2.2.2 PE_0 (0-day plate inoculation efficiency) was determined by taking an appropriate amount of cell suspension, diluting it in a gradient to 8 cells/mL, and inoculating a 96-well plate (0.2 mL per well, i.e. an average of 1.6 cells/well) with 1~2 plates of each dose. The number of wells with colony growth per plate was counted.

6.2.2.3 Expression: The cell suspensions obtained in step 6.2.2.1 were cultured for 2 d. Cell densities were counted daily and maintained at

106/mL or less.

6.2.2.4 PE_2 (plate inoculation efficiency at day 2): After the end of the expression culture at day 2, take an appropriate amount of cell suspension, dilute it in a gradient according to step 6.2.2.2 and inoculate a 96-well plate, counting the number of wells with colony growth per plate after 12 days of incubation.

6.2.2.5 TFT resistance mutation frequency (MF) assay: At the end of the 2nd day of expression culture, an appropriate amount of cell suspension was taken, the cell density was adjusted to 1×104/mL, TFT (trifluorothymidine, final concentration 3μg/mL) was added, mixed well and inoculated into 96-well plates (0.2 mL per well, i.e. an average of 2000 cells/well), each dose was made into 2~4 Plates were incubated at 37°C, 5% CO_2 and saturated humidity for 12 d. The number of wells with mutant colonies was counted.

6.2.2.6 Calculation

6.2.2.6.1 Flat efficiency (PE_0 and PE_2)

$$PE = \frac{-\ln(EW/TW)}{1.6}$$

where EW is the number of holes with no colony growth; TW is the total number of holes.

1.6 is the number of cells inoculated per well

6.2.2.6.2 Relative Survival Rate (%RS)

$$\text{Relative Survival Rate (\%RS)} = \frac{PE_0\ (\text{treatment})}{PE_0\ (\text{control})} \times 100$$

6.2.2.6.3 Mutation Frequency (MF)

$$MF\ (x\ ^{10\text{-}6}) = \frac{-\ln(EW/TW)/n}{PE2}$$

where EW is the number of holes with no colony growth; TW is the total number of holes.

n is the number of cells inoculated per well (2000)

7 Evaluation of results

A positive result for a subject in this test system can be determined in either of the following two situations.

（1） Subjects caused a statistically significant, dose-related increase in mutation frequency.

（2） The subject elicits a statistically significant and reproducible positive response at any one dose condition. A negative result is determined by the absence of mutation frequency at a %RS of ±20% (i.e. significant cytotoxicity has been produced)

It should only be made when there is a significant increase. A combination of biological and statistical significance should be considered in the evaluation.

Positive results indicate that the test substance can cause mutations in the mammalian cells used. Reproducible positive dose-response relationships are more significant.

The negative results indicate that under the conditions of this test, the subjects do not cause mutations in the mammalian cells used.

8 Test reports

The test report shall include the following.

（1） The name of the test substance, the relevant physicochemical properties, the solvent used and its preparation, the choice of dose (the method of determination of the cytotoxicity of the test substance, its dissolution, etc. should be indicated).

（2） The name of the cell line.

（3） Experimental conditions and methods

(i) Metabolic activation systems: inducers used in the preparation of $_{S9}$, animal species and sources, formulation of $_{S9}$ mix.

② Controls: name and concentration used for positive controls; name and concentration used for negative (solvent) controls.

(iii) Culture fluid: name of culture fluid used, type of serum and concentration used.

④ The cell density at the time of inoculation and the size of the culture flask used.

⑤ Handling time: the contact time of the subject with the experimental system.

(vi) Time of expression.

(vii) Methods of outcome evaluation.

（4） Results

① Determination of the highest dose of the test substance and results: including determination of cytotoxicity; dissolution; effect on pH and osmolyte concentration (if any).

②Test results: frequency of mutations and statistical results for the test and control groups.

(iii) Range of mutation frequencies, means and standard deviations for positive and negative controls (including commonly used solvents such as DMSO) in the history of this laboratory (indicate number of samples).

（5） Conclusion.

XI. Mammalian bone marrow cell chromosome aberration test

In Vivo Mammalian Bone Marrow Cell Chromosome Aberration Test

1 Scope

This specification specifies the basic principles, requirements and methods of the mammalian bone marrow cell chromosome aberration test. This specification applies to the testing of cosmetic raw materials and their products for genotoxicity.

2 Normative references

OECD Guidelines for Testing of Chemicals（No.475，April 1997）

3 Purpose of the test

This test is a mutagenicity test that detects chromosomal aberrations in bone marrow cells of whole animals to evaluate the mutagenic potential of the subject.

4 Definition

Chromosome-type aberration: Structural damage to a chromosome, manifested by a break or breakage at the same site on both chromosomes.

Chromatid-type aberration: Structural damage to chromosomes, manifested by damage to chromosome breaks or recombination of chromosome breaks.

Numerical-type aberration: alterations in the number of chromosomes in mammalian cells.

5 Basic principles of testing

Mammals (e.g. rats or mice) are poisoned orally or by other suitable routes, treated with a mid-cell division blocker prior to execution and post-execution chromosome specimens of bone marrow cells are prepared for analysis of chromosomal aberrations.

This method is particularly suitable for the analysis of chromosomal aberrations that need to be taken into account following in vivo metabolic activation.

This method is not applicable if there is evidence that the substance to be tested or its metabolites do not reach the bone marrow.

6 Test method

6.1 Experimental animals and housing environment.

Healthy adult rodents, rats or mice are recommended, with at least 5 of each sex in each group. The animals should be acclimatised in the laboratory for at least 3-5 days and the difference in weight between

each sex at the start of the experiment should be limited to ±20%.

Laboratory animals and laboratory animal rooms should comply with the corresponding national regulations.

6.2 Subjects

6.2.1 Preparation of the test substance: The solid test substance should be dissolved or suspended in a suitable solvent and diluted to a certain concentration. Liquid reagents may be used directly or diluted. The test substance should be freshly prepared prior to use, otherwise it is necessary to verify that storage does not affect its stability.

6.2.2 Choice of solvent: The solvent does not cause toxic effects and does not react chemically with the test substance at the concentration chosen. The preferred solvent is a water-soluble solvent.

6.2.3 Dose setting: A pre-test should be performed to select the highest dose. When toxic, the highest dose can be determined as an indicator of death or inhibition of the mitotic index of bone marrow cells (above 50%). For the first sample collection, 3 doses should be set for analysis and for the second sample collection, only the highest dose group should be set.

If a single dose of 2000 mg/kg bw does not cause toxic effects, then only the 2000 mg/kg bw dose group will be established.

If the potential (desired) human exposure is too high, choose 2000 mg/kg/BW/d for 14 days or 1000mg/kg/BW/d Infection for >14 days was tested.

6.3 Controls: In each test, there should be a negative control group and a positive control group for each sex. Treatments are the same as for the subject group, except that no subjects are used.

6.3.1 Negative control: In addition to a solvent control (i.e. solvent only), a blank control should be provided if there is no documented or historical information confirming that the solvent used does not have a deleterious or mutagenic effect.

6.3.2 Positive control: The positive control should cause a significantly higher rate of structural chromosomal aberrations than the background information. The route of contamination may be different from that of the test. The positive control chosen should preferably be related to the type of subject. The following may be used: triethylenemelamine, ethyl methanesulphonate, ethyl nitrosonrea, mytomycin C and cyclophosphamide. cyclophosphamide.

6.4 The method of poisoning can be oral or any other suitable method of poisoning. It is usually done in a single dose, but if the dose is too high, it is possible to dose several times in one day, but each time should be several hours apart.

In general, the animals are poisoned once, but specimens are collected twice, i.e. each group is divided into two subgroups, subgroup 1 is killed 12h-18h after poisoning and the first specimen is collected; subgroup 2 collects the second specimen 24h after subgroup 1 is killed. If multiple staining is used, specimens are collected 12h to 18h after the last staining. A mid-cell division blocker (e.g. colchicine, administered at 4mg/kg body weight 4h prior to execution) is injected intraperitoneally prior to collection of specimens. The appropriate treatment time is 3h-5h if the animal is a mouse and 4h-5h if the animal is a Chinese hamster).

6.5 Test procedure

6.5.1 The animal is executed by cervical dislocation, the femur is removed and muscle and other tissues are removed.

6.5.2 The ends of the femur were cut off and 5mL of saline was drawn from one end of the femur using a syringe and a 10mL centrifuge tube was used to pick up the bone marrow cell suspension from the other end of the femur.

6.5.3 The cell suspension was centrifuged at 1000rpm for 5min-7min and the supernatant was removed.

6.5.4 Add 7mL of 0.075mol/L KCl solution, mix the cells gently with a dropper, place in a 37°C water bath for 7min, add 1mL~2mL of fixative (glacial acetic acid: methanol=1:3), mix well, centrifuge at 1000rpm for 5min~7min, discard the supernatant.

6.5.5 Add 7mL of fixing solution, mix well, fix for 15min, centrifuge at 1000rpm for 7min and discard the supernatant.

6.5.6 Fix 1 or 2 more times in the same way and discard the supernatant.

6.5.7 Add a few drops of fresh fixative and mix well.

6.5.8 Tablets are prepared by air-drying or flame-drying using drops in suspension.

6.5.9 Stained with Kimsa stain.

6.6 Film reading and results processing

6.6.1 Determination of mitotic index: includes all treatment groups, positive and negative controls (500-1000 counts per animal)

(one cell).

6.6.2 Counting aberrant cells: For each animal, select at least 100 well-dispersed mid-phase divisions and read them under the oil microscope. The number of chromosomes observed in the midphase should be limited to 2n±2 due to the loss of chromosomes in the midphase due to mechanical disruptions such as hypotrophy. The number of chromosomes in each observed cell should be recorded during the reading, and for aberrant cells the position of the coordinates of the microscopic field and the type of aberration should also be recorded. Gaps should be recorded and listed separately and are not usually counted as structural aberrations of chromosomes. The resulting chromosomal aberration rates for each group are statistically processed using, for example, the X^2 test to assess whether there is a significant difference between the test and control groups.

6.7 Evaluation of results

Each animal is treated as a test unit and data for each animal should be tabulated for statistical analysis. The rate of structurally aberrated cells (%) and the number of chromosomal aberrations per cell may be used as indicators for evaluation. There are several criteria for statistical analysis, mutagenicity is determined when the subject causes a statistically significant increase in the number of chromosomal aberrations with a dose-related increase or when there is a significant increase in the number of chromosomal aberrant cells in a single dose group, in a single time sampling of the test.

A combination of biological and statistical significance should be considered in the evaluation and further tests should be carried out with changes to the test conditions if no definite conclusions can be made.

7 Test reports

The report should include the following items

（1） The name of the test substance, its physical and chemical properties, the solvent used and its preparation.

（2） Species and strain of animal, weight, number, sex, source (specify certificate of conformity number and class of animal).

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal house certificate number.

（4） Doses and groups: principles of dose selection, doses and groups, negative and positive controls and doses.

（5） Test conditions and methods: route and protocol of transfection, methods of cytotoxicity determination, mid-cell division blockers used, their doses and sampling times, brief description of methods of chromosome preparation.

（6） The number of cells observed and analysed.

（7） Type and number of distortions and distortion rates.

（8） Conclusion.

8 Interpretation of results

Positive results demonstrate the ability of the test substance to cause chromosomal aberrations in the bone marrow cells of this species of animal.

The negative result indicates that the test substance did not cause chromosomal aberrations in the bone marrow cells of this species under the conditions of this test.

XII. In vivo mammalian cell micronucleus assay

Mammalian Erythrocyte Micronucleus Test

1 Scope

This specification specifies the basic principles, requirements and methods for the mammalian erythrocyte micronucleus test. This specification applies to the detection of chromosomal aberrations in cosmetic materials.

2 Normative references

GB14924 Standard for Laboratory Animals and Feed

OECD Guidelines for Testing of Chemicals（No.474，Adopted：21，July 1997）

3 Definition

Micronucleus: A chromatid monomer or a chromosome with a non-attachment point break, or an entire chromosome lost due to damage to the spindle, remains in the cytoplasm during late cell division. After the terminal phase, one or several regular secondary nuclei form alone and are contained within the cytoplasm of the daughter cell, called micronucleus because they are smaller than the main nucleus.

4 Principle

Any chemical that breaks chromosomes or damages chromosome and spindle junctions can be detected by the micronucleus test. Micronuclei can form in all types of bone marrow cells, but nucleated cells have little cytoplasm and micronuclei are difficult to distinguish from normal nuclear lobes and protrusions of the nucleus. Multistained erythrocytes are a stage in the development of late dividing erythrocytes from juvenile to mature erythrocytes, when the main nucleus of the erythrocyte has been expelled and the Kimsa stain is grey-blue due to the presence of ribosomes in the cytoplasm, while the ribosomes of mature erythrocytes have disappeared and are stained a pale orange-red. The presence of sufficient numbers of multistained erythrocytes in the bone marrow, the ease with which micronuclei can be identified and the low spontaneous rate of micronuclei make them the preferred cell population for micronucleus testing.

If the animal has been contaminated for more than 4 weeks, a micronucleus test may also be performed on peripheral blood positive stained red cells from the same endpoint. This method is not applicable if there is evidence that the substance to be tested or its metabolites do not reach the bone marrow.

5 Basic principles of the test

The animal is exposed to the test substance by appropriate means and after a certain period of time the animal is executed, the bone marrow (or peripheral blood) is removed, a smear is prepared, fixed, stained and the multistained erythrophils (red blood cells) containing micronuclei are counted under a microscope.

6 Instruments and apparatus

Biological microscope, dissecting scissors, forceps, haemostat, syringe, gavage needle, slides, coverslips (24mm x

(50mm), plastic suction bottles, gauze, filter paper, etc.

7 Reagents

7.1 Calf serum (inactivated)

The filtered calf serum is inactivated by holding it in a constant temperature water bath at 56°C for 30min. The inactivated calf serum is usually stored in a refrigerator freezer.

7.2 Giemsa staining solution

Ingredients: Kimsa dye	3.8g
Methanol	375mL
Glycerine	125mL

Preparation: grind the dyestuff and a small amount of methanol in a emulsion, add methanol to 375mL and glycerol, mix well and keep in a constant temperature oven at 37°C for 48h. During this period, shake several times to dissolve the dyestuff, remove and filter, use after two weeks.

7.3 1/15 mol/L phosphate buffer (pH 7.4)

Ingredients: Disodium hydrogen phosphate (Na2HPO4-12H2O)	19.077g
Potassium dihydrogen phosphate (KH_2PO_4)	1.814g
Add distilled water to	1000mL

Preparation: Dissolve the above two ingredients in distilled water. Check the pH value with a pH test paper.

7.4 Kimsa Application Solution

Mix one part of Giemsa dye with 6 parts of 1/15 mol/L phosphate buffer. Ready to use.

8 Laboratory animals and housing environment

Suitable mammals are suitable for this experiment, with mice or rats being recommended. The mouse is the usual animal for the micronucleus test. The animals should be acclimatised in the laboratory for at least 3-5 days and the difference in weight between the two sexes should be limited to ±20% at the beginning of the experiment.

Laboratory animals and laboratory animal rooms should comply with the corresponding national regulations.

9 Dose grouping

Doses of 1/2, 1/5, 1/10, 1/20 of the LD_{50} of the subject are usually taken to obtain a dose-response curve for the micronucleus. When the LD_{50} is greater than 5g/kg body weight, the highest dose of 5g/kg body weight should be taken, usually at least 3 doses. Each dose group should consist of 10 animals, 50/50 females and males. A solvent control and a positive control group should also be used. Cyclophosphamide is commonly used as a positive control at a dose of 40mg/kg bw.

If the potential (desired) human exposure is too high, choose 2000 mg/kg/BW/d for 14 days or 1000mg/kg/BW/d Infection for >14 days was tested.

The solvent to be used for the test substance is determined by the physicochemical properties of the test substance (water-soluble and/or fat-soluble), usually water, vegetable oil or edible starch etc.

10 Ways and means of contamination

The route of contamination depends on the purpose of the experiment and is recommended to be by oral gavage. The 30h double dose method is used, i.e. 24h between doses and 6h after the second dose.

11 Test method

11.1 Sample preparation

After the animal has been dislocated and executed, the thoracic cavity is opened, the sternal stalk is cut

along the junction with the ribs, the muscle attached to it is stripped away, the blood is wiped away, the sternum is cut horizontally to expose the marrow cavity, and then the marrow fluid is squeezed out with haemostatic forceps.

Peripheral blood samples for prolonged poisoning should be taken from the caudal or ear vein, usually in two separate sessions between 18-24h and 36-48h after the first poisoning.

11.2 Smear

Bone marrow fluid (peripheral blood) is applied to a drop of calf serum at one end of the slide and mixed carefully. Generally speaking, it is appropriate to apply one slice of marrow from two sternum sections. The smear is then applied in the usual blood smear method and dried in the air for approximately 2cm to 3cm. If staining is done immediately, it needs to be placed above the flame of an alcohol lamp and slightly baked.

11.3 Fixed

The dried smear is fixed in methanol for 5 min. and should be fixed and stored even if not stained on the same day.

11.4 Dyeing

The fixed smears were placed in Giemsa application solution, stained for 10 min to 15 min and then immediately rinsed with 1/15 mol/L phosphate buffer.

11.5 Cover film

Dry the water droplets on the back of the stained piece with filter paper, then gently press the stained piece with a double layer of filter paper to absorb the residual water on the stained piece, then shake it several times in the air to dry it as soon as possible, then put it into xylene for 5 min transparently, remove it and apply an appropriate amount of optical resin glue, cover the coverslip and write the label.

11.6 Observation and counting

The cells are first examined cursorily with low magnification and then with high magnification, and areas of uniform distribution, undamaged cells and appropriate staining are selected and counted under oil immersion microscopy. Although nucleated cells containing micronuclei are not counted, the morphological integrity of the nucleated cells should be used as a criterion for judging the quality of the production.

This method looks at red blood cells containing micronuclei. Multistained red blood cells are grey-blue in colour and mature red blood cells are pale orange in colour. The micronuclei are mostly single and round, with smooth and neat margins, and the chromophobia is consistent with the nucleoplasm, which is purplish or bluish-purple.

A minimum of 2000 multistained erythrocytes (erythrocytes) are counted per animal. The micronucleus rate refers to the number of erythrocytes containing micronuclei and is expressed in thousands (‰). If two or more micronuclei are present in one pleomorphic erythrocyte, the count is still based on one micronucleated cell.

Micronucleus tests performed by automated image analysis systems with flow cytometry, validated or confirmed by the Cosmetic Standards Committee, are acceptable as an alternative to this method.

12 Data processing and judgement of results

12.1 Data processing

Means and standard deviations of micronuclei rates for each group are reported and micronuclei rates for each dose group of subjects are compared with solvent controls using appropriate statistical methods such as the Poinsson distribution u test.

If there is no evidence of gender differences in the data obtained, data from both sexes can be combined for statistical analysis.

12.2 Result determination

A combination of biological and statistical significance should be considered in the evaluation. A positive micronucleus test may be considered if there is a statistically significant increase in the micronucleus rate for a single dose method compared to a solvent control group, and if there is a statistically significant difference between the dose groups for multiple dose methods and a dose-response relationship.

13 Test reports

The test report shall include the following.

（1） Name of the test substance, physical and chemical properties, method of preparation, solvent used.

（2） Species and strain of animal, weight, number, sex, source (specify certificate of conformity number and class of animal).

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal room qualification number.

（4） Dose groupings, routes and modes of contamination.

（5） Test methods: brief description of the operational steps, statistical methods used, criteria for determining results.

（6） RESULTS: The incidence of micronuclei in the bone marrow cells of the animals is reported in tabular form for the subjects (Table 1).

（7） Conclusion.

Table 1 Incidence of multistained erythrophilic micronuclei in bone marrow of ××× vs.

Group	Dosage (g/kg body weight)	Number of animals (only)	Number of cells examined (pcs)	Number of micronucleated cells (pcs)	Micronucleus rate (‰)	P-value
Subjects						
Solvent control						
Positive control	(mg/kg body weight)					

XIII. Testicular germ cell chromosome aberration test

Testicle Cells Chromosome Aberration Test

1 Scope

This specification specifies the basic principles, requirements and methods for chromosomal aberration tests on primary spermatocytes of the mammalian testis. This specification applies to the genotoxicity testing of cosmetic raw materials.

2 Normative references

GB15193 Procedures and methods for the toxicological evaluation of food safety GB15193.8-2003

3 Purpose of the test

Detection of chromosomal damage in germ cells of male animals to evaluate the mutagenic potential of the test substance.

4 Basic principles of the test

The animals were exposed to the subjects by appropriate routes and after a certain period of time the animals were executed, treated with a mid-cell division blocker prior to execution and after execution chromosome specimens of primary testicular spermatocytes were prepared and observed under the microscope for chromosomal aberrations.

This method is particularly suitable for the analysis of chromosomal aberrations that need to be taken into account following in vivo metabolic activation.

This method is not applicable if there is evidence that the substance to be tested or its metabolites do not reach the testes.

5 Instruments and apparatus

Biological microscope, centrifuge, dissecting scissors, forceps, centrifuge tubes, flat dishes, syringes, gavage needles, slides, coverslips (24mm x 50mm), etc.

6 Reagents

6.1 Colchicine 0.04%: 40mg of colchicine in saline to 100mL.

6.2 Trisodium citrate 1%: Take 1g of trisodium citrate and add distilled water to 100mL.

6.3 0.075 mol/L potassium chloride solution: take 5.59 g of potassium chloride and add distilled water to 1000 mL.

6.4 Methanol/glacial acetic acid (3:1, v/v) fixative: ready to use.

6.5 60% glacial acetic acid: take 60mL of glacial acetic acid and add distilled water to 100mL, all freshly prepared.

6.6 pH 6.8 phosphate buffer.

6.6.1 1/15 mol/L disodium hydrogen phosphate solution: disodium hydrogen phosphate ($_{Na2HPO4}$) 9.47 g, add distilled water to 1000 mL.

6.6.2 l/15 mol/L potassium dihydrogen phosphate solution: 9.07 g of potassium dihydrogen phosphate ($_{KH2PO4}$), add distilled water to 1000 mL.

6.6.3 Mix 50mL of disodium hydrogen phosphate solution with 50mL of potassium dihydrogen phosphate solution.

6.7 Kimsa dye solution.

6.7.1 The solution was dissolved and then 125 mL of pure glycerol was added and kept at 37°C for 48h, during which time it was shaken several times and then filtered for 1-2 weeks.

6.7.2 Kimsa application solution: add lmL of stock solution to 10mL of pH 6.8 phosphate buffer.

6.8 Physiological saline, methanol.

7 Laboratory animals and housing environment

Suitable male rodents are used for this experiment. It is recommended to use mice, 6 to 8 weeks of age, weighing 30 g to

The animals should be acclimatised in the laboratory for at least 5 days and the difference in weight at the start of the experiment should be limited to ±20%. Laboratory animals and laboratory animal houses should comply with the appropriate national regulations.

8 Dose grouping

Subjects are given at least three dose groups. Dose 1/2, 1/5, 1/10 or 1/20 LD_{50} respectively. When the LD_{50} is greater than 5 g/kg body weight, the highest dose is 5 g/kg body weight. A negative (solvent) control group and a positive control group are also available. In the positive control group, cyclophosphamide (40mg/kg bw) or mitomycin C (1.5mg/kg bw to 2mg/kg bw) is administered intraperitoneally. At least 5 surviving animals in each group.

The solvent to be used for the test substance is determined by the physicochemical properties of the test substance (water-soluble and/or fat-soluble), usually water, vegetable oil or edible starch etc.

9 Ways and means of contamination

Depending on the purpose of the experiment, oral gavage is the recommended method of administration. The animals should be poisoned once a day (if the dose is too high, it is possible to poison them several times a day, but at intervals of several hours) for 5 d. The animals should be executed between 12 and 14 d after the first poisoning. The animals were injected intraperitoneally with a 0.04% colchicine solution at a dose of 4mg/kg bw 6h before execution.

10 Test method

10.1 Collection of materials

The testes were removed from both sides, cleaned of fat, washed in saline to remove hair and blood and placed in a small flat dish containing an appropriate amount of 1% trisodium citrate or 0.075 mol/L potassium chloride solution.

10.2 Production

10.2.1 Hypotonicity: the peritoneum is torn open with ophthalmic forceps and the varicose tubes are gently separated and hypotonic at room temperature, the duration of hypotonicity depends on the temperature and other specific conditions, usually between 15min and 25min.

10.2.2 Fixation: carefully aspirate the hypotonic solution and add 10mL of fixative (methanol: glacial acetic acid = 3:1) to fix. Fix for no more than 15 min the first time, pour off the fixative and then add a new fixative for more than 20 min.

10.2.3 Centrifugation: aspirate all the fixative, add 1mL to 2mL of 60% ice acetic acid, add twice the amount of fixative as soon as most of the spermatozoa have softened, beat well, transfer to a centrifuge tube, centrifuge at 1000rpm for 10min and fix again.

10.2.4 The cell suspension is then evenly dripped onto an ice-cold water slide and air-dried or flame-dried to make the slides.

10.2.5 Staining: Stain with 1:10 Kimsa stain (pH 6.8) for 20 min to 40 min.

10.3 Cover film

Dry the water droplets on the back of the stained piece with filter paper, then gently press the stained piece with a double layer of filter paper to absorb the residual water on the stained piece, then shake it

several times in the air to dry it as soon as possible, then put it into xylene for 5 min transparently, remove it and apply an appropriate amount of optical resin glue, cover the coverslip and write the label.

10.4 Read the film

10.4.1 Film review requirements

Look for intermediate split phases with a clear background, good dispersion and moderate chromosome shrinkage in sequence under low magnification, then analyse them under oil microscopy. The number of chromosomes observed in the mid-phase should be n pairs of bivalents, and at least 100 primary spermatocytes in the mid-phase division should be analysed per animal, as mechanical disruptions such as hypotonicity can lead to loss of chromosomes in the mid-phase. The position of the coordinates of the microscopic field and the type of aberration should also be recorded for aberrant cells.

10.4.2 Observation Items

10.4.2.1 Changes in chromosome structure

10.4.2.1.1 Breakage: damage greater than the width of the chromosome.

10.4.2.1.2 Microsomes: smaller and more rounded than broken pieces.

10.4.2.1.3 Polyvalent: During meiosis, chromosomal translocations can produce ring-like polyvalents, or chain-like polyvalents. The rate of spontaneous translocations in control adults is very low, less than 0.01%. There may be a slight increase in older animals.

10.4.2.2 X-Y and autosomal monovalents

Monovalent X-Y and autosomal chromosomes are also known as premature segregation. In control animals, X-Y monosomy is more common, with about 0-10%. The separation of X and Y can often cause sterility. Monosomy of autosomes is caused by non-association (absence of paired congeners between homologous segments), or loss of association (separation due to crossover failure); they are less common in control animals because crossovers form at the bilineage stage and normally paired associations continue until the end of Intermediate I. It often occurs in the smallest pair of autosomes.

11 Data processing and judgement of results

The percentage of cells with aberrant chromosome structure (%), X-Y and autosomal monovalents were calculated separately. Statistical treatment was performed using the ^{X2} test or other appropriate significance test. A positive test result was judged when there was a significant increase in aberrant cell rate in each dose group compared to the negative (solvent) control group with a dose-response relationship; or when there was a significant and reproducible increase in only one dose group.

A combination of biological and statistical significance should be considered in the evaluation. When no definitive conclusion can be made, further tests should be carried out with changes to the test conditions.

12 Test reports

The test report shall include the following.

（1） The name of the test substance, its physical and chemical properties, the method of preparation, the solvent used and the formulation.

（2） Species and strain of animal, weight, number, source (specify the certificate of conformity number and class of animal).

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal room qualification number.

（4） Doses and groups: principles of dose selection, doses and groups, negative and positive controls and doses.

（5） Test conditions and methods: route and protocol of staining, mid-cell division blockers used and their doses and sampling times, brief description of the method of chromosome preparation, statistical methods used.

（6） The cell types and cell numbers observed and analysed.

（7） Type and number of distortions and distortion rates.

（8） Conclusion.

13 Interpretation of results

Positive results demonstrate the ability of the test substance to cause chromosomal aberrations in the testicular germ cells of this species.

The negative results indicate that the test subjects did not cause chromosomal aberrations in the testicular germ cells of this species under the conditions of this test.

XIV. Subchronic oral toxicity test

Subchronic Oral Toxicity Test

1 Scope

This specification specifies the basic principles, requirements and methods for subchronic oral toxicity testing in rodents. This specification applies to the detection of subchronic oral toxicity of cosmetic ingredients.

2 Normative references

OECD Guidelines for Testing of Chemicals（No.408，Sep. 1998）

3 Purpose of the test

In the estimation and evaluation of the toxicity of cosmetic ingredients, information on the acute toxicity of the test substance is followed by a sub-chronic oral toxicity test. This test not only provides information on the health effects caused by repeated exposure to the test substance over a certain period of time, the target organ and the accumulation capacity of the test substance, but also allows the estimation of the level of no harmful effects of the exposure, which can be used to select and determine the level of exposure for chronic tests and the preliminary calculation of the safety level for population exposure.

4 Definition

4.1 Subchronic oral toxicity

It is an adverse reaction caused by repeated daily oral exposure to the test substance during part of the survival period of the experimental animal.

4.2 No-adverse-effect level

It is the maximum dose of the test that does not cause any harmful effects and can be expressed as the weight of the test substance per unit of animal body weight per day (mg/kg). When the test substance is mixed into the animal's feed or drinking water for poisoning, the weight of the test substance per kilogram of feed or per millilitre of drinking water (mg/kg, mg/mL) can be expressed.

5 Basic principles of the test

Groups of animals were orally administered with different doses of the test substance daily for 90 d. One dose was used for each group. The animals are observed daily for toxic reactions during the period of exposure. Animals that die during the period of toxicity are subjected to necropsy. All surviving animals are put to death at the end of the period of toxicity and are subjected to post-mortem examination and appropriate pathological histological examination.

6 Test method

6.1 Laboratory animals and housing environment

6.1.1 Selection of animal breeding lines

Rodents are routinely selected, with rats being preferred. Rats between 6 and 8 weeks of age are generally used. Animal weights should not vary by more than 10% of the average animal weight. If the test is a preparatory test to a chronic test, the same strain of animals should be used in both tests.

6.1.2 Sex and number of animals

There should be at least 20 animals (half male and half female) in each dose group, but given the importance of sub-chronic trials, the number of male and female animals in each group should be increased as appropriate. If animals are planned to be killed during the course of the test, the number of animals planned to be killed should be increased. The number of animals at the end of the test needs to be such that the toxic effects of the test substance can be effectively evaluated. In addition, a follow-up group of 20 animals (half males and half females) may be established and given the highest dose of the test substance for 90 d. Observation should continue for a period of time (usually not less than 28 d) after the full dose has been administered to determine the persistence, reversibility or delayed toxic effects.

6.1.3 Rearing environment

Laboratory animals and laboratory animal houses should comply with the corresponding national regulations. Conventional feed is selected and water is not restricted.

6.2 Dose grouping

The test should be carried out with at least three contaminated groups and one control group. The control group should be subjected to the same conditions as the test group, except that it is not exposed to the test substance. The maximum dose should be designed to cause toxic effects without causing excessive animal mortality, otherwise the evaluation of the results will be affected. The low dose group should not show any toxic effects. If population exposure levels are known, the lowest dose should be higher than the actual population exposure level. The intermediate dose group should cause milder observable toxic effects. If multiple intermediate dose groups are established, the doses in each group should cause different levels of toxic effects. In the intermediate and low dose groups and in the control group, animal mortality should be low to ensure that meaningful conclusions can be drawn.

For those substances of lower toxicity, special care should be taken when poisoning by feed to ensure that the mixing of large quantities of the test substance does not affect the normal nutrition of the animal. Special instructions should be given for other methods of poisoning. If gavage is used, the dose should be administered at the same time each day and adjusted regularly (weekly) by body weight to maintain a constant level of toxicity per body weight.

In this test, if no observable toxic effect is produced at exposure levels above 1000 mg/kg and the toxicity of the subject can be expected based on the structural compound of interest, a full test observation at three dose levels may be considered unnecessary.

6.3 Test procedure

At least 5 d should be allowed for the animals to acclimatise to the laboratory environment before the start of the infection. The animals are randomly grouped. Subjects can be contaminated by mixing with feed or water, by direct feeding and by gavage. The animals are contaminated 7 d per week. All animals should be contaminated in exactly the same way during the test. If other solvents or additives are added for the purpose of poisoning, these should not affect the absorption or cause toxic effects.

6.4 Clinical observations

The observation period should be at least 90 d. A further 28 d should be added to the follow-up group without any treatment to understand the reversibility, persistence and delayed toxic effects.

Any signs of toxicity in the animal during observation should be recorded, including the time of occurrence, extent and duration. Observations should include at least the following: changes in skin and coat, eye and mucous membrane changes, respiratory, circulatory, vegetative and central nervous system, limb movements and behavioural activity. Weekly feed consumption (or drinking water consumption when poisoned through drinking water) should be calculated and weekly weight changes recorded.

6.5 Clinical Examination

6.5.1 Ophthalmic examinations

An ophthalmic examination using an ophthalmoscope or other relevant equipment should preferably be carried out on all experimental animals, at least in the highest dose group and in the control group, before and after the animals have been poisoned. All animals should be examined if ophthalmic changes are found.

6.5.2 Blood tests

Blood cell volume, haemoglobin concentration, red blood cell count, total white blood cell count and classification should be measured before, during and at the end of the period of poisoning and at the end of

the follow-up period.

6.5.3 Clinical blood biochemistry tests

The tests are carried out before, during and at the end of the period of poisoning and at the end of the follow-up period and include electrolyte balance, carbohydrate metabolism, liver and kidney function. Other specific tests may be selected depending on the form of action of the test. Recommended tests include: calcium, phosphorus, chloride, sodium, potassium, fasted blood glucose (different fasting periods for different animal strains), serum glutamic aminotransferase, serum glutamic oxaloacetic aminotransferase, ornithine decarboxylase, glutamyl transpeptidase, urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein. Analysis of lipids, hormones, acid-base balance, nor-iron haemoglobin and cholinesterase activity may be measured if necessary. In addition, other broader clinical biochemical tests may be performed based on the toxic effects observed to allow for a full toxicity evaluation.

6.5.4 Urine test

This is not normally required and urine testing is only required when toxic effects are suspected or observed.

6.6 Pathological examination

6.6.1 Gross autopsy

All animals should undergo a full gross necropsy covering the animal's appearance, all orifices, the thoracic and abdominal cavities and their contents. The liver, kidneys, adrenal glands, testes, epididymis, uterus, ovaries, thymus, spleen, brain and heart should be weighed as soon as possible after separation to prevent loss of water. The following tissues and organs should be preserved in fixative for later pathological histological examination: all organs with abnormal gross anatomical presentation, brain (including medulla oblongata/pontine, cerebellum and cerebral cortex, pituitary gland), thyroid/parathyroid, thymus, lung/trachea, heart, aorta, salivary glands*, liver, spleen, kidney, adrenal glands, pancreas, gonads, uterus, reproductive appendages*, skin*, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, prostate, representative lymph nodes, female breast*, thigh muscles*, peripheral nerves, sternum (including bone marrow), eye*, femur (including articular surfaces)*, spinal cord (including neck, chest, lumbar region)* and lacrimal gland*.

*These organs need to be examined only if toxic effects are suggested or if they are the target organ being studied.

6.6.2 Pathological histological examination

The following organs and tissues should be examined.

（1） Significant and potentially damaged organs or tissues of all animals in the highest dose group and the control group should be extended to corresponding organs and tissues in other dose groups if there are pathological histological lesions in the organs or tissues of animals in the high dose group.

（2） Gross anatomy of organs or tissues with abnormalities was seen in all dose groups.

（3） Target organs of animals in other dose groups.

（4） In the follow-up group, those tissues and organs that exhibit toxic effects in the infected group should be examined.

7 Evaluation of test results

7.1 Processing of results

The results of the test may be summarised in tabular form showing the number of animals in each group at the start of the test, the number of animals showing injury, the type of injury and the percentage of animals with each type of injury. All data should be evaluated using an appropriate statistical method, which should be determined at the time of test design.

7.2 Evaluation of test results

The results of the sub-chronic oral toxicity test should be evaluated in conjunction with the results of the previous tests, taking into account the indicators of toxic effects and the results of the autopsy and pathological histological examination. The toxicity evaluation should include the relationship between the dose at which the subject was infected and the presence or absence of toxic reactions, the incidence of toxic reactions and their magnitude. These reactions include behavioural or clinical abnormalities, visible damage, target organs, changes in body weight, mortality effects and other general or specific toxic effects. Successful subchronic trials should be able to present statistically significant levels of no harmful effects.

7.3 Test reports

The test report shall include the following.

（1） Name of the test substance, physicochemical properties, method of preparation, concentration used.

（2） The species, strain and origin of the experimental animal (indicating the certificate of conformity number and animal class).

（3） The laboratory animal housing environment, including feed source, room temperature, relative humidity, single cage housing or group feeding, and laboratory animal housing certification number.

（4） Test methods.

（5） Toxicity response data by sex and dose.

（6） The time at which the animal died during the test or whether the animal survived at the end of the contamination.

（7） Toxic effects or other effects.

（8） The timing of each abnormal symptom observed and its regression.

（9） Food intake and animal weight information.

（10） Ophthalmic findings.

（11） Haematological findings.

（12） Clinical biochemical findings.

（13） What the autopsy found.

（14） A detailed description of what is seen on pathological histological examination.

（15） Statistical methods for the processing of the results.

（16） Conclusion.

8 Interpretation of test results

The sub-chronic oral toxicity test can provide information on the toxic effects of a test substance at repeated oral exposure. The test results can be extrapolated to a very limited extent to humans, but it can provide useful information for determining the NOAEL and Permissible Exposure Level (PEL) for population exposure.

XV. Subchronic percutaneous toxicity test

Subchronic Dermal Toxicity Test

1 Scope

This specification specifies the basic principles, requirements and methods for subchronic percutaneous toxicity testing in rodents. This specification applies to the detection of subchronic percutaneous toxicity of cosmetic ingredients.

2 Normative references

OECD Guidelines for Testing of Chemicals（No.411，May 1981）

3 Purpose of the test

When estimating and evaluating the toxicity of cosmetic ingredients, the acute percutaneous toxicity of the test substance is followed by a sub-chronic percutaneous toxicity test. This test not only provides information on the health effects that may be caused by repeated exposure over a certain period of time, but also provides a basis for evaluating the percutaneous permeability of the test substance, the target organ of action and the dose selection for chronic dermal toxicity tests.

4 Definition

4.1 Subchronic dermal toxicity

It is an adverse reaction caused by repeated daily percutaneous exposure to the test substance during part of the survival period of the experimental animal.

4.2 No-adverse-effect level

It is the maximum dose of toxicity that does not cause any harmful effects in the test. It is expressed as the weight of the test substance given per unit of animal body weight per day (mg/kg).

5 Basic principles of the test

The animals were given daily percutaneously at different doses for 90 d. One dose was used for each group. The animals were observed daily for toxic reactions during the period of exposure. Animals that died during the period of toxicity were subjected to necropsy. At the end of the period, all surviving animals are put to death and post-mortem examination and appropriate pathological histology is performed.

6 Test method

6.1 Subjects

If the subject is a solid, it should be crushed and well moistened with water (or an appropriate medium) to ensure good contact between the subject and the skin. If a medium is used, consideration should be given

to the effect of that medium on the skin permeability of the subject. Liquid subjects generally do not need to be diluted.

6.2 Laboratory animals and housing environment

6.2.1 Selection of animal breeding lines

Adult rats, rabbits or guinea pigs may be used for the test, or animals of other species may be used. When the subchronic test is used as a preparatory test to the chronic test, the same strain of animal should be used in both tests.

6.2.2 Sex and number of animals

There should be at least 20 animals (half male and half female) with healthy skin in each dose group. If animals are planned to be killed in the course of the test, the number of animals planned to be killed should be increased. In addition, a follow-up group of 20 animals (half male and half female) may be used to administer the highest dose for 90 d and continue to be observed for a period of time (usually not less than 28 d) after the full dose has been administered to determine the persistence, reversibility or late onset of toxic effects.

6.2.3 Rearing environment

Laboratory animals and laboratory animal houses should comply with the corresponding national regulations. Conventional feed is selected and water is not restricted.

6.3 Dose grouping

The test should be carried out with at least three contaminated groups and one control group. The control group should be subjected to the same conditions as the test group, except that it is not exposed to the test substance. The maximum dose should be designed to cause toxic effects without causing excessive animal mortality, otherwise the evaluation of the results will be affected. The low dose group should not show any toxic effects. If population exposure levels are known, the lowest dose should be higher than the actual population exposure level. The intermediate dose group should cause milder observable toxic effects. If multiple intermediate dose groups are established, the doses in each group should cause different levels of toxic effects. In the intermediate and low dose groups and in the control group, animal mortality should be low to ensure that meaningful conclusions can be drawn.

If the test substance causes severe skin irritation, the concentration of the test substance should be reduced, although this may lead to a reduction or disappearance of other toxic effects originally seen at higher doses. If the skin of the animal is severely damaged early in the test, it may be necessary to terminate the test and restart it at a lower concentration.

In this test, if no observable toxic effect is produced at exposure levels above 1000 mg/kg and the toxicity of the subject can be expected based on the structural compound of interest, a full test observation at three dose levels may be considered unnecessary.

6.4 Test procedure

The animals should be acclimatised in a laboratory environment for at least 5 d prior to testing. 24h prior to the test, the coat is cut or shaved from the dorsal area of the trunk. The infected area should be debrided approximately weekly. Care should be taken when using scissors or razors to prevent damage to the animal's skin and consequent changes in skin permeability. The area of the area to be contaminated should not be less than 10% of the animal's body surface area and should be determined by measuring the animal's body weight. If the test is more toxic, the area may be relatively small, but the test should be applied as thinly and evenly as possible over the entire area. Cellophane and non-irritating tape should be used to hold the test in place during the staining operation to ensure good contact with the skin and to prevent licking by the animal.

During the 90-d test period, the animals are exposed for 6 h per day, 7 days per week, while the follow-up group is observed for an additional 28 d in order to understand the persistence, reversibility and delayed toxic effects.

6.5 Clinical observations

A careful clinical examination should be carried out at least once a day during the trial.

Any signs of toxicity in the animal during observation should be recorded, including the time of occurrence, extent and duration. Cageside observations should include at least the following: changes in skin and coat, eye and mucous membrane changes, changes in respiration, circulation, vegetative and central nervous system, limb movements and behavioural activity. Weekly feed consumption should be calculated and weekly weight changes recorded.

6.6 Clinical Examination

6.6.1 Ophthalmic examinations

An ophthalmic examination using an ophthalmoscope or other relevant equipment should preferably be

carried out on all experimental animals, at least in the highest dose group and in the control group, before and after the animals have been poisoned. All animals should be examined if ophthalmic changes are found.

6.6.2 Blood tests

Measurements should be taken before, during and at the end of the period of poisoning and at the end of the follow-up period, including haematocrit, haemoglobin concentration, red blood cell count, total white blood cell count and classification, and coagulation, such as clotting time, prothrombin time, prothrombin time or platelet count, if necessary.

6.6.3 Clinical blood biochemistry tests

The tests are carried out before, during and at the end of the period of exposure and at the end of the follow-up period and include electrolyte balance, carbohydrate metabolism, liver and kidney function. Other specific tests may be selected depending on the form of action of the test. Recommended tests include: calcium, phosphorus, chloride, sodium, potassium, fasted blood glucose (different fasting periods are used for different animal strains), serum glutamic aminotransferase, serum glutamic oxaloacetic aminotransferase, ornithine decarboxylase, glutamyl transpeptidase, urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein. Analysis of lipids, hormones, acid-base balance, nor-iron haemoglobin and cholinesterase activity may be measured if necessary. In addition, other broader clinical biochemical tests may be performed based on the toxic effects observed to allow for a full toxicological

Sexual evaluation.

6.6.4 Urine test

This is not normally required and urine testing is only required when toxic effects are suspected or observed.

6.7 Pathological examination

6.7.1 Gross autopsy

All animals should undergo a full gross necropsy, covering the appearance of the body, all orifices, the thoracic and abdominal cavities and their contents. The liver, kidneys, adrenal glands and testes, epididymis, uterus, ovaries, thymus, spleen, brain and heart should be weighed as soon as possible after separation to prevent loss of water. The following tissues and organs should be preserved in fixative for later pathological histological examination: all organs with abnormal gross anatomical presentation, brain (including medulla oblongata/pontine, cerebellum and cortex, pituitary gland), thyroid/parathyroid, thymus, lung/trachea, heart, aorta, salivary glands*, liver, spleen, kidney, adrenal glands, pancreas, gonads, uterus, reproductive appendages*, skin*, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, prostate, representative lymph nodes, female breast*, thigh muscles*, peripheral nerves, sternum (including bone marrow), eye*, femur (including articular surfaces)*, spinal cord (including neck, chest, lumbar region)* and lacrimal gland*.

*These organs need to be examined only if toxic effects are suggested or if they are the target organ being studied.

6.7.2 Pathological histological examination

Pathological histological examination should be performed on the following organs and tissues.

（1） Significant and potentially damaged organs or tissues of all animals in the highest dose group and the control group, and if there are pathological histological lesions in organs or tissues of animals in the high dose group extend to the corresponding organs and tissues of the other dose groups.

（2） Gross anatomy of organs or tissues with abnormalities was seen in all dose groups.

（3） Target organs of animals in other dose groups.

（4） For the follow-up group, those tissues and organs that exhibit toxic effects in the infected group should be examined.

7 Evaluation of test results

7.1 Processing of results

The results of the test may be summarised in tabular form showing the number of animals in each group at the start of the test, the number of animals showing injury, the type of injury and the percentage of animals with each type of injury. All data should be evaluated using an appropriate statistical method, which should be determined at the time of test design.

7.2 Evaluation of test results

The results of the subchronic percutaneous toxicity test should be evaluated in conjunction with the results of the previous tests, taking into account the indicators of toxic effects and the results of the autopsy and pathological histological examination. The toxicity evaluation should include the relationship between the dose at which the subject was infected and the presence or absence of toxic reactions, the incidence of toxic reactions and their magnitude. These reactions include behavioural or clinical abnormalities, visible

damage, target organs, changes in body weight, mortality effects and other general or specific toxic effects. Successful subchronic trials should be able to present statistically significant levels of no harmful effects.

7.3 Test reports

The test report shall include the following.

（1） The name of the test substance, its physical and chemical properties, the method of preparation, the dose of poisoning, the area of poisoning and the manner of poisoning.

（2） The species, strain and origin of the experimental animal (indicating the certificate of conformity number and animal class).

（3） The housing environment, including source of feed, room temperature, relative humidity, single cage housing or group feeding, and laboratory animal house conformation number.

（4） Test methods.

（5） Toxicity response data by sex and dose.

（6） The time at which the animal died during the experiment or whether the animal survived at the end of the experiment.

（7） Toxic effects or other effects.

（8） The time at which each abnormal symptom was observed and its regression.

（9） Food intake and animal weight information.

（10） Ophthalmic findings.

（11） Haematological findings.

（12） Clinical biochemical findings.

（13） What the autopsy found.

（14） A detailed description of what is seen on pathological histological examination.

（15） Statistical methods for the processing of the results.

（16） Conclusion.

8 Interpretation of test results

The sub-chronic percutaneous toxicity test can provide information on the toxic effects of a test substance at repeated percutaneous exposures. The results of the test can be extrapolated to a very limited extent to humans, but it can provide useful information for determining the NOAEL and Permissible Exposure Level (PEL) for population exposure.

XVI. Teratogenicity test

Teratogenicity Test

1 Scope

This specification specifies the basic principles, requirements and methods of animal teratogenicity testing. This specification is used to test the teratogenicity of cosmetic ingredients.

2 Normative references

OECD Guidelines for Testing of Chemicals (No. 414, January 2001) Procedures and Methods for the Toxicological Evaluation of Food Safety (GB15193.14-2003).

3 Purpose of the test

To test for the possibility of fetal malformations in pregnant animals following exposure to cosmetic ingredients.

4 Definition

Teratogenicity: The property of a chemical that causes permanent structural and functional abnormalities in fetal mice during embryonic development.

5 Basic principles of testing

Pregnant animals are poisoned during the organogenesis phase of embryonic development, executed before birth and removed for examination of skeletal and visceral malformations.

6 Test method

6.1 Reagents

6.1.1 Formaldehyde, glacial acetic acid, 2,4,6-trinitrophenol, potassium hydroxide, glycerol, chloral hydrate, alizarin red.

6.1.2 Alizarin Red stock solution: Alizarin Red saturated solution, 50% acetic acid saturated solution 5.0mL, glycerol 10.0mL, 1% chloral hydrate 60.0mL mixed in a brown bottle.

6.1.3 Alizarin Red Application Solution: Take 3mL to 5mL of stock solution and dilute to 1000mL with 1g to 2g/100mL of potassium hydroxide solution in a brown bottle.

6.1.4 Alizarin Red solution: Alizarin Red 0.1g, potassium hydroxide 10g, distilled water 1000mL.

6.1.5 Clear Solution A: 200mL of glycerine, 10g of potassium hydroxide, 790mL of distilled water.

6.1.6 Clear Liquid B: Glycerine mixed with distilled water in equal parts.

6.1.7 Fixative (Bouins solution): 2,4,6-trinitrophenol (picric acid saturated solution) 75 parts, formaldehyde 20 parts, glacial acetic acid 5 parts

Portions.

6.2 Laboratory animals and housing environment

Animal selection: Healthy, sexually mature rats are preferred.

Laboratory animals and laboratory animal rooms should comply with the corresponding national regulations.

6.3 Dosing and grouping

At least three dose groups should be established and the highest dose should cause some toxicity in females, but should not cause mortality in more than 10% of the animals. The lowest dose should not cause observable toxic reactions. A negative control group should be established. At least 12 pregnant rats in each group. When teratogenicity tests are first carried out or when new animal species and strains are used, a positive control group must be set up at the same time.

6.4 Test procedure

6.4.1 Detection of "pregnant mice" and timing of administration of subjects

Female and male rats are caged 1:1 (or 2:1) together and the clot (or vaginal smear) is observed every morning. The day on which the clot (or sperm) is detected is defined as day zero of the gestation period. If no 'fertilised rat' is detected within 5 d, the female should be switched. The "fertilized rats" detected are grouped at random. The test is given orally daily from 6d to 15d of gestation. Pregnant rats are weighed at 0, 6, 10, 15 and 20 d of gestation and the amount of substance administered is adjusted according to body weight.

6.4.2 Execution and general examination of pregnant rats

The rats were executed on the 20th day of gestation. The ovaries were examined for the number of corpus luteum, the uterus was removed and weighed; the number of live fetuses, early resorptions and stillbirths were examined.

6.4.3 Live fetal rat examination

Record the weight, body length and tail length of the fetus one by one, and check the appearance of the fetus for any abnormalities such as bulging brain, exposed brain, small head, small ears, small eyes, no eyes and open eyes, harelip, cleft jaw, abdominal wall cleft, umbilical hernia, curved spine, small limbs, short limbs, parallel toes, multiple toes, no toes and other deformities, short tail, curled tail, no tail, and atresia of the anus.

6.4.4 Preparation and examination of fetal rat bone specimens

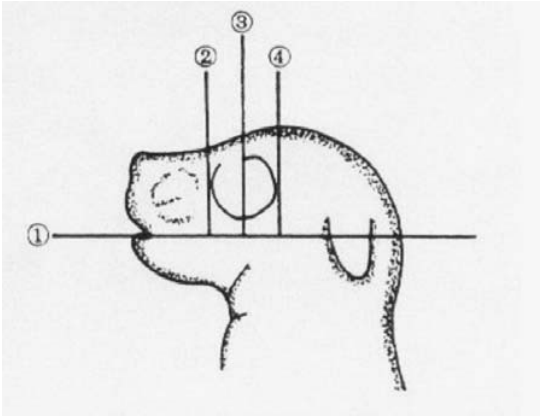
The fetuses were removed from the litter (or the skin, viscera and fat could be removed), rinsed in running water for a few minutes and placed in 1g-2g/100mL of potassium hydroxide solution (at least 5 times the volume of the litter) for 8h-72h, then transparently stained in alizarin red application solution for 6h-48h and shaken gently. Shake gently 1 to 2 times/d until the skull is red. After the bones have stained red and the soft tissues have faded, the specimen can be stored in glycerine. The fetal rat can also be skinned, gutted and fat removed, then stained in alizarin red solution, shaking the glass jar 2-3 times on the same day until the bones are red. Place the fetal rat in Clear Solution A for 1 to 2 days and then switch to Clear Solution B for 2 to 3 days. When the fetal rat bones have stained red and the purplish colour of the soft tissues has largely faded, the specimen can be stored in glycerine. (skinning method)

The specimen is placed in a small flat dish and viewed as a whole under a body microscope using a transilluminating light source, followed by a step-by-step examination of the bones. The size of the fontanelle, the width of the sagittal suture, the absence of the parietal and posterior cephalic bones are measured, followed by the number of sternum, missing or fused (6 sternum, with the 5th sternum missing first and the 2nd sternum second in cases of incomplete ossification). Ribs are usually 12-13 pairs, with common deformities such as fused ribs, bifurcated ribs, undulating ribs, short ribs, multiple ribs, missing ribs and interrupted ribs. Spinal development and number of vertebrae

(7 cervical, 12-13 thoracic, 5-6 lumbar, 4 basal and 3-5 caudal vertebrae), for fusion, longitudinal fractures, etc. Finally, the bones of the extremities are examined.

6.4.5 Visceral examination of fetal rats

One half of each litter was placed in Bouins' fluid and fixed for a fortnight before examination of the internal organs. The fixative is washed off with tap water, the rat is placed on its back on a paraffin board, the limbs and tail are cut off, and a razor blade is used to cut the rat crosswise or longitudinally from the



head to the tail. The size, shape and relative position of the organs are observed in different sections. Normal sections are shown in Fig.

（1） Transverse section through the mouth from the tongue and both corners of the mouth towards the occiput (section 1), looking at the brain, mesencephalon, medulla oblongata, tongue and palpebral fissure.

（2） A vertical longitudinal section on the anterior surface of the eye (section 2) reveals the nose.

（3） A longitudinal cut is made vertically from the head through the centre of the eye (section 3).

（4） A transverse cut (section 4) is made across the head at its greatest transverse position.

The purpose of the above sections is to visualise lingual fissures, cleft palate, ocular malformations, brain and ventricular anomalies.

（5） A transverse section is made along the level of the mandible through the middle of the neck, allowing observation of the trachea, oesophagus and extended brain or spinal cord.

The thoracic and abdominal cavities are later cut open from the midline of the abdomen and the size and location of the heart, lungs, diaphragm, liver, stomach and intestines are examined in turn. The kidneys, ureters, bladder, uterus or testes are then examined for their location and development. The kidney is then cut open and observed for hydronephrosis and enlargement.

6.5 Statistical methods and assessment of results

Various rates were examined using the $^{X2\ test}$, weight gain of pregnant rats using ANOVA or non-parametric statistics, and fetal length, weight and litter mean live births using the T test. The results should be able to be derived for maternal and embryotoxicity, teratogenicity and, preferably, the minimum teratogenic dose of the test substance.

In order to compare the teratogenic strength of different harmful substances, the teratogenic index can be calculated, with the teratogenic index below 10 being non-teratogenic, 10 to

100 is teratogenic and above 100 is strongly teratogenic. To indicate the magnitude of the hazard to humans, a teratogenic hazard index can be calculated.

If the index is greater than 300 it means that the test substance is less hazardous to humans, 100 to 300 is moderate and less than 100 is hazardous.

$$\text{Teratogenicity Index} = \frac{LD50\ for\ \text{female rats}}{\text{Minimum Teratogenic Dose}}$$

$$\text{Teratogenic hazard index} = \frac{\text{Maximum non-teratogenic dose}}{\text{Maximum possible intake}}$$

7 Test reports

The test report shall include the following.

（1） Name of the test substance, physicochemical properties, method of preparation, dose of poisoning.

（2） Animal species, strain, source (indicating the certificate of conformity number and animal class), weight.

（3） The environment in which the laboratory animals are kept, including the source of feed, room temperature, relative humidity, and the laboratory animal house certificate number.

（4） Doses and groups: principles of dose selection, doses and groups, negative and positive controls and doses.

（5） Test conditions and methods.

（6） Toxic reactions in animals, time of onset and mortality.

（7） Weight gain and gestation in pregnant rats.

（8） Results: Absorption of fetus and foetus, presence of visceral and skeletal malformations and no effect dose.

（9） Conclusion.

8 Interpretation of results

When interpreting the results of teratogenic tests, attention must be paid to species differences. The extrapolation of test results from animals to humans is of limited validity.

XVII. Chronic toxicity/carcinogenicity combination test

Combined Chronic Toxicity/Carcinogenicity Test

1 Scope

This specification specifies the basic principles, requirements and methods for the combined chronic toxicity/carcinogenicity test in animals. This specification applies to the testing of cosmetic ingredients for chronic toxicity and carcinogenicity.

2 Normative references

GB14924 Standard for Laboratory Animals and Feed

OECD Guidelines for Testing of Chemicals（No.453，Adopted：12 May 1981）

3 Definition

3.1 Chronic toxicity (chronic toxicity)

Adverse reactions caused by exposure to the test substance during most of the animal's normal life span.

3.2 maximal no-adverse effect level

The highest dose at which the test substance is exposed to the body in a certain way over a certain period of time and no damaging effect is observed using modern detection methods or sensitive observational indicators.

3.3 慢性有害作用阈剂量（chronic adverse effect threshed level）

The smallest dose required to cause an abnormality in a sensitive observable, even if it is the lowest dose at which the organism shows a toxic reaction, when the test substance is exposed to the organism in a certain way for a certain period of time, using modern detection methods or sensitive observables.

3.4 Chemical carcinogen (chemical carcinogen)

Chemicals that can cause tumours, or increase the incidence of tumours.

4 Principle

The accumulation of chemical substances in the body is the basis for the development of chronic toxicity. The chronic toxicity test is a test in which animals are exposed in a certain way to a toxic reaction caused by the test substance over a long period of time.

When a chemical substance has been shown to be potentially carcinogenic in short-term screening tests, or when its chemical structure closely resembles that of a known carcinogen, and the chemical substance has some practical application, it is further verified by a carcinogenicity test. Animal carcinogenicity tests provide information on the likelihood of long-term human exposure to the substance causing tumours.

5 Basic principles of the test

The animals are poisoned in a certain way for most of their lives and are observed to show signs of intoxication. Biochemical, haematological and histological tests are carried out to clarify the chronic toxicity of the chemical.

The test chemical is treated in such a way that the number, type, site and time of occurrence of tumours are examined during most or all of the animal's life and after death, compared with control animals, to clarify whether the chemical is carcinogenic.

6 Laboratory animals and housing environment

6.1 Selection of species and strains

To select suitable animals (species and strains), relevant acute, subacute and toxicokinetic tests should be carried out. Mice and rats are commonly used in the evaluation of carcinogenicity, while rats and dogs are commonly used for chronic toxicity tests.

For chronic toxicity/carcinogenicity combination tests, rats are generally used, but this does not preclude the use of other species. The choice of

The strain should be sensitive to the carcinogenic and toxic effects of the test substance.

6.2 Sex and age at the start of the experiment

Both sexes should be used, most often using young animals, recently weaned or weaned, for long-term biological tests of chronic toxicity and carcinogenicity.

The trial should be started as soon as possible after the rodent has been weaned and acclimatised, preferably before 6 weeks of age.

6.3 Number of animals in the experimental group

The reliability of the test results and the ability to perform statistical processing should be ensured, and the experimental and control groups of animals, should be randomly assigned.

Each group should have a sufficient number of animals for detailed biological and statistical analysis.

There should be at least 50 male and 50 female animals in each dose group and corresponding control group, excluding early dissection

The number of animals killed. If pathological changes other than tumours are to be observed, an additional dose group of 20 animals of each sex can be set up, with the relative

Ten animals of each sex in the corresponding control group.

6.4 Animal management, feed and water

Strict control of environmental conditions and reasonable animal management measures are required. Laboratory animals and laboratory animal rooms should comply with the appropriate national regulations.

7 Dose groups and frequency of administration of subjects

In order to evaluate the carcinogenicity test, at least three experimental dose groups and a corresponding control group should be set up. Some milder toxic effects may occur in the higher dose groups, but they do not significantly reduce the life span of the animals. These may be in the form of altered serum enzyme levels or a mild inhibition of body weight gain (less than 10%).

The low dose should not cause any toxic reactions and should not affect the normal growth, development and life span of the animal. It should not normally be less than 10% of the high dose.

The medium dose should fall between the high and low doses and can be determined by the toxicokinetic properties of the chemical.

In combination with the chronic toxicity test, an experimental group and corresponding control group should be added. The highest dose should be capable of producing significant toxicity. The test substance is normally given daily. If the chemical given is mixed in drinking water or feed, continuous administration should be ensured.

The frequency of administration of subjects can also be adjusted for changes in their toxicokinetics.

There should be a corresponding control group, which should have the same conditions as the experimental group, except that it is not exposed to the test substance.

8 Route of administration to the subject

Oral administration, dermal contact and inhalation are the three main routes of administration. The

choice of route depends on the physicochemical properties of the test substance and the mode of exposure that is representative of the human population.

The frequency of administration may vary according to the route and mode of administration chosen and should be adjusted, if possible, according to the toxicokinetic changes of the test substance.

8.1 Oral trials

The oral route is preferred if the test substance is absorbed through the gastrointestinal tract. The test substance should be mixed into the feed, dissolved in drinking water or given to the animal continuously by tube feeding for the test period indicated in the test period (9). The maximum concentration of the test substance to be mixed into the feed should not exceed 5%. Interruptions in the administration of the test substance 7 days a week may allow the animals to recover or the toxicity to abate, thus affecting the results and subsequent evaluation.

8.2 Skin tests

The choice of dermal exposure is used as a primary route to mimic human exposure to the substance of interest and as a test model for the induction of skin lesions. Special tests concerning the induction of skin tumours are not described in this specification.

Inhalation is not the primary route of exposure for cosmetic products and therefore the inhalation test is not described in this specification.

9 Test period

Twenty experimental animals/per sex in the additional group and 10 corresponding control animals/per sex should be maintained until at least 12 months of age. These animals should be dissected and killed for the evaluation of pathological changes related to the subject, but not caused by age-related alterations. The duration of the carcinogenicity test must cover the majority of the normal life span of the subject.

A few guidelines for determining the duration of the test.

（1） In general, the end of the test should be at 18 months for mice and hamsters and 24 months for rats; however, for

Certain strains of animals with a longer life span or low spontaneous tumour rate, up to 24 months in mice and hamsters and up to 30 months in rats.

（2） The test may also be terminated when only 25% of the animals in the lowest dose and control group survive. For tests with significant gender differences, the timing of the end of the test should be different for each gender. In some cases where only the high dose group dies prematurely due to apparent toxic effects, the test should not be terminated.

The negative test should meet the following criteria.

1. Losses of animals due to autolysis, being eaten by their own kind, or due to management problems must not be higher than 10% in any group.
2. At 18 months for mice and hamsters and 24 months for rats, no less than 50% of the animals in each group should survive.

10 Test method

10.1 Watch

The condition of the animals should be checked at least once a day. There should also be several purposeful observations per day, such as dissecting dead animals or storing them in the refrigerator, separating sick or dying animals or putting them to death. Timely detection of the onset of all toxic effects and their changes, and the ability to reduce animal losses due to disease, autolysis or ingestion by the same species.

Detailed documentation of the animals' symptoms including neurological and ocular changes, the time of appearance and change of all toxic effects including suspected tumours, and death.

Weights were taken once a week for the first 13 weeks of the trial and every 4 weeks thereafter. During the first 13 weeks of the trial

The animals' food intake is checked weekly and then every 3 months if there are no abnormal changes in health or body weight.

10.2 Haematology

Haematological examinations (haemoglobin content, haematocrit, red blood cell count, white blood cell count, platelets, or other haemagglutination tests) should be carried out at 3 months, 6 months and every 6 months thereafter and at the end of the experiment, with 20 rats of each sex in each group. Blood specimens should be collected from the same rats each time. The highest dose and control rats should have their white blood cells sorted at the same intervals, the medium dose group rats only if necessary.

During the test, if gross observations indicate deterioration in the health of the animals, a blood cell sorting count should be performed on the animals concerned. A blood cell sorting count should be

performed on the animals in the higher dose and control groups. If there is a significant difference between the two groups, the lower dose group should be

Animals are counted for blood cell sorting.

10.3 Urinalysis

Urine samples from 10 rats of each sex in each group should be collected for analysis, preferably at the same time as the blood test and from the same rats. The following indicators should be measured, either individually or by mixing urine specimens from each group of the same sex.

Analytical parameters: appearance; urine volume and specific gravity for each animal; protein, sugar, ketone bodies, occult blood (semi-quantitative); microscopic examination of sediment (semi-quantitative).

10.4 Clinical Chemistry

Every 6 months and at the end of the experiment, blood specimens were collected from 10 rats of each sex in each group for clinical chemistry, taking the same rats at each time interval as far as possible. Plasma was isolated and the following parameters were measured.

Total protein concentration; albumin concentration; liver function tests (e.g. alkaline phosphatase, alanine aminotransferase, glutamate aminotransferase, glutamyl transpeptidase, ornithine decarboxylase); glucose metabolism, e.g. glucose tolerance; renal function, e.g. blood urea nitrogen.

10.5 Pathological examination

Visual and pathological examination is often the basis of the chronic/carcinogenicity combination test.

10.5.1 Visual autopsy

All animals, including those that die during the experiment or are put to death because they are in a dying state, should be examined visually. Blood samples should be collected for blood cell sorting counts before all animals are put to death. Preserve all tumours visible to the naked eye or suspected to be tumours.

All organs or tissues should be retained for microscopic examination. This generally includes the following organs and tissues: brain* (medulla/bridge, cerebellar cortex, cerebral cortex), pituitary gland, thyroid (including parathyroid), thymus, lungs (including trachea), heart, salivary glands, liver*, spleen, kidney*, adrenal glands*, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, lymph nodes, pancreas, gonads*, genital appendages, breast, skin, muscles, peripheral nerves, spinal cord (cervical, thoracic, lumbar), sternum or femur (including joints) and eye. The lungs, muscles, peripheral nerves, spinal cord (cervical, thoracic, lumbar), sternum or femur (including joints) and eyes. Lungs and bladders are better preserved by filling them with fixative.

10.5.2 Histopathological examination

All tumours and other lesions that are visible to the naked eye should be examined pathologically. In addition the following should be noted.

（1） Microscopic examination of all preserved organs and tissues with a detailed description of all lesions found.

①Includes animals that died or were executed during the course of the experiment.

② All animals in the highest dose group and the control group.

（2） In the lower dose groups, organs or tissues that are or may be abnormal due to the test substance should also be examined.

All organs marked with *, including the thyroid and parathyroid glands, of non-rodents should be weighed.

11 Data processing and evaluation of results

11.1 Incidence of tumours

The incidence of tumours is the percentage of the total number of tumour-bearing animals out of the total number of valid animals at the end of the entire experiment. The total number of valid animals is the total number of animals alive at the earliest appearance of tumours.

$$\text{Tumour incidence} = \frac{\text{Total number of tumour-bearing animals at the end of the experiment}}{\text{Total number of active animals}} \times 100$$

11.2 Criteria for determining a positive cancer induction test

The four criteria for a positive carcinogenicity test, as proposed by the World Health Organization of the combined countries, were used.

（1） Tumours occurred only in the animals in the test group, with no tumours in the control group.

（2） Tumours occurred in both test and control animals, but the incidence was high in the test group.

（3） Multiple tumours were evident in the test animals and none or only a few animals in the control group had multiple tumours.

（4） There was no significant difference in the incidence of tumours between the test and control

animals, but the tumours occurred earlier in the test group. A statistically significant difference between the test and control groups for any of the four entries above is considered

The test was positive for carcinogenicity.

11.3 Establishment of a negative carcinogenicity test result

If the size of the animal experiment is two species, two sexes and at least three dose levels, one of which is close to the maximum tolerated

The dose was considered negative only if the number of animals in each group was at least 50 and the incidence of tumours in the experimental group did not differ from that in the control group.

12 Test reports

The test report shall include the following.

（1） The name of the test substance, its physical and chemical properties, and the method of preparation.

（2） The species, strain, sex, weight, number and source of the experimental animal (indicating the certificate of conformity number and animal class).

（3） Laboratory animal housing environment, including feed source, room temperature, relative humidity, single cage housing or group feeding, and laboratory animal room qualification number.

（4） Test method: route of infection and duration of test, dose grouping.

（5） Food intake and animal weight information.

（6） Data on toxic effects by sex and dose, appearance and timing of abnormal symptoms in animals.

（7） Results of haematological tests, urinalysis, clinical chemistry, etc.

（8） Gross autopsy and histopathological examination by sex and dose, indicating the nature of lesions visible to the naked eye and microscopic examination.

（9） Data processing and evaluation of results, including criteria for determining tumour incidence, positive tests for carcinogenicity, statistical methods for processing results.

（10） Conclusion.

Part III Hygienic chemical test methods

I. General Provisions

General Principles

1 Scope

This specification specifies the requirements for hygienic chemical testing methods for prohibited and restricted ingredients in cosmetics. This specification applies to the detection of prohibited and restricted ingredients in cosmetic products.

2 Definition

2.1 Volume: Dilute to scale in a volumetric flask with water or other solvent.

2.2 Limit of detection: the lowest amount of the test substance that can be detected. This specification defines the detection limits for each type of test method as shown in Table 1.

2.3 Lower limit of quantification: The lowest concentration or mass that can be accurately quantified for the substance being tested is known as the lower limit of quantification for the method. This specification defines the lower limit of quantification for each type of test method as shown in Table 1.

Table 1 Definition of detection limits and lower limits of quantification

	Detection limit (corresponding mass, concentration)	Lower limit of quantification (corresponding mass, concentration)
AAS/AES	3 SD	10 SD
GC	3x blank noise	10x blank noise
HPLC	3x blank noise	10x blank noise
Spectrophotometry	0.005 A	0.015 A
Capacity method	X(1)+3 SD	X(1)+10 SD

(1) X is the average of the smallest volumes of reagent that show a perceptible change near the endpoint

2.4 Detection concentration: The concentration of the test substance corresponding to the detection limit of the method when operated according to the standard method.

2.5 Minimum quantification concentration: the concentration of the test substance corresponding to the lower limit of quantification when operating according to the regulated method.

3 Reagents used in this specification

Whenever specifications are not specified, they are analytical purity (AR). Where other specifications are required, these will be stated separately. However, no specification is given for indicators and biological

dyes. Where no solvent is specified, the reagent solution is prepared in pure water.

4 Water used in this specification

Where no specification is given, this refers to pure water. It includes, for example, distilled or deionised water as described below. Special requirements for pure water are specified separately.

4.1 Distilled water: water prepared by distillation in a still.

4.2 Deionised water: water prepared by means of anionic and cationic resin exchange beds.

4.3 Distilled deionised water: water prepared by passing distilled water through anionic and cationic resin exchange beds.

5 Concentration representation

5.1 Concentration of substance B: The amount of substance B divided by the volume of the mixture.

$$c(B) = \frac{nB}{V}$$; common unit: mol/L.

5.2 Mass concentration of substance B: Mass of substance B divided by the volume of the mixture.

$$(B) = \frac{mB}{V}$$; common units: g/L, mg/L, g/L.

5.3 Mass fraction of substance B: Ratio of the mass of substance B to the mass of the mixture.

$$(B) = \frac{mB}{m}$$

The unit is dimensionless and can be expressed as a % concentration, or as mg/kg, g/g, etc.

5.4 Volume fraction of substance B: Volume of substance B divided by the volume of the mixture.

$$(B) = \frac{VB}{V}$$

The concentration is often expressed in %.

5.5 Volume to volume concentration: two liquids are mixed by volume V_1 and V_2 respectively. Whenever the name of the solvent is not specified, it refers to pure water. When two or more specific liquids are mixed with water, water must be specified. For example: HCl (1+2), methanol + tetrahydrofuran + water + perchloric acid = (250+450+300+0.2).

5.6 Mass ratio of the fixative used in gas chromatography: The mass ratio between the fixative and the carrier.

6 Calibration and verification of gauges

Balances, volumetric flasks, burets, non-indexing pipettes, graduated pipettes, etc. are checked and calibrated according to the relevant national regulations and protocols.

7 Choice of test methods

If there are two or more test methods for the same project, you can choose to use them according to the equipment and technical conditions, but the first method is the arbitration method.

8 Testing of cosmetic products

In general, newly developed cosmetic products should be tested according to their category to assess their safety before being placed on the market.

9 Sampling of cosmetic products

The sampling process for cosmetic products should, as far as possible, take into account the representativeness and homogeneity of the samples so that the analytical results correctly reflect the quality of the cosmetics. Samples should be registered upon receipt in the laboratory and the integrity of the seal should be checked. Before taking a sample for analysis, the sample should be visually inspected for properties and characteristics and allowed to mix thoroughly. After opening the package, the part to be measured should be removed for analysis as quickly as possible. If the sample must be stored, the container should be kept airtight under an inert gas. If the sample is sold in a special manner and cannot be sampled according to the above method or if no sampling method is readily available, a reasonable sampling method may be developed and the actual sampling procedure recorded and attached to the original record.

9.1 Liquid samples

These are mainly make-ups, emollients, etc. consisting of oil, alcohol and water solutions. The container should be shaken vigorously before opening and closed after removing the sample to be analysed.

9.2 Semi-fluid samples

This refers mainly to cream, honey and gel products. For samples in thin-necked containers, discard at least 1cm of the initial sample, squeeze out the required amount of sample and close the container immediately. For samples in wide neck containers, the surface layer should be scraped off, the desired sample removed and the container closed immediately.

9.3 Solid samples

These are mainly powder molasses, powders, lipsticks, etc. Of these, powder molasses samples should be shaken vigorously to remove the test portion before opening. Powder and lipstick samples should be taken after scraping off the surface layer.

9.4 Samples of other dosage forms can be sampled using appropriate methods according to the sampling principles.

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II. Mercury

Mercury

1 Scope

This specification specifies a method for the determination of total mercury in cosmetics by cold atomic absorption and hydride atomic fluorescence photometry. This specification applies to the determination of total mercury in cosmetics.

First method Cold atomic absorption method

2 Methodology Summary

Mercury vapour has a characteristic absorption of ultraviolet light at a wavelength of 253.7 nm. The absorption value is proportional to the concentration of mercury vapour over a range of concentrations. The sample is digested and reduced to convert the mercury in the chemical state to the atomic state and then the absorption value is measured with a carrier gas in a mercury meter and compared to a standard series for quantification. The method has a detection limit of 0.01 g and a lower limit of quantification of 0.04 g. If a 1 g sample is taken, the detection concentration is 0.01 g/g and the lowest quantification concentration is 0.04 g/g.

3 Reagents

3.1 Nitric acid ($_{20}$ = 1.42 g/mL), ultrapure.

3.2 Sulphuric acid ($_{20}$ = 1.84 g/mL), ultrapure.

3.3 Hydrochloric acid ($_{20}$ = 1.19 g/mL), ultrapure.

3.4 Hydrogen peroxide [($_{H2O2}$) = 30%].

3.5 Vanadium pentoxide.

3.6 Sulphuric acid [($_{H2SO4}$) = 10%]: take 10mL of sulphuric acid (3.2), add slowly to 90mL of water and mix well.

3.7 Hydroxylamine hydrochloride solution (120g/L): Take 12.0g of hydroxylamine hydrochloride and 12.0g of sodium chloride and dissolve in 100mL of water.

3.8 Stannous chloride solution (200g/L): weigh 20g of stannous chloride into a 250mL beaker, add 20mL of hydrochloric acid (3.3), heat slightly to promote dissolution if necessary, and then dilute to 100mL with water.

3.9 Potassium dichromate solution (100g/L): weigh 10g of potassium dichromate and dissolve in 100mL of water.

3.10 Potassium dichromate - nitric acid solution: Take 5mL of potassium dichromate solution (3.9), add

50mL of nitric acid (3.1) and dilute to 1L with water.

3.11 Octanol.

3.12 Mercury standard solutions

3.12.1 Mercury standard solution [(Hg)=100mg/L]: weigh 0.1354g of mercury chloride ($_{HgCl2}$) into a 100mL beaker and dissolve in potassium dichromate-nitric acid solution (3.10). Transfer to a 1000mL volumetric flask and dilute to the scale with potassium dichromate-nitric acid solution (3.10).

3.12.2 Mercury Standard Solution [(Hg)=10mg/L]: Take 10.0mL of Mercury Standard Solution (3.12.1) in a 100mL volumetric flask and dilute to the scale with potassium dichromate-nitric acid solution (3.10). It can be stored for one month.

3.12.3 Mercury Standard Solution [(Hg)=1mg/L]: Take 10.0mL of Mercury Standard Solution (3.12.2) in a 100mL volumetric flask and dilute to the scale with potassium dichromate - nitric acid solution (3.10). Prepare before use.

3.12.4 Mercury standard solution [(Hg)=0.1mg/L]: Take 10.0mL of the mercury standard solution (3.12.3) in a 100mL volumetric flask and dilute to the scale with potassium dichromate-nitric acid solution (3.10).

4 Instruments

4.1 Stoppered cuvettes, 50mL, 10mL.

4.2 Conical flask, 100mL.

4.3 Glass reflux unit (mill-mouth spherical condenser tube), 250mL.

4.4 Dissolving sample cup.

4.5 Water baths (or open type electrically heated thermostats)

4.6 Cold atomic absorption mercury meter.

4.7 Mercury vapour generating bottle.

4.8 Pressure self-contained microwave digestion system.

4.9 High-pressure, airtight digestion tank.

4.10 Polytetrafluoroethylene dissolving sample cup.

5 Analysis steps

5.1 Sample pre-treatment (either one)

5.1.1 Wet reflux digestion

Accurately weigh approximately 1.00 g of the mixed sample in a 250 mL round bottom flask. A reagent blank is made with the sample. If the sample contains organic solvents such as ethanol, evaporate first in a water bath or on an electric hot plate at low temperature (do not dry out).

Add 30mL of nitric acid (3.1) Note 1, 5mL of water, 5mL of sulphuric acid (3.2) and a few glass beads. Place on an electric stove and connect

A spherical condenser is placed on top and circulated through the condensate. Heat and reflux the solution for 2 h. The solution is usually slightly yellow or yellow in colour. Fill the upper port of the condenser with 10mL of water, continue heating for 10mim and leave to cool. Remove solids by filtering the digestion solution through a pre-wetted filter paper. For specimens containing large amounts of grease and wax, the digestion solution can be frozen in advance to solidify the grease and wax. Wash the filter paper several times with distilled water and combine the washing solution in the filtrate. Add 1.0 mL of hydroxylamine hydrochloride solution (3.7) and set aside with water to 50 mL.

5.1.2 Wet catalytic digestion

Accurately weigh approximately 1.00 g of the mixed sample in a 100 mL conical flask. Make a reagent blank with the sample. If the sample contains organic solvents such as ethanol, evaporate first in a water bath or on an electric hot plate at low temperature (do not dry out).

Add 50mg of vanadium pentoxide (3.5) and 7mL of nitric acid (3.1) and place in a sand bath or on an electric hot plate and heat over a light flame until slightly boiling. Remove from the heat, add 5.0mL of sulphuric acid (3.2), place a small glass funnel at the mouth of the conical flask, continue the digestion at 135°C to 140°C and add a small amount of nitric acid (3.1) if necessary, and digest until the solution appears transparent blue-green or orange-red. After cooling, add a small amount of water and continue boiling for about 2 min to drive off the nitrogen dioxide. Add 1.0mL of hydroxylamine hydrochloride solution (3.7), set in water to 50mL and reserve.

5.1.3 Extraction method (only for wax-free cosmetics)

Accurately weigh approximately 1.00 g of the mixed sample and place in a 50 mL stoppered cuvette. Make a reagent blank with the sample. If the sample contains organic solvents such as ethanol, evaporate

first in a water bath or on a hotplate at low temperature (do not dry out).

Add 5.0 mL of nitric acid (3.1) and 2 mL of hydrogen peroxide (3.4) and mix well. If the sample produces a large amount of foam, add a few drops of octanol (3.11). Heat in a boiling water bath for 2h, remove, add 1.0mL of hydroxylamine hydrochloride (3.7), leave for 15min-20min, add sulphuric acid (3.6), set with water to 25mL and reserve.

5.1.4 Microwave Ablation Method Note 2

Weigh approximately 0.5g to 1g of the mixed sample into a cleaned Teflon sample cup. Cosmetics containing volatile materials such as ethanol, such as perfumes, mousse, body lotions, hair dyes, serums, shaving lotions, face masks, etc., should first be volatilised in a temperature-adjustable 100ºC electric heater or water bath (do not steam dry). For dry substances such as lipstick, mascara, eyebrow pencil, rouge, lip liner, powder, eye shadow, talcum powder, prickly heat powder, etc., add 0.5mL to 1.0mL of water after sampling and wetting and shaking well.

Depending on the ease of sample digestion, samples or pre-treated samples were first added to nitric acid (3.1) 2.0mL to 3.0mL and left to stand overnight. Then add hydrogen peroxide (3.4) 1.0mL to 2.0mL and shake the sample cup several times to fully submerge the sample. Place in a boiling water bath or thermostatic heating apparatus at an adjustable temperature for 20 min at 100ºC and remove. If the body of the solution

Replenish with water if the volume is less than 3mL. Also follow the Microwave Dissolution System operating instructions.

Place the sample cup into a clean, high pressure, airtight lysimeter prepared in advance and screw on the lid (note: do not over-tighten).

Table 1 shows the pressure - time procedure for general cosmetic products for decomposition. If the cosmetics are oils, herbs or detergents, the sensitivity of the explosion-proof system can be increased appropriately to increase safety.

Depending on the ease of sample digestion, the digestion can be completed within 5 min to 20 min, the sample is cooled down, the can is opened and the sample cup containing the digested sample is placed in a boiling water bath or an electric heater at an adjustable temperature of 100ºC for a few minutes to remove excess nitrogen oxides from the sample so as not to interfere with the determination.

Table 1 Pressure during digestion - time procedure

Pressure gear	Pressure (Mpa)	Holding pressure accumulation time (min)
1	0.5	1.5
2	1.0	3.0
3	1.5	5.0

Transfer the sample to a 10mL stoppered cuvette, wash the dissolution cup several times with water, combine the washing solution, add 0.5mL of Hydroxylamine Hydrochloride solution (3.7), fix the volume with water to 10mL and set aside.

5.2 Preparation of calibration curves

5.2.1 Pipette 0, 0.10, 0.30, 0.50, 0.70, 1.00, 2.00 mL of the mercury standard solution (3.12.4) into 100 mL of

In a conical flask or mercury vapour generating flask, set to a certain volume with sulphuric acid (3.6).

5.2.2 Adjust the mercury meter according to the instrument instructions. Add the standard series to the mercury vapour generating bottle and add the stannous chloride solution

(3.8) 2mL Quickly stopper the bottle tightly. Open the instrument air valve. When the indication reaches the highest reading, record the reading. Plot the calibration curve or calculate the regression equation.

5.3 Measurement

Pipette a quantity of blank and sample solution into a mercury vapour generating flask and add sulphuric acid (3.6) to a certain volume. Press 5.2.2

The measurements were carried out.

6 Calculation

$$(Hg) = \frac{(m_1 - m_0)V}{mV_1}$$

where: (Hg) - mass fraction of mercury in the sample, g/g; m_1 - mass of mercury in the test solution, g;

m_0 - mass of mercury in the blank solution, g; V - total volume of the sample digest, mL.

V_1 - volume of sample digest dispensed, mL.

m - Sample size, g.

Note 1 Powder containing carbonates in the sample should be added slowly when adding acid to prevent the carbon dioxide gas from being produced too vigorously.

Note 2 Caution.

1. If the pressure setting is set to 1 and the time between the start of microwave heating and the setting of 1 in the table exceeds 1 min, cut off the microwave immediately and check for leaks in the dissolution tank or insufficient volume of dissolved sample.

2. Prevent damage to the digestion tank: after the local surface of the digestion tank had been contaminated, or the residual trace of moisture in the digestion tank, under the action of microwave, will make the digestion tank local heating; or insufficient pressure caused by excessive heating time, these can make the local temperature of the digestion tank exceed its temperature limit and soften or even melt. At this point, the pressure difference between the inside and outside of the tank makes the local deformation of the tank (such as bulging) or blowing up. In the process of pressurisation, the display figures not only do not rise, but also do not move or fall, and the microwave should be switched off immediately to prevent burning of the dissolution jar. Check that the sample cup is well sealed and that the sample is in good condition.

Forget the gasket; whether the elastomer in the lid of the dissolution tank has failed.

3. After microwave heating, do not rush to open the door, but turn off the microwave switch and then idle for 2min, in order to eliminate the nitrogen oxides in the furnace, and make the pressure in the tank drop, after the 2min is over, you can open the door, take out the dissolving sample tank, put it in the fume hood to cool down, wait until the reflector returns to its original shape, at this time there is basically no pressure in the tank, then you can take out the dissolving sample cup.

Second method Hydride atomic fluorescence photometry

7 Methodology Summary

After the sample has been digested, the mercury is dissolved out of the sample. The mercury ions react with potassium borohydride to form atomic mercury, which is carried into the atomiser by the carrier gas (argon). Under the irradiation of a special mercury hollow cathode lamp, the ground state mercury atoms are excited to the high energy state, de-activated back to the ground state and emit fluorescence at characteristic wavelengths, the intensity of which is proportional to the mercury content over a range of concentrations and quantified by comparison with a standard series. The method has a detection limit of 0.1 g/L and a lower limit of quantification of 0.3 g/L. For a sample size of 0.5 g, the detection concentration is 0.002 g/g and the lowest quantification concentration is 0.006 g/g.

8 Reagents

8.1 Potassium hydroxide solution (5g/L): weigh 5g of potassium hydroxide and dissolve in 1L of water.

8.2 Potassium borohydride solution (20g/L): weigh 20g of potassium borohydride (95%) and dissolve in 1L of potassium hydroxide solution (8.1). Store in the refrigerator for up to one week.

8.3 Hydrochloric acid [(HCl) = 10%]: take 10mL of hydrochloric acid (3.3), add 90mL of water and mix well.

8.4 Mercury standard solution [(Hg)=0.01mg/L]: Take 10.0mL of the mercury standard solution (3.12.4) in a 100mL volumetric flask and dilute to the scale with potassium dichromate - nitric acid solution (3.10).

9 Instruments

9.1 The glassware used was soaked overnight in dilute nitric acid and rinsed. The tubes were baked in an oven at 105°C for 2h.

9.2 Stoppered cuvettes, 10mL, 25mL, 50mL.

9.3 Atomic fluorescence photometer.

10 Analysis steps

10.1 Sample pre-treatment (either one)

10.1.1 The microwave digestion method is the same as 5.1.4.

10.1.2 The wet reflux digestion method is the same as 5.1.1.

10.1.3 The wet catalytic digestion method is the same as 5.1.2.

10.1.4 Extraction method

Accurately weigh approximately 1.00 g of the mixed sample and place in a 50 mL stoppered cuvette. Make a reagent blank with the sample. If the sample contains organic solvents such as ethanol, evaporate first in a water bath or on a hotplate at low temperature (do not dry out).

Add 5.0 mL of nitric acid (3.1) and 2.0 mL of hydrogen peroxide (3.4) and mix well. If the sample produces a large amount of foam, add a few drops of octanol (3.11). Heat in a boiling water bath for 2h, remove, add 1.0mL of hydroxylamine hydrochloride (3.7), leave for 15min-20min, add water to build up to 25mL and reserve.

10.2 Preparation of calibration curves

Pipette 0, 0.50, 1.25, 2.50, 5.00 mL of mercury standard solution (8.4) into a 25 mL stoppered cuvette, add 2.5 mL of hydrochloric acid (8.3) and add water to the scale. Shake well with cap (corresponding concentrations 0, 0.20, 0.50, 1.00, 2.00 g/L).

Atomic fluorescence measurements were carried out according to 10.3.

10.3 Measurement

10.3.1 Instrument reference conditions

Photomultiplier tube negative high voltage 300 V, mercury elemental lamp current 15 mA, atomiser temperature 300 °C, height 8.0 mm; argon gas flow rate: carrier gas 300 mL/min, shielding gas 700 mL/min; measurement method: standard curve method; reading method: peak area, reading delay time 2 s, reading time 12 s; test sample injection volume and The sample injection volume and the potassium borohydride solution (8.2) spiking volume (1:1 ratio) can be set between 0.5mL and 0.8mL.

10.3.2 Measurement methods

Set the instrument conditions according to 10.3.1, enter the relevant parameters including sample dilution multiples and concentration units, preheat the instrument, and after the instrument has stabilised, take an appropriate volume of the digestion volume (2mL to 5mL), dilute to 10mL with hydrochloric acid (8.3), shake well, number and place on the instrument feed rack, and determine the standard curve first and then the sample under the same conditions.

11 Calculation

$$(Hg) = \frac{(_{1-0})V}{} \; m1000$$

where: (Hg) - the mass fraction of mercury in the sample, g/g.

$_1$ - the mass concentration of mercury in the test solution, g/L.

$_0$ - mass concentration of mercury in blank solution, g/L; V - total volume of sample digest, mL; m - -sample sampling volume, g.

12 Linearity range, precision and accuracy

The linear range of the method was 0 g/L to 10 g/L; the recovery was 95%; and the relative standard deviation of multiple determinations was 1.2%.

III. Arsenic

Arsenic

1 Scope

This specification specifies a method for the determination of total arsenic in cosmetics by hydride atomic fluorescence photometry, spectrophotometry and hydride atomic absorption.

This specification applies to the determination of total arsenic in cosmetics.

First method Hydride atomic fluorescence photometry

2 Methodology Summary

Under acidic conditions, pentavalent arsenic is reduced by thiourea-ascorbic acid to trivalent arsenic, which then reacts with a large amount of neo-ecological hydrogen produced by the action of sodium borohydride with acid to produce gaseous arsine, which is fed into a quartz tube furnace by the carrier gas and decomposed by heat into the atomic state of arsenic, which is excited by the emission spectrum of an arsenic hollow cathode lamp to produce atomic fluorescence, the fluorescence intensity of which, within a certain concentration range, is The fluorescence intensity is proportional to the arsenic content over a certain concentration range and is quantified by comparison with a standard series. The method has a detection limit of 4.0 g/L and a lower limit of quantification of 13.3 g/L. If 1 g of sample is taken, the detection concentration is 0.01 g/g and the lowest quantification concentration is 0.04 g/g.

3 Reagents

3.1 Nitric acid ($_{20}$ = 1.42 g/mL), ultrapure.

3.2 Sulphuric acid ($_{20}$ = 1.84 g/mL), ultrapure.

3.3 Magnesium oxide.

3.4 Magnesium nitrate hexahydrate solution (500g/L): weigh 500g of magnesium nitrate hexahydrate, add water to dissolve and dilute to 1L. 3.5 Hydrochloric acid (1+1): Take 100mL of superior pure hydrochloric acid ($_{20}$ = 1.19g/mL), add 100mL of water and mix well. 3.6 Hydrogen peroxide [($_{H2O2}$) = 30%].

3.7 Thiourea-ascorbic acid solution: Weigh 12.5g of thiourea [($_{NH2}$)2CS], add about 80mL of water, heat and dissolve, add 12.5g of ascorbic acid after cooling, dilute to 100mL and store in a brown bottle for up to one month.

3.8 Sodium hydroxide solution (1g/L): weigh lg of sodium hydroxide dissolve in water and dilute to 1L.

3.9 Sodium borohydride solution (7g/L): weigh 7g of sodium borohydride and dissolve in 1L of sodium

hydroxide solution (3.8).

3.10 Sodium hydroxide solution (100g/L): weigh 100g of sodium hydroxide dissolve in water and dilute to 1L.

3.11 Sulphuric acid (1+9): take 10mL of sulphuric acid (3.2) and slowly add to 90mL of water.

3.12 Phenolphthalein indicator (1g/L ethanol solution): weigh 0.1g of phenolphthalein and dissolve in 50mL of 95% ethanol with water to 100mL.

3.13 Arsenic standard stock solution [(As)=1g/L]: weigh 0.6600g of arsenic trioxide (As_2O_3) dried at 150°C for 2h, dissolve in 10mL of sodium hydroxide solution (3.8), add 2 drops of phenolphthalein indicator (3.12), neutralise with sulphuric acid (3.11), add sulphuric acid (3.11) to 10mL, transfer to a 500mL volumetric flask, add water to the scale and mix well. Add 10mL of sulphuric acid (3.11), transfer to a 500mL volumetric flask, add water to the scale and mix well.

3.14 Arsenic standard solution [(As)=10mg/L]: Dispense 1.00mL of arsenic standard stock solution (3.13) into a 100mL volumetric flask, add water to the scale and mix well.

3.15 Arsenic standard working solution [(As) = 1mg/L]: 10.0mL of arsenic standard solution (3.14) in 100mL when ready to use

In a volumetric flask, add water to the scale and mix well.

4 Instruments

4.1 Atomic fluorescence photometer.

4.2 Electric heating plate.

4.3 Box-type electric furnace.

4.4 Conical flask, 150mL.

4.5 Stoppered cuvettes, 10mL, 25mL.

4.6 Pressure self-contained microwave digestion system.

4.7 High-pressure, airtight digestion tank.

4.8 Polytetrafluoroethylene dissolving sample cup.

4.9 Water baths (or open type electrically heated thermostats)

4.10 Crucible, 50mL.

5 Analysis steps

5.1 Sample pre-treatment

5.1.1 HNO_3-H_2SO_4 wet digestion method

Accurately weigh approximately 1.00g of the mixed sample and place in a 150mL conical flask. Also make a reagent blank. If the sample contains a solvent such as ethanol, the solvent should be evaporated (not dried out) after weighing the sample. Add several glass beads, add nitric acid (3.1) 10mL to 20mL, leave for a few moments, heat slowly, remove the heat source when the reaction starts, cool slightly and add sulphuric acid (3.2) 2mL. continue to heat the solution for digestion, if the solution appears brown during digestion, add a little nitric acid (3.1) for digestion, repeat until the solution is clarified or slightly yellow. Allow to cool, then add 20mL of water and continue to boil until white smoke is produced.

5.1.2 Dry ashing method

Accurately weigh approximately 1.00 g of the mixed sample and place in a 50 mL crucible while making a reagent blank. Add 1g of magnesium oxide (3.3) and 2mL of magnesium nitrate hexahydrate solution (3.4), stir well, evaporate on a water bath and then charcoal over a slight fire until no smoke is emitted, transfer to a box furnace, ash at 550°C for 4h-6h, remove, add a little water to the ash to moisten, then use hydrochloric acid (1+1)

(3.5) 20mL Dissolve the ash in several portions, add water to a volume of 25mL and reserve.

5.1.3 Microwave digestion

Weigh accurately about 0.5g to 1g of the mixed sample and place it in a cleaned Teflon dissolution cup. For cosmetics containing volatile raw materials such as ethanol, such as perfume, mousse, body lotion, hair dye, serum, shaving lotion, face mask, etc., place in a temperature adjustable 100ºC electric heater or water bath to evaporate (do not steam dry), for dry substances such as lipstick, mascara, eyebrow pencil, rouge, lip liner, powder, eye shadow, talcum powder, prickly heat powder, etc., add water after taking the sample Add 0.5mL to 1.0mL of water, moisten and shake well.

Depending on the ease of sample digestion, samples or pre-treated samples are first added to nitric acid (3.1) 2.0mL to 3.0mL and left to stand overnight for full action. Then add hydrogen peroxide (3.6) 1.0mL to 2.0mL and shake the sample cup several times to fully submerge the sample. Place in a boiling water bath or thermostatic electric heating apparatus at an adjustable temperature for 20min at 100ºC and remove. If the

volume of solution is less than 3mL, replenish with water. Follow the Microwave Dissolution System operating instructions to the letter.

Place the sample cup into a clean, high pressure, airtight lysimeter prepared in advance and screw on the lid (note: do not over-tighten).

Table 1 shows the pressure - time procedure for general cosmetic products for decomposition. If the cosmetics are oils, herbs or detergents, the sensitivity of the explosion-proof system can be increased appropriately to increase safety.

Depending on the ease of sample digestion, the digestion can be completed within 5 min to 20 min, the sample is cooled down, the can is opened and the sample cup containing the digested sample is placed in a boiling water bath or an electric heater at an adjustable temperature of 100ºC for a few minutes to remove excess nitrogen oxides from the sample so as not to interfere with the determination.

Table 1 Pressure - time procedures during digestion

Pressure gear	Pressure (Mpa)	Holding pressure accumulation time (min)
1	0.5	1.5
2	1.0	3.0
3	1.5	5.0

Transfer the sample to a 10mL stoppered cuvette, wash the sample cup several times with water, combine the washings, and set the volume with water to 10mL for use.

5.2 Instrument conditions

Switch on the instrument and adjust the operating conditions according to the instrument instructions.

5.2.1 Reference condition 1.

Lamp current: 45mA; Photomultiplier negative high voltage: 340V; Atomiser height: 8.5mm; Carrier gas flow rate: 500mL Ar/min; Shielding gas flow rate: 1000mL Ar/min; Measurement method: Calibration curve method; Reading time: 12s; Potassium borohydride addition time: 8s; Injection volume: 2mL.

5.2.2 Reference condition 2 (with flow injection).

Lamp current: 45mA; Photomultiplier negative high voltage: 340V; Atomiser height: 8.5mm; Argon gas pressure: 0.03Mpa; Carrier gas flow: 300mL Ar/min; Shielding gas flow: 600mL Ar/min; Measurement method: Calibration curve method; Reading time: 12s; Potassium borohydride addition time: 10s; Injection volume: 1mL.

5.3 Preparation of calibration curves

Arsenic standard working solution (3.15) 0, 0.10, 0.30, 0.50, 1.00, 1.50, 2.00 mL was pipetted into a 25 mL stoppered cuvette, water was added to 5 mL, hydrochloric acid (1+1) solution (3.5) 5.0 mL was added, then thiourea-ascorbic acid solution was added

(3.7) 2.0 mL, mix well, aspirate 2.0 mL of each standard series solution into the hydride generator, add a certain amount of sodium borohydride solution (3.9), measure the fluorescence intensity and plot the calibration curve with the fluorescence intensity as the vertical coordinate and the arsenic content (g/L) as the horizontal coordinate.

5.4 Measurement

Add 2.0 mL of thiourea-ascorbic acid solution (3.7) to a 25 mL stoppered cuvette, mix well and aspirate 2.0 mL. Determine the fluorescence intensity of the sample according to the procedure for preparing the calibration curve (see 5.3).

6 Calculation

$$(As) = \frac{(_1 - _0)V}{m1000}$$

where: (As) - mass fraction of arsenic in the sample, g/g.

$_1$ - the mass concentration of arsenic in the test solution, g/L.

$_0$ - the mass concentration of arsenic in the blank solution, g/L.

V - total volume of sample digest, mL; m - sample volume taken, g.

7 Precision and Accuracy

The intra-batch relative standard deviation for each concentration was 1.1 when the arsenic content in the samples ranged from 0.24g/g to 4.59g/g.

The average relative standard deviations of the three laboratories were 5.1%, 4.3% and 3.2%, respectively. The mean relative standard deviations of the three laboratories were 5.1%, 4.3% and 3.2% respectively. The average recoveries of the samples were 100.3% when 0.3g/g-4.5g/g of arsenic was added to the samples.

The mean spiked recoveries were 99.0 %, 98.1 % and 98.5 %, respectively.

Second method Spectrophotometric method

8 Methodology Summary

After ashing or ablation of the specimen, the pentavalent arsenic in the sample solution is reduced to trivalent in the presence of potassium iodide and stannous chloride. The trivalent arsenic is passed through lead acetate cotton to remove hydrogen sulphide interference by generating arsine gas with neo-ecological hydrogen. It then interacts with a silver nitrate solution containing polyvinyl alcohol and ethanol to produce yellow colloidal silver. Colourimetric, quantitative. Silver, chromium, cobalt, nickel, selenium, lead, bismuth, antimony and mercury interfere with the measurement of arsenic, but generally do not interfere with the levels in cosmetics. The method has a detection limit of 0.03g and a lower limit of quantification of 0.1g. If 1g of sample is taken, the method has a detection concentration of 0.03g/g and a minimum quantification concentration of 0.1g/g.

9 Reagents

9.1 Sulphuric acid (1 + 1): Take 100mL of sulphuric acid (3.2) and add slowly to 100mL of water.

9.2 Sulphuric acid (1 mol/L): take 55.5mL of sulphuric acid (3.2) and add slowly to 944.5mL of water.

9.3 Hydrochloric acid ($_{20}$ = 1.19 g/mL), ultrapure.

9.4 Sodium hydroxide solution (200g/L): weigh 200g of sodium hydroxide and dissolve in 1L of water.

9.5 Phenolphthalein indicator (1g/L ethanol solution): weigh 0.1g of phenolphthalein and dissolve in 50mL of 95% ethanol, add water to 100mL.

9.6 Magnesium nitrate solution (100g/L): weigh 100g of magnesium nitrate and dissolve in 1L of water.

9.7 Potassium iodide solution (150g/L): weigh 150g of potassium iodide and dissolve in 1L of water.

9.8 Stannous chloride solution (400g/L): weigh 40g of stannous chloride, dissolve in 40mL of hydrochloric acid (9.3), add water to 100mL and add a few pellets of tin.

9.9 Arsenic-free zinc granules, 10-20 mesh.

9.10 Lead acetate solution (100g/L): weigh 100g of lead acetate and dissolve in 1L of water.

9.11 Lead acetate cotton: Immerse the skimmed cotton in lead acetate solution (9.10), remove after 2h, dry and puff.

9.12 Nitric acid - silver nitrate solution: weigh 4.0g of silver nitrate, add 15mL of nitric acid (3.1) and set with water to 500mL.

9.13 Polyvinyl alcohol solution (2g/L): weigh 1.0g of polyvinyl alcohol (average degree of polymerisation 1750±50) and add slowly until there is 520mL

The final volume is 500 mL.

9.14 Anhydrous ethanol.

9.15 Absorbent: Mix nitric acid-silver nitrate solution (9.12) + polyvinyl alcohol solution (9.13) + ethanol (9.14) in (1+1+2).

10 Instruments

10.1 Kjeldahl flask (250mL) or conical flask (125mL).

10.2 Porcelain evaporating dish (50mL) or crucible.

10.3 Spectrophotometer.

10.4 The arsenic determination device (see Figure 1).

11 Analysis steps

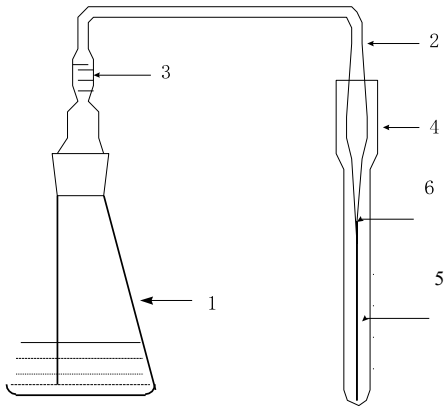
11.1 Sample pre-treatment (either one)

11.1.1 HNO_3-H_2SO_4 wet digestion method

Samples containing solvents such as ethanol should be pre-evaporated (not dried out). Samples containing particularly high levels of glycerol should be disinfected with particular care for safety.

Accurately weigh approximately 1.00 g of the mixed sample and place in a 250 mL nitrogen fixation flask or 125 mL conical flask. Also make a reagent blank. Add a few glass beads, 5mL of water and 10mL-15mL of nitric acid (3.1) and leave for a few moments. After cooling, add 5mL of sulphuric acid (3.2) and continue to heat for digestion. If during the digestion the solution is

Add a small amount of nitric acid (3.1) and continue to digest until the solution is clear or slightly yellow. Cool, add 20mL of water, heat and boil again until white smoke is present. This solution is equivalent to 2.0mL of sulphuric acid (1+1) per 10mL.



1: 125mL arsenical generation bottle.

2: Air guide tubes.

3: Lead acetate cotton.

4: 5mL graduated absorption tubes.

5: Silver nitrate-polyvinyl alcohol-ethanol absorbent; 6: PTFE tube 0.4mm inner diameter

Fig. 1 Arsenic determination device

11.1.2 Dry ashing method

Accurately weigh approximately 1.00 g of the mixed sample and place in a 50 mL porcelain evaporating dish. Also make a reagent blank. Add 10mL of magnesium nitrate solution (9.6) and 1g of magnesium oxide (3.3), mix well. Evaporate in a water bath, charring on low heat until no smoke, transfer to a box furnace, ash at 550°C for 4h, cool and remove. Add a little water to moisten. Add 20mL of hydrochloric acid (3.5) in several portions to dissolve the ash and wash the evaporating vessel, combine and transfer to a 50mL volumetric flask with water to scale and set aside. Each 10mL of this solution is equivalent to 2.0mL of hydrochloric acid (1+1).

11.2 Preparation of calibration curves

Pipette 0.00, 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 mL of the arsenic standard working solution (3.15) into the arsenical generation bottle. Add 10 mL of sulphuric acid (9.1) for samples treated by the wet digestion method (11.1.1) or 10 mL of hydrochloric acid (3.5) for samples treated by the dry ashing method (11.1.2). add water to a total volume of 50 mL, add 2.5 mL of potassium iodide solution (9.7) and 2 mL of stannous chloride solution (9.8) to each and shake well. After 10 min, add about 5 g of zinc pellets (9.9), immediately connect to an air guide tube stuffed with lead acetate and insert into an absorbent tube with 5.0 mL of absorbent solution (9.15) and react at room temperature for 1 h. If the volume of absorbent solution decreases after the reaction, add anhydrous ethanol (9.14) to 5.0 mL. The absorbance was measured at 410 nm using a 1 cm cuvette and the calibration curve was plotted.

11.3 Measurement

Take an appropriate amount of sample solution (11.1.1 or 11.1.2) and blank solution and place in an arsenical generation flask. Add sulphuric acid

Add 2.5 mL of potassium iodide solution (9.7) and 2 mL of stannous chloride solution (9.8). shake well for

10 min, add about 5 g of zinc pellets (9.9), immediately connect to an air guide tube stuffed with lead acetate and insert it into an absorbent tube filled with absorbent solution (9.15) to 5.0 mL. At the end of the reaction, add ethanol (9.14) to 5.0 mL if the volume of absorbent decreases.

12 Calculation

$$(As) = \frac{(_{1-0})V}{mV1}$$

where: (As) - mass fraction of arsenic in the sample, g/g.

$_1$ - the mass of arsenic in the test solution, g.

$_0$ - mass of arsenic in the blank solution, g; V - total volume of sample solution, mL; $_{V1}$ - - volume of sample solution dispensed, mL; m - volume of sample taken, g.

13 Precision and Accuracy

The relative standard deviation of the results for the 2.00g sample was 8.2% to 8.7%. The relative standard deviations (RSDs) for the 5.00g sample were 5.9% to 8.7%. The recoveries ranged from 91.2% to 94.7% at 2.00g. The recoveries ranged from 89.8% to 97.2% at 5.00g.

Third method Hydride generation by atomic absorption

14 Methodology Summary

After pretreatment of the sample, the arsenic in the sample solution is reduced to trivalent arsenic by potassium iodide-ascorbic acid under acidic conditions and then to arsine by the neo-ecological hydrogen produced by the interaction of sodium borohydride with the acid, which is introduced into the heated "T" quartz tube atomiser by the carrier gas and atomised. The characteristic spectral lines emitted by an arsenic hollow cathode lamp. The absorbance is proportional to the arsenic content of the sample over a range of concentrations. Comparison with standard series for quantification. The minimum limits of detection and quantification for this method were 1.7 ng and 5.7 ng, respectively, and 0.17 g/g and 0.57 g/g, respectively, for a 1 g sample.

15 Reagents

15.1 Hydrochloric acid [(HCl) = 10%]: take 10mL of hydrochloric acid (9.3) and add 90mL of water, mix well.

15.2 Potassium iodide (150g/L) - ascorbic acid solution (20g/L): 15g of potassium iodide and 2g of ascorbic acid, dissolved in water and diluted to 100mL.

15.3 Sodium borohydride solution (5g/L): Dissolve 0.5g of sodium hydroxide in 100mL of water, add 0.5g of sodium borohydride, dissolve and filter, store in a plastic bottle in the refrigerator.

16 Instruments

16.1 Atomic absorption spectrophotometer with hydride generator.

16.2 Stoppered colorimetric tube, 50mL.

16.3 Constant temperature oven.

17 Analysis steps

17.1 Sample pre-treatment (either method available)

17.1.1 $_{HNO3-H2SO4}$ wet digestion method

Accurately weigh approximately 1.00g of the mixed sample into a 125mL conical flask and make a reagent blank. If the sample contains a solvent such as ethanol, the solvent should be evaporated (not dried out) after weighing the sample. Add several glass beads, add nitric acid (3.1) 10mL to 20mL, leave for a few moments, heat slowly, remove the heat source after the reaction starts, cool slightly and add sulphuric acid (3.2) 2mL. continue to heat the solution for digestion, if the solution appears brown during digestion, add a little nitric acid (3.1) for digestion, repeat until the solution is clarified or slightly yellow. After cooling, add 20mL of water and continue to boil until white smoke is produced. Transfer the digestion solution quantitatively to a 50mL stoppered cuvette, add 5mL of potassium iodide-ascorbic acid solution (15.2), add water and allow to settle to the scale for 10min.

17.1.2 Dry ashing method

Accurately weigh approximately 1.00 g of the mixed sample and place in a 50 mL crucible while making a reagent blank. Add 1g of magnesium oxide (3.3) and 2mL of magnesium nitrate solution (9.6), stir well, evaporate on a water bath and char on a light flame until no smoke is emitted. Remove from the oven and add a little water to the ash to make it wet, then use hydrochloric acid (1+1)

(3.5) 20mL Dissolve the ash in several portions, add potassium iodide-ascorbic acid solution (15.2) 5mL, add water to build up to 50mL and allow to stand for 10min before measuring.

17.1.3 Pressure digestion tank digestion method

Accurately weigh approximately 1.00 g of the mixed sample and place it in a PTFE liner while making a reagent blank. If the sample contains a large amount of solvent such as ethanol, the solvent should be evaporated in advance on a water bath. Add 10mL-15mL of nitric acid (3.1) or 6mL of nitric acid (3.1) and 6mL of hydrogen peroxide (3.6), leave for a few moments, cover with the inner Teflon lid, place in the stainless steel cylinder of the digestion tank, cover with the inner stainless steel lid, inner gasket and outer lid in turn, tighten the outer lid with the tightening handle. Place in a constant temperature oven at 100C for 2h, increase temperature to 140C-150C for 4h, cool and remove. Transfer the sample solution to a 50mL beaker, wash the liner several times with water and combine the wash solutions. Add 5mL of sulphuric acid (9.2) and heat on a hotplate to drive out the nitric acid until white smoke is produced. Cool, add 20mL of water, transfer to a 50mL volumetric flask, add 5mL of potassium iodide-ascorbic acid solution (15.2) and add water to the scale. Allow to stand for 10min before measuring.

17.2 Preparation of calibration curves

Adjust the apparatus and hydride generator according to the apparatus instructions and Table 2. Adjust the apparatus and hydride generator according to the apparatus instructions and Table 2.

Table 2 Reference analytical conditions for the determination of arsenic

Wave length	Passband	Lamp current	Negative high pressure	Gain	Mode
193.7nm	0.4nm	1.5mA	588V	×2	Peak area
Points	Carrier Gas	Carrier gas flow	C2H2/air	Sodium borohydride solution	
9s	Nitrogen	1.0L/min	1.0/5.0	2mL	

Take 5mL of each standard solution into a hydride reaction flask, pass carrier gas to drive air out of the gas path to bring the absorbance to zero. Turn off the gas, add 2.0mL of sodium borohydride solution (15.3), ventilate and record the absorbance. The waste solution was drained and washed. The concentration-absorbance curve is plotted.

17.3 Measurement

Pipette 0.5 mL of sample solution and 4.5 mL of hydrochloric acid (15.1) into a hydride reaction flask and carry out the determination according to 17.2.

18 Calculation

$$(As) = \frac{(_{1\text{-}0})\ _{VVS} \times 1000}{mV1}$$

where: (As) - mass fraction of arsenic in the sample, g/g.

$_1$ - the mass concentration of arsenic in the test solution, g/L.

$_0$ - concentration of arsenic in blank solution, g/L; V - total volume of sample solution, mL; $_{VS}$ - volume of standard solution removed for determination, mL; V1 - volume of sample solution removed for determination, mL; m - volume of sample taken, g. - volume of standard solution removed for the determination, mL; $_{V1}$ - volume of sample solution removed for the determination, mL; m - volume of sample taken, g.

19 Precision and Accuracy

The relative standard deviations of the samples for each concentration ranged from 3.1% to 7.1 % when the arsenic content in the samples ranged from 2.09 g/g to 12.12 g/g.

%. The relative standard deviations of the three laboratories ranged from 3.7% to 9.0%.

The spiked recoveries were 94.3% when 2.5g/g to 10g/g of arsenic was added to the samples, and the spiked recoveries determined by the three laboratories ranged from 84.2% to 103%.

IV. Lead

Lead

1 Scope

This specification specifies a method for the determination of lead in cosmetics by flame atomic absorption spectrophotometry, differential potential dissolution and dithizone extraction spectrophotometry.

This specification applies to the determination of lead in cosmetics.

First method Flame atomic absorption spectrophotometry

2 Methodology Summary

The sample is pretreated so that the lead is present in the sample solution in an ionic state. After the lead ions in the sample solution have been atomised, the base state lead atoms absorb resonance lines from a lead hollow cathode lamp and their absorbance is proportional to the amount of lead in the sample. Quantification is based on the measurement of the intensity of the absorbed spectral lines, compared to a standard series, all other things being equal. The method has a detection limit of 0.15 mg/L and a lower limit of quantification of 0.50 mg/L. If 1 g of sample is taken for determination and the volume is fixed to 10 mL, the detection concentration of the method is

1.5g/g with a minimum quantitative concentration of 5g/g.

3 Reagents

3.1 Nitric acid ($_{20}$ = 1.42 g/mL), ultrapure.

3.2 Perchloric acid [($_{HClO4}$) = 70% to 72%], superior pure.

3.3 Hydrogen peroxide [($_{H2O2}$) = 30%].

3.4 Nitric acid (1+1): Take 100mL of nitric acid (3.1), add 100mL of water and mix well.

3.5 Mixed acids: Nitric acid (3.1) and perchloric acid (3.2) mixed at 3+1.

3.6 Octanol.

3.7 Hydroxyammonium Hydrochloride Solution (120g/L): Take 12.0g of Hydroxyammonium Hydrochloride and 12.0g of Sodium Chloride and dissolve in 100mL of water.

3.8 Lead standard solutions

3.8.1 Lead standard solution [(Pb)=1g/L]: weigh 1.000g of lead metal of 99.99% purity, add 20mL of nitric acid solution (3.4), heat to dissolve, transfer to a 1L volumetric flask and dilute to the scale with water.

3.8.2 Lead standard solution [(Pb)=100mg/L]: Take 10.0mL of lead standard solution (3.8.1) in a 100mL volumetric flask, add 2mL of nitric acid solution (3.4) and dilute to the scale with water.

3.8.3 Lead standard solution [(Pb)=10mg/L]: Take 10.0mL of lead standard solution (3.8.2) in a 100mL volumetric flask, add 2mL of nitric acid solution (3.4) and dilute to the scale with water.

3.9 Methyl isobutyl ketone (MIBK).

3.10 Hydrochloric acid solution (7 mol/L): Take 30 mL of concentrated hydrochloric acid ($_{20}$ = 1.19 g/mL) in excellent purity and add water to 50 mL.

4 Instruments

4.1 Atomic absorption spectrophotometer and accessories.

4.2 Centrifuge.

4.3 Stiff glass digestion tubes or small nitrogen fixing digestion bottles.

4.4 Stoppered cuvettes, 10mL, 25mL, 50mL.

4.5 Dispensing funnel, 100mL.

4.6 Evaporation dish.

4.7 Pressure self-contained microwave digestion system.

4.8 High-pressure, airtight digestion tank.

4.9 Polytetrafluoroethylene dissolving sample cup.

4.10 Water baths (or open type electrically heated thermostats)

5 Analysis steps

5.1 Sample pre-treatment (either method available)

5.1.1 Wet digestion method

Weigh approximately 1.00g to 2.00g of the mixed sample in the digestion tube and make a reagent blank. For samples containing organic solvents such as ethanol, evaporate at low temperature in a water bath or on an electric hot plate. For cream-type samples, pre-heat in a water bath to melt the sample on the walls of the bottle into the bottom of the bottle. Add several glass beads, then add nitric acid (3.1) 10mL Note 1 and heat the digestion from low to high temperature. When the digestion volume is reduced to 2mL to 3mL, remove the heat source and cool. Add perchloric acid (3.2) 2mL to 5mL

Note 2: Continue to heat the digestion, shaking slowly from time to time to make it homogeneous, and digest until white smoke is present and the digestion solution is light yellow or colourless. Concentrate the digestion

The solution is brought to approximately 1mL. Cool to room temperature and transfer quantitatively to a 10mL (or 25mL for powder samples) stoppered cuvette, set to scale with water and reserve. If the sample is cloudy, the supernatant can be removed by centrifugation and used for determination.

5.1.2 Microwave digestion

Weigh approximately 0.5g to 1g of the mixed sample into a cleaned Teflon sample cup. Cosmetics containing volatile materials such as ethanol, such as perfumes, mousse, body lotions, hair dyes, serums, shaving lotions, face masks, etc., should first be volatilised in a temperature adjustable 100ºC electric heater or water bath (do not steam dry). For dry substances such as lipstick, mascara, eyebrow pencil, rouge, lip liner, powder, eye shadow, talcum powder, prickly heat powder, etc., add 0.5mL to 1.0mL of water after sampling and wetting and shaking well.

Depending on the ease of sample digestion, samples or pre-treated samples are first added to nitric acid (3.1) 2.0mL to 3.0mL and allowed to stand overnight for full action. Then add hydrogen peroxide (3.3) 1.0mL to 2.0mL in sequence and shake the sample cup several times to fully submerge the sample. Place in a boiling water bath or thermostatic heating apparatus at an adjustable temperature for 20 min at 100ºC and remove. If the volume of solution is less than 3mL, replenish with water. Follow the Microwave Dissolution System operating instructions to the letter.

Place the sample cup into a clean, high pressure, airtight lysimeter prepared in advance and screw on the lid (note: do not over-tighten).

Table 1 shows the pressure - time procedure for general cosmetic products for decomposition. If the cosmetics are oils, herbs or detergents, the sensitivity of the explosion-proof system can be increased appropriately to increase safety.

Depending on the ease of sample digestion, the digestion can be completed within 5 min to 20 min, the sample is cooled down, the can is opened and the sample cup containing the digested sample is placed in a boiling water bath or an electric heater at an adjustable temperature of 100ºC for a few minutes to remove excess nitrogen oxides from the sample so as not to interfere with the determination.

Table 1 Pressure time program during digestion

Pressure gear	Pressure (Mpa)	Holding pressure accumulation time (min)
1	0.5	1.5
2	1.0	3.0
3	1.5	5.0

Transfer the sample to a 10mL stoppered cuvette, wash the dissolution cup several times with water, combine the washing solution, add hydroxylamine hydrochloride solution (3.7) 0.5mL Note 3, set the volume with water to 10mL and reserve.

5.1.3 Extraction method (only for wax-free cosmetics)

Accurately weigh approximately 1.00 g of the mixed sample and place in a 50 mL stoppered cuvette. Make a reagent blank with the sample. For samples containing organic solvents such as ethanol, evaporate first in a water bath or on a hotplate at low temperature. For cream samples, preheat in a water bath to melt the sample on the wall into the bottom of the tube. Add 5.0mL of nitric acid (3.1) and 2.0mL of hydrogen peroxide (3.3), mix well and

If large amounts of foam appear, add a few drops of octanol (3.6). Heat in a boiling water bath for 2 h. Remove, add hydroxylammonium hydrochloride solution (3.7) 1.0 mL Note 3, leave for 15 min to 20 min and set with water to 25 mL.

5.2 Measurement

5.2.1 Pipette 0, 0.50, 1.00, 2.00, 4.00, 6.00 mL of the lead standard solution (3.8.3) into a 10 mL stoppered cuvette and add water to the scale. The analytical conditions of the instrument were adjusted to optimum conditions according to the instrument operating procedure. The calibration curve series, the blank and the sample solution were measured under background absorption. If the iron content of the sample solution exceeds the lead content by a factor of 100, the

The deuterium lamp method of background deduction should not be used, but the Seeman effect method of background deduction should be used, or iron should be removed in advance according to 5.2.2. Plotting concentrations

--Absorbance curve to calculate the sample content.

5.2.2 The standard, blank and sample solutions were transferred to an evaporating dish and evaporated to dryness on a water bath. Dissolve the residue by adding 10 mL of hydrochloric acid (3.10), transfer to a separatory funnel and extract twice with an equal amount of MIBK (3.9), retaining the hydrochloric acid solution. The solution is then extracted with hydrochloric acid

(3.10) Wash 5 mL of the MIBK layer, combine the hydrochloric acid solutions, drive out the acid if necessary and fix the volume. Follow the instrument procedure and carry out the determination.

6 Calculation

$$(Pb) \qquad \frac{(_{10})\ V}{m}$$

where: (Pb) - mass fraction of lead in the sample, g/g.

$_1$ - the mass concentration of lead in the test solution, mg/L.

$_0$ - mass concentration of lead in blank solution, mg/L; V - total volume of sample digest, mL; m - sample sampling volume , g.

Second method Differential Potential Dissolution Method

7 Methodology Summary

The sample is pretreated so that the lead is present in the solution in an ionic state. The lead is enriched at the appropriate reduction potential at a glassy mercury film electrode. In acidic solutions, there is a sensitive dissolution peak for lead ions at -0.46V (relative to the saturated mercury electrode), the peak height being proportional to its content. Other things being equal, the dissolved peak is measured and quantified by comparison with a standard series. The method has a detection limit of 0.056 g and a lower limit of quantification of 0.19 g. If 1 g of sample is taken, the detection concentration is 0.56 g/g and the

lowest quantification concentration is 1.9 g/g.

8 Reagents

8.1 Electrolytic mercury plating solution: weigh $Hg(_{NO3})2$-H2O 68.5mg and $_{KNO3}$ 25.3g in water, add nitric acid (3.1)

0.63mL, volume to 1L.

8.2 Hydrochloric acid (1+1): Take 100mL of superior pure hydrochloric acid ($_{20}$ = 1.19g/mL), add 100mL of water and mix well.

8.3 Ethanol (1+1): Take 100mL of anhydrous ethanol, add 100mL of water and mix well.

8.4 Phenolphthalein indicator (1g/L ethanol solution): Dissolve 0.1g of phenolphthalein in 50mL of anhydrous ethanol, add 50mL of water and mix well.

8.5 Ammonia (1+1): take 100mL of ammonia, add 100mL of water and mix well.

9 Instruments

9.1 Electric sand bath.

9.2 Conical flask, 100mL.

9.3 Volumetric flask, 100mL.

9.4 Glass beaker, 50mL.

9.5 Differential Potential Dissolution Analyser and accessories.

10 Analysis steps

10.1 Sample pre-treatment

If a standard addition method is used, take two identical samples, add the appropriate amount of lead ions to one of the samples, disinfect both samples equally and then volumize to 100 mL.

10.2 Preparation of standard working solutions

Take 0, 0.050, 0.10, 0.20, 0.40, 0.70 and 1.00 mL of lead standard solution (3.8.3), place in 6 conical flasks, disperse simultaneously with the sample and allow to build up to 100 mL.

10.3 Electrode pre-treatment

The glassy carbon electrodes were soaked in nitric acid (1+1) (3.4) before use, rinsed with water, wiped with filter paper dripping with ethanol (1+1) (8.3) and then rinsed with water. The three electrodes are inserted into the mercury plating solution (8.1), plated with mercury according to the parameters in Table 2 and then set aside for use.

10.4 Measurement

Add 2 drops of phenolphthalein indicator (8.4) and adjust with ammonia (1+1) (8.5) until the solution is slightly red, then add 0.3 mL of hydrochloric acid (1+1) (8.2), set the volume with water to the scale, transfer to a 50 mL beaker and insert into a triple The solution was transferred to a 50mL beaker, inserted into three electrodes and measured according to the parameters in Table 2.

For the working curve method, a mass-solution peak height curve is plotted and the sample content is calculated. For the standard addition method, the peak height and addition amount are calculated directly.

Table 2 Instrument reference parameters

Instrument parameters	Mercury plating	Measurement
Electrolytic voltage (V)	-1.10	-1.10
Electrode speed (rpm)	2500	2000
Enrichment time (s)	40	60
Dissolved lower limit voltage (V)	-0.20	-0.20
Dissolved upper voltage (V)	-0.90	-1.00
Resting time (s)	30	30
Sensitivity	20	20
Electrode wash time (s)	20	10 to 20

11 Calculation

11.1 Working curve method.

$$(Pb) = \frac{(m_1 - m_0)V}{mV_1}$$

Where: (Pb) - mass fraction of lead in the sample, g/g; m_1 - mass of lead in the test solution, g; m0 -- mass of lead in the blank solution, g; V --total volume of sample solution, mL; V1--volume of sample solution taken, mL V - total volume of sample solution, mL; V1 - volume of sample solution dispensed, mL; m - sample volume taken, g.

11.2 Standard accession method.

$$(Pb) = \frac{h1 \times m1}{(h2-h1) \times m}$$

Where: (Pb) - mass fraction of lead in the sample, g/g; $h1$ - peak height of lead in the sample solution; $h2$ - - the peak height of the sample of lead in the solution after the addition of the standard; $m1$ - the mass of the lead standard added, g; m - the sample sampling volume, g.

12 Precision and Accuracy

Four types of cosmetic samples (powder, water, honey and oil) with different matrix types were spiked at three concentrations (high, medium and low) and the relative standard deviations ranged from 2.05% to 7.96%, with the recoveries ranging from 82.7% to 103.0%.

Third method Dithizone extraction spectrophotometry

13 Methodology Summary

The sample was pretreated and the lead in the sample solution under weak alkalinity to form a red chelate with dithizone, extracted with chloroform and quantified colourimetrically. The presence of large amounts of tin interferes with the determination. This method is not applicable to samples containing titanium oxide and bismuth compounds. The detection limit of this method is 0.3 g and the lower limit of quantification is 1.0 g. If 1 g of sample is taken, the detection concentration is 0.3 g/g and the minimum quantification concentration is 1 g/g.

14 Reagents

14.1 Chloroform, oxide free.

14.2 Phenol Red Indicator Solution (1g/L ethanol solution): Dissolve 0.1g of phenol red in 100mL of anhydrous ethanol and mix well.

14.3 Dithizone stock solution (1 g/L chloroform solution): store in a cold, dark place. If necessary, purify as follows: weigh 0.5 g of finely ground dithizone and dissolve in 50 mL of chloroform. If not completely dissolved, filter through 950 mL of filter paper into a separatory funnel. Use ammonia

(1+1) (8.5) Extract 3 times, 100mL each time, and combine the extracts. The ammonia solution was then washed 2 times with l0mL of chloroform. Use hydrochloric acid

(1+1) (8.2) was adjusted to acidity and the precipitated dithizone was extracted 2-3 times with chloroform at 100 mL each. the chloroform layers were combined and chloroform was added to a total volume of 500 mL.

14.4 Dithizone use solution (0.01 g/L chloroform solution): take 1 mL of the dithizone stock solution

(14.3) and dilute to 100 mL with chloroform.

14.5 Nitric acid [(HNO_3) = 1%]: take 10mL of nitric acid (3.1), add 990mL of water and mix well.

14.6 Hydroxylamine hydrochloride solution (200g/L): take 20g of hydroxylamine hydrochloride, add 50mL of water, add 2 drops of phenol red indicator solution (14.2), add ammonia (1+1) (8.5) to pH 8.5~9.0, extract with dithizone chloroform solution (14.4) until the chloroform layer remains green, then wash the aqueous layer twice with chloroform (14.1). The aqueous layer was then washed twice with chloroform (14.1). The aqueous layer was adjusted to acidity with hydrochloric acid (1+1) (8.2) and water was added to 100 mL and set aside.

14.7 Ammonium citrate solution (200g/L): Take 50g of ammonium citrate and dissolve in 100mL of water, add 2 drops of phenol red indicator solution (14.2), add ammonia (1+1) (8.5) to pH 8.5~9.0, extract several times with dithizone chloroform solution (14.4), 10mL~20mL each time, until the chloroform layer remains green. The chloroform layer was discarded and the aqueous layer was diluted to 250 mL with water.

14.8 Potassium cyanide solution (100g/L) (note that it is highly toxic): If the reagent contains lead and needs to be purified, 10g of potassium cyanide should first be dissolved in 20mL of water and then diluted to 100mL after the following purification according to the method described in 14.3.

14.9 Lead-free skimmed cotton: medical skimmed cotton, if necessary with dithizone chloroform solution to remove lead.

15 Instruments

15.1 Dispensing funnel (125mL): pre-soaked in dilute acid and washed with water.

15.2 Spectrophotometer.

16 Analysis steps

16.1 Sample pre-treatment is as in 5.1.1.

16.2 Measurement

Add water to a 125 mL separatory funnel to a total volume of 50 mL, then add 0, 0.10, 0.20, 0.30, 0.40 and 0.50 mL of lead standard solution (3.8.3) to a 125 mL separatory funnel and add nitric acid solution (14.5) to a total volume of 50 mL. Then add 2 mL of ammonium citrate solution (14.7), 1 mL of hydroxylamine hydrochloride solution (14.6) and 2 drops of phenol red indicator solution (14.2) to the sample solution, blank solution and lead standard solution, and adjust with ammonia (8.5) until red colour appears. Add 2 mL of potassium cyanide solution (14.8) to each separatory funnel and mix well. Add 5mL of dithizone solution (14.4) accurately, shake vigorously for lmin and leave to stratify. A small amount of lead-free desiccated cotton (14.9) was stuffed into the lower neck of the partition funnel and the chloroform layer was then filtered into a cuvette. Zeroed with chloroform, the absorbance was measured at a wavelength of 5l0nm and a standard curve was plotted.

17 Calculation

$$(Pb) = \frac{(m_1 - m_0)\,V_{mV1}}{}$$

Where: (Pb) - mass fraction of lead in the sample, g/g; $_{m1}$ - mass of lead in the test solution, g; $_{m0}$ -- mass of lead in the blank solution, g; V -- total volume of sample solution, mL; $_{V1}$ -- volume of sample solution dispensed, mL ;. m - the volume of sample taken, g.

Note 1 For samples containing carbonate-based powders, nitric acid should be added slowly to prevent the production of carbon dioxide gas too vigorously.

NOTE 2 Perchloric acid is explosive if used improperly. For the safe use of perchloric acid, the following points should be noted.

1. Spilled perchloric acid should be rinsed immediately with water.

2. Fume hoods, air ducts and other devices for the removal of perchloric acid vapours should be made of chemically inert substances and flushed with water after digestion. Exhaust systems should be installed in a safe location.

3. Avoid the use of organic or other fume-producing substances in fume hoods where perchloric acid digestion is used.

4. Operators should use goggles, shields and other personal protective equipment. Use polyvinyl chloride gloves, not rubber gloves.

5. When using perchloric acid for wet digestion, unless otherwise stated, samples should first be destroyed by nitric acid to destroy easily oxidised organic matter and care should be taken to avoid burning them dry.

6. Perchloric acid (constant boiling mixture, boiling point 203°C) is stable at a concentration of 72%. If perchloric acid is dehydrated (e.g. in contact with strong dehydrating agents), anhydrous perchloric acid will be formed and its stability will be very significantly reduced, at which point it will explode when exposed to heat, impact or to organic matter or reducing agents (e.g. paper, wood or rubber).

Note 3 This addition of hydroxylamine hydrochloride is waived if the sample is not to be measured for mercury.

V. Methanol

Methanol

1 Scope

This specification specifies a gas chromatographic method for the determination of methanol in cosmetics. This specification applies to the determination of methanol in cosmetics containing ethanol or isopropanol.

2 Methodology Summary

The samples are pretreated (by distillation or by gas-liquid equilibration) and then tested and quantified by gas chromatography. The method has a detection concentration of 15 g/g and a minimum quantitative concentration of 50 g/g.

3 Reagents

3.1 Methanol-free ethanol: 1.0L should be injected into the chromatograph and no spurious peaks should appear.

3.2 Ethanol [(C2H5OH)=75%]: 75mL of methanol-free ethanol (3.1) was taken and diluted to 100mL with water.

3.3 Chromatographic stretcher GDX-102 (60 mesh to 80 mesh), gas chromatographic reagent.

3.4 Chromatographic fixative polyethylene glycol 1540 (or 1500), gas chromatography reagent.

3.5 Methanol Standard Solution

3.5.1 Sample preparation for 5.3.1: Take 1.00mL of chromatographically pure methanol in a 100mL volumetric flask and fix to the mark with 75% ethanol (3.2). Store in a refrigerator.

3.5.2 For 5.3.2 and 5.3.3 sample preparation: take approximately 1.00 g of chromatographically pure methanol in a 100 mL volumetric flask and fix to the mark with 75% ethanol (3.2), this standard contains 10 g/L of methanol. store in a refrigerator.

3.6 Sodium chloride.

3.7 Defoamer: emulsified silicone oil.

4 Instruments

4.1 Gas chromatograph with hydrogen flame ionisation detector.

4.2 Chromatographic column: 2m2mm, filled with GDX-102, suitable for samples without dimethyl ether.

4.3 Chromatographic column: 2m4mm, filled with GDX-102 (3.3) stretcher coated with 25% polyethylene glycol 1540 (or 1500). Suitable for samples containing dimethyl ether.

4.4 All-glass, ground-mouth water distillation unit.

4.5 Super thermostat bath: temperature range 0°C to 100°C, temperature control accuracy ±0.5°C.

4.6 Headspace bottle: 20mL to 65mL.

4.7 Syringes: 0.5L, 1L, 1mL.

5 Analysis steps

5.1 Chromatographic reference conditions

Start the chromatograph and make the necessary adjustments to achieve optimum operating conditions for the instrument, the chromatographic conditions being selected on a case-by-case basis.

The reference conditions are.

Chromatographic conditions for column 1 (4.2) (for samples without dimethyl ether) Column temperature: 170°C; gas chamber temperature: 180°C; detector temperature: 180°C.

Nitrogen flow rate: 40 mL/min; hydrogen flow rate: 40 mL/min; air flow rate: 500 mL/min. Chromatographic conditions for column 2 (4.3) (for samples containing dimethyl ether)

Column temperature: 75°C; gasification chamber temperature: 90°C; detector temperature: 150°C.

Nitrogen flow rate: 30mL/min; Hydrogen flow rate: 30mL/min; Air flow rate: 300mL/min.

5.2 Sample taking

Direct sampling of cosmetic products without propellant. Samples containing propellants, such as hairspray, are sampled as follows: take a quantity of 75% ethanol (3.2) in a headspace bottle or distillation bottle, fit a syringe needle to the nozzle of the hairspray bottle, connect it to a thin Teflon tube, insert the other end of this tube below the ethanol level and slowly press the nozzle so that the hairspray flows out of the needle into the ethanol solution via the thin Teflon tube. If it is difficult to press out the sample, the sample can be cooled in the refrigerator and then squeezed to take the sample. Calculate the sample size by subtracting the difference.

5.3 Sample pre-treatment

5.3.1 Direct method (this method is only applicable to non-hairspray, low-viscosity cosmetics): direct sampling for determination or taking a certain sample with

Dilute with 75% ethanol (3.2) and measure (filter if necessary).

5.3.2 Distillation method (this method is applicable to all types of cosmetics): Take about 10 g of the sample in a distillation flask (4.4), add 50 mL of water, 2 g of sodium chloride (3.6), 1 drop of antifoaming agent (3.7) and 30 mL of methanol-free ethanol (3.1), distill in a boiling water bath, collect the distillate until it no longer evaporates, add methanol-free ethanol to a volume of 50 mL and use this as the sample solution.

5.3.3 Gas-liquid equilibrium method (this method is not applicable to hairspray cosmetics): take about 5g of sample in a headspace bottle and add 75% ethanol

(3.2) 5 mL, sealed and equilibrated in a constant temperature water bath at 40°C for 20 min. The gas on the liquid after equilibration was taken as the sample to be measured.

5.4 Preparation of calibration curves

5.4.1 For samples pretreated according to 5.3.1: Take 7 50mL volumetric flasks and add 0.25, 0.50, 1.00, 2.00, 4.00, 7.00, 10.0mL of methanol standard solution (3.5.1) and then add 75% ethanol (3.2) to the scale, the standard series contains 0.005, 0.010, 0.020, 0.040, 0.080, 0.140, 0.200% methanol (v/v). This standard series contains 0.005, 0.010, 0.020, 0.040, 0.080, 0.140, 0.200% methanol (v/v). The standard solution was then injected into the gas chromatograph and the peak area was recorded and the peak area - methanol concentration (%, v/v) curve was plotted.

5.4.2 For samples pretreated according to 5.3.2: Take 7 50mL volumetric flasks and add 0.25, 0.50, 1.00, 2.00, 4.00, 7.00, 10.0mL of methanol standard solution (3.5.2), followed by 75% ethanol (3.2) to the scale, this standard series contains 0.050, 0.10, 0.20, 0.40, 0.80, 1.40, 2.00g/L of methanol. The standard solution was then injected into the gas chromatograph and the peak area was recorded and the peak area-methanol concentration (g/L) curve was plotted.

5.4.3 For samples pretreated according to 5.3.3: take the methanol standard solution (3.5.2) 0, 0.10, 0.50, 1.00, 2.00

3.00, 4.00mL in a headspace flask, add 75% ethanol (3.2) to 10.0mL and prepare to 0, 0.10, 0.50, 1.00, 2.00, 2.00

The standard series of 3.00 and 4.00 g/L were sealed and equilibrated in a constant temperature water bath at 40°C for 20 min. 1 mL of gas was taken from the liquid in turn

Injected into the gas chromatograph, the peak area of each chromatogram was noted and a peak area - methanol concentration (g/L) curve was plotted.

5.5 Measurement

1L (or 1mL of gas on liquid) of the sample solution to be measured was injected into the gas chromatograph in turn and the peak area was recorded for each chromatographic run. The methanol content of the sample solution was obtained from the peak area - methanol concentration curve.

6 Calculation

$$(CH_3OH) = \frac{V1000\ m}{}$$

where: (CH3OH) - mass fraction of methanol in the sample, g/g.

--mass concentration of methanol in the test solution, g/L; V - volume of sample volume, mL.
m - volume of sample taken, g.

If the sample is fed directly according to 3.5.1, the calculation can be made according to the following formula. If necessary, convert to mass fraction based on methanol and sample density.

(CH3OH)= 1100K

where: (CH3OH) - volume fraction of methanol concentration in the sample, $^{10-6}$.

$_1$ - concentration of methanol in the test solution, % (v/v).

K - sample dilution times.

7 Chromatograms

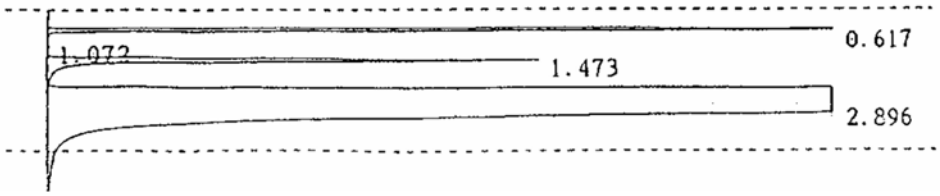


Fig. 1 Chromatogram of methanol and dimethyl ether (chromatographic condition 2) 1 Dimethyl ether (0.617min) 2 Methanol (1.473min)

VI. Free hydroxide

Free Hydroxide

1 Scope

This specification specifies a method for the determination of free hydroxide (both sodium and potassium hydroxide as sodium hydroxide) in cosmetics.

This specification applies to the determination of free hydroxides in various different types of straight hair products.

2 Citation Standards

The following standards contain provisions which, by reference in this specification, constitute the provisions of this specification, the versions shown are valid at the time of publication of this specification, all standards are subject to revision and parties using this specification should explore the possibility of using the latest versions of the following standards.

GB/T601-2002 Preparation of standard titration solutions for chemical reagents, section 4.2.

3 Methodology Summary

The hydroxide in the sample is neutralised with hydrochloric acid and the electrode potential changes. The end point of the titration is determined as pH 9.2. The detection limit for hydroxide in this method is 0.20 mg. If a 2 g sample is taken, the minimum detection concentration is 0.01%.

4 Reagents

4.1 Standard solution of hydrochloric acid [c(HCl) = 0.100 mol/L], prepared and calibrated as described in GB/T601-2002, section 4.2.

5 Instruments

5.1 Acid micro-buret.

5.2 Precision acidity meter.

5.3 Composite electrodes or glass electrodes with saturated glycury electrodes.

5.4 Magnetic stirrer.

5.5 Beakers, 150mL, 25mL.

6 Analysis steps

6.1 Qualitative tests

6.1.1 Sample preparation Weigh 1g into 9mL of water, place in a 25mL beaker, add a stirrer and stir on a magnetic stirrer until the sample is uniformly dispersed in the water (if not, sonicate the sample for another 5min-10min) as a pH qualitative assay solution.

6.1.2 pH measurement

If the pH of the solution to be measured is greater than or equal to 11, the following quantitative determination is carried out using a calibrated pH meter.

6.2 Quantification

6.2.1 Weigh 1g~2g (to 1mg) of the mixed sample, place in a 150mL beaker, add a few small pumice or small glass beads if it contains ammonia odour, place in a vacuum desiccator, pump with a vacuum pump for 3h (about 4h if pumped) until the sample no longer has ammonia odour, add 100mL of water, add a stirrer, stir on a magnetic stirrer until the sample is uniformly dispersed in Add 100mL of water, add the stirrer and stir on a magnetic stirrer until the sample is uniformly dispersed in the water (if not uniformly, the sample is then ultrasonically dispersed on an ultrasonic cleaner for 5min~10min) to be measured, while stirring.

Titrate the standard solution (4.1) (the titration rate should not be fast), titrate slowly as the pH approaches 9.6, stirring more, stop stirring when the pH reaches 9.2 and take an accurate reading of the amount of hydrochloric acid standard solution.

6 Calculation

$$(NaOH) = \frac{40cV100\ m1000}{}$$

where: (NaOH) - the mass fraction of hydroxide in the sample, %; c - the concentration of the standard solution of hydrochloric acid, mol/L; V - - volume of hydrochloric acid standard solution consumed for the titration, mL; m - volume of sample taken, g.

40 - Molar mass of hydroxide, g/mol.

VII. pH

pH

1 Scope

This specification specifies the potentiometric method for determining the pH value of cosmetics. This specification applies to the determination of the pH value of cosmetics.

2 Methodology Summary

A glass electrode is used as the indicator electrode and a saturated mercury electrode as the reference electrode, which are inserted into the solution being measured to form a cell. The potential difference generated by this cell is related to the pH of the solution being measured and the relationship between them is in accordance with the Nernst equation.

$$E = E_0 + 0.059 \lg[H^+] \ (25°C) \quad E = E_0 - 0.059\ pH$$

where E_0 - constant

At 25°C, each unit of pH is equivalent to 59.1mV of potential difference. This means that for every 59.1mV change in potential difference, the pH in the solution changes by 1 unit. The pH value can be read directly from the instrument.

3 Reagents

The reagents used in this specification are of superior purity unless otherwise stated. Water used refers to deionised water free of CO_2.

3.1 Potassium hydrogen benzodicarboxylate standard buffer solution: 10.12 g of potassium hydrogen benzodicarboxylate ($KHC_8H_4O_4$), dried at 105°C for 2h, was dissolved in water and diluted to 1L and stored in a plastic bottle. This solution has a pH of 4.00 at 20°C.

3.2 Phosphate standard buffer solution: 3.40g of potassium dihydrogen phosphate (KH_2PO_4) and 3.55g of disodium hydrogen phosphate (Na_2HPO_4), dried at 105°C for 2h, were dissolved in water and diluted to 1L and stored in a plastic bottle. This solution has a pH of 6.88 at 20°C.

3.3 Sodium borate standard buffer solution: Weigh 3.81 g of sodium tetraborate (NaB4O7-10H2O), dissolve in water, dilute to 1 L and store in a plastic bottle. This solution has a pH of 9.22 at 20°C.

The pH values of the above three standard buffer solutions vary slightly with temperature, see Appendix A.

4 Instruments

4.1 Precision acidity meter.

4.2 Composite electrodes or glass electrodes and glymeric electrodes.

4.3 Magnetic stirrer (with heating control)

4.4 Beaker, 50mL.

5 Analysis steps

5.1 Sample pre-treatment

5.1.1 Dilution method

Weigh 1 sample (to 0.1g), add 10 parts of deionised water without $_{CO2}$, heat to 40°C, stir continuously until homogeneous and cool to room temperature.

For products with a high oil content, heat to 70°C to 80°C, cool and remove the oil for use; powdered products can be precipitated and filtered for use.

5.1.2 Direct measurement (not applicable to powders, oily cosmetics and water-in-oil emulsions)

Place the appropriate amount of sample from the packaging container into the beaker to be used or remove the cap from the small packaging and insert the electrode directly into it.

5.2 Measurement

5.2.1 Electrode activation Composite electrodes or glass electrodes (4.2) should be soaked in water for at least 24h before use.

5.2.2 Calibrate the instrument according to the factory instructions (4.1) with two standard buffer solutions close to the pH of the sample at the specified temperature or under temperature compensation conditions.

5.2.3 After washing the electrode with water and blotting it with filter paper, insert the electrode into the sample to be measured, start the stirrer and stop the stirrer after 1 min of stable acidity meter reading and read the pH value directly from the instrument. Test twice with a margin of error of ±0.1 and take the average reading. After the measurement, the electrode is rinsed with water and the glass electrode is immersed in water.

6 Precision

The relative standard deviations of the four laboratories for the 19 commercially available cosmetic samples were 0.16% to 1.94% for 6 to 22 parallel determinations using the dilution method.

Appendix A

Table 1 pH values of standard buffer solutions at different temperatures

Temperature ° C	pH of the standard buffer solution		
	Benzenedicarboxylic acid salt	Phosphate	Borates
0	4.01	6.98	9.46
5	4.01	6.95	9.39
10	4.00	6.92	9.33
15	4.00	6.90	9.27
20	4.00	6.88	9.22
25	4.01	6.86	9.18
30	4.01	6.85	9.14
35	4.02	6.84	9.10
40	4.02	6.84	9.07
45	4.03	6.83	9.04

VIII. Cadmium

Cadmium

1 Scope

This specification specifies a method for the determination of total cadmium in cosmetics by flame atomic absorption spectrophotometry and differential potential dissolution. This specification applies to the determination of total cadmium in cosmetics.

First method Flame atomic absorption spectrophotometry

2 Methodology Summary

The sample is pretreated so that the cadmium is present in solution in an ionic state. After the cadmium ions in the sample solution have been atomised, the ground state atoms absorb the resonance lines from the cadmium hollow cathode lamp in an amount proportional to the amount of cadmium in the sample. Quantification is based on comparison of the measured absorption values with a standard series, all other conditions being equal. The method has a detection limit of 0.007 mg/L and a lower limit of quantification of 0.023 mg/L. If 1 g of sample is taken, the detection concentration is 0.18 g/g and the lowest quantitative concentration is 0.59 g/g.

3 Reagents

3.1 Nitric acid ($_{20}$ = 1.42 g/mL), ultrapure.

3.2 Perchloric acid [($_{HClO4}$) = 70% to 72%], superior pure.

3.3 Hydrogen peroxide [($_{H2O2}$) = 30%], ultrapure.

3.4 Nitric acid (1+1): Take 100mL of nitric acid (3.1), add 100mL of water and mix well.

3.5 Mixed acids: Nitric acid (3.1) and perchloric acid (3.2) mixed at (3+1).

3.6 Cadmium standard solution

3.6.1 Cadmium standard solution [(Cd) = 1 g/L]: weigh 1.000 g of cadmium metal [(Cd) = 99.99%] and add nitric acid (1+1)

(3.4) 20mL in a 250mL beaker and heat to dissolve. Transfer to a 1L volumetric flask and dilute to scale with water.

3.6.2 Cadmium Standard Solution [(Cd)=100mg/L]: Pipette 10.0mL of Cadmium Standard Solution (3.6.1) into a 100mL volumetric flask, add nitric acid (1+1) (3.4) 2mL and dilute to the scale with water.

3.6.3 Cadmium Standard Solution [(Cd)=10mg/L]: Dispense 10.0mL of Cadmium Standard Solution

(3.6.2) into a 100mL volumetric flask, add nitric acid (1+1) (3.4) 2mL and dilute to the scale with water.

3.7 Methyl isobutyl ketone (MIBK).

3.8 Hydrochloric acid (7 mol/L): Take 30 mL of concentrated hydrochloric acid ($_{20}$ = 1.19 g/mL) in excellent purity and add water to 50 mL.

3.9 Hydroxylamine hydrochloride solution (120g/L): Take 12.0g of hydroxylamine hydrochloride and 12.0g of sodium chloride and dissolve in 100mL of water.

3.10 Octanol.

4 Instruments

4.1 Atomic absorption spectrophotometer and accessories.

4.2 Stiff glass ablation tubes or tall beakers.

4.3 Stoppered cuvettes, 10mL, 25mL.

4.4 Electric hotplates or water baths.

4.5 Pressure controlled closed microwave dissolution oven.

4.6 High-pressure, airtight digestion tank.

4.7 Polytetrafluoroethylene dissolving sample cup.

5 Analysis steps

5.1 Sample pre-treatment

5.1.1 Wet digestion method

Accurately weigh approximately 1.00g to 2.00g of the mixed sample into the digestion tube and make a reagent blank. If the sample contains organic solvents such as ethanol, evaporate first in a water bath or on a hotplate at low temperature. For cream samples, preheat in a water bath to melt the sample on the walls of the bottle into the bottom of the bottle. Add several glass beads, then add 10mL of nitric acid (3.1) and heat the digestion from low to high temperature. When the volume of digested liquid is reduced to 2mL-3mL, remove the heat source and cool. Add perchloric acid (3.2) 2mL to 5mL and continue to heat the digestion, shaking slowly from time to time to make it uniform. Concentrate the digestion solution to about 1mL. Cool to room temperature and transfer quantitatively to a 10mL (or 25mL for powder samples) stoppered cuvette, set in water to the mark and set aside. If the sample solution is cloudy, the supernatant can be removed by centrifugation for determination.

5.1.2 Microwave digestion

Weigh approximately 0.5g to 1g of the mixed sample into a cleaned Teflon sample cup. Cosmetics containing volatile materials such as ethanol, such as perfumes, mousse, body lotions, hair dyes, serums, shaving lotions, face masks, etc., should first be volatilised in a temperature adjustable 100ºC electric heater or water bath (do not steam dry). For dry substances such as lipstick, mascara, eyebrow pencil, rouge, lip liner, powder, eye shadow, talcum powder, prickly heat powder, etc., add 0.5mL to 1.0mL of water after sampling and wetting and shaking well.

Depending on the ease of sample digestion, samples or pre-treated samples are first added to nitric acid (3.1) 2.0mL to 3.0mL and allowed to stand overnight for full action. Then add hydrogen peroxide (3.3) 1.0mL to 2.0mL in sequence and shake the sample cup several times to fully submerge the sample. Place in a boiling water bath or thermostatic heating apparatus at an adjustable temperature for 20 min at 100ºC and remove. If the volume of solution is less than 3mL, replenish with water. Follow the Microwave Dissolution System operating instructions to the letter.

Place the sample cup into a clean, high pressure, airtight lysimeter prepared in advance and screw on the lid (note: do not over-tighten).

Table 1 shows the pressure - time procedure for general cosmetic products for decomposition. If the cosmetics are oils, herbs or detergents, the sensitivity of the explosion-proof system can be increased appropriately to increase safety.

Depending on the ease of sample digestion, the digestion can be completed within 5 min to 20 min, the sample is cooled down, the can is opened and the sample cup containing the digested sample is placed in a boiling water bath or an electric heater at an adjustable temperature of 100ºC for a few minutes to remove excess nitrogen oxides from the sample so as not to interfere with the determination.

Table 1 Pressure during digestion - time procedure

Pressure gear	Pressure (Mpa)	Holding pressure accumulation time (min)
1	0.5	1.5
2	1.0	3.0
3	1.5	5.0

Transfer the sample to a 10mL stoppered cuvette, wash the dissolution cup several times with water, combine the washing solution, add hydroxylamine hydrochloride solution (3.9) 0.5mL Note 1, fix the volume with water to 10mL and set aside.

5.1.3 Extraction method (only for wax-free cosmetics)

Accurately weigh approximately 1.00 g of the mixed sample and place in a 50 mL stoppered cuvette. Make a reagent blank with the sample. For samples containing organic solvents such as ethanol, evaporate first in a water bath or on a hotplate at low temperature. For cream samples, preheat in a water bath to melt the sample on the wall into the bottom of the tube. Add 5.0 mL of nitric acid (3.1) and 2.0 mL of hydrogen peroxide (3.3), mix well and add a few drops of octanol (3.10) if large bubbles appear. Heat in a boiling water bath for 2 h. Remove and add hydroxylammonium hydrochloride solution

(3.9) 1.0mL Note 1, leave for 15min to 20min and fix with water to 25mL.

5.2 Measurement

5.2.1 Pipette 0, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00 mL of Cadmium Standard Solution (3.6.3) into a 50 mL volumetric flask, add 1 mL of Nitric Acid (1+1) (3.4) and dilute to the scale with water. This is equivalent to 0, 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00mg/L of cadmium respectively. The analytical conditions of the instrument were adjusted to optimum conditions according to the instrument operating procedure. The calibration curve series, blank and sample solutions were determined under deduction of background absorption. If the iron content of the sample solution exceeds the cadmium content by 100

The deuterium lamp method should not be used for background deduction, but the Seeman effect method should be used for background deduction, or the iron should be removed in advance according to 5.2.2. The concentration-absorbance curve should be plotted and the sample content calculated.

5.2.2 Transfer the standard, blank and sample solutions to an evaporating dish and evaporate to dryness on a water bath. Dissolve the residue by adding 10 mL of hydrochloric acid (3.8), transfer to a separatory funnel and extract twice with an equal amount of MIBK (3.7), retaining the hydrochloric acid solution. The solution is then extracted with hydrochloric acid

(3.8) Wash 5 mL of the MIBK layer, combine the hydrochloric acid solutions, drive out the acid if necessary and fix the volume. Determine according to the operating procedures of the instrument.

6 Calculation

$$(Cd) = \frac{(_{1}-_{0})V}{m}$$

where: (Cd) - mass fraction of cadmium in the sample, g/g.

$_{1}$ - the mass concentration of cadmium in the test solution, mg/L.

$_{0}$ - mass concentration of cadmium in blank solution, mg/L; V - total volume of sample solution, mL; m - sample sampling volume, m g.

7 Precision and Accuracy

The relative standard deviations (RSDs) of the four laboratories were 0.73%-8.73% for the determination of different types of cosmetic samples, including creams, pastes, powders and aqueous preparations containing 0.25g/g-1.00g/g cadmium, using the wet digestion method. The recoveries of the 228 samples ranged from 85.8% to 101.3%.

The relative standard deviations (RSDs) of the four laboratories were 0.69%-6.90% for the determination of different types of cosmetic samples including creams, pastes, powders and aqueous preparations containing 0.25g/g-1.00g/g of cadmium by the leaching method; the recoveries of 252 samples ranged from 85.6% to 102.0%. The recoveries of the 252 samples ranged from 85.6% to 102.0%.

Second method Differential Potential Dissolution Method

8 Methodology Summary

The sample is pretreated so that the cadmium is present in the solution in an ionic state. $^{Cd2+}$ was enriched on a glassy mercury film in an electrolytic cell at a suitable reduction potential. In acidic solutions, Cd has a sensitive dissolution peak at -0.62 V (relative to the saturated glycogen electrode), the peak height being proportional to its content. All other conditions being equal, the dissolved peak is measured and compared with a standard series for quantification. The limits of detection (LOD) and the lower limit of quantification (LOQ) for this method were 0.025 g and 0.082 g respectively, giving a LOD of 0.25 g/g and a LOQ of 0.82 g/g for a 1 g sample.

9 Reagents

9.1 $Hg(_{NO3})$2-H2O 68.5mg and $_{KNO3}$ 25.3g mixed in water, add nitric acid (3.1) 0.63mL and dissolve in water to 1L.

9.2 Hydrochloric acid (1+1): Take 100mL of superior pure hydrochloric acid ($_{20}$ = 1.19g/mL), add 100mL of water and mix well.

9.3 Ethanol (1+1): Take 100mL of anhydrous ethanol, add 100mL of water and mix well.

9.4 Phenolphthalein indicator (1g/L ethanol solution): Dissolve 0.1g of phenolphthalein in 50mL of anhydrous ethanol, add 50mL of water and mix well.

9.5 Ammonia (1+1): take 100mL of ammonia, add 100mL of water and mix well.

10 Instruments

10.1 Electric sand bath.

10.2 Conical flask, 100mL.

10.3 Volumetric flask, 100mL.

10.4 Glass beaker, 50mL.

10.5 Differential Potential Dissolution Analyser and accessories.

11 Analysis steps

11.1 Sample pre-treatment

As in 5.1.1, volume to 100 mL.

11.2 Preparation of standard working solutions

Cadmium standard solutions (3.6.3) of 0, 0.050, 0.10, 0.20, 0.40, 0.70 and 1.00 mL were placed in 7 conical flasks, digested simultaneously with the samples and transferred to a 100 mL volumetric flask and fixed with water.

11.3 Electrode pre-treatment

The glassy carbon electrodes were soaked in nitric acid (1+1) (3.4) before use, rinsed with water, wiped with filter paper dripping with ethanol (1+1) (9.3), rinsed with water, the three electrodes were inserted into the mercury plating solution, plated with mercury according to the parameters in Table 2 and then left to use.

Table 2 Instrument reference parameters

Instrument parameters	Mercury plating	Measurement
Electrolytic voltage (V)	-1.10	-1.10
Electrode speed (rpm)	2500	2000
Enrichment time (s)	40	60
Dissolved lower limit voltage (V)	-0.20	-0.20
Dissolved upper voltage (V)	-0.90	-1.00
Resting time (s)	30	30

Sensitivity	20	20
Electrode wash time (s)	20	10 to 20

11.4 Measurement

Add 2 drops of phenolphthalein indicator to a 25mL volumetric flask and adjust with ammonia (1+1) (9.5) until the solution is slightly red, then add 0.3mL of hydrochloric acid (1+1) (9.2), set the volume with water to the scale, transfer to a 50mL beaker, insert the triple electrode and determine according to Table 2. The parameters were determined according to Table 2.

For the working curve method, the peak height is recorded and the mass - dissolved peak height curve is plotted to calculate the sample content. For the standard addition method, the peak height and the standard addition amount are calculated directly.

12 Calculation

12.1 Standard curve method

$$(Cd) = \frac{(m_{1}-m_{0})\times V}{m\times V_{1}}$$

Where: (Cd) - mass fraction of cadmium in the sample, g/g; $_{m1}$ - mass of cadmium in the test solution, g; $_{m0}$ --mass of cadmium in the blank solution, g; V --total volume of sample solution, mL; V1--volume of sample solution taken, mL V - the total volume of the sample solution, mL; V1 - the volume of the sample solution dispensed, mL; m - the volume of the sample taken, g.

12.2 Standard accession method.

$$(Cd) = \frac{h1 \times m1}{(h2-h1) \times m}$$

Where: (Cd) - mass fraction of cadmium in the sample, g/g; $_{h1}$ - peak height of cadmium in the sample solution; $_{h2}$ - - peak height of cadmium in the sample solution after adding the standard; $_{m1}$ - mass of cadmium standard added, g; m - sample sampling volume, g.

13 Precision and Accuracy

The recoveries of the four major cosmetic samples (powder, water, honey and oil) were spiked at three concentrations (high, medium and low) with the relative standard deviations ranging from 2.44% to 9.35% and the recoveries ranging from 81.3% to 104%.

Note 1 This addition of hydroxylamine hydrochloride is dispensed with if the sample is not to be measured for mercury.

IX. Strontium

Strontium

1 Scope

This specification specifies a flame atomic absorption spectrophotometric method and an ion chromatographic method for the determination of strontium in toothpaste. This specification applies to the determination of strontium in different types of toothpaste.

First method Flame atomic absorption spectrophotometry

2 Methodology Summary

The interfering substances aluminium and silicon in the toothpaste form insoluble oxides at 600°C. The strontium in the ashed specimen is dissolved in a nitric acid solution. Under the high temperature of the flame, the strontium is atomised and absorbs the resonance lines from the strontium hollow cathode lamp in an amount proportional to the amount of strontium in the sample. The measured absorption values are compared with the standard solution and quantified. Interference from calcium and magnesium in the sample solution is eliminated by the addition of disodium EDTA or lanthanum solution. The detection limit of the method is 0.06 mg/L and the lower limit of quantification is 0.2 mg/L. If 1 g of sample is taken, the detection concentration of the method is 3 g/g and the minimum quantification concentration is 10 g/g.

3 Reagents

3.1 Magnesium oxide (MgO).

3.2 Nitric acid ($_{20}$ = 1.42 g/mL), ultrapure.

3.3 Disodium EDTA solution (0.2 mol/L): weigh 74.4 g of disodium EDTA ($_{Na2}$ C10H14N2O8-2H2O) and 8.0 g of sodium hydroxide (NaOH), dissolve in water and dilute to 1 L.

3.4 Lanthanum nitrate solution (50g/L): weigh 117g of lanthanum nitrate [La($_{NO3}$)3-6H2O], dissolve in water and dilute to 1L, or weigh 58.65g of lanthanum oxide, slowly add 250mL of concentrated hydrochloric acid (20=1.19g/mL) of superior purity and dissolve and dilute to 1L with water.

3.5 Strontium standard reserve solution [(Sr) = 1 g/L]: weigh 2.4153 g of spectrally pure strontium nitrate [Sr($_{NO3}$)$_2$], dissolve in water, add 20 mL of nitric acid (3.2), fix with water to 1 L and calibrate with GBW(E) 080242 standard solution.

3.6 Strontium standard use solution [(Sr) = 100 mg/L]: take 10.0 mL of strontium standard reserve solution (3.5) in 100 mL at the time of use

Add 1 mL of nitric acid (3.2) to the volumetric flask and set to scale with water.

4 Instruments

4.1 Stoppered cuvettes (10mL, 50mL) and porcelain crucibles (50mL): all vessels must be soaked in nitric acid (1+4) for 6h

Rinse well with water.

4.2 Atomic Absorption Spectrophotometer.

4.3 Strontium hollow cathode lamp.

4.4 High temperature chamber resistance furnace.

5 Analysis steps

5.1 Sample pre-treatment

Take a sample of toothpaste, squeeze out about 20mm of paste and discard, then weigh about 1.00g of toothpaste into a 50mL porcelain crucible

(The sample with high strontium content can be taken in small quantities), add 0.50g of magnesium oxide (3.1) to cover the toothpaste evenly, and make a reagent blank and parallel sample. Carbonise on an electric stove over low heat until no smoke is emitted (if the toothpaste is dilute evaporate the water on a water bath and then carbonise). After cooling, remove from the furnace and add a little water to the crucible to moisten the ash, then add 10mL of nitric acid (1+3) (or 10mL of nitric acid (1+1), but a white precipitate will appear when disodium EDTA is added for 1h, but this will not affect the determination), heat to dissolve and transfer to a 50mL cuvette. Dissolve by heating, transfer to a 50mL cuvette, set to the scale, shake well and reserve.

5.2 Instrument reference conditions

Analysis line: 460.7nm; Slit: 1.3nm; Flame height: 7.5mm; Air: 1.$^{60kg/cm2}$ (9.4L/min); Acetylene: 0.$^{25kg/cm2}$ (2.2L/min Use high purity acetylene). The atomic absorption spectrophotometer can also be adjusted to the optimum state for strontium measurement according to the instrument instructions. Measurements are made using the standard calibration curve method (or the standard addition calibration curve method); calculations can be made by direct measurement of absorbance or by integration over 5s.

5.3 Preparation of calibration curves

Six 10mL stoppered cuvettes were added to the standard solutions (3.6) 0, 0.10, 0.30, 0.50, 0.70, 0.70 and 0.70 respectively.

1.00mL, add nitric acid (1+3) 2mL, add water and dilute to the scale, this standard series is equivalent to those containing strontium 0.00, 1.00, 1.00

3.00, 5.00, 7.00 and 10.0 mg/L. Add 0.2 mL each of disodium EDTA solution (3.3) or lanthanum nitrate solution (3.4)

0.2 mL, shake well. The calibration curve series was determined with background absorption deducted. The concentration-absorbance curve was plotted.

5.4 Measurement

Add 10.0 mL each of the reagent blank and sample solution of 5.1 to 0.2 mL of EDTA disodium solution (3.3) or 0.2 mL of lanthanum nitrate solution (3.4) and shake well. Determine under the deduction of background absorption. The amount of strontium in the test solution was found from the calibration curve.

6 Calculation

$$(Sr) = \frac{(m_1-m_0)V}{mV_1}$$

where: (Sr) - mass fraction of strontium in the sample, g/g; $_{m1}$ - mass of strontium in the test solution, g; $_{m0}$ --mass of strontium in the blank solution, g.

V - total volume of sample solution, mL; $_{V1}$ - volume of sample solution dispensed, mL; m - sample volume taken, g.

7 Precision and Accuracy

Four laboratories determined the strontium content in toothpaste from 21.6 g/g to 1485 g/g with relative standard deviations of 0.36% to 5.1% and recoveries ranging from 96.4% to 106%.

Second method Ion chromatography

8 Methodology Summary

The sample is pretreated so that strontium is present in the sample solution in an ionic state. After adjusting the sample solution to neutral, the strontium ions are separated from other inorganic ions by an ion exchange column, the background conductance is reduced using an ion chromatograph suppressor, the conductance values are determined with a conductivity detector, compared with the standard solution, characterised by retention time and quantified by peak area or peak height. Interference of calcium in the sample solution can be excluded by the addition of an appropriate amount of NaF solution. The detection limit of this method is 0.006 mg/L and the lower limit of quantification is 0.02 mg/L. If 0.5 g of sample is taken, the detection concentration of this method is 0.6 μg/g and the minimum quantification concentration is 2 μg/g.

9 Reagents

9.1 Pure water: conductivity <1.0μs/cm.

9.2 Hydrogen peroxide [($_{H2O2}$) = 30%].

9.3 Hydrochloric acid (0.12 mol/L): Take 10 mL of concentrated hydrochloric acid ($_{\rho 20}$ = 1.19 g/mL) in its purest form and dilute to 1 L with water.

9.4 Sodium hydroxide solution (0.1 mol/L): Weigh 4 g of pure sodium hydroxide, dissolve in water and set to 1 L with water.

9.5 Sodium fluoride solution (0.1 mol/L): weigh 0.42 g of sodium fluoride, dissolve in water and fix the volume with water to 100 mL.

9.6 Methanesulfonic acid aqueous solution (20mmol/L): take 1.3mL of methanesulfonic acid (99%) and fix the volume with water to 1L.

10 Instruments

10.1 Stoppered cuvettes (10mL, 50mL) and volumetric flasks: all vessels must be soaked in nitric acid (1+4) for at least 6h and rinsed with water.

10.2 Pressure-controlled closed microwave lysis system, including high-pressure closed digestion tank and lysis cup.

10.3 Open type electrically heated thermostatic furnace.

10.4 Ion chromatograph with isocratic pump, conductivity detector, cation suppressor and integrator or chromatography workstation.

10.5 Vortex oscillator.

10.6 Ultrasonic cleaners

10.7 High-speed centrifuge.

10.8 0.25μm filter membrane.

11 Analysis steps

11.1 Sample pre-treatment

11.1.1 Microwave digestion

Take a sample of toothpaste, squeeze out about 20mm of the paste and discard it, then weigh about 0.5g of the toothpaste sample into a cleaned Teflon dissolution cup and make a reagent blank with the sample. Add 2mL to 3mL of nitric acid (3.2) and leave overnight or place in a thermostatic heater at an adjustable temperature of 100°C for 1h, remove and cool, add 1.0mL of nitric acid (3.2) and 2.0mL of hydrogen peroxide (9.2), cover with an inner lid and follow the operating procedures in the microwave lysis system manual. After the sample has been digested, the sample is removed and cooled, the canister is opened and the sample cup containing the digested sample is placed in an electric heater and heated at 200°C to drive the acid to dryness. Add 2mL to 3mL of hydrochloric acid (9.3), evaporate to near dryness at 100°C, add 10mL of water, bring to the boil and transfer to a 50mL cuvette, add sodium hydroxide solution (9.4) dropwise and adjust pH to neutral. Set the volume to 25mL and shake well.

Dilute 5.00mL of the above solution to 10.0mL with water, centrifuge at 10,000rpm for 10min, remove the supernatant, pass through a 0.25μm filter tip and feed the sample. For samples with high calcium content, add sodium fluoride solution (9.5) slowly drop by drop, shake well, observe the degree of turbidity, stop adding drop by drop if turbidity is obvious, dilute to 10mL with water, centrifuge, filter and inject sample.

11.1.2 Wet digestion method

Weigh about 0.5g~1.0g of toothpaste sample into a 50mL digestion tube and make a reagent blank with the sample. Add several glass beads, then add 5mL of nitric acid (3.2) and place in an electric heater at a constant temperature, first at 90°C for 1h, then at 180°C for 5h, and finally at 240°C to drive the acid to dryness. Add 2mL to 3mL of hydrochloric acid (9.3), evaporate to near dryness at 100°C, add 10mL of water, bring to the boil, remove from heat, cool, add sodium hydroxide solution (9.4) dropwise and adjust pH to neutral. Allow to build up to 25mL, shake well.

Dilute 5mL of the above solution to 10mL with water, centrifuge at 10,000rpm for 10min, remove the supernatant, pass through a 0.25μm filter tip and feed the sample. For samples with high calcium content, add sodium fluoride solution (9.5) slowly drop by drop, shake well, observe the degree of turbidity, stop adding drop by drop if turbidity is obvious, dilute to 10mL with water, centrifuge, filter and inject sample.

11.1.3 Ultrasonic leaching with dilute nitric acid (not applicable to samples containing fluorine)

Weigh 0.5g~1.0g of toothpaste sample into a 50mL stoppered cuvette, add 25mL of 1% (v/v) nitric acid solution, shake and disperse the sample at high speed, then extract the sample by ultrasonication for 20min~30min, with the sample being taken out at 10min intervals. Dilute to 10mL with water and centrifuge at 10,000rpm for 10min, remove the supernatant, pass through a 0.25μm filter tip and sample. For samples with high calcium content, slowly add sodium fluoride solution (9.5) drop by drop, shake well, observe the degree of turbidity, stop adding drop by drop if turbidity is obvious, dilute to 10mL with water, centrifuge, filter and inject sample.

11.2 Chromatographic reference conditions

Chromatographic column: IonPac CS12 (250mm x 4mm), CG12 (50mm x 4mm), CSRS®-ULTRA Inhibitor Leaching solution: 20mmol/L methanesulfonic acid

Leaching solution flow rate: 0.70 mL/min Injection volume: 50 μL

Range step: 3μS

Nitrogen flow rate (pressure): 7psi

Automatic regeneration of electrolytic water with automatic current suppression 50mA

Column temperature: room temperature

11.3 Measurement

11.3.1 Preparation of calibration curves

The standard series is equivalent to 0.00, 0.50, 1.00, 4.00, 8.00 and 10.0 mg/L. Adjust the instrument to the optimum condition and inject 0.5 mL~1.0mL into the ion chromatograph and plot the concentration-peak area curve.

11.3.2 Sample determination

The concentration of strontium in the solution to be measured was found from the calibration curve based on the peak area by injecting 0.5mL to 1.0mL of the solution to be measured into the ion chromatograph.

12 Calculation (Sr) = $\frac{VN\ M}{\quad}$

where: (Sr) - mass fraction of strontium in the sample, g/g.

- mass concentration of strontium in the test solution, mg/L; V - volume of the sample volume, mL; N - dilution factor.

m - Sample size, g.

13 Chromatograms

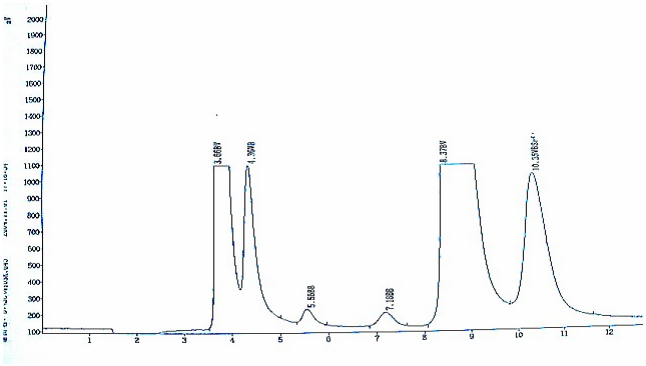


Figure 1 Ion chromatogram of toothpaste sample solution

1: $^{Na+}$(3.66min); 2: $^{NH+}$(4.36min); 3: $^{K+}$(5.56min); 4: $^{Mg2+}$(7.18min); 5: $^{Ca2+}$(8.37min); 6: $^{Sr2+}$(10.35min)

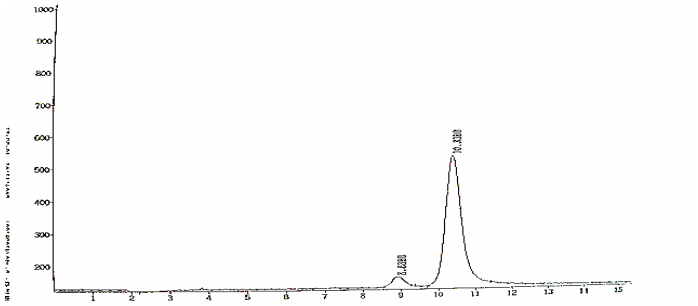


Figure 2 Ion chromatogram of strontium standard solution

1: $^{Ca2+}$(8.88min); 2: $^{Sr2+}$(10.33min)

X. Total Fluorine

Total Fluorine

1 Scope

This specification specifies a spectrophotometric method for the determination of total fluoride in oral hygiene products. This specification applies to the determination of total fluoride in oral hygiene products.

2 Methodology Summary

The samples were separated from fluorine by distillation under acidic conditions, and the fluoride ion was complexed with alizarinone in a pH 5.2 acetate buffer solution

The reaction of the complex of lanthanum (ALC) and lanthanum produced a blue ternary complex, the colour of which was proportional to the concentration of the fluoride ion. The method has a detection limit of 5 g and a lower limit of quantification of 17 g. If 0.5 g of sample is taken, the detection concentration is 0.01% (w/w) and the lowest quantitative concentration is 0.034% (w/w).

3 Reagents

3.1 Sodium hydroxide solution (80g/L): weigh 8g of sodium hydroxide, dissolve in water and dilute to 100mL.

3.2 Sulphuric acid (1+1): Take 100mL of superior pure sulphuric acid ($_{20}$ = 1.84g/mL), add slowly to 100mL of water and mix well.

3.3 Hydrochloric acid (0.1 mol/L): Take 0.83 mL of concentrated hydrochloric acid ($_{20}$ = 1.19 g/mL) in superior purity and add water to 100 mL. 3.4 Acetic acid (20=1.049g/mL).

3.5 Alizarin complex ketone solution (3.85 g/L): weigh 0.385 g of alizarin complex ketone (ALC, molecular formula $C_{19}H_{15}NO_8$, commonly known as fluorine reagent) with about 10 mL of water, add a few drops of sodium hydroxide solution (3.1) to dissolve it and adjust with hydrochloric acid solution (3.3) so that the solution turns from purple to red, at which point the pH of the solution is 4.5. Dilute to 100 mL with water and store in a brown bottle, refrigerate. Dilute to 100mL with water and store in a brown bottle under refrigeration.

3.6 Buffer solution: Weigh 100 g of sodium acetate (CH3COONa-3H2O) in about 200 mL of water and add acetic acid (3.4)

11 mL, adjust pH to 5.2 with sodium or acetic acid using an acidity meter and dilute to 1 L with water.

3.7 Lanthanum nitrate solution (4.33g/L): weigh 4.33g of lanthanum nitrate (LaNO3-6H2O) dissolve in water and dilute to 1L.

3.8 Acetone.

3.9 Fluorine standard stock solution [($_{F^-}$)=100mg/L]: accurately weigh 0.221g of sodium fluoride which has been baked at 120°C for 2h, dissolve in water and transfer to a 1L volumetric flask and dilute to the scale.

3.10 Fluorine standard use solution: [($_{F^-}$) = 10mg/L]: Accurately draw up 10.0mL of fluorine standard reserve solution (3.9) and place in

In a 100mL volumetric flask, dilute to the scale with water and store in a polyethylene bottle.

4 Instruments

4.1 Spectrophotometer.

4.2 Acid meter.

4.3 Distillation unit: see Figure 1.

5 Analysis steps

5.1 Sample pre-treatment

Weigh 0.5 g to 5.0 g of the mixed sample and add 3.0 mL of sodium hydroxide solution (3.1). shake while heating over low heat for about 5 min. then wash with 50 mL of water into a double-necked flask. Add a few glass beads and 40mL of sulphuric acid solution (3.2) and connect the distillation apparatus according to Figure 1. Heat the distillation. When the temperature in the flask has risen to 130°C, start to introduce steam and control the distillation temperature at 140°C to 150°C. Collect the distillate in a 200mL volumetric flask which has been pre-watered to approximately 20mL. When the solution in the volumetric flask

Stop distillation at approximately 180mL. Add water to the scale, mix well and use as the solution to be measured.

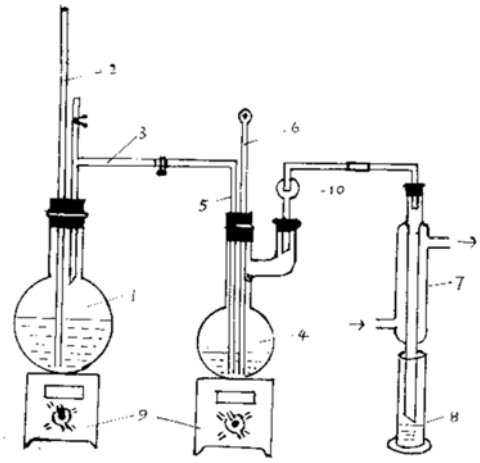


Fig. 1 Distillation unit

1: 1000mL vapour generating flask; 2: 1m long glass tube; 3: T-tube; 4: 350mL double-necked flask; 5: curved vapour tube; 6: 200C thermometer; 7: condenser tube; 8: absorption flask (200mL volumetric flask); 9: electric furnace; 10: nitrogen bulb

5.2 Preparation of calibration curves

Aspirate 0, 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00mL of the standard use solution (3.10) into a 50mL colorimetric tube, equivalent to 0, 5.00, 10.0, 20.0, 30.0, 40.0, 50.0g of F Add 1.00mL of alizarin complex ketone solution (3.5), 5.0mL of buffer solution (3.6), 1.0mL of lanthanum nitrate solution (3.7) and 15.0mL of acetone (3.8), add water to the scale and mix well. The absorbance was measured at 620nm wavelength using a 1cm cuvette with a blank solution as reference. The absorbance was used as the vertical coordinate and the concentration as the horizontal coordinate to draw the calibration curve.

5.3 Measurement

Take 20mL of the solution to be measured (5.1) into a 50mL cuvette and proceed as in 5.2 to find out the fluorine content from the calibration curve. If the chloride content is high, add silver sulphate to eliminate interference.

6 Calculation

$$(F) = \frac{m_1 V_2}{m V_1 10000}$$

Where: (F) - mass fraction of fluorine in the sample, % (w/w); m_1 - mass of fluorine in the test solution, g; V_1 - volume of distillate drawn during colourimetry, mL; V_2 - total volume of distillate when fixed, mL; m - sample volume taken, g.

7 Precision and Accuracy

The relative standard deviations of the four laboratories were 1.30% to 4.10% for the determination of

cosmetic fluorine content from 0.04% (w/w) to 0.08% (w/w); the recoveries were 93.0% to 106.5%.

xi. total selenium

Total Selenium

1 Scope

This specification specifies a fluorescence spectrophotometric method for the determination of total selenium in cosmetics. This specification applies to the determination of total selenium in cosmetics.

2 Methodology Summary

The cosmetic was digested by nitric acid-perchloric acid, in which the selenium was free and oxidized, and then the hexavalent selenium was reduced to tetravalent selenium by hydrochloric acid, and reacted with 2,3-diaminonaphthalene at pH 1.5-2.0 to produce 4,5-benzoselenium brain green fluorescent substance. The method has a detection limit of 2.1 x $^{10-3}$ g and a lower limit of quantification of 7.0 x $^{10-3}$ g. If 1 g of the sample is taken, the detection concentration is 2.1 x $^{10-3}$ g/g and the lowest quantification concentration is 7.0 x $^{10-3}$ g/g.

3 Reagents

3.1 Nitric acid ($_{20}$ = 1.42 g/mL), ultrapure.

3.2 Perchloric acid [($_{HClO4}$) = 70% to 72%], superior pure.

3.3 Hydrochloric acid ($_{20}$ = 1.19 g/mL), ultrapure.

3.4 Hydrochloric acid (1+4): measure 50mL of hydrochloric acid (3.3) and add to 200mL of water.

3.5 Hydrochloric acid (0.1 mol/L): measure 8.3 mL of hydrochloric acid (3.3) and dilute to 1000 mL with water. 3.6 Ammonia ($_{20}$ = 0.892 g/mL).

3.7 Disodium EDTA solution (50g/L): weigh disodium EDTA (C10H14N2O8Na2-2H2O, abbreviated as (EDTA-2Na) 50 g in a small amount of water, dissolved by heating, cooled and diluted to 1 L.

3.8 Hydroxylamine hydrochloride solution (100g/L): Weigh 100g of hydroxylamine hydrochloride (NH2OH-HCl), dissolve in water and dilute to 1L.

3.9 Precision pH test paper, pH 0.5 to 5.0.

3.10 Cresol Red solution (2g/L): weigh 0.2g of Cresol Red (C22H18O5S) dissolve in a small amount of water, add a drop of ammonia (3.6) to dissolve completely and dilute to 100mL with water.

3.11 Disodium EDTA - Hydroxylamine Hydrochloride - Cresol Red Mix: Before use, take 50mL of Disodium EDTA (3.7), 50mL of Hydroxylamine Hydrochloride (3.8) and 2.5mL of Cresol Red (3.10), dilute to 500mL with water and mix well.

3.12 Ammonia (1+1): measure 100mL of ammonia (3.6) and add to 100mL of water.

3.13 Cyclohexane: No fluorescent impurities, to be used after re-distillation if necessary.

3.14 2,3-Diaminonaphthalene solution [$_{C10H6(NH2)2}$, DAN] (2 g/L): (Do the following in a dark room.)

Weigh 200 mg of 2,3-diaminonaphthalene in a 250 mL conical flask, add 100 mL of hydrochloric acid (3.5) and shake until completely dissolved.

(approx. 15min). Add 20 mL of cyclohexane and shake for 5 min, transfer to a separatory funnel with glass wool (or skimmed cotton) at the bottom, leave to stratify and return the aqueous phase to the original conical flask, then extract with cyclohexane and repeat until the cyclohexane phase has the lowest fluorescence value. The purified 2,3-diaminonaphthalene solution was stored in a brown bottle with a layer of cyclohexane approximately 1 cm thick to insulate it from air and stored in a refrigerator. If necessary, extract again with cyclohexane before use.

3.15 Defoamer: Octanol or other equivalent defoamer.

3.16 Selenium standard stock solution [(Se)=100mg/L]: weigh 0.1000g of selenium metal, dissolve in a small amount of nitric acid (3.1), add perchloric acid (3.2) 2mL. heat on a boiling water bath to evaporate the nitric acid (about 3h~4h), cool slightly, add hydrochloric acid (3.4) 8.4mL, continue heating for 2min, then then set to 1L with water.

3.17 Selenium Standard Use Solution [(Se)=0.1mg/L]: Take a quantity of Selenium Standard Reserve Solution (3.16) and dilute with hydrochloric acid (3.5) to 1.00mL containing 0.100g of selenium. Store in the refrigerator and reserve.

4 Instruments

All glassware used for the first time must be soaked in nitric acid (1+1) for at least 4h and rinsed with water. Glassware used in this method is treated and cleaned as if it were being used for the first time after being rinsed in tap water, soaked in detergent solution and rinsed in tap water.

4.1 Conical flask, 100mL.

4.2 4cm diameter funnel.

4.3 Stoppered colorimetric tube, 50mL.

4.4 Electric sand bath.

4.5 Water bath.

4.6 Fluorescence spectrophotometer.

5 Analysis steps

5.1 Sample pre-treatment

Accurately weigh approximately 1.00g to 2.00g of the mixed sample into the digestion tube and make a reagent blank. If the sample contains organic solvents such as ethanol, evaporate first in a water bath or on a hotplate at low temperature. For cream samples, preheat in a water bath to melt the sample on the walls of the bottle into the bottom of the bottle. Add several glass beads, then add 10mL of nitric acid (3.1) and heat the digestion from low to high temperature. When the volume of digested liquid is reduced to 2mL-3mL, remove the heat source and cool. Add perchloric acid (3.2) 2mL to 5mL and continue to heat the digestion, shaking slowly from time to time to make it uniform. After cooling slightly add 4mL of hydrochloric acid (3.4) and continue to heat until white smoke is produced, remove immediately.

5.2 Preparation of standard working solutions

Take 0, 0.10, 0.25, 0.50, 0.75, 1.00, 2.00mL of selenium standard use solution (3.17) in 100mL of

in a conical flask and disintegrate simultaneously with the sample.

5.3 Measurement

Transfer the digested sample solution and the standard working solution into separate 50mL cuvette tubes. Add 10mL of the reagent mixture (3.11) to each tube and shake well, the solution should be peachy red. Adjust to a light orange colour with ammonia (3.12), adding a small amount of hydrochloric acid (3.4) if necessary, at which point the pH of the solution should be 1.5 to 2.0 [the solution can also be tested with pH 0.5 to 5.0 precision paper (3.9)].

Add 1 mL of 2,3-diaminonaphthalene solution (3.14) to each of the above tubes, shake well, place in a boiling water bath for 5 min (from the time of placement in the boiling water bath), remove and cool. Add 4.0 mL of cyclohexane (3.13) to each tube, cover tightly with a stopper and shake for 2 min. Leave to stratify for measurement. The fluorescence intensity of the cyclohexane phase in each tube was measured with a fluorescence spectrophotometer at 379 nm excitation and 519 nm emission wavelengths.

The working curve was plotted and the selenium content of the sample was found from the curve.

6 Calculation

$$(Se) = \frac{m_1 - m_0}{m}$$

Where: (Se) - mass fraction of selenium in the sample, g/g; m_1 - mass of selenium in the test solution, g; m_0 --mass of selenium in the blank solution, g; m --sample size, g.

7 Precision and Accuracy

Four types of cosmetic products (water, powder, honey and oil) were used for spiked recovery experiments at three levels (high, medium and low) with an accuracy of 92.0% to 98.0% and a precision of 4.9% to 8.0%.

XII. Boric acid and borates

Boric Acid and Borate

1 Scope

This specification specifies a methylene-H spectrophotometric method for the determination of boric acid and borates in cosmetics. This specification applies to the determination of boric acid and borates in cosmetics.

2 Methodology Summary

After the extraction of boric acid and borates in the sample, boron forms a yellow complex with methylene-H, the colour of which is linearly related to the concentration of boron in a certain range. The detection limit of this method is 1.17 g and the lower limit of quantification is 3.86 g. If a 1 g sample is taken, the detection concentration is 11.7 g/g and the lowest quantification concentration is 38.6 g/g.

3 Reagents

3.1 Acetic acid-ammonium acetate buffer solution (pH=6.0): weigh 50g of ammonium acetate, 4.5g of disodium EDTA, add 150mL of water and dissolve, then add 3.5mL of glacial acetic acid and shake well.

3.2 Methylimine-H solution

3.2.1 Synthesis of methylimine-H solution: Dissolve 18g of H acid monosodium salt [$_{NH2C10H4}$(OH)(SO3H)SO3Na-3/2H2O] in 1L of water, heat slightly to dissolve completely, neutralise with 10% sodium hydroxide solution to neutral, slowly add 10mL of concentrated hydrochloric acid under stirring to make pH 1.5. Add 20mL of salicylic aldehyde, hold at 40°C under stirring for Leave for 1h and after 16h filter the precipitate (golden yellow methane-H) through a cloth funnel, draw it dry and then wash it 3-4 times with a small amount of anhydrous ethanol. After complete evaporation of the anhydrous ethanol from the drained golden yellow powder, it was placed in a desiccator for drying or in an oven below 80°C for 2h to 3h and stored in a desiccator.

3.2.2 Methylimine-H solution (5 g/L): weigh 0.5 g of methylimine-H (3.2.1) and 2.0 g of ascorbic acid, add 100 mL of water and heat slightly (<50°C) to dissolve completely. This solution is prepared ready for use.

3.3 Sodium carbonate solution (10g/L): weigh 1g of sodium carbonate and dissolve in 100mL of water.

3.4 Hydrochloric acid (1+9): Take 100mL of superior pure hydrochloric acid ($_{20}$ = 1.19g/mL), add 900mL of water and mix well.

3.5 Ethanol (1+1): Take 100mL of anhydrous ethanol, add 100mL of water and mix well.

3.6 Boric acid standard solution

3.6.1 Boric acid standard solution [($_{H3BO3}$) = 1 g/L]: weigh 1.000 g of anhydrous boric acid ($_{H3BO3}$) in a 250 mL beaker and dissolve with water. Transfer to a 1L volumetric flask, dilute to the scale with water and place in a polyethylene bottle.

3.6.2 Boric acid standard solution for use [($_{H3BO3}$) = 20 mg/L]: Pipette 10.0 mL of the boric acid standard solution (3.6.1) into a 500 mL volumetric flask, dilute to the scale with water and place in a polyethylene

bottle.

4 Instruments

4.1 Spectrophotometer.

4.2 Boron-free colorimetric tube, 25mL.

5 Analysis steps

5.1 Sample pre-treatment

5.1.1 Powder: Weigh approximately 1.0g of the sample, place in a 200mL volumetric flask, add an appropriate amount of water and shake vigorously for 3min, then add water to set the volume to the scale, shake well, filter or centrifuge, discard the initial filtrate, and continue filtrate as the sample solution to be tested.

5.1.2 Creams and other types (either of the following two methods can be used)

5.1.2.1 Method 1

Accurately weigh the mixed sample of about 1g~2g, place it in a 30mL porcelain evaporating dish, add sodium carbonate solution (3.3) 5mL, steam dry on a water bath, carbonise the porcelain evaporating dish on an electric stove, then move it into a high temperature stove, ash it at 500°C, cool it and add hydrochloric acid (3.4) 10mL to the ash to dissolve it, move it into a 100mL volumetric flask, fix the volume with water to the scale and make the sample solution to be tested.

5.1.2.2 Method 2

Weigh approximately 1.0g of the mixed sample accurately, place in a triangular flask, add an appropriate amount of ethanol (3.5), shake vigorously (or heat slightly) to completely disperse the paste in the solution, transfer into a 200mL volumetric flask and set to the scale with ethanol (3.5), shake well, remove part of the solution and centrifuge at 5000rpm for half an hour, take the clarified solution as the sample solution. If the solution to be measured is turbid, the effect of turbidity can be eliminated by double beam dual wavelength spectrophotometry, or by using the extinction value of the measured sample minus the extinction value of the sample blank (without colour developer).

5.2 Determination

Pipette 0, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, 10.0 mL of Boric Acid Standard Solution (3.6.2) (equivalent to 0, 10.0, 20.0, 40.0, 80.0, 120, 160, 200 g of boric acid, respectively), the appropriate amount of sample solution (5.1.1 or 5.1.2) and blank solution into a 25 mL colorimetric tube and add water to 10 mL. Add 2.0 mL of acetic acid-ammonium acetate buffer solution (3.1), respectively, and shake well. Add 2.0 mL of methylamine-H solution (3.2.2) and shake well. React for 80 min at room temperature (25°C) and allow to settle. The absorbance was measured at 415 nm on a 1 cm cuvette with water as a reference. The mass-absorbance curve was plotted and the sample content was calculated.

6 Calculation

$$(H\ BO\ ) = \frac{(m_1-m_0)V}{33\ m\ V_1}$$

where: $(_{H3BO3})$ - mass fraction of boric acid in the sample, g/g; $_{m1}$ - mass of boric acid in the test solution, g g; $_{m0}$ - mass of boric acid in the blank solution, g; V - total volume of the sample solution, mL.

$_{V1}$ - the volume of sample solution aspirated for the determination, mL; m - the volume of sample taken, g.

7 Precision and Accuracy

The relative standard deviations of the five laboratories for the determination of boric acid concentrations of 0.003% to 2.05% ranged from 0.67% to 5.9%, with recoveries of 81.2% to 117.7% for powder samples and 68% to 90% for creams and other samples using the alkali ashing method and 76% to 99% using the ethanol and water leaching method.

XIII. Selenium disulfide

Selenium Disulfide

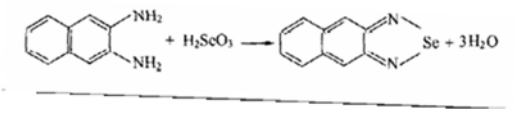
1 Scope

This specification specifies a fluorescence spectrophotometric method for the determination of selenium disulphide in dandruff shampoo cosmetics. This specification applies to the determination of selenium (Ⅳ) in selenium disulphide contained in anti-dandruff shampoo cosmetics.

2 Methodology Summary

Selenium disulfide contained in the anti-dandruff shampoo sample was extracted with perchloric acid-hydrogen peroxide and incubated with 2,3-diaminonaphthalene at pH 1.5 to 1.5.

2.0 Reaction to produce 4,5-benzoselenium brain green fluorescent substance with the following equation.



The reaction products were extracted with cyclohexane, and the fluorescence intensity was measured by fluorescence spectrophotometer, compared with the standard solution and quantified. The minimum detection limit of this method is 4.8 x 10-3 g and the lower limit of quantification is 1.6 x 10-2 g. If 1 g of sample is taken, the detection concentration of this method is 4.8 x 10-3 g/g and the minimum quantification concentration is 1.6 x $_{10\text{-}2\ g/g.}$ g/g.

3 Reagents

3.1 Nitric acid ($_{20}$ = 1.42 g/mL), ultrapure.

3.2 Perchloric acid [($_{HClO4}$) = 70% to 72%], superior pure.

3.3 Hydrogen peroxide [($_{H2O2}$) = 30%], ultrapure.

3.4 Perchloric acid (1+9): measure 10mL of perchloric acid (3.2) add 90mL of water and mix.

3.5 Perchloric acid-hydrogen peroxide mixture: perchloric acid (1+9) (3.4) + hydrogen peroxide (3.3) = 4+1

3.6 Hydrochloric acid ($_{20}$ = 1.19 g/mL), ultrapure.

3.7 Hydrochloric acid (1+4): measure 50mL of hydrochloric acid (3.6) and add to 200mL of water.

3.8 Hydrochloric acid (0.1 mol/L): measure 8.3 mL of hydrochloric acid (3.6) and dilute to 1000 mL with water.

3.9 Disodium EDTA solution (50g/L): weigh disodium EDTA (C10H14N2O8Na2-2H2O, abbreviated as (EDTA-2Na) 50 g in a small amount of water, dissolved by heating, cooled and diluted to 1 L.

3.10 Hydroxylamine hydrochloride solution (100g/L): Weigh 100g of hydroxylamine hydrochloride

(NH2OH-HCl), dissolve in water and dilute to 1L.

3.11 Precision pH test paper: pH 0.5 to 5.0.

3.12 Cresol Red solution (2g/L): weigh 0.2g of Cresol Red (C22H18O5S) in a small amount of water, add a drop of ammonia (3.14) to dissolve completely and dilute to 100mL with water.

3.13 Disodium EDTA - Hydroxylamine Hydrochloride - Cresol Red Mix: Before use, take 50mL of Disodium EDTA (3.9), 50mL of Hydroxylamine Hydrochloride (3.10) and 2.5mL of Cresol Red (3.12), dilute to 500mL with water and mix well.

3.14 Ammonia (1+1): measure 100mL of ammonia and add to 100mL of water.

3.15 Cyclohexane, with no fluorescent impurities, to be used after re-distillation if necessary.

3.16 2,3-Diaminonaphthalene solution [$C_{10}H_6(NH_2)_2$, DAN] (2 g/L): (Do the following in a dark room.) Weigh 200 mg of 2,3-diaminonaphthalene in a 250 mL conical flask, add 100 mL of hydrochloric acid (3.8) and shake until completely dissolved.

(approx. 15min). Add 20 mL of cyclohexane (3.15) and shake for 5 min. Transfer to a separatory funnel with glass wool (or skimmed cotton) at the bottom. The purified 2,3-diaminonaphthalene solution was stored in a brown bottle with a layer of approximately 1 cm thick

Store in cyclohexane in an airtight layer in the refrigerator. If necessary, extract again with cyclohexane before use.

3.17 Defoamer: Octanol or other equivalent defoamer.

3.18 Selenium standard stock solution [(Se)=100mg/L]: weigh 0.1000g of selenium metal, dissolve in a small amount of nitric acid (3.1), add perchloric acid (3.2) 2mL. heat on a boiling water bath to evaporate the nitric acid (about 3h~4h), cool slightly, add hydrochloric acid (3.7) 8.4mL, continue to heat for 2min, use water Allow to build up to 1L.

3.19 Selenium Standard Use Solution [(Se)=0.1mg/L]: Take a quantity of Selenium Standard Reserve Solution (3.18) and dilute with hydrochloric acid (3.8) to 1.00mL containing 0.100g of selenium. Store in the refrigerator and reserve.

4 Instruments

All glassware used for the first time must be soaked in nitric acid (1+1) for at least 4h and rinsed with water. Glassware used in this method is treated and cleaned as if it were being used for the first time after being rinsed in tap water, soaked in detergent solution and rinsed in tap water.

4.1 Conical flask, 100mL.

4.2 4cm diameter funnel.

4.3 50mL stoppered cuvette.

4.4 Electric sand bath.

4.5 Water bath.

4.6 Fluorescence spectrophotometer.

4.7 Centrifuge.

5 Analysis steps

5.1 Sample pre-treatment

5.1.1 Shampoo samples: weigh 1.00g~2.00g of anti-dandruff shampoo into a 50mL tube and add antifoaming agent.

(3.17) 5 drops, add perchloric acid-hydrogen peroxide mixture (3.5) 10mL to 20mL, shake for 3min, leave overnight and leave to measure.

5.1.2 Cream sample: Weigh 1.00g~2.00g of anti-dandruff shampoo into a 50mL cuvette, add 5 drops of antifoaming agent (3.17), add 20mL~40mL of perchloric acid-hydrogen peroxide mixture (3.5), leave for 4h, shake for 3min, leave overnight and filter, take 10.0mL~20.0mL of filtrate for measurement.

5.2 Preparation of standard working solutions

Take 0, 0.10, 0.50, 0.70, 1.00, 1.50, 2.00mL of selenium standard use solution (3.19) in 50mL of

The colorimetric tube is operated simultaneously with the sample and is left to be measured.

5.3 Measurement

Transfer the sample solution and the standard working solution into separate 50mL tubes. Add the reagent mix to each tube separately

(3.13) 10mL, shake well and the solution should be peachy red. Tone with ammonia (1+1) (3.14) to a light orange colour and add a small amount of hydrochloric acid (1+4) (3.7) if necessary, at which point the pH of the solution should be pH 1.5 to 2.0, or use pH 0.5 to 5.0 precision test paper (3.11) to test.

(The following steps should be carried out in a dark room.) Add 1 mL of 2,3-diaminonaphthalene solution (3.16) to each of the above tubes, shake well, place in a boiling water bath for 5 min (from the time of placement in the boiling water bath), remove and cool.

The fluorescence intensity was measured by fluorescence spectrophotometer at excitation wavelength 379 nm and emission wavelength 519 nm. The working curve was plotted and the amount of selenium (IV) in the sample was found from the curve.

6 Calculation

$$(SeS2) = \frac{(m1-m0)V}{mV1} 1.812$$

where: $(SeS2)$ - mass fraction of $SeS2$ in the sample, g/g; $m1$ - mass of selenium (IV) in the test solution, g; m0 - mass of selenium (IV) in the blank solution, g g; $m0$ - mass of selenium(IV) in the blank solution, g.

V - total volume of the sample extracted with the perchloric acid-hydrogen peroxide solution, mL; $V1$ - volume of the sample extracted with the perchloric acid-hydrogen peroxide solution, mL; m - volume of the sample extracted with the perchloric acid-hydrogen peroxide solution, mL the volume of sample taken, g.

1.812 - Conversion factor of $^{Se+4}$ to $SeS2$.

7 Precision and Accuracy

The three levels (high, medium and low) of the anti-dandruff shampoo and anti-dandruff shampoo were spiked and recovered with an accuracy of 84.0%-94.0% and a precision of 6.4%-8.9%.

XIV. Formaldehyde

Formaldehyde

1 Scope

This specification specifies a spectrophotometric method for the determination of formaldehyde in cosmetics using acetylacetone.

This specification applies to the determination of formaldehyde in cosmetics. This specification does not apply to the determination of formaldehyde in nail polish containing toluene sulfonamide resin.

2 Methodology Summary

In the presence of excess ammonium salt, formaldehyde interacts with acetylacetonate and ammonia to produce yellow 3,5-diacetyl-1,4 dihydrolucentidine, which is quantified by colourimetric shades. The reaction equation is as follows.

H2

OOOOCO

The Sightings Browsing ‖ ‖ ‖ in

HCH+NH3+2CH3-C-CH2-C-CH3-→CH3-C-CH2 -CC-CH2C-CH3+3H2O

The "S" is the name of the company.

HCCH

Browsing／N H

The method has a detection limit of 1.8 g and a lower limit of quantification of 6.0 g. For a 1 g sample, the detection concentration is 18 g/g and the lowest quantitative concentration is 60 g/g.

3 Reagents

3.1 Sodium sulphate solution (250g/L): weigh 25g of anhydrous sodium sulphate in a beaker and dissolve in water to 100mL.

3.2 Ammonium acetate solution of acetylacetone [(acetylacetone) = 0.2%]: weigh 25 g of ammonium acetate dissolved in water and add glacial acetic acid

3mL and acetylacetone 0.2mL, add water to 100mL, mix well and transfer to a brown bottle and store in the refrigerator for up to one month.

3.3 Ammonium acetate solution (250g/L): weigh 25g of ammonium acetate in water, add 3mL of glacial acetic acid, then add water to 100mL and mix well.

3.4 Sodium hydroxide solution (40g/L): weigh 4g of sodium hydroxide, dissolve in a small amount of water, add water to 100mL and mix well. 3.5 Sulphuric acid [($_{H2SO4}$) = 3%]: Take 3mL of superior pure sulphuric acid ($_{20}$ = 1.84g/mL), add slowly to 97mL of water and mix well.

3.6 Sulphuric acid [($_{H2SO4}$) = 10%]: take 10mL of superior pure sulphuric acid ($_{20}$ = 1.84g/mL) and slowly

add to 90mL

In water, mix well.

3.7 Starch solution (10g/L): weigh 1g of soluble starch and make a solution with 5mL of water, add 95mL of boiling water, boil and add 0.1g of salicylic acid or 0.4g of zinc chloride to prevent corrosion.

3.8 Iodine standard solution (0.05 mol/L): weigh 13.0 g of iodine and 35 g of potassium iodide, add 100 mL of water, dissolve and add hydrochloric acid 3

Dilute to 1L with water, filter and transfer to a brown bottle.

3.9 Potassium dichromate standard solution [$c(_{1/6K2Cr2O7})$ = 0.1000 mol/L]: weigh accurately 4.9031 g of potassium dichromate dried to a constant weight in an electric oven at 120 °C ± 2 °C, dissolve in water and transfer to a 1 L volumetric flask, fix the volume to the scale and shake well.

3.10 Sodium thiosulphate solution (0.1 mol/L): weigh 26 g of sodium thiosulphate ($_{Na2S2O3}$.5H2O) or 16 g of anhydrous sodium thiosulphate in 1 L of freshly boiled and cooled water, add 0.4 g of sodium hydroxide or 0.2 g of anhydrous sodium carbonate, shake well, store in a brown bottle, leave for a fortnight, filter and then calibrate its exact concentration as follows.

25.00 mL of potassium dichromate standard solution (3.9) was accurately drawn into a 500 mL iodine measuring flask and 2.0 g of potassium iodide and sulphuric acid were added.

Add 150 mL of water and titrate with sodium thiosulphate solution (3.10) until the solution is light yellow, then add 2 mL of starch solution (3.7) and continue titrating until the blue colour changes to bright green. A blank test was also performed. Calculate the concentration of the sodium thiosulphate solution using the following formula.

$$c(_{Na2S2O3}) = \frac{c' \times 25.00}{(V_1'-V_0')}$$

Where: $c(_{Na2S2O3})$ - the concentration of sodium thiosulphate standard solution, mol/L; c' - the concentration of potassium dichromate standard solution [$c(_{1/6K2Cr2O7})$], mol/ L; V_1' - the amount of sodium thiosulphate solution, mL; V_0' - the amount of sodium thiosulphate solution for the blank test, mL.

3.11 Formaldehyde standard reserve solution: weigh about 1 g of formalin solution (analytically pure) and dilute to 1 L with water as a standard reserve solution, which can be stored in a refrigerator for three months. The exact concentration of formaldehyde (HCHO) contained in the reserve solution is calibrated according to the following method.

Add 50 mL of iodine standard solution (3.8) and 15 mL of sodium hydroxide solution (3.4) to a 250 mL iodine flask, stopper, shake well for 15 min, add 20 mL of sulphuric acid [$(_{H2SO4})$=3%] (3.5), stopper immediately, mix well and leave in a dark place for another 15 min. Add 2mL of starch solution (3.7) and continue titrating until the blue colour has just faded, record the volume of sodium thiosulphate. At the same time, replace the formaldehyde solution with water and do a blank test using the same procedure. Calculate the concentration of formaldehyde according to the following formula.

$$(HCHO) = \frac{(V_1-V_0)\times c\times 15\times 1000}{V}$$

where: (HCHO) - concentration fraction of formaldehyde solution, mg/L.

V - volume of formaldehyde sampled, mL; V_0 - sodium thiosulphate solution consumed in blank, mL.

V_1 - the sodium thiosulphate solution consumed for the calibration of formaldehyde, mL; c - the molar concentration of the sodium thiosulphate solution, mol/L; 15 - the molar mass of formaldehyde (1/ 2HCHO) molar mass, g/mol.

3.12 Formaldehyde standard use solution: Take an appropriate amount of formaldehyde reserve solution (3.11) and dilute it step by step with water to the required concentration (1mg/L～).

(4mg/L) for standard use. This solution is prepared at the time of use.

4 Instruments

4.1 Stoppered colorimetric tube, 50mL.

4.2 Stoppered colorimetric tube, 10mL.

4.3 Glass funnel.

4.4 Water bath.

4.5 Centrifuge.

4.6 Spectrophotometer.

5 Analysis steps

5.1 Sample pre-treatment

Weigh 1.0 g of sample accurately into a 50 mL stoppered cuvette. Add 25mL of sodium sulphate solution (3.1), shake, add water to the scale and leave in a water bath at 40°C for 1h (shaking occasionally). The sample solution was cooled quickly and transferred to a centrifuge

The tubes were centrifuged at 3000 rpm. Filter through a glass funnel. The filtrate is used as the solution to be tested.

5.2 Measurement

Add 5.00mL of the solution to be measured to a 10mL stoppered cuvette. Add 5.00mL of ammonium acetate solution (3.2) with acetylacetonate, shake well, heat in a water bath at 40°C for 30min and leave to cool at room temperature for 30min. Add 5.00mL of ammonium acetate solution (3.3) to 5.00mL of the solution to be measured, shake well, heat and cool in the same way as before, and use as a reference solution for colourimetric analysis. The absorbance was measured at 414nm using a 1cm cuvette and the difference between the absorbance of the solution to be measured and that of the reference solution was taken as A.

The formaldehyde standard solution and water were each taken at 5.00 mL and added to 5.00 mL of ammonium acetate solution of acetylacetone (3.2), heated and cooled in the same way as the sample. To ensure the accuracy of the results, the concentration of formaldehyde in the sample solution should be similar to that in the standard solution.

6 Calculation

$$(HCHO) = \frac{A-Ao}{As-Aom} \times \frac{1}{} \times V \times$$

where: (HCHO) - mass fraction of formaldehyde in the sample, g/g.

A - the difference between the absorbance of the solution to be measured and the reference solution; $_{AS}$ - the absorbance of the standard solution of formaldehyde using water as reference; A0 - the absorbance of the blank solution using water as reference $_{A0}$ - the absorbance of the blank solution with water as reference; V - the volume of the sample volume, mL.

m - Sample size, g.

7 Interference removal

For samples containing more sulphide, add an appropriate amount of 10% zinc acetate solution under weak alkaline conditions to produce a zinc sulphide precipitate, filter the precipitate and remove the solution for determination.

XV. Thioglycolic acid

Thioglycollic Acid

1 Scope

This specification specifies an ion chromatographic and chemical titration method for the determination of thioglycolic acid in cosmetics.

This specification applies to the determination of thioglycolic acid and its salts and esters in hair removal, perm and other hair cosmetics.

First method Ion chromatography

2 Methodology Summary

Thioglycolic acid in cosmetics is extracted by dissolving in water, separated from inorganic ions by an ion exchange column and the conductivity detector determines the instant conductivity value, qualitative by retention time and quantitative by peak area.

The limit of detection for mercaptoacetic acid is 5.8 ng and the lower limit of quantification is 20 ng. If 0.5 g is sampled according to this method, the detection concentration is 46 g/g and the lowest quantification concentration is 0.15 mg/g.

3 Reagents

3.1 Thioglycolic acid, ultrapure.

3.2 Methanol, superior grade pure.

3.3 Trichloromethane, analytical purity.

3.4 Sulphuric acid [(H_2SO_4) = 10%]: Take 10mL of sulphuric acid ($_{20}$ = 1.84g/mL), add slowly to 90mL of water and mix well.

3.5 Hydrochloric acid [(HCl) = 10%]: take 10mL of hydrochloric acid ($_{20}$ = 1.19g/mL), add to 90mL of water and mix well.

3.6 Starch solution (10g/L): weigh 1g of soluble starch and make a solution with 5mL of water, add 95mL of boiling water, boil and add 0.1g of salicylic acid or 0.4g of zinc chloride to prevent corrosion.

3.7 Sodium hydroxide solution (500g/L): 50g of pure sodium hydroxide in round granular form, dissolved in water and added to 100mL, then aspirated and diluted to the lysate concentration with ultrasonically degassed water.

3.8 Potassium dichromate standard solution [c ($_{1/6K2Cr2O7}$) = 0.1000 mol/L]: weigh 4.9031 g of potassium dichromate dried to a constant weight in an electric oven at 120 °C ± 2 °C, dissolve in water and transfer to a 1000 mL volumetric flask, fix the volume to the scale and shake well.

3.9 Sodium thiosulfate solution (0.1 mol/L): 26 g of sodium thiosulfate ($_{Na2S2O3}$.5H2O) (or 16 g of anhydrous sodium thiosulfate) is dissolved in 1000 mL of freshly boiled and cooled water, 0.4 g of sodium hydroxide or 0.2 g of anhydrous sodium carbonate is added, shaken well, stored in a brown bottle, placed for a fortnight, filtered and calibrated with potassium dichromate standard solution. The exact concentration is calibrated as follows.

Add 25.00 mL of potassium dichromate standard solution (3.8) to a 500 mL iodine flask, add 2.0 g of potassium iodide and 20 mL of sulphuric acid solution (3.4), stopper immediately, shake well and leave in a dark place for 10 min. add 150 mL of water and titrate with sodium thiosulphate solution until the solution is pale yellow, then add 2 mL of starch solution (3.6). Continue titrating until the blue colour changes to bright green. A blank test is also performed. Calculate the concentration of the sodium thiosulphate solution according to the following formula.

$$c(_{Na2S2O3}) = \frac{C' \times 25.00}{(V_1 - V_0)}$$

Where $c(_{Na2S2O3})$ - the concentration of sodium thiosulphate standard solution, mol/L; C' - the concentration of potassium dichromate standard solution [$c(_{1/6K2Cr2O7})$], mol/ L; V_1 - the amount of sodium thiosulphate solution, mL; V_0 - the amount of sodium thiosulphate solution for the blank test, mL.

3.10 Iodine standard solution (0.05 mol/L): weigh 13.0 g of iodine and 35 g of potassium iodide, add 100 mL of water, dissolve, add 3 drops of hydrochloric acid, dilute with water to 1000 mL, filter and transfer to a brown bottle, calibrate its exact concentration with sodium thiosulphate solution (3.9), calibrate as follows.

Add 150 mL of water and titrate with sodium thiosulphate standard solution (3.9). When the solution becomes lighter in colour near the end point, add 2 mL of starch solution (3.6) and continue titrating until the blue colour disappears.

A blank test is also carried out: 175 mL of water is taken, 0.05-0.20 mL of iodine standard solution and 2 mL of starch solution (3.6) are added and titrated with sodium thiosulphate standard solution (3.9) until the blue colour disappears. Calculate the concentration of the iodine standard solution according to the formula below.

$$c(_{1/2I2}) = \frac{(V_{2}-V_0')\times c_1}{V_3-V_4}$$

where $c(_{1/2I2})$ - concentration of iodine standard solution, mol/L; c_1 - concentration of sodium thiosulphate standard solution, mol/L; V_2 - V_3 - volume of iodine standard solution, mL; V_0' - volume of sodium thiosulphate standard solution for the blank test, mL; V_4 - the exact value of the volume of iodine standard solution added in the blank test, mL.

3.11 Standard solution of thioglycolic acid (1000mg/L): weigh 0.5g of thioglycolic acid standard (3.1), dilute it with water and transfer it to a 500mL volumetric flask, add 1mL of formaldehyde, add water to fix the volume to obtain the standard reserve solution, then use the iodometric method to calibrate the standard reserve solution and dilute it to the standard use solution with the contents of 0.50, 1.00, 2.00, 5.00, 10.0, 20.0, 50.0, 80.0mg/L respectively. The calibration method is as follows.

Add 25.0 mL of thioglycolic acid standard stock solution to a 250 mL iodine flask, add 25 mL of water and 20 mL of hydrochloric acid, then add 2 mL of starch solution (3.6) and titrate with iodine standard solution (3.10), the end point is when the colour of the solution changes from colourless to light blue. At the same time, do a blank test and calculate the concentration of thioglycolic acid standard solution according to the following formula.

$$c(HSCH2COOH) = \frac{92.1 \times c \times (V_1 - V_0) \times 2 \times 1000 \times 1000}{V \times 1000}$$

Where: c(HSCH2COOH) - concentration of thioglycolic acid in the sample, μg/mL; c - concentration of iodine solution, mol/L; V_1 - titration V1 - consumption of iodine solution after titration, mL; V_0 - amount of sodium thiosulphate standard solution for blank test, mL; V - volume of thioglycolic acid standard solution, mL.

92.1 - molar mass of thioglycolic acid, g/mol.

2 - Molecular coefficient for the reaction of iodine with thioglycolic acid.

4 Instruments

4.1 Ion chromatograph.

4.2 Vortex oscillator.

4.3 Ultrasonic cleaners.

4.4 High-speed centrifuge.

5 Analysis steps

5.1 Sample pre-treatment

Weigh 0.5g of sample into a 100mL stoppered cuvette, add water to the scale, shake the paste well with a vortex shaker, extract with an ultrasonic cleaner for 20min, add 2mL of trichloromethane (3.3), shake gently and leave to stand. For turbid samples, centrifuge the sample at 14000 rpm for 15 min and pass the supernatant through a 0.25m membrane as the sample to be measured.

5.2 Chromatographic reference conditions

Chromatographic columns: AS11-HC (250 x 4 mm I. D.), AG11-HC (50 x 4 mm I. D.), packed with a strongly basic ion exchange resin with alkanol quaternary ammonium as functional group.

Suppressor: ASRS-ULTRA.

Leaching solution: 25 mmol/L NaOH + 1% methanol mixture; Leaching solution flow rate: 0.85 mL/min.

Inhibition mode: external water 1.0mL/min, automatic inhibition current 50mA; nitrogen flow rate (pressure): 5psi.

Column temperature: room temperature; Injection volume: 25 L.

Detector: Suppression type conductivity detector.

5.3 Preparation of calibration curves

After injection, the retention time and peak area of the peaks were recorded and calculated by the chromatography workstation, and the calibration curve of peak area-concentration of thioglycolic acid was plotted.

5.4 Sample determination

Aspirate 0.5mL to 1mL of the prepared sample (5.1) into the injection tube of the ion chromatograph. After injection, the retention time and peak area of the peaks were recorded and calculated by the chromatographic workstation, and the concentration of mercaptoacetic acid was obtained from the calibration curve.

6 Calculation

Calculate the concentration of thioglycolic acid (in thioglycolic acid) using the following formula.

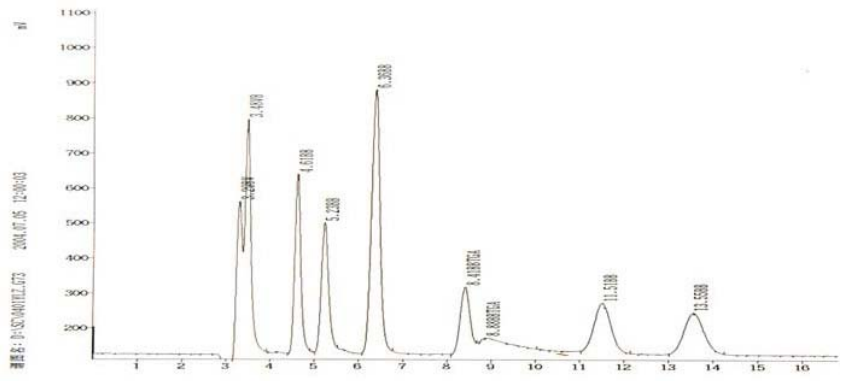
$$\text{(Thioglycolic acid)} = \frac{\times V}{} \text{ m}$$

where: (thioglycolic acid) - mass fraction of thioglycolic acid in the sample, g/g.

--mass concentration of thioglycolic acid in the test solution, mg/L; V - volume of sample volume, mL; m - sample volume taken , g.

7 Chromatograms

mv



min

Figure 1 Ion chromatogram of the mixed standard solution

Chromatographic column: AS11-HC,AG11-HC; Leaching solution: 25 mmol/L NaOH+1% methanol mixture, flow rate 0.85 mL/min; Suppressed conductivity detection: ASRS-ULTRA suppressor, external water auto suppression current 50 mA; Injection volume: 25 μL; Peak: thioglycolic acid (TR=8.43 min)

Second method Chemical titration

8 Methodology Summary

Cosmetics containing thioglycolic acid and its salts and esters are pretreated and quantified by titration with iodine standard solutions. The reaction equation is as follows.

$2HSCH_2COOH + I_2 \rightarrow HOOCH_2C\text{-}S\text{-}S\text{-}CH_2COOH + 2HI$

The method has a detection limit of 0.46 mg of thioglycolic acid, with a minimum concentration of 0.023% (w/w) for a 2 g sample.

9 Reagents

Same as the first method.

10 Instruments

10.1 Acid burette.

10.2 Electromagnetic stirrers: do not wrap the outer layer of the stirrer in plastic.

11 Analysis steps

11.1 Sample pre-treatment

Add 20mL of hydrochloric acid (3.5) and 50mL of water, heat slowly to boiling, cool, add 5mL of trichloromethane (3.3), stir with an electromagnetic stirrer for 5min and reserve as the solution to be measured. For perm products with little organic interference, the acid and water can be added and measured directly.

11.2 Sample determination

Add 2mL of starch solution (3.6) as an indicator and titrate the solution to be measured with iodine standard solution (3.10) until the solution changes colour abruptly or the blue colour does not disappear within 1min.

12 Calculation

$$(HSCH2COOH) = \frac{92.1 \times c \times V \times 2 \times 100\ m \times 1000}{}$$

where: (HSCH2COOH) - mass fraction of thioglycolic acid in the sample, % (w/w).

c - concentration of iodine standard solution, mol/L; V - amount of iodine standard solution used in the titration, mL; m - amount of sample taken, g.

92.1 - molar mass of thioglycolic acid, g/mol.

2 - Molecular coefficient for the reaction of iodine with thioglycolic acid.

13 Interference

Compounds containing free sulfhydryl groups such as mercaptopropionic acid and cysteine interfere with the chemical titration method.

XVI. Hydroquinone, phenol

Hydroquinone and Phenol

1 Scope

This specification specifies high performance liquid chromatography with diode array detector, gas chromatography and high performance liquid chromatography with ultraviolet detector methods for the determination of hydroquinone and phenol in cosmetics.

This specification applies to the determination of the content of hydroquinone and phenol in blemish removing cosmetics and shampoos.

First method High Performance Liquid Chromatography - Diode Array Detector Method

2 Methodology Summary

Hydroquinone and phenol in cosmetics were extracted with methanol and analysed by high performance liquid chromatography (HPLC). The method was characterised by retention time and ultraviolet absorption spectra, quantified by peak height or peak area, and confirmed by gas chromatography-mass spectrometry. The detection limit of this method is 0.001 g for phenol and 0.003 g for hydroquinone; the lower limit of quantification is 0.003 g for phenol and 0.01 g for hydroquinone. The lowest limit of quantification was 7 g/g for phenol and 23 g/g for hydroquinone.

3 Reagents

3.1 Methanol, superior grade pure.

3.2 Hydroquinone standard solution [(hydroquinone) = 1 g/L]: weigh accurately 0.1000 g of chromatographically pure or distilled hydroquinone in a beaker, dissolve with a small amount of methanol (3.1), transfer to a 100 mL volumetric flask and dilute to the scale with methanol. This solution is stable for one month when stored in the dark at 4°C.

3.3 Phenol Standard Solution [(phenol) = 1 g/L]: weigh 0.1000 g of chromatographically pure phenol accurately, place in a beaker, dissolve in a small amount of methanol (3.1), transfer to a 100 mL volumetric flask and dilute to the scale with methanol. The solution is stable for one month when stored in the dark at 4°C.

4 Instruments

4.1 High performance liquid chromatograph with isovolume pump and diode array detector.

4.2 Ultrasonic cleaners.

4.3 0.45m filter membrane.

4.4 Gas chromatography-mass spectrometer.

5 Analysis steps

5.1 Sample pre-treatment

Weigh approximately 1.0g of the sample into a stoppered cuvette and distill on a water bath to remove volatile organic solvents such as ethanol if necessary. The sample was extracted with methanol (3.1) to a volume of 10mL, sonicated at room temperature for 15min, and the supernatant was filtered through a 0.45m membrane.

5.2 Chromatographic reference conditions

Chromatographic column: $_{C18}$ column, 150 mm 3.9 mm, 5 m; mobile phase: methanol + water = 60 + 40.

Flow rate: 1.0 mL/min; column temperature: room temperature.

Detector: Diode array detector, detection wavelength 280nm.

5.3 Preparation of calibration curves

The solutions in (3.2) and (3.3) were used to prepare mixed standard solutions containing 10.0, 50.0, 100 and 200 mg/L of hydroquinone and phenol. The peak areas were recorded and the peak area-hydroquinone and phenol concentration (mg/L) curves were plotted.

5.4 Measurement

The concentration of hydroquinone and phenol in the solution to be measured was determined from the calibration curve based on the peak retention time and the UV spectra of the peaks.

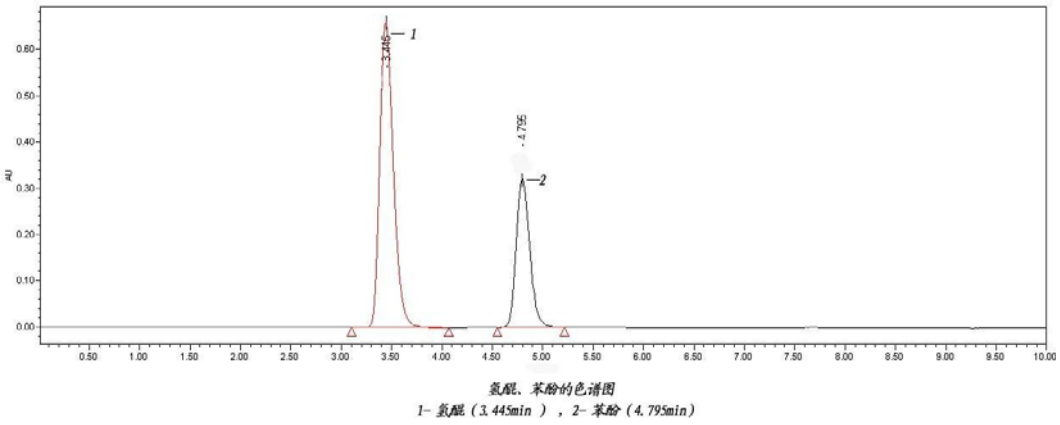
6 Calculation

$$(\text{hydroquinone or phenol}) = \frac{\times V}{m}$$

where: (hydroquinone or phenol) - the mass fraction of hydroquinone or phenol in the sample, g/g.

--mass concentration of hydroquinone and phenol in the test solution, mg/L; V - volume of sample volume, mL; m - sample volume, g Volume, g.

7 Chromatograms



氨醌、苯酚的色谱图

1- 氨醌（3.445min），2- 苯酚（4.795min）

8 Confirmation of positive results

A positive result during the determination must be confirmed by gas chromatography-mass spectrometry. Gas chromatography reference conditions.

Chromatographic column: DB-1 30m0.25mm; column chamber temperature: 50C (1min), ramp up to 190C at 6C/min

(2min); inlet temperature: 250C; interface temperature: 230C; split ratio: 1:30; pre-column pressure: 100kPa. mass spectrometry reference conditions.

Mass number range: 30 to 300; scan speed: 50amu/s; solvent cutting time: 4min; start acquisition time: 5min; detection port voltage: 1.4kV.

Second method Gas Chromatography

9 Methodology Summary

Hydroquinone and phenol in cosmetics were extracted with ethanol and analysed by gas chromatography. The retention time was used for the determination and the peak height or peak area for the quantification of the standards. The detection limit of this method is 0.03 g for phenol and 0.05 g for hydroquinone, and the lower limit of quantification is 0.10 g for phenol and 0.16 g for hydroquinone. The lowest limit of quantification was 500 g/g for phenol and 830 g/g for hydroquinone.

10 Reagents

10.1 Ethanol [(ethanol) = 99.9%].

10.2 Hydroquinone standard solution [(hydroquinone) = 4g/L]: accurately weigh 0.400g of chromatographically pure hydroquinone in a beaker, dissolve in a small amount of ethanol, transfer to a 100mL volumetric flask and dilute to the scale with ethanol. This standard solution is stable for one month.

10.3 Phenol standard solution [(phenol) = 2g/L]: accurately weigh 0.200g of chromatographically pure phenol in a beaker, dissolve in a small amount of ethanol and transfer to a 100mL volumetric flask and dilute to the scale with ethanol. This standard solution is stable for one month.

11 Instruments

Gas chromatograph with hydrogen flame ionisation detector.

12 Analysis steps

12.1 Sample pre-treatment

Weigh 1.0g of the sample into a 10mL stoppered cuvette, dissolve in ethanol (10.1), shake for 1min, dilute with ethanol (10.1) to the scale, and then inject the supernatant into the chromatograph to determine the peak height or peak area.

12.2 Chromatographic reference conditions

Chromatographic column: rigid glass column (2 m long, 3 mm inner diameter).

Stationary phase: 10% SE-30, stretcher: Chromosorb W AW DMCS 60-80 mesh; column chamber temperature: 220°C; vapour chamber temperature: 280°C.

Carrier gas: nitrogen.

Gas flow: Nitrogen 30mL/min, Hydrogen 50mL/min, Air 500mL/min.

12.3 Preparation of calibration curves

A 5 mL pipette was used to accurately dispense 0, 1.50, 2.00, 2.50 and 3.00 mL of the hydroquinone standard solution (10.2) into a 10 mL volumetric flask and the solution was fixed to the scale with ethanol (10.1) to prepare a series of 0, 0.60, 0.80, 1.00 and 1.20 g/L hydroquinone standards respectively.

Using a 5mL pipette, accurately pipette the phenol standard solution (10.3) 0, 0.50, 1.00, 2.00, 3.00, 4.00, and

5.00mL in a 10mL volumetric flask, fixed to the scale with ethanol (10.1) and prepared to 0, 0.10, 0.20, 0.40, 0.40, 0.00mL and 0.00mL respectively.

Standard series of phenol at 0.60, 0.80 and 1.00g/L.

An accurate 2.0L of hydroquinone or phenol standard series was injected into the chromatograph using a 10L microsampler. The hydroquinone or phenol content is measured as

(g/L) as the horizontal coordinate and peak height or peak area as the vertical coordinate for the standard curve.

12.4 Sample determination

2.0L of sample solution was accurately aspirated using a microsampler and injected into the chromatograph. Each sample was repeated three times and the peak height or peak area was measured and averaged.

13 Calculation

$$(\text{hydroquinone or phenol}) = \frac{\times V \times 1000}{m}$$

where: (hydroquinone or phenol) - the mass fraction of hydroquinone or phenol in the sample, g/g.

- the mass concentration of hydroquinone and phenol in the solution to be measured, g/L, as found from the calibration curve.

V - volume of sample volume, mL; m - volume of sample taken, g.

14 Chromatograms

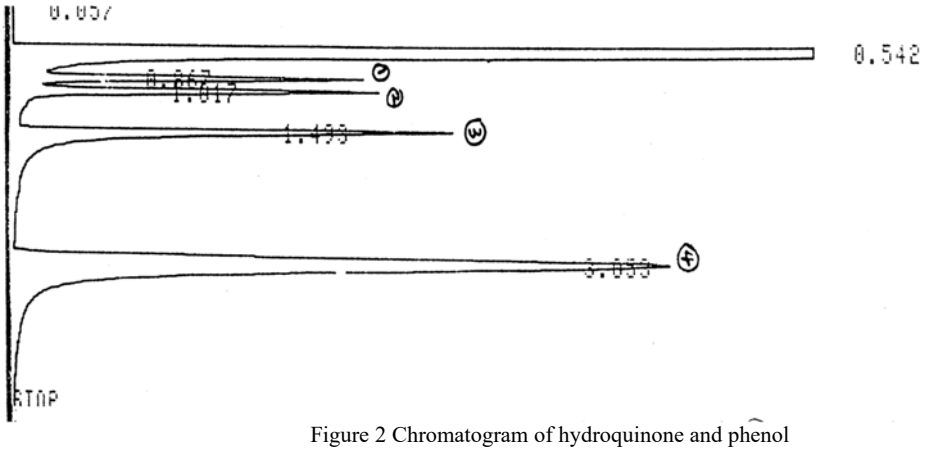


Figure 2 Chromatogram of hydroquinone and phenol

1 Phenol; 3 Hydroquinone

Third method High performance liquid chromatography with UV detector method

15 Methodology Summary

Hydroquinone and phenol in cosmetics were extracted with methanol and analysed by high performance liquid chromatography (HPLC), characterised by retention time and quantified by peak height or peak area. The detection limit of this method is 0.045 g for phenol and 0.09 g for hydroquinone, and the lower limit of quantification is 0.15 g for phenol and 0.3 g for hydroquinone. The lowest quantitative limit is 300g/g for phenol and 600g/g for hydroquinone.

16 Reagents

Same as the first method.

17 Instruments

17.1 High performance liquid chromatograph with isovolumetric pump and UV detector.

17.2 Ultrasonic cleaners.

17. 30.45m filter membrane.

18 Analysis steps

18.1 The samples were pre-treated as in the first method.

18.2 Chromatographic reference conditions

The chromatographic column, mobile phase, flow rate and column temperature are the same as those of the first method; detector: UV detector, detection wavelength 280 nm.

18.3 Measurement

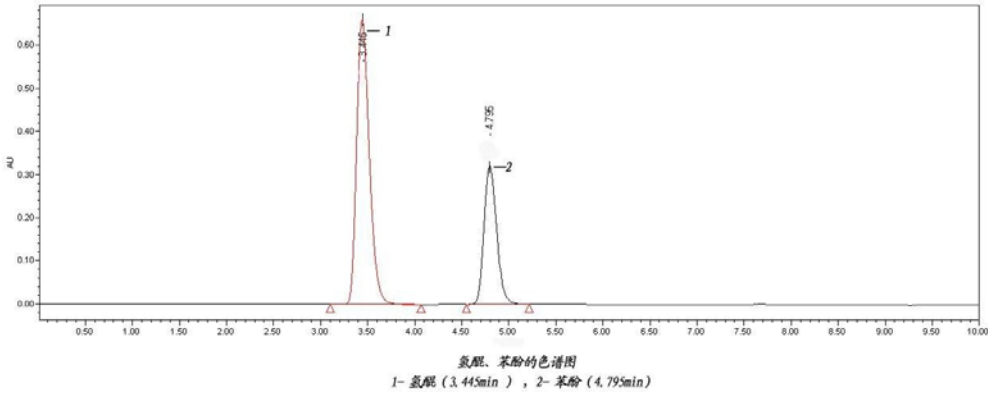
The concentration of hydroquinone and phenol in the solution to be measured was determined from the calibration curve based on the peak area. If necessary, a second method was used to support this.

18.4 The calibration curve was prepared as in the first method.

19 Calculation

Same as the first method.

20 Chromatograms



氢醌、苯酚的色谱图

1- 氢醌（3.445min），2- 苯酚（4.795min）

XVII. Sex hormones

Sexual Hormones

1 Scope

This specification specifies a high performance liquid chromatography (HPLC) method with diode array detector, a high performance liquid chromatography (HPLC) method with ultraviolet detector/fluorescence detector and a gas chromatography/mass spectrometry (GC/MS) method for the determination of seven sex hormones including estriol in cosmetics.

This specification applies to the detection and identification of seven sex hormones, including estriol, in cosmetics.

First method High Performance Liquid Chromatography - Diode Array Detector Method

2 Methodology Summary

The sex hormones in cosmetics were extracted with organic solvents and analysed by high performance liquid chromatography (HPLC), characterised by retention time and UV absorption spectra or fluorescence spectra and quantified by peak area. The limits of detection for each hormone and the concentration at which 1 g of sample was taken are shown in Table 1.

Table 1 Detection limits and concentrations for each hormone

Hormone components	Estriol	Estrone	Hexenestrol	Estradiol	Testosterone	Methyltestosterone	Progesterone
Detection limit, g	0.02	0.04	0.01	0.02	0.002	0.002	0.003
Detected concentration, g/g	40	80	20	40	4	4	6

3 Reagents

The reagents used in this standard are of superior purity unless otherwise stated.

3.1 Methanol.

3.2 Saturated sodium chloride solution.

3.3 Cyclohexane.

3.4 Sulphuric acid [($_{H2SO4}$) = 2%]: Take 2mL of sulphuric acid ($_{20}$ = 1.84g/mL), add slowly to 98mL of water and mix well.

3.5 Hormone standard solutions

3.5.1 Estrogen Standard Solution [(estrone, estradiol, estriol, hexenestrol) = 2 g/L]: 0.200 g of each of estrone, estradiol, estriol and hexenestrol were weighed, dissolved in a small amount of methanol (3.1), transferred to a 100 mL volumetric flask and diluted to the scale with methanol.

3.5.2 Androgen standard solution [(testosterone, methyltestosterone) = 600mg/L]: weigh 0.600g of each of testosterone and methyltestosterone, dissolve with a small amount of methanol (3.1), transfer to a 100mL volumetric flask and dilute to the scale with methanol. 1mL of this solution contains 6.00mg of the above two androgens. 10.0mL of this standard solution was placed in a 100mL volumetric flask and diluted with methanol.

(3.1) Dilute to the scale.

3.5.3 Progesterone Standard Solution [(progesterone) = 600mg/L]: Weigh 0.600g of progesterone, dissolve in a small amount of methanol (3.1), transfer to a 100mL volumetric flask and dilute to the scale with methanol. 1mL of this solution contains 6.00mg of progesterone. 10.0mL of this standard solution is placed in a 100mL volumetric flask and diluted to the scale with methanol (3.1). Dilute to the mark with methanol (3.1).

3.5.4 Mixed standard solutions: 50.0 mL of estrogen standard solution (3.5.1), 5.00 mL of androgen standard solution (3.5.2) and 5.00 mL of progestin standard solution (3.5.3) were pipetted into a 100 mL volumetric flask and diluted to the mark with methanol (3.1). 1 mL of this solution contained 1.00 mg of each of the four estrogens, 30.0 g of each of the two androgens and 30.0 g of one progestin. 1 mL of this solution contains 1.00 mg of each of the 4 oestrogens, 30.0 g of each of the 2 androgens and 30.0 g of the 1 progestin.

4 Instruments

4.1 High performance liquid chromatograph with isovolume pump, diode array detector or fluorescence detector.

4.2 Centrifuge.

4.3 Stoppered colorimetric tube, 10mL.

5 Analysis steps

5.1 Sample pre-treatment

5.1.1 Sample in solution: Weigh 1 g to 2 g of sample in a 10 mL stoppered cuvette, distill on a water bath to remove volatile organic solvents such as ethanol, dilute to 10 mL with methanol (3.1) and set aside.

5.1.2 Paste and emulsion samples: Weigh 1 g to 2 g of sample in a 100 mL conical flask, add 50 mL of saturated sodium chloride solution (3.2) and 2 mL of sulphuric acid (3.4), shake to dissolve and transfer to a 100 mL separatory funnel. Extract in three portions with 30 mL of cyclohexane (3.3) and centrifuge if necessary. Combine the cyclohexanes and distill on a water bath. Dissolve the residue in methanol (3.1), transfer to a 10 mL stoppered cuvette and dilute to the mark with methanol. Mix well and filter through a 0.45m membrane and reserve the filtrate.

5.2 Chromatographic reference conditions

Chromatographic column: $_{C18}$ column, 250 x 4.6 mm, 10 m.

Detection wavelength: Diode array detector (estrogen - 204nm, androgen - 245nm) or fluorescence detector

(excitation wavelength 280 nm, emission wavelength 310 nm); mobile phase: methanol + water = 60 + 40.

Flow rate: 1.3mL/min.

5.3 Preparation of calibration curves

Pipette 0.00, 1.00, 2.00 and 5.00 mL of the hormone standard solution (3.5.4) into a 10 mL stoppered cuvette and dilute to the scale with methanol (3.1). Adjust the instrument to the optimum condition, take a 5L sample and inject it into the HPLC and plot the calibration curve using the peak area of the standard.

5.4 Measurement

5L of the solution to be measured was injected into a high performance liquid chromatograph and characterised according to the retention time of the peaks and the UV absorption spectra or fluorescence spectra, and the mass concentration of the hormone in the solution to be measured was found from the curve according to the peak area.

6 Calculation

$$(\text{Hormones}) = \frac{\text{—} \times V}{m}$$

where: (hormone) - mass fraction of hormone in the sample, g/g.

- the mass concentration of the hormone in the test solution from the curve, mg/L.

V - volume of sample volume, mL; m - volume of sample taken, g.

7 Chromatograms

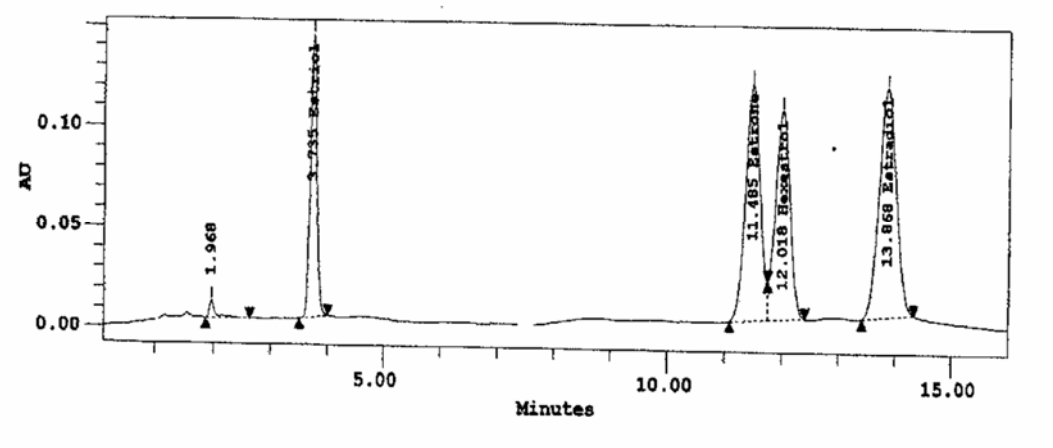


Fig. 1 Chromatogram of estrogen

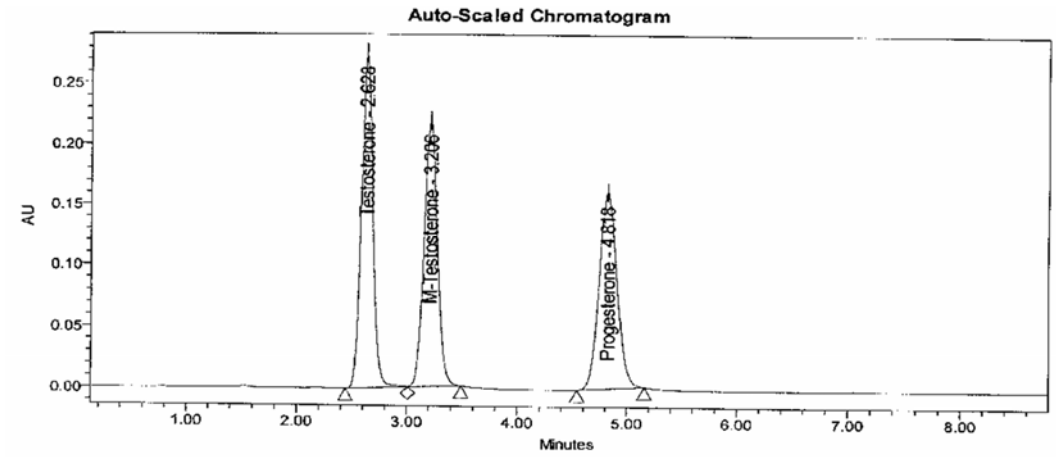


Fig. 2 Chromatogram of androgens

Second method High Performance Liquid Chromatography - UV Detector Method / Fluorescence Detector Method

8 Methodology Summary

The sex hormones in cosmetics were extracted with organic solvents and analysed by high performance liquid chromatography (HPLC), characterised by retention time and quantified by peak area. The limits of detection for each hormone and the concentrations at which 1 g of the sample was taken are shown in Table 2.

Table 2 Detection limits and concentrations for each hormone

Hormone	Estriol	Estrone	Hexenestro	Estradiol	Testoste	Methyltestost	Progester

components			l		rone	erone	one
Detection limit, g	0.05	0.4	0.03	0.035	0.002	0.002	0.004
Detected concentration, g/g	100	800	60	70	4	4	8

9 Reagents

Same as the first method.

10 Instruments

10.1 High performance liquid chromatograph: with isovolumetric pump, UV detector or fluorescence detector.

10.2 Centrifuge.

10.3 Stoppered colorimetric tube, 10mL.

11 Analysis steps

11.1 The samples were pre-treated as in the first method.

11.2 Chromatographic reference conditions

Chromatographic column: C_{18} column, 250 mm x 4.6 mm, 10 m.

Detection wavelength: UV detector (detection wavelength 254 nm) or fluorescence detector (excitation wavelength 280 nm, emission wavelength 310 nm).

Mobile phase: methanol + water = 80 + 20; column temperature: 45 °C.

Flow rate: 0.6mL/min.

11.3 Preparation of calibration curves

Pipette 0, 1.0, 2.0 and 5.0 mL of the hormone standard (3.5.4) into a 10 mL stoppered test tube and dilute to the mark with methanol (3.1). Adjust the instrument to the optimum state, take 5L of sample and inject into the HPLC and plot the calibration curve using the peak area of the standard.

11.4 Measurement

5L of the solution to be measured was injected into a high performance liquid chromatograph and the mass concentration of the hormone in the solution to be measured was determined from the retention time of the peaks and the peak area from the curve.

12 Calculation

Same as the first method.

13 Chromatograms

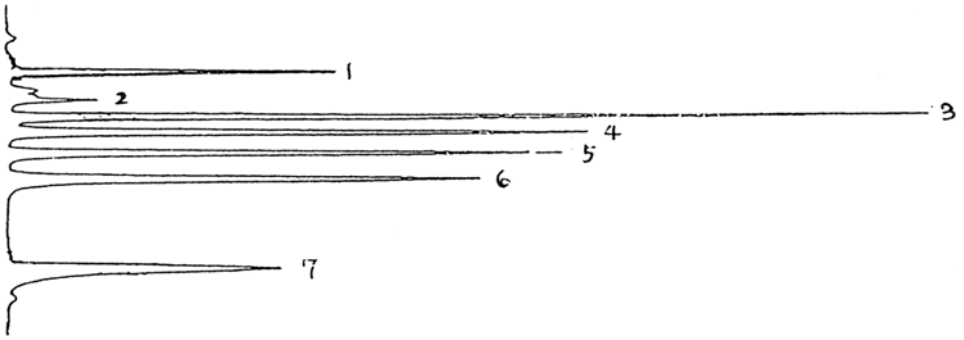


Fig. 3 Chromatogram of sex hormones

1: Estriol (2.68min); 2: Estrone (3.62min); 3: Hexestrol (4.22min); 4: Estradiol (4.81min).

5: Testosterone (5.58min); 6: Methyltestosterone (6.54min); 7: Progesterone (9.74min)

Third method Gas chromatography-mass spectrometry identification

14 Methodology Summary

A gas chromatography/mass spectrometry (GC-MS) coupled technique was used to simultaneously analyse seven hormones in aqueous cosmetics. Samples were extracted, defatted, cleaned up using a C18 solid phase extraction column and the targets were derivatised with heptafluorobutyric anhydride and analysed by GC-MS-SIM.

15 Reagents

15.1 Aether.

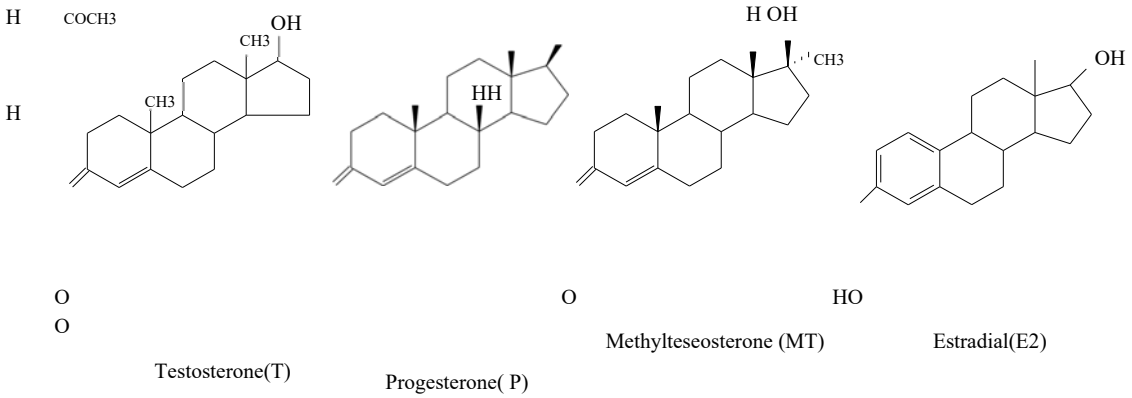
15.2 Acetonitrile, chromatographically pure.

15.3 Methanol, chromatographically pure.

15.4 Heptafluorobutyric anhydride (HFBA), chromatographically pure.

15.5 7 sex hormone standards: testosterone (T), progesterone (P), methyltestosterone (MT), estradiol (E2), estriol

(E3), estrone (E1) and hexestrol (DES) (see Figure 4 for the structural formulae of the seven compounds).



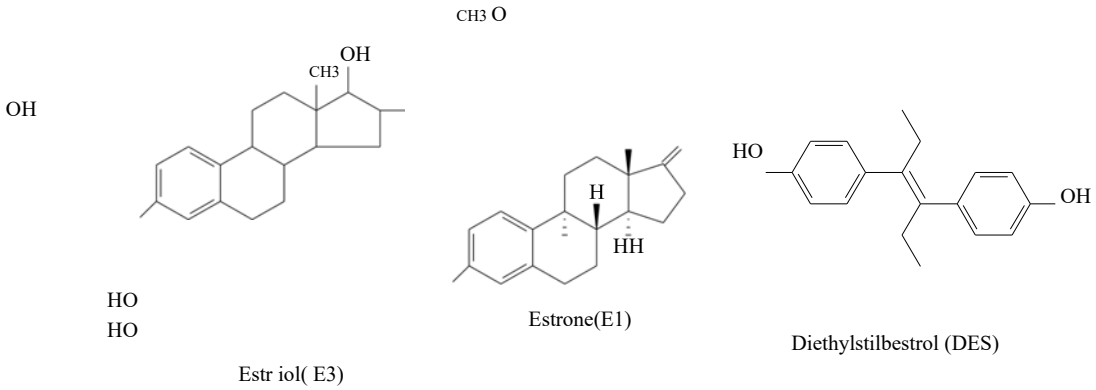


Fig. 4 Chemical structure formulae of the seven hormones

15.6 Hormone standard solutions

15.6.1 Estrogen Standard Solution [(estrone, estradiol, estriol, hexenestrol) = 1 g/L]: 0.100 g of each of estrone, estradiol, estriol and hexenestrol were weighed, dissolved in a small amount of methanol (15.3), transferred to a 100 mL volumetric flask and diluted to the scale with methanol.

15.6.2 Androgen standard solution [(testosterone, methyltestosterone) = 1 g/L]: weigh 0.100 g each of testosterone and methyltestosterone, dissolve with a small amount of methanol (15.3), transfer to a 100 mL volumetric flask and dilute to the scale with methanol.

15.6.3 Progesterone Standard Solution [(progesterone) = 1g/L]: weigh 0.100g of progesterone, dissolve in a small amount of methanol (15.3), transfer to a 100mL volumetric flask and dilute to the scale with methanol.

15.6.4

5.00 mL of Progesterone Standard Solution (15.6.2) and 5.00 mL of Progesterone Standard Solution (15.6.3) were placed in a 500 mL volumetric flask and diluted to the scale with methanol (15.3).

15.6.5 Mixed standard solution [= 1 g/L]: accurately pipette 10.0 mL of the mixed standard use solution (15.6.4) into 100 mL of

In a volumetric flask, dilute to the scale with methanol (15.3)

16 Instruments

16.1 Gas Chromatography-Mass Spectrometer.

16.2 Chromatographic column: DB-5MS capillary column (30m x 0.25mm x 0.25m)

16.3 Solid phase extraction system.

16.4 Nitrogen blowing concentrator.

16.5 $_{C18}$ extraction column.

16.6 Micro-derivative bottle.

17 Analysis steps

17.1 Sample pre-treatment

The sample was extracted 3 times with 2 mL of ether (15.1), combined and blown dry with nitrogen, then removed by ultrasonication with 1 mL of acetonitrile (15.2), washed with 0.5 mL of acetonitrile (15.2), combined and blown dry with nitrogen. The residue was dissolved by sonication with 0.5 mL of methanol (15.3) followed by 3.5 mL of water, mixed and activated by adsorption on a $_{C18}$ column [the small column was pre-activated by sequential elution with 3 mL of methanol (15.3) in water, 5 mL, and 3 mL of methanol + water (1+7)], then washed with 3 mL of acetonitrile + water (1+4) and vacuum dried. The eluate was finally eluted with acetonitrile (15.2) 7 mL and the eluate was finally collected in a derivatization vial, blown dry at 35°C under nitrogen and set aside. Addition of heptafluorobutyric anhydride (HFBA)

(15.4) 40L at a constant temperature of 60°C for 65 min. cool to room temperature and inject 1.0L into the sample.

17.2 Colour quality reference conditions

Carrier gas: Helium, constant flow rate 1.0 mL/min.

Inlet temperature: 270 °C, MS transmission line temperature: 280 °C, column temperature: programmed ramp-up from an initial temperature of 120 °C (2 min) to 200 °C (2 min) at 20 °C/min and then to 280 °C (5 min) at 3 °C/min.

Injection method: non-split injection, injection volume 1.0 L; EI source: electron bombardment energy 70 eV.

Solvent delay time: 10min.

Scanning method: Single Ion Scanning (SIM)

17.3 Measurement

Take 1.0 mL of the mixed standard solution (15.6.5) in a derivatization vial and blow dry under nitrogen. Add 40L of heptafluorobutyric anhydride (HFBA) (15.4) together with the blown dry sample and leave at a constant temperature of 60°C for 65 min. Cool to room temperature and inject 1.0L into the sample.

18 Atlas

18.1 Total ion diagram for the seven target substances (see Figure 5).

18.2 Selection of characteristic ions

The characteristic fragmentation of the derived product into the ion source (see Figure 6).

The ions with low interference and good selectivity were selected as characteristic ions according to their corresponding mass spectra (see Table 3).

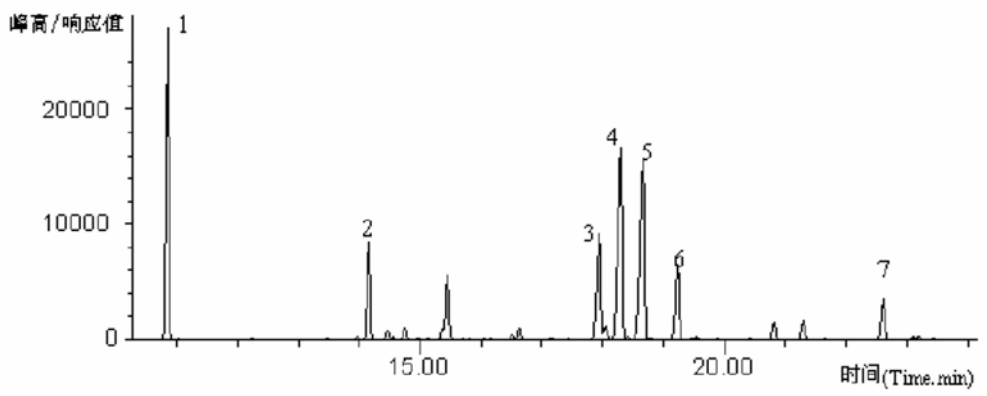
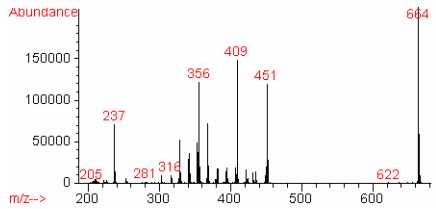
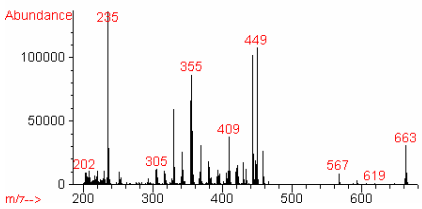


Figure 5 Total ion flow chromatogram of the derivatives of the mixed standards

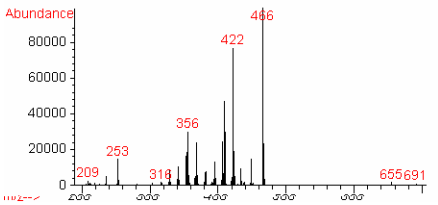
1 DES; 2 MT; 3 T; 4 E2; 5 E3; 6 E1; 7 P



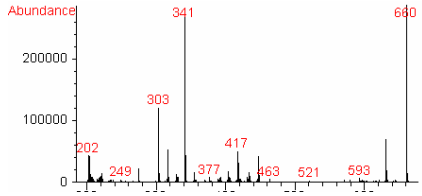
Estradiol



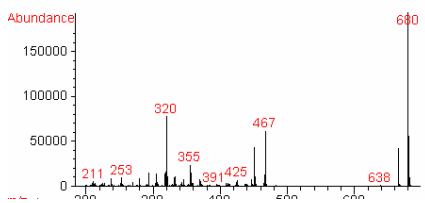
Estriol



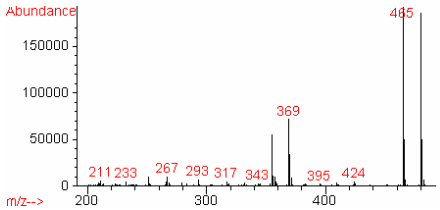
Estrone



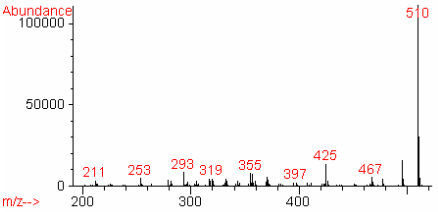
Hexenestrol



Testosterone



Methyltestosterone



Progesterone

Figure 6 Full mass spectral scans of the seven derivatised products

Table 37 Retention times and characteristic ions of the derivatised products

Substance name	Retention time (min)	Characteristic ion (m/z)
Diethylstilbestrol (DES)	10.87	341 447 660
Methyltestosterone (MT)	14.17	369 465 480
Testosterone (T)	17.96	320 467 680
Estradiol (E2)	18.31	409 451 664
Estriol (E3)	18.69	449 663
Estrone (E1)	19.24	409422 466
Progesterone (P)	22.50	370425 510

Note: Underlined parts are molecular ions.

19 Identification criteria

19.1 The retention time of each hormone measured was consistent with the standard, both selected detection ions peaked, and the relative error of the ratio of the intensities of the two detection ions to the ratio of the intensities of the two ions in the standard mass spectra was <30%.

19.2 If the area of the peak is three times larger than the noise and the above conditions are met, the substance is judged to be the same as the standard.

XVIII. Sunscreens

UV filters

First method High performance liquid chromatography with diode array detector - gradient elution

1 Scope

This specification specifies a high performance liquid chromatographic method for the determination of sunscreens in cosmetics.

This specification applies to 15 sunscreens including phenylbenzimidazole sulfonic acid, diphenylketone-4 and diphenylketone-5, p-aminobenzoic acid, diphenylketone-3, isoamyl p-methoxycinnamate, 4-methylbenzylidene camphor, ethylhexyl PABA, butylmethoxydibenzoylmethane, oxytetracycline, ethylhexyl methoxycinnamate, ethylhexyl salicylate, humulanilide, ethylhexyl triazinone, methylenebisbenzotriazolyl tetramethylbutyl phenol, bis-ethylhexoxyphenol methoxyphenyl triazine. The test of 15 sunscreens including methylenebisbenzotriazolyl tetramethylbutyl phenol, bis-ethylhexoxyphenol methoxyphenyl triazine.

2 Methodology Summary

The various sunscreens in cosmetics can be separated by reversed-phase high-performance liquid chromatography due to their structural differences. They are characterised on the basis of their retention time and UV absorption spectra and quantified by peak area. The detection limits, detection concentrations, lower limits of quantification and lowest quantitative concentrations of the method are shown in Table 1.

Table 1 Limit of detection, concentration, lower limit of quantification and minimum concentration for this method

Preface No.	Name of sunscreen	Detection limit (ng)	Detected concentration (%)	Lower limit of quantification ng	Minimum ration Concentration (%)
1	Phenylbenzimidazole sulfonic acid	2	0.02	7	0.07
2	Diphenylketone-4 and diphenylketone-5	3	0.03	10	0.10
3	P-aminobenzoic acid	2	0.02	7	0.07

4	Diphenylketone-3	3	0.03	10	0.10
5	Isoamyl p-methoxycinnamate	3	0.03	10	0.10
6	4-Methylbenzylidene camphor	2.5	0.025	8	0.08
7	PABA ethylhexyl ester	3	0.03	10	0.10
8	Butylmethoxydibenzoylmethane	12	0.12	40	0.40
9	Oaklein	5	0.05	17	0.17
10	Ethylhexyl methoxycinnamate	3	0.03	10	0.10
11	Ethylhexyl salicylate	20	0.20	67	0.67
12	Humulanate	20	0.20	67	0.67
13	Ethylhexyltriazinone	2	0.02	7	0.07
14	Methylenebis-benzotriazolyltetramethylbutyl phenol	5	0.05	17	0.17
15	Bis-ethylhexoxyphenol methoxyphenyl triazine	5	0.05	17	0.17

3 Reagents

3.1 Methanol, chromatographically pure.

3.2 Tetrahydrofuran, chromatographically pure.

3.3 Perchloric acid [($HClO_4$) = 70% to 72%], ultrapure.

3.4 Mixed solution: methanol (3.1) + tetrahydrofuran (3.2) + water + perchloric acid (3.3) = 250 + 450 + 300 + 0.2.

3.5 Standard reserve solution of sunscreen: Weigh each UV absorber according to Table 2 and dissolve and dilute with the solvent shown in the table to

100 mL was prepared as a standard stock solution for each UV absorber, the concentrations of which are shown in Table 2.

3.6 The UV absorber standard solutions were prepared by transferring 1.00mL of each UV absorber standard stock solution into a 100mL volumetric flask and using the mixture (3.4) to build up a volume of 100mL. The concentrations of the UV absorbers contained in this mixed standard solution are shown in Table 2.

Table 2 Preparation of standard stock solutions and mixed standard solutions

Preface No.	Name of sunscreen	Sample size (g)	Constant volume soluble Agent[1]	Stock solutions Concentration (g/L)	Mixed standard solutions Concentration (mg/L)
1	Phenylbenzimidazole sulfonic acid[2]	0.300	3.4	3	30
2	Diphenylketone-4 and diphenylketone-5	1.000	3.4	10	100
3	P-aminobenzoic acid	0.300	3.4	3	30
4	Diphenylketone-3	1.000	3.4	10	100
5	Isoamyl p-methoxycinnamate	1.000	3.4	10	100
6	4-Methylbenzylidene camphor	0.600	3.4	6	60
7	PABA ethylhexyl ester	1.000	3.4	10	100
8	Butylmethoxydibenzoylmethane	3.000	3.2	30	300
9	Oaklein	1.450	3.2	14.5	100[3]
10	Ethylhexyl methoxycinnamate	1.000	3.2	10	100
11	Ethylhexyl salicylate	5.000	3.2	50	500
12	Humulanate	5.000	3.2	50	500
13	Ethylhexyltriazinone	0.500	3.2	5	50

14	Methylenebis-benzotriazolyltetramethylbutyl phenol	1.000	3.2	10	100
15	Bis-ethylhexoxyphenol methoxyphenyl triazine	1.000	3.2	10	100

[1] For the designation of the fixing solvent, see Reagent 3 in this paper; [2] a small amount of NaOH solution is added in advance to dissolve it before adding the fixing solvent, and then the fixing solvent is used to fix the volume; [3] has been converted from ester to acid.

4 Instruments

4.1 High performance liquid chromatograph with ternary pump, diode array detector and integrator or chromatography workstation.

4.2 Ultrasonic cleaners.

4.3 Microsampler (10L), or autosampler.

4.4 0.45m filter membrane.

5 Analysis steps

5.1 Chromatographic conditions

Chromatographic column: $_{C18}$ column, 250 mm × 4.6 mm, 5 m; UV detection wavelength: 311 nm.

Flow rate: 1.0 mL/min; Mobile phase.

Solution A: Methanol (3.1), filtered through a 0.45m filter membrane and degassed under vacuum before use.

Solution B: Tetrahydrofuran (3.2), filtered through a 0.45m membrane and degassed under vacuum before use.

Solution C: water + perchloric acid (3.3) (300 + 0.2), filtered through a 0.45m membrane and degassed under vacuum before use. The gradient procedure is shown in Table 3.

Table 3 Gradient procedure for mobile phases

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0.00	25	45	30
13.00	25	45	30
14.00	45	50	5
20.00	45	50	5
22.00	25	45	30

5.2 Sample pre-treatment

5.2.1 For non-waxy cosmetics such as skin care products, shampoos, powders, etc.: accurately weigh approximately 0.25 g of sunscreen cosmetics into a 25 mL stoppered cuvette, add Mixing Solution (3.4), fix the volume, mix well and shake ultrasonically for 20 min to 30 min. take 1.00 mL of this shaking solution, dilute to 10.0 mL with Mixing Solution (3.4), mix well and filter through a 0.45 m The filtrate was then filtered through a 0.45 m membrane and set aside.

5.2.2 For cosmetics containing waxes such as lipstick and lipstick, weigh approximately 0.25 g of sunscreen into a 25 mL stoppered cuvette, add tetrahydrofuran (3.2), fix the volume, mix well and shake ultrasonically for 20 min to 30 min. The filtrate was then filtered through a 0.45 m filter membrane and set aside.

5.3 Preparation of calibration curves

Pipette 0, 0.20, 1.00, 5.00 and 10.0 mL of the sunscreen mixture (3.6) into a 10 mL stoppered cuvette and dilute to the scale with the mixture (3.4). The calibration curve was obtained by plotting the peak area against the UV absorber content.

5.4 Measurement

A 10L sample solution was measured using a microsampler or autosampler and injected into a high performance liquid chromatograph. The sample was characterised by its retention time (if necessary by UV absorption spectroscopy with a diode array detector) and the peak area was quantified.

6 Calculation

(UV absorber) =

$$\frac{V10\text{-}4}{m}$$

where: (UV absorber) - mass fraction of sunscreen in the sample, %.

- the mass concentration of sunscreen in the test solution from the curve, mg/L.

V - volume of sample volume, mL; m - volume of sample taken, g.

7 Chromatograms

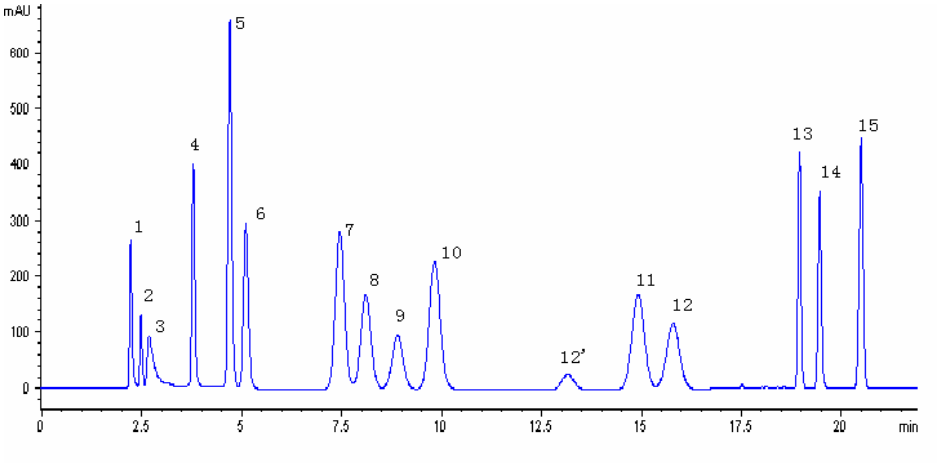


Fig. 1 Chromatogram of sunscreen standards

1: Phenylbenzimidazole sulfonic acid; 2: Diphenylketone-4 and Diphenylketone-5; 3: p-aminobenzoic acid; 4: Diphenylketone-3; 5: Isopentyl p-methoxycinnamate; 6: 4-methylbenzylidene camphor; 7: PABA ethylhexyl ester; 8: Butylmethoxydiphenylmethane; 9: Octolin; 10: Ethylhexyl methoxycinnamate; 12 ': tautomer of peak 12; 11: Ethylhexyl salicylate; 12: Humolyl ester; 13: Ethylhexyl triazinone; 14: Methylenebis-benzotriazolyl tetramethylbutyl phenol; 15: Bis-ethylhexoxyphenol methoxyphenyl triazine

Second method High performance liquid chromatography with UV detector method

8 Scope

This specification specifies a high performance liquid chromatographic method for the determination of sunscreens in cosmetics.

This specification applies to 15 sunscreen agents in sunscreen cosmetics: phenylbenzimidazole sulfonic acid, diphenylketone-4 and diphenylketone-5, p-aminobenzoic acid, diphenylketone-3, isoamyl p-methoxycinnamate, 4-methylbenzylidene camphor, ethylhexyl PABA, butylmethoxydibenzoylmethane, oxytetracycline, ethylhexyl methoxycinnamate, ethylhexyl salicylate, humulanilide, ethylhexyl triazinone, methylenebisbenzotriazolyl tetramethylbutyl phenol, bis-ethylhexoxyphenol methoxyphenyl triazine. The 15 sunscreens were tested for methylenebisbenzotriazolyl tetramethylbutyl phenol and bis-ethylhexoxyphenol methoxyphenyl triazine.

9 Methodology Summary

Various sunscreens in cosmetics can be separated by reversed-phase high-performance liquid chromatography due to their structural differences. They are characterised according to their retention times and quantified by their peak areas. The detection limit, the concentration of detection, the lower limit of quantification and the minimum concentration of quantification of this method are the same as those of the first method.

10 Reagents

10.1 Mixed solution 1: methanol + tetrahydrofuran + water + perchloric acid = 250 + 450 + 300 + 0.2.

10.2 Mixed solution 2: methanol + tetrahydrofuran + water + perchloric acid = 450 + 500 + 50 + 0.5.

11 Instruments

11.1 High performance liquid chromatograph with ternary pump, ultraviolet absorption detector and integrator or chromatographic workstation.

11.2 Ultrasonic cleaners.

11.3 Microsampler (10L), or autosampler.

11. 40.45m filter membrane.

12 Analysis steps

12.1 Chromatographic conditions

Chromatographic column: $_{C18}$ column, 250 mm x 4.6 mm, 5 m.

Mobile phase: Mix 1 (10.1); Mix 2 (10.2); Flow rate: 1.0 mL/min.

UV detection wavelength: 311 nm.

12.2 The samples were pre-treated as in the first method.

12.3 The calibration curve was prepared as in the first method.

12.4 Measurement

12.4.1 The first 12 sunscreens in Table 1 can be separated simultaneously using Mix 1 (10.4) as the mobile phase.

12.4.2 The last 3 sunscreens in Table 1 can be separated simultaneously using Mix 2 (10.5) as the mobile phase.

12.4.3 A microsampler or autosampler was used to measure 10L of the sample solution and injected into a high performance liquid chromatograph. The retention time was determined and the peak area was quantified.

13 Calculation

Same as the first method.

14 Chromatograms

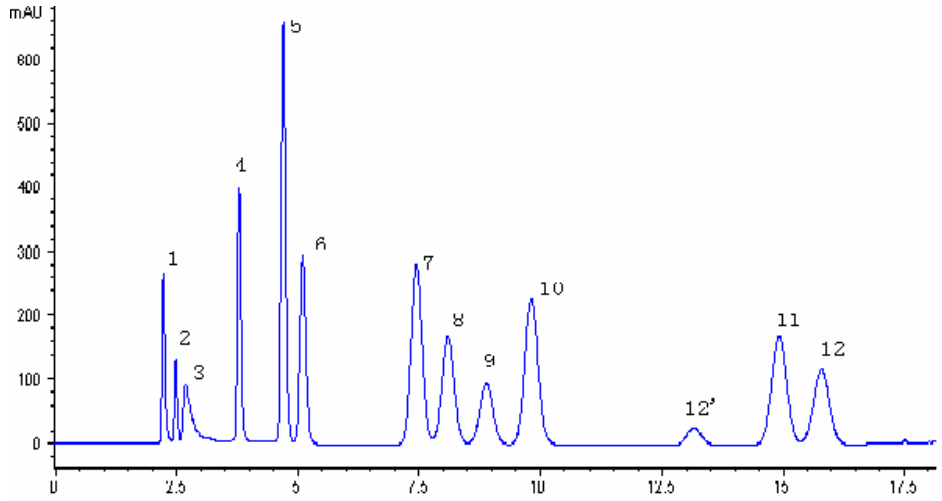


Figure 2 Chromatogram of sunscreen standards with mobile phase as Mix 1 (10.1) 1: phenylbenzimidazole sulfonic acid; 2: diphenylketone-4 and diphenylketone-5; 3: p-aminobenzoic acid; 4: diphenylketone-3; 5: isoamyl p-methoxycinnamate; 6: 4-methylbenzylidene camphor; 7: PABA ethylhexyl ester; 8: butyl methoxydiphenylmethane; 9: oxycodylic acid; 10: ethylhexyl methoxycinnamate; 12': isomer of peak 12; 11: ethylhexyl salicylate; 12: humulanate 10: Ethylhexyl methoxycinnamate; 12': isomer of peak 12; 11: Ethylhexyl salicylate; 12: Humulanilide

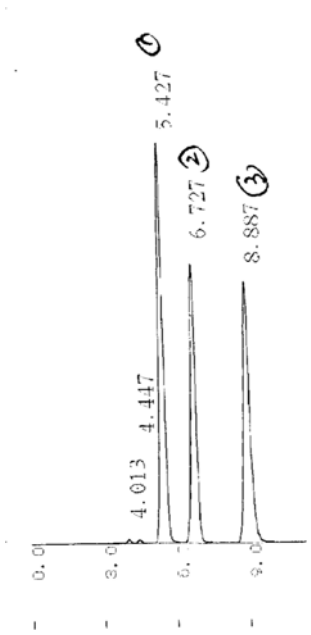


Fig. 3 Chromatogram of sunscreen standards with mobile phase as Mix 2 (10.2) 1: ethylhexyltriazinone; 2: methylenebis-benzotriazolyltetramethylbutyl phenol; 3: bis-ethylhexoxyphenol methoxyphenyltriazine

XIX. Preservatives

Preservatives

1 Scope

This specification provides for the determination of 12 preservatives, including 2-bromo-2-nitropropane-1,3-diol, in cosmetics by high performance liquid chromatography (HPLC).

Law.

This specification applies to the examination of 12 preservatives, including 2-bromo-2-nitropropane-1,3-diol, in cosmetics.

2 Methodology Summary

The 12 preservatives including 2-bromo-2-nitropropane-1,3-diol in cosmetics were extracted with methanol and analysed by high performance liquid chromatography (HPLC), characterised by retention time and UV absorption spectra, and quantified by peak height or peak area. The limits of detection (LOD) and lower limits of quantification (LOQ) for each preservative, as well as the concentration of detection and the minimum concentration of quantification when 1g of sample was taken, are shown in Table 1.

Table 1 Limit of detection, lower limit of quantification and concentration of detection, minimum quantitative concentration for each preservative

Name of preservative	Methyl chloride 2-bromo-2-nitro Methyl iso Clonidine	Isothiazole -1,3-Diol	Propane based Ketones	Thiazoline Ethanol	Benzyl alcohol methyl ester	Phenoxy 4-hydroxy Ethyl ester	Benzoic acid 4-Hydroxy Isopropyl ester	Benzoic acid 4-Hydroxy Propyl ester	Benzoic acid 4-Hydroxy Isobutyl ester	Benzoic acid 4-Hydroxy Butyl ester
Limit of detection (g)	0.002	0.15	0.002 0.1	0.1	0.05	0.005	0.005	0.005	0.015	0.015
Lower limit of quantification (g)		0.5	0.007	0.34	0.17	0.017	0.017	0.017	0.05	0.05
			(g/g)							
			(g/g)							

3 Reagents

3.1 Methanol, chromatographically pure.

3.2 Sodium dihydrogen phosphate, superiorly pure.

3.3 Acetonitrile, chromatographically pure.

3.4 Trimethylamine hexadecane chloride, ultrapure.

3.5 Preservative standard solutions: Use methanol (3.1) as the solvent, dissolve the appropriate amount of each preservative standard, transfer it to a 100mL volumetric flask and fix the volume. The standard reserve solution was prepared at the concentrations shown in Table 2, and then the standard reserve solution was used to prepare a mixed standard series.

Table 2 Concentrations of each preservative stock solution and standard series concentrations

Name of standard product	Isothiazole Clonidine	Propane based -1,3-Diol	Thiazoline Benzyl alcohol Ketones	Ethanol	Benzoic acid methyl ester	Benzoic acid Ethyl ester	Benzoic acid Isopropyl ester	Benzoic acid Propyl ester	Benzoic acid Isobutyl ester	Benzoic acid Butyl ester
Stock solution concentration	25.0	25.0	25.0	25.0	10.0	1.0	10.0	1.0	1.0	1.0	2.5	2.5
(g/L)	250	250	0	250	250	100	0	100	10	0	10	25	25

(mg/L)

4 Instruments

4.1 High performance liquid chromatograph with diode array detector.

4.2 Ultrasonic cleaners.

4.3 Water bath.

4.4 pH meter.

4. 50.45m filter membrane.

5 Analysis steps

5.1 Sample pre-treatment

Weigh approximately 1.00g of the sample accurately in a stoppered cuvette and, if necessary, remove volatile organic solvents such as ethanol in a water bath. Add methanol (3.1) to 10 mL, shake, extract with ultrasound for 15 min and centrifuge. The sample was filtered through a 0.45m membrane and the filtrate was used as the sample solution to be measured.

5.2 Chromatographic reference conditions

Chromatographic column: C_{18} column, 250 mm x 4.6 mm, 10 m.

Mobile phase: 0.05 mol/L sodium dihydrogen phosphate + methanol + acetonitrile = 50 + 35 + 15 with the addition of hexadecyltrimethylamine chloride to a final concentration of 0.002 mol/L and pH adjusted to 3.5 with phosphoric acid.

Flow rate: 1.5 mL/min; column temperature: room temperature.

Detector: Diode array detector, methylchloroisothiazolinone and methylisothiazolinone at 280nm, other components at 254nm.

5.3 Preparation of calibration curves

A standard series of preservative (3.5) 5L was injected into a high performance liquid chromatograph and a calibration curve was plotted for each preservative peak area - concentration.

5.4 Sample determination

A sample solution (5.1) of 5L was injected into a high performance liquid chromatograph and characterised based on the retention time of the peaks and the UV spectrogram. The peak areas were recorded and the corresponding preservative concentrations were obtained from the calibration curve.

6 Calculation

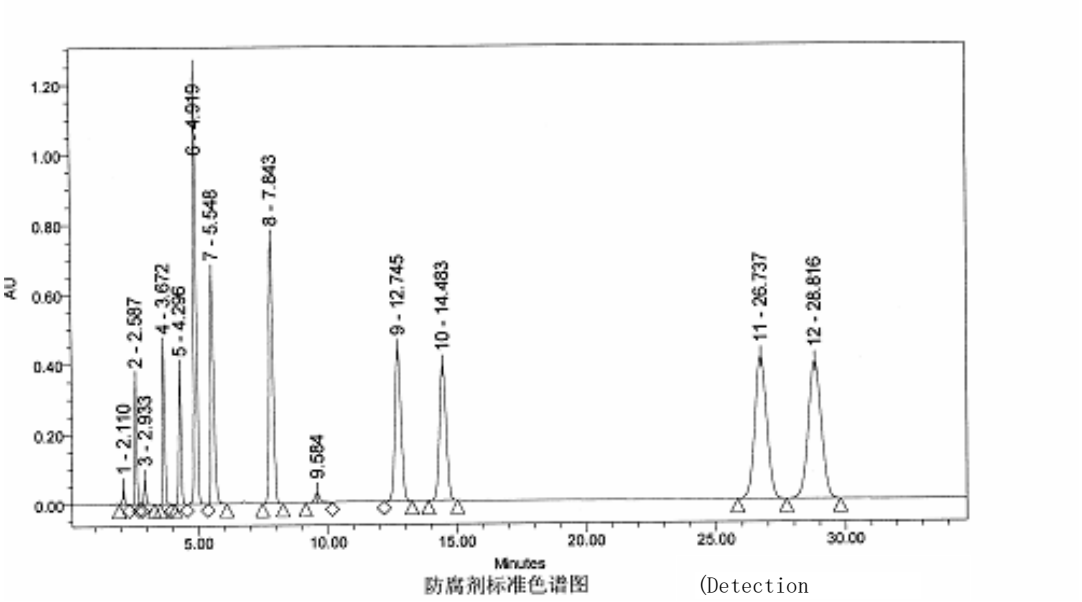
$$(\text{Preservative}) = \frac{\times V}{} \text{ m}$$

where: (preservative) - mass fraction of preservative in the sample, g/g.

- the mass concentration of the preservative in the test solution from the curve, mg/L.

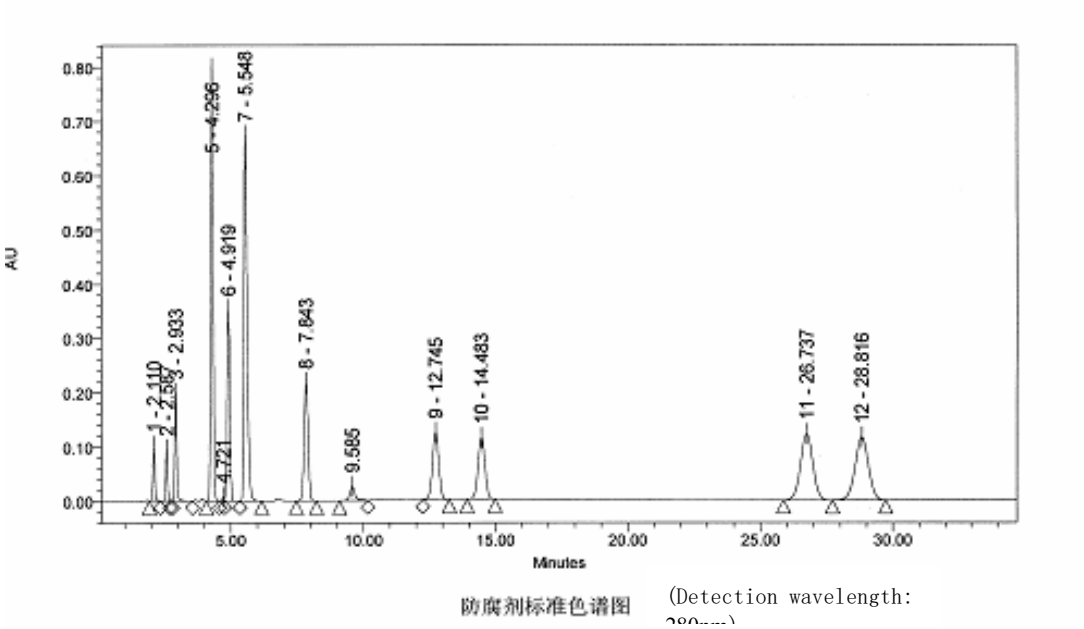
V - volume of sample volume, mL; m - volume of sample taken, g.

7 Chromatograms



防腐剂标准色谱图 (Detection

1: Methylchloroisothiazolinone (2.110); 2: 2-bromo-2-nitropropane-1,3-diol (2.587); 3: Methylisothiazolinone (2.933) 4: benzyl alcohol (3.672); 5: phenoxyethanol (4.296); 6: methyl 4-hydroxybenzoate (4.919); 7: benzoic acid (5.548); 8. Ethyl 4-hydroxybenzoate (7.843); 9: Isopropyl 4-hydroxybenzoate (12.745); 10: Propyl 4-hydroxybenzoate (14.483); 11. Isobutyl 4-hydroxybenzoate (26.737); 12:Butyl 4-hydroxybenzoate (28.816)



防腐剂标准色谱图 (Detection wavelength:

1: Methylchloroisothiazolinone (2.110); 2: 2-bromo-2-nitropropane-1,3-diol (2.587); 3: Methylisothiazolinone (2.933); 4: Benzyl alcohol (3.672); 5: Phenoxyethanol (4.296); 6: Methyl 4-hydroxybenzoate (4.919); 7: Benzoic acid (5.548); 8: 4-Hydroxybenzoic acid ethyl ester (7.843); 9: isopropyl 4-hydroxybenzoate (12.745); 10: propyl 4-hydroxybenzoate (14.483); 11: isobutyl 4-hydroxybenzoate (26.737); 12: butyl 4-hydroxybenzoate (28.816)

XX. Dyes in oxidative hair dyes

Oxidative Hair Dyes

1 Scope

This specification specifies a high performance liquid chromatographic method for the determination of oxidative dyes in hair dyes.

This specification applies to the determination of the content of 8 dyestuff components, including p-phenylenediamine, in hair-dyeing cosmetics.

2 Methodology Summary

Eight dyestuffs, including p-phenylenediamine, were extracted from cosmetics with 95% ethanol and water (1 + 1) and analysed by high performance liquid chromatography (HPLC), characterised by retention time and UV absorption spectra, and quantified by peak height or peak area. The limits of detection (LOD) and lower limits of quantification (LOQ) for each of the dyestuff components, as well as the concentrations at which 0.5 g of the sample was taken and the lowest quantitative concentration are shown in Table 1 below.

Table 1 Limit of detection, lower limit of quantification and concentration of detection and minimum quantitative concentration for each dye component

Dye components	p-Phenylenediamine	Hydroquinone	Phenol	m-aminoamino o-Phenylenediamine	p-Phenol	Toluene 2,5-Diamines	m-benzene Diphenol	p-Methylamine Phenol-based
Detection limit, g	0.08	0.015	0.02	0.03	0.025	0.05	0.025	0.05
Lower limit of quantification, g	0.27	0.05	0.067	0.10	0.083	0.17	0.083	0.17
Detected concentration, g/g	800	150	200	300	250	500	250	500
Minimum quantitative concentration, g/g	2700	500	670	1000	830	1700	830	1700

3 Reagents

3.1 Ethanol [(CH3CH2OH)=95 %], ultrapure.

3.2 Ethanol (1+1): take equal parts of ethanol (3.1) and mix with water.

3.3 Triethanolamine.

3.4 Phosphoric acid [$_{20}(H_4PO_3)$ = 1.83 g/mL], ultrapure.

3.5 Acetonitrile, chromatographically pure.

3.6 Sodium sulphite.

3.7 Dye component standard solution [(dye component) = 5g/L]: weigh approximately 0.5g of each of the 8 dye components including p-phenylenediamine, add 0.1g of sodium sulphite (3.6) (or sodium sulphite solution equivalent to 0.1g of sodium sulphite), dissolve in 95% ethanol (3.1) and set in 100mL (If toluene 2,5-diamine sulphate and p-methylaminophenol sulphate are used as standards, they should be dissolved in water).

4 Instruments

4.1 High performance liquid chromatograph: with isovolume pump and diode array detector.

4.2 Ultrasonic cleaners.

4.3 pH meter.

4.4 Stoppered colorimetric tube, 25mL.

4. 50.45m filter membrane.

5 Analysis steps

5.1 Chromatographic reference conditions

Chromatographic column: $_{C18}$ column, 250 x 4.6 mm, 10 m.

Mobile phase: add 10mL of triethanolamine to 980mL of water, add phosphoric acid to make the solution pH 7.7 and add water to 1 L. Mix 950mL of this solution with 50mL of acetonitrile (3.5) to form a phosphate buffer solution containing 5% acetonitrile.

Flow rate: 2.0 mL/min; column temperature: 20 °C.

Detector: Diode array detector, wavelength 280nm.

5.2 Sample pre-treatment

Place 0.5g of sample in a 25mL stoppered cuvette with 1.0mL of 1% sodium sulphite solution and add ethanol (1+1).

(3.2) to 25 mL, extracted by ultrasonication for 15 min, centrifuged, filtered through a 0.45 m membrane and the filtrate was used as the sample solution to be tested.

5.3 Preparation of calibration curves

The standard solutions (3.7) 1.00mL, 2.50mL and 5.00mL of each component should be dispensed into 3 100mL volumetric flasks and diluted to the scale with 95% ethanol (3.1) to make a mixture of 50, 125 and 250mg/L of each dye component. The standard working solutions should be prepared before use. The working solutions should be prepared before use.

5.4 Measurement

Sample solution (5.2) 5L was injected into a high performance liquid chromatograph and analysed. It was characterised according to its retention time and UV absorption spectrogram and the peak area was quantified.

6 Calculation

$$\text{(dye component)} = \frac{\times V\ m}{}$$

where: (dye fraction) - mass fraction of dye fraction in the sample, g/g.

- the mass concentration of a dye component in the test solution from the curve, mg/L.

V - volume of sample volume, mL; m - volume of sample taken, g.

7 Chromatograms

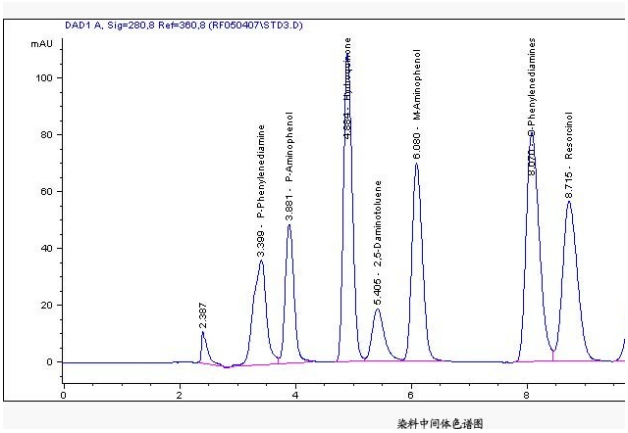


Figure 1 Liquid chromatogram of dye intermediates

1: p-phenylenediamine (3.399min); 2: p-aminophenol (3.881min); 3: hydroquinone (4.884min); 4: toluene 2,5-diamine (6.080min).

5: m-aminophenol (5.405min); 6: o-phenylenediamine (8.070min); 7: resorcinol (8.715min); 8: p-methylaminophenol (9.848min)

XXI. Azadirachtin

Chlormethine

1 Scope

This specification specifies a gas chromatographic method for the determination of nitrogen mustard in cosmetics. This specification applies to the determination of nitrogen mustard in hair care cosmetics.

2 Methodology Summary

Nitrogen mustard in cosmetics was extracted with trichloromethane under alkaline conditions and determined by gas chromatography with a hydrogen flame ionisation detector. The method is characterised by retention time and quantified by peak height or peak area. The method has a detection limit of 0.3 ng and a lower limit of quantification of 1.0 ng for nitrogen mustard; if 5 g of sample is taken, the detection concentration is 0.3 g/g and the lowest quantification concentration is 1 g/g.

3 Reagents

3.1 High purity nitrogen (99.999%)

3.2 High purity hydrogen (99.999%)

3.3 Oil-free compressed air, purified by a purification tube with 5Å molecular sieve.

3.4 Trichloromethane: Redistillation.

3.5 Anhydrous sodium sulphate.

3.6 Hydrochloric acid solution (1 mol/L): take 8.3 mL of concentrated hydrochloric acid ($_{20}$ = 1.19 g/mL) and add water to 100 mL.

3.7 Sodium hydroxide solution (2 mol/L): weigh 8 g of sodium hydroxide, dissolve in water, fix the volume to 100 mL and mix well.

3.8 Sodium carbonate.

3.9 Nitrogen Mustard Standard Reserve Solution [(CH3N(CH2Cl)$_2$)=1g/L]: weigh 0.1234g of Nitrogen Mustard Hydrochloride [CH3N(CH2Cl)2HCl] in water, fix to 100mL and store in a glass bottle.

3.10 Standard use solution of nitrogen mustard [(CH3N(CH2Cl)$_2$)=10mg/L]: aspirate standard stock solution of nitrogen mustard (3.9)

1.00mL in a 100mL volumetric flask, set to scale with water.

4 Instruments

4.1 Gas Chromatograph: Gas chromatograph with hydrogen flame ionisation detector.

4.2 Injection device: Micro syringe, glass, 10L.

4.3 Chromatographic column: DB-225 capillary column (0.25mm 30m).

5 Analysis steps

5.1 Sample pre-treatment

Place approximately 5g of sample in a 25mL separatory funnel, add 5mL of water and mix well. Adjust pH to below 2 with hydrochloric acid solution (3.6), add 5mL of trichloromethane (3.4), shake for 30s and leave to stratify (centrifuge if necessary), discard the organic phase. Adjust the aqueous phase to neutral with sodium hydroxide (3.7), add about 50 mg of sodium carbonate (3.8), extract with 5 mL of trichloromethane (3.4), shake for 30 s and allow to stratify (centrifuge if necessary), place the organic phase in a graduated tube, add trichloromethane to 5 mL, dry with anhydrous sodium sulphate (3.5) and leave to determine. The standard solution for nitrogen mustard should be treated in the same way as above before determination.

5.2 Chromatographic reference conditions

Temperature: Inlet temperature 170C, detection port temperature 200C, column temperature, 50C (1min), 8C/min to 160C

(10min).

Gas flow rate: high purity nitrogen 60mL/min, high purity hydrogen 50mL/min, compressed air 500mL/min; split ratio: 1:50.

5.3 Measurement

1L of the above sample pre-treatment solution should be taken into the sample for determination. For quantification by the single point external standard method, the volume of the treated solution for use with the nitrogen mustard standard should be the same as the sample solution and its peak area should be within the same order of magnitude as the sample peak area.

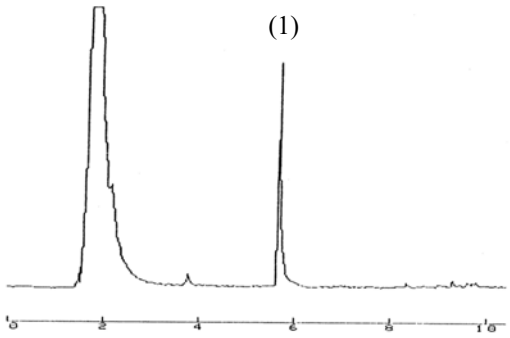
6 Calculation

(Azadirachtin) = $\frac{A_1 V}{A_0 m}$

where: (nitrogen mustard) - mass concentration of nitrogen mustard in the sample, g/g.

A_1 - peak area of nitrogen mustard in the test solution; A_0 - peak area of nitrogen mustard in the test solution V - volume of sample volume, mL; m - sample volume, g.

7 Chromatograms



Standard chromatogram of Azadirachta indica

(1) Nitrogen mustard

XXII. Zebularine

Cantharidin

1 Scope

This specification specifies a gas chromatographic method for the determination of zebranin in cosmetics. This specification applies to the determination of zebularin content in hair care cosmetics.

2 Methodology Summary

Zebularine in cosmetics was extracted with trichloromethane and determined by gas chromatography with a hydrogen flame ionisation detector. It was characterised by retention time and quantified by peak height or peak area. The method has a detection limit of 0.6 ng and a lower limit of quantification of 2.0 ng for zebularin; if 5 g of sample is taken, the detection limit is 0.6 g/g and the lowest quantitative concentration is 2 g/g.

3 Reagents

3.1 High purity nitrogen (99.999%)

3.2 High purity hydrogen (99.999%)

3.3 Oil-free compressed air, purified by a purification tube with 5Å molecular sieve.

3.4 Trichloromethane, redistilled.

3.5 Anhydrous sodium sulphate.

3.6 Zebularin standard stock solution [($C_{10}H_{12}O_4$)=1g/L]: weigh 0.1000g of zebularin, dissolve in trichloromethane, fix the volume to 100mL and store in a glass bottle.

3.7 Zebularin Standard Solution [($C_{10}H_{12}O_4$)=10mg/L]: Pipette 1.00mL of Zebularin Standard Reserve Solution (3.6) into a 100mL volumetric flask and set to scale with trichloromethane.

4 Instruments

4.1 Gas Chromatograph: Gas chromatograph with hydrogen flame ionisation detector.

4.2 Injection device: Micro syringe, glass, 10L.

4.3 Chromatographic column: DB-5 capillary column (0.25mm 30m).

5 Analysis steps

5.1 Sample pre-treatment

Weigh 5g of the sample into a 25mL separatory funnel, add 5mL of water and mix well. Add 5mL of trichloromethane, shake for 30s and leave to stratify (centrifuge if necessary), place the organic phase in a graduated tube, top up with trichloromethane to 5mL, add the appropriate amount of anhydrous sodium

sulphate (3.5) and dry, pending determination.

5.2 Chromatographic reference conditions

Temperature: Inlet temperature 230C, detection port temperature 250C, column temperature, 60C (1min), 10C/min to 230C

(10min).

Gas flow rate: high purity nitrogen 60mL/min, high purity hydrogen 50mL/min, compressed air 500mL/min; split ratio: 1:50.

5.3 Measurement

A sample of 1L of the above sample preparation solution should be taken for determination. For quantification by the single point external standard method, the volume of the standard solution (3.7) should be the same as the sample solution and the peak area should be within the same order of magnitude as the sample peak area.

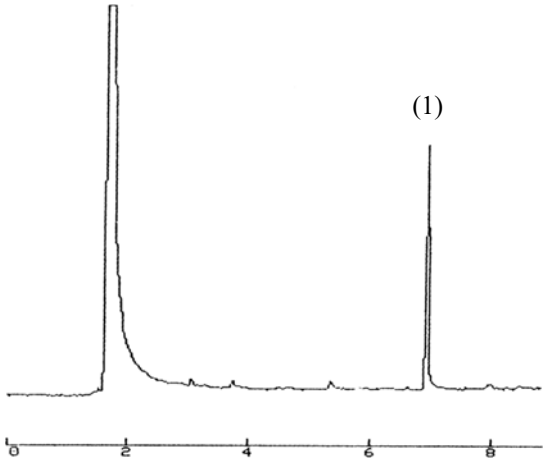
6 Calculation

$$(\text{zebularine}) = \frac{VA_1}{mA_0}$$

where: (zebracho) - concentration concentration of zebracho in the sample, g/g.

A_0 - the peak area of zebularine in the standard solution; V - the volume of the sample volume, mL; m - the sample volume, g. V - volume of sample volume, mL; m - sample volume, g.

7 Chromatograms



Standard chromatogram of zebularin

(1) Zebularine

XXIII.-Hydroxy acids

-Hydroxy Acid

1 Scope

This specification specifies high performance liquid chromatography, ion chromatography and gas chromatography methods for the determination of -hydroxy acids in shampoo, hair care and skin care cosmetics.

This specification applies to the determination of -hydroxy acid content in shampoo, hair care and skin care cosmetics.

First method High Performance Liquid Chromatography

2 Methodology Summary

The five -hydroxy acid fractions in cosmetics were extracted with water and analysed by high performance liquid chromatography (HPLC), characterised by retention time and quantified by peak area. The limits of detection and lower limits of quantification of the various -hydroxy acids in this method are shown in Table 1, as well as the detection and minimum quantification concentrations for 1g of sample.

Table 1 Limit of detection, lower limit of quantification and concentration of detection, minimum quantitative concentration of each -hydroxy acid

-Hydroxy acid component	Tartaric acid	Glycolic acid	Malic acid	Lactic acid	Citric acid
Detection limit (g)	0.1	0.35	0.2	0.4	0.25
Lower limit of quantification (g)	0.33	1.17	0.67	1.33	0.83
Detected concentration (g/g)	200	700	400	800	500
Minimum quantitative concentration (g/g)	660	2340	1340	2660	1660

3 Reagents

3.1 Ammonium dihydrogen phosphate.

3.2 Phosphoric acid, superiorly pure.

3.3 Standard solutions of hydroxy acids: Weigh the appropriate amount of various -hydroxy acid standards, dissolve and transfer to a 100mL volumetric flask and fix the volume. The standard reserve solution was prepared at the concentrations shown in Table 2, and then the standard reserve solution was used to prepare a mixed standard series.

Table 2 Concentrations of reserve solutions and standard series of concentrations for each -hydroxy acid

-Hydroxy acid component	Tartaric acid	Glycolic acid	Malic acid	Lactic acid	Citric acid
Concentration of stock solution, g/L	5.0	8.0	20.0	40.0	20.0
Standard series concentration, mg/L	100	160	400	800	400
	250	400	1000	2000	1000
	500	800	2000	4000	2000

4 Instruments

4.1 High performance liquid chromatograph with diode array detector.

4.2 Ultrasonic cleaners.

4.3 Water bath.

4.4 High-speed centrifuge.

4.5 pH meter.

5 Analysis steps

5.1 Sample pre-treatment

Weigh 1g of the sample into a 10mL stoppered cuvette, remove the volatile organic solvent in a water bath, add water to 10mL and extract the sample by ultrasonication for 20min, centrifuge the sample at 10000rpm for 15min and pass the supernatant through a 0.45m filter membrane.

5.2 Chromatographic reference conditions

Chromatographic column: C_8 column, 250 mm 4.6 mm, 10 m.

Mobile phase: 0.1 mol/L ammonium dihydrogen phosphate solution, pH adjusted to 2.45 with phosphoric acid; flow rate: 0.8 mL/min.

Column temperature: room temperature.

Detector: Diode array detector with a detection wavelength of 214 nm.

5.3 Preparation of calibration curves

The peak areas of the six hydroxy acid components were recorded and the calibration curves were plotted for each hydroxy acid component. The peak areas of each -hydroxy acid component were recorded and the calibration curve was plotted.

5.4 Sample determination

5L of the solution to be measured (5.1) was injected into a high performance liquid chromatograph and characterised on the basis of the retention time of the peaks and the UV spectrogram. The peak area was recorded and the concentration of the corresponding -hydroxy acid component was found from the calibration curve.

6 Calculation

$$(\text{-hydroxy acid}) = \frac{V}{\quad} \; m$$

where: (-hydroxy acid) - mass fraction of -hydroxy acid fraction in the sample, g/g.

- the mass concentration of -hydroxy acid in the test solution from the curve, mg/L.

V - volume of sample volume, mL; m - volume of sample taken, g.

7 Chromatograms

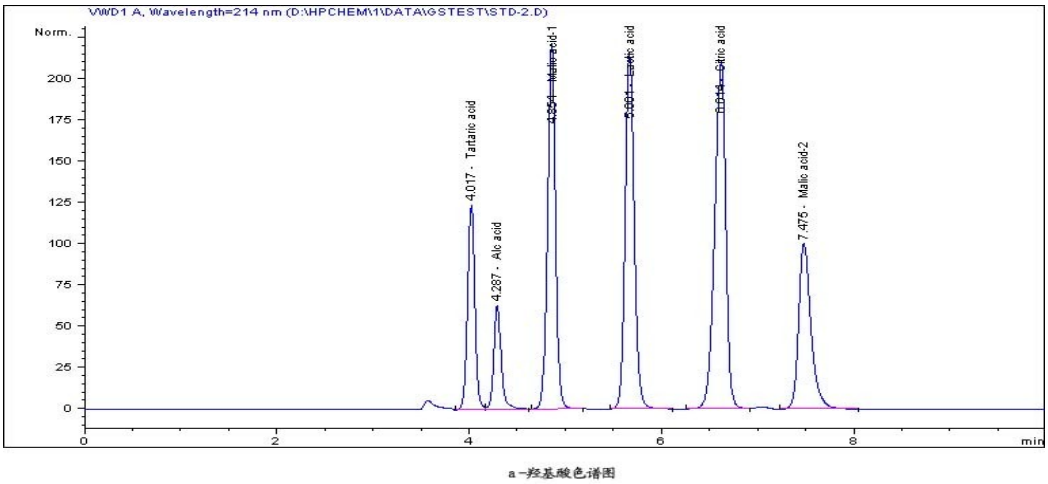


Fig. 1 - Standard liquid chromatogram of hydroxy acids

1: Tartaric acid; 2: Glycolic acid; 3: Malic acid 1; 4: Lactic acid; 5: Citric acid; 6: Malic acid 2

Second method Ion chromatography

8 Methodology Summary

The five -hydroxy acids in cosmetics were extracted with water, separated by ion chromatography and detected by conductivity detector. The limits of detection and lower limits of quantification of the various -hydroxy acids in this method are shown in Table 3, as well as the detection and minimum quantification concentrations for 0.5 g of sample.

Table 3 Limit of detection, lower limit of quantification and concentration of detection, minimum quantitative concentration of each -hydroxy acid

-Hydroxy acid component	Tartaric acid	Citric acid	Malic acid	Glycolic acid	Lactic acid
Detection limit (ng)	0.94	1.1	0.83	0.90	1.7
Lower limit of quantification (ng)	20	8.0	9.0	8.5	10
Detected concentration (g/g)	3.8	4.4	3.3	3.6	6.8
Quantitative concentration (g/g)	80	32	36	34	40

9 Reagents

9.1 Hydrochloric acid, superiorly pure.

9.2 Sodium hydroxide.

9.3 High purity nitrogen.

9.4 Hydroxy acid standard solution: Use water as solvent, weigh the appropriate amount of five -hydroxy acid standards, dissolve and transfer to a 100mL volumetric flask, and fix the volume to the scale. The standard reserve solution was prepared at the concentrations shown in Table 4, and then the standard reserve solution was used to prepare a mixed standard series.

Table 4 Concentrations of reserve solutions and standard series of concentrations for each -hydroxy acid

-Hydroxy acid component	Tartaric acid	Citric acid	Malic acid	Glycolic acid	Lactic acid
Reserve solution concentration, mg/L	1000	1000	1000	1000	2000
	2.00	0.45	0.50	0.60	1.00
	5.00	5.00	4.00	5.00	4.00
Standard series concentration, mg/L	10.0	10.0	10.0	10.0	10.0

	30.0	40.0	40.0	40.0	60.0
	70.0	50.0	80.0	70.0	120

10 Instruments

10.1 Ion chromatograph.

10.2 Vortex oscillator.

10.3 Ultrasonic cleaners.

10.4 High-speed centrifuge.

11 Analysis steps

11.1 Sample pre-treatment

The sample was then centrifuged at 19000 rpm for 10 min. The supernatant was passed through a 0.25 μm filter membrane and used as the sample solution to be measured.

11.2 Chromatographic reference conditions

Chromatographic column: ICE-AS6 (9 x 250 mm), suppressor AMMS-ICE II.

Leaching solution: 0.4 mmol/L hydrochloric acid solution.

Chemically inhibited regeneration solution: 5 mmol/L sodium hydroxide solution; drenching solution flow rate: 1.0 mL/min.

Regeneration fluid flow rate: 1.5 mL/min; nitrogen flow rate (pressure): 5 psi; column temperature: room temperature.

Injection volume: 25 μL.

Detector: Chemically suppressed conductivity detector.

11.3 Preparation of calibration curves

After injection, the retention time and peak area of the peaks were recorded and calculated by the chromatographic workstation, and the calibration curves of peak area-concentration of various α-hydroxy acid components were plotted.

11.4 Sample determination

After the sample solution (11.1) was injected into the ion chromatograph injection tube at 0.5mL~1.0mL, the retention time and peak area of the peaks were recorded and calculated by the chromatography workstation, and the concentration of the corresponding α-hydroxy acid component was calculated from the calibration curve.

12 Calculation

$$(\text{-hydroxy acid}) = \frac{V}{\quad} m$$

where: (-hydroxy acid) - mass fraction of -hydroxy acid fraction in the sample, g/g.

- the mass concentration of -hydroxy acid in the test solution from the curve, mg/L.

V - volume of sample volume, mL; m - volume of sample taken, g.

13 Chromatograms

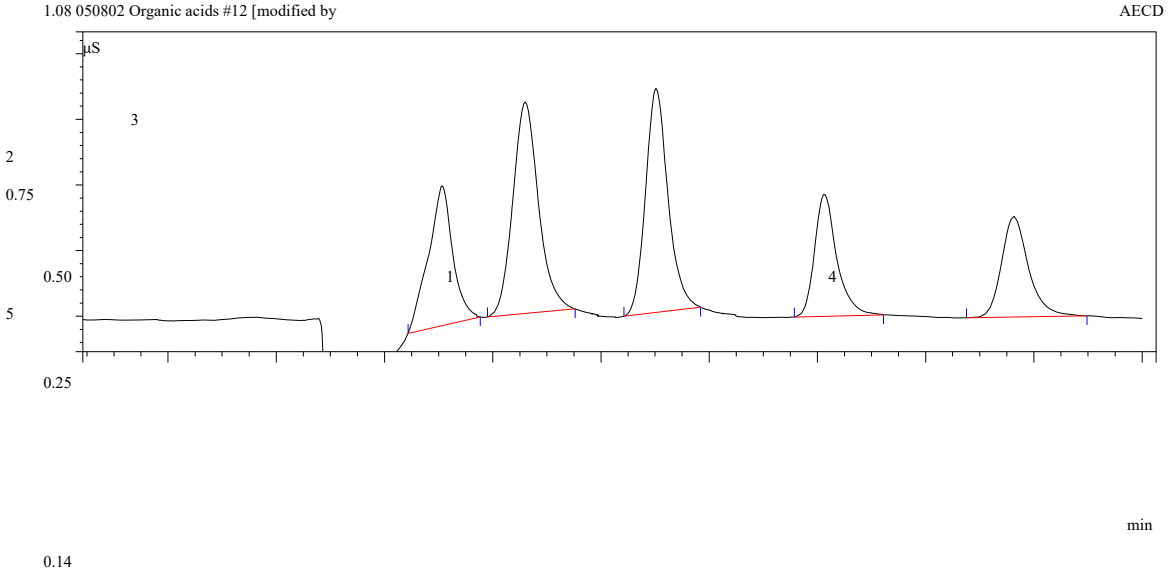


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Fig. 2 Standard ion chromatogram of α-hydroxy acids

1: Tartaric acid 6.0mg/L; 2: Citric acid 5.0mg/L; 3: Malic acid 5.0mg/L; 4: Ethanoic acid 5.0mg/L; 5: Lactic acid 5.0mg/L

Third method Gas Chromatography

14 Methodology Summary

The five α-hydroxy acids in cosmetics were extracted with N,N-dimethylformamide, derivatised with trimethylsilyl trifluoroacetamide and analysed by gas chromatography for the determination of retention time and quantification of peak area or peak height.

15 Reagents

15.1 Trimethylsilyl trifluoroacetamide (BSTFA).

15.2 N,N-Dimethylformamide (DMF).

15.3 α-Hydroxy acid standard solution [ρ(α-hydroxy acid)=10g/L]: weigh 500mg each of lactic acid, glycolic acid, malic acid, tartaric acid and citric acid in a 50mL volumetric flask, dissolve with DMF (15.2) and dilute to the scale.

16 Instruments

16.1 Gas chromatograph with hydrogen flame ionisation detector.

16.2 High-speed ultrasonic cleaner.

16.3 Derivative bottle with cap, 2mL.

16.4 Volumetric flask, 50mL.

16.5 Stoppered colorimetric tube, 10mL.

17 Analysis steps

17.1 Chromatographic reference conditions

Chromatographic column: CP-Sil8CB (30m x 0.32mm, 0.25μm).

Temperature: column temperature, 60 °C (1 min), rising to 310 °C (5 min) at 10 °C/min, inlet and detector temperature 330 °C; gas flow rate: carrier gas (high-purity nitrogen) 50 mL/min, high-purity hydrogen 35 mL/min, air 350 mL/min.

Diversion ratio: 1:50.

17.2 Sample pre-treatment

Weigh 0.1g~0.5g of sample into a 10mL stoppered cuvette, add DMF (15.2), dissolve and volumize to 10mL. extract with ultrasound for 20min, pass the supernatant through a 0.45μm filter membrane, take 50μL of the solution into a 2mL capped derivative bottle, add BSTFA

(15.1) 100 μL, derivatised at 80°C for 20 min, this solution was used as the sample solution to be tested.

17.3 Preparation of calibration curves

The working solutions of 50.0mg/L, 100mg/L, 300mg/L and 1000mg/L were prepared separately. After the same treatment as the sample, 1μL was injected into the gas chromatograph and the peak area or peak height of each chromatographic peak was recorded and the standard curves were plotted.

17.4 Sample determination

A 1 μL sample of the solution to be measured was injected into a gas chromatograph and analysed. Qualify the sample according to its retention time and determine the peak area or peak height

Quantity.

18 Calculation

$$(\text{-hydroxy acid}) = \frac{V}{m}$$

where: (-hydroxy acid) - mass fraction of -hydroxy acid fraction in the sample, g/g.

- the mass concentration of -hydroxy acid in the test solution from the curve, mg/L.

V - volume of sample volume, mL; m - volume of sample taken, g.

19 Chromatograms

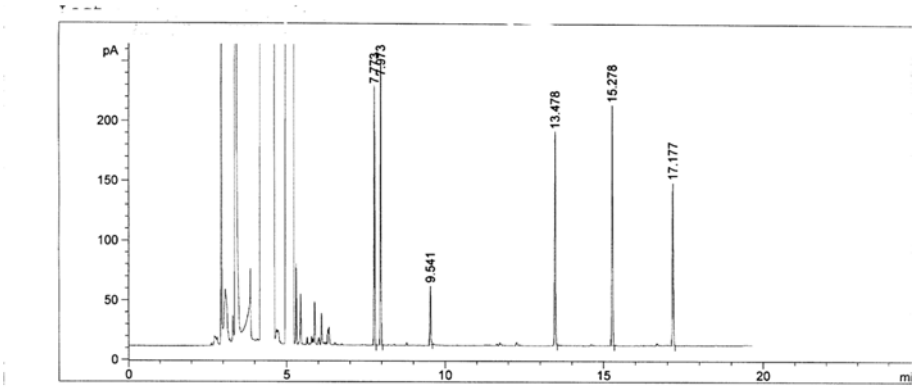


Fig. 3 Standard gas chromatogram of α-hydroxy acids

1: lactic acid (7.773); 2: ethanoic acid (7.937); 3: malic acid (13.478); 4: tartaric acid (15.278); 5: citric acid (17.177)

XXIV. Anti-dandruff agents

Antidangdruff agents

1 Scope

This specification specifies a high performance liquid chromatographic method for the determination of anti-dandruff agents (preservatives) such as salicylic acid, ketoconazole, clomiprazole and pyrrolizone ethanolamine salts in anti-dandruff shampoo cosmetics.

This specification applies to the determination of the content of anti-dandruff agents (preservatives) such as salicylic acid, ketoconazole, clomiprazole and pyrrolizone ethanolamine salt in anti-dandruff shampoo cosmetics.

2 Methodology Summary

Salicylic acid and other anti-dandruff agents in anti-dandruff shampoo cosmetics were extracted with acetonitrile + methanol = 95 + 5 and analysed by high performance liquid chromatography (HPLC) with retention time and UV absorption spectroscopy for characterisation and peak area for quantification. The limits of detection (LOD) and lower limits of quantification (LOQ) for each component of the method are shown in Table 1, as well as the concentration at which 0.5g of the sample was taken and the lowest quantitative concentration.

Table 1 Limit of detection, lower limit of quantification and concentration of detection, minimum quantitative concentration for each component

Anti-dandruff agent components	Salicylic acid	Ketoconazole	Clomiprazole	Piroctone ethanolamine salt
Detection limit (ng)	1.0	1.5	2.0	4.5
Lower limit of quantification (ng)	3.3	5.0	6.7	15.0
Detected concentration (g/g)	20.0	30.0	40.0	90.0
Minimum quantitative concentration (g/g)	66.7	100	133	300

3 Reagents

3.1 Methanol, chromatographically pure.

3.2 Acetonitrile, chromatographically pure.

3.3 Phosphoric acid, superiorly pure.

3.4 Potassium dihydrogen phosphate.

3.5 Disodium ethylenediaminetetraacetate.

3.6 Standard solution of the de-scaling agent: weigh the appropriate amount of each de-scaling agent standard, add 85mL of acetonitrile + methanol = 95 + 5 Note 1 solution, sonicate, transfer to a 100mL volumetric flask after complete dissolution, and fix the volume with acetonitrile + methanol = 95 + 5. The standard stock solution was then diluted with acetonitrile + methanol = 95 + 5 to form a mixed standard series.

Table 2 Concentrations of stock solutions and standard series of concentrations for each debris remover

Anti-dandruff agent components	Salicylic acid	Ketoconazole	Clomiprazole	Piroctone ethanolamine salt
Reserve solution concentration (mg/L)	500	500	1000	1000
Standard series concentration (mg/L)	50	50	100	100
	100	100	200	200
	200	200	400	400
	400	400	800	800
	500	500	1000	1000

4 Instruments

4.1 High performance liquid chromatograph with diode array detector.

4.2 Ultrasonic cleaners.

4.3 pH meter.

4. 40.45m filter membrane.

5 Analysis steps

5.1 Sample pre-treatment

Add acetonitrile + methanol = 95 + 5 Note 1 to the scale, shake and extract with ultrasound for 40 min. Filter through a 0.45m membrane and use the filtrate as the solution to be measured.

5.2 Chromatographic reference conditions

Chromatographic column: C_{18} column, 150 mm 4.6 mm, 5 m.

Mobile phase: acetonitrile + methanol + 0.01 mol/L aqueous potassium dihydrogen phosphate (disodium EDTA added to a final concentration of 0.5 mmol/L and the pH of the aqueous solution adjusted to 4.0 with phosphoric acid) = 50 + 10 + 40.

Flow rate: 1.0 mL/min; column temperature: room temperature.

Detector: Diode array detector with a detection wavelength of 230 nm. for the determination of salicylic acid and pyrrolizone ethanolamine salts with interfering samples it is recommended that the detection wavelength be adjusted to 300 nm.

5.3 Preparation of calibration curves

A standard series (3.6) of 5L of de-scaling agent was injected into a high performance liquid chromatograph and a calibration curve was plotted for each de-scaling agent peak area - concentration.

5.4 Sample determination

A sample solution (5.1) of 5L was injected into a high performance liquid chromatograph and characterised on the basis of peak retention times and UV spectra. The peak area was recorded and the corresponding concentration of the de-flaking agent was obtained from the calibration curve.

6 Calculation

(Anti-dandruff agent) = $\frac{\times V}{}$ m

where: (debris remover) - mass fraction of debris remover in the sample, g/g.

--mass concentration of debris remover in the test solution, mg/L; V - volume of sample volume, mL; m - sample volume taken, m g.

7 Chromatograms

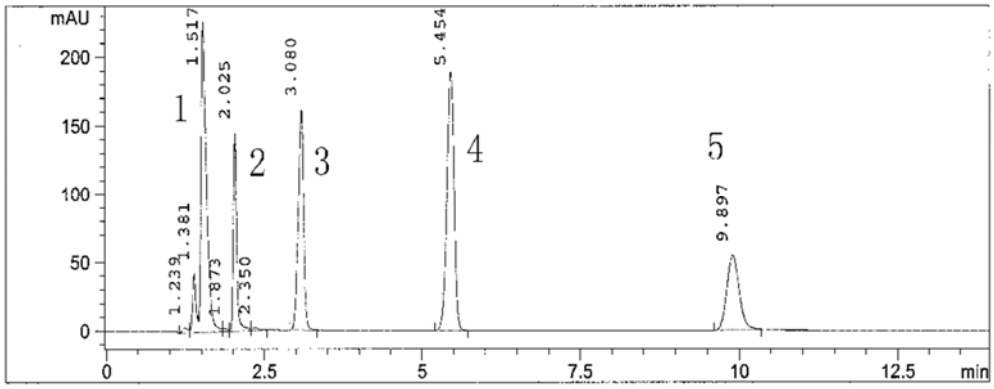


Fig. 1 Standard chromatogram of a crumb remover with a detection wavelength of 230 nm

1: Salicylic acid (1.517); 2: --; 3: Ketoconazole (3.080); 4: Clomiprazole (5.454); 5: Piroctone ethanolamine salt (9.897)

xxv. antibiotics, metronidazole

Antibiotics and Metronidazole

1 Scope

This specification specifies a high performance liquid chromatographic (HPLC) method for the determination of six antibiotics and metronidazole in acne and mite removal cosmetics: methomycin hydrochloride, oxytetracycline dihydrate, tetracycline hydrochloride, chlortetracycline hydrochloride, doxycycline hydrochloride and chloramphenicol.

This specification applies to the determination of the content of memantine hydrochloride, hygromycin dihydrate, tetracycline hydrochloride, chlortetracycline hydrochloride, doxycycline hydrochloride, chloramphenicol and metronidazole in acne and mite removal cosmetics.

2 Methodology Summary

Methomycin hydrochloride, oxytetracycline dihydrate, tetracycline hydrochloride, chlortetracycline hydrochloride, doxycycline hydrochloride, chloramphenicol and metronidazole have UV absorption at 268 nm and can be separated by reversed-phase high performance liquid chromatography (RP-HPLC) and quantified by retention time and UV spectra. The limits of detection for each component and the concentrations at 1g of sample are shown in Table 1.

Table 1 Detection limits and concentrations for each component

Meprobamate hydrochloride		Metronidazole	Secondary water and soil	Hydrochloric acid IV	Gold hydrochloride	Hydrochloric acid poly	Chloromycetes
Name of substance	Manomycin	Azole	Mycin	Cyclin	Mycin	Ciclosporin	Vegetables
Detection limit (ng)	50	50	1	1	1	1	1
Lower limit of quantification (ng)	150	150	3.3	3.3	3.3	3.3	3.3
Detected concentration (g/g)	50	50	1	1	1	1	1
Minimum quantitative concentration (g/g)	150	150	3.3	3.3	3.3	3.3	3.3

3 Reagents

3.1 Methanol, chromatographically pure.

3.2 Acetonitrile, chromatographically pure.

3.3 Oxalic acid, analytical pure.

3.4 Hydrochloric acid (0.1 mol/L): Take 8.3 mL of concentrated hydrochloric acid ($_{20}$ = 1.19 g/mL) in excellent purity and add water to 1 L.

3.5 Mixed standard stock solution: Weigh 0.1000g of each of memantine hydrochloride, oxytetracycline dihydrate, tetracycline hydrochloride, chlortetracycline hydrochloride, doxycycline hydrochloride, chloramphenicol and metronidazole, dissolve with a little methanol (3.1) and hydrochloric acid (3.4), transfer into a 100mL volumetric flask, fix the volume of methanol to the scale and shake well to make a mixed standard solution with a mass concentration of 1.00g/L of each component. The mixture of standard solutions was prepared at a concentration of 1.00g/L.

4 Instruments

4.1 High performance liquid chromatograph with diode array detector, chromatography processor or chromatography workstation.

4.2 Microsampler or autosampling unit.

4.3 Ultrasonic cleaners.

5 Analysis steps

5.1 Sample pre-treatment

The sample was weighed 1 g in a 10 mL stoppered cuvette, added with methanol (3.1) + hydrochloric acid (3.2) = 1+1 to the scale, shaken and extracted by ultrasonication for 20 min-30 min. The filtrate was filtered through a 0.45 m membrane and used as the solution to be tested.

5.2 Chromatographic reference conditions

Chromatographic column: C_{18} column, 250 mm × 4.6 mm I.D., 5 m; detector: diode array detector, detection wavelength 268 nm.

Mobile phase: 0.01 mol/L oxalic acid solution (pH of aqueous solution adjusted to 2.0 by phosphoric acid) + methanol + acetonitrile = 67 + 11 + 22 (HPLC)

(filtered through a 0.45m membrane and degassed under vacuum before analysis); flow rate: 0.8mL/min.

Column temperature: room temperature.

5.3 Preparation of calibration curves

The standard solution (3.5) was accurately pipetted into a 10mL stoppered cuvette, diluted to the scale with the mobile phase and shaken well. Filter through a 0.45m membrane and set aside. 10L of each solution was analyzed under the set chromatographic conditions. The calibration curve was plotted against the mass concentration and peak area of the standard series.

5.4 Sample determination

A 10L sample solution (5.1) is analysed under the set chromatographic conditions. If the sample is too high, it should be diluted with the mobile phase and determined. The mass concentration of the corresponding component is found from the calibration curve based on the peak area.

6 Calculation

$$(\text{antibiotics, metronidazole}) = \frac{\text{×V}}{\text{m}}$$

where: (antibiotic, metronidazole) - mass fraction of antibiotic, metronidazole in cosmetics, g/g.

--mass concentration of antibiotic, metronidazole in the test solution, mg/L.

V - volume of sample volume, mL; m - volume of sample taken, g.

7 Chromatograms

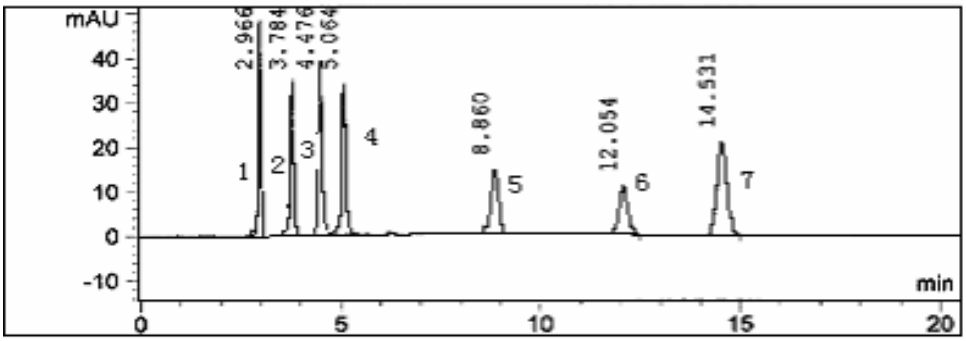


Fig. 1 Colour spectrum of antibiotics, metronidazole Fig. 1: memantine hydrochloride; 2: metronidazole; 3: hygromycin dihydrate; 4: tetracycline hydrochloride.

5: Chlortetracycline Hydrochloride; 6: Doxycycline Hydrochloride; 7: Chloramphenicol

XXVI. Vitamin $_{D2}$, vitamin $_{D3}$ vitamin $_{D2}$, vitamin $_{D3}$

1 Scope

This specification specifies a high performance liquid chromatographic method for the determination of vitamin $_{D2}$ and vitamin $_{D3}$ in cosmetics. This specification applies to the determination of the content of vitamin $_{D2}$ and vitamin $_{D3}$ in cosmetics.

2 Methodology Summary

Vitamin $_{D2}$ and vitamin $_{D3}$ have UV absorption at 265 nm and can be separated by reversed-phase high performance liquid chromatography and quantified by retention time and UV spectrogram. The limits of detection (LOD), lower limits of quantification (LOQ) and the lowest quantitative concentrations of vitamin $_{D2}$ and vitamin $_{D3}$ at 0.5 g of sample are shown in Table 1.

Table 1 Limit of detection and concentration of each vitamin

Group name	Vitamin $_{D2}$	Vitamin $_{D3}$	
Limit of detection (ng)		0.	580.32
Lower limit of quantification (ng)		21	
Detected concentration (g/g)	2.	61.3	
Minimum quantitative concentration (g/g)		84	

3 Reagents

3.1 Methanol, chromatographically pure.

3.2 Acetonitrile, chromatographically pure.

3.3 Mixed standard stock solution: accurately weigh 0.1000g each of vitamin $_{D2}$ and vitamin $_{D3}$ standards and transfer to 100mL

Dissolve in a volumetric flask with methanol (3.1) and set to scale, shake well and use as a standard stock solution.

4 Instruments

4.1 High performance liquid chromatograph with diode array detector, chromatography processor or chromatography workstation.

4.2 Microsampler or autosampling unit.

4.3 Ultrasonic cleaners.

4.4 UV spectrophotometer.

5 Analysis steps

5.1 Sample pre-treatment

Weigh approximately 0.5 g of the sample into a 10 mL stoppered cuvette, bring to scale with the mobile phase, shake well and extract with ultrasound.

The filtrate was filtered through a 0.45m membrane and used as the sample solution for the test.

5.2 Chromatographic reference conditions

Chromatographic column: $_{C18}$ column, 250 mm x 4.6 mm, 5 m.

Detector: Diode array detector, detection wavelength 265 nm.

Mobile phase: methanol + acetonitrile = 90 + 10 (filtered through a 0.45m membrane and degassed under vacuum prior to HPLC analysis); flow rate: 1.0mL/min.

Column temperature: room temperature.

5.3 Preparation of calibration curves

5.3.1 Purity correction: As vitamin $_{D2}$ and vitamin $_{D3\ are}$ unstable to light, a purity correction by UV spectrophotometry is required prior to the preparation of standard solutions as follows: vitamin $_{D2}$ and vitamin $_{D3}$ have maximum UV absorption at 263 nm, while ethanol has no absorption at this wavelength. In 95% ethanol, the absorbance coefficient of $_{1\%\ for}$ a 1% mass fraction of vitamin $_{D2\ solution}$ is 460 and for a 1% mass fraction of vitamin D solution is 485. The absorbance ^{1cm3} coefficient is equal to the absorbance/concentration, so the absorbance can be measured to obtain the exact concentration of the prepared solution. The absorbance coefficient corresponds to the absorbance/concentration and therefore the exact concentration of the prepared solution can be determined by measuring the absorbance and thus correcting for purity.

100A

$$P = \frac{}{C1\% \quad L}$$

1cm

Where: P - purity of vitamin $_{D2}$ and $_{D3}$, %; A - absorbance of vitamin $_{D2}$ and $_{D3;}$ C - mass fraction of vitamin $_{D2}$ and $_{D3}$ L - optical diameter of the cuvette, cm.

$^{1\%}$ --The absorbance coefficient of vitamin D, D $_{1cm25}$

5.3.2 The standard solutions were prepared by accurately aspirating 1.00 mL each of the standard stock solutions (3.3) for vitamins $_{D2}$ and $_{D3}$ and diluting to 10.0 mL with methanol (3.1), this mixed standard solution contained approximately 100.0 mg/L of vitamins $_{D2}$ and $_{D3.\ The}$ solution was stable at room temperature for more than 2 weeks provided it was well protected from light. The mixed standard solution was diluted to a series of 50.0 mg/L, 20.0 mg/L, 10.0 mg/L, 2.00 mg/L, 0.50 mg/L mass concentration solutions using mobile phase.

5.3.3 Calibration curve: 5μL of the standard series were taken for HPLC analysis under the set chromatographic conditions. The standard curve was plotted using the mass concentration of the standard series as the horizontal coordinate and the peak area as the vertical coordinate.

5.4 Sample determination

5μL of the solution was analyzed by HPLC under set chromatographic conditions. The mass concentration of each component in the solution to be measured is obtained from the calibration curve based on the peak area of the solution to be measured.

6 Calculation

$$(\text{Vitamin}_{D2,\ D3}) = \frac{\times V\ M}{}$$

where: (vitamins $_{D2,\ D3}$) - mass fraction of vitamins $_{D2}$ and $_{D3}$ in cosmetics, g/g.

--mass concentration of vitamins $_{D2}$ and $_{D3}$ in the test solution, mg/L.

V - volume of sample volume, mL; m - volume of sample taken, g.

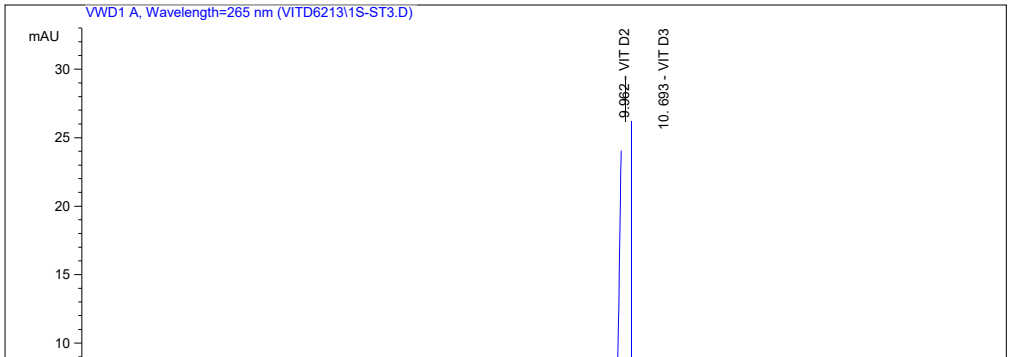


Fig. 1 Liquid chromatogram of the vitamin mixture standard solution 1: Vitamin $_{D2}$; 2: Vitamin $_{D3}$

XXVII. Soluble zinc salts

Dissolvable zinc salt

1 Scope

This specification specifies a flame atomic absorption spectrophotometric method for the determination of soluble zinc salts in deodorant cosmetics. This specification applies to the determination of soluble zinc salts in deodorant cosmetics.

2 Methodology Summary

The atomic energy of zinc in the base state in cosmetics absorbs resonance lines emitted from a hollow cathode lamp of the same metallic element and its absorption intensity is proportional to the amount of that element in the sample. Quantification is based on the measured absorbance intensity and comparison with a standard series. The method has a detection limit of $8.^{210\text{-}3}$ g and a lower limit of quantification of $2.^{710\text{-}2}$ g. If 1 g of sample is taken, the detection concentration of the method is $8.^{210\text{-}3}$ g/g and the lowest quantitative concentration is 2.710-2g/g.

3 Reagents

3.1 Nitric acid ($_{20}$ = 1.42 g/mL), ultrapure.

3.2 Nitric acid (1.5 + 998.5): take 1.5mL of nitric acid (3.1) and add water to 1000mL.

3.3 Zinc standard stock solution [(Zn) = 1.00 g/L]: weigh 1.000 g of zinc metal with a purity greater than 99.9% and add 20 mL

In nitric acid (3.1), fix the volume with water to 1L, shake well and set aside. 1.00mL of this solution contains 1.00mg of zinc.

3.4 Zinc Standard Use Solution [(Zn) = 20.0mg/L]: Take 2.00mL of Zinc Standard Reserve Solution (3.3) in a 100mL volumetric flask and dilute to 100mL with nitric acid (3.1).

4 Instruments

All glassware must be soaked in nitric acid (1+1) for at least 4h and rinsed with water before use.

4.1 Atomic Absorption Spectrophotometer.

4.2 Centrifuge.

4.3 Ultrasonic cleaners.

4.4 Stoppered cuvettes, 10mL, 25mL.

5 Analysis steps

5.1 Sample pre-treatment

Weigh 1.00g~2.00g of the sample into a 25mL stoppered cuvette, dilute to 10mL with water, mix well, sonicate for 20min and centrifuge at 5000rpm for 40min. 2.00mL~5.00mL of the sample centrifuge solution was diluted to 10.0mL with nitric acid (3.2) and set aside.

5.2 Preparation of standard solutions

Prepare the zinc standard series by diluting 0.00, 0.50, 1.00, 2.00, 3.00 and 5.00 mL of the standard use solution (3.4) in a 100 mL volumetric flask with nitric acid (3.2) to the scale.

5.3 Measurement

5.3.1 The standard solution (3.4) and the blank solution were alternately sprayed into the flame and the absorbance was measured. The calibration curve was plotted using the concentration and absorbance of the standard solution.

5.3.2 Under the same instrumental conditions, the absorbance of the sample solution was determined. From the absorbance of the solution to be measured, the mass concentration of zinc in the solution to be measured is derived from the calibration curve.

6 Calculation

$$(Zn) \quad \frac{(_{10})\ V}{m} \quad \frac{V2}{V1}$$

where: (Zn) - mass fraction of zinc in the sample, g/g.

$_1$ - the mass concentration of zinc in the test solution, mg/L.

$_0$ - mass concentration of zinc in the blank solution, mg/L; V - total volume of the sample solution, mL; $_{V1}$ - volume of the dispensed sample $_{V2}$ - volume of diluted sample solution, mL; m - volume of sample taken, g.

7 Accuracy and precision

The recoveries of the deodorant cosmetics were 97.0%~98.5% with a precision of 1.26% for both high and low levels.

XXVIII. Instrumental method for determining the resistance of cosmetics to UVA

Test in vitro of protection against UVA

1 Scope

This specification specifies an instrumental method for the determination of the UVA (320nm~400nm) resistance of cosmetics. This specification applies to the determination of the UVA resistance of sunscreen cosmetics.

2 Methodology Summary

The samples were applied to 3M film or poly(methyl methacrylate) plates with a gross surface and the critical wavelengths $_C$ and C were determined using an SPF meter.

UVA/UVB ratio R.

The critical wavelength ($_C$) is the absorbance of UVA at 90% of the total absorbance of UVA + UVB (290nm~400nm)

End wavelength (nm). Calculated by the following formula.

$$90\% \quad \int_{290}^{c} A()d \Big/ \int_{290}^{400} A()d$$

where A () - absorbance UVA/UVB ratio at wavelength (R).

$$R \quad A()d \Big/ \int_{290}^{400} A()d$$

3 Instrumentation

3.1 SPF meter: SPF-290 Analyzer or similar device from Optometrics Group with $_C$ measurement and recording capabilities.

3.2 3M film or polymethylmethacrylate (PMMA) sheet 5 x 5cm, (Europlast, France).

3.3 Latex Medical Finger Covers, a product of Shanghai Latex Factory Changzheng Branch or a similar

product.

3.4 Thermohygrometer.

3.5 Quality control samples

SPF15 standard: $_C$ = 366 nm. the $_C$ value for this standard should be between 365 nm and 367 nm.

4 Analysis steps

4.1 Sample preparation

The sample should be drawn by pressure or pumping with a special syringe and applied uniformly in dots or strips to the 3M film or the gross surface of the PMMA, then the sample should be applied with a finger wearing a latex medical finger cover to make a uniform surface. The actual amount of sample added to each plate should be between 1.$^{8mg/cm2}$ and 2.$^{2mg/cm2.}$ Results on PMMA plates are for negative determination only. Confirmation with 3M film results is required when positive results are obtained.

4.2 Sample determination

Calibration of the instrument and determination of the time space using a quartz plate with a load strip and 3M film or a polymethylmethacrylate plate according to the instrument instructions

White calibration. The samples coated according to step 4.1 were then placed at room temperature (20°C-30°C) and 40%-60% relative humidity for 20 min before being measured on the SPF meter, with no less than 4 points per sample.

5 Quality assurance

5.1 Instruments

Calibration and measurement of the intensity of the light source, wavelength accuracy and UV absorption of the loaded sample glass plate in accordance with the instrument's instructions, all of which must meet the requirements of the instrument's instructions.

5.2 Sample preparation

5.2.1 The sample must not contain air bubbles (this can be observed by squeezing the sample with two microscope coverslips).

5.2.2 The sample must be repeatedly coated back and forth after addition to ensure uniformity, with at least 4 test points on the same slide and the relative standard deviation of $_{C}$ between test points must not be greater than 1%, otherwise the result is invalid.

5.2.3 Two or more slides must be applied to each sample for determination. The $_{C}$ difference between the two slides must not be greater than 2nm, otherwise it should be redone.

6 Results Report

The report should contain the following elements.

6.1 Instruments and numbers used.

6.2 Parallel sample $_{C}$.

6.3 Quality control sample $_{C}$.

6.4 Expression of results

C≥370nm Markable broad spectrum

Part 4 Microbiological testing methods

I. General Provisions

General Principles

1 Scope

This specification sets out the basic requirements for the microbiological examination of cosmetics.

This specification applies to the collection, preservation and preparation of cosmetic samples for testing.

2 Instruments and equipment

2.1 Scales.

2.2 Autoclave.

2.3 Oscillator.

2.4 Triangular bottle, 250mL.

2.5 Glass beads.

2.6 Glass rods.

2.7 Graduated pipettes, 1mL, 10mL.

2.8 Grinder or homogeniser.

2.9 Constant temperature water bath.

3 Culture media and reagents

3.1 Sanitary saline

Ingredients: Sodium chloride 8.5g

Distilled water added to 1000mL

After dissolution, dispense into triangular bottles with glass beads, 90 mL each, 103.43 kPa (121°C 15 lb) for 20 min

Autoclave.

3.2 SCDLP Liquid Medium

Ingredients: Casein peptone	17g
Soybean peptone	3g
sodium chloride	5g
Dipotassium hydrogen phosphate	2.5g
Glucose	2.5g

Lecithin	1g
Twain 80	7g
Distilled water	1000mL

Preparation: Dissolve lecithin in a small amount of distilled water by heating, then mix with other ingredients, dissolve by heating, adjust pH to 7.2-7.3 and autoclave for 20 min at 103.43 kPa (121°C 15 lb). Shake carefully to mix well with the Tween 80 in the bottom layer and cool to about 25°C for use.

Note: If casein peptone and soy peptone are not available, they can also be replaced by peptone.

3.3 Sterilised liquid paraffin.

3.4 Sterilise Tween 80.

4 Sample collection and precautions

4.1 The samples collected should be representative, generally depending on the size of each batch of cosmetics, the corresponding number of packages should be taken at random

Packed units. For testing, a total of 10g or 10mL should be taken from two or more packing units respectively.

The sampling volume may be increased by the number of sample packs as appropriate.

4.2 Samples for testing should be kept strictly in their original packaging. The container should not be broken and should not be opened before the test to prevent contamination of the sample.

4.3 Upon receipt of the sample, it should be registered immediately, the test No. should be prepared and the sample should be tested as soon as possible according to the test requirements. If the sample cannot be tested in time, it should be kept in a cool, dry place at room temperature and not refrigerated or frozen.

4.4 If only one sample is available and multiple analyses are required at the same time, e.g. bacteriological, toxicological, chemical, etc., it is advisable to remove part of the sample for bacteriological testing and then the remaining sample for other analyses.

4.5 In the testing process, from the opening of the packaging to the end of all testing operations, are required to prevent the re-contamination and spread of micro-organisms, the utensils and materials used should be pre-sterilised, all operations should be carried out in a sterile room, or under the appropriate conditions, according to the provisions of aseptic operation.

4.6 If faecal coliform or other pathogenic bacteria are detected, the strain and the sample tested should be kept for one month from the date of the report.

5 Preparation of samples for testing

5.1 Liquid samples

5.1.1 For water-soluble liquids, measure 10mL into 90mL of sterilised saline or, if the sample is less than 10mL, follow the 10-fold dilution method. For 5mL, add to 45mL of sterilised saline, mix well and make a 1:10 test solution.

5.1.2 For oily liquid samples, take 10mL of sample, add 5mL of sterilised liquid paraffin and mix well, then add 10mL of sterilised Tween 80, shake and mix for 10min in a water bath at 40°C-44°C, add 75mL of sterilised saline (pre-warmed in a water bath at 40°C-44°C), emulsify in a water bath at 40°C-44°C and make a 1:10 suspension. The suspension was made into a 1:10 suspension.

5.2 Semi-solid samples of creams, pastes and emulsions

5.2.1 For hydrophilic samples, weigh 10g, add to a triangular flask containing glass beads and 90mL of sterilised saline, shake well and allow to stand for 15min. use the supernatant as a 1:10 test solution.

5.2.2 For hydrophobic samples: weigh 10g, place in a sterilised mortar, add 10mL of sterilised liquid paraffin, grind to a viscous consistency, then add 10mL of sterilised Tween 80, grind until dissolved, add 70mL of sterilised saline and mix thoroughly in a water bath at 40°C to 44°C to make a 1:10 test solution.

5.3 Solid samples

Weigh 10g, add to 90mL of sterilised saline, shake well and mix to disperse and suspend, then leave to stand and take the supernatant as a 1:10 test solution.

If a homogeniser is available, add 10g of the above water-soluble creams, pastes and powders to 90mL of sterilised saline and homogenise for 1min-2min; for hydrophobic creams, pastes, eyebrow pencils and lipsticks, add 10mL of sterilised liquid paraffin, 10mL of Tween 80 and 70mL of sterilised saline and homogenise for 3min-5min. Mix with 10mL of sterilised liquid paraffin, 10mL of Tween 80 and 70mL of sterilised saline for 3min-5min.

II. Total number of bacteria

Aerobic Bacterial Count

1 Scope

This specification specifies the test method for the total number of bacteriological colonies in cosmetics. This specification applies to the determination of the total number of bacteriological colonies in cosmetics.

2 Definition

The following definitions are used in this specification

Aerobic bacterial count is the total number of colonies contained in 1g (1mL) of a cosmetic sample after treatment and incubation under certain conditions (e.g. medium composition, incubation temperature, incubation time, pH value, aerobic nature, etc.). The results obtained include only the total number of aerobic colonies of aerobic bacteria grown under the conditions specified in this method.

The determination of the total bacterial count facilitates the determination of the degree of bacterial contamination of the sample and is a comprehensive basis for the overall hygienic evaluation of the sample.

According to.

3 Instruments and equipment

3.1 Triangular bottle, 250mL.

3.2 Measuring cylinder, 200mL.

3.3 pH meter or precision pH test paper.

3.4 Autoclave.

3.5 Test tube: 15 x 150mm.

3.6 Sterilised flat dish: 9cm diameter.

3.7 Sterilised graduated pipettes, 10mL, 1mL.

3.8 Alcohol lamp.

3.9 Constant temperature incubator: 36°C ± 1°C.

3.10 Magnifying glass.

4 Culture media and reagents

4.1 Physiological saline: see 3.1 in the General Regulations.

4.2 Lecithin, Tween 80 - Nutrient Agar Medium

4.2.1 Ingredients: Peptone 20g

Beef Paste 3g

Sodium chloride 5g

Agar 15g

Lecithin 1g

Tween 807g

Distilled water 1000mL

4.2.2 Preparation: Add lecithin to a small amount of distilled water, heat to dissolve, add Tween 80, add the other ingredients (except agar) to the rest of the distilled water and dissolve. Add the dissolved lecithin and Tween 80, mix well, adjust pH to 7.1-7.4, add agar, autoclave for 20 min at 103.43 kPa (121°C 15 lb) and store in a cold dark place.

4.3 0.5% 2,3,5-triphenyl terazolium chloride (TTC) Ingredients: TTC 0.5g

Distilled water	100mL

Dissolve, filter, autoclave at 103.43 kPa (121°C for 15 lb) for 20 min and store in a brown reagent bottle at 4°C in the refrigerator.

5 Operating steps

5.1 Aspirate 2mL of the 1:10 dilution into two sterilised dishes of 1mL each, using a sterilised pipette. 1mL into a 9mL sterilised saline tube (do not allow the pipette to touch the surface), replace with another pipette and mix well to make a 1:100 solution. If the sample has a high bacterial content, it can be diluted to 1:1000, 1:10,000, etc., with one pipette for each dilution.

5.2 Pour melted and chilled lecithin Tween 80 nutrient agar medium at 45°C to 50°C into dishes, approximately one per dish.

15mL, then turn the dish to mix the sample well with the medium, and after the agar has solidified, turn the dish over and place it at 36°C.

An empty sterilised dish without sample was added with approximately 15mL of Tween 80 nutrient agar medium, and after the agar had solidified, the dish was turned over and incubated in an incubator at 36°C ± 1°C for 48h ± 2h as a blank control.

5.3 To differentiate between particles and colonies in cosmetics, add 1mL of 0.5% TTC solution to every 100mL of Tween 80 nutrient agar with lecithin, and if bacteria are present, the colonies will be red after incubation, while the colour of the cosmetic particles will not change.

6 Colony counting method

Count the number of colonies with the naked eye and then check with a magnifying glass at 5x to 10x magnification to prevent missing. After recording the number of colonies in each dish, find out the average number of colonies growing in each dish at the same dilution. If there are flaky colonies in the dish or the spreading colonies, the dish should not be counted. If the flake colony is less than half of the dish, and the remaining half of the number of colonies in the distribution is very uniform, then the half dish colony count can be multiplied by 2, to represent the whole dish colony count.

7 Colony counting and reporting methods

7.1 Firstly, a range of 30 to 300 average colonies was selected for the determination of the total number of colonies. When the average colony count of only one dilution falls within this range, the number of colonies in that dish is multiplied by its dilution (see example 1 in Table 1).

7.2 If there are two dilutions, both with an average colony count between 30 and 300, the ratio of the two colony counts should be determined, and if the ratio is less than or equal to 2, the average should be reported, or if greater than 2, the colony count of the less diluted dish should be reported (see examples 2 and 3 in Table 1).

7.3 If the average number of colonies at all dilutions is greater than 300, the average number of colonies at the highest dilution should be reported multiplied by the dilution factor (see example 4 in Table 1).

7.4 If the average number of colonies at all dilutions is less than 30, the average number of colonies at the lowest dilution should be reported multiplied by the dilution factor (see Table 1, Example 5).

7.5 If the average number of colonies for all dilutions is not between 30 and 300, and one dilution is

greater than 300 and the other adjacent dilution is less than 30, the average number of colonies close to 30 or 300 is reported multiplied by the dilution (see example 6 in Table 1).

7.6 If all dilutions are free of bacterial growth, the number reported is less than 10 CFU per g or per mL.

7.7 For reporting colony counts, the number of colonies within 10 is reported as the actual value, and for values greater than 100, two significant digits are used and the value following the two significant digits should be rounded off. To reduce the number of zeros after a number, an exponent of 10 may be used (see Table 1, column on reporting methods). When reporting the number of colonies as 'not countable', the dilution of the sample should be indicated.

Table 1 Bacterial count results and reporting methods

Example	Average number of colonies at different dilutions 10^{-3}	10^{-1}	10^{-2}	Ratio of the number of bacteria at two dilutions	Total number of colonies (CFU/mL or CFU/g)	Reporting Method (CFU/mL or CFU/g)
1	1365	164	20	-	16400	16000 or 1.6×10^{4}
2	2760	295	46	1.6	38000	38000 or 3.8×10^{4}
3	2890	271	60	2.2	27100	27000 or 2.7×10^{4}
4	Not countable	4650	513	-	513000	510000 or 5.1×10^{5}
5	27	11	5	-	270	270 or 2.7×10^{2}
6	Not countable	305	12	-	30500	31000 or 3.1×10^{4}
7	0	0	0	-	$<1\times10$	

*CFU: Colony forming unit.

III. Fecal coliform

Fecal Coliforms

1 Scope

This specification specifies the test method for faecal coliforms in cosmetics. This specification applies to the testing of faecal coliforms in cosmetics.

2 Definition

The following definitions are used in this specification

Fecal coliforms are a group of aerobic and partly anaerobic Gram-negative non-bacteriophages that are found at 44.5

Incubated at ℃±0.5℃ for 24h-48h, it can ferment lactose to produce acid and gas. The bacterium is directly derived from faeces and is an important hygienic indicator bacterium.

3 Instruments

3.1 Constant temperature water bath or compartmentalised thermostat: 44°C ± 0.5°C.

3.2 Thermometer.

3.3 Microscope.

3.4 Slides.

3.5 Inoculation ring.

3.6 Induction hob.

3.7 Triangular bottle, 250mL.

3.8 Test tube: 15 x 150mm.

3.9 Small inverted tube.

3.10 pH meter or pH test paper.

3.11 Autoclave.

3.12 Sterilised pipettes, 10mL, 1mL.

3.13 Sterilised flat dish: 90mm diameter.

4 Culture media and reagents

4.1 Double lactose bile salt (with neutralising agent) medium

Ingredients: Peptone 40g

Pig bile salt	10g
Lactose	10g
0.4% Bromocresol Violet aqueous solution	5mL
Lecithin	2g
Twain 80	14g
Distilled water	1000mL

Preparation: Dissolve lecithin and Tween 80 in a small amount of distilled water. Dissolve peptone, bile salts and lactose in the rest of the distilled water, add together and mix, adjust pH to 7.4, add 0.4% aqueous bromocresol violet, mix well and dispense into test tubes (add a small inverted tube to each test tube). 68.95 kPa (115°C 10 lb) for 20 min.

4.2 Erythromelan (EMB) agar

Ingredients: Peptone 10g

Lactose	10g
Dipotassium hydrogen phosphate	2g
Agar	20g
2% eosin aqueous solution	20mL
0.5% Melphalan aqueous solution	13mL
Distilled water	1000mL

Preparation: Add the agar to 900mL of distilled water, heat and dissolve, then add dipotassium phosphate peptone, mix and dissolve. The pH was corrected to 7.2-7.4 and divided into triangular flasks at 103.43kPa.

(121°C 15 lb) for 15 min Autoclave and reserve. When ready to use, add lactose and heat to melt agar. Cool to about 60°C. Add sterilised Erythromax Blue solution aseptically and shake well. Pour into a flat dish and reserve.

4.3 Peptone water (for indigo substrate test)

Ingredients: peptone (or tryptone) 20g

Sodium chloride 5g

Distilled water 1000mL

Preparation: Melt the above ingredients, adjust the pH value to 7.0-7.2, dispense in small test tubes and autoclave for 15 min at 103.43 kPa (121°C 15 lb).

4.4 Indigo Matrix Reagent

Test method: Inoculate bacteria in peptone water and incubate at 44°C±0.5°C for 24h±2h. Add Kovac's test along the wall of the tube.

The test tube was gently shaken with 0.3mL to 0.5mL of reagent. Positive ones show a deep rose red colour in the reagent layer.

Note: Peptones should be rich in tryptophan and each batch of peptones should be identified with known strains before use.

4.5 Gram staining solution.

4.5.1 Dye preparation

4.5.1.1 Crystalline violet staining solution.

Crystalline Violet 1g

95% ethanol 20mL

1 % aqueous ammonium oxalate solution 80mL

The crystalline violet was dissolved in ethanol and then mixed with a solution of ammonium oxalate.

4.5.1.2 Gram's iodine solution.

Iodine 1g

Potassium iodide	2g
Distilled water added to	300mL

Mix iodine with potassium iodide, add a little distilled water, shake thoroughly, and then add distilled water until completely dissolved.

300mL.

4.5.1.3 Decolourisation solution: 95% ethanol.

4.5.1.4 Re-staining solution.

（1） Sandy yellow re-staining solution.

Sandy Yellow	0.25g
95% ethanol	10mL
Distilled water	90mL

Dissolve the saxifrage in ethanol, then dilute with distilled water.

（2） Dilute sarcocarbonate reddish solution: weigh 10g of basic reddish, grind finely, add 95% ethanol 100mL, leave overnight and filter through filter paper. Take 10mL of this solution, add 5% aqueous solution of carbolic acid 90mL and mix, that is, carbolic acid reddish solution. Then take 10mL of this solution and add 90mL of water, that is, dilute stannous carbonic acid reddish solution.

4.5.2 Staining method

4.5.2.1 The smear was fixed on the flame, stained with crystalline violet dropwise for 1 min and washed with water.

4.5.2.2 Add a drop of Gram's iodine solution for 1 min and wash with water.

4.5.2.3 Decolourise by adding 95% ethanol dropwise for approx. 30s, or fill the entire smear with ethanol and immediately decant, then fill the entire smear with ethanol dropwise for 10s and wash with water.

4.5.2.4 Add re-staining solution dropwise, re-stain for 1min, wash with water, leave to dry and microscopically examine.

4.5.3 Staining results

Gram-positive bacteria are purple in colour and Gram-negative bacteria are red in colour.

Note: If a 1:10 dilution of Paraffin Red stain is used for re-staining, the re-staining time is only 10s.

5 Operating steps

5.1 Add 10mL of 1:10 diluted test solution to 10mL of double lactose bile salt (with neutralising agent) medium and set at 44

Incubate for 24h-48h in an incubator at ℃±0.5°C. If there is no acid production or gas production, then report as negative for faecal coliforms.

5.2 If acid and gas are produced, inoculate the plate with Erythromax blue agar and incubate at 36°C±1°C for 18h-24h. At the same time, inoculate 1 to 2 drops of the culture solution into peptone water and incubate at 44°C±0.5°C for 24h±2h.

After incubation, the plates were observed for typical colony growth on the above plates. Typical colonies of faecal coliforms on Erythromax Blue agar medium are dark purple-black, round, with neat edges and a smooth, moist surface, often with a metallic lustre. There are also purple-black colonies with no or slightly metallic lustre, or pinkish-purple colonies with a darker centre, which are also often faecal coliforms and should be selected for.

5.3 The above suspicious colonies were picked and stained for Gram stain microscopy.

5.4 Add approximately 0.5mL of indigo substrate reagent to the peptone water culture solution and observe the indigo substrate reaction. The surface of the positive reaction is rose-red; the surface of the negative reaction is the colour of the reagent.

6 Report of test results

The detection of faecal coliforms in the sample examined is reported on the basis of acid production and gas production from fermented lactose, typical colonisation of the plate and confirmation of Gram-negative short bacilli with a positive indigo matrix test.

IV. Pseudomonas aeruginosa

Pseudomonas Aeruginosa

1 Scope

This specification specifies the test method for Pseudomonas aeruginosa in cosmetics. This specification applies to the testing of Pseudomonas aeruginosa in cosmetics.

2 Definition

The following definitions are used in this specification.

Pseudomonas aeruginosa belongs to the genus Pseudomonas and is a Gram-negative bacterium, positive for oxidase and capable of producing pseudomonas aeruginosa. It also liquefies gelatine, reduces nitrate to nitrite and grows at 42°C ± 1°C.

The bacterium is pathogenic to humans and can cause septicemia, etc. by causing septicemia in the wounded area.

3 Instruments

3.1 Incubator: 42°C ± 1°C, 36°C ± 1°C.

3.2 Triangular bottle, 250mL.

3.3 Test tube: 15 x 150 mm.

3.4 Sterilised flat dish: 90mm diameter.

3.5 Sterilised graduated pipettes, 10mL, 1mL.

3.6 Microscope.

3.7 Slides.

3.8 Inoculation needle, inoculation ring.

3.9 Induction hob.

3.10 Autoclave.

4 Culture media and reagents

4.1 For SCDLP liquid media, see 3.2 in the general rules.

4.2 Hexadecyltrimethylammonium bromide medium

Ingredients:	Beef paste	3g
	Peptones	10g

Sodium chloride	5g
Hexadecyltrimethylammonium bromide	0.3g
Agar	20g
Distilled water	1000mL

Preparation: Dissolve the above ingredients, except agar, by heating, adjust pH to 7.4-7.6, add agar, 68.95 kPa

(115°C 10 lb) for 20 min, then sterilise and prepare the plates.

4.3 Acetamide medium

Ingredients: Acetamide	10.0g
Sodium chloride	5.0g
Dipotassium hydrogen phosphate anhydrous	1.39g

Potassium dihydrogen phosphate anhydrous 0.73g

Magnesium sulfate ($_{MgSO4}$.7H2O) 0.5g

Phenol Red 0.012g

Agar 20g

Distilled water 1000mL

Preparation: Add all ingredients except agar and phenol red to distilled water, dissolve by heating, adjust pH to 7.2, add agar and phenol red, sterilize by autoclaving for 20 min at 103.43 kPa (121°C for 15 lb) and make plates for use.

4.4 Media for Pseudomonas aeruginosa assay

Ingredients: Peptone 20g

Magnesium chloride 1.4g

Potassium sulphate 10g

Agar 18g

Glycerine (chemically pure) 10g

Distilled water 1000mL

Preparation: Add peptone, magnesium chloride and potassium sulphate to distilled water, dissolve by heating, adjust pH to 7.4, add agar and glycerol, dissolve by heating, dispense in test tubes, autoclave for 20 min at 68.95 kPa (115°C 10 lb), make slant and reserve.

4.5 Gelatine medium

Ingredients: Beef paste 3g

Peptone 5g

Gelatine 120g

Distilled water 1000mL

Preparation: Add the ingredients to distilled water and soak for 20 min, stir at any time to dissolve, adjust pH to 7.4, dispense in test tubes, sterilize at 68.95 kPa (115°C 10 lb) for 20 min, then stand upright to make a high level and reserve.

4.6 Nitrate Peptone Water Medium

Ingredients: Peptone 10g

Yeast Infusion 3g

Potassium nitrate 2g

Sodium nitrite 0.5g

Distilled water 1000mL

Preparation: Add peptone and yeast extract to distilled water, heat to dissolve, adjust pH to 7.2, boil and filter, add potassium nitrate and sodium nitrite, dissolve and mix well, divide into test tubes with small inverted tubes, 68.95kPa (115)

(℃ 10 lb) for 20 min and then sterilise and prepare for use.

4.7 Plain agar slant medium

Ingredients: Peptone		10g
Beef Paste	3g	
Sodium chloride	5g	
Agar		15g
Distilled water		1000mL

Preparation: Dissolve all ingredients except agar in distilled water, adjust pH to 7.2-7.4, add agar, dissolve by heating, dispense in test tubes, autoclave for 20 min at 103.43 kPa (121°C 10 lb) and make a slant and reserve.

5 Operating steps

5.1 Culture: add 10mL of 1:10 sample dilution to 90mL of SCDLP liquid medium at 36°C±1°C.

If Pseudomonas aeruginosa grows, there is a thin film on the surface of the culture, and the culture is often yellow-green or blue-green.

5.2 Pseudomonas aeruginosa on this medium, the colonies are flat and amorphous, spreading or slightly spreading to the periphery, the surface is moist, the colonies are greyish-white, the medium around the colonies often spread with water-soluble pigments, this medium is selective. E. coli cannot grow, Gram-positive bacteria grow poorly.

In the absence of hexadecane-trimethylammonium bromide agar can also be isolated by acetamide medium, the bacterial solution will be inoculated in line on the plate, put 36 ℃ ± 1 ℃ incubation 24h ± 2h, Pseudomonas aeruginosa in this medium grows well, the colony is flat, the edge is not neat, the medium around the colony is slightly pink, other bacteria do not grow.

5.3 Stain microscopy: pick suspicious colonies, smear, Gram stain, and perform oxidase test if microscopy is Gram-negative.

5.4 Oxidase test: Take a small piece of clean white filter paper and place it in a sterilised dish. Pick a suspicious colony of Pseudomonas aeruginosa on the filter paper with a sterile glass rod, then add a drop of freshly prepared 1% dimethyl-p-phenylenediamine solution on it.

5.5 Pseudomonas aeruginosa test: take 2 to 3 suspect colonies, inoculate them on Pseudomonas aeruginosa assay medium and place them at 36°C.

Incubate at ±1°C for 24h±2h, add 3mL-5mL of chloroform, shake thoroughly to dissolve the Pseudomonas aeruginosa in the chloroform solution, when the chloroform extract is blue, transfer the chloroform to another test tube with a pipette and add 1mL of 1mol/L hydrochloric acid, shake and leave for a moment. The presence of Pseudomonas aeruginosa in the upper hydrochloric acid solution is considered positive if the upper layer appears pink to purple in colour.

5.6 Nitrate reduction and gas production test: Pick a pure culture of suspected Pseudomonas aeruginosa, inoculate it in nitrate peptone medium, incubate it at 36°C±1°C for 24h±2h and observe the results. If there is gas in the small inverted tube in the nitrate peptone medium, it is positive, indicating that the bacterium can reduce nitrate and decompose nitrite to produce nitrogen gas.

5.7 For gelatin liquefaction test, take pure culture of Pseudomonas aeruginosa suspected colonies, puncture and inoculate in gelatin medium, incubate at 36℃±1℃ for 24h±2h, take out and put in the refrigerator for 10min~30min, if it is still dissolved or the surface is dissolved, it is positive for gelatin liquefaction test; if it is solidified and insoluble, it is negative.

5.8 42°C growth test: Pick a pure culture of suspected Pseudomonas aeruginosa, inoculate it on normal agar slant medium, place it in an incubator at 42°C±1°C and incubate for 24h-48h. Pseudomonas aeruginosa is positive if it can grow, while Pseudomonas fluorescens, which is a close approximation, cannot grow.

6 Report of test results

Pseudomonas aeruginosa can be reported if the sample is confirmed to be a Gram-negative bacillus with a positive oxidase and pseudomonas aeruginosa test; if the pseudomonas aeruginosa test is negative but the liquefied gelatin, nitrate reduction gas and 42°C growth tests are all positive, Pseudomonas aeruginosa can still be reported.

V. Staphylococcus aureus

Staphylococcus Aureus

1 Scope

This specification specifies the test method for Staphylococcus aureus in cosmetics. This specification applies to the testing of Staphylococcus aureus in cosmetics.

2 Definition

The following definitions are used in this specification

Staphylococcus aureus is a gram-positive coccus with a grape-like arrangement, no budding cells, no pods, able to break down mannitol and positive for plasma coagulase.

This bacterium is the most pathogenic of the staphylococci in humans, causing localised septic lesions in humans and in severe cases septicaemia.

3 Instruments and equipment

3.1 Microscope.

3.2 Constant temperature incubator: 36°C ± 1°C.

3.3 Centrifuge.

3.4 Sterilised pipettes, 1mL, 10mL.

3.5 Sterilised test tubes: 15 x 150mm.

3.6 Slides.

3.7 Alcohol lamp.

4 Culture media and reagents

4.1 For SCDLP liquid media, see 3.2 in the general rules.

4.2 7.5% sodium chloride broth

Ingredients: Peptone 10g

Beef Paste 3g

Sodium chloride 75g

Distilled water added to 1000mL

Preparation: Dissolve the above ingredients by heating, adjust the pH to 7.4, dispense and autoclave for

15 min at 103.43 kPa (121°C 15 lb).

4.3 Baird Parker Flats

Ingredients: Tryptone	10g
Beef Paste	5g
Yeast infusion	1g
Sodium pyruvate	10g
Glycine	12g
Lithium chloride (LiCl-6H2O)	5g

Agar 20g

Distilled water 950mL

pH 7.0±0.2

Preparation of bacterium enhancer: 50mL of 30% yolk saline mixed with 10mL of decontaminated and filtered 1% potassium tellurite solution, stored in the refrigerator.

Preparation: Add the ingredients to distilled water, boil to dissolve completely, cool to 25°C ± 1°C and correct pH. 95mL per bottle, autoclave at 103.43kPa (121°C for 15 lb) for 15min. when ready to use, dissolve the agar by heating, add 5mL of potassium yolk tellurite booster per 95mL, shake well and pour onto the plate. Shake well and pour onto the plates. The medium should be dense and opaque. Do not store in the refrigerator for more than 48h±2h before use.

4.4 Blood agar medium

Ingredients: Nutrient agar 100mL

Defibrinated sheep blood (or rabbit blood) 10mL

Preparation: Melt the nutrient agar, leave to cool to about 50°C, add the defibrinated sheep's blood aseptically, shake well, make a plate and set aside in the refrigerator.

4.5 Mannitol fermentation medium

Ingredients: Peptone 10g

Sodium chloride 5g

Mannitol 10g

Beef Paste 5g

0.2% Muscovitol Blue solution 12mL

Distilled water 1000mL

Preparation: Add peptone, sodium chloride and beef paste to distilled water, dissolve by heating, adjust pH 7.4, add mannitol and indicator, mix well and dispense in test tubes, sterilize at 68.95 kPa (115°C 10 lb) for 20 min and set aside.

4.6 Rabbit (human) plasma preparation

Autoclave 3.8% sodium citrate solution at 103.43 kPa (121°C 15 lb) for 30 min, add 1 portion to 4 portions of rabbit (human) whole blood, mix well and leave to stand; centrifuge at 2000 rpm to 3000 rpm for 3 min to 5 min. blood cells sink and take the top plasma.

5 Operating steps

5.1 Bacterial growth: Inoculate a 1:10 dilution of the sample into 90mL of SCDLP liquid medium, incubate at 36°C±1°C and incubate for 24h±2h.

Note: If this medium is not available, 7.5% NaCl broth can also be used.

5.2 The colonies on the blood agar plate are golden yellow, large and raised, round, opaque, smooth and surrounded by a haemolytic circle. On Baird Parker's medium they are round, smooth, raised, moist, 2mm to 3mm in diameter, grey to black in colour with a pale margin and surrounded by a cloudy band with a transparent band in the outer layer. The colonies appear to have the softness of cream gum when touched with an inoculating needle. Occasionally, similar colonies are encountered that are not lipolytic, but without

the cloudy band or hyaline band. Individual colonies were picked and purified on a blood agar plate and placed on a 36-cm plate.

Incubate at °C ± 1°C for 24h ± 2h.

5.3 Staining and microscopy: Pick a pure colony, smear, Gram stain and microscopy. Staphylococcus aureus is a Gram-positive bacterium, arranged in a grape shape, without budding cells, without entrapment, pathogenic staphylococcus, with a small body, about 0.5m to 1m in diameter.

5.4 Mannitol fermentation test: Inoculate the above pure colonies into mannitol fermentation medium, add 2mm~3mm of sterilised liquid paraffin on the surface of the medium and incubate at 36°C±1°C for 24h±2h. Staphylococcus aureus should be able to ferment mannitol to produce acid.

5.5 Plasma coagulase test: aspirate 0.5mL of fresh 1:4 plasma into a sterilised tube, add 0.5mL of broth culture of the bacterium to be tested for 24h±2h. Mix well, place in a 36°C±1°C thermostat or constant temperature water bath, observe every half hour, if a clot is present within 6h, the test is positive. At the same time, 0.5mL of broth culture and 0.5mL of broth medium of known plasma coagulase-positive and negative strains were added to 0.5mL of sterilised 1:4 plasma and mixed as control.

6 Report of test results

Where there is suspicious colony growth on the above selected plates, proven by stain microscopy to be Gram-positive Staphylococcus and capable of fermenting mannitol to produce acid, and a positive plasma coagulase test, the sample examined can be reported as having detected Staphylococcus aureus.

VI. Moulds and yeasts

Molds and Yeast Count

1 Scope

This specification specifies a method for the detection of mould and yeast counts in cosmetics. This specification applies to the counting of moulds and yeasts in various cosmetics.

2 Definition

The following definitions are used in this specification.

Determination of molds and yeast count (Determination of molds and yeast count) is the number of live moulds and yeasts contaminated in 1g or 1mL of cosmetic products after incubation under certain conditions, in order to determine the degree of contamination of cosmetics by moulds and yeasts and their general hygiene.

This method is based on the specific morphological and cultural characteristics of moulds and yeasts, and the number of moulds and yeasts grown is calculated by incubating them on Tiger Red medium at 28°C ± 2°C for 72h.

3 Instruments and equipment

3.1 Incubator: 28°C ± 2°C.

3.2 Oscillator.

3.3 Scales.

3.4 Triangular bottle, 250mL.

3.5 Test tube: 15 x 150mm.

3.6 Flatware: 9cm diameter.

3.7 Pipette, 1mL, 10mL.

3.8 Measuring cylinder, 200mL.

3.9 Alcohol lamp.

3.10 Autoclave.

4 Culture media and reagents

4.1 Sanitary saline

See 3.1 in the General Conditions.

4.2 Tiger Red (Bengal Red) Medium

Ingredients: Peptone 5g

Glucose	10g	
Potassium dihydrogen phosphate	1g	
Magnesium sulphate (containing 7H2O)		0.5g
Agar	20g	
1/3000 Tiger Red solution	100mL	
(Tetrachlorotetraiodofluorescein)		
Distilled water	1000mL	
Chloramphenicol	100mg	

Preparation: Dissolve the above ingredients (except Tiger Red) in distilled water, then add Tiger Red solution. After dispensing, the

103.43 kPa (121°C 15 lb) for 20 min, autoclave, dissolve chloramphenicol in a small amount of ethanol, filter and dissolve, then add to Pe

If chloramphenicol is not available, add 30mg of streptomycin per 1000mL.

5 Operating steps

5.1 Sample dilution

See 6.1 in the determination of the total number of bacteria.

5.2 Take 1mL of 1:10, 1:100 and 1:1000 test solution into sterilised dishes, using 2 dishes for each dilution, fill with Tiger Red medium melted and cooled to about 45°C±1°C and shake well. After solidification, turn the plates over and incubate at 28°C±1°C for 72h±2h and count the number of moulds and yeasts growing in the plates. If there is mould spreading, to avoid affecting other moulds and yeast counting, the plate should be taken out for counting at 48h±2h.

5.3 Calculation method: Count the number of moulds and yeast colonies growing on each plate and find out the average number of colonies per dilution. When determining the results, the number of colonies should be selected within the range of 5 to 50 dishes counted, multiplied by the dilution, that is, each g (or each mL) of the sample contains the number of moulds and yeasts. For other ranges, the colony count should be reported in the same way as the colony count.

5.4 The number of moulds and yeasts per g (or per mL) of cosmetic product is expressed in CFU/g (mL).

Part 5 Methods of Safety and Efficacy Evaluation in Human

I. General Provisions

General principles

1 Scope

This specification specifies the human test items and requirements for the safety and efficacy evaluation of cosmetics. This specification applies to the human safety and efficacy evaluation of cosmetic end-products.

2 The basic principles of cosmetic human testing

2.1 Selection of an appropriate subject population with a certain number of cases.

2.2 Cosmetics human testing should be completed before the necessary toxicological tests and written proof, toxicological tests failed samples are no longer human testing.

2.3 The human patch test for cosmetics is suitable for testing sunscreen, spot removal and deodorant cosmetics.

2.4 The human safety test for cosmetics is suitable for testing bodybuilding, breast beauty, hair care and hair removal cosmetics.

2.5 The sun protection test for sunscreen cosmetics applies to the determination of the Sun Protection Factor (SPF), the SPF

Waterproof test and determination of the Protection Factor of UVA (PFA value).

II. Human skin patch test

Human Skin Patch Test

1 Scope

This specification specifies the basic principles, purpose, requirements, methods and interpretation of results of the human skin patch test. This specification applies to the detection of potential adverse reactions of cosmetic end-products and their raw materials on human skin.

2 Citation Standards

Diagnostic criteria and principles for the treatment of cosmetic dermatoses General (GB17149.1-1997)
Diagnostic criteria and principles for the treatment of cosmetic contact dermatitis (GB17149.2-1997)

3 Purpose

To test the potential of the test substance to cause adverse skin reactions in humans.

4 Basic Principles

4.1 Eligible volunteers were selected as test subjects.

4.2 A human skin patch test should be carried out using standardised patch test materials.

4.3 In principle, depending on the nature of the cosmetic product, the original cosmetic end-product can be used for the closed skin patch test, i.e. skin washes and/or hair cleansers should be diluted to 1% aqueous solution; the original cosmetic end-product can be used for the open skin patch test, i.e. skin washes and/or hair cleansers should be diluted to 5% aqueous solution, and hair removal agents to 10% dilution. The test substance may be a cosmetic end-product, i.e. a skin wash and/or hair cleanser diluted to 5% aqueous solution and a depilatory agent diluted to 10%.

5 Subject selection

5.1 Volunteers aged 18 to 60 years who met the requirements of the trial were selected as subjects.

5.2 A person cannot be selected as a subject who has

5.2.1 Those who have used antihistamines in the last week or immunosuppressive drugs in the last month.

5.2.2 Those who have had any anti-inflammatory drugs applied to the test site within the last two months

5.2.3 Subjects with clinically unresolved inflammatory skin diseases.

5.2.4 Insulin-dependent diabetics.

5.2.5 Patients with asthma or other chronic respiratory conditions undergoing treatment.

5.2.6 Those who have received anti-cancer chemotherapy within the last 6 months.

5.2.7 Patients with immune deficiencies or autoimmune diseases.

5.2.8 Women who are breastfeeding or pregnant.

5.2.9 Bilateral mastectomies and bilateral axillary lymph node dissection

5.2.10 Persons whose test results are affected by scarring, pigmentation, atrophy, macules or other defects in the area of skin to be tested.

5.2.11 Participants in other clinical trial studies.

5.2.12 Highly sensitive individuals.

5.2.13 Non-volunteer participants or those who are unable to complete the required content as required by the test.

6 Methods

6.1 The skin patch test can be divided into a closed skin patch test and an open skin patch test. The closed skin patch test is suitable for most cosmetic products and for a small number of cosmetic products that require pre-treatment. The open skin patch test is used for products that cannot be tested directly on the original cosmetic product and to verify the skin reaction results of the closed skin patch test.

6.2 Closed skin patch test

6.2.1 A minimum of 30 participants were selected for the trial according to subject entry criteria.

6.2.2 Select a qualified spot test material. Place the test substance in the spotter at a dosage of approximately 0.020g to 0.025g (solid or semi-solid) or 0.020mL to 0.025mL (liquid, which can be added dropwise to the filter paper attached to the spotter and placed in the spotter). When the test substance is the original cosmetic end-product, the control well is a blank control (no substance is placed) and when the test substance is a diluted cosmetic, the diluent of the cosmetic is used in the control well. The spot test device with the test substance is applied to the back or the curved side of the forearm of the subject with non-irritating tape and is applied to the skin with gentle pressure with the palm of the hand for 24h.

6.2.3 The skin reaction was observed 30 min after removal of the subject's patch test apparatus and after the indentation had disappeared. If the result is negative, observe again at 24h and 48h after the patch test. The results were recorded in accordance with Table 1 (Standard Scale for Grading Adverse Skin Reactions).

Table 1 Grading criteria for adverse skin reactions

Response level	Rating scale	Skin reactions
-	0	Negative reaction
±	1	Suspicious reaction; faint erythema only
+	2	Weakly positive reaction (erythematous reaction); erythema, infiltrates, oedema, may have papules
+ + +	3	Strongly positive reaction (herpetic reaction); erythema, infiltration, oedema, papules, herpes; reactions Should be able to extend beyond the test area
+ + + +	4	Very strong positive reaction (fused herpetic reaction); marked erythema, severe infiltration, edema, Fusion herpes; reaction beyond the test area

6.3 Open skin patch test

6.3.1 A minimum of 30 participants were selected for the trial according to subject entry criteria.

6.3.2 The test area should be 5 x 5 $^{cm2\ on\ the}$ flexor side of the forearm, the mastoid area or the site of application, and should be kept dry and away from other topical preparations.

6.3.3 Apply 03g to 05g (mL) of the test substance evenly to the test site twice a day for 7 days while observing skin reactions, and if skin reactions occur during this process, decide whether to continue the test according to the specific situation.

6.3.4 Skin reactions are judged according to the skin reaction criteria of the open patch test, see Table 2.

6.3.5 The concentration of the test substance should be determined by the actual concentration and method of use of the cosmetic product, i.e. when diluting the product, the diluent or excipient should be applied to the opposite side of the test area as a control.

Table 2 Open patch test skin reaction assessment criteria table

Response level	Rating scale	Skin reactions
-	0	Negative reaction
±	1	Faint erythema, dry, wrinkled skin
+	2	Erythema, oedema, papules, bumps, flaking, fissures
+ + +	3	Visible erythema, edema, blisters
+ + + +	4	Severe erythema, oedema, blistering, vesicles, hyperpigmentation or hypopigmentation, acne-like changes
		Change

7 Interpretation of results

7.1 Interpretation of the results of the skin-closing patch test: A dermal adverse reaction is considered to be present in humans if more than 5 out of 30 subjects have a Grade 1 dermal adverse reaction, or more than 2 have a Grade 2 dermal adverse reaction (more than 5 have a Grade 2 reaction in the patch test for deodorant products), or if any of the subjects have a Grade 3 or more dermal adverse reaction.

7.2 Interpretation of the results of the open skin patch test: If there are 5 or more Grade 1 dermal adverse reactions, 2 or more Grade 2 dermal adverse reactions, or 1 or more Grade 3 or higher dermal adverse reactions in any of the 30 subjects, the test substance is considered to have a significant adverse effect on humans.

III. Human trial test safety evaluation

Safety Evaluation of Using Tests of Cosmetics on Human Body

1 Principles

Human testing of cosmetics should conform to the basic principles of the International Declaration of Helsinki by requiring subjects to sign an informed consent form and to take the necessary medical precautions to protect the interests of the subjects to the greatest extent possible.

2 Scope

The human test safety evaluation applies to special purpose cosmetics as defined in the Cosmetics Hygiene Supervision Regulations, which currently include bodybuilding, breast care, hair care and hair removal cosmetics.

3 Purpose of the test

The main test is the potential of the test substance to cause adverse skin reactions in humans.

4 Subject selection

4.1 Volunteers aged 18 to 60 years who met the requirements of the trial were selected as subjects.

4.2 A person cannot be selected as a subject who has

4.2.1 Those who have used antihistamines in the last week or immunosuppressive drugs in the last month.

4.2.2 Any anti-inflammatory drug applied to the test site within the last two months.

4.2.3 Subjects with clinically untreated inflammatory skin disease.

4.2.4 Insulin-dependent diabetics.

4.2.5 Patients with asthma or other chronic respiratory conditions who are receiving treatment.

4.2.6 Those who have received anti-cancer chemotherapy within the last 6 months.

4.2.7 Patients with immunodeficiency or autoimmune diseases.

4.2.8 Women who are breastfeeding or pregnant.

4.2.9 Bilateral mastectomy and bilateral axillary lymph node dissection.

4.2.10 Where the test results are affected by scarring, pigmentation, atrophy, macules or other defects on the skin area to be tested.

4.2.11 Participants in other clinical trials.

4.2.12 Highly sensitive individuals.

4.2.13 Non-volunteer participants or those who are unable to complete the required content as required by the test.

5 See Table 1 for **grading criteria for skin reactions**.

Table 1 Grading criteria for adverse skin reactions in human trial tests

Adverse skin reactions	Grading
No response	0
Faint erythema	1
Erythema, infiltrates, papules	2
Erythema, oedema, papules, blisters	3
Erythema, edema, blistering	4

6 Test method

6.1 Haircare products

More than 30 patients with hair loss were selected according to the subject entry criteria and the subjects were allowed to use the tested products directly according to the characteristics and methods of use stated on the cosmetic product label. Subjects were observed or followed up by telephone once a week for skin reactions, and the results were recorded according to the classification criteria for adverse skin reactions in Table 1, and the duration of the trial should not be less than 4 weeks.

6.2 Bodybuilding products

At least 30 cases of simple obesity were selected according to the criteria for the selection of subjects, and the subjects were allowed to use the product directly according to the characteristics and methods of use stated on the cosmetic product label. The subjects were observed once a week or followed up by telephone for any systemic adverse reactions such as anorexia, diarrhoea or fatigue, etc. Skin reactions at the sample application site were observed and the results were recorded according to the classification of skin adverse reactions in Table 1.

6.3 Breast Beauty Products

Thirty or more normal female subjects were selected according to the subject selection criteria, and the subjects were allowed to use the tested product directly according to the characteristics and methods of use stated on the cosmetic product label. The subjects were observed once a week or followed up by telephone for any systemic adverse reactions such as nausea, fatigue, menstrual disorders and other discomforts, etc. Skin reactions at the site of application of the sample were observed and the results were recorded according to the classification criteria for skin adverse reactions in Table 1. The trial period should not be less than 4 weeks.

6.4 Hair removal products

Thirty or more volunteer subjects were selected according to the subject selection criteria, and the subjects were allowed to use the tested product directly according to the characteristics and methods of use stated on the cosmetic product label. After the trial, the doctor in charge observed the local skin reactions and recorded the results according to the classification criteria for adverse skin reactions in Table 1.

7 Results safety evaluation

If there are more than 2 cases (excluding 2 cases) of Grade 1 skin adverse reactions, or more than 1 case (excluding 1 case) of Grade 2 skin adverse reactions, or any 1 case of Grade 3 or above skin adverse reactions among 30 subjects of hair care, body building and breast beauty products, the test substance is considered to have an adverse skin reaction to humans; if there are more than 3 cases (excluding 3 cases) of Grade 1 skin adverse reactions, or more than 2 cases (including 2 cases) of Grade 2 skin adverse reactions among 30 subjects of hair removal products, the test substance is considered to have a significant adverse skin reaction to humans. If more than 3 cases (excluding 3 cases) of Grade 1 skin adverse reactions or more than 2 cases (excluding 2 cases) of Grade 2 skin adverse reactions or any 1 case of Grade 3 or more skin adverse reactions occur in 30 subjects of hair removal products, the test substance is considered to have significant adverse reactions in humans.

Fourth, sunscreen cosmetics sun protection effect of human testing

Tests *in vivo* of UV Protection Efficacy of Cosmetic Sunscreens

1 Principles

Human trials of the sunscreen effects of sunscreen cosmetics should conform to the basic principles of the International Declaration of Helsinki by requiring subjects to sign an informed consent form and to take the necessary medical precautions to protect the interests of the subjects to the greatest extent possible.

2 Scope

The human test on the sun protection effect of sunscreen cosmetics is applicable to sunscreen cosmetics as defined in the Cosmetics Hygiene Supervision Regulations and currently includes the determination of the sun protection index (SPF), the SPF water resistance test and the long-wave violet ray protection index (PFA).

3 Test conclusions and reporting requirements

Sunscreen cosmetic sunscreen effect test should give specific test results or conclusions. The test report should include the following: general information about the subject including sample number, name, production batch number, production and delivery unit, description of the physical state of the sample and the test start and end time, test purpose, materials and methods, test results and conclusions. The test results section is generally expressed in a table and should include general information about the subject, test conditions, standard control samples, all raw test data and statistical results. The test report should be signed by the examiner, the checker and the technical director respectively, and stamped with the official seal of the testing unit.

(i) Sunscreen cosmetics sun protection index (SPF) determination method

1 Scope

This specification specifies a method for the determination of SPF values for sunscreen cosmetics. This specification applies to the determination of the SPF value of sunscreen cosmetics.

2 Normative references

（1） The US Food and Drug Administration (FDA) method for determining the sun protection index of sunscreen products

(Testing Procedure, Federal Register, 21 CFR. Part 352. 70-73, 1999)

（2） International SPF determination methods (COLIPA Europe, CTFA South Africa and JCIA Japan)

(International Sun Protection Factor (SPF) Test Method, 2006)

3 Definition

3.1 Ultraviolet wavelength

Short-wave ultraviolet (UVC): 200nm～290nm Medium-wave ultraviolet (UVB): 290nm～320nm
Long-wave ultraviolet (UVA): 320nm～400nm

3.2 Minimal erythema dose (MED): The minimum dose ($^{J/m2}$) or minimum time (seconds) of UV exposure required to cause erythema of the skin to the edge of the point of exposure.

3.3 Sun protection factor (SPF): The ratio of the MED required to cause erythema on skin protected by a sunscreen to the MED required to cause erythema on unprotected skin is the SPF of the sunscreen, which can be expressed as follows.

$$SPF = \frac{\text{MED for skin protection with sunscreen cosmetics}}{\text{MED for unprotected skin}}$$

4 SPF measurement method

4.1 Light source: The artificial light source used must be a xenon arc lamp daylight simulator with a

filtering system.

4.1.1 Nature of UV radiation: UV daylight simulators should emit a continuous spectrum with no gaps or peaks in the UV region.

4.1.2 The light source output should be stable and homogeneous over the entire beam cross-section (especially important for single beam sources).

4.1.3 The light source must be equipped with an appropriate filtering system so that the output spectrum meets the requirements of Table 1. The spectral characteristics are described in terms of the cumulative erythema effect in the continuous band from 290 nm to 400 nm. The erythemal effect in each band can be expressed as a percentage of the total erythemal effect from 280 nm to 400 nm, i.e. the Relative Cumulative Erythemal Effectiveness (%RCEE). The %RCEE requirements for the light source output are shown in Table 1.

Table 1 Acceptable limits of %RCEE for the output of UV daylight simulator light sources

Spectral range (nm)	Measured %RCEE	
	Lower limit	Upper limit
<290		<1.0
290-300	1.0	8.0
290-310	49.0	65.0
290-320	85.0	90.0
290-330	91.5	95.5
290-340	94.0	97.0
290-400	99.9	100.0

4.1.4 The light source output should be checked by a UV auxiliary illuminator prior to the test and the light source spectrum should be systematically calibrated once a year, with similar calibrations carried out each time the main optical components are replaced. Independent experts are required to carry out this annual monitoring work.

4.2 Subject selection

4.2.1 Healthy volunteer subjects aged 18 to 60 years, both sexes, were selected.

4.2.2 No previous history of photoreceptor disease and no recent use of medication that affects photoreceptors.

4.2.3 Subjects with skin types I, II or III, i.e. those who are sensitive to sunlight or UV radiation and prone to sunburn but not to hyperpigmentation after exposure.

4.2.4 The skin of the subject area should be free of hyperpigmentation, inflammation, scarring, pigmented nevi and hirsutism.

4.2.5 Subjects should be excluded if they are pregnant, breastfeeding, taking anti-inflammatory drugs such as oral or topical corticosteroids, or have undergone a similar trial within the last month.

4.2.6 The method specifies a minimum number of 10 cases and a maximum number of 25 cases for each sunscreen cosmetic.

4.3 See Appendix I for the preparation of SPF standards.

4.4 MED measurement method

4.4.1 Subject position: irradiated posteriorly, either in a forward leaning or prone position.

4.4.2 The sample is coated with an area of not less than ^{30cm2}.

4.4.3 Sample dosage and application method: Weigh the sample at a dosage of $^{2mg/cm2}$, apply the sample evenly to the test area using a latex finger sleeve and wait for 15 minutes.

4.4.4 Subject MED: This should be done 24 hours before the product is tested. An irradiated area is selected on the back of the subject's skin and 5 points are irradiated with different doses of UV light and the results are observed after 16-24 hours. The lowest dose or shortest exposure time at which the skin becomes erythematous is the MED of the subject's normal skin.

4.4.5 Determination of the SPF of the sample to be tested: The MED is to be determined on the day of the

test in all three cases simultaneously.

4.4.5.1 Determination of the subject's unprotected skin MED: The UV dose was adjusted according to the predicted MED value in 4.4.4 and the subject's unprotected skin MED was determined again on the day of the test.

4.4.5.2 The MED of the subject's skin under product protection was determined by applying the test product to the subject's skin and then determining the MED of the skin under product protection as described in 4.4.4. In selecting the increase in dose at the 5-point test site, reference was made to the range of SPF values for which the sunscreen product was formulated: for products with an SPF of ≤15, the incremental dose at the five points was 25%; for products with an SPF >15, the incremental dose at the five points was at least 12%. For SPF >15, the dose increment at the five exposure points should be at least 12%.

4.4.5.3 To determine the MED of the subject's skin under the protection of the standard sample: apply the SPF standard sample to the subject area. For SPF values

For products with ≤15, choose a low SPF standard, for products with SPF >15, a high SPF standard is preferable (P2)

(or P3). The MED of the skin under the protection of the standard sample was determined in the same way as in 4.4.4.

4.5 Exclusion criteria: If no red spots appear on any of the 5 test points, or if red spots appear on all 5 test points, or if red spots appear randomly on the test points, the results should be ruled invalid and the instrumentation should be calibrated and re-measured.

4.6 Calculation of SPF values

The SPF of the sample for an individual subject is calculated using the following equation.

$$\text{Individual SPF} = \frac{\text{Sample MED for skin protection}}{\text{MED for unprotected skin}}$$

The arithmetic mean of the SPF values of all subjects protected by the sample is calculated and the integer part is the SPF value for that assay sample. The sampling error of the mean can be estimated by calculating the standard deviation and standard error of the data for the group. The 95% confidence interval (95% CI) of the mean is required to be no more than 17% of the mean (if the mean is 10, the 95% CI should be between 8.3 and 11.7), otherwise the number of subjects should be increased (to no more than 25) until the above requirements are met.

5 Inspection reports

The report should include the following: general information on the subject including sample number, name, production batch number, production and delivery units, sample physical description and test start and end time, test purpose, materials and methods, test results, conclusions. The inspection report should be the inspector, checker and technical person in charge of the signature, and stamped with the official seal of the inspection unit. The test results are given in the form of a table (see Table 2).

Table 2 Results of SPF determination for standard controls and samples

Subject number	Gender	Skin Type	Age	Standard SPF	Sample to be tested SPF value
01					
02					
03					
04					
05					
06					
07					
08					
09					
10					
Mean X					

Standard deviation SD	
95% CI	

Appendix I Preparation of Low-SPF Standards

Ⅰ1 When determining the SPF of sunscreen products, it is necessary to measure sunscreen standards as controls at the same time to ensure the validity and consistency of the test results.

I2 The sunscreen standard is an 8% trimethylene ring already ester salicylate product with a mean SPF of 4.47 and a standard deviation of 1.297.

I3 The SPF value of the standard measured must lie within the standard deviation of the known SPF value, i.e. 4.47 ± 1.297, and must include SPF value 4 within the 95% confidence limit of the measured SPF value.

I4 The standards were prepared as follows.

Table 3 Preparation of sunscreen standards

	Ingredients	Weight ratio %
Phase A.		
	Humosalate (trimethylcyclic acid salicylate, Homosalate)	8.00
	Lanolin (Lanolin)	5.00
	Stearic acid (Stearic acid)	4.00
	White petrolatum	2.50
	Propyl paraben (Propylparaben)	0.05
Phase B.		
	Purified water	74.30
	1,2-Propanediol (Propylene glycol)	5.00
	Triethanolamine	1.00
	Methyl p-hydroxybenzoate (Methylparaben)	0.10
	Disodium EDTA (EDTA)	0.05

Preparation method: Heat phase A and phase B to 72℃～82℃ respectively and stir continuously until all components are dissolved. Add phase A to phase B while stirring and continue stirring until the resulting emulsion cools to room temperature (15℃～30℃), resulting in 100g of sunscreen standard.

Appendix II Preparation of high-SPF standards (P2, P3)

The specific formulation, production process and quality standards for the high SPF standards (P2, P3) are described in the international SPF determination methods

(Annex V of the International Sun Protection Factor (SPF) Test Method, 2006).

(ii) Determination method for water resistance of sunscreen cosmetics

1 Introduction

The history of sunscreen cosmetics shows that water and sweat resistance is a classic attribute of sunscreen products. As sunscreens, especially those with high SPF values, are often used during outdoor sports in summer, the season and the environment in which they are used require them to be water and sweat resistant, i.e. to maintain a certain level of protection from the sun even under sweat or swimming conditions.

Products that are waterproof are often labelled as "waterproof and sweatproof" and "suitable for outdoor activities such as swimming".

2 Normative references

The U.S. Food and Drug Administration (FDA) has established a Testing Procedure for the determination of the sun protection index of sunscreen products (Testing Procedure.

(Federal Register/Vol 64, No98/1999)

3 Equipment requirements

Indoor pools, whirlpools or flowing bathtubs are all acceptable, water temperature should be maintained at 23°C to 32°C and the water should be fresh. Record water and room temperatures and relative humidity.

4 Test methods

4.1 Tests on the general water resistance of sunscreens

If the product is claimed to be water resistant, the SPF value indicated shall be the SPF value of the product as measured by the following 40min water resistance test.

4.1.1 Apply sunscreen to the tested area of the skin and wait 15min or as required by the label instructions.

4.1.2 Subjects were moved in the water at a moderate level or the water was rotated at a moderate level for 20min.

4.1.3 Take a 20min break out of the water (do not wipe the test area with a towel).

4.1.4 Into the water for a further 20min of moderate activity.

4.1.5 End the water activity and wait for the skin to dry (do not rub the test area with a towel).

4.1.6 UV exposure and measurement according to the SPF determination method specified in this specification.

4.2 Test for superior water resistance of sunscreens

If the SPF value of a product is claimed to be very water resistant, the SPF value indicated should be the SPF value of the product measured after the following 80min water resistance test.

4.2.1 Apply sunscreen to the tested area of the skin and wait 15min or as required by the label instructions.

4.2.2 Subjects were active in the water for 20min at a moderate level.

4.2.3 Take a 20min break out of the water (do not wipe the test area with a towel).

4.2.4 Into the water for a further 20min of moderate activity.

4.2.5 Take a 20min break out of the water (do not wipe the test area with a towel).

4.2.6 Into the water for a further 20min of moderate activity.

4.2.7 Take a 20min break out of the water (do not wipe the test area with a towel).

4.2.8 Into the water for a further 20min of moderate activity.

4.2.9 End the water activity and wait for the skin to dry (do not rub the test area with a towel).

4.2.10 UV exposure and measurement according to the SPF determination method specified in this specification.

5 Markings

Indicate the value measured after bathing. If the SPF value measured after bathing is reduced by more than 50%, the product must not be labelled as water resistant, with reference to the SPF value marked before the water resistance test or the predicted SPF value.

(iii) Sunscreen cosmetics long-wave UV protection index (PFA value) determination method

1 Introduction

Labelling and advertising UVA protection or broad-spectrum sun protection is becoming increasingly common in the sunscreen cosmetics market. Human testing of PFA values or PA + to PA + + + + expressions on sunscreen cosmetic labels is more commonly used and is recognised by most countries, cosmetic companies and consumers internationally.

2 Normative references

UVA Protection Effect Measurement Method Standard (Japan Cosmetic Industry Federation: UV Protection Effect of Cosmetics for UV Protection, 2003)

3 Definition

3.1 Ultraviolet wavelength

Short-wave ultraviolet (UVC): 200nm～290nm Medium-wave ultraviolet (UVB): 290nm～320nm
Long-wave ultraviolet (UVA): 320nm～400nm

3.2 Minimal persistent pigment darkening dose (MPPD): The minimum dose or duration of UV irradiation required to produce a slight darkening of the skin over the entire exposure area 2 to 4 hours after irradiation. The MPPD should be observed at a fixed point within 2 to 4 hours of exposure, in a well-lit room, and by at least two trained observers.

3.3 Protection factor of UVA (PFA): The ratio of the MPPD required to cause darkening of the skin protected by a sunscreen to the MPPD required to cause darkening of unprotected skin is the PFA value of the sunscreen. This can be expressed as follows.

$$\text{PFA} = \frac{\text{MPPD for skin protection with sunscreen cosmetics}}{\text{MPPD for unprotected skin}}$$

4 Test method

4.1 Selection of subjects and test sites

4.1.1 Healthy people aged 18 to 60, both men and women.

4.1.2 Subjects with skin types III and IV, i.e. those with varying degrees of skin pigmentation following UV exposure.

4.1.3 Subjects should have no history of photosensitive skin conditions.

4.1.4 No medications such as anti-inflammatory drugs, antihistamines etc. were taken prior to the trial.

4.1.5 The test site was the back. The skin was uniformly coloured, with no pigmented nevi or other discolourations, etc.

4.2 Number of Subjects

The number of subjects in each trial should be 10 or more, with a maximum of 20.

4.3 Sample dose used

The sample should be applied accurately and evenly to the skin of the test area in the same manner as the actual application. The skin of the test area should be marked with a marker to indicate the boundaries and different weighing and application methods may be used for different dosage forms.

4.4 Sample application area

Approximately ^{30cm2} or more. To minimise errors in sample weighing, the sample coating area or total sample volume should be as large as possible.

4.5 Waiting time

After application of the sample you should wait 15min for the sample to moisten the skin or dry on the skin.

4.6 Ultraviolet light sources

Artificial light should be used and the following conditions should be met.

4.6.1 Emits a continuous spectrum of light in the UVA region close to daylight. The light source output should remain stable and relatively homogeneous in the beam irradiation plane.

4.6.2 To avoid UV burns, UV light with wavelengths shorter than 320 nm should be filtered out using appropriate filters. Visible and infrared light at wavelengths greater than 400 nm should also be filtered out to avoid their blackening and thermogenic effects.

4.6.3 These conditions should be regularly monitored and maintained. The irradiance of the light source should be measured by a UV irradiometer, the results of regular monitoring should be recorded, the irradiance should be measured promptly each time a major optical component is replaced and the irradiometer should be calibrated by the manufacturer at least once a year. Changes in the intensity and spectrum of the light source can cause changes in the subject's MPPD and should therefore be carefully monitored and the light bulb replaced if necessary.

4.7 Minimum irradiated area

The minimum irradiated area of a single spot should not be less than 0.5 ^{cm2} (8 mm). The irradiated area should be the same for unprotected skin and sample protected skin.

4.8 Incremental UV irradiation dose

The maximum increase in UV irradiation should not exceed 25% for multi-point increments. The smaller the increase, the more accurate the measured PFA value.

4.9 The PFA value is calculated using the following formula.

$$PFA = \frac{MPPDp}{MPPDu}$$

where MPPDp is the MPPD of the skin protected by the test product; MPPDu is the MPPD of the unprotected skin.

The arithmetic mean of the PFA values of all subjects protected by the sample is calculated and the integer part is the PFA value of the sample measured. The sampling error of the mean can be estimated by calculating the standard deviation and standard error of the data for the group. It is required that the standard error should be less than 10% of the mean, otherwise the number of subjects should be increased (to no more than 20) until the above requirements are met.

5 Marking methods for UVA protection

UVA protection is indicated on the product label by the UVA protection level PA according to the size of the measured PFA value

The PF rating should be identified together with the SPF value of the product; the PFA value is taken as an integer only and converted to a PA rating by the following formula.

PFA value less than 2	No UVA protection
PFA value 2 to 3	PA+
PFA value 4 to 7	PA++
PFA value of 8 or more	PA + + +

Appendix: Preparation of standards

The standard formulations are shown in Table 1.

Table 1 Standard product formulations

	Ingredients	Weight ratio %
Phase A.		
	Purified water	57.13
	Dipropylene glycol	5.00
	Phenoxyethanol (Phenoxyethanol)	0.30
	Potassium hydroxide (Potassium hydroxide)	0.12
	Trisodium EDTA (Trisodium edetate)	0.05
Phase B.		
	Glyceryl tri-2-ethylhexanoate	15.00
	Cetearyl alcohol (sixteen/octadecanol)	5.00
	Butyl methoxydibenzoylmethane (BMDM)	5.00
	Mineral fat or petroleum jelly (Petrolatum)	3.00
	Stearic acid (Stearic acid)	3.00
	Ethylhexyl methoxycinnamate	3.00
	Glyceryl monostearate (selfmulsifying)	3.00
	Methyl p-hydroxybenzoate (Methylparaben)	0.20
	Ethyl p-hydroxybenzoate (Ethylparaben)	0.20

Preparation process: Weigh out the raw materials in phase A separately, dissolve in pure water and heat to 70°C. Weigh out the raw materials in phase B separately and heat to 70°C until completely dissolved. Add phase B to phase A, mix, emulsify, stir and cool. The standard prepared by the above method has a PFA value of 3.75 with a standard deviation of 1.01.

EXHIBIT 3

(化妆品安全技术规范 - 2015 年版)

Safety and Technical Standards for Cosmetics (2015)

Translation of Chapter I overview and Chapter VI toxicological test methods

第一章 概述

1 范围

本规范规定了化妆品的安全技术要求，包括通用要求、禁限用组分要求、准用组分要求以及检验评价方法等。

本规范适用于中华人民共和国境内生产和经营的化妆品（仅供境外销售的产品除外）。

Chapter I overview

1 Range

This Standards specify the safety technical requirements of cosmetics, including general requirements, prohibited and restricted components requirements, approved components requirements, inspection and evaluation methods, etc.

This Standards are applicable to cosmetics produced and operated within the territory of the People's Republic of China (except products only for overseas sales).

2 术语和释义

下列术语和释义适用于本规范。

2.1 化妆品原料：化妆品配方中使用的成分。

2.2 化妆品新原料：在国内首次使用于化妆品生产的天然或人工原料。

2.3 禁用组分：不得作为化妆品原料使用的物质。

2.4 限用组分：在限定条件下可作为化妆品原料使用的物质。

2.5 防腐剂：以抑制微生物在化妆品中的生长为目的而在化妆品中加入的物质。

2.6 防晒剂：利用光的吸收、反射或散射作用，以保护皮肤免受特定紫外线所带来的伤害或保护产品本身而在化妆品中加入的物质。

2.7 着色剂：利用吸收或反射可见光的原理，为使化妆品或其施用部位呈现颜色而在化妆品中加入的物质，但不包括第三章表 7 中规定的染发剂。

2.8 染发剂：为改变头发颜色而在化妆品中加入的物质。

2 Terms and interpretation

The following terms and interpretation apply to this specification.

2.1 Cosmetic raw materials: ingredients used in cosmetic formula.

2.2 New cosmetics raw materials: natural or artificial raw materials first used in cosmetics production in China.

2.3 Prohibited components: substances that cannot be used as cosmetic raw materials.

2.4 Restricted components: substances that can be used as cosmetic raw materials under limited conditions.

2.5 Preservative: substance added to cosmetics for the purpose of inhibiting the growth of microorganisms in cosmetics.

2.6 Sunscreen: a substance added to a cosmetic product to protect the skin from specific ultraviolet rays or to protect the product itself by the absorption, reflection, or scattering of light.

2.7 Colorant: a substance added to a cosmetic to make the cosmetic or its application part appear color by using the principle of absorption or reflection of visible light, but excluding the hair dye specified in Table 7 of chapter III.

2.8 Hair dye: substance added to cosmetics to change the color of hair.

2.9 淋洗类化妆品：在人体表面（皮肤、毛发、甲、口唇等）使用后及时清洗的化妆品。

2.10 驻留类化妆品：除淋洗类产品外的化妆品。

2.11 眼部化妆品：宣称用于眼周皮肤、睫毛部位的化妆品。

2.12 口唇化妆品：宣称用于嘴唇部的化妆品。

2.13 体用化妆品：宣称用于身体皮肤（不含头面部皮肤）的化妆品。

2.14 肤用化妆品：宣称用于皮肤上的化妆品。

2.15 儿童化妆品：宣称适用于儿童使用的化妆品。

2.16 专业使用：在专门场所由经过专业培训的人员操作使用。

2.17 包装材料：直接接触化妆品原料或化妆品的包装容器材料。

2.18 安全性风险物质：由化妆品原料、包装材料、生产、运输和存储过程中产生或带入的，暴露于人体可能对人体健康造成潜在危害的物质。

2.9 Rinsing cosmetics: cosmetics that are ished in time after use on the surface of human body (skin, hair, nail, lips, etc.).

2.10 Resident cosmetics: cosmetics other than rinsing products.

2.11 Eye cosmetics: cosmetics claimed to be used for the skin around the eyes and eyelashes.

2.12 Lip cosmetics: cosmetics claimed to be used on the lips.

2.13 Body cosmetics: cosmetics claimed to be used for the skin of the body (excluding the skin of the head and face).

2.14 Skin cosmetics: cosmetics claimed to be used on the skin.

2.15 Children's cosmetics: cosmetics claimed to be suitable for children.

2.16 Professional use: it is operated and used by specially trained personnel in special places.

2.17 Packaging materials: packaging materials that directly contact cosmetics raw materials or cosmetics.

2.18 Safety risk substances: substances generated or brought in by cosmetics raw materials, packaging materials, production, transportation and storage, which may cause potential harm to human health when exposed to human body.

3 化妆品安全通用要求

3.1 一般要求

3.1.1 化妆品应经安全性风险评估，确保在正常、合理的及可预见的使用条件下，不得对人体健康产生危害。

3.1.2 化妆品生产应符合化妆品生产规范的要求。化妆品的生产过程应科学合理，保证产品安全。

3.1.3 化妆品上市前应进行必要的检验，检验方法包括相关理化检验方法、微生物检验方法、毒理学试验方法和人体安全试验方法等。

3.1.4 化妆品应符合产品质量安全有关要求，经检验合格后方可出厂。

3 General requirements for cosmetics safety

3.1 General requirements

3.1.1 Cosmetics shall be subject to safety risk assessment to ensure that under normal, reasonable and foreseeable conditions of use, they shall not cause harm to human health.

3.1.2 The production of cosmetics shall meet the requirements of the cosmetics production specifications. The production process of cosmetics should be scientific and reasonable to ensure product safety.

3.1.3 Necessary inspection shall be carried out before cosmetics are put on the market, including relevant physical and chemical inspection methods, microbial inspection methods, toxicology test methods and human safety test methods.

3.1.4 Cosmetics shall meet the relevant requirements of product quality and safety, and can be delivered only after passing the inspection.

3.2 配方要求

3.2.1 化妆品配方不得使用本规范第二章表 1 和表 2 所列的化妆品禁用组分。若技术上无法避免禁用物质作为杂质带入化妆品时，国家有限量规定的应符合其规定；未规定限量的，应进行安全性风险评估，确保在正常、合理及可预见的适用条件下不得对人体健康产生危害。

3.2.2 化妆品配方中的原料如属于本规范第二章表 3 化妆品限用组分中所列的物质，使用要求应符合表中规定。

3.2.3 化妆品配方中所用防腐剂、防晒剂、着色剂、染发剂，必须是对应的本规范第三章表 4 至表 7 中所列的物质，使用要求应符合表中规定。

3.3 微生物学指标要求

化妆品中微生物指标应符合表 1 中规定的限值。

3.2 Formula requirements

3.2.1 The prohibited components of cosmetics listed in Table 1 and table 2 of the second chapter of this specification shall not be used in cosmetics formula.

If it is technically impossible to avoid prohibited substances being brought into cosmetics as impurities, the national limited provisions shall be complied with; If no limit is specified, safety risk assessment shall be carried out to ensure no harm to human health under normal, reasonable and foreseeable applicable conditions.

3.2.2 If the raw materials in the cosmetic formula belong to the substances listed in Table 3 of Chapter II of this specification, the use requirements shall meet the provisions in the table.

3.2.3 Preservatives, sunscreens, colorants and hair dyes used in the cosmetics formula must be the substances listed in tables 4 to 7 in Chapter III of this specification, and the use requirements shall meet the regulations in the table.

3.3 Microbiological index requirements

Microbiological indicators in cosmetics shall meet the limits specified in Table 1.

表 1 化妆品中微生物指标限值

微生物指标	限值	备注
菌落总数（CFU/g 或 CFU/ml）	≤500	眼部化妆品、口唇化妆品和儿童化妆品
	≤1000	其他化妆品
霉菌和酵母菌总数（CFU/g 或 CFU/ml）	≤100	
耐热大肠菌群/g（或 ml）	不得检出	
金黄色葡萄球菌/g（或 ml）	不得检出	
铜绿假单胞菌/g（或 ml）	不得检出	

Table 1 Limit values of microbiological indicators in cosmetics

Microbial index	Limit value	Remarks
Total number of colonies (CFU / g or CFU / ml)	≤ 500	Eye cosmetics, lip cosmetics
	≤ 1000	children's cosmetics
Total number of mold and yeast (CFU / g or CFU / ml)	≤ 100	other cosmetics
Thermotolerant coliforms / g (or ml)	shall not be detected	
Staphylococcus aureus / g (or ml)	shall not be detected	
Pseudomonas aeruginosa / g (or ml)	shall not be detected	

3.4 有害物质限值要求

化妆品中有害物质不得超过表 2 中规定的限值。

表 2 化妆品中有害物质限值

有害物质	限值（mg/kg）	备注
汞	1	含有机汞防腐剂的眼部化妆品除外
铅	10	
砷	2	
镉	5	
甲醇	2000	
二噁烷	30	
石棉	不得检出*	

3.4 Limit requirements for hazardous substances

Hazardous substances in cosmetics shall not exceed the limits specified in Table 2.

Table 2 limits of harmful substances in cosmetics

Hazardous substances	Limit (mg / kg)	Remarks
mercury	1	Except for eye cosmetics containing organomercury preservatives
lead	10	
arsenic	2	
cadmium	5	
methanol	2000	
Dioxane	30	
asbestos	No detection*	

3.5 包装材料要求

直接接触化妆品的包装材料应当安全，不得与化妆品发生化学反应，不得迁移或释放对人体产生危害的有毒有害物质。

3.6 标签要求

3.6.1 凡化妆品中所用原料按照本技术规范需在标签上标印使用条件和注意事项的，应按相应要求标注。

3.6.2 其他要求应符合国家有关法律法规和规章标准要求。

3.7 儿童用化妆品要求

3.7.1 儿童用化妆品在原料、配方、生产过程、标签、使用方式和质量安全控制等方面除满足正常的化妆品安全性要求外，还应满足相关特定的要求，以保证产品的安全性。

3.7.2 儿童用化妆品应在标签中明确适用对象。

3.5 Packaging material requirements

Packaging materials in direct contact with cosmetics shall be safe, shall not react with cosmetics, and shall not migrate or release toxic and harmful substances harmful to human body.

3.6 Label requirements

3.6.1 If the raw materials used in cosmetics need to be marked with the use conditions and precautions according to this technical specification, they shall be marked according to the corresponding requirements.

3.6.2 Other requirements shall meet the requirements of relevant national laws, regulations and standards.

3.7 Cosmetic requirements for children

3.7.1 In addition to the normal safety requirements of cosmetics, children's cosmetics should also meet the relevant specific requirements in terms of raw materials, formula, production process, label, use mode and quality safety control, so as to ensure the safety of products.

3.7.2 Children's cosmetics should be clearly identified in the label.

3.8 原料要求

3.8.1 化妆品原料应经安全性风险评估，确保在正常、合理及可预见的使用条件下，不得对人体健康产生危害。

3.8.2 化妆品原料质量安全要求应符合国家相应规定，并与生产工艺和检测技术所达到的水平相适应。

3.8.3 原料技术要求内容包括化妆品原料名称、登记号（CAS 号和/或 EINECS 号、INCI 名称、拉丁学名等）、使用目的、适用范围、规格、检测方法、可能存在的安全性风险物质及其控制措施等内容。

3.8.4 化妆品原料的包装、储运、使用等过程，均不得对化妆品原料造成污染。

直接接触化妆品原料的包装材料应当安全，不得与原料发生化学反应，不得迁移或释放对人体产生危害的有毒有害物质。

对有温度、相对湿度或其他特殊要求的化妆品原料应按规定条件储存。

3.8.5 化妆品原料应能通过标签追溯到原料的基本信息（包括但不限于原料标准中文名称、INCI 名称、CAS 号和/或 EINECS 号）、生产商名称、纯度或含量、生产批号或生产日期、保质期等中文标识。

属于危险化学品的化妆品原料，其标识应符合国家有关部门的规定。

3.8.6 动植物来源的化妆品原料应明确其来源、使用部位等信息。

动物脏器组织及血液制品或提取物的化妆品原料，应明确其来源、质量规格，不得使用未在原产国获准使用的此类原料。

3.8.7 使用化妆品新原料应符合国家有关规定。

3.8 Raw material requirements

3.8.1 Cosmetic raw materials shall be subject to safety risk assessment to ensure that under normal, reasonable and foreseeable conditions of use, there is no harm to human health.

3.8.2 The quality and safety requirements of cosmetics raw materials shall comply with the relevant national regulations and be compatible with the level achieved by the production process and detection technology.

3.8.3 The technical requirements for raw materials include the name of cosmetic raw materials, registration number (CAS number and / or EINECS number, INCI name, Latin scientific name, etc.), purpose of use, scope of application, specifications, testing methods, potential safety risk substances and control measures.

3.8.4 The packaging, storage, transportation and use of cosmetics raw materials shall not cause pollution to cosmetics raw materials.

Packaging materials that directly contact cosmetic raw materials shall be safe, shall not have chemical reaction with raw materials, and shall not migrate or release toxic and harmful substances harmful to human body.

Cosmetics raw materials with temperature, relative humidity or other special requirements shall be stored according to the specified conditions.

3.8.5 Cosmetic raw materials shall be able to trace the basic information of raw materials (including but not limited to the Chinese name of raw material standard, inci name, CAS number and / or EINECS number), manufacturer name, purity or content, production batch number or production date, shelf life and other Chinese marks through labels.

The identification of cosmetics raw materials belonging to hazardous chemicals shall conform to the regulations of relevant national departments.

3.8.6 The raw materials of cosmetics from animals and plants should be clear about their sources, using parts and other information.

The source and quality specification of cosmetics raw materials for animal organ tissue and blood products or extracts shall be specified. Such raw materials not approved for use in the country of origin shall not be used.

3.8.7 The use of new cosmetic raw materials shall comply with the relevant regulations of the state.

第六章 毒理学试验方法

1 毒理学试验方法总则

General Principles

1 范围

本部分规定了化妆品原料及其产品安全性评价的毒理学检测要求。本部分适用于对化妆品原料及其产品的安全性评价。

Chapter VI Toxicological Test Methods

1 General principles of toxicological test methods

General Principles

1 Range

This part specifies the toxicological test requirements for the safety evaluation of cosmetics raw materials and products. This part is applicable to the safety evaluation of cosmetics raw materials and products.

2 化妆品原料的安全性评价的毒理学检测

2.1 评价原则

化妆品原料在正常以及合理的、可预见的使用条件下，不得对人体健康产生危害。

2.2 毒理学检测项目的选择原则

化妆品的新原料，一般需进行下列毒理学试验：

（1）急性经口和急性经皮毒性试验；

（2）皮肤和急性眼刺激性/腐蚀性试验；

（3）皮肤变态反应试验；

（4）皮肤光毒性和光敏感试验※（原料具有紫外线吸收特性需做该项试验）；

（5）致突变试验（至少应包括一项基因突变试验和一项染色体畸变试验）；

（6）亚慢性经口和经皮毒性试验；

（7）致畸试验；

（8）慢性毒性/致癌性结合试验；

（9）毒物代谢及动力学试验※；

（10）根据原料的特性和用途，还可考虑其他必要的试验。如果该新原料与已用于化妆品的原料化学结构及特性相似，则可考虑减少某些试验。

本规定毒理学试验为原则性要求，可以根据该原料理化特性、定量构效关系、毒理学资料、临床研究、人群流行病学调查以及类似化合物的毒性等资料情况，增加或减免试验项目。

*试验方法参照 GB7919-87 化妆品安全性评价程序和方法；
OECD 化学物质试验指南(OECD Guidelines for Testing of Chemicals)

2 Toxicological test for safety evaluation of cosmetic raw materials

2.1 Evaluation principle

Under normal, reasonable and foreseeable conditions of use, cosmetic raw materials shall not cause harm to human health.

2.2 Selection principle of toxicology test items

The following toxicological tests are generally required for the new raw materials of cosmetics:

(1) Acute oral and dermal toxicity test;

(2) Skin and acute eye irritation / corrosiveness test;

(3) Skin allergy test;

(4) Skin phototoxicity and light sensitivity test ※ (this test is required for raw materials with UV absorption characteristics);

(5) Mutagenicity test (including at least one gene mutation test and one chromosome aberration test);

(6) Subchronic oral and percutaneous toxicity tests;

(7) Teratogenesis test;

(8) Chronic toxicity / carcinogenicity combination test;

(9) Toxicant metabolism and kinetics test ※;

(10) Other necessary tests can be considered according to the characteristics and use of raw materials. If the chemical structure and characteristics of the new raw material are similar to those of the raw material used in cosmetics, some tests may be considered to be reduced.

The toxicology test in this regulation is a principle requirement, and the test items can be increased or reduced according to the physical and chemical characteristics of the raw material, quantitative structure-activity relationship, toxicology data, clinical research, population epidemiological investigation and toxicity of similar compounds.

*The test methods refer to GB7919-87 cosmetics safety evaluation procedures and methods; OECD guidelines for testing of chemicals

3 化妆品产品安全性评价的毒理学检测

3.1 评价原则

在一般情况下，新开发的化妆品产品在投放市场前，应根据产品的用途和类别进行相应的试验，以评价其安全性。

3.2 检测项目的选择原则

3.2.1 由于化妆品种类繁多，在选择试验项目时应根据实际情况确定。

3.2.2 每天使用的化妆品需进行多次皮肤刺激性试验，进行多次皮肤刺激性试验者不再进行急性皮肤刺激性试验，间隔 1 日或数日使用和用后冲洗的化妆品进行急性皮肤刺激性试验。

3.2.3 与眼接触可能性小的产品不需进行急性眼刺激性试验。

3 Toxicological test for safety evaluation of cosmetic products

3.1 Evaluation principle

In general, before the newly developed cosmetic products are put on the market, corresponding tests should be carried out according to the use and category of the products to evaluate their safety.

3.2 Selection principle of test items

3.2.1 Due to the wide variety of cosmetics, the test items should be selected according to the actual situation.

3.2.2 The cosmetics used every day need to undergo multiple skin irritation tests. Those who have conducted multiple skin irritation tests will no longer undergo the acute skin irritation test, and the cosmetics used and rinsed after use will undergo the acute skin irritation test at an interval of 1 day or several days.

3.2.3 It is not necessary to carry out acute eye irritation test for products with low possibility of eye contact.

2 急性经口毒性试验

Acute Oral Toxicity Test

1 范围

本规范规定了动物急性经口毒性试验的基本原则、要求和方法。本规范适用于化妆品原料安全性毒理学检测。

2 试验目的

急性经口毒性试验是评估化妆品原料毒性特性的第一步，通过短时间经口染毒可提供对健康危害的信息。试验结果可作为化妆品原料毒性分级和标签标识以及确定亚慢性毒性试验和其他毒理学试验剂量的依据。

2 Acute oral toxicity test

1 Range

This Standards specifies the basic principles, requirements and methods of animal acute oral toxicity test. This Standards is applicable to the safety toxicology test of cosmetic raw materials.

2 Test purpose

Acute oral toxicity test is the first step to evaluate the toxicity characteristics of cosmetic raw materials, which can provide information on health hazards through short-term oral exposure. The test results can be used as the basis for toxicity classification and labeling of cosmetic raw materials, as well as for determining the dose of subchronic toxicity test and other toxicological tests.

3 定义

3.1 急性经口毒性 acute oral toxicity

一次或在 24h 内多次经口给予实验动物受试物后，动物在短期内出现的健康损害效应。

3.2 经口 LD50 半数致死量 medium lethal dose

经口一次给予受试物后，引起实验动物总体中半数死亡的毒物的统计学剂量。以单位体重接受受试物的重量(mg/kg 或 g/kg)来表示。

4 试验的基本原则

以管饲法经口给予各试验组动物不同剂量的受试物，每组用一个剂量，染毒剂量的选择可通过预试验确定。染毒后观察动物的毒性反应和死亡情况。试验期间死亡的动物要进行尸检，试验结束时仍存活的动物要处死并进行尸检。本方法主要适用于啮齿类动物的研究，但也可用于非啮齿类动物的研究。

3 Definition

3.1 Acute oral toxicity

The effect of health damage in a short period of time after one or more times of oral administration of test substance in 24 hours.

3.2 Medium lethal dose

The statistical dose of the toxicant that causes half of the total deaths of the experimental animals after one oral administration of the test substance. The weight of the test substance received per unit weight (mg / kg or g / kg).

4 Basic principles of test

Different doses of test substance are given orally by tube feeding method to each experimental group. Each group is given one dose. The selection of dose could be determined by pre-test. The toxicity and death of the animals are observed. The animals that died during the experiment should be autopsied, and the animals that survived at the end of the experiment should be executed and autopsied. This method is mainly suitable for the study of rodents, but it can also be used for the study of non-rodents.

5 试验方法

5.1 受试物

受试物应溶解或悬浮于适宜的介质中，建议首选水，其次是植物油(如玉米油)，或考虑使用其他介质（如羧甲基纤维素、明胶、淀粉等）。对非水溶性介质，应了解其毒理特性，否则应在试验前先确定其毒性。每次经口染毒液体的最大容量取决于实验动物的

大小，对啮齿类动物所给液体容量一般为 1mL/100g， 水溶液可至 2mL/100g。通过调整受试物溶液浓度使各剂量组经口染毒的容量一致。

5.2 实验动物和饲养环境

首选健康成年大鼠和小鼠，也可选用其他敏感动物。使用雌性动物应是未孕和未曾产仔的。实验动物体重之间相差不得超过平均体重的 20%。试验前动物要在实验动物房环境中至少适应 3—5d 时间。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Test substance

The test substance should be dissolved or suspended in a suitable medium. Water is the prior choice, followed by vegetable oil (such as corn oil), or other medium (such as carboxymethylcellulose, gelatin, starch, etc.). For non-water soluble media, the toxicological characteristics should be understood, otherwise the toxicity should be determined before the test. The maximum volume of liquid for each oral exposure depends on the size of experimental animals. The volume of liquid for rodents is generally 1ml / 100g, and the volume of water solution can reach 2ml / 100g. By adjusting the concentration of the test solution, the volume of each dose group is the same.

5.2 Laboratory animals and feeding environment

Healthy adult rats and mice are prior choice, and other sensitive animals can also be selected. Female animals should not be pregnant and have never been given birth. The difference between the weight of experimental animals shall not exceed 20% of the average weight. Before the experiment, the animals should adapt to the environment of the laboratory for at least 3-5 days.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Standard compound feed is selected, and drinking water is not limited.

5.3 剂量水平

根据所选方法的要求，原则上应设 4—6 个剂量组，每组动物一般为 10 只，雌雄各半。

各剂量组间距大小以兼顾产生毒性大小和死亡为宜，通常以较大组距和较少量动物进行预试。如果受试物毒性很低，也可采用一次限量法，即用 10 只动物（雌雄各半）口服 5000mg/kg 体重剂量，当未引起动物死亡，可考虑不再进行多个剂量的急性经口毒性试验。

5.3 Dose level

According to the requirements of the selected method, 4-6 dose groups should be set up in principle, with 10 animals in each group, half male and half female.

The distance of each dose group is suitable for both toxicity and death, and the larger group distance and the smaller number of animals are usually used for pre test. If the toxicity of the test substance is very low, the one-time limit method can also be used, that is to say, 10 animals (half male and half female) can be used to orally take 5000mg / kg body weight dose. When the animal does not die, it can be considered not to carry out multiple doses of acute oral toxicity test.

5.4 试验步骤

5.4.1 试验前，实验动物禁食过夜，不限制饮水。若采用代谢率高的其他动物，禁食时间可以适当缩短。

5.4.2 正式试验时，称量动物体重，随机分组，然后对各组动物用管饲法一次进行染毒，若估计受试物毒性很低，一次给予容量太大，也可在 24h 内分 2—3 次染毒，但合并作为一次剂量计算。染毒后继续禁食 3 h—4 h。若采用分批多次染毒，根据染毒间隔长短，必要时可给动物一定量的食物和水。

5.4.3 染毒后，对每只动物都应有单独全面的记录，染毒第 1 d 要定时观察实验动物的中毒表现和死亡情况，其后至少每天进行一次仔细的检查。详细记录被毛和皮肤、眼睛和粘膜，呼吸、循环、自主神经和中枢神经系统、肢体活动和行为等改变。特别注意是否出现震颤、抽搐、流涎、腹泻、嗜睡和昏迷等症状。应记录毒作用体征出现和消失的时间和死亡时间。

5.4 Test procedure

5.4.1 Before the experiment, the experimental animals are fasted overnight, but drinking water is not restricted. If other animals with high metabolic rate are used, the fasting time can be shortened appropriately.

5.4.2 In the formal test, the animals are weighed and randomly divided into groups. Then the animals in each group are exposed to the drug once by tube feeding method. If the toxicity of the test substance is estimated to be very low and the capacity of one-time administration is too large, it can also be exposed to the drug 2-3 times in 24 hours, but it is combined as one-time dose calculation. Fasting continues for 3-4 hours. If it is used for multiple times in batches, a certain amount of food and water can be given to the animals if necessary according to the length of poisoning interval.

5.4.3 After poisoning, each animal shall be recorded separately and comprehensively. On the first day of poisoning, the poisoning performance and death of experimental animals shall be regularly observed, and then at least one careful inspection shall be conducted every day. The changes of hair and skin, eyes and mucous membrane, respiration, circulation, autonomic and central nervous system, limb activity and behavior are recorded in detail. Pay special attention to the symptoms of tremor, convulsion, salivation, diarrhea, drowsiness and coma. The time of appearance and disappearance of signs of toxic action and the time of death should be recorded.

5.4.4 观察期限一般不超过 14d，但观察时间并非一成不变，要视动物中毒反应的严重程度、症状出现快慢和恢复期长短而定。若有死亡延迟迹象，可延长观察时间。

观察期内存活动物每周称重，观察期结束存活动物应称重，处死后进行尸检。

5.4.5 对实验动物进行大体解剖学检查，并记录全部大体病理改变。对死亡和存活 24h 和 24h 以上动物并存在大体病理改变的器官应进行病理组织学检查。

5.4.6 可采用多种方法测定 LD50，建议采用一次最大限度试验、霍恩氏法、上-下法、概率单位-对数图解法和寇氏法等。

5.5 试验结果评价

评价试验结果时，应将 LD50 与观察到的毒性效应和尸检所见相结合考虑，LD50 值是受试物毒性分级和标签标识以及判定受试物经消化道摄入后引起动物死亡可能性大小的依据。引用 LD50 值时一定要注明所用实验动物的种属、性别、染毒途径、观察期限

等。评价应包括动物接触受试物与动物异常表现（包括行为和临床改变、大体损伤、体重变化、致死效应及其他毒性作用)的发生率和严重程度之间的关系。

毒性分级见表 1。

5.4.4 Generally, the observation period is not more than 14 days, but the observation time is not invariable. It depends on the severity of animal poisoning reaction, the speed of symptoms and the length of recovery period. If there are signs of death delay, the observation time can be prolonged.

During the observation period, the active objects are weighed every week, and the living animals should be weighed at the end of the observation period, and then the autopsy is performed.

5.4.5 The gross anatomy of experimental animals was examined and all gross pathological changes were recorded. Pathologic examination should be carried out on organs that have died and survived for 24 hours or more and have gross pathological changes.

5.4.6 LD50 can be determined by many methods. It is suggested to use one maximum test, horn's method, up-down method, probability unit logarithm diagram method and Coriolis method.

5.5 Evaluation of test results

When evaluating the test results, LD50 should be considered in combination with the observed toxic effects and autopsy findings. LD50 value is the basis of toxicity classification and label identification of the test substance, as well as the determination of the possibility of animal death caused by ingestion of the test substance through the digestive tract.When quoting LD50 value, it is necessary to indicate the species, sex, route of exposure, observation period, etc. of the experimental animals used.The evaluation should include the relationship between the incidence and severity of the animal's exposure to the test substance and the animal's abnormal performance (including behavior and clinical changes, general injury, weight change, lethal effect and other toxic effects).

See Table 1 for toxicity classification.

表 1 经口毒性分级

LD_{50} (mg/kg)	毒性分级
≤50	高毒
51—500	中等毒
501—5000	低毒
>5000	实际无毒

6 试验结果的解释

通过急性经口毒性试验和 LD50 的测定可评价受试物的毒性。其结果外推到人类的有效性很有限。

Table 1 oral toxicity classification

LD50 (mg/kg)	Toxicity classification
Less than 50	Highly toxic
51—500	Moderate toxicity
501—5000	Low toxicity
>5000	Practically non-toxic

6 Interpretation of test results

The toxicity of the test substance can be evaluated by acute oral toxicity test and LD50 determination. The validity of extrapolation of the results to humans is very limited.

3 急性经皮毒性试验

Acute Dermal Toxicity Test

1 范围

本规范规定了动物急性皮肤毒性试验的基本原则、要求和方法。本规范适用于化妆品原料安全性毒理学检测。

2 试验目的

急性皮肤毒性试验可确定受试物能否经皮肤吸收和短期作用所产生的毒性反应，可为化妆品原料毒性分级和标签标识以及确定亚慢性毒性试验和其他毒理学试验剂量提供依据。

3 Acute percutaneous toxicity test

1 Range

This specification specifies the basic principles, requirements and methods of animal acute skin toxicity test. This specification is applicable to the safety toxicology test of cosmetic raw materials.

2 Test purpose

The acute skin toxicity test can determine whether the test substance can be absorbed by the skin and the toxic reaction produced by the short-term effect. It can provide the basis for the toxicity classification and labeling of cosmetics raw materials, as well as the determination of sub chronic toxicity test and other toxicological test doses.

3 定义

3.1 急性皮肤毒性 acute dermal toxicity

经皮一次涂敷受试物后，动物在短期内出现的健康损害效应。

3.2 经皮 LD50 半数致死量 mdium lethal dose

经皮一次涂敷受试物后，引起实验动物总体中半数死亡的毒物的统计学剂量。以单位体重涂敷受试物的重量(mg/kg 或 g/kg)来表示。

4 试验的基本原则

受试物以不同剂量经皮给予各组实验动物，每组用一个剂量。染毒后观察动物的毒性反应和死亡情况。试验期间死亡的动物要进行尸检，试验结束时仍存活的动物要处死并进行尸检。若已知受试物具有腐蚀性或强刺激性可不进行急性经皮毒性试验。

3 Definition

3.1 Acute dermal toxicity

The effect of health damage on animals in a short period of time after a single application of the test substance.

3.2 Medium lethal dose

The statistical dose of the toxicant that causes half of the total deaths in the experimental animals after a single skin application of the test substance. The weight of the coated test substance per unit weight (mg / kg or g / kg).

4 Basic principles of test

The test substance is given to each group of experimental animals in different doses through skin, with one dose for each group. The toxicity and death of the animals are observed. The animals that die during the experiment should be autopsied, and the animals that survived at the end of the experiment should be executed and autopsied. If the test substance is known to be corrosive or highly irritating, acute percutaneous toxicity test may not be carried out.

5 试验方法

5.1 受试物

液体受试物一般不需稀释。若受试物为固体，应研磨成细粉状，并用适量水或无毒、无刺激性、不影响受试物穿透皮肤、不与受试物反应的介质混匀，以保证受试物与皮肤有良好的接触。常用的介质有橄榄油、羊毛脂、凡士林等。

5.2 实验动物和饲养环境

可选用健康成年大鼠、家兔或豚鼠作为实验动物，也可使用其他种属动物进行试验。使用雌性动物应是未孕和未曾产仔的。建议实验动物体重范围为：大鼠 200g—300g；家兔 2kg—3kg；豚鼠 350g—450g。实验动物皮肤应健康无破损。试验前动物要在实验动物房环境中至少适应 3d—5d 时间。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Test substance

In general, the liquid test substance does not need to be diluted. If the test substance is solid, it shall be ground into fine powder, and mix with appropriate amount of water or non-toxic, non irritant medium, which does not affect the penetration of the test substance into the skin and does not react with the test substance, so as to ensure good contact between the test substance and the skin. Commonly used media are olive oil, lanolin, Vaseline, etc.

5.2 Laboratory animals and feeding environment

Healthy adult rats, rabbits or guinea pigs can be selected as experimental animals, or other species of animals can be used for the test. Female animals should not be pregnant and have not given birth. It is suggested that the weight range of experimental animals be: 200g-300g for rats, and 200g-300g for rabbits 2kg-3kg; 350g-450g for guinea pigs. The skin of experimental animals should be healthy without damage. Before the experiment, the animals should adapt to the environment of the laboratory for at least 3-5 days.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Standard compound feed is selected, and drinking water is not limited.

5.3 剂量水平

根据所选用的方法要求，原则上应设 4—6 个剂量组，每组动物一般为 10 只，雌雄各半。各剂量组间距大小以兼顾产生毒性大小和死亡为宜，通常以较大组距和较少量动物进行预试。如果受试物毒性很低，可采用一次限量法，即用 10 只动物（雌雄各半）皮肤涂抹 2000mg/kg 体重剂量，当未引起动物死亡，可考虑不再进行多个剂量的急性经皮毒性试验。

5.3 Dose level

According to the requirements of the selected method, in principle, 4-6 dosage groups should be set up, each group of animals is generally 10 animals with half male and half female. The distance between different dosage groups is suitable for both toxicity and death. Usually, a larger group distance and a smaller number of animals are used for pre-test. If the toxicity of the tested substance is very low, a one-time limit method can be adopted, i.e. the skin of 10 animals (half male and half female) is smeared with a dose of 2000mg/kg body weight. When no animal death is caused, multiple doses of acute percutaneous toxicity tests can be considered no longer.

5.4 试验步骤

5.4.1 试验开始前 24h，剪去或剃除动物躯干背部拟染毒区域的被毛，去毛时应非常小心，不要损伤皮肤以免影响皮肤的通透性。涂皮面积约占动物体表面积的 10%，应根据动物体重确定涂皮面积。体重为 200g—300g 的大鼠约为 30cm2—40cm2，体重为 2kg—3kg 的家兔约为 160 cm2—210 cm2,体重为 350g—450g 的豚鼠约为 46 cm2—54 cm2。

5.4.2 将受试物均匀涂敷于动物背部皮肤染毒区，然后用一层薄胶片覆盖，无刺激胶布固定，防止动物舔食。若受试物毒性较高，可减少涂敷面积，但涂敷仍需尽可能薄而均匀。一般封闭接触 24h。

5.4.3 染毒结束后，应使用水或其他适宜的溶液清除残留受试物。

5.4.4 观察期限一般不超过 14d，但要视动物中毒反应的严重程度、症状出现快慢和恢复期长短而定。若有延迟死亡迹象，可考虑延长观察时间。

5.4 Test procedure

5.4.1 24 hours before the start of the test, cut or shave the coat of the area to be poisoned on the back of the animal trunk. When removing the coat, take great care not to damage the skin to avoid affecting the permeability of the skin. The coated area is about 10% of the surface area of the animal. The coated area should be determined according to the weight of the animal. The body weight of rats is about 30cm2-40cm2, that of rabbits is about 160cm2-210cm2, and that of guinea pigs is about 46cm2-54cm2.

5.4.2 The test substance shall be evenly applied to the toxic area of the skin on the back of the animal, and then covered with a thin film, fixed with non irritating adhesive tape, so as to prevent the animal from licking. If the toxicity of the test substance is high, the coating area can be reduced, but the coating should be as thin and uniform as possible. Generally, it is closed for 24 hours.

5.4.3 At the end of the exposure, water or other suitable solution shall be used to remove the residual test substance.

5.4.4 The duration of observation generally does not exceed 14 days, but depends on the severity of the animal poisoning reaction, the speed of symptoms and the length of recovery period. If there are signs of delayed death, extended observation time can be considered.

5.4.5 对每只动物都应有单独全面的记录，染毒第 1 d 要定时观察实验动物的中毒表现和死亡情况，其后至少每天进行一次仔细的检查。包括被毛和皮肤、眼睛和粘膜以及呼吸、循环、自主神经和中枢神经系统、肢体运动和行为活动等的改变。特别注意观察动物是否出现震颤、抽搐、流涎、腹泻、嗜睡、和昏迷等症状。死亡时间的记录应尽可能准确。

观察期内存活动物每周称重、观察期结束存活动物应称重，处死后进行尸检。

5.4.6 对实验动物进行大体解剖学检查，并记录全部大体病理改变。对死亡和存活 24h 和 24h 以上动物并存在大体病理改变的器官应进行病理组织学检查。

5.4.7 可采用多种方法测定 LD50，建议采用一次最大限度试验法、霍恩氏法、上-下法、概率单位-对数图解法和寇氏法等。

5.4.5 Each animal shall be recorded separately and comprehensively. The poisoning performance and death of the experimental animal shall be observed regularly on the first day after exposure, and then at least one careful inspection shall be carried out every day. It includes the changes of coat and skin, eyes and mucous membrane, respiration, circulation, autonomic and central nervous system, limb movement and behavior. Special attention shall be paid to observe whether the animals have tremor, convulsion, salivation, diarrhea, drowsiness, coma and other symptoms. The time of death should be recorded as accurately as possible.

During the observation period, the living animals should be weighed every week. After the end of the observation period, the autopsy should be carried out.

5.4.6 The gross anatomy of experimental animals was examined and all gross pathological changes were recorded. Pathologic examination should be carried out on organs that have died and survived for 24 hours or more and have gross pathological changes.

5.4.7 LD50 can be determined by many methods. It is suggested to use the method of one-time maximum test, horn's method, up-down method, probability unit logarithm diagram method and Coriolis method.

5.5 试验结果评价

评价试验结果时，应将经皮 LD50 与观察到的毒性效应和尸检所见相结合考虑，LD50 值是受试物毒性分级和标签标识以及判定受试物经皮肤吸收后引起动物死亡可能性大小的依据。引用 LD50 值时一定要注明所用实验动物的种属、性别、染毒途径、观察期限等。评价应包括动物接触受试物与动物异常表现（包括行为和临床改变、大体损伤、体重变化、致死效应及其他毒性作用）的发生率和严重程度之间的关系。

毒性分级见表 1。

5.5 Evaluation of test results

When evaluating the test results, we should consider the dermal LD50 combined with the observed toxic effects and autopsy findings. The LD50 value is the basis of the toxicity classification and label identification of the test substance and the determination of the possibility of animal death caused by the absorption of the test substance through the skin. When quoting LD50 value, it is necessary to indicate the species, sex, route of exposure, observation period, etc. of the experimental animals used. The evaluation should include the relationship between the incidence and severity of the animal's exposure to the test substance and the animal's abnormal performance (including behavior and clinical changes, general injury, weight change, lethal effect and other toxic effects).

See Table 1 for toxicity classification.

表1 皮肤毒性分级

LD_{50}（mg/kg）	毒性分级
＜5	剧毒
5—＜44	高毒
44—＜350	中等毒
350—＜2180	低毒
≥2180	微毒

6 试验结果的解释

急性经皮毒性试验研究和经皮 LD50 的确定提供了受试物经皮染毒的毒性。其结果外推到人类的有效性很有限。急性经皮毒性试验的结果应与经其他途径染毒的急性毒性试验结果相结合进行综合评价。

Table 1 skin toxicity classification

LD50（mg/kg）	Toxicity classification
＜5	Extremely toxic
5—＜44	Highly toxic
44—＜350	Moderate toxicity
350—＜2180	Low toxicity
≥2180	Micro toxicity

6 Interpretation of test results

The acute dermal toxicity test and the determination of dermal LD50 provided the toxicity of the test substance. The validity of extrapolation of the results to humans is very limited. The results of acute percutaneous toxicity test should be combined with the results of acute toxicity test of other routes for comprehensive evaluation.

4 皮肤刺激性/腐蚀性试验

Dermal Irritation/Corrosion Test

1 范围

本规范规定了动物皮肤刺激性或腐蚀性试验的基本原则、要求和方法。本规范适用于化妆品原料及其产品安全性毒理学检测。

2 试验目的

确定和评价化妆品原料及其产品对哺乳动物皮肤局部是否有刺激作用或腐蚀作用及其程度。

4 Dermal Irritation/Corrosion Test

1 Range

This Test specifies the basic principles, requirements and methods of animal skin irritation or Corrosion test. This Test is applicable to the safety toxicology test of cosmetics raw materials and products.

2 Test purpose

To determine and evaluate whether cosmetic raw materials and their products have irritating or corrosive effects on mammalian skin and their degree.

3 定义

3.1 皮肤刺激性 dermal irritation
皮肤涂敷受试物后局部产生的可逆性炎性变化。

3.2 皮肤腐蚀性 dermal corrosion
皮肤涂敷受试物后局部引起的不可逆性组织损伤。

4 试验的基本原则

将受试物一次(或多次)涂敷于受试动物的皮肤上，在规定的时间间隔内，观察动物皮肤局部刺激作用的程度并进行评分。采用自身对照，以评价受试物对皮肤的刺激作用。急性皮肤刺激性试验观察期限应足以评价该作用的可逆性或不可逆性。

动物如果在试验的任何阶段出现严重抑郁、痛苦的表现，则应当给予人道地处死。依据试验情况对受试物进行适当评价。

3 Definition

3.1 Dermal irritation
Reversible inflammatory changes in the local area of the skin after application of the test substance.

3.2 Dermal corrosion
Irreversible tissue damage caused by local application of the test substance on the skin.

4 Basic principles of test

The test substance is applied to the skin of the test animal once (or several times), and the degree of local skin irritation is observed and scored within the specified time interval. Self control is used to evaluate the skin irritation of the test substance. The

observation period of acute skin irritation test should be enough to evaluate the reversibility or irreversibility of the effect.

If animals show severe depression and pain at any stage of the trial, they should be executed humanely. The test object shall be evaluated properly according to the test situation.

5 试验方法

5.1 受试物

液体受试物一般不需稀释，可直接使用原液。若受试物为固体，应将其研磨成细粉状，并用水或其他无刺激性溶剂充分湿润，以保证受试物与皮肤有良好的接触。使用其他溶剂，应考虑到该溶剂对受试物皮肤刺激性的影响。需稀释后使用的产品，先进行产品原型的皮肤刺激性/腐蚀性试验，如果试验结果显示中度以上的刺激性，可按使用浓度为受试物再进行皮肤刺激性/腐蚀性试验。

受试物为强酸或强碱（pH 值 ≤2 或≥11.5），可以不再进行皮肤刺激试验。此外，若已知受试物有很强的经皮吸收毒性，经皮 LD50 小于 200mg/kg 体重或在急性经皮毒性试验中受试物剂量为 2000mg/kg 体重仍未出现皮肤刺激性作用，也无需进行急性皮肤刺激性试验。

5.2 实验动物和饲养环境

多种哺乳动物均可被选为实验动物，首选白色家兔。应使用成年、健康、皮肤无损伤的动物，雌性和雄性均可，但雌性动物应是未孕和未曾产仔的。实验动物至少要用 4 只，如要澄清某些可疑的反应则需增加实验动物数。实验动物应单笼饲养，试验前动物要在实验动物房环境中至少适应 3d 时间。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Test substance

Generally, the liquid test substance does not need to be diluted, and the original solution can be used directly. If the test substance is a solid, it shall be ground into a fine powder and moistened with water or other non irritant solvent to ensure good contact between the test substance and the skin. When using other solvents, the effect of the solvent on the skin irritation of the test object should be considered. For the products to be diluted and used, the skin irritation / corrosivity test of the product prototype shall be carried out first. If the test results show moderate or above irritation, the skin irritation / corrosivity test can be carried out according to the use concentration of the test substance.

If the test substance is strong acid or strong base (pH value ≤ 2 or ≥ 11.5), so skin irritation test can be stopped. In addition, if it is known that the test substance has a strong percutaneous absorption toxicity, the LD50 of the test substance is less than 200mg / kg body weight or the dose of the test substance is 2000mg / kg body weight in the acute percutaneous toxicity test, there is no need for the acute dermal irritation test.

5.2 Laboratory animals and feeding environment

A variety of mammals can be selected as experimental animals, white rabbits are the first choice. Adult, healthy and skin free animals should be used, both female and male, but female animals should not be pregnant and have not given birth. At least 4 experimental animals should be used. To clarify some suspicious reactions, the number of experimental animals should be increased. The experimental animals should be raised in single cage. Before the experiment, the animals should adapt to the environment of the laboratory for at least three days.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, drinking water is not limited.

5.3 急性皮肤刺激性试验步骤

5.3.1 试验前约 24 h ，将实验动物背部脊柱两侧毛剪掉，不可损伤表皮，去毛范围左、右各约 3cm×3cm。

5.3.2 取受试物约 0. 5 mL（g）直接涂在皮肤上，然后用二层纱布（2.5cm×2.5cm）和一层玻璃纸或类似物覆盖，再用无刺激性胶布和绷带加以固定。另一侧皮肤作为对照。采用封闭试验，敷用时间为 4h。对化妆品产品而言，可根据人的实际使用和产品类型，延长或缩短敷用时间。对用后冲洗的化妆品产品，仅采用 2 h 敷用试验。试验结束后用温水或无刺激性溶剂清除残留受试物。

如怀疑受试物可能引起严重刺激或腐蚀作用，可采取分段试验，将三个涂布受试物的纱布块同时或先后敷贴在一只家兔背部脱毛区皮肤上，分别于涂敷后 3 min、60min 和 4 h 取下一块纱布，皮肤涂敷部位在任一时间点出现腐蚀作用，即可停止试验。

5.3.3 于清除受试物后的 1、24、48 和 72 h 观察涂抹部位皮肤反应，按表 1 进行皮肤反应评分，以受试动物积分的平均值进行综合评价，根据 24、48 和 72 h 各观察时点最高积分均值，按表 2 判定皮肤刺激强度。

5.3.4 观察时间的确定应足以观察到可逆或不可逆刺激作用的全过程，一般不超过 14d。

5.3 Acute skin irritation test procedure

5.3.1 About 24 hours before the experiment, the hair on both sides of the back spine of the experimental animal is cut off, and the epidermis could not be damaged. The hair removal range is about 3cm × 3cm on the left and 3cm on the right.

5.3.2 Take about 0.5ml (g) of the test substance and apply it directly to the skin, then cover it with two layers of gauze (2.5cm × 2.5cm) and a layer of cellophane or similar substance, then fix it with non irritating adhesive tape and bandage. The other side of the skin served as a control. The application time is 4 hours. For cosmetic products, the application time can be prolonged or shortened according to the actual use and product type of people. For the cosmetics washed after use, only 2 hours application test is used. At the end of the test, the residual test substance is removed with warm water or non irritant solvent.

If it is suspected that the test substance may cause serious irritation or corrosion, a sectional test can be carried out. Three gauze blocks coated with the test substance can be applied to the depilated area skin of a rabbit's back at the same time or successively. One gauze can be removed at 3 min, 60 min and 4 h after the application respectively. The test can be stopped if there is corrosion at any time point on the coated area of the skin.

5.3.3 Observe the skin reaction of the smear site at 1, 24, 48 and 72 hours after the test substance is removed, score the skin reaction according to table 1, comprehensively evaluate according to the average value of the integral of the test animals, and determine the skin stimulation intensity according to table 2 according to the highest integral mean value of each observation time point at 24, 48 and 72 hours.

5.3.4 The observation time should be enough to observe the whole process of reversible or irreversible stimulation, generally no more than 14 days.

5.4 多次皮肤刺激性试验步骤

5.4.1 试验前将实验动物背部脊柱两侧被毛剪掉，去毛范围各为 3cm×3cm，涂抹面积 2.5cm×2.5cm。

5.4.2 取受试物约 0.5mL(g)涂抹在一侧皮肤上，当受试物使用无刺激性溶剂配制时，另一侧涂溶剂作为对照，每天涂抹 1 次，连续涂抹 14d。从第二天开始，每次涂抹前应剪毛，用水或无刺激性溶剂清除残留受试物。一小时后观察结果，按表 1 评分，对照区和试验区同样处理。

5.4.3 结果评价：按下列公式计算每天每只动物平均积分，以表 2 判定皮肤刺激强度。

$$\text{每天每只动物平均积分} = \frac{\sum \text{红斑和水肿积分}}{\text{受试动物数}} \Big/ 14$$

5.4 Multiple skin irritation test procedures

5.4.1 Before the experiment, the two sides of the back spine of the experimental animal are cut off, the hair removal range is 3cm × 3cm, and the application area is 2.5cm×2.5cm。

5.4.2 Take about 0.5ml (g) of the test substance and apply it on one side of the skin. When the test substance is prepared with non irritant solvent, apply the solvent on the other side as a control, once a day for 14 days. From the next day, the hair shall be cut before each application, and the residual test substance shall be removed with water or non irritant solvent. One hour later, the observation results are scored according to table 1, and the control area and the test area are treated the same.

5.4.3 Results evaluation: calculate the average score of each animal every day according to the following formula, and determine the skin irritation intensity according to table 2.

Average score per animal per day = $\sum$ Erythema and edema integral/Number of animals tested /14

表 1 皮肤刺激反应评分

皮肤反应	积分
红斑和焦痂形成	
无红斑	0
轻微红斑（勉强可见）	1
明显红斑	2
中度—重度红斑	3
严重红斑（紫红色）至轻微焦痂形成	4
水肿形成	
无水肿	0
轻微水肿（勉强可见）	1
轻度水肿（皮肤隆起轮廓清楚）	2
中度水肿（皮肤隆起约 1mm）	3
重度水肿（皮肤隆起超过 1mm，范围扩大）	4
最高积分	8

表 2 皮肤刺激强度分级

积分均值	强度
0 — ＜ 0.5	无刺激性
0.5— ＜ 2.0	轻刺激性
2.0 — ＜ 6.0	中刺激性
6.0 — 8.0	强刺激性

6 试验结果的解释

急性皮肤刺激试验结果从动物外推到人的可靠性很有限。白色家兔在大多数情况下对有刺激性或腐蚀性的物质较人类敏感。若用其他品系动物进行试验时也得到类似结果，则会增加从动物外推到人的可靠性。试验中使用封闭式接触是一种超常的实验室条件下的试验，在人类实际使用化妆品过程中很少存在这种接触方式。

Table 1 skin irritation response score

Skin reaction	integral
Erythema and eschar formation	
No erythema	0
Slight erythema (barely visible)	1
Obvious erythema	2
Moderate to severe erythema	3

Severe erythema (purplish red) to slight eschar formation	4
Edema formation	
No edema	0
Slight edema (barely visible)	1
Mild edema (clear contour of skin bulge)	2
Moderate oedema (skin swelling about 1mm)	3
Severe edema (skin swelling more than 1mm, expanded range)	4
Highest integral	8

Table 2 classification of skin irritation intensity

Integral mean	Strength
0 — ＜ 0.5	Non irritant
0.5— ＜ 2.0	Light irritant
2.0 — ＜ 6.0	Moderate irritant
6.0 — 8.0	Strong irritant

6 Interpretation of test results

The reliability of extrapolation of acute skin irritation test results from animal to human is very limited. In most cases, white rabbits are more sensitive to irritant or corrosive substances than human beings. If similar results are obtained when testing with other strains of animals, it will increase the reliability of extrapolation from animals to humans. The use of closed contact in the experiment is an extraordinary laboratory test, which is rarely used in the actual use of cosmetics.

5 急性眼刺激性/腐蚀性试验

Acute Eye Irritation/Corrosion Test

1 范围

本规范规定了动物急性眼刺激性或腐蚀性试验的基本原则、要求和方法。本规范适用于化妆品原料及其产品安全性毒理学检测。

2 试验目的

确定和评价化妆品原料及其产品对哺乳动物的眼睛是否有刺激作用或腐蚀作用及其程度。

3 定义

3.1 眼睛刺激性 eye irritation
眼球表面接触受试物后所产生的可逆性炎性变化。

3.2 眼睛腐蚀性 eye corrosion
眼球表面接触受试物后引起的不可逆性组织损伤。

5 Acute eye irritation / Corrosion test

1 Range

This Test specifies the basic principles, requirements and methods of animal acute eye irritation or corrosion test. This Test is applicable to the safety toxicology test of cosmetics raw materials and products.

2 Test purpose

To determine and evaluate whether cosmetic raw materials and their products have irritating or corrosive effects on mammalian eyes and their degree.

3 Definition

3.1 Eye irritation
Reversible inflammatory changes on the surface of the eyeball after contact with the test substance.

3.2 Eye corrosion
Irreversible tissue damage caused by the contact of the eyeball surface with the test object.

4 试验的基本原则

受试物以一次剂量滴入每只实验动物的一侧眼睛结膜囊内，以未作处理的另一侧眼睛作为自身对照。在规定的时间间隔内，观察对动物眼睛的刺激和腐蚀作用程度并评分，以此评价受试物对眼睛的刺激作用。观察期限应能足以评价刺激效应的可逆性或不可逆性。

动物如果在试验的任何阶段出现严重抑郁、痛苦的表现，应当给予人道地处死，依据试验情况对受试物进行适当评价。动物出现角膜穿孔、角膜溃疡、角膜 4 分超过 48h、缺乏光反射超过 72h、结膜溃疡、坏疽、腐烂等情况，通常为不可逆损伤的症状，也应当给予人道地处死。

4 Basic principles of test

The test substance is dripped into conjunctival sac of one eye of each experimental animal in a single dose, and the untreated other eye is used as self-control. In the specified time interval, the degree of eye irritation and corrosion is observed and scored to evaluate the eye irritation of the test object. The duration of observation should be sufficient to evaluate the reversibility or irreversibility of the stimulus effect.

If the animal shows severe depression and pain at any stage of the test, it shall be executed humanely, and the test object shall be properly evaluated according to the test situation. Animals with corneal perforation, corneal ulcer, cornea 4 points over 48h, lack of light reflection over 72h, conjunctival ulcer, gangrene, rot and other conditions, usually irreversible injury symptoms, should also be given a humane execution.

5 试验方法

5.1 受试物

液体受试物一般不需稀释，可直接使用原液，染毒量为 0.1mL。若受试物为固体或颗粒状，应将其研磨成细粉状，染毒量应为体积 0.1mL 或重量不大于 100mg（染毒量应进行记录）。

受试物为强酸或强碱（pH 值≤ 2 或≥11.5），或已证实对皮肤有腐蚀性或强刺激性时，可以不再进行眼刺激性试验。

气溶胶产品需喷至容器中，收集其液体再使用。

5.2 实验动物和饲养环境

首选健康成年白色家兔。至少使用 3 只家兔。试验前动物要在实验动物房环境中至少适应 3d 时间。在试验开始前的 24h 内要对试验动物的两只眼睛进行检查（包括使用荧光素钠检查）。有眼睛刺激症状、角膜缺陷和结膜损伤的动物不能用于试验。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Test substance

In general, the liquid test substance does not need to be diluted, and the original solution can be used directly, with a dose of 0.1ml. If the test substance is solid or granular, it shall be ground into fine powder, and the amount of poisoning shall be 0.1ml in volume or no more than 100mg in weight (the amount of poisoning shall be recorded).

When the test substance is strong acid or alkali (pH value ≤ 2 or ≥ 11.5), or it has been confirmed that it is corrosive or strong irritant to the skin, eye irritation test can be stopped.

Aerosol products need to be sprayed into containers to collect their liquid for reuse.

5.2 Laboratory animals and feeding environment

The first choice is healthy adult white rabbits. At least 3 rabbits are used. Before the experiment, the animals should adapt to the environment of the laboratory for at least three days. Two eyes of the test animal shall be examined within 24 hours before the start of the test (including the use of fluorescein sodium). Animals with eye irritation, corneal defects, and conjunctival damage should not be tested.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Standard compound feed is selected, and drinking water is not limited.

5.3 试验步骤

5.3.1 轻轻拉开家兔一侧眼睛的下眼睑，将受试物 0.1 mL（100mg）滴入(或涂入)结膜囊中，使上、下眼睑被动闭合 1s，以防止受试物丢失。另一侧眼睛不处理作自身对照。滴入受试物后 24h 内不冲洗眼睛。若认为必要，在 24h 时可进行冲洗。

5.3.2 若上述试验结果显示受试物有刺激性，需另选用 3 只家兔进行冲洗效果试验，即给家兔眼滴入受试物后 30s，用足量、流速较快但又不会引起动物眼损伤的水流冲洗至少 30s。

5.3.3 临床检查和评分：在滴入受试物后 1、24、48、72h 以及第 4d 和第 7d 对动物眼睛进行检查。如果 72 h 未出现刺激反应，即可终止试验。如果发现累及角膜或有其他眼刺激作用，7d 内不恢复者，为确定该损害的可逆性或不可逆性需延长观察时间，一般不超过 21d，并提供 7d、14d 和 21d 的观察报告。除了对角膜、虹膜、结膜进行观察外，其他损害效应均应当记录并报告。在每次检查中均应按表 1 眼损害的评分标准记录眼刺激反应的积分。

可使用放大镜、手持裂隙灯、生物显微镜或其他适用的仪器设备进行眼刺激反应检查。在 24h 观察和记录结束之后，对所有动物的眼睛应用荧光素钠作进一步检查。

5.3.4 对用后冲洗的产品（如洗面奶、发用品、育发冲洗类）只做 30s 冲洗试验，即滴入受试物后，眼闭合 1s，至第 30s 时用足量、流速较快但又不会引起动物眼损伤的水流冲洗 30s，然后按 5.3.3 进行检查和评分。

5.3.5 对染发剂类产品，只做 4s 冲洗试验，即滴入受试物后，眼闭合 1s，至第 4s 时用足量、流速较快但又不会引起动物眼损伤的水流冲洗 30s，然后按 5.3.3 进行检查和评分。

5.3 Test procedure

5.3.1 Gently open the lower eyelid of one eye of the rabbit, drop (or apply) 0.1ml (100mg) of the test substance into the conjunctival sac, make the upper and lower eyelids passively closed for 1s, so as to prevent the loss of the test substance. The other side of the eye is not treated as self-control. Do not wash eyes within 24 hours after dropping the test substance. If necessary, flush at 24 hours.

5.3.2 If the above test results show that the test substance is irritant, another 3 rabbits shall be selected for the flushing effect test, that is, 30 s after the test substance is dripped into the rabbit's eyes, and at least 30 s after the test substance is flushed with sufficient water with fast flow rate but without eye injury.

5.3.3 Clinical examination and score: the eyes of the animals are examined at 1, 24, 48, 72 hours, and at the 4th and 7th days after the infusion of the test substance. If there is no stimulation reaction in 72 hours, the test can be terminated. In case of corneal involvement or other eye irritation, the observation

time shall be extended to determine the reversibility or irreversibility of the damage, generally not more than 21 days, and the observation reports of 7 days, 14 days and 21 days shall be provided. In addition to the observation of cornea, iris and conjunctiva, other damage effects should be recorded and reported. In each examination, the score of eye irritation should be recorded according to the scoring standard of eye damage in Table 1.

Eye irritation can be examined with a magnifying glass, hand-held slit lamp, biomicroscope, or other suitable equipment. After 24 hours observation and recording, the eyes of all animals are further examined with fluorescein sodium.

5.3.4 For the products washed after use (such as facial cleanser, hair products and hair care washing), only 30 s washing test shall be conducted, i.e. after dropping into the test object, the eyes shall be closed for 1 s, and then washed for 30 s with sufficient water with fast flow rate but without eye injury of animals, and then the inspection and scoring shall be conducted according to 5.3.3.

5.3.5 For hair dye products, only 4-s washing test is conducted, i.e. after dropping into the test substance, the eyes are closed for 1 s, and then washed for 30 s with enough water with fast flow rate but without eye injury of animals, and then inspected and scored according to 5.3.3.

表 1 眼损害的评分标准

眼损害	积分
角膜：混浊（以最致密部位为准）无溃疡形成或混浊	0
散在或弥漫性混浊，虹膜清晰可见半透明区易分辨，虹膜模糊不清	1
出现灰白色半透明区，虹膜细节不清，瞳孔大小勉强可见	2
角膜混浊，虹膜无法辨认	3
	4
虹膜：正常	0
皱褶明显加深，充血、肿胀、角膜周围有中度充血，瞳孔对光仍有反应	1
出血、肉眼可见破坏，对光无反应（或出现其中之一反应）	2

结膜：充血（指睑结膜、球结膜	
部位）血管正常	0
血管充血呈鲜红色	1
血管充血呈深红色，血管不易分辨弥漫性充血呈紫红色	2 3
水肿	
无	
轻微水肿（包括瞬膜）	0
明显水肿，伴有部分眼睑外翻	1 2

眼损害	积分
水肿至眼睑近	3
半闭合水肿至眼睑大半闭合	4

6 结果评价

化妆品原料—以给受试物后动物角膜、虹膜或结膜各自在 24、48 和 72h 观察时点的刺激反应积分的均值和恢复时间评价，按表 2 眼刺激反应分级判定受试物对眼的刺激强度。

Table 1 scoring standard of eye damage

Eye damage	integral
Cornea: turbid (subject to the densest part) without ulcer formation or turbidity	0
Scattered or diffuse turbidity, clear and translucent iris area, easy to distinguish, unclear iris	1 2
Gray white translucent area appears, iris details are unclear, pupil size is barely visible	3 4
The cornea is cloudy and the iris is illegible	

Iris: normal	0
The wrinkles are obviously deepened, hyperemia, swelling, moderate hyperemia around the cornea, and the pupil still responded to the light	1
Bleeding, visible damage, no response to light (or one of them)	2
Conjunctiva: normal blood vessels in hyperemia (palpebral conjunctiva and bulbar conjunctiva)	0
Blood vessel congestion is bright red	1
Blood vessel congestion is dark red, blood vessel is indistinguishable diffuse congestion is purplish red	2
edema	3
nothing	0
Mild edema (including blink film)	1
Obvious edema with partial ectropion	2

Eye damage	integral
Dropsy to near closure of eyelid	3
dropsy to greater closure of eyelid	4

6 Result evaluation

Cosmetic raw materials: the mean value and recovery time of the stimulus response scores of cornea, iris or conjunctiva at 24, 48 and 72 hours after the test substance is given to the animals, and the stimulus intensity of the test substance

to the eyes is determined according to the eye stimulus response classification in Table 2.

表 2 原料眼刺激性反应分级

可逆眼损伤	2A 级（轻刺激性） 2/3 动物的刺激反应积分均值：角膜浑浊≥1；虹膜≥1；结膜充血≥2；结膜水肿≥2 和上述刺激反应积分在≤7 天完全恢复 2B 级（刺激性） 2/3 动物的刺激反应积分均值：角膜浑浊≥1；虹膜≥1；结膜充血≥2；结膜水肿≥2 和上述刺激反应积分在＜21 天完全恢复
不可逆眼损伤	任 1 只动物的角膜、虹膜和/或结膜刺激反应积分在 21 天的观察期间没有完全恢复 2/3 动物的刺激反应积分均值：角膜浑浊≥3 和/或虹膜＞1.5

注：当角膜、虹膜、结膜积分为 0 时，可判为无刺激性，界于无刺激性和轻刺激性之间的为微刺激性。

化妆品产品—以给受试物后动物角膜、虹膜或结膜各自在 24、48 或 72h 观察时点的刺激反应的最高积分均值和恢复时间评价，按表 3 眼刺激反应分级判定受试物对眼的刺激强度。

表 3 产品眼刺激性反应分级

可逆损伤	微刺激性	动物的角膜、虹膜积分=0；结膜充血和/或结膜水肿积分≤2，且积分在＜7 天内降至 0
	轻刺激性	动物的角膜、虹膜、结膜积分在≤7 天降至 0
	刺激性	动物的角膜、虹膜、结膜积分在 8—21 天内降至 0
不可眼损伤	腐蚀性	①动物的角膜、虹膜和/或结膜积分在第 21 天时＞0 ,2/3 动物的眼刺激反应积分：角膜浑浊≥3 和/或虹膜=2

注：当角膜、虹膜、结膜积分为 0 时，可判为无刺激性。

7 试验结果的解释

急性眼刺激性试验结果从动物外推到人的可靠性很有限。白色家兔在大多数情况下对有刺激性或腐蚀性的物质较人类敏感。若用其他品系动物进行试验时也得到类似结果，则会增加从动物外推到人的可靠性。

Table 2 classification of eye irritation response of raw materials

Reversible eye injury	Class 2A (mild irritation) 2/3 The mean score of stimulus response in animals: corneal turbidity ≥ 1; iris ≥ 1; conjunctival hyperemia ≥ 2; conjunctival edema ≥ 2 and the above stimulus response score recovered completely in ≤ 7 days Grade 2B (irritant) 2/3 The mean score of stimulus response in animals: corneal turbidity ≥ 1; iris ≥ 1; conjunctival hyperemia ≥ 2; conjunctival edema ≥ 2 and the above stimulus response score recovered completely in < 21 days
Irreversible eye injury	1. The scores of corneal, iris and / or conjunctival irritation in any animal did not fully recover during the 21 day observation period 2. 2/3 The mean score of stimulus response in animals: corneal turbidity ≥ 3 and / or iris > 1.5

Note: When the score of cornea, iris and conjunctiva is 0, it can be judged as non irritant, and the one between non irritant and light irritant is micro irritant.

Cosmetic products - to evaluate the maximum integral mean value and recovery time of the stimulus response of the cornea, iris or conjunctiva of the animal at the observation time point of 24, 48 or 72 hours after the test object is given, and determine the stimulus intensity of the test object to the eye according to the eye stimulus response classification in Table 3.

Table 3 Classification of eye irritation response

Reversible eye injury	Microstimulation	The score of cornea and iris is 0; the score of conjunctival congestion and / or conjunctival edema is ≤ 2, and the score is < 0 in 7 days
	Light irritant	The scores of cornea, iris and conjunctiva of animals decreased to 0 at ≤ 7 days
	thrill	The scores of cornea, iris and conjunctiva of animals decreased to 0 in 8-21 days
Irreversible eye injury	corrosive	① The score of cornea, iris and / or conjunctiva in animals is > 0 on the 21st day ,2/3 The score of eye stimulation response in animals: corneal turbidity ≥ 3 and / or iris = 2

Note: when the score of cornea, iris and conjunctiva is 0, it can be judged as non irritant.

7 Interpretation of test results

The reliability of acute eye irritation test results extrapolated from animal to human is very limited. In most cases, white rabbits are more sensitive to irritant or corrosive substances than human beings. If similar results are obtained when testing with other strains of animals, it will increase the reliability of extrapolation from animals to humans.

6 皮肤变态反应试验

Skin Sensitisation Test

1 范围

本规范规定了动物皮肤变态反应试验的基本原则、要求和方法。本规范适用于化妆品原料及其产品安全性毒理学检测。

2 试验目的

确定重复接触化妆品及其原料对哺乳动物是否可引起变态反应及其程度。

6 Skin Sensitisation test

1 Range

This specification specifies the basic principles, requirements and methods of animal skin allergy test. This specification is applicable to the safety toxicology test of cosmetics raw materials and products.

2 Test purpose

To determine whether and to what extent repeated contact with cosmetics and their ingredients may cause allergic reactions to mammals.

3 定义

3.1 皮肤变态反应（过敏性接触性皮炎）skin sensitization，allergic contact dermatitis

是皮肤对一种物质产生的免疫源性皮肤反应。在人类这种反应可能以瘙痒、红斑、丘疹、水疱、融合水疱为特征。动物的反应不同，可能只见到皮肤红斑和水肿。

3.2 诱导接触 induction exposure

指机体通过接触受试物而诱导出过敏状态的试验性暴露。

3.3 诱导阶段 induction period

指机体通过接触受试物而诱导出过敏状态所需的时间，一般至少一周。

3.4 激发接触 challenge exposure

机体接受诱导暴露后，再次接触受试物的试验性暴露，以确定皮肤是否会出现过敏反应。

3 Definition

3.1 Skin sensitization (allergic contact dermatitis)

It is the skin's immune response to a substance. In humans, this response may be characterized by pruritus, erythema, papules, vesicles, and fusion vesicles. The reaction of animals is different, and only erythema and edema may be seen.

3.2 Induction exposure

It refers to the experimental exposure that the body induces the allergic state by contacting the test substance.

3.3 Induction period

It refers to the time required for the body to induce the allergic state by contacting the test substance, generally at least one week.

3.4 Challenge exposure

After the body receives the induced exposure, it is exposed to the test substance again to determine whether the skin will have allergic reaction.

4 试验的基本原则

实验动物通过多次皮肤涂抹（诱导接触）或皮内注射受试物 10d—14d（诱导阶段）后，给予激发剂量的受试物，观察实验动物并与对照动物比较对激发接触受试物的皮肤反应强度。

4.1 实验动物和饲养环境

一般选用健康、成年雄性或雌性豚鼠，雌性动物应选用未孕或未曾产仔的。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制，需注意补充适量 Vc。

4.2 动物试验前准备

试验前动物要在实验动物房环境中至少适应 3d—5d 时间。将动物随机分为受试物组和对照组，按所选用的试验方法，选择适当部位给动物去毛，避免损伤皮肤。试验开始和结束时应记录动物体重。

4.3 无论在诱导阶段或激发阶段均应对动物进行全面观察包括全身反应和局部反应，并作完整记录。

4.4 试验方法可靠性的检查

使用已知的能引起轻度/中度致敏的阳性物每隔半年检查一次。局部封闭涂皮法至少有

30%动物出现皮肤过敏反应；皮内注射法至少有 60%动物出现皮肤过敏反应。阳性物一般采用 2,4-二硝基氯代苯，肉桂醛，2-巯基苯并噻唑或对氨基苯酸乙酯。

4 Basic principles of test

After several times of skin application (induced contact) or intradermal injection of the test substance for 10-14 days (induction stage), the test substance is given with excitation dose. The skin reaction intensity of the test substance is observed and compared with that of the control animal.

4.1 Laboratory animals and feeding environment

Generally, healthy, adult male or female guinea pigs shall be selected, and female animals shall be those who are not pregnant or have not given birth.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, unlimited drinking water, and pay attention to supplement appropriate VC.

4.2 Preparation before animal test

Before the experiment, the animals should adapt to the environment of the laboratory for at least 3-5 days. The animals are randomly divided into test substance group and control group. According to the selected test method, the appropriate parts are selected to remove hair to avoid skin damage. Animal weight shall be recorded at the beginning and end of the test.

4.3 In both induction and stimulation stages, the animals should be observed comprehensively, including systemic and local reactions, and a complete record should be made.

4.4 Test method reliability check

Use known positive substances that can cause mild / moderate sensitization to check every six months. Local sealing coating method at least has

30% of the animals had skin anaphylaxis; at least 60% of the animals had skin anaphylaxis by intradermal injection.2,4-dinitrochlorobenzene, cinnamaldehyde, 2-Mercaptobenzothiazole or ethyl p-aminobenzoate are generally used as the positive substance.

5 试验方法

5.1 局部封闭涂皮试验（Buehler Test, BT）

5.1.1 动物数

试验组至少 20 只，对照组至少 10 只。

5.1.2 剂量水平

诱导接触受试物浓度为能引起皮肤轻度刺激反应的最高浓度，激发接触受试物浓度为不能引起皮肤刺激反应的最高浓度。试验浓度水平可以通过少量动物（2—3 只）的预试验获得。

水溶性受试物可用水或用无刺激性表面活性剂作为赋形剂，其他受试物可用 80%乙醇或丙酮等作赋形剂，并设溶剂对照。

5.1.3 试验步骤

5.1.3.1 试验前约 24 h ，将豚鼠背部左侧去毛，去毛范围为 4 cm2—6 cm2 。

5.1.3.2 诱导接触：将受试物约 0.2mL（g）涂在实验动物去毛区皮肤上，以二层纱布和一层玻璃纸覆盖，再以无刺激胶布封闭固定 6 h。第 7d 和第 14d 以同样方法重复一次。

5.1.3.3 激发接触：末次诱导后 14—28d，将约 0.2mL 的受试物涂于豚鼠背部右侧 2cm×2cm

去毛区（接触前 24h 脱毛），然后用二层纱布和一层玻璃纸覆盖，再以无刺激胶布固定 6h。

5.1.3.4 激发接触后 24h 和 48h 观察皮肤反应，按表 1 评分。

5.1.3.5 试验中需设阴性对照组，使用 5.1.3.2 和 5.1.3.3 的方法，在诱导接触时仅涂以溶剂作为对照，在激发接触时涂以受试物。对照组动物必须和受试物组动物为同一批。在实验室开展变态反应试验初期，或使用新的动物种属或品系时，需同时设阳性对照组。

5 test method

5.1 Buehler test (BT)

5.1.1 Number of animals

At least 20 in the experimental group and 10 in the control group.

5.1.2 Dose level

The concentration of induced contact test substance is the highest concentration that can cause mild skin irritation, and the concentration of induced contact test substance is the highest concentration that can not cause skin irritation.The test concentration level can be obtained by a small number of animals (2-3).

Water soluble test substance can be used as excipient with water or non irritant surfactant, other test substance can be used as excipient with 80% ethanol or acetone, etc., and solvent control is set.

5.1.3 Test procedure

5.1.3.1 About 24 hours before the experiment, the left side of the guinea pig's back is depilated in the range of 4-6 cm2.

5.1.3.2 Induced contact: Apply about 0.2ml (g) of the test substance on the skin of the depilated area of the experimental animal, cover it with two layers of gauze and one layer of cellophane, and then seal it with non irritating adhesive tape for 6 hours. The 7th and 14th day are repeated in the same way.

5.1.3.3 Excitation contact: at 14-28 days after the last induction, about 0.2ml of test substance is coated on the right side of guinea pig's back 2cm × 2cm

Depilated area (depilated 24 hours before contact), then covered with two layers of gauze and one layer of cellophane, and then fixed with non irritating adhesive tape for 6 hours.

5.1.3.4 The skin reaction is observed 24 hours and 48 hours after stimulation, and the score is according to table 1.

5.1.3.5 The negative control group should be set up in the test. The methods of 5.1.3.2 and 5.1.3.3 should be used. Only the solvent should be used as the control when inducing contact and the test substance should be used when stimulating contact.The

control group animals must be the same batch as the test group animals.In the early stage of allergy test in the laboratory, or when using new animal species or strains, a positive control group should be set up at the same time.

表 1 变态反应试验皮肤反应评分

皮肤反应	积分
红斑和焦痂形成	
无红斑	0
轻微红斑（勉强可见）	1
明显红斑（散在或小块红斑）	2
中度—重度红斑	3
严重红斑（紫红色）至轻微焦痂形成	4
水肿形成	
无水肿	0
轻微水肿（勉强可见）	1
中度水肿（皮肤隆起轮廓清楚）	2
重度水肿（皮肤隆起约 1mm 或超过 1mm）	3
最高积分	7

Table 1 skin reaction score of allergy test

Skin reaction	integral
Erythema and eschar formation	
No erythema	0
Slight erythema (barely visible)	1
Marked erythema (scattered or small erythema)	2
Moderate to severe erythema	3
Severe erythema (purplish red) to slight eschar formation	4
Edema formation	
No edema	0
Slight edema (barely visible)	1
Moderate edema (clear contour of skin bulge)	2
Severe edema (skin swelling of about 1mm or more)	3

Highest integral	7

5.1.4 结果评价

5.1.4.1 当受试物组动物出现皮肤反应积分≥2 时，判为该动物出现皮肤变态反应阳性，按表 3 判定受试物的致敏强度。

5.1.4.2 如激发接触所得结果仍不能确定，应于第一次激发后一周，给予第二次激发，对照组作同步处理或按 5.2 的方法进行评价。

5.1.4 Result evaluation

5.1.4.1 When the skin reaction score of the animal in the subject group is ≥2, the animal is judged to be positive for skin allergy, and the sensitization intensity of the subject is judged according to Table 3.

5.1.4.2 If the result of excitation contact is still uncertain, the second excitation should be given one week after the first excitation, and the control group should be treated synchronously or evaluated according to the method of 5.2.

5.2 豚鼠最大值试验 (Guinea Pig Maximinatim Test , GPMT)

采用完全福氏佐剂 (Freund Complete Adjvant, FCA) 皮内注射方法检测致敏的可能性。

5.2.1 动物数

试验组至少用 10 只，对照组至少 5 只。如果试验结果难以确定受试物的致敏性，应增加动物数，试验组 20 只，对照组 10 只。

5.2.2 剂量水平

诱导接触受试物浓度为能引起皮肤轻度刺激反应的最高浓度，激发接触受试物浓度为不能引起皮肤刺激反应的最高浓度。试验浓度水平可以通过少量动物（2—3 只）的预试验获得。

5.2 Guinea Pig Maximinatim Test (GPMT)

The possibility of sensitization is detected by intradermal injection of Freund complete adjvant (FCA).

5.2.1 Number of animals

At least 10 in the experimental group and 5 in the control group. If the test results are difficult to determine the sensitization of the test substance, the number of animals should be increased, 20 in the test group and 10 in the control group.

5.2.2 Dose level

The concentration of induced contact test substance is the highest concentration that can cause mild skin irritation, and the concentration of induced contact test substance is the highest concentration that can not cause skin irritation. The test concentration level can be obtained by a small number of animals (2-3).

5.2.3 试验步骤

5.2.3.1 诱导接触（第 0d）

受试物组：将颈背部去毛区（2cm×4cm）中线两侧划定三个对称点，每点皮内注射 0.1mL 下述溶液。

第 1 点 1：1（V/V）FCA/水或生理盐水的
混合物第 2 点 耐受浓度的受试物
第 3 点 用 1：1（v/v）FCA/水或生理盐水配制的受试物，浓度与第 2
点相同对照组：注射部位同受试物组
第 1 点 1：1（V/V）FCA/水或生理盐水的
混合物第 2 点 未稀释的溶剂
第 3 点 用 1：1（v/v）FCA/水或生理盐水配制的浓度为 50%（w/v）的溶剂

5.2.3 Test procedure

5.2.3.1 Induced contact (0d)

Test substance group: draw three symmetrical points on both sides of the midline of the neck back hair removal area (2cm × 4cm), inject 0.1ml of the following solution into the skin at each point.

Point 1 1:1 (V / V) FCA / water or saline
mixture point 2 tolerance concentration of
test substance
Point 3: test substance prepared with 1:1 (V / V) FCA / water or
normal saline at the same concentration as point 2 control group:
injection site is the same as test substance group
Point 1 1:1 (V / V) FCA / water or saline
mixture point 2 undiluted solvent
Point 3 50% (w / V) solvent prepared with 1:1 (V / V) FCA / water or normal saline

5.2.3.2 诱导接触（第 7d）：

将涂有 0.5g(mL)受试物的 2cm×4cm 滤纸敷贴在上述再次去毛的注射部位，然后用两层纱布，一层玻璃纸覆盖，无刺激胶布封闭固定 48h。对无皮肤刺激作用的受试物，可加强致敏，于第二次诱导接触前 24h 在注射部位涂抹 10%十二烷基硫酸钠（SLS）0.5mL。对照组仅用溶剂作诱导处理。

5.2.3.3 激发接触（第 21d）

将豚鼠躯干部去毛，用涂有 0.5g(mL)受试物的 2cm×2cm 滤纸片敷贴在去毛区，然后再用两层纱布，一层玻璃纸覆盖，无刺激胶布封闭固定 24h。对照组动物作同样处理。如激发接触所得结果不能确定，可在第一次激发接触一周后进行第二次激发接触。对照组作同步处理。

5.2.3.2 Induced contact (day 7):

The 2cm × 4cm filter paper coated with 0.5g (ML) test substance is applied to the injection site where the hair is removed again, and then two layers of gauze and one layer of cellophane are used to cover the injection site, which is closed and fixed for 48h without stimulating adhesive tape. For the test substance without skin irritation, sensitization can be enhanced, and 0.5ml of 10% sodium dodecyl sulfate (SLS) can be applied to the injection site 24 hours before the second induced contact. The control group is treated with solvent only.

5.2.3.3 Excitation contact (21d)

Remove the hair from the trunk of guinea pig, apply the 2cm × 2cm filter paper coated with 0.5g (ML) test substance to the hair removal area, and then use two layers

of gauze, one layer of cellophane covered, sealed and fixed for 24h without stimulating adhesive tape. The control group is treated with the same method. If the result of excitation contact is uncertain, the second excitation contact can be carried out one week after the first excitation contact. The control group is treated synchronously.

5.2.4 观察及结果评价

激发接触结束，除去涂有受试物的滤纸后 24、48 和 72h，观察皮肤反应，（如需要清除受试残留物可用水或选用不改变皮肤已有反应和不损伤皮肤的溶剂）按表 2 评分。当受试物组动物皮肤反应积分≥1 时，应判为变态反应阳性，按表 3 对受试物进行致敏强度分级。

5.2.4 Observation and result evaluation

At the end of the stimulation contact, 24, 48 and 72 hours after removing the filter paper coated with the test substance, observe the skin reaction (if it is necessary to remove the test residue, use water or choose the solvent that does not change the existing skin reaction and does not damage the skin) according to table 2.When the skin reaction score of the animal in the test substance group is ≥ 1, it shall be judged as allergic reaction positive, and the test substance shall be graded according to the sensitization intensity in Table 3.

表 2 变态反应试验皮肤反应评分

评分	皮肤反应
0	未见皮肤反应
1	散在或小块红斑
2	中度红斑和融合红斑
3	重度红斑和水肿

Table 2 skin reaction score of allergy test

score	Skin reaction
0	No skin reaction
1	Scattered or small erythema
2	Moderate and confluent erythema
3	Severe erythema and edema

表 3 致敏强度

致敏率%	致敏强度
0—8	弱
9—28	轻
29— 64	中
65— 80	强
81—00	极强

注：当致敏率为 0 时，可判为未见皮肤变态反应。

Table 3 sensitization intensity

Sensitization rate%	Sensitization intensity
0 - 8	weak
9 - 28	light
29— 64	medium
65— 80	strong
81—00	Extremely strong

Note: When the sensitization rate is 0, it can be judged as no skin allergy.

6 试验结果的解释

试验结果应能得出受试物的致敏能力和强度。这些结果只能在很有限的范围内外推到人类。引起豚鼠强烈反应的物质在人群中也可能引起一定程度的变态反应，而引起豚鼠较弱反应的物质在人群中也许不能引起变态反应。

6 Interpretation of test results

The test results should be able to obtain the sensitization ability and strength of the test substance. These results can only be extrapolated to humans in a very limited range. The substances that cause the guinea pig's strong reaction may also cause allergic reaction to a certain extent in the crowd, while the substances that cause the guinea pig's weak reaction may not cause allergic reaction in the crowd.

7 皮肤光毒性试验

Skin Phototoxicity Test

1 范围

本规范规定了皮肤光毒性试验的基本原则，要求和方法。本规范适用于化妆品原料及其产品安全性毒理学检测。

2 试验目的

评价化妆品原料及其产品引起皮肤光毒性的可能性。

7 Skin phototoxicity test

1 Range

This specification specifies the basic principles, requirements and methods of skin phototoxicity test. This specification is applicable to the safety toxicology test of cosmetics raw materials and products.

2 Test purpose

To evaluate the possibility of skin phototoxicity caused by cosmetic materials and products.

3 定义

光毒性 phototoxicity

皮肤一次接触化学物质后，继而暴露于紫外线照射下所引发的一种皮肤毒性反应，或者全身应用化学物质后，暴露于紫外线照射下发生的类似反应。

4 试验的基本原则

将一定量受试物涂抹在动物背部去毛的皮肤上，经一定时间间隔后暴露于 UVA 光线下，观察受试动物皮肤反应并确定该受试物有否光毒性。

3 Definition

Phototoxicity

A skin toxic reaction caused by exposure of skin to chemicals at one time and then to ultraviolet radiation, or a similar reaction caused by exposure to ultraviolet radiation after application of chemicals in the whole body.

4 Basic principles of test

Apply a certain amount of test substance on the depilated skin on the back of the animal, after a certain time interval, expose to UVA light, observe the skin reaction of the test animal and determine whether the test substance has phototoxicity.

5 试验方法

5. 1 受试物

液体受试物一般不用稀释，可直接使用原液。若受试物为固体，应将其研磨成细粉状并用水或其他溶剂充分湿润，在使用溶剂时，应考虑到溶剂对受试动物皮肤刺激性的影响。对于化妆品产品而言，一般使用原霜或原液，所用受试物浓度不能引起皮肤刺激反应 (可通过预试验确定)。阳性对照物选用 8—甲氧基补骨脂 （8-methoxypsoralen，8—Mop）。

5. 2 实验动物和饲养条件

使用成年白色家兔或白化豚鼠，尽可能雌雄各半。选用 6 只动物进行正式试验。试验前动物要在实验动物房环境中至少适应 3d—5d 时间。

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制，需注意补充适量 Vc。

5. 3 UV 光源

5.3.1 UV 光源：波长为 320nm—400nm 的 UVA，如含有 UVB，其剂量不得超过 0.1J/cm2。

5.3.2 强度的测定：用前需用辐射计量仪在实验动物背部照射区设 6 个点测定光强度

（mW/cm2），以平均值计。

5 test method

5. 1 Test substance

Generally, the liquid test substance does not need to be diluted, and the original solution can be used directly. If the test substance is a solid, it shall be ground into a fine powder and fully wetted with water or other solvents. When using solvents, the effects of solvents on the skin irritation of the test animals shall be taken into account. For cosmetic products, the original cream or solution is generally used, and the concentration of the test substance cannot cause skin irritation reaction (it can be determined by pre test).8-methoxypsoralen (8-MOP) is selected as the positive control.

5. 2 Laboratory animals and feeding conditions

Use adult white rabbits or albino guinea pigs, half male and half female as much as possible. Six animals are selected for formal test. Before the experiment, the animals should adapt to the environment of the laboratory for at least 3-5 days.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, unlimited drinking water, and pay attention to supplement appropriate VC.

5.3 UV light source

5.3.1 UV light source: UVA with a wavelength of 320nm-400nm, if containing UVB, its dose shall not exceed 0.1j/cm2.

5.3.2 Determination of intensity: 6 points shall be set in the back irradiation area of experimental animals to measure the light intensity before use (MW / cm2), in average.

5.3.3 照射时间的计算：照射剂量为 10J/cm2，按下式计算照射时间。

$$照射时间(sec) = \frac{照射剂量(10000mJ/cm^2)}{光强度(mJ/cm^2/sec)}$$

注：$1\ mW/cm^2 = 1\ mJ/cm^2/sec$

5.3.3 Calculation of irradiation time: The irradiation dose is 10J/cm2, and the irradiation time is calculated according to the following formula.

Exposure time (sec) = irradiation dose (10000mJ/ cm2) / Light intensity (mJ/cm2/sec)

Note: 1 mW/cm2 = 1 mJ/cm2/sec

5.4 试验步骤

5.4.1 进行正式光毒试验前 18h—24h，将动物脊柱两侧皮肤去毛，试验部位皮肤需完好，无损伤及异常。备 4 块去毛区（见图 1），每块去毛面积约为 2cm×2cm。

5.4.2 将动物固定，按表 1 所示，在动物去毛区 1 和 2 涂敷 0.2mL(g)受试物，30min 后，左侧（去毛区 1 和 3）用铝箔复盖，胶带固定，右侧用 UVA 进行照射。

5.4.3 结束后分别于 1、24、48 和 72h 观察皮肤反应，根据表 2 判定每只动物皮肤反应评分。

5.4.4 为保证试验方法的可靠性，至少每半年用阳性对照物检查一次。即在去毛区 1 和 2 涂阳性对照物，方法同 5.4.2。

5.4 Test procedure

5.4.1 18-24 hours before the official phototoxicity test, the skin on both sides of the spine of the animal shall be depilated, and the skin at the test site shall be intact without damage or abnormality. Prepare 4 depilated areas (see Figure 1), each with an area of about 2cm × 2cm.

5.4.2 Fix the animal, as shown in Table 1, apply 0.2ml (g) of test substance to the depilated area 1 and 2 of the animal, 30 minutes later, cover the left side (depilated area 1 and 3) with aluminum foil, fix it with adhesive tape, and irradiate the right side with UVA.

5.4.3 The skin reaction is observed at 1, 24, 48 and 72 hours after the end of the treatment, and the skin reaction score of each animal is determined according to table 2.

5.4.4 In order to ensure the reliability of the test method, the positive control substance shall be checked at least once every six months. That is to say, positive control substances are applied to depilation areas 1 and 2 in the same way as in 5.4.2.

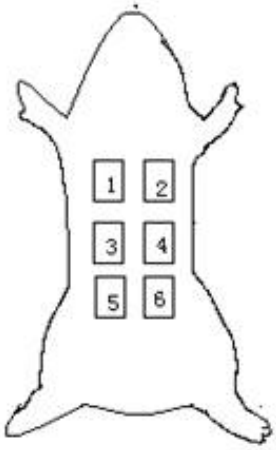


图 1 动物皮肤去毛区位置示意图

Fig. 1 location of hair removal area of animal skin

表 1 动物去毛区的试验安排

去毛区编号	试验处理
1	涂受试物，不照射
2	涂受试物，照射
3	不涂受试物，不照射
4	不涂受试物，照射

Table 1 experimental arrangement of animal hair removal area

Depilation Area No	Test treatment
1	Coated test substance, not irradiated
2	Coating test substance, irradiation
3	No test substance, no irradiation

4	Uncoated, irradiated

表 2 皮肤刺激反应评分

皮肤反应	积 分
红斑和焦痂形成	
无红斑	0
轻微红斑（勉强可见）	1
明显红斑	2
中度—重度红斑	3
严重红斑（紫红色）至轻微焦痂形成	4
水肿形成	
无水肿	0
轻微水肿（勉强可见）	1

皮肤反应	积 分
轻度水肿（皮肤隆起轮廓清楚）	2
中度水肿（皮肤隆起约 1mm）	3
重度水肿（皮肤隆起超过 1mm，范围扩大）	4
最高积分	8

Table 2 skin irritation response score

Skin reaction	Integral
Erythema and eschar formation	
No erythema	0
Slight erythema (barely visible)	1
Obvious erythema	2
Moderate to severe erythema	3
Severe erythema (purplish red) to slight eschar formation	4
Edema formation	
No edema	0
Slight edema (barely visible)	1

Skin reaction	Integral
Mild edema (clear contour of skin bulge)	2
Moderate oedema (skin swelling about 1mm)	3
Severe edema (skin swelling more than 1mm, expanded range)	4
Highest integral	8

6 结果评价

单纯涂受试物而未经照射区域未出现皮肤反应，而涂受试物后经照射的区域出现皮肤反应分值之和为 2 或 2 以上的动物数为 1 只或 1 只以上时，判为受试物具有光毒性。

6 Result evaluation

When the total number of animals with skin reaction score of 2 or more in the irradiated area is 1 or more, the test substance is deemed to have phototoxicity.

8 鼠伤寒沙门氏菌 / 回复突变试验

Salmonella Typhimurium / Reverse Mutation Assay

1 范围

本规范确定了鼠伤寒沙门氏菌 / 回复突变试验的基本原则、要求和方法。本规范适用于化妆品原料及其产品的基因突变检测。

8 Salmonella Typhimurium / Reverse Mutation Assay

1 Range

This specification defines the basic principles, requirements and methods of Salmonella typhimurium / reverse mutation test.This specification is applicable to gene mutation detection of cosmetic raw materials and products.

2 定义

2.1 回复突变 reverse mutation

细菌在化学致突变物作用下由营养缺陷型回变到原养型(prototroph)。

2.2 基因突变 gene mutation

在化学致突变物作用下细胞 DNA 中碱基对的排列顺序发生变化。

2.3 碱基置换突变 base substitution mutation

引起 DNA 链上一个或几个碱基对的置换。

碱基置换有转换(transition)和颠换(transversion)两种形式。

转换是 DNA 链上的一个嘧啶被另一嘧啶所替代，或一个嘌呤被另一嘌呤所代替。颠换是 DNA 链上的一个嘧啶被另一嘌呤所替代，或一个嘌呤被另一嘧啶所代替。

2.4 移码突变 frameshift mutation

引起 DNA 链上增加或缺失一个或多个碱基对。

2.5 鼠伤寒沙门氏菌/回复突变试验 salmonella typhimurium/reverse mutation assay

利用一组鼠伤寒沙门氏组氨酸缺陷型试验菌株测定引起沙门氏菌碱基置换或移码突变的化学物质所诱发的组氨酸缺陷型(his-)→原养型(his+)回复突变的试验方法。

2.6 S9

经多氯联苯(PCB 混合物)或苯巴比妥钠和 β-萘黄酮结合诱导的大鼠制备肝匀浆，在 9000g 下离心 10min 后的肝匀浆上清液。

2 Definition

2.1 Reverse mutation

Under the action of chemical mutagens, bacteria changed from deficient to prototroph.

2.2 Gene mutation

Under the action of chemical mutagens, the sequence of base pairs in cell DNA changes.

2.3 Base substitution mutation

Causes the replacement of one or more base pairs on a DNA strand.

There are two forms of base substitution: transition and transversion.

Transformation is the replacement of one pyrimidine in the DNA chain by another pyrimidine, or the replacement of one purine by another purine.

Transversion is the substitution of one pyrimidine in a DNA strand by another purine, or one purine by another pyrimidine.

2.4 Frameshift mutation

Causes the addition or deletion of one or more base pairs on the DNA strand.

2.5 Salmonella typhimurium / reverse mutation assay

A group of Salmonella typhimurium histidine deficient test strains are used to determine the test method of histidine deficient (his -) prototrophic (his) + revertant mutation induced by chemicals that cause base replacement or frameshift mutation of Salmonella.

2.6 S9

The liver homogenate is prepared by the combination of PCBs or sodium phenobarbital with β - naphthalene flavone

The supernatant of liver homogenate is centrifuged at 9000g for 10min.

3 原理

鼠伤寒沙门氏组氨酸营养缺陷型菌株不能合成组氨酸，故在缺乏组氨酸的培养基上，仅少数自发回复突变的细菌生长。假如有致突变物存在，则营养缺陷型的细菌回复突变成原养型，因而能生长形成菌落，据此判断受试物是否为致突变物。

某些致突变物需要代谢活化后才能引起回复突变，故需加入经诱导剂诱导的大鼠肝制备的 S9 混合液。

4 仪器和设备

培养箱、恒温水浴、振荡水浴摇床、压力蒸汽消毒器、干热烤箱、低温冰箱(-80℃)或液氮生物容器、普通冰箱、天平(精密度 0.1g 和 0.0001g)、混匀振荡器、匀浆器、菌落计数器、低温高速离心机，玻璃器皿等。

3 Principle

The histidine deficient strains of Salmonella typhimurium could not synthesize histidine, so only a few bacteria with spontaneous reverse mutation grew on the medium lacking histidine. If there is a mutagen, the bacteria of the nutritional deficiency type will revert to the prototrophic type, so that they can grow and form colonies, so as to judge whether the test substance is a mutagen.

Some mutagens need to be metabolized and activated to cause reverse mutation, so it is necessary to add S9 mixture prepared by rat liver induced by inducer.

4 Instruments and equipment

Incubator, constant temperature water bath, oscillating water bath shaker, pressure steam sterilizer, dry heat oven, low temperature refrigerator (- 80 ℃) or liquid nitrogen biological container, general refrigerator, balance (precision 0.1g and 0.0001g), mixing oscillator, homogenizer, colony counter, low temperature high-speed centrifugal machine, glassware, etc.

5 培养基和试剂

5.1 0.5mmol/L 组氨酸-0.5mmol/L 生物素溶液

成分： L-组氨酸(MW 155) 78mg

D-生物素(MW 244) 122mg

加蒸馏水至 1000mL

配制：将上述成分加热，以溶解生物素，然后在 0.068MPa 下高压灭菌 20min。贮于 4℃冰箱。

5.2 顶层琼脂培养基

成分： 琼脂粉 1.2g

氯化钠 1.0g

加蒸馏水至 200mL

配制：上述成分混合后，于 0.103MPa 下高压灭菌 30min。实验时，加入 0.5mmol/L 组氨酸—0.5mmol/L 生物素溶液 20mL。

5 Media and reagents

5.1 0.5mmol/l histidine-0.5mmol/l biotin solution

Ingredient: L-histidine (MW 155): 78mg

D-biotin (MW 244): 122mg

Add distilled water to: 1000mL

Preparation: The above ingredients are heated to dissolve biotin, and then autoclaved at 0.068MPa for 20min minutes. Store in 4℃ refrigerator.

5.2 Top agar medium

Ingredients: agar powder 1.2g

Sodium chloride 1.0g

Add distilled water to 200ml

Preparation: after mixing the above ingredients, autoclave at 0.103mpa for 30min.In the experiment, 20ml of 0.5mmol/l histidine-0.5mmol/l biotin solution is added.

5.3 Vogel-Bonner (V-B) 培养基 E

成分：

枸橼酸(C6H8O7·H2O) 100g

磷酸氢二钾(K2HPO4) 500g

磷酸氢铵钠(NaNH4HPO4·4H2O) 175g

硫酸镁(MgSO4·7H2O) 10g

加蒸馏水至 1000mL

配制：先将前三种成分加热溶解后，再将溶解的硫酸镁缓缓倒入容量瓶中，加蒸馏水至 1000mL。于 0.103MPa 下高压灭菌 30min。储于 4℃冰箱。

5.4 20%葡萄糖溶液

成分：葡萄糖 200g

加蒸馏水至 1000mL

配制：加少量蒸馏水加温溶解葡萄糖，再加蒸馏水至 1000mL。于 0.068MPa 下高压灭菌 20min。储于 4℃冰箱。

5.3 Vogel Bonner (V-B) medium e

Ingredient:

citric acid (C6H8O7 · H2O) 100g

Potassium hydrogen phosphate (K2HPO4) 500g
Sodium hydrogen phosphate (nanh4hpo4 · 4H2O) 175g
Magnesium sulfate (MgSO4 · 7H2O) 10g
Add distilled water to 1000ml

Preparation: first heat and dissolve the first three ingredients, then slowly pour the dissolved magnesium sulfate into the volumetric flask, add distilled water to 1000ml.Autoclave at 0.103mpa for 30min.Store in 4 ℃ refrigerator.

5.4 20% glucose solution

Ingredient: glucose 200g
Add distilled water to 1000ml

Preparation: add a small amount of distilled water, warm and dissolve glucose, and then add distilled water to 1000ml.Autoclave at 0.068mpa 20min. Store in 4 ℃ refrigerator.

5.5 底层琼脂培养基

成分：琼脂粉 7.5g
蒸馏水 480mL
V-B 培养基 E 10mL
20%葡萄糖溶液 10mL

配制：首先将前两种成分于 0.103MPa 下高压灭菌 30min 后，再加入后两种成分，充分混匀倒底层平板。按每皿 25mL 制备平板，冷凝固化后倒置于 37℃培养箱中 24h，备用。

5.6 营养肉汤培养基

成分：牛肉膏 2.5g
胰 胨 5.0g
磷酸氢二钾(K2HPO4) 1.0g
加蒸馏水至 500mL

配制：将上述成分混合后，于 0.103MPa 下高压灭菌 30min。储于 4℃冰箱。

5.5 Basal agar medium

Ingredients: agar powder 7.5G
Distilled water 480ml
V-B medium e 10ml
20% glucose solution 10ml

Preparation: first sterilize the first two ingredients under high pressure at 0.103mpa for 30min, then add the last two ingredients, mix them well and pour them into the bottom plate.Prepare the plate according to 25ml of each dish, after condensation and solidification, invert it into a 37 ℃ incubator for 24h, for standby.

5.6 Nutrient broth

Ingredients: beef paste 2.5G
Tryptone 5.0g
Dipotassium hydrogen phosphate (K2HPO4) 1.0g
Add distilled water to 500ml

Preparation: after mixing the above ingredients, autoclave at 0.103mpa for 30min.Store in 4 ℃ refrigerator.

5.7 盐溶液(1.65mol/L KCl+0.4mol/L MgCl2)

成分： 氯化钾(KCl) 61.5g

氯化镁(MgCl2·6H2O) 40.7g

加蒸馏水至 500mL

配制：在水中溶解上述成分后，于 0.103MPa 下高压灭菌 30min。储于 4℃冰箱。

5.8 0.2mol/L 磷酸盐缓冲液(pH7.4)

成分： 磷酸二氢钠(NaH2PO4·2H2O) 2.965g

磷酸氢二钠(Na2HPO4·12H2O) 29.015g

加蒸馏水至 500mL

配制：溶解上述成分后，于 0.103MPa 下高压灭菌 30min。储于 4℃冰箱。

5.9 S9 混合液

成分 每毫升 S9 混合液

肝 S9 100ml

盐溶液 20ml

灭菌蒸馏水 380ml

0.2mol/L 磷酸盐缓冲液 500ml

辅酶 II(NADP) 4mmol

6-磷酸葡萄糖(G-6-P) 5mmol

配制：将辅酶 II 和 6-磷酸葡萄糖置于灭菌三角瓶内称重，然后按上述相反的次序加入各种成分， 使肝 S9 加到已有缓冲液的溶液中。该混合液必须临用现配，并保存于冰水浴中。实验结束，剩余 S9 混合液应该丢弃。

5.7 Salt solution (1.65mol/l KCl + 0.4mol/l MgCl2)

Ingredient: potassium chloride (KCl) 61.5g

Magnesium chloride (MgCl2 · 6H2O) 40.7g

Add distilled water to 500ml

Preparation: after dissolving the above ingredients in water, autoclave at 0.103mpa for 30min.Store in 4 ℃ refrigerator.

5.8 0.2 mol/l phosphate buffer (pH7.4)

Ingredient: sodium dihydrogen phosphate (NaH2PO4 · 2H2O) 2.965g

Disodium hydrogen phosphate (Na2HPO4 · 12H2O) 29.015g

Add distilled water to 500ml

Preparation: after dissolving the above ingredients, autoclave at 0.103mpa for 30min.Store in 4 ℃ refrigerator.

5.9 S9 mixture

Ingredients S9 mixture / ml

Liver S9 100ml

Salt solution 20ml

Sterilized distilled water 380ml

0.2mol/l phosphate buffer 500ml

Coenzyme II (NADP) 4mmol

6-Glucose phosphate (g-6-p) 5mmol

Preparation: put coenzyme II and glucose-6-phosphate into a sterilized triangular flask and weigh them, then add various components in the reverse order, so that liver S9 is added to the solution of the existing buffer solution. The mixture must be prepared temporarily and stored in an ice water bath. At the end of the experiment, the remaining S9 mixture should be discarded.

5.10 菌株鉴定用和特殊用途试剂

5.10.1 组氨酸—生物素平板

成分：琼脂粉 15g

蒸馏水 944mL

(V-B)培养基 E 20mL

20%葡萄糖 20mL

灭菌盐酸组氨酸水溶液(0.5g/100mL) 10mL

灭菌 0.5mmol/L 生物素溶液 6mL

配制：高压灭菌琼脂和水后，将灭菌 20%葡萄糖，V-B 培养基和组氨酸溶液加进热的琼脂溶液中。待溶液稍为冷却后，加入灭菌生物素，混匀，浇制平板。

5.10.2 氨苄青霉素平板和氨苄青霉素/四环素平板

成分：琼脂粉 15g

蒸馏水 940mL

(V-B)盐溶液 20mL

20%葡萄糖 20mL

灭菌盐酸组氨酸溶液（0.5g/100mL） 10mL

灭菌 0.5mmol/L 生物素溶液 6mL

氨苄青霉素溶液(8mg/mL 于 0.02mol/LNaOH 中) 3.15mL

四环素溶液(8mg/mL 于 0.02mol/L HCl 中) 0.25mL

配制：琼脂和水高压灭菌 20min，将无菌的葡萄糖、VB 盐溶液和组氨酸—生物素溶液加进热的溶液中去，混匀。冷却至大约 50℃，无菌条件下加入四环素溶液和/或氨苄青霉素溶液。

应该在倾注琼脂平板后几天内，制备主平板。

5.10.3 营养琼脂平板

成份：琼脂粉 7.5g

营养肉汤培养基 500mL

配制：于 0.103MPa 下高压灭菌 30min 后倾注平板。

5.10 Reagents for strain identification and special use

5.10.1 Histidine biotin plate

Ingredients: agar powder 15g

Distilled water 944ml

(V-B) medium e 20ml

20% glucose 20ml

Sterilized histidine hydrochloride aqueous solution (0.5g / 100ml) 10ml

Sterilization 0.5mmol/l biotin solution 6ml

Preparation: after high-pressure sterilization of agar and water, sterilized 20% glucose, V-B medium and histidine solution are added into hot agar solution.After the solution is slightly cooled, add the sterilized biotin, mix well, and pour the plate.

5.10.2 Ampicillin plate and ampicillin / tetracycline plate

Ingredients: agar powder 15g

Distilled water 940ml

(V-B) salt solution 20ml

20% glucose 20ml

Sterilization histidine hydrochloride solution (0.5g / 100ml) 10ml

Sterilization 0.5mmol/l biotin solution 6ml

Ampicillin solution (8mg / ml in 0.02mol/lnaoh) 3.15ml

Tetracycline solution (8mg / ml in 0.02mol/l HCl) 0.25ml

Preparation: sterilize agar and water under high pressure for 20min, add sterile glucose, VB salt solution and histidine biotin solution into hot solution, mix well.Cool to about 50 ℃, and add tetracycline solution and / or ampicillin solution in sterile condition.

The main plate should be prepared within a few days of pouring the agar plate.

5.10.3 Nutrient agar plate

Ingredients: agar powder 7.5G

Nutrient broth medium 500ml

Preparation: pour the plate after 30 minutes of high-pressure sterilization at 0.103mpa.

6 试验菌株及其 Th 物学特性鉴定

6.1 试验菌株

采用 TA97、TA98、TA100 和 TA102 一组标准测试菌株。

6.2 生物学特性鉴定

新获得的或长期保存的菌种，在试验前必须进行菌株的生物特性鉴定。菌株鉴定的判断标准，如表 1 所示。

6 Test strains and identification of Th physical properties

6.1 Test strain

Ta97, TA98, TA100 and TA102 are used to test the strains.

6.2 Identification of biological characteristics

For the newly obtained or long-term preserved strains, the biological characteristics of the strains must be identified before the test. The judgment criteria for strain identification are shown in Table 1.

表 1 试验菌株鉴定的判断标准

菌株	组氨酸缺陷	脂多糖屏障缺损	氨苄青霉素抗性	切除修复缺损	四环素抗性	自发回变菌落数*
TA97	+	+	+	+	-	90—180
TA98	+	+	+	+	-	30—50
TA100	+	+	+	+	-	100—200
TA102	+	+	+	-	+	240—320
注	“+”表示需要组氨酸	“+”表示具有 rfa 突变	“+”表示具有 R 因子	“+”表示具有△uvrB 突变	“+”表示具有 pAQ1 质粒	* 在体外代谢活化条件下自发回变菌落数略增

Table 1 criteria for identification of test strains

Strain	Histidine deficiency	Lipopolysaccharide barrier defect	Ampicillin resistance	Excision and repair of defect	tetracycline resistance	Spontaneous reversion colony number*
TA97	+	+	+	+	-	90—180
TA98	+	+	+	+	-	30—50
TA100	+	+	+	+	-	100—200
TA102	+	+	+	-	+	240—320
notes	"+" indicates the need for histidine	"+" indicates RFA mutation	"+" means having R factor	"+" indicates a mutation of △uvrb	"+" indicates the presence of paq1 plasmid	*Under the condition of metabolic activation in vitro, the number of self returning colonies increased slightly

6.2.1 组氨酸缺陷

原理：组氨酸缺陷型试验菌株本身不能合成组氨酸，只能在补充组氨酸的培养基上生长，而在缺乏组氨酸的培养基上，则不能生长。

鉴定方法：将测试菌株增菌液分别于含组氨酸培养基平板和无组氨酸平板上划线，于

37℃下培养 24h 后观察结果。

结果判断：组氨酸缺陷型菌株在含组氨酸平板上生长，而在无组氨酸平板上则不能生长。

6.2.1 Histidine deficiency

Principle: histidine deficient test strains can't synthesize histidine by themselves, and can only grow on the medium supplemented with histidine, but can't grow on the medium lacking histidine.

Identification method: mark the enrichment solution of test strain on the plate containing histidine and the plate without histidine, respectively

The results are observed 24 hours after incubation at 37℃.

The results showed that the histidine deficient strain could not grow on the histidine containing plate, but on the histidine free plate.

6.2.2 脂多糖屏障缺损

原理：具有深粗糙（rfa）的菌株，其表面一层脂多糖屏障缺损，因此一些大分子物质如结晶紫能穿透菌膜进入菌体，从而抑制其生长，而野生型菌株则不受其影响。

鉴定方法：吸取待测菌株增菌液 0.1mL 于营养琼脂平板上划线，然后将浸湿的0.1%结晶紫溶液滤纸条与划线处交叉放置。37℃下培养 24h 后观察结果。

结果判断：假若待测菌在滤纸条与划线交叉处出现一透明菌带，说明该待测菌株具有 rfa 突变。

6.2.2 Lipopolysaccharide barrier defect

Principle: for strains with deep roughness (RFA), there is a layer of lipopolysaccharide barrier defect on the surface. Therefore, some macromolecular substances such as crystal violet can penetrate the bacterial membrane and enter into the bacteria, thus inhibiting its growth, while wild-type strains are not affected by it.

Identification method: draw 0.1ml of enrichment solution of the strain to be tested and scribe it on the nutrient agar plate, then place the filter paper strip of 0.1% crystal violet solution soaked in water across the scribe. The results are observed at 37 ℃ for 24 hours.

Results Judgment: If a transparent bacterial band appears at the intersection of the filter paper strip and the scribe line, the strain to be tested has rfa mutation.

6.2.3 氨苄青霉素抗性

原理：含 R 因子的试验菌株对氨苄青霉素有抗性。因为 R 因子不太稳定，容易丢失，故用氨苄青霉素确定该质粒存在与否。

鉴定方法：吸取待测菌株增菌液 0.1mL，在氨苄青霉素平板上划线，37℃下培养 24h 后观察结果。

结果判断；假若测试菌在氨苄青霉素平板上生长，说明该测试菌具有抗氨苄青霉素作用，表示含 R 因子，否则，表示测试菌不含 R 因子或 R 因子丢失。

6.2.4 紫外线敏感性

原理：具有△uvrB 突变的菌株对紫外线敏感，当受到紫外线照射后，不能生长，而具有野生型切除修复酶的菌株，则能照常生长。

鉴定方法：吸取待测菌株增菌液 0.1mL 于营养琼脂平板上划线，用黑纸盖住平板的一半，置紫外灯下照射（15W，距离 33cm）8 秒钟。置 37℃下孵育 24h 后观察结果。

结果判断：具有△uvrB 突变的菌株对紫外线敏感，经辐射后细菌不生长，而具有完整的切除修复系统的菌株，则照常生长。

6.2.3 Ampicillin resistance

Principle: the test strain containing R factor is resistant to ampicillin. Because R factor is not stable and easy to be lost, ampicillin is used to determine the existence of the plasmid.

Identification method: take 0.1ml of enrichment solution of the strain to be tested, draw lines on the ampicillin plate, and culture at 37 ℃ for 24h, then observe the results.

Results: if the test bacteria grow on the ampicillin plate, it indicates that the test bacteria has the effect of anti-ampicillin, indicating that it contains R factor, otherwise, it indicates that the test bacteria does not contain R factor or R factor is lost.

6.2.4 UV sensitivity

Principle: the strain with △ uvrB mutation is sensitive to UV, and can't grow when it is irradiated by UV, while the strain with wild type excision and repair enzyme can grow as usual.

Identification method: draw 0.1ml of enrichment solution of the strain to be tested, scribe on the nutrient agar plate, cover half of the plate with black paper, and irradiate under the ultraviolet lamp (15W, distance 33cm) for 8 seconds. The results are observed after incubation at 37 ℃ for 24 hours.

The results showed that the strains with △ uvrB mutation are sensitive to UV, and the bacteria did not grow after irradiation, but the strains with complete removal and repair system grew as usual.

6.2.5 四环素抗性

原理：具有 pAQI 的菌株对四环素有抗性。

鉴定方法：吸取待测菌株增菌液 0.1mL 于氨苄青霉素/四环素平板上划线，置 37℃下孵育 24h 后观察结果。

结果判断：假若测试菌照常在氨苄青霉素/四环素平板上生长，表明该测试菌株对氨苄青霉素和四环素两者有抗性，具有 pAQI 质粒，否则，说明测试菌株不含 pAQI 质粒。

6.2.6 自发回变

原理：每种试验菌株都以一定的频率自发地产生回变，称为自发回变。这种自发回变是每种试验菌株的一项特性。

鉴定方法：将待测菌株增菌液 0.1mL 加到 2mL 含组氨酸—生物素的顶层琼脂培养基的试管内，混匀后铺到于底层琼脂平板上，待琼脂固化后，置 37℃培养箱中孵育 48h 后记数每皿回变菌落数。

结果判断：每种标准测试菌株的自发回变菌落数应符合表 1 要求。经体外代谢活化后的自发回变菌落数，要比直接作用下的略高。

6.2.5 tetracycline resistance

Principle: strains with pAQI are resistant to tetracycline.

Identification method: take 0.1ml of enrichment solution of the strain to be tested, draw a line on the ampicillin / tetracycline plate, incubate at 37 ℃ for 24h, and observe the results.

The results showed that if the test strain grew on the ampicillin / tetracycline plate as usual, the test strain is resistant to both ampicillin and tetracycline, and had pAQI plasmid, otherwise, the test strain did not contain pAQI plasmid.

6.2.6 Spontaneous reversion

Principle: each test strain produces spontaneous reversion at a certain frequency, which is called spontaneous reversion. This spontaneous reversion is a characteristic of each test strain.

Identification method: add 0.1ml of enrichment solution to 2ml of top agar medium containing histidine biotin, mix well and spread it on the bottom agar plate. After the agar solidifies, incubate in 37 ℃ incubator for 48h and count the number of changed colonies in each dish.

Results judgment: the number of spontaneous revertant colonies of each standard test strain should meet the requirements of Table 1.The number of spontaneous revertant colonies after in vitro metabolism activation is slightly higher than that under direct action.

6.2.7 回变特性—诊断性试验

原理：每种试验菌株对诊断性诱变剂回变作用的性质以及 S9 混合液的效应不一。

鉴定方法：按照平板掺入试验的操作步骤进行。将受试物换成诊断性诱变剂。

结果判断：标准菌株对某些诊断性诱变剂特有的回变结果参见表 2。

6.2.7 Return characteristic diagnostic test

Principle: the nature of each test strain's reversion to diagnostic mutagen and the effect of S9 mixture are different.

Identification method: according to the operation steps of plate mixing test. Replace the test substance with diagnostic mutagen.

Results judgment: see Table 2 for the specific reversion results of standard strains to some diagnostic mutagens.

表 2 测试菌株的回变性

诱变剂	剂量(mg)	S9	TA97	TA98	TA100	TA102

柔毛霉素	6.0	-	124	3123	47	592
叠氮化钠	1.5	-	76	3	3000	188
ICR—191	1.0	-	1640	63	185	0
链霉黑素	0.25	-	inh	inh	inh	2230
丝裂霉素 C	0.5	-	inh	inh	inh	2772
2,4,7-三硝基-9-芴酮	0.20	-	8377	8244	400	16
4-硝基-O-次苯二胺	20	-	2160	1599	798	0
4-硝基喹啉-N-氧化物	0.5	-	528	292	4220	287
甲基磺酸甲酯	1.0	-	174	23	2730	6586
2-氨基芴	10	+	1742	6194	3026	261
苯并（a）芘	1.0	+	337	143	937	255

inh 表示抑菌。表中数值均已扣除溶剂对照回变菌落数。

Table 2. Reversion of tested strains

Mutagen	Dose (mg)	S9	TA97	TA98	TA100	TA102	
Pilomycin	6.0	-	124	3123	47	592	
sodium azide	1.5	-	76	3	3000	188	
ICR-191	1.0	-	1640	63	185		0
Streptomycin melanin	0.25	-	inh	inh	inh	2230	
Mitomycin C	0.5	-	inh	inh	inh	2772	
2,4,7-Trinitro-9-fluorenone	0.20	-	8377	8244	400	16	
4-Nitro-o-phenylenediamine	20	-	2160	1599	798		0
4-Nitroquinoline-n-oxide	0.5	-	528	292	4220	287	
methyl sulfonate	1.0	-	174	23	2730	6586	
2-Aminofluorene	10	+	1742	6194	3026	261	
Benzo (a) pyrene	1.0	+	337	143	937	255	

INH indicates bacteriostasis. The number of revertant colonies of solvent control has been deducted from the values in the table.

7 大鼠肝微粒体酶的诱导和 S9 的制备

7.1 诱导

选择健康雄性成年大鼠，体重 200g 左右。将多氯联苯（PCB 混合物）溶于玉米油中，浓度为 200mg/mL，按 500mg/kg 体重一次腹腔注射，5d 后处死动物，处死前禁食 12h。

也可采用苯巴比妥钠和 β-萘黄酮联合诱导的方法进行制备。经口或腹腔注射给予 80mg/kg 苯巴比妥钠和 80mg/kgβ-萘黄酮，连续 3 天，处死前禁食 16h。

7 Induction of rat liver microsomal enzyme and preparation of S9

7.1 Induce

Healthy male adult rats are selected with a body weight of about 200g.The polychlorinated biphenyls (PCB mixture) are dissolved in corn oil at a concentration of 200mg / ml, and injected intraperitoneally once at a weight of 500mg / kg. The animals are killed five days later and fasted for 12 hours before the death.

It can also be prepared by the combined induction method of phenobarbital sodium and beta-naphthoflavone. 80mg/kg phenobarbital sodium and 80mg/kgβ- naphthoflavone were given orally or intraperitoneally for 3 consecutive days and fasting for 16 hours before execution.

7.2 S9 制备

首先，用 75%酒精消毒动物皮毛，剖开腹部。在无菌条件下，取出肝脏，去除肝脏的结缔组织，用冰浴的 0.15mol/L 氯化钾溶液淋洗肝脏，放入盛有 0.15mol/L 氯化钾溶液的烧杯里。按每克肝脏加入 0.15mol/L 氯化钾溶液 3mL。用电动匀浆器制成肝匀浆，再在低温高速离心机上，在 4℃条件下，以 9000g 离心 10min，取其上清液（S9）分装于塑料管中。每管装 2 mL —3 mL。储存于液氮生物容器中或-80℃冰箱中备用。

上述全部操作均在冰水浴中和无菌条件下进行。制备肝 S9 所用一切手术器械、器皿等，均经灭菌消毒。S9 制备后，其活力需经诊断性诱变剂进行鉴定。

7.2 S9 preparation

First, disinfect animal fur with 75% alcohol and cut open the abdomen. Under sterile condition, take out the liver, remove the connective tissue of the liver, wash the liver with 0.15mol/l potassium chloride solution of ice bath, and put it into a beaker containing 0.15mol/l potassium chloride solution. Add 3ml of 0.15mol/l potassium chloride solution per gram of liver. The liver homogenate is made by electric homogenizer, and then centrifuged at 4 ℃ for 10 minutes at 9000g on a low-temperature high-speed centrifuge. The supernatant (S9) is separated into plastic tubes.2-3 ml for each tube. Store in liquid nitrogen biological container or refrigerator at - 80 ℃ for standby.

All the above operations are carried out in ice water bath and sterile condition. All surgical instruments and utensils used in the preparation of liver S9 are sterilized. After S9 preparation, its activity needs to be identified by diagnostic mutagen.

8 溶剂的选择

如果受试物为水溶性，可用灭菌蒸馏水作为溶剂；如为脂溶性，应选择对试验菌株毒性低且无致突变性的有机溶剂，常用的有二甲基亚砜（DMSO）、丙酮、95%乙醇。一般操作中，为了减少误差和溶剂的影响，常按每皿使用剂量用同一溶剂配成不同的浓度，固定加入量为 100ml。

8 Solvent selection

If the test substance is water-soluble, sterilized distilled water can be used as the solvent; if it is fat soluble, the organic solvent with low toxicity to the test strain and no mutagenicity should be selected, commonly used are DMSO, acetone and 95% ethanol. In general operation, in order to reduce the error and the influence of solvent, the same solvent is often used to prepare different concentrations according to the dosage of each dish, and the fixed dosage is 100ml.

9 剂量的设计

决定受试物最高剂量的标准是对细菌的毒性及其溶解度。自发回变数的减少，背景菌变得清晰或被处理的培养物细菌存活数减少，都是毒性的标志。

对原料而言，一般最高剂量组可为 5mg/皿或 5ul/皿。对产品而言，有杀菌作用的受试物，最高剂量可为最低抑菌浓度，无杀菌作用的受试物，最高剂量可为原液。受试物至少应设四个剂量组。每个剂量均做三个平行平板。

9 Dose design

The standard for determining the maximum dose of test substance is its toxicity to bacteria and its solubility. The decrease of spontaneous return variable, the clear background bacteria or the decrease of bacterial survival in the treated culture are all signs of toxicity.

For raw materials, the general maximum dose group can be 5mg / dish or 5ul / dish. For the product, the highest dose of the test substance with bactericidal effect can be the lowest bacteriostatic concentration, the highest dose of the test substance without bactericidal effect can be the original solution. There should be at least four dose groups for the test substance. Three parallel plates are made for each dose.

10 试验操作步骤

10.1 增菌培养

取营养肉汤培养基 5mL，加入无菌试管中，将主平板或冷冻保存的菌株培养物接种于营养肉汤培养基内，37℃振荡（100 次/min）培养 10h。该菌株培养物应每毫升不少于 1—2×109 活菌数。

10.2 平板掺入法

实验时，将含 0.5mmol/L 组氨酸-0.5mmol/L 生物素溶液的顶层琼脂培养基 2.0mL 分装于试管中，45℃水浴中保温，然后每管依次加入试验菌株增菌液 0.1mL，受试物溶液 0.1mL 和 S9 混合液 0.5mL（需代谢活化时），充分混匀，迅速倾入底层琼脂平板上，转动平板，使之分布均匀。水平放置待冷凝固化后，倒置于 37℃培养箱里孵育 48h。记数每皿回变菌落数。

实验中，除设受试物各剂量组外，还应同时设空白对照、溶剂对照、阳性诱变剂对照和无菌对照。

10 Test operation steps

10.1 Enrichment culture

Take 5ml of nutrient broth culture medium, add it into sterile test tube, inoculate the main plate or frozen preserved strain culture into nutrient broth culture medium, and

culture for 10h under 37 ℃ shaking (100 times / min).The number of viable strains should be no less than 1-2 × 109 per ml.

10.2 Plate incorporation method

In the experiment, the top layer agar medium containing 0.5mmol/l histidine-0.5mmol/l biotin solution is divided into 2.0ml tubes, which are kept warm in a 45 ℃ water bath, and then 0.1ml of enrichment solution, 0.1ml of test solution and S9 mixture are added to each tube in turn0.5ml (when metabolic activation is required), mix it well, pour it into the bottom agar plate quickly, and rotate the plate to make it evenly distributed. After condensation and solidification, it is placed horizontally and incubated in 37 ℃ incubator for 48h.Count the number of changed colonies per dish.

In the experiment, in addition to each dose group of test substance, blank control, solvent control, positive mutagen control and sterile control should also be set.

11 数据处理和结果判断

记录受试物各剂量组、空白对照（自发回变）、溶剂对照以及阳性诱变剂对照的每皿回变菌落数，并求平均值和标准差。

如果受试物的回变菌落数是溶剂对照回变菌落数的两倍或两倍以上，并呈剂量-反应关系者，则该受试物判定为致突变阳性；受试物在任何一个剂量条件下，出现阳性反应并有可重复性，则该受试物判定为致突变阳性。

受试物经上述四个试验菌株测定后，只要有一个试验菌株，无论在加 S9 或未加 S9 条件下为阳性，均可报告该受试物对鼠伤寒沙门氏菌为致突变阳性。

如果受试物经四个试验菌株检测后，无论加 S9 和未加 S9 均为阴性，则可报告该受试物为致突变阴性。

11 Data processing and result judgment

The colony number of each dish of each dose group, blank control (spontaneous reversion), solvent control and positive mutagen control are recorded, and the mean value and standard deviation are calculated.

If the number of revertant colonies of the test substance is twice or more than that of the solvent control, and there is a dose-response relationship, the test substance is determined to be mutagenic positive; if the test substance has a positive reaction and repeatability under any dose condition, the test substance is determined to be mutagenic positive.

After the test substance is tested by the above four test strains, as long as one test strain is positive with or without S9, the test substance can be reported to be mutagenic positive for salmonella typhimurium.

If the test substance is negative with or without S9 after being tested by four test strains, the test substance can be reported as mutagenic negative.

9 体外哺乳动物细胞染色体畸变试验

In Vitro Mammalian Cells Chromosome Aberration Test

1 范围

本规范规定了体外哺乳动物细胞染色体畸变试验的基本原则、要求和方法。本规范适用于检测化妆品原料及其产品的致突变性。

2 试验目的

本试验是用于检测培养的哺乳动物细胞染色体畸变，以评价受试物致突变的可能性。

9 In Vitro Mammalian Cells Chromosome Aberration Test

1 Range

This specification specifies the basic principles, requirements and methods of chromosome aberration test of mammalian cells in vitro. This specification is applicable to the detection of mutagenicity of cosmetic raw materials and products.

2 Test purpose

The purpose of this study is to detect chromosome aberrations in cultured mammalian cells and to evaluate the possibility of mutagenicity of the test substance.

3 定义

3.1 结构畸变 structural aberration

在细胞分裂的中期相阶段，用显微镜检出的染色体结构改变，表现为缺失、断片、互换等。结构畸变可分为以下两类。

3.1.1 染色体型畸变 chromosome-type aberration

染色体结构损伤，表现为在两个染色单体相同位点均出现断裂或断裂重组的改变。

3.1.2 染色单体型畸变 chromatid-type aberration

染色体结构损伤，表现为染色单体断裂或染色单体断裂重组的损伤。

3.2 有丝分裂指数 mitotic index

中期相细胞数与所观察的细胞总数之比值；是一项反映细胞增殖程度的指标。

3 Definition

3.1 Structural aberration

In the metaphase of cell division, the chromosome structural changes detected by microscope are deletion, fragment, exchange, etc. Structural aberration can be divided into the following two categories.

3.1.1 Chromosome-type aberration

The damage of chromosome structure shows the change of breakage or recombination at the same site of two chromatids.

3.1.2 chromatid-type aberration

The damage of chromosome structure is the damage of chromatid breaking or chromatid breaking and recombination.

3.2 Mitotic index

The ratio of the number of metaphase cells to the total number of observed cells is an index reflecting the degree of cell proliferation.

4 试验基本原则

在加入和不加入代谢活化系统的条件下，使培养的哺乳动物细胞暴露于受试物中。用中期分裂相阻断剂（如秋水仙素或秋水仙胺）处理，使细胞停止在中期分裂相，随后收获细胞，制片，染色，分析染色体畸变。

大部分的致突变剂导致染色单体型畸变，偶有染色体型畸变发生。虽然多倍体的增加可能预示着有染色体数目畸变的可能，但本方法并不适合用于测定染色体的数目畸变。

4 Basic principles of test

The cultured mammalian cells are exposed to the test substance with or without metabolic activation system. The metaphase blocker (such as colchicine or colchicine) is used to stop the cell from metaphase, and then the cell is harvested, sectioned, stained and analyzed for chromosomal aberrations.

Most of the mutagens lead to chromosomal aberrations, with occasional chromosomal aberrations. Although the increase of polyploidy may indicate the possibility of chromosome number aberrations, this method is not suitable for the determination of chromosome number aberrations.

5 试验方法

5.1 试剂和受试物制备

5.1.1 阳性对照物：可根据受试物的性质和结构选择适宜的阳性对照物，阳性对照物应是已知的断裂剂，能引起可检出的、并可重复的阳性结果。当外源性活化系统不存在时，可使用甲磺酸甲酯（methyl methanesulphonate (MMS)）、甲磺酸乙酯（ethylmethanesulphonate(EMS)）、乙基亚硝基脲(ethyl nitrosourea)、丝裂霉素

C(mitomycin C)、4-硝基喹啉-N-氧化物（4-nitroquinoline-N-oxide）。当外源性活化系统存在时，可使用苯并（a）芘[benzo(a)pyrene]、环磷酰胺(cyclophosphamide)。

5.1.2 阴性对照物：应设阴性对照，即仅含和受试物组相同的溶剂，不含受试物，其他处理和受试物组完全相同。此外，如未能证实所选溶剂不具有致突变性，溶剂对照与本实验室空白对照背景资料有明显差异，还应设空白对照。

5 test method

5.1 Reagent and test substance preparation

5.1.1 Positive control substance: A suitable positive control substance can be selected according to the nature and structure of the test substance. The positive control substance should be a known breaking agent, which can cause detectable and repeatable positive results. When the exogenous activation system does not exist, methyl methanesulphonate (MMS), ethylmethanesulphonate (EMS), ethyl nitrosourea, mitomycin C, 4- nitroquinoline -N- oxide can be used. When an exogenous activation system is present, benzo (a) pyrene [benzo(a)pyrene], cyclophosphamide can be used.

5.1.2 Negative control substance: negative control substance shall be set, that is, only containing the same solvent as the test substance group, excluding the test substance, and other treatments are exactly the same as the test substance group. In addition, if it is not confirmed that the selected solvent does not have mutagenicity, the background data of solvent control and blank control in our laboratory are significantly different, and blank control should be set up.

5.1.3 受试物

5.1.3.1 受试物的配制：固体受试物需溶解或悬浮于溶剂中，用前稀释至适合浓度；液体受试物可以直接加入试验系统和/或用前稀释至适合浓度。受试物应在使用前新鲜配制，否则就必须证实贮存不影响其稳定性。

5.1.3.2 溶剂的选择：溶剂必须是非致突变物，不与受试物发生化学反应，不影响细胞存活和 S9 活性。首选溶剂是培养液（不含血清）或水。二甲基亚砜（DMSO）也是常用溶剂，使用时浓度不应大于 0.5%。

5.1.3 Test substance

5.1.3.1 Preparation of test substance: the solid test substance shall be dissolved or suspended in the solvent and diluted to the appropriate concentration before use; the liquid test substance can be directly added into the test system and / or diluted to the appropriate concentration before use. The test substance shall be freshly prepared before use, otherwise it must be confirmed that storage does not affect its stability.

5.1.3.2 Selection of solvent: the solvent must be non mutagenic, not react with the test substance, not affect cell survival and S9 activity. The preferred solvent is culture medium (without serum) or water. Dimethyl sulfoxide (DMSO) is also a common solvent, the concentration should not be more than 0.5% when used.

5.1.3.3 受试物浓度设置

（1）最高浓度的选择：

决定最高浓度的因素是细胞毒性、受试物在试验系统中的溶解度以及 pH 或渗克分子浓度(osmolality)的改变。

（2）细胞毒性的确定：

应使用指示细胞完整性和生长情况的指标，在活化系统存在或不存在的两种条件下确定细胞毒性，例如细胞覆盖程度（degree of confluency）、存活细胞计数(viable cell counts)或有丝分裂指数(mitotic index)。应在预试验中确定细胞毒性和溶解度。

（3）剂量设置：

①至少应设置 3 个可供分析的浓度。当有细胞毒性时，其浓度范围应包括从最大毒性至几乎无毒性；通常浓度间隔系数不大于 2 —√10。

②在收获细胞时，最高浓度应能明显降低细胞覆盖程度、细胞计数或有丝分裂指数（均应大于 50%）。

③对于那些相对无细胞毒性的化合物，最高浓度应是 5μl/mL，5mg/mL 或 0.01mol/L。

④对于相对不溶解的物质，当浓度低于不溶解浓度时仍无毒性，则最高剂量应是，当处理期结束时，在最终培养液中溶解度限值以上的一个浓度。在某些情况下（即仅当高于最低不溶解浓度时才发生细胞毒性），应使用一个以上可看见沉淀的浓度。最好在试验处理开始和结束时均评价溶解度，因为由于细胞、S9 等的存在，在试验系统内在暴露过程中溶解度可能变化。不溶解性可用肉眼鉴别，但沉淀不能影响观察。

5.1.4 培养液：采用 MEM(Eagle)，并加入非必需氨基酸和抗菌素（青、链霉素，按 100IU/mL），胎牛血清或小牛血清按 10%加入。也可选用其他合适的培养液。

5.1.3.3 Test substance concentration setting

(1) Selection of the highest concentration:

The factors that determine the highest concentration are cytotoxicity, solubility of the test substance in the test system, and changes in pH or osmolality.

(2) Determination of cytotoxicity:

Cell toxicity should be determined under two conditions, with or without activation system, using indicators indicating cell integrity and growth, such as cell coverage, viable cell counts, or mitotic index. Cytotoxicity and solubility should be determined in the pre-test.

(3) Dose setting:

① At least 3 concentrations for analysis shall be set. When there is cytotoxicity, its concentration range should include from maximum toxicity to almost no toxicity; Usually the concentration interval coefficient is not more than 2-√ 10.

② When harvesting cells, the highest concentration should be able to significantly reduce cell coverage, cell count or mitotic index (all should be greater than 50%).

③ For those relatively non cytotoxic compounds, the maximum concentration should be 5 μ L / ml, 5mg / ml or 0.01mol/l.

④ For relatively insoluble substances, when the concentration is lower than the insoluble concentration, it is still non-toxic. The maximum dose should be a concentration above the solubility limit in the final culture solution at the end of the treatment period. In some cases (i.e. cytotoxicity occurs only when above the minimum insoluble concentration), more than one concentration of visible precipitates should be used. It is best to evaluate solubility at the beginning and end of the treatment because solubility may change during exposure within the test system due to the presence of

cells, S9, etc. The insolubility can be identified by naked eyes, but the precipitation can not affect the observation.

5.1.4 Culture medium: MEM (Eagle), non essential amino acids and antibiotics (penicillin and streptomycin, 100iu / ml) are added, and fetal bovine serum or calf serum is added at 10%.Other suitable culture medium can also be selected.

5.1.5 活化系统

通常使用的是 S9 混合物（S9 mix）。S9 是从经酶诱导剂（Aroclor 1254 或苯巴比妥钠和 β-萘黄酮联合使用）处理的啮齿动物肝脏获得的。S9 的制备同 Ames 试验。S9 的使用浓度为 1%—10%（终浓度）。S9 mix 中所加辅助因子的量由各实验室自行决定，但需对 S9 mix 的活性进行鉴定，必须能明显活化阳性对照物。也可使用下述

S9 0.125ml
MgC12 （0.4 mol/L） 0.02 ml
KC1（1.65mol/L） 0.02 ml
葡萄糖-6-磷酸 1.791mg
辅酶Ⅱ（氧化型，NADP） 3.0615mg
用无血清 MEM 培养液补足至 1mL.

5.1.5 Activation system

Usually S9 mix is used. S9 is obtained from rodent liver treated with enzyme inducer (Aroclor 1254 or sodium phenobarbital combined with β-naphthoflavone). The preparation of S9 was the same as Ames test. The concentration of S9 is 1%-10% (final concentration). The amount of auxiliary factors added in S9 mix is determined by each laboratory, but the activity of S9 mix must be identified and the positive control substance must be activated obviously. The following can also be used

S9 0.125ml
MgC12 （0.4 mol/L） 0.02 ml
KC1（1.65mol/L） 0.02 ml
Glucose-6-phosphate 1.791mg
Coenzyme Ⅱ (oxidation type, NADP) 3.0615mg
Make up to 1ml with serum-free MEM medium

5.2 试验步骤

5.2.1 细胞：可使用已建立的细胞株或细胞系，也可使用原代培养细胞。所使用的细胞应该在生长性能、染色体数目和核型、自发的染色体畸变率等方面有一定的稳定性。推荐使用中国地鼠卵巢（CHO）细胞株或中国地鼠肺（CHL）细胞株。

5.2.2 试验时，应同时设阳性对照物，阴性对照物和至少 3 个可供分析的受试物浓度组。

5.2.3 试验前一天，将一定数量的细胞接种于培养皿（瓶）中，放 CO2 培养箱内培养。

5.2.4 试验需在加入和不加入 S9 mix 的条件下进行。试验时，吸去培养皿（瓶）中的培养液，加入一定浓度的受试物、S9 mix（不加 S9 mix 时，需用培养液补足）以及一定量不含血清的培养液，放培养箱中处理 3h—6h。结束后，吸去含受试物的培养液，用 Hanks 液洗细胞 3 次，加入含 10%胎牛血清的培养液，放回培养箱，于 24h 内收获细胞。于收获前 2h—4h，加入细胞分裂中期阻断剂（如用秋水仙素，作用时间为 4h，终浓度为 1μg/mL）。

当受试物为原料时，如果在上述加入和不加入 S9 mix 的条件下均获得阴性结果，则尚需补加另外的试验，即在不加 S9 mix 的条件下，使受试物与试验系统的接触时间延长至 24h。

当难以得出明确结论时，应更换试验条件，如改变代谢活化条件、受试物与试验系统接触时间等重复试验。

5.2 Test procedure

5.2.1 Cell: the established cell line or cell line can be used, or the primary culture cell can be used. The cells used should have certain stability in growth performance, chromosome number and karyotype, spontaneous chromosome aberration rate, etc.I t is recommended to use Chinese hamster ovary (CHO) cell line or Chinese hamster lung (CHL) cell line.

5.2.2 During the test, positive control substance, negative control substance and at least three test substance concentration groups that can be analyzed shall be set at the same time.

5.2.3 On the day before the experiment, a certain number of cells are inoculated in a culture dish (bottle) and cultured in a CO2 incubator.

5.2.4 The test shall be conducted with and without S9 mix. During the test, the culture medium in the culture dish (bottle) is sucked away, and a certain concentration of test substance, S9 mix (if not, it needs to be supplemented with culture medium) and a certain amount of culture medium without serum are added, and then it is put into the incubator for 3h-6h.After that, the culture medium containing the test substance is sucked out, the cells are washed with Hanks solution three times, the culture medium containing 10% fetal bovine serum is added, and the cells are returned to the incubator and harvested within 24 hours. Two hours to four hours before harvest, add the medium-term blocker of cell division (such as colchicine, the action time is four hours, the final concentration is 1 μ g / ml).

When the test object is used as the raw material, if negative results are obtained under the above conditions with and without S9 mix, additional tests need to be added, i.e. the contact time between the test object and the test system is extended to 24h without S9 mix.

When it is difficult to reach a definite conclusion, the test conditions should be changed, such as changing metabolic activation conditions, contact time between the test object and the test system, etc.

5.2.5 收获细胞时，用 0.25%胰蛋白酶溶液消化细胞，待细胞脱落后，加入含 10%胎牛或小牛血清的培养液终止胰蛋白酶的作用，混匀，放入离心管以 1000r/min—1200r/min 的速度离心 5min—7min，弃去上清液，加入 0.075mol/L KC1 溶液低渗处理，继而以新配制的甲醇和冰醋酸液（容积比为 3:1）进行固定。空气干燥或火焰干燥法制片常规制片，用姬姆萨染液染色。

5.2.6 作染色体分析时，对化妆品终产品，每一处理组选择 100 个分散良好的中期分裂相（染色体数为 2n±2）进行染色体畸变分析。对化妆品原料，则每一处理组选 200 个（阳性对照可选 100 个）。在分析时应记录每一观察细胞的染色体数目，对于畸变细胞还应记录显微镜视野的坐标位置及畸变类型。

5.3 统计处理：对染色体畸变细胞率用 X2 检验，以评价受试物的致突变性。

5.4 结果评价：在下列两种情况下可判定受试物在本试验系统中具有致突变性：

（1）受试物引起染色体结构畸变数具有统计学意义，并有剂量相关性。

（2）受试物在任何一个剂量条件下，引起具有统计学意义的增加，并有可重复性。在评价时应把生物学和统计学意义结合考虑。

5.2.5 When harvesting the cells, digest the cells with 0.25% trypsin solution. After the cells fall off, add the culture medium containing 10% fetal bovine or calf serum to stop the trypsin effect, mix well, put it into a centrifuge tube and centrifugate at the speed of 1000r / min-1200r / min for 5min-7min, discard the supernatant and add 0.075mol/l KC1The solution is treated with low permeability, and then fixed with newly prepared methanol and glacial acetic acid solution (volume ratio of 3:1).The films are made by air drying or flame drying and dyed with Giemsa dye solution.

5.2.6 For chromosomal analysis, 100 well dispersed metaphase (chromosome number 2n ± 2) are selected for chromosomal aberration analysis in each treatment group. For cosmetic raw materials, 200 are selected for each treatment group (100 for positive control).The chromosome number of each observed cell should be recorded during the analysis, and the coordinate position and distortion type of the microscopical field of vision should also be recorded for the aberrant cells.

5.3 Statistical treatment: x2 test is used to evaluate the mutagenicity of the test substance.

5.4 Results evaluation: the mutagenicity of the test substance in the test system can be determined under the following two conditions:

(1) The number of chromosomal structural aberrations caused by the test substance is statistically significant and dose-dependent.

(2) Under any dose condition, the test substance causes a statistically significant increase and has repeatability. Biological and statistical significance should be considered in the evaluation.

6 结果解释

阳性结果表明受试物引起培养的哺乳动物体细胞染色体结构畸变。

阴性结果表明在本试验条件下，受试物不引起培养的哺乳动物体细胞染色体结构畸变。

6 Interpretation of results

The positive results showed that the test substance caused chromosome structural aberrations in cultured mammalian somatic cells.

The negative results showed that under the conditions of this experiment, the test substance did not cause the chromosome structure distortion of the cultured mammalian somatic cells.

10 体外哺乳动物细胞基因突变试验

In Vitro Mammalian Cell Gene Mutation Test

1 范围

本规范规定了体外哺乳类细胞基因突变试验的基本原则、要求和方法。本规范适用于检测化妆品原料及其产品的致突变性。

2 试验目的

该测试系统用于检测化妆品原料及其产品引起的突变，包括碱基对突变、移码突变和缺失等，从而评价受试物引起突变的可能性。

10 In Vitro Mammalian Cell Gene Mutation Test

1 Range

This specification specifies the basic principles, requirements and methods of gene mutation test of mammalian cells in vitro. This specification is applicable to the detection of mutagenicity of cosmetic raw materials and products.

2 Test purpose

The test system is used to detect the mutation caused by cosmetic raw materials and products, including base pair mutation, frameshift mutation and deletion, so as to evaluate the possibility of mutation caused by the test substance.

3 定义

3.1 正向突变 forward mutation
从原型至突变子型的基因突变，这种突变可引起酶和功能蛋白的改变。

3.2 突变频率 mutant frequency
所观察到的突变细胞数与存活细胞数之比值。

4 试验原理

在加入和不加入代谢活化系统的条件下，使细胞暴露于受试物一定时间，然后将细胞再传代培养。胸苷激酶正常水平的细胞对三氟胸苷（trifluorothymidine，TFT）等敏感，因而在培养液中不能生长分裂，突变细胞则不敏感，在含有 6-硫代鸟嘌呤（6-thioguanine，

6-TG）、 8-azaguanine (AG)或 TFT 的选择性培养液中能继续分裂并形成集落。基于突变集落数，计算突变频率以评价受试物的致突变性。

3 Definition

3.1 Forward mutation

Mutations in genes from prototypes to mutants that cause changes in enzymes and functional proteins.

3.2 Mutation frequency

The ratio of the number of mutant cells to the number of viable cells observed.

4 Test principle

Under the condition of adding or not adding the metabolic activation system, the cells are exposed to the test substance for a certain period of time, and then the cells are subcultured. The cells with normal thymidine kinase level are sensitive to trifluorotoxidine (TFT), so they can't grow and divide in the culture medium, while the mutant cells are not sensitive. They can continue to divide and form colonies in the selective culture medium containing 6-thioguanine (6-TG), 8-azaguanine (Ag) or TFT. Based on the number of mutation colonies, the mutation frequency is calculated to evaluate the mutagenicity of the test substance.

5 试验方法

5.1 试剂和受试物制备

5.1.1 受试物

5.1.1.1 受试物的配制：固体受试物需溶解或悬浮于溶剂中，用前稀释至适合浓度；液体受试物可以直接加入试验系统/或用前稀释至适合浓度。受试物应在使用前新鲜配制，否则就必须证实储存不影响其稳定性。

5.1.1.2 溶剂的选择：溶剂必须是非致突变物，不与受试物发生化学反应，不影响细胞存活和 S9 活性。首选溶剂是水或水溶性溶剂。二甲基亚砜（DMSO）也是常用溶剂，但使用时浓度不应大于 0.5%。

5.1.1.3 受试物浓度设置

5.1.1.3.1 最高浓度的选择：决定最高浓度的因素是细胞毒性、受试物在试验系统中的溶解度以及 pH 或渗克分子浓度（osmolality）的改变。

5.1.1.3.2 细胞毒性的确定：应使用指示细胞完整性和生长情况的指标，在活化系统存在或不存在两种条件下确定细胞毒性，例如相对集落形成率或相对细胞总生长情况（ total growth）。应在预试验中确定细胞毒性和溶解度。

5 test method

5.1 Reagent and test substance preparation

5.1.1 Test substance

5.1.1.1 Preparation of test substance: the solid test substance shall be dissolved or suspended in the solvent and diluted to the appropriate concentration before use; the liquid test substance can be directly added to the test system / or diluted to the appropriate

concentration before use. The test substance shall be freshly prepared before use, otherwise it must be confirmed that storage does not affect its stability.

5.1.1.2 Selection of solvent: the solvent must be non mutagenic, not react with the test substance, not affect cell survival and S9 activity. The preferred solvent is water or water-soluble. DMSO is also a common solvent, but the concentration should not be more than 0.5%.

5.1.1.3 Test substance concentration setting

5.1.1.3.1 Selection of the highest concentration: the factors determining the highest concentration are cytotoxicity, solubility of the test substance in the test system and the change of pH or osmolality.

5.1.1.3.2 Determination of cytotoxicity: indicators indicating cell integrity and growth should be used to determine cytotoxicity in the presence or absence of an activation system, such as relative colony-forming rate or total cell growth
growth）。Cytotoxicity and solubility should be determined in the pre-test.

5.1.1.3.3 剂量设置

至少应设置 4 个可供分析的浓度。当有细胞毒性时，其浓度范围应包括从最大毒性至几乎无毒性。通常浓度间隔系数在 2-√ 10 之间。

对于那些细胞毒性很低的化合物，最高浓度应是 5μL/mL, 5mg/mL 或 0.01mol/L。

如最高浓度是基于细胞毒性，那么该浓度组的细胞相对存活率（相对集落形成率）或相对细胞总生长情况应为 10%—20%（不低于 10%）。

对于相对不溶解的物质，其最高浓度应达到或超过在细胞培养状态下的溶解度限值。最好在试验处理开始和结束时均评价溶解度，因为由于 S9 等的存在，试验系统内在暴露过程中溶解度可能发生变化。不溶解性可用肉眼鉴别，但沉淀不应影响观察。

5.1.2 对照：在每一项试验中，在代谢活化系统存在和不存在的条件下均应设阳性对照和阴性（溶剂）对照。

5.1.1.3.3 Dose setting

At least 4 concentrations for analysis shall be set. When there is cytotoxicity, the concentration range should include from maximum toxicity to almost no toxicity. Usually the concentration interval coefficient is between 2 and √ 10.

For those compounds with low cytotoxicity, the maximum concentration should be 5 μ L / ml, 5mg / ml or 0.01mol/l.

If the highest concentration is based on cytotoxicity, the relative survival rate (relative colony formation rate) or relative total cell growth of the concentration group should be 10% - 20% (not less than 10%).

For relatively insoluble substances, the highest concentration should reach or exceed the solubility limit in cell culture. It is best to evaluate solubility at the beginning and end of the test treatment as solubility may change during exposure within the test system due to the presence of S9, etc. The insolubility can be identified by naked eyes, but the precipitation should not affect the observation.

5.1.2 Control: in each test, positive control and negative (solvent) control should be set under the condition that metabolic activation system exists or does not exist.

5.1.2.1 阳性对照：当使用代谢活化系统时，阳性对照物必须是要求代谢活化、并能引起突变的物质 。 在没有代谢活化系 统时 ， 阳性对照物可用甲磺酸乙酯（ethyl methanesulfonate-EMS，HPRT 试验）、甲磺酸甲酯（methyl methanesulphonate，MMS,TK 试验），乙基亚硝基脲（ethyl nitrosourea-ENU，HPRT 试验）等。在有代谢活化系统时，可以使用 3- 甲基胆蒽（ 3-methylcholanthrene， HPRT 试验； TK 试 验）、 环磷酰胺（cyclophosphamide,TK 试验）N-亚硝基胍（N-nitroso-dimethylamine，HPRT 试验）、7,12-二甲基苯蒽（HPRT 试验）等。也可使用其他适宜的阳性对照物。

5.1.2.2 阴性对照物:阴性对照(包括溶剂对照)除不含受试物外，其他处理应与受试物相同。此外，当不具有实验室历史资料证实所用溶剂无致突变作用和无其他有害作用时，还应 设空白对照。

5.1.2.1 Positive control: When using metabolic activation system, the positive control must be a substance that requires metabolic activation and can cause mutation. When there is no metabolic activation system, the positive contrast substance can be ethyl methanesulfonate-EMS (HPRT test), methyl methanesulfonate (MMS,TK test), ethyl nitrosourea-ENU (HPRT test), etc. When there is a metabolic activation system, 3-methylcholanthrone (HPRT test; TK test), cyclophosphamide (TK test), n-nitrosodimethylamine (HPRT test), 7,12- dimethylbenzanthracene (HPRT test), etc. Other suitable positive controls may also be used.

5.1.2.2 Negative control substance: the negative control substance (including solvent control substance) shall be treated the same as the test substance except for the test substance. In addition, when there is no laboratory historical data to prove that the solvent used has no mutagenic effect or other harmful effects, a blank control should be set up.

5.1.3 细胞：HPRT 位点突变分析常用中国仓鼠肺细胞株（V-79）和中国仓鼠卵巢细胞株（CHO）。TK 位点突变分析常用小鼠淋巴瘤细胞株（L5178Y）和人类淋巴母细胞株（TK6）。细胞在使用前应进行有无支原体污染的检查。

5.1.4 培养液：应根据实验所用系统和细胞类型来选择适宜的培养基。对于 V-79 或 CHO 细胞，常用 MEM（Eagle）培养基加入 10%胎牛血清和适量抗菌素。对于 L5178Y 或 TK6 细胞，常用 RPMI 1640 培养基加入 10%马血清和适量抗菌素。

5.1.5 活化系统：同体外哺乳类细胞染色体畸变试验。

5.1.6 选择剂：6-硫代鸟嘌呤（6-TG）：建议使用终浓度为 5mg/mL—10μg/mL，用碳酸氢钠溶液配制（0.5%）。三氟胸苷（TFT）：建议使用终浓度为 3mg /mL。

5.1.7 预处理培养基: THMG/ THG

为减少细胞的自发突变率,在试验前，先将细胞加在含 THMG 的培养液中培养 24h，杀灭自发的突变细胞，然后将细胞再接种于 THG（不含氨甲喋呤的 THMG 培养液）中培养 1—3d。

THMG 含除培养液成份外的各物质终未浓度如下：

胸苷 5´10-6mol/L

次黄嘌呤 5´10-5mol/L

氨甲喋呤 4´10-7mol/L

甘氨酸 1´10-4mol/L

5.1.3 Cell: HPRT site mutation analysis is commonly used for Chinese hamster lung cell strain (V-79) and chinese hamster ovary strain (CHO). TK site mutation analysis is commonly used for mouse lymphoma cell line (L5178Y) and human

lymphoblastic cell line (TK6). Cells should be checked for mycoplasma contamination before use.

5.1.4 Culture medium: the appropriate culture medium should be selected according to the system and cell type used in the experiment.For V-79 or CHO cells, 10% fetal bovine serum and appropriate amount of antibiotics are added to MEM (Eagle) medium. For L5178Y or TK6 cells, RPMI 1640 medium is used to add 10% horse serum and appropriate amount of antibiotics.

5.1.5 Activation system: in vitro mammalian cell chromosome aberration test.

5.1.6 Selector: 6-thioguanine (6-TG): it is recommended to use the final concentration of 5mg / ML-10 μ g / ml, prepared with sodium bicarbonate solution (0.5%).TFT: the recommended final concentration is 3mg / ml.

5.1.7 Pretreatment medium: thmg / THG

In order to reduce the spontaneous mutation rate of cells, before the test, the cells were added to THMG-containing culture solution for 24 hours to kill spontaneous mutant cells, and then the cells were inoculated into THG (THMG-containing culture solution without methotrexate) for 1-3 days.

The final concentration of THMG is as follows

Thymidine 5’10-6 mol/l

Hypoxanthine 5’10-5 mol/l

Methotrexate 4’10-7mol/l

Glycine 1’10-4mol/l

5.2 试验步骤

5.2.1 HPRT 位点突变分析

5.2.1.1 试验前 1d，接种细胞于培养瓶中，置于 37℃孵箱培养。

5.2.1.2 试验时吸去培养瓶中的培养液，加入一定浓度的受试物、S9-mix（不加入 S9-mix 的样品，用培养液补足）及一定量的不含血清培养液，置孵箱中处理 3h—6h 后，吸去培养液，用 Hank's 液洗细胞三次，加入含胎牛血清的培养液。

5.2.1.3 在受试物与细胞作用后当天和第 3d 将细胞按低密度分种，在第 7d 接种细胞，每个剂量 3 瓶。7d 后染色以测定细胞存活率。另将一定数量细胞接种于每个培养瓶中，每个剂量 8 瓶，3h 后加入 6-TG（终浓度为 5mg /mL），10d 后染色，计数突变细胞集落。

5.2 Test procedure

5.2.1 Mutation analysis of HPRT site

5.2.1.1 One day before the experiment, the cells are inoculated into culture flask and incubated in 37 ℃ incubator.

5.2.1.2 During the test, the culture solution in the culture flask was sucked out, and a certain concentration of the test substance, S9-mix (no S9-mix sample was added, and the culture solution was supplemented with the culture solution) and a certain amount of serum-free culture solution were added. after treatment for 3-6 hours in the incubator, the culture solution was sucked out, the cells were washed with Hank's solution three times, and the culture solution containing fetal bovine serum was added.

5.2.1.3 The cells are divided into low density groups on the same day and the third day after the interaction between the test substance and the cells. The cells are

inoculated on the seventh day with 3 bottles of each dose.7 days later, the cell survival rate is determined by staining. In addition, a certain number of cells are inoculated into each culture bottle, each dose is 8 bottles, and 6-TG is added 3 hours later (the final concentration is 5mg / ml), then stained 10 days later, and the mutant cell colonies are counted.

5.2.1.4 试验结果用 x 2 检验进行统计分析。

5.2.2 TK 位点突变分析（L5178Y 细胞，96 孔板法）

5.2.2.1 处理：取生长良好的细胞，调整密度为 5×105/mL，按 1%体积加入受试物，37℃震摇处理 3 小时。离心，弃上清液，用 PBS 或不含血清的培养基洗涤细胞 2 遍，重新悬浮细胞于含 10%马血清的 RPMI 1640 培养液中，并调整细胞密度为 2×105/mL。

5.2.2.2 PE0（0 天的平板接种效率）测定：取适量细胞悬液，作梯度稀释至 8 个细胞/mL，接种 96 孔板（每孔加 0.2 mL，即平均 1.6 个细胞/孔），每个剂量作 1—2 块板，37℃，5% CO2，饱和湿度条件下培养 12d，计数每块平板有集落生长的孔数。

5.2.2.3 表达：步骤 6.2.2.1 所得细胞悬液作 2d 表达培养，每天计数细胞密度并保持密度在 10^6/ml 以下。

5.2.1.4 The test results are analyzed by x2 test.

5.2.2 TK site mutation analysis (L5178Y cells, 96 well plate method)

5.2.2.1 Treatment: take the cells with good growth, adjust the density to 5 × 105 / ml, add the test substance by 1% volume, shake at 37 ℃ for 3 hours.After centrifugation, the supernatant is discarded and ished twice with PBS or serum-free medium, the cells are resuspended in RPMI 1640 medium containing 10% horse serum, and the cell density is adjusted to 2 × 105 / ml.

5.2.2.2 PE0(0-day plate inoculation efficiency) determination: take appropriate cell suspension, make gradient dilution to 8 cells /mL, inoculate 96-well plate (0.2 mL per well, i.e. 1.6 cells/well on average), make 1-2 plates for each dose, culture for 12 days at 37℃, 5% CO2 and saturated humidity, and count the number of wells with colony growth on each plate.

5.2.2.3 Expression: the cell suspension obtained in step 6.2.2.1 is cultured for 2D expression, and the cell density is counted every day and kept below 10^6/ ml.

5.2.2.4 PE2（第 2d 的平板接种效率）测定：第 2d 表达培养结束后，取适量细胞悬液，按步骤 6.2.2.2 作梯度稀释并接种 96 孔板，培养 12d 后计数每块平板有集落生长的孔数。

5.2.2.5 TFT 抗性突变频率（MF）测定：第 2d 表达培养结束后，取适量细胞悬液，调整细胞密度为 1×104/mL，加入 TFT（三氟胸苷，终浓度为 3μg/mL），混匀，接种 96 孔板（每孔加 0.2 mL，即平均 2000 个细胞/孔），每个剂量作 2—4 块板，37℃，5% CO2，饱和湿度条件下培养 12d，计数有突变集落生长的孔数。

5.2.2.4 Pe2 (the second day of plate inoculation efficiency) measurement: after the second day of expression and culture, take appropriate amount of cell suspension, make gradient dilution according to step 6.2.2.2 and inoculate 96 well

plates, and count the number of holes with colony growth in each plate after 12 days of culture.

5.2.2.5 Determination of TFT resistance mutation frequency (MF): after the end of 2d expression culture, take appropriate amount of cell suspension, adjust the cell density to 1×104/mL, add TFT (trifluorothymidine, final concentration is 3g/ml), mix well, inoculate 96-well plate (0.2 mL per well, i.e. an average of 2000 cells/well), make 2-4 plates for each dose, culture at 37℃, 5% CO2 under saturated humidity for 12 days, and count the number of wells with mutation colony growth.

5.2.2.6 计算

5.2.2.6.1 平板效率（PE0 和 PE2）

$$PE = \frac{-\ln（EW/TW）}{1.6}$$

式中：EW 为无集落生长的孔数；TW 为总孔数；

1.6 为每孔接种细胞数

5.2.2.6.2 相对存活率（RS%）

$$相对存活率（RS\%）= \frac{PE0（处理）}{PE0（对照）} \times 100\%$$

5.2.2.6.3 突变频率（MF）

$$MF(\times 10\text{-}6) = \frac{-\ln（EW/TW）/n}{PE2}$$

式中：EW 为无集落生长的孔数；TW 为总孔数；

n 为每孔接种细胞数（2000）

5.2.2.6 Calculation

5.2.2.6.1 Plate efficiency (Pe0 and Pe2)

$$PE = \frac{-\ln（EW/TW）}{1.6}$$

Where: EW is the number of pores without colony growth; TW is the total number of pores;

1.6 Number of cells inoculated per pore

5.2.2.6.2 Relative survival rate (RS%)

$$Relative\ survival\ rate\ (RS\%) = \frac{Pe0\ (processing)}{Pe0\ (control)} * 100\%$$

5.2.2.6.3 Mutation frequency (MF)

$$MF(\times 10\text{-}6) = \frac{-\ln(EW/TW)/n}{PE2}$$

Where: EW is the number of pores without colony growth; TW is the total number of pores;
N is the number of cells inoculated per pore (2000)

6 结果评价

在下列两种情况下可判定受试物在本试验系统中为阳性结果：
(1) 受试物引起突变频率具有统计学意义、并与剂量相关的增加。
(2) 受试物在任何一个剂量条件下，引起具有统计学意义，并有可重复性的阳性反应。阳性结果表明受试物可引起所用哺乳类细胞的基因突变。可重复的阳性剂量—反应关系意义更大。阴性结果表明在本试验条件下，受试物不引起所用哺乳类细胞的基因突变。

6 Result evaluation

In the following two cases, it can be determined that the test substance is a positive result in this test system:
(1) The frequency of mutation caused by test substance is statistically significant and dose-related.
(2) Under any dose condition, the tested substance causes a positive reaction with statistical significance and repeatability. Positive results show that the tested substance can cause gene mutation of mammalian cells used. Repeated positive dose-response relationship is of greater significance. Negative results showed that under the test conditions, the tested substance did not cause gene mutation in the mammalian cells used.

11 哺乳动物骨髓细胞染色体畸变试验

In Vivo Mammalian Bone Marrow Cell Chromosome Aberration Test

1 范围

本规范规定了哺乳动物骨髓细胞染色体畸变试验的基本原则、要求和方法。本规范适用于检测化妆品原料及其产品的遗传毒性。

2 试验目的

本试验是一项致突变性试验，检测整体动物骨髓细胞染色体畸变，以评价受试物致突变的可能性。

11 In Vivo Mammalian Bone Marrow Cell Chromosome Aberration Test

1 Range

This specification specifies the basic principles, requirements and methods of chromosome aberration test for mammalian bone marrow cells. This specification is applicable to the detection of genetic toxicity of cosmetic raw materials and products.

2 Test purpose

This test is a mutagenicity test to detect chromosomal aberrations of bone marrow cells of whole animals, so as to evaluate the possibility of mutagenicity of test substance.

3 定义

3.1 染色体型畸变 chromosome-type aberration

染色体结构损伤，表现为在两个染色单体的相同位点均出现断裂或断裂重组的改变。

3.2 染色单体型畸变 chromatid-type aberration

染色体结构损伤，表现为染色单体断裂或染色单体断裂重组的损伤。

3.3 染色体数目畸变 numerical-type aberration

哺乳动物细胞染色体数目的改变。

3 Definition

3.1 chromosome-type aberration

The damage of chromosome structure is manifested by the change of breakage or recombination at the same site of two chromatids.

3.2 chromatid-type aberration

The damage of chromosome structure is the damage of chromatid breaking or chromatid breaking and recombination.

3.3 numerical-type aberration

Changes of chromosome number in mammalian cells.

4 试验基本原则

使哺乳动物（如大鼠或小鼠）经口或其他适宜途径染毒，动物处死前用细胞分裂中期阻断剂处理，处死后制备骨髓细胞染色体标本，分析染色体畸变。

本方法特别适用于需考虑体内代谢活化后的染色体畸变分析。

若有证据表明待测物或其代谢产物不能到达骨髓，则不适用于本方法。

4 Basic principles of test

To infect mammals (such as rats or mice) orally or by other appropriate ways. Before the animals are killed, they are treated with the medium-term blocker of cell division. After the animals are killed, the chromosome samples of bone marrow cells are prepared and the chromosome aberrations are analyzed.

This method is especially suitable for the analysis of chromosomal aberrations after metabolic activation in vivo.

If there is evidence that the substance to be tested or its metabolites cannot reach the bone marrow, this method is not applicable.

5 试验方法

5.1 实验动物和饲养环境：

选用健康成年啮齿类动物，推荐使用大鼠或小鼠，每组每种性别至少 5 只，动物在实验室中至少应适应 5 天，实验开始时每一性别动物的体重差异应控制在±20%内。实验动物及实验动物房应符合国家相应规定。

5 test method

5.1 Laboratory animals and feeding environment:

Healthy adult rodents are selected, and rats or mice are recommended. There are at least 5 animals of each sex in each group. The animals should be adapted for at least 5 days in the laboratory. At the beginning of the experiment, the weight difference of animals of each sex should be controlled within+/-20%. Laboratory animals and laboratory animal houses shall conform to the corresponding regulations of the state.

5.2 受试物

5.2.1 受试物配制：固体受试物应溶解或悬浮于适合的溶剂中，并稀释至一定浓度。液体受试物可直接使用或予以稀释。受试物应在使用前新鲜配制，否则就必须证实贮存不影响其稳定性。

5.2.2 溶剂的选择：溶剂在所选用浓度下，不引起毒性效应，不与受试物发生化学反应。水为首选溶剂。

5.2.3 剂量设置：应进行预试验以选择最高剂量。当有毒性时，可以引起死亡或者抑制骨髓细胞有丝分裂指数（50%以上）为指标确定最高剂量。在第一次采集样品时，需设置 3 个可供分析的剂量，在第二次采集样品时，则仅需设置最高剂量组。

如果一次剂量为 2000mg/kg 体重时仍未引起毒性效应，则只设 2000mg/kg 体重剂量组。如果人类的可能（期望）暴露量过大，可选择 2000mg/kg/BW/d 染毒 14 天，或选择 1000mg/kg/B W/d 染毒大于 14 天进行试验。

5.2 Test substance

5.2.1 Preparation of test substance: the solid test substance shall be dissolved or suspended in a suitable solvent and diluted to a certain concentration. The liquid test substance can be used directly or diluted. The test substance shall be freshly prepared before use, otherwise it must be confirmed that storage does not affect its stability.

5.2.2 Selection of solvent: under the selected concentration, the solvent will not cause toxic effect or chemical reaction with the test substance. Water is the preferred solvent.

5.2.3 Dose setting: a pretest should be performed to select the highest dose. When it is toxic, it can cause death or inhibit the mitosis index of bone marrow cells (more than 50%) to determine the maximum dose. In the first sample collection, three doses for analysis need to be set. In the second sample collection, only the highest dose group needs to be set.

If a single dose of 2000mg/kg body weight does not cause toxic effects, only a dose group of 2000mg/kg body weight is set. If the possible (expected) exposure of human is too large, the test can be carried out by selecting 2000mg/kg/BW/d for 14 days or 1000mg/kg/B W/d for more than 14 days.

5.3 对照：在每项试验中，对每种性别均应设阴性对照组和阳性对照组。除不使用受试物外，其他处理与受试物组一致。

5.3.1 阴性对照：除设溶剂对照（即仅含溶剂）外，如果没有文献资料或历史性资料证实所用溶剂不具有有害作用或致突变作用，还应设空白对照组。

5.3.2 阳性对照：阳性对照物应能引起染色体结构畸变率明显高于背景资料。染毒途径可以不同于受试物。所选用的阳性对照物最好与受试物类别有关。可以使用下述物质：三亚乙基密胺（triethylenemelamine）、甲磺酸乙酯（ethyl methanesulphonate）、乙基亚硝基脲（ethylnitrosonrea）、丝裂霉素 C（mytomycin C）和环磷酰胺（cyclophosphamide）。

5.3 Control: in each test, negative control group and positive control group shall be set for each sex.The other treatments are the same as the test substance group except that the test substance is not used.

5.3.1 Negative control: in addition to solvent control (i.e. only containing solvent), if there is no literature or historical data to prove that the solvent used does not have harmful or mutagenic effects, a blank control group should also be set.

5.3.2 Positive control: Positive control should cause chromosome structural aberration rate significantly higher than background data. The route of exposure may be different from that of the subject. The selected positive control substance is preferably related to the type of test substance. The following substances can be used: triethylenemelamine, ethyl methanesulphonate, ethylnitrosonrea, Mitomycin C and cyclophosphamide.

5.4 染毒方式：可采用经口或其他适宜的染毒方式。一般染毒为一次完成，如剂量过大时，一天内染毒数次也是可以的，但每次应间隔数小时。

一般情况下，染毒 1 次，但分两次采集标本,即每组动物分两个亚组，亚组 1 于染毒后 12h—18h 处死并采集第一次标本；亚组 2 于亚组 1 处死后 24h 采集第二次标本。如果采用多次染毒，于末次染毒后 12h—18h 采集标本。于处死动物采集标本前腹腔注射细胞分裂中期阻断剂（如用秋水仙素，于处死前 4h，按 4mg/kg 体重给药。若使用动物为小鼠，适宜的处理时间为 3—5 h，若使用动物为中国仓鼠，适宜的处理时间为 4—5 h。

5.4 Method of poisoning: oral or other appropriate methods can be used. Generally, the poisoning is completed at one time. If the dosage is too large, it is OK to be poisoned several times a day, but the interval should be several hours each time.

In general, the animals in each group are divided into two subgroups. Subgroup 1 is collected after the poisoning

In general, the animals were exposed once, but the specimens were collected twice, i.e. each group of animals was divided into two subgroups. subgroup 1 was killed 12-18 hours after exposure and the first specimen was collected. Subgroup 2 collected the second sample 24 hours after subgroup 1 was executed. If multiple exposures are used, specimens are collected 12-18 hours after the last exposure. Cell division metaphase blockers (such as colchicine, 4mg/kg body weight) were injected intraperitoneally before the animals were killed to collect specimens. If the animal used is a mouse, the appropriate treatment time is 3-5 h, and if the animal used is a Chinese hamster, the appropriate treatment time is 4-5 h.

5.5 试验步骤

5.5.1 用颈椎脱臼法处死动物，取出股骨，剔除肌肉等组织。

5.5.2 剪去股骨两端，用注射器吸取 5mL 生理盐水，从股骨一端注入，用 10mL 离心管，从股骨另一端接取流出的骨髓细胞悬液。

5.5.3 将细胞悬液以 1000r/min 的速度离心 5 min—7 min，去除上清液。

5.5.4 加入 0.075mol/L KCl 溶液 7ml，用滴管将细胞轻轻地混匀，放入 37℃水浴中低渗处理 7min，加入 1—2mL 固定液（冰醋酸:甲醇=1:3），混匀，以 1000r/min 速度离心 5 min—7min，弃去上清液。

5.5.5 加入 7mL 固定液，混匀，固定 15min，以 1000r/min 的速度离心 7min，弃去上清液。

5.5.6 用同法再固定 1—2 次，弃去上清液。

5.5.7 加入数滴新鲜固定液，混匀。

5.5.8 用混悬液以空气干燥或火焰干燥法制片。

5.5.9 用姬姆萨染液染色。

5.5 Test procedure

5.5.1 Animals are killed by cervical dislocation, femurs are removed and muscles are removed.

5.5.2 Cut off both ends of the femur, use a syringe to suck 5ml of normal saline, inject it from one end of the femur, use a 10ml centrifuge tube, and connect the marrow cell suspension from the other end of the femur.

5.5.3 The cell suspension is centrifuged at a rate of 1000 R / min for 5-7 min to remove the supernatant.

5.5.4 Add 7ml of 0.075mol/l KCl solution, mix the cells gently with a burette, put them into a 37 ℃ water bath for 7 min with low permeability treatment, add 1-2ml of fixed solution (glacial acetic acid: methanol = 1:3), mix them evenly, centrifugate at a speed of 1000r / min for 5-7min, and discard the supernatant.

5.5.5 Add 7ml of fixing liquid, mix well, fix for 15min, centrifugate at the speed of 1000r / min for 7min, and discard the supernatant.

5.5.6 Use the same method to fix again 1-2 times, and discard the supernatant.

5.5.7 Add a few drops of fresh fixative and mix well.

5.5.8 The suspension is used for air drying or flame drying.

5.5.9 Dye with Giemsa dye.

5.6.1 确定有丝分裂指数：包括所有处理组、阳性和阴性对照组（每只动物计数 500—1000 个细胞）。

5.6.2 计数畸变细胞：对每只动物至少选择 100 个分散良好的中期分裂相，在显微镜油镜下进行读片。由于低渗等机械作用的破坏，会导致处于中期的染色体发生丢失，所以，观察的中期相染色体数目应控制在 2n±2 内。在读片时应记录每一观察细胞的染色体数目，对于畸变细胞还应记录显微镜视野的坐标位置及畸变类型。裂隙（Gap）应单独记录并列出，通常不作为染色体结构畸变计算。所得各组的染色体畸变率用 X2 检验等进行统计学处理，以评价试验组和对照组之间是否有显著差异。

5.6.1 Determine mitotic index: including all treatment groups, positive and negative control groups (500-1000 cells per animal).

5.6.2 Count aberrant cells: select at least 100 well dispersed metaphase of each animal, and read the film under the microscope oil microscope. The number of metaphase chromosomes should be controlled within 2n ± 2 because of the loss of metaphase chromosomes due to the mechanical effects such as hypotonic. The chromosome number of each observation cell should be recorded during the reading of the film, and the coordinate position and distortion type of the microscopical field of vision should also be recorded for the aberrant cells. Gap should be recorded and listed separately, which is not usually used as the calculation of chromosome structural aberrations. In order to evaluate whether there is significant difference between the experimental group and the control group.

5.7 结果评价

每个动物作为一个试验单位，在统计分析时，每个动物的数据应列表进行。可把结构畸变细胞率（%）和每细胞内的染色体畸变数作为评价指标。统计分析的标准有几个，当受试物引起染色体畸变数具有统计学意义，并有与剂量相关的增加或者在一个剂量组、单一时间点采样的试验中出现染色体畸变细胞数明显增高，则判定具有致突变性。

在评价时应综合考虑生物学意义和统计学意义，不能作出明确结论时，应改变试验条件进一步进行测试。

5.7 Result evaluation

Each animal as a test unit, in statistical analysis, the data of each animal should be tabulated. The cell rate (%) of structural aberration and chromosome aberration per cell can be used as evaluation indexes. There are several criteria for statistical analysis. Mutagenicity is determined when the number of chromosome aberration cells caused by the test object has statistical significance, and there is a dose-related increase or the number of chromosome aberration cells is significantly increased in a dose group and a single time point sampling test.

The biological and statistical significance should be taken into account in the evaluation, and the test conditions should be changed for further testing if no clear conclusion can be made.

6 结果解释

阳性结果证明受试物具有引起该种受试动物骨髓细胞染色体畸变的能力。

阴性结果表明在本试验条件下受试物不引起该种受试动物骨髓细胞染色体畸变。

6 Interpretation of results

The positive results showed that the test substance had the ability to cause chromosomal aberrations in bone marrow cells of this kind of animal.

The negative results showed that the test substance did not cause chromosomal aberrations of bone marrow cells of this kind of animal under the test conditions.

12 体内哺乳动物细胞微核试验

Mammalian Erythrocyte Micronucleus Test

1 范围

本规范规定了哺乳动物红细胞微核试验的基本原则、要求和方法。本规范适用于化妆品原料的染色体畸变检测。

2 定义

微核 micronucleus

染色单体或染色体的无着丝点断片，或因纺锤体受损而丢失的整个染色体，在细胞分裂后期，仍然遗留在细胞质中。末期之后，单独形成一个或几个规则的次核，被包含在子细胞的胞质内，因比主核小，故称为微核。

12 Mammalian Erythrocyte Micronucleus Test

1 Range

This specification specifies the basic principles, requirements and methods of mammalian micronucleus test. This specification is applicable to chromosome aberration detection of cosmetic raw materials.

2 Definition

Micronucleus

At the later stage of cell division, the chromatid or chromosome acentric fragment, or the whole chromosome lost due to the damage of spindle, is still left in the cytoplasm. After the end of the period, one or several regular subnuclei are formed, which are

contained in the cytoplasm of daughter cells. Because they are smaller than the main nucleus, they are called micronuclei.

3 原理

凡能使染色体发生断裂或使染色体和纺锤体联结损伤的化学物，都可用微核试验来检测。各种类型的骨髓细胞都可形成微核，但有核细胞的胞质少，微核与正常核叶及核的突起难以鉴别。嗜多染红细胞是分裂后期的红细胞由幼年发展为成熟红细胞的一个阶段，此时红细胞的主核已排出，因胞质内含有核糖体，姬姆萨染色呈灰蓝色，成熟红细胞的核糖体已消失，被染成淡桔红色。骨髓中嗜多染红细胞数量充足，微核容易辨认，而且微核自发率低，因此，骨髓中嗜多染红细胞成为微核试验的首选细胞群。

若动物染毒的时间达 4 周以上，也可选同一终点的外周血正染红细胞进行微核试验。若有证据表明待测物或其代谢产物不能到达骨髓，则不适用于本方法。

3 principle

Micronucleus test can be used to detect chemicals that can break chromosomes or damage the connection between chromosomes and spindles. All kinds of bone marrow cells can form micronucleus, but the cytoplasm of nucleated cells is few, and it is difficult to distinguish micronucleus from normal nuclear leaves and nuclear processes. Polychromatic erythrocytes are a stage in which erythrocytes develop from infancy to mature erythrocytes at the later stage of division. At this time, the main nucleus of erythrocytes has been discharged. Because ribosomes are contained in the cytoplasm, Giemsa staining is gray blue, and the ribosomes of mature erythrocytes have disappeared and dyed light orange red. The number of polychromatic erythrocytes in bone marrow is sufficient, the micronucleus is easy to identify, and the spontaneous rate of micronucleus is low. Therefore, polychromatic erythrocytes in bone marrow become the preferred cell group for micronucleus test.

If the exposure time of the animal is more than 4 weeks, the micronucleus test can also be performed on the peripheral blood positive erythrocytes at the same end point. If there is evidence that the substance to be tested or its metabolites cannot reach the bone marrow, this method is not applicable.

4 试验的基本原则

通过适当的途径使动物接触受试物，一定时间后处死动物，取出骨髓，制备涂片，经固定、染色，在显微镜下计数含微核的嗜多染红细胞。

5 仪器和器械

生物显微镜、解剖剪、镊子、止血钳、注射器、灌胃针头、载玻片、盖玻片（24mm×50mm）、塑料吸瓶、纱布、滤纸等。

4 Basic principles of test

The animals are exposed to the test substance through appropriate ways. After a certain period of time, the animals are killed, the bone marrow is taken out, the smear is prepared, fixed and stained, and the polychromatic erythrocytes containing micronucleus are counted under the microscope.

5 Instruments and apparatus

Biological microscope, dissecting scissors, forceps, hemostatic forceps, syringes, stomach filling needles, slides, cover slides (24mm × 50mm), plastic suction bottles, gauze, filter paper, etc.

6 试剂

6.1 小牛血清（灭活）

将滤菌的小牛血清置于 56℃恒温水浴保温 30min 灭活。灭活的小牛血清通常保存于冰箱冷冻室里。

6.2 姬姆萨（Giemsa）染液

成分：Giemsa 染料 3.8g

甲醇 375mL

甘油 125mL

配制：将染料和少量甲醇于乳钵里仔细研磨，再加入甲醇至 375mL 和甘油，混合均匀，放置 37℃恒温箱中保温 48h。保温期间，振摇数次，促使染料的充分溶解，取出过滤，两周后用。

6.3 1/15mol/L 磷酸盐缓冲液（pH6.8）

磷酸二氢钾（KH2PO4） 4.50 g

磷酸氢二钠（Na2HPO4·12H2O） 11.81 g

加蒸馏水至 1000mL

6.4 Giemsa 应用液

取一份 Giemsa 染液与 6 份 1/15mol/L 磷酸盐缓冲液混合而成。现用现配。

6 reagent

6.1 Calf serum (inactivated)

The calf serum is inactivated in a constant temperature water bath at 56 ℃ for 30 minutes. Inactivated calf serum is usually stored in a refrigerator freezer.

6.2 Giemsa dye solution

Ingredient: Giemsa dye 3.8g

Methanol 375ml

Glycerin 125ml

Preparation: carefully grind the dye and a small amount of methanol in a mortar, add methanol to 375ml and glycerin, mix evenly, and place in a 37 ℃ incubator for 48h.During the heat preservation period, shake several times to make the dye fully dissolved, take it out and filter it, and use it two weeks later.

6.3 1 / 15mol / L phosphate buffer (ph6.8)

Potassium dihydrogen phosphate (KH2PO4) 4.50 G

Disodium hydrogen phosphate (Na2HPO4 · 12H2O) 11.81 G

Add distilled water to 1000ml

6.4 Giemsa solution

One part of Giemsa dye solution is mixed with six parts of 1 / 15mol / L phosphate buffer solution. Matching only before use.

7 实验动物和饲养环境

适宜的哺乳动物均适用于本实验，推荐使用小鼠或大鼠。小鼠是微核试验的常规动物。体重为 25g —30g。也可选用成年大鼠，体重为 150g —200g。动物在实验室中至少应适应 3-5 天，实验开始时每一性别动物的体重差异应控制在±20%内。

实验动物及实验动物房应符合国家相应规定。

7 Laboratory animals and feeding environment

Suitable mammals are suitable for this experiment, and mice or rats are recommended. Mice are routine animals for micronucleus test. The body weight is 25g-30g.Adult rats with a weight of 150g-200g can also be selected. The animals should adapt for at least 3-5 days in the laboratory. At the beginning of the experiment, the weight difference of each sex should be controlled within ± 20%.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations.

8 剂量分组

一般取受试物 LD50 的 1/2、1/5、1/10、1/20 等剂量，以求获得微核的剂量-反应关系曲线。当受试物的 LD50 大于 5g/kg 体重时，可取 5g/kg 体重为最高剂量，一般至少设 3 个剂量。每个剂量组 10 只动物，雌、雄性各半。另外，还应设溶剂对照和阳性物对照组。常用环磷酰胺作为阳性物对照，剂量可为 40mg/kg 体重。

如果人类的可能（期望）暴露量过大，可选择 2000mg/kg/BW/d 染毒 14 天，或选择 1000mg/kg/B W/d 染毒大于 14 天进行试验。

根据受试物的理化性质（水溶性和/或脂溶性）确定受试物所用的溶剂，通常用水、植物油或食用淀粉等。

8 Dose grouping

Generally, 1 / 2, 1 / 5, 1 / 10 and 1 / 20 of LD50 are taken to obtain the dose-response curve of micronucleus. When LD50 of the test substance is greater than 5g / kg body weight, 5g / kg body weight can be taken as the highest dose, generally at least 3 doses are set.10 animals in each dose group, half female and half male. In addition, a solvent control group and a positive control group should be set up. Cyclophosphamide is commonly used as a positive control, and the dosage can be 40mg / kg body weight.

If the possible (expected) exposure of human is too large, the test can be carried out by selecting 2000mg/kg/BW/d for 14 days or 1000mg/kg/B W/d for more than 14 days.

According to the physical and chemical properties (water-soluble and / or fat soluble) of the test object, the solvent used for the test object is determined, usually water, vegetable oil or edible starch, etc.

9 染毒途径和方式

染毒途径视实验目的而定，建议采用经口灌胃方式。采用 30h 两次给药法，即两次给受试物间隔 24h，第二次给受试物后 6h 取材。

10 试验方法

10.1 样本的制取

动物颈椎脱臼处死后，打开胸腔，沿着胸骨柄与肋骨交界处剪断，剥掉附着其上的肌肉，擦净血污，横向剪开胸骨，暴露骨髓腔，然后用止血钳挤出骨髓液。

长时间染毒的外周血样本从尾或耳静脉采血，一般应在未次染毒的 18—24h、36—48h 之间分两次进行。

10.2 涂片

将骨髓液滴在载玻片一端的小牛血清液滴里，仔细混匀。一般来讲，两节胸骨髓液涂一张片子为宜。然后，按血常规涂片法涂片，长度约 2cm —3cm。在空气中晾干。若立即染色，需在酒精灯火焰上方，稍微烘烤一下。

9 Ways and means of poisoning

The route of poisoning depends on the purpose of the experiment, and oral gavage is recommended. The drug is given twice in 30h, i.e. the interval between two times is 24h, and the material is taken 6h after the second time.

10 test method

10.1 Sample preparation

After cervical vertebrae dislocated and killed, open the chest cavity, cut along the junction of the sternal stalk and rib, strip the muscle attached to it, wipe off the blood stain, cut the sternum horizontally, expose the marrow cavity, and then use hemostatic forceps to extrude the marrow fluid.

Peripheral blood samples exposed for a long time should be collected from tail or ear vein in two times between 18-24 hours and 36-48 hours without secondary exposure.

10.2 smear

Drop the marrow into the calf serum drop at one end of the slide and mix it carefully. Generally speaking, it's better to apply one film of two sections of thoracic marrow fluid. Then, smear according to the blood routine smear method, the length is about 2cm-3cm.Dry in the air. If dyeing immediately, it is necessary to bake slightly above the flame of alcohol lamp.

10.3 固定

将干燥的涂片放入甲醇液中固定 5min。即使当日不染色，也应固定后保存。

10.4 染色

将固定过的涂片放入 Giemsa 应用液中，染色 10min —15min，然后立即用 1/15mol/L 磷酸盐缓冲液冲洗。

10.5 封片

用滤纸及时擦干染片背面的水滴，再用双层滤纸轻轻按压染片，以吸附染片上残留的水分，再在空气中晃动数次，以促其尽快晾干，然后放入二甲苯中透明 5min，取出滴上适量光学树脂胶，盖上盖玻片，写好标签。

10.6 观察与计数

先用低倍镜，后用高倍镜粗略检查，选择细胞分布均匀，细胞无损，着色适当的区域，再在油浸镜下计数。虽然不计数含微核的有核细胞，但需用有核细胞形态染色完好做好判断制片优劣的标准。

本法观察含微核的嗜多染红细胞。嗜多染红细胞呈灰蓝色，成熟红细胞呈淡桔红色。微核大多数呈单个圆形，边缘光滑整齐，嗜色性与核质相一致，呈紫红色或蓝紫色。

每只动物至少计数 2000 个嗜多染红细胞。微核率指含有微核的嗜多染红细胞数，以千分率（‰）表示之。若一个嗜多染红细胞中出现两个或两个以上微核，仍按一个有微核细胞计数。

经过化妆品标准委员会验证或证实的图像自动分析系统与流式细胞仪进行的微核试验，可接受为本方法的替代试验。

10.3 fixed

Put the dried smear into methanol solution and fix it for 5min. Even if it is not stained on that day, it should be fixed and stored.

10.4 dyeing

Put the fixed smear into Giemsa application solution, dye for 10min-15min, and then immediately wash it with 1 / 15mol / L phosphate buffer.

10.5 Seals

Wipe the water drop on the back of the dye pad with filter paper in time, press the dye pad gently with double-layer filter paper to absorb the residual water on the dye pad, shake it in the air for several times to promote it to dry as soon as possible, then put it into xylene for 5min, take out the appropriate amount of optical resin glue, cover the cover glass and write the label.

10.6 Observation and counting

First use low power microscope, then use high power microscope to roughly check, select the area with uniform cell distribution, no damage and proper coloring, and then count under oil immersion microscope. Although there is no count of nucleated cells with micronucleus, it is necessary to make a good standard to judge the quality of the production.

Polychromatic erythrocytes with micronucleus are observed. Polychromatic erythrocytes are gray blue, mature erythrocytes are light orange red. Most of the micronuclei are single and round, with smooth and neat edges. The color preference is consistent with the nucleoplasm, and they are purplish red or blue purple.

At least 2000 polychromatic erythrocytes are counted in each animal. Micronucleus rate refers to the number of polychromatic erythrocytes containing

micronucleus, expressed in thousands (‰).If there are two or more micronuclei in a polychromatic red blood cell, it is still counted as one with micronuclei.

The micronucleus test conducted by the image automatic analysis system and flow cytometer verified or confirmed by the cosmetics standards committee can be accepted as an alternative test of this method.

11 数据处理和结果判断

11.1 数据处理

报告各组微核细胞率的均数和标准差，利用适当的统计学方法如 Poinsson 分布 u 检验比较受试物各剂量组与溶剂对照组的微核率。

若无证据表明所得的数据有性别间的差异，则可将两性别的数据合并进行统计分析。

11.2 结果判定

在评价时应综合考虑生物学意义和统计学意义。如果受试物试验组与溶剂对照组相比，单一剂量法微核率有明显增高；多剂量法的剂量组在统计学上有显著性差异，并有剂量—反应关系则可认为微核试验阳性。

11 Data processing and result judgment

11.1 data processing

The mean and standard deviation of micronucleus cell rate of each group are reported. The micronucleus rate of each dose group is compared with that of the solvent control group by using appropriate statistical methods such as Poisson distribution U test.

If there is no evidence that the data obtained are different in sex, the data of gender can be combined for statistical analysis.

11.2 Result determination

Biological significance and statistical significance should be considered in the evaluation. If the micronucleus rate of the test substance test group is significantly higher than that of the solvent control group, the micronucleus rate of the multi dose test group is statistically significant, and there is a dose-response relationship, it can be considered that the micronucleus test is positive.

13 睾丸 Th 殖细胞染色体畸变试验

Testicle Cells Chromosome Aberration Test

1 范围

本规范规定了哺乳动物睾丸生殖细胞染色体畸变试验的基本原则、要求和方法。本规范适用于化妆品原料的遗传毒性检测。

2 试验目的

检测雄性动物生殖细胞染色体损伤，以评价受试物在生殖细胞诱导可遗传的致突变的可能性。

13 Testicle Cells Chromosome Aberration Test

1 Range

This specification specifies the basic principles, requirements and methods of chromosome aberration test for mammalian testicular germ cells. This specification is applicable to the genotoxicity test of cosmetic raw materials.

2 Test purpose

In order to evaluate the possibility of inducing heritable mutagenesis in germ cells, chromosome damage of germ cells of male animals is detected.

3 定义

3.1 染色体型畸变 chromosome-type aberration
染色体结构损伤，表现为两个染色单体的相同位点均出现断裂或断裂重接。

3.2 染色单体型畸变 chromatid-type aberration
染色体结构损伤，表现为染色单体断裂或染色单体断裂重接。

3.3 染色体数目畸变 numerical-type aberration
染色体数目发生改变，不同于正常二倍体核型，包括整倍体和非整倍体。

3 Definition

3.1 chromosome-type aberration
The damage of chromosome structure shows that the same sites of two chromatids are broken or reconnected.

3.2 chromatid-type aberration
The chromosome structure is damaged, which is manifested as chromatid breaking or chromatid breaking and reconnecting.

3.3 numerical-type aberration
The number of chromosomes changed, which is different from normal diploid karyotype, including aneuploid and aneuploid.

4 试验的基本原则

通过适当的途径使动物接触受试物，一定时间后处死动物，动物处死前用细胞分裂中期阻断剂处理，处死后制备睾丸初级精母细胞染色体标本，在显微镜下观察染色体畸变。

本方法特别适用于需考虑体内代谢活化后的染色体畸变分析。

若有证据表明待测物或其代谢产物不能到达睾丸，则不适用于本方法。

4 Basic principles of test

The animals are exposed to the test substance through appropriate ways. The animals are killed after a certain period of time. Before the animals are killed, they are treated with the medium-term cell division blocker. After the animals are killed, the chromosome samples of primary spermatocytes of testis are prepared, and the chromosome aberrations are observed under the microscope.

This method is especially suitable for the analysis of chromosomal aberrations after metabolic activation in vivo.

If there is evidence that the substance to be tested or its metabolites cannot reach the testis, this method is not applicable.

5 仪器和器械

生物显微镜、离心机、解剖剪、镊子、离心管、平皿、注射器、灌胃针头、载玻片、盖玻片（24mm×50mm）等。

5 Instruments and apparatus

Biological microscope, centrifuge, dissecting scissors, tweezers, centrifuge tubes, plates, syringes, stomach filling needles, slides, cover slides (24mm × 50mm), etc.

6 试剂

6.1 0.04%秋水仙素：取 40mg 秋水仙素，加生理盐水至 100mL。

6.2 1%柠檬酸三钠：取 1g 柠檬酸三钠，加蒸馏水至 100mL。

6.3 0.075mol/L 氯化钾溶液：取氯化钾 5.59g，加蒸馏水至 1000mL。

6.4 甲醇/冰醋酸（3：1，v/v）固定液：临用现配。

6.5 60%冰乙酸：取 60mL 冰乙酸，加蒸馏水至 100mL，均宜新鲜配制。

6.6 pH6.8 磷酸盐缓冲液

1/15mol/L 磷酸盐缓冲液（pH6.8）

磷酸二氢钾（KH_2PO_4） 4.50 g

磷酸氢二钠（$Na_2HPO_4 \cdot 12H_2O$） 11.81 g

加蒸馏水至 1000mL

6.7 姬姆萨染液

姬姆萨（Giemsa）染液

成分：Giemsa 染料 3.8g

甲醇 375mL

甘油 125mL

配制：将染料和少量甲醇于乳钵里仔细研磨，再加入甲醇至 375mL 和甘油，混合均匀，放置 37℃恒温箱中保温 48h。保温期间，振摇数次，促使染料的充分溶解，取出过滤，两周后用。

姬姆萨应用液：取 1mL 储备液加入 10mL pH6.8 磷酸缓冲液。

6.8 生理盐水、甲醇。

6 reagent

6.1 0.04% colchicine: take 40 mg colchicine and add normal saline to 100 ml.

6.2 1% trisodium citrate: take 1g trisodium citrate, add distilled water to 100ml.

6.3 0.075mol/l potassium chloride solution: take 5.59g of potassium chloride, add distilled water to 1000ml.

6.4 Fixed solution of methanol / glacial acetic acid (3:1, V / V): ready to use.

6.5 60% glacial acetic acid: take 60ml glacial acetic acid, add distilled water to 100ml, which should be prepared fresh.

6.6 Ph6.8 phosphate buffer

1 / 15mol / L phosphate buffer (ph6.8)

Potassium dihydrogen phosphate (KH2PO4) 4.50 G

Disodium hydrogen phosphate (Na2HPO4 · 12H2O) 11.81 G

Add distilled water to 1000ml

6.7 Giemsa dye

Giemsa dye

composition:

Giemsa dye 3.8g

Methanol 375ml

Glycerin 125ml

Preparation: carefully grind the dye and a small amount of methanol in a mortar, add methanol to 375ml and glycerin, mix evenly, and place in a 37 ℃ incubator for 48h.During the heat preservation period, shake several times to make the dye fully dissolved, take it out and filter it, and use it two weeks later.

Giemsa application solution: take LML stock solution and add 10ml ph6.8 phosphoric acid buffer solution.

6.8 Normal saline, methanol.

7 实验动物和饲养环境

适宜的雄性啮齿类动物均适用于本实验。推荐使用小鼠，6 周—8 周龄，体重为 30g—35g。动物在实验室中至少应适应 5 天，实验开始时动物的体重差异应控制在±20%内。实验动物及实验动物房应符合国家相应规定。

7 Laboratory animals and feeding environment

Suitable male rodents are all suitable for this experiment. It is recommended to use mice, 6-8 weeks old, weighing 30g—35g. Animals should adapt for at least 5 days in the laboratory, and the weight difference of animals should be controlled within+/-20% at the beginning of the experiment. Laboratory animals and laboratory animal houses shall conform to the corresponding regulations of the state.

8 剂量分组

受试物至少设三个剂量组。分别取 1/2、1/5、1/10 或 1/20LD50 剂量。当受试物的 LD50 大于 5g/kg 体重时，可取 5g/kg 体重为最高剂量。另外设阴性（溶剂）对照组和阳性物对照组。阳性对照组用环磷酰胺（40mg/kg 体重）或丝裂霉素 C（1.5mg/kg 体重—2mg/kg 体重），腹腔注射。每组至少有 5 只存活动物。

根据受试物的理化性质（水溶性和/或脂溶性）确定受试物所用的溶剂，通常用水、植物油或食用淀粉等。

8 Dose grouping

There are at least three dose groups for the tested substance. Doses of 1/2, 1/5, 1/10 or 1/20LD50 were taken respectively. When the LD50 of the test substance is greater than 5g/kg body weight, 5g/kg body weight is preferable as the highest dose. In addition, negative (solvent) control group and positive control group were set up. Positive control group was injected intraperitoneally with cyclophosphamide (40mg/kg body weight) or mitomycin C(1.5mg/kg body weight-2 mg/kg body weight). There are at least 5 living animals in each group.

According to the physical and chemical properties (water-soluble and / or fat soluble) of the test substance, the solvent used for the test substance is usually water, vegetable oil or edible starch.

9 染毒途径和方式

染毒途径视实验目的而定，建议采用经口灌胃方式。每天 1 次染毒（如剂量过大时，一天内染毒数次也是可以的，但每次应间隔数小时），连续 5d。于第 1 次染毒后的第 12d—14d 将受试动物处死。处死动物前 6h，腹腔注射 0.04%秋水仙素溶液，剂量为 4mg/kg 体重。

9 Ways and means of poisoning

The route of poisoning depends on the purpose of the experiment, and oral gavage is recommended. Once a day (if the dose is too large, several times a day is OK, but the interval should be several hours), for 5 days. The animals are killed from the 12th to the 14th day after the first exposure. Six hours before the animals are killed, 0.04% colchicine solution is injected intraperitoneally at a dose of 4 mg / kg body weight.

10 试验方法

10.1 取材

取出两侧睾丸，去净脂肪，于生理盐水中洗去毛和血污，放入盛有适量 1% 柠檬酸三钠或 0.075mol/L 氯化钾溶液的小平皿中。

10.2 制片

10.2.1 低渗：以眼科镊撕开被膜，轻轻地分离曲细精管，加入 1%柠檬酸三钠溶液 10mL，用滴管吹打曲细精管，室温下静止 20min。

10.2.2 固定：仔细吸尽低渗液，加固定液（甲醇：冰乙酸＝3∶1）10 mL 固定。第一次不超过 15 min，倒掉固定液后，再加入新的固定液固定 20min 以上 。如在冰箱（0℃-4℃）过夜固定更好。

10.2.3 离心：吸尽固定液，加 60% 冰乙酸 1—2 mL，待大部分曲细精管软化完后，立即加入倍量的固定液，打匀、移入离心管，以 1000 r/min 离心 10 min 。

10.2.4 滴片：弃去大部分上清液，留下约 0.5—1.0 mL ，充分打匀制成细胞混悬液，将细胞混悬液均匀地滴于冰水玻片上。每个样本制得 2—3 张。空气干燥或微热烘干。

10.2.5 染色：用 1∶10 Giemsa 液（PH 6.8）染色 10min（根据室温染色时间不同），用蒸馏水冲洗、晾干。

10 test method

10.1 Draw materials

Take out the testicles on both sides, remove the fat, ish the hair and blood stains in normal saline, and put them into a small dish containing a proper amount of 1% trisodium citrate or 0.075mol/l potassium chloride solution.

10.2 Production

10.2.1 Hypotonic: tear the tunica with ophthalmic forceps, gently separate the seminiferous tubules, add 10ml of 1% trisodium citrate solution, blow the seminiferous tubules with dropper, and keep still at room temperature for 20min.

10.2.2 Fixation: carefully suck up the hypotonic solution, and add 10 ml of fixed solution (methanol: glacial acetic acid = 3:1) for fixation. For the first time, it shall not be more than 15 min. after pouring out the fixing liquid, add new fixing liquid for more than 20 min. Overnight fixation in refrigerator (0 ℃ - 4 ℃) would be better.

10.2.3 Centrifugation: suck up the fixed solution, add 1-2 ml of 60% glacial acetic acid, after most of the fine convoluted tubes are softened, immediately add multiple amounts of fixed solution, mix and transfer into the centrifuge tube, and centrifugate at 1000 R / min for 10 min.

10.2.4 Drop: discard most of the supernatant, leave about 0.5-1.0ml, mix well to make cell suspension, and drop the cell suspension evenly on the ice water slide. Make 2-3 pieces of each sample. Air drying or slight heat drying.

10.2.5 Dyeing: dye with 1:10 Giemsa solution (pH 6.8) for 10min (according to different dyeing time at room temperature), wash with distilled water and dry in the air.

10.4 阅片

10.4.1 阅片要求

在低倍镜下按顺序寻找背景清晰、分散良好、染色体收缩适中的中期分裂相，然后在油镜下进行分析。由于低渗等机械作用的破坏，会导致处于中期的染色体发生丢失，所以，观察的中期相染色体数目应是 n 对双价体，每只动物至少分析 100 个中期分裂相的初级精母细胞。计数的细胞应含染色体数为 1n＋1 的中期相细胞。有对于畸变细胞还应记录显微镜视野的坐标位置及畸变类型。

10.4.2 染色体分析

除了可见到裂隙、短片、微小体外，还要分析互相易位、X-Y 和常染色体的单价体

10.4 Observation

10.4.1 Observation requirements

At low magnification, the metaphase with clear background, good dispersion and moderate chromosome contraction is found in sequence, and then analyzed under oil microscope. Due to the destruction of low permeability and other mechanical effects, the metaphase chromosomes will be lost. Therefore, the number of metaphase chromosomes observed should be n-pair bivalents. Each animal should analyze at least 100 primary spermatocytes of metaphase. The cells counted should contain the metaphase cells with chromosome number of 1n + 1.For aberrant cells, we should also record the coordinate position and aberrance type of microscope field.

10.4.2 Chromosome analysis

In addition to cracks, short, and tiny bodies, the univalent of translocation, X-Y, and autosomes should be analyzed

11 数据处理和结果判断

所得各组染色体畸变率用 χ2 检验，或其他适当的显著性检验方法进行统计学处理。当各剂量组与阴性(溶剂)对照组相比，畸变细胞率有显著性意义的增加，并有剂量-反应关系时；或仅一个剂量组有显著性意义的增加，经重复试验证实后，可判为试验结果阳性。

11 Data processing and result judgment

The chromosome aberration rate of each group is statistically analyzed by χ 2 test or other appropriate significance test. When there is a significant increase in the rate of aberrant cells and a dose-response relationship between each dose group and the negative (solvent) control group, or only one dose group has a significant increase, it can be judged as a positive test result after the retest verification.

12 结果解释

阳性结果证明受试物具有引起该种动物睾丸生殖细胞染色体畸变的能力。

阴性结果表明在本试验条件下受试物不引起该种动物睾丸生殖细胞染色体畸变。

12 Interpretation of results

The positive results showed that the test substance had the ability to cause chromosomal aberrations in testicular germ cells of this kind of animal.

The negative results showed that the test substance did not cause chromosomal aberrations in the testicular germ cells of this kind of animal.

14 亚慢性经口毒性试验

Subchronic Oral Toxicity Test

1 范围

本规范规定了啮齿类动物亚慢性经口毒性试验的基本原则、要求和方法。本规范适用于检测化妆品原料的亚慢性经口毒性。

2 试验目的

在估计和评价化妆品原料的毒性时，获得受试物急性毒性资料后，还需进行亚慢性经口毒性试验。通过该试验不仅可获得一定时期内反复接触受试物后引起的健康效应、受试物作用靶器官和受试物体内蓄积能力资料，并可估计接触的无有害作用水平，后者可用于选择和确定慢性试验的接触水平和初步计算人群接触的安全性水平。

14 Subchronic oral toxicity test

1 Range

This specification specifies the basic principles, requirements and methods of subchronic oral toxicity test for rodents. This specification is applicable to the detection of subchronic oral toxicity of cosmetic raw materials.

2 Test purpose

When estimating and evaluating the toxicity of cosmetic raw materials, subchronic oral toxicity test should be carried out after obtaining the acute toxicity data of the test substance. Through this experiment, we can not only obtain the data of health effect, target organ and accumulation capacity of the test object caused by repeated contact with the test object in a certain period of time, but also estimate the non harmful level of contact. The latter can be used to select and determine the contact water level of the chronic experiment and preliminarily calculate the safety level of human contact.

3 定义

3.1 亚慢性经口毒性 subchronic oral toxicity

是指在实验动物部分生存期内，每日反复经口接触受试物后所引起的不良反应。

3.2 未观察到有害作用的剂量水平 no observed adverse effect level(NOAEL）

在规定的试验条件下，用现有的技术手段或检测指标未观察到任何与受试物有关的毒性作用的最大剂量。

3.3 观察到有害作用的最低剂量水平 Lowest observed adverse effect level(LOAEL)

在规定的试验条件下，受试物引起实验动物组织形态、功能、生长发育等有害效应的最低剂量。

3.4 靶器官 Target organ

实验动物出现由受试物引起的明显毒性作用的器官。

3 Definition

3.1 Subchronic oral toxicity

It refers to the adverse reactions caused by repeated oral contact with the test substance every day during the partial survival period of the experimental animal.

3.2 No observed adverse effect level (NOAEL)

Under the specified test conditions, no maximum dose of toxic effect related to the test substance is observed by using the existing technical means or detection indicators.

3.3 Lowest observed adverse effect level (LOAEL)

Under the specified test conditions, the lowest dose of test substance causing harmful effects such as tissue morphology, function, growth and development of experimental animals.

3.4 Target organ

The organs of experimental animals with obvious toxic effects caused by the test substance.

4 试验的基本原则

以不同剂量受试物每日经口给予各组实验动物，连续染毒 90d，每组采用一个染毒剂量。染毒期间每日观察动物的毒性反应。在染毒期间死亡的动物要进行尸检。染毒结束后所有存活的动物均要处死，并进行尸检以及适当的病理组织学检查。

4 Basic principles of test

The experimental animals in each group are given different doses of the test substance orally every day for 90 days, and each group is given one dose. The toxic reaction of animals is observed every day during the period of exposure. Necropsy is required for animals that die during exposure. At the end of the exposure, all the surviving animals are killed and necropsy and appropriate histopathological examination are carried out.

5 试验方法

5.1 实验动物和饲养环境

5.1.1 动物种系的选择

常规选择啮齿类动物，首选大鼠。一般选用 6 周—8 周龄的大鼠。动物体重的变动范围不应超出平均动物体重的 20%。若该试验为慢性试验的预备试验，则在两个试验中所用的动物种系应当相同。

5.1.2 动物的性别和数量

每一剂量组实验动物至少应有 20 只（雌雄各半），但是考虑到亚慢性试验的重要性，应适当增加每组雌雄动物数。若计划在试验过程中处死动物，则应增加计划处死的动物数。试验结束时的动物数需达到能够有效评价受试物毒性作用的数量。此外，可另设一追踪观察组，选用 20 只动物（雌雄各半），给予最高剂量受试物，染毒 90d，在全程染毒结束后继续观察一段时间（一般不少于 28d），以了解毒性作用的持续性、可逆性或迟发毒作用。

5.1.3 饲养环境

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5 test method

5.1 Laboratory animals and feeding environment

5.1.1 Selection of animal species

Rodents are selected as the first choice. Generally, rats aged 6-8 weeks are selected. The variation range of animal weight should not exceed 20% of the average animal weight. If the test is a preliminary test for a chronic test, the animal strains used in both tests should be the same.

5.1.2 Sex and number of animals

Each dose group should have at least 20 experimental animals (half male and half female), but considering the importance of subchronic tests, the number of male and female animals in each group should be appropriately increased. If it is planned to kill animals during the experiment, the number of animals to be killed should be increased. At the end of the test, the number of animals should reach the number that can effectively evaluate the toxicity of the tested substance. In addition, another follow-up observation group can be set up. 20 animals (half male and half female) are selected and given the highest dose of the tested substance for 90 days, and the observation period (generally not less than 28 days) is continued after the whole course of exposure to understand the persistence, reversibility or delayed toxicity of the toxic effect.

5.1.3 Feeding environment

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, drinking water is not limited.

5.2 剂量分组

试验时至少要设三个染毒组和一个对照组。除不接触受试物外，对照组的其他条件均与试验组相同。最高染毒剂量的设计应在引起中毒效应的前提下又不致造成动物过多死亡，否则将会影响结果的评价。低剂量组应不出现任何毒性作用。若掌握人群接触水平，则最低染毒剂量应高于人群的实际接触水平。中间剂量组应引起较轻的可观察到的毒性作用。若设多个中间剂量组，则各组的染毒剂量应引起不同程度毒性作用。在中、低剂量组和对照组中，动物死亡率应很低，以保证得到有意义的评价结论。

对那些毒性较低的物质来说，当通过饲料染毒时应特别注意确保大量的受试物混入不会对动物正常营养产生影响。对其他的染毒方式要加以特殊说明。若采用灌胃方式染毒，则每日染毒时点应相同，并定期（每周）按体重调整染毒剂量，维持单位体重染毒水平不变。

本项试验中，如果接触水平超过 1000mg/kg 时仍未产生可观测到的毒性效应，而且可以根据相关结构化合物预期受试物毒性时，可以考虑不必进行三个剂量水平的全面试验观察。

5.2 Dose grouping

At least three exposure groups and one control group should be set up in the experiment. The other conditions of the control group are the same as those of the test group except for the non-contact with the test substance. The design of the maximum dose should not lead to excessive death of animals on the premise of toxic effect, otherwise it will affect the evaluation of the results. There should be no toxic effect in the

low dose group. If the exposure level of the population is known, the lowest exposure dose should be higher than the actual exposure level of the population. The intermediate dose group should cause less observable toxicity. If there are more than one middle dose group, the dose of each group should cause different degrees of toxicity. In the middle and low dose group and the control group, the mortality rate of animals should be very low, so as to ensure a meaningful evaluation conclusion.

For those substances with low toxicity, special attention should be paid to ensure that a large number of test substances will not affect the normal nutrition of animals when they are exposed to feed. Other ways of poisoning should be specified. If the drug is administered by gavage, the time point of daily administration should be the same, and the dose should be adjusted regularly (weekly) according to the body weight to maintain the same level of unit body weight.

In this test, if the exposure level is more than 1000mg / kg, there is no observed toxic effect, and the toxicity of the test substance can be expected according to the related structural compounds, it can be considered that there is no need to conduct three dose levels of comprehensive test observation.

5.3 试验步骤

染毒开始前至少要有 5d 时间使实验动物适应实验室饲养环境。实验动物随机分组。受试物可通过混入饲料或饮水、直接喂饲以及灌胃进行染毒。动物每周 7d 染毒。试验期间所有动物染毒的方式应完全相同。若为染毒目的加入其他溶剂或添加剂，这些溶剂或添加剂不应影响受试物的吸收或引起毒性作用。

5.3 Test procedure

There should be at least 5 days before the start of poisoning to make the experimental animals adapt to the laboratory feeding environment. The experimental animals are randomly divided into groups. The test substance can be poisoned by mixing feed or drinking water, direct feeding and gavage. Animals are poisoned 7 days a week. All animals should be exposed in exactly the same way during the test. If other solvents or additives are added for the purpose of poisoning, they shall not affect the absorption of the test substance or cause toxicity.

5.4 临床观察

观察时间应至少为 90d。追踪观察组还要增加 28d，但不作任何处理，以了解毒性作用的可逆性、持续性及迟发毒作用。

观察期间对动物的任何毒性表现均应记录，记录内容包括发生时间、程度和持续时间。观察应至少包括如下内容：皮肤和被毛的改变、眼和粘膜变化、呼吸、循环、植物神经和中枢神经系统、肢体运动和行为活动等改变。应计算每周饲料消耗量（或当通过饮水染毒时的饮水消耗量），记录每周体重变化。

5.4 clinical observation

The observation time should be at least 90 days. In the follow-up observation group, 28 days are added, but no treatment is done to understand the reversibility, persistence and delayed toxicity of the toxicity.

Any toxicity to animals during the observation period shall be recorded, including the occurrence time, degree and duration. The observation should at least include the following contents: changes of skin and coat, changes of eyes and mucosa, changes of breath, circulation, autonomic and central nervous system, changes of limb movement and behavior. The weekly feed consumption (or water consumption when poisoned by drinking water) shall be calculated and the weekly weight change shall be recorded.

5.5 临床检查

5.5.1 眼科检查

在动物染毒前和染毒后，最好对所有实验动物，至少应对最高剂量组和对照组动物，使用眼科镜或其他有关设备进行眼科检查。若发现动物有眼科变化则应对所有动物进行检查。

5.5.2 血液检查

在染毒前、染毒中期、染毒结束及追踪观察结束时应测定血球容积、血红蛋白浓度、红细胞数、白细胞总数和分类，必要时测定凝血功能如凝血时间、凝血酶原时间、凝血激酶时间或血小板数等指标。

5.5.3 临床血液生化检查

在染毒前、染毒中期、染毒结束及追踪观察结束时进行，检查指标包括电解质平衡、碳水化合物代谢、肝、肾功能。可根据受试物作用形式选择其他特殊检查。推荐的指标包括：钙、磷、氯、钠、钾、禁食血糖（不同动物品系采用不同的禁食期）、血清谷丙转氨酶、血清谷草转氨酶、鸟氨酸脱羧酶、g 谷氨酰转肽酶、尿素氮、白蛋白、血液肌酐、总胆红素及总血清蛋白。必要时可进行脂肪、激素、酸碱平衡、正铁血红蛋白、胆碱酯酶活性的分析测定。此外，还可根据所观察到的毒性作用进行其他更大范围的临床生化检查，以便进行全面的毒性评价。

5.5 clinical examination

5.5.1 Ophthalmic examination

Before and after the animals are poisoned, it is better to use ophthalmoscope or other relevant equipment for ophthalmic examination on all experimental animals, at least the animals in the highest dose group and the control group.If eye changes are found in animals, all animals should be examined.

5.5.2 Blood test

The blood cell volume, hemoglobin concentration, the number of red blood cells, the total number of white blood cells and their classification should be measured before, during, after and at the end of follow-up observation. If necessary, the coagulation function such as coagulation time, prothrombin time, coagulation kinase time or platelet number should be measured.

5.5.3 Clinical blood biochemical examination

Before, during, at the end of exposure and at the end of follow-up observation, the examination indexes include electrolyte balance, carbohydrate metabolism, liver and kidney function. Other special examinations can be selected according to the acting form of the test object. The recommended indexes include: calcium, phosphorus, chlorine, sodium, potassium, fasting blood glucose (different fasting periods are adopted for different animal strains), serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, ornithine decarboxylase, G glutamyl transpeptidase, urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein. When

necessary, fat, hormone, acid-base balance, ferrohemoglobin and cholinesterase activity can be analyzed and determined. In addition, other clinical biochemical tests in a wider range can be carried out according to the observed toxic effects so as to carry out a comprehensive toxicity evaluation.

5.5.4 尿液检查

一般不需要进行，只有当怀疑存在或观察到相关毒性作用时方需进行尿液检查。

5.6 病理检查

5.6.1 大体尸检

所有动物均应进行全面的大体尸检，内容包括动物的外观、所有孔道，胸腔、腹腔及其内容物。肝、肾、肾上腺、睾丸、附睾、子宫、卵巢、胸腺、脾、脑和心脏应在分离后尽快称重以防水分丢失。应将下列组织和器官保存在固定液中，以备日后进行病理组织学检查：所有大体解剖呈现异常的器官、脑（包括延髓/脑桥、小脑和大脑皮层、脑垂体）、甲状腺/甲状旁腺、胸腺、肺/气管、心脏、主动脉、唾液腺*、肝、脾、肾、肾上腺、胰、性腺、子宫、生殖附属器官*、皮肤*、食管、胃、十二指肠、空肠、回肠、盲肠、结肠、直肠、膀胱、前列腺、有代表性的淋巴结、雌性乳腺*、大腿肌肉*、周围神经、胸骨（包括骨髓）、眼*、股骨（包括关节面）*、脊髓（包括颈部、胸部、腰部）*和泪腺*。

* 只有当毒性作用提示或作为被研究的靶器官时才需要检查这些器官。

5.5.4 Urinalysis

Generally, it is not necessary to carry out a urine test only when the presence or observation of related toxic effects is suspected.

5.6 Pathological examination

5.6.1 General autopsy

All animals should undergo a comprehensive gross autopsy, including the animal's appearance, all channels, chest cavity, abdominal cavity and its contents. Liver, kidney, adrenal gland, testis, epididymis, uterus, ovary, thymus, spleen, brain and heart should be weighed as soon as possible after separation to prevent water loss. The following tissues and organs shall be preserved in the fixative. For future histopathological examination: all organs with gross anatomical abnormalities, brain (including medulla oblongata/pons, cerebellum and cerebral cortex, pituitary gland), thyroid gland/parathyroid gland, thymus gland, lung/trachea, heart, aorta, salivary gland *, liver, spleen, kidney, adrenal gland, pancreas, gonad, uterus, reproductive accessory organs *, skin *, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, prostate, Representative lymph nodes, female mammary glands *, thigh muscles *, peripheral nerves, sternum (including bone marrow), eyes *, femur (including articular surface) *, spinal cord (including neck, chest, waist) *, and lacrimal gland *.

* These organs need to be examined only when toxic effects are indicated or when they are target organs to be studied.

5.6.2 病理组织学检查

应对下述器官和组织进行检查：

(1) 所有最高剂量组和对照组动物的重要的和可能受到损伤的器官或组织，如高剂量组动物的器官或组织有病理组织学的病变，则应扩展至其他剂量组的相应的器官和组织。

(2) 各剂量组大体解剖见有异常的器官或组织。

(3) 其他剂量组动物的靶器官。

(4) 在追踪观察组，应对那些在染毒组呈现毒性作用的组织和器官进行检查。

5.6.2 Histopathological examination

The following organs and tissues should be examined:

(1) All the important and possibly damaged organs or tissues of the highest dose group and the control group animals, such as the organs or tissues of the high dose group animals with pathological changes, should be extended to the corresponding organs and tissues of other dose groups.

(2) The gross anatomy of each dose group showed abnormal organs or tissues.

(3) Target organs of other dose groups.

(4) In the follow-up group, the tissues and organs with toxic effects in the exposed group should be examined.

6 试验结果的评价

6.1 结果的处理

可通过表格形式总结试验结果，显示试验开始时各组动物数、出现损伤的动物数、损伤的类型和每种损伤的动物百分比。对所有数据应采用适当的统计学方法进行评价，统计学方法应在试验设计时确定。

6.2 结果评价

亚慢性经口毒性试验结果应结合前期试验结果，并考虑到毒性效应指标和尸检及病理组织学检查结果进行综合评价。毒性评价应包括受试物染毒剂量与是否出现毒性反应、毒性反应的发生率及其程度之间的关系。这些反应包括行为或临床异常、肉眼可见的损伤、靶器官、体重变化情况、死亡效应以及其他一般或特殊的毒性作用。在综合分析的基础上得出 90 天经口毒性的 LOAEL 和（或）NOAEL，为慢性毒性试验的剂量、观察指标的选择提供依据。

6 Evaluation of test results

6.1 Treatment of results

The test results can be summarized in tabular form, showing the number of animals in each group at the beginning of the test, the number of animals with injury, the type of injury and the percentage of animals with each injury. All data should be evaluated using appropriate statistical methods, which should be determined at the time of trial design.

6.2 Result evaluation

The results of subchronic oral toxicity test should be combined with the results of previous tests, and the toxicity index and the results of autopsy and histopathology should be considered for comprehensive evaluation. The toxicity evaluation should include the relationship between the dose of the test substance and whether there is toxic reaction, the incidence and degree of toxic reaction. These reactions include behavioral or clinical abnormalities, visible damage to the naked eye, target organs,

weight changes, death effects, and other general or special toxic effects. Based on the comprehensive analysis, the LOAEL and / or NOAEL of 90 day oral toxicity are obtained, which provided the basis for the selection of dose and observation index of chronic toxicity test.

7 试验结果的解释

亚慢性经口毒性试验能够提供受试物在经口反复接触时的毒性作用资料。其试验结果可在很有限的程度上外推到人，但它可为确定人群的允许接触水平提供有用的信息。

7 Interpretation of test results

Subchronic oral toxicity test can provide the toxicity data of the tested substance in repeated oral exposure. The test results can be extrapolated to human to a very limited extent, but it can provide useful information for determining the allowable exposure level of the population.

15 亚慢性经皮毒性试验

Subchronic Dermal Toxicity Test

1 范围

本规范规定了啮齿类动物亚慢性经皮毒性试验的基本原则、要求和方法。本规范适用于检测化妆品原料的亚慢性经皮毒性。

2 试验目的

在估计和评价化妆品原料的毒性时，获得受试物急性经皮毒性资料后，还需进行亚慢性经皮毒性试验。通过该试验不仅可获得在一定时期内反复接触受试物后可能引起的健康影响资料，而且为评价受试物经皮渗透性、作用靶器官和慢性皮肤毒性试验剂量选择提供依据。

15 Subchronic Dermal Toxicity Test

1 Range

This specification specifies the basic principles, requirements and methods of subchronic percutaneous toxicity test for rodents. This specification is applicable to the detection of subchronic transdermal toxicity of cosmetic raw materials.

2 Test purpose

In the estimation and evaluation of the toxicity of cosmetic raw materials, the subchronic dermal toxicity test should be carried out after obtaining the acute dermal toxicity data of the test substance. Through this test, we can not only obtain the data of the possible health effects caused by repeated exposure to the test substance in a certain period of time, but also provide the basis for the evaluation of the percutaneous permeability of the test substance, the target organ and the dose selection of the chronic dermal toxicity test.

3 定义

3.1 亚慢性经皮毒性 subchronic dermal toxicity

是指在实验动物部分生存期内，每日反复经皮接触受试物后所引起的不良反应。

3.2 未观察到有害作用的剂量水平 no observed adverse effect level(NOAEL）

在规定的试验条件下，用现有的技术手段或检测指标未观察到任何与受试物有关的毒性作用的最大剂量。

3.3 观察到有害作用的最低剂量水平 lowest observed adverse effect level(LOAEL）

在规定的试验条件下，受试物引起实验动物组织形态、功能、生长发育等有害效应的最低剂量。

3.4 靶器官 target organ

实验动物出现由受试物引起的明显毒性作用的器官。

3 Definition

3.1 Subchronic dermal toxicity

It refers to the adverse reactions caused by repeated skin contact with the test substance every day during the partial survival period of the experimental animal.

3.2 No observed adverse effect level (NOAEL)

Under the specified test conditions, no maximum dose of toxic effect related to the test substance is observed by using the existing technical means or detection indicators.

3.3 Lowest observed adverse effect level (LOAEL)

Under the specified test conditions, the lowest dose of test substance causing harmful effects such as tissue morphology, function, growth and development of experimental animals.

3.4 Target organ

The organs of experimental animals with obvious toxic effects caused by the test substance.

4 试验的基本原则

以不同剂量受试物每日经皮给予各组实验动物，连续染毒 90d，每组采用一个染毒剂量。染毒期间每日观察动物的毒性反应。在染毒期间死亡的动物要进行尸检。染毒结束后对所有存活的动物均要处死，并进行尸检以及适当的病理组织学检查。

4 Basic principles of test

The experimental animals in each group are given different doses of the test substance by skin every day for 90 days, and each group is given one dose. The toxic reaction of animals is observed every day during the period of exposure. Necropsy is required for animals that

die during exposure. At the end of the exposure, all the surviving animals are killed, and necropsy and appropriate histopathological examination are carried out.

5 试验方法

5.1 受试物

若受试物为固体，应将其粉碎并用水（或适当的介质）充分湿润，以保证受试物与皮肤有良好的接触。若采用介质，应考虑该介质对受试物皮肤通透性的影响。液体受试物一般不用稀释。

5.2 实验动物和饲养环境

5.2.1 动物种系的选择

可采用成年大鼠、家兔或豚鼠进行试验，也可使用其他种属的动物。当亚慢性试验作为慢性试验的预备试验时，则在两项试验中所使用的动物种系应当相同。

5.2.2 动物的性别和数量

每一剂量组实验动物至少应有 20 只（雌雄各半），皮肤健康。若计划在试验过程中处死动物，则应增加计划处死的动物数。此外，可另设一追踪观察组，选用 20 只动物（雌雄各半），给予最高剂量受试物，染毒 90d，全程染毒结束后继续观察一段时间（一般不少于 28d），以了解毒性作用的持续性、可逆性或迟发毒作用。

5 test method

5.1 Test substance

If the test substance is solid, it shall be crushed and moistened with water (or appropriate medium) to ensure good contact between the test substance and the skin.If the medium is used, the influence of the medium on the skin permeability of the test object shall be considered. In general, the liquid test substance does not need to be diluted.

5.2 Laboratory animals and feeding environment

5.2.1 Selection of animal species

Adult rats, rabbits or guinea pigs can be used for the test, and other species of animals can also be used. When the subchronic test is used as the preliminary test of the chronic test, the animal species used in the two tests should be the same.

5.2.2 Sex and number of animals

There should be at least 20 animals (half male and half female) in each dose group with healthy skin. If animals are planned to be killed during the test, the number of animals planned to be killed shall be increased. In addition, a follow-up observation group can be set up to select 20 animals (half male and half female) and give them the highest dose of test substance for 90 days. After the whole course of exposure, continue to observe for a period of time (generally no less than 28 days) to understand the persistence, reversibility or delayed toxicity of toxicity.

5.2.3 饲养环境

实验动物及实验动物房应符合国家相应规定。选用标准配合饲料，饮水不限制。

5.3 剂量分组

试验时至少要设三个染毒组和一个对照组。除不接触受试物外，对照组的其他条件均与试验组相同。最高染毒剂量的设计应在引起中毒效应的前提下又不致造成动物过多死亡，否则将会影响结果的评价。低剂量组应不出现任何毒性作用。若掌握人群接触水平，则最低染毒剂量应高于人群的实际接触水平。中间剂量组应引起较轻的可观察到的毒性作用。若设多个中间剂量组，则各组的染毒剂量应引起不同程度毒性作用。在中、低剂量组和对照组中，动物死亡率应很低，以保证得到有意义的评价结论。

若受试物引起严重的皮肤刺激效应，则应降低受试物的使用浓度，尽管这样可导致原来在高剂量下出现的其他毒性作用减弱或消失。若在试验早期动物的皮肤受到严重损伤，则有必要终止试验，并使用较低的浓度重新开始试验。

本项试验中，如果接触水平超过 1000mg/kg 时仍未产生可观测到的毒性效应，而且可以根据相关结构化合物预期受试物毒性时，可以考虑不必进行三个剂量水平的全面试验观察。

5.2.3 Feeding environment

The laboratory animal and laboratory animal room shall comply with the relevant national regulations. Select standard compound feed, drinking water is not limited.

5.3 Dose grouping

At least three exposure groups and one control group should be set up in the experiment. The other conditions of the control group are the same as those of the test group except for the non-contact with the test substance. The design of the maximum dose should not lead to excessive death of animals on the premise of toxic effect, otherwise it will affect the evaluation of the results. There should be no toxic effect in the low dose group. If the exposure level of the population is known, the lowest exposure dose should be higher than the actual exposure level of the population. The intermediate dose group should cause less observable toxicity. If there are more than one middle dose group, the dose of each group should cause different degrees of toxicity. In the middle and low dose group and the control group, the mortality rate of animals should be very low, so as to ensure a meaningful evaluation conclusion.

If the test substance causes severe skin irritation, the concentration of the test substance should be reduced, although this may lead to the weakening or disappearance of other toxic effects that originally occurred at high doses. If the skin of the animal is severely damaged in the early stage of the test, it is necessary to terminate the test and restart the test with a lower concentration.

In this test, if the exposure level is more than 1000mg / kg, there is no observed toxic effect, and the toxicity of the test substance can be expected according to the related structural compounds, it can be considered that there is no need to conduct three dose levels of comprehensive test observation.

5.4 试验步骤

动物在试验前至少要在实验室饲养环境中适应 5d 时间。染毒前 24h，将动物躯干背部染毒区的被毛剪掉或剃除。大约每周要对染毒部位去毛。在使用剪刀或剃刀进行去毛时应特别小心，以防损伤动物的皮肤从而引起皮肤通透性的改变。染毒部位的面积不应小于动物体表面积的 10%，应通过对动物体重的测定确定染毒部位的面积。若受试物毒性较大，则可相对减小染毒区域的面积，但受试物应尽可能薄而均匀地涂敷于整个染

毒区域。在染毒操作期间应使用玻璃纸和无刺激的胶带将受试物固定，以保证受试物与皮肤有良好的接触，并防止动物舔食。

在 90d 试验期间，实验动物每周 7d 每天染毒 6h。追踪观察组则要多进行 28d 观察，以了解毒性作用的持续性、可逆性及迟发毒作用。

5.4 Test procedure

Before the experiment, the animals should adapt to the laboratory environment for at least 5 days.24 hours before the exposure, the hairs in the exposed area on the back of the animal's trunk are cut off or shaved off. Hair should be removed from the infected area about every week. Special care should be taken when using scissors or razors for hair removal to prevent damage to the animal's skin that may cause changes in skin permeability. The area of the infected part shall not be less than 10% of the body surface area of the animal, and the area of the infected part shall be determined by the measurement of the weight of the animal. If the toxicity of the test substance is large, the area of the contaminated area can be relatively reduced, but the test substance should be applied to the whole contaminated area as thin and even as possible. During the poisoning operation, cellophane and non irritating tape shall be used to fix the test substance to ensure good contact between the test substance and the skin and prevent animals from licking.

During the 90-day test, the animals were exposed to the virus for 6 hours every day, 7 days a week. In the follow-up observation group, 28 days more observation was needed to understand the persistence, reversibility and delayed toxicity of toxic effects.

5.5 临床观察

试验中每天至少应进行一次仔细的临床检查。

观察期间对动物的任何毒性表现均应记录，记录内容包括发生时间、程度和持续时间。笼边观察应至少包括如下内容：皮肤和被毛的改变、眼和粘膜变化、呼吸、循环、植物神经和中枢神经系统、肢体运动和行为活动等改变。应计算每周饲料消耗量，记录每周体重变化。

5.6 临床检查

5.6.1 眼科检查

在动物染毒前和染毒后，最好对所有实验动物，至少应对最高剂量组和对照组动物，使用眼科镜或其他有关设备进行眼科检查。若发现眼科变化则应对所有动物进行检查。

5.6.2 血液检查

在染毒前、染毒中期、染毒结束及追踪观察结束时应测定包括血球容积、血红蛋白浓度、红细胞数、白细胞总数和分类、必要时测定凝血功能，如凝血时间、凝血酶原时间、凝血激酶时间或血小板数等指标。

5.5 clinical observation

Careful clinical examination should be carried out at least once a day in the trial.

Any toxicity to animals during the observation period shall be recorded, including the occurrence time, degree and duration. Cage side observation should at least include the following contents: changes of skin and coat, changes of eyes and mucosa,

changes of breath, circulation, autonomic and central nervous system, changes of limb movement and behavior. Weekly feed consumption should be calculated and weekly weight changes recorded.

5.6 clinical examination

5.6.1 Ophthalmic examination

Before and after the animals are poisoned, it is better to use ophthalmoscope or other relevant equipment for ophthalmic examination on all experimental animals, at least the animals in the highest dose group and the control group. If eye changes are found, all animals should be examined.

5.6.2 Blood test

Before, during, after and after exposure, blood cell volume, hemoglobin concentration, red blood cell number, total number and classification of white blood cells, and coagulation function, such as coagulation time, prothrombin time, thrombokinase time or platelet number, should be determined.

5.6.3 临床血液生化检查

染毒前、染毒中期、染毒结束及追踪观察结束时进行，检查指标包括电解质平衡、碳水化合物代谢、肝、肾功能。可根据受试物作用形式选择其他特殊检查。推荐的指标包括：钙、磷、氯、钠、钾、禁食血糖（不同动物品系采用不同的禁食期）、血清谷丙转氨酶、血清谷草转氨酶、鸟氨酸脱羧酶、g 谷氨酰转肽酶、尿素氮、白蛋白、血液肌酐、总胆红素及总血清蛋白。必要时可进行脂肪、激素、酸碱平衡、正铁血红蛋白、胆碱酯酶活性的分析测定。此外，还可根据所观察到的毒性作用进行其他更大范围的临床生化检查，以便进行全面的毒性评价。

5.6.4 尿液检查

一般不需要进行，只有当怀疑存在或观察到相关毒性作用时方需进行尿液检查。

5.6.3 Clinical blood biochemical examination

Before, during, after and at the end of follow-up observation, electrolyte balance, carbohydrate metabolism, liver and kidney functions are examined. Other special inspection can be selected according to the action form of test substance. The recommended indexes include: calcium, phosphorus, chlorine, sodium, potassium, fasting blood glucose (different fasting periods are adopted for different animal strains), serum GPT, serum GST, ornithine decarboxylase, g-glutamyltranspeptidase, urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein. If necessary, fat, hormone, acid-base balance, methemoglobin and cholinesterase activity can be determined. In addition, a wider range of clinical biochemical tests can be carried out according to the observed toxic effects in order to carry out a comprehensive toxicity evaluation.

5.6.4 Urinalysis

Generally, it is not necessary to carry out a urine test only when the presence or observation of related toxic effects is suspected.

5.7 病理检查

5.7.1 大体尸检

所有动物均应进行全面的大体尸检，内容包括机体的外观、所有孔道，胸腔、腹腔及其内容物。肝、肾、肾上腺、睾丸、附睾、子宫、卵巢、胸腺、脾、脑和心脏应

在分离后尽快称重以防水分丢失。应将下列组织和器官保存在固定液中，以便日后进行病理组织学检查：所有大体解剖呈现异常的器官、脑（包括延髓/脑桥、小脑和大脑皮层、脑垂体）、甲状腺/甲状旁腺、胸腺、肺/气管、心脏、主动脉、唾液腺*、肝、脾、肾、肾上腺、胰、性腺、子宫、生殖附属器官*、皮肤、食管、胃、十二指肠、空肠、回肠、盲肠、结肠、直肠、膀胱、前列腺、有代表性的淋巴结、雌性乳腺*、大腿肌肉*、周围神经、胸骨（包括骨髓）、眼*、股骨（包括关节面）*、脊髓（包括颈部、胸部、腰部）*和泪腺*。

* 只有当毒性作用提示或作为被研究的靶器官时才需要检查这些器官。

5.7 Pathological examination

5.7.1 autopsy

All animals should undergo a comprehensive gross autopsy, including the animal's appearance, all channels, chest cavity, abdominal cavity and its contents. Liver, kidney, adrenal gland, testis, epididymis, uterus, ovary, thymus, spleen, brain and heart should be weighed as soon as possible after separation to prevent water loss. The following tissues and organs shall be preserved in the fixative. For future histopathological examination: all organs with gross anatomical abnormalities, brain (including medulla oblongata/pons, cerebellum and cerebral cortex, pituitary gland), thyroid gland/parathyroid gland, thymus gland, lung/trachea, heart, aorta, salivary gland *, liver, spleen, kidney, adrenal gland, pancreas, gonad, uterus, reproductive accessory organs *, skin *, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, prostate, Representative lymph nodes, female mammary glands *, thigh muscles *, peripheral nerves, sternum (including bone marrow), eyes *, femur (including articular surface) *, spinal cord (including neck, chest, waist) *, and lacrimal gland *.

5.7.2 病理组织学检查

应对下述器官和组织进行病理组织学检查：

(1) 所有最高剂量组和对照组动物的重要的和可能受到损伤的器官或组织，如高剂量组动物的器官或组织有病理组织学病变则应扩展至其他剂量组的相应的器官和组织。

(2) 各剂量组大体解剖见有异常的器官或组织。

(3) 其他剂量组动物的靶器官。

(4) 对追踪观察组，应对那些在染毒组呈现毒性作用的组织和器官进行检查。

5.7.2 Histopathological examination

The following organs and tissues shall be examined for histopathology:

(1) All the important and possibly damaged organs or tissues of the highest dose group and the control group animals, such as the organs or tissues of the high dose group animals with pathological changes, should be extended to the corresponding organs and tissues of other dose groups.

(2) The gross anatomy of each dose group showed abnormal organs or tissues.

(3) Target organs of other dose groups.

(4) For the follow-up observation group, the tissues and organs with toxic effects in the exposed group should be examined.

6 试验结果的评价

6.1 结果的处理

可通过表格形式总结试验结果，显示试验开始时各组动物数、出现损伤的动物数、损伤的类型和每种损伤的动物百分比。对所有数据应采用适当的统计学方法进行评价，统计学方法应在试验设计时确定。

6.2 试验结果的评价

亚慢性经皮毒性试验结果应结合前期试验结果，并考虑到毒性效应指标和尸检及病理组织学检查结果进行综合评价。毒性评价应包括受试物染毒剂量与是否出现毒性反应、毒性反应的发生率及其程度之间的关系。这些反应包括行为或临床异常、肉眼可见的损伤、靶器官、体重变化情况、死亡效应以及其他一般或特殊的毒性作用。在综合分析的基础上得出 90 天经皮毒性的 LOAEL 和（或）NOAEL，为慢性毒性试验的剂量、观察指标的选择提供依据。

6 Evaluation of test results

6.1 Treatment of results

The test results can be summarized in tabular form, showing the number of animals in each group at the beginning of the test, the number of animals with injury, the type of injury and the percentage of animals with each injury. All data should be evaluated using appropriate statistical methods, which should be determined at the time of trial design.

6.2 Evaluation of test results

The results of subchronic percutaneous toxicity test should be combined with the results of previous tests, and the toxicity index, autopsy and histopathological examination results should be considered for comprehensive evaluation. The toxicity evaluation should include the relationship between the dose of the test substance and whether there is toxic reaction, the incidence and degree of toxic reaction. These reactions include behavioral or clinical abnormalities, visible damage to the naked eye, target organs, weight changes, death effects, and other general or special toxic effects. Based on the comprehensive analysis, the LOAEL and / or NOAEL of 90 days' percutaneous toxicity are obtained, which provided the basis for the selection of dose and observation index of chronic toxicity test.

7 试验结果的解释

亚慢性经皮毒性试验能够提供受试物在经皮反复接触时的毒性作用资料。其试验结果可在很有限的程度上外推到人，但它可为确定人群的允许接触水平提供有用的信息。

7 Interpretation of test results

Subchronic percutaneous toxicity test can provide the toxicity data of the tested substance in repeated percutaneous contact. The test results can be extrapolated to human to a very limited extent, but it can provide useful information for determining the allowable exposure level of the population.

16 致畸试验

Teratogenicity Test

1 范围

本规范规定了动物致畸试验的基本原则，要求和方法。本规范用于检测化妆品原料的致畸性。

2 试验目的

检测妊娠动物接触化妆品原料后引起胎鼠畸形的可能性。

16 Teratogenicity test

1 Range

This specification specifies the basic principles, requirements and methods of animal teratogenesis test. This specification is used to detect the teratogenicity of cosmetic raw materials.

2 Test purpose

To detect the possibility of fetal rat deformity caused by the contact of pregnant animals with cosmetic materials.

3 定义

致畸性 Teratogenicity
在胚胎发育期引起胎仔永久性结构和功能异常的化学物质特性。

4 试验基本原则

在胚胎发育的器官形成期给妊娠动物染毒，在胎鼠出生前将妊娠动物处死，取出胎鼠检查其骨骼和内脏畸形。

3 Definition

Teratogenicity

Chemical properties that cause permanent structural and functional abnormalities of the fetus during embryonic development.

4 Basic principles of test

Pregnant animals are poisoned in the organogenesis stage of embryo development, and are killed before birth, and their skeleton and visceral deformities are examined.

5 试验方法

5.1 试剂

5.1.1 甲醛、冰乙酸、2，4，6-三硝基酚、氢氧化钾、甘油、水合氯醛、茜素红。

5.1.2 茜素红贮备液：茜素红饱和液，50% 乙酸饱和液 5.0 mL，甘油 10.0 mL，1% 水合氯醛 60.0 mL 混合，放入棕色瓶中。

5.1.3 茜素红应用液：取贮备液 3—5 mL，用 1—2g/100 mL 氢氧化钾液稀释至 1000 mL，存于棕色瓶中。

5.1.4 茜素红溶液：茜素红 0.1g，氢氧化钾 10g，蒸馏水 1000mL。

5.1.5 透明液 A：甘油 200mL，氢氧化钾 10g 蒸馏水 790mL.

5.1.6 透明液 B：甘油与蒸馏水等量混合。

5.1.7 固定液（Bouins 液）：2,4,6-三硝基酚（苦味酸饱和液）75 份、甲醛 20 份、冰乙酸 5 份

5 test method

5.1 reagent

5.1.1 Formaldehyde, glacial acetic acid, 2,4,6-trinitrophenol, potassium hydroxide, glycerin, chloral hydrate, alizarin red.

5.1.2 Alizarin red stock solution: alizarin red saturated solution, 50% acetic acid saturated solution 5.0ml, glycerin 10.0ml, 1% chloral hydrate 60.0ml mixed, put into a brown bottle.

5.1.3 Alizarin red application solution: take 3-5ml of stock solution, dilute to 1000ml with 1-2g / 100ml potassium hydroxide solution, and store in brown bottle.

5.1.4 Alizarin red solution: 0.1g alizarin red, 10g potassium hydroxide, 1000ml distilled water.

5.1.5 Transparent solution A: Glycerin 200ml, potassium hydroxide 10g distilled water 790ml

5.1.6 Transparent solution B: mix glycerin and distilled water in equal amount.

5.1.7 Fixed solution (bouins solution): 75 parts of 2,4,6-trinitrophenol (picric acid saturated solution), 20 parts of formaldehyde, 5 parts of glacial acetic acid

5.2 实验动物和饲养环境

动物选择：首选为健康的性成熟大鼠。

实验动物及实验动物房应符合国家相应规定。

5.3 剂量和分组

至少设三个剂量组，最高剂量应能引起母鼠某些毒性反应，但不应引起 10%以上动物的死亡。最低剂量不会出现可观察到的毒性反应。另设阴性对照组。每组至少 12 只孕鼠。当初次进行致畸试验或使用新的动物种属和品系时，必须同时设阳性对照组，阳性对照物可选用敌枯双、维生素 A 等。

5.2 Laboratory animals and feeding environment

Animal selection: the first choice is healthy sexually mature rats.

The laboratory animal and laboratory animal room shall comply with the relevant national regulations.

5.3 Dose and grouping

There should be at least three dose groups. The highest dose should be able to cause some toxic reactions in female mice, but it should not cause more than 10% of animal deaths. There will be no observed toxicity at the lowest dose. Another negative control group is set up. At least 12 pregnant rats in each group. When the first teratogenesis test is carried out or new animal species and strains are used, a positive control group must be set up at the same time. The positive control materials can be diclofenac, vitamin A, etc.

5.4 试验步骤

5.4.1 “孕鼠”的检出和给受试物时间

雌鼠和雄鼠按 1：1（或 2：1）同笼，每日晨观察阴栓（或阴道涂片），查出阴栓（或精子）的当天定为孕期零天。如果 5 d 内没查出“受精鼠”，应调换雌鼠。检出的“受精鼠”按随机分组。在孕期 6d—15d，每天经口给予受试物。孕鼠于孕期 0、6、10、15 和 20 d 称重，并根据体重调整给受试物量。

5.4.2 孕鼠处死和一般检查

大鼠于妊娠第 20 d 处死。剖腹检查卵巢内黄体数，取出子宫，称重；检查活胎、早期吸收和死胎数。

5.4.3 活胎鼠检查

逐一记录胎鼠体重、体长、尾长、检查胎鼠外观有无异常，如头部有无脑膨出、露脑、小头、小耳、小眼、无眼和睁眼、兔唇、下颌裂，躯干部有无腹壁裂、脐疝、脊柱弯曲，四肢有无小肢、短肢、并趾、多趾、无趾等畸形，尾部有无短尾、卷尾、无尾、肛门有无闭锁。

5.4 Test procedure

5.4.1 Detection of "pregnant mice" and time of test substance administration

Female rats and male rats are caged in the same cage according to 1:1 (or 2:1). Observe the Yin suppository (or vaginal smear) every morning, and the day of finding out the Yin suppository (or sperm) is determined as the zero day of pregnancy. If no "fertilized rat" is found within 5 days, the female rat should be replaced. The detected "fertilized mice" are randomly divided into groups. During the 6-15 days of pregnancy, the test substance is given orally every day. Pregnant rats are weighed at 0, 6, 10, 15 and 20 days of pregnancy, and the amount of test substance is adjusted according to the weight.

5.4.2 Execution and general examination of pregnant rats

The rats are killed on the 20th day of pregnancy. The number of corpus luteum in the ovary is examined by laparotomy, the uterus is taken out and weighed; the number of live fetus, early absorption and stillbirth are examined.

5.4.3 Live fetal rat examination

Record the body weight, body length and tail length of fetal rats one by one, and check whether the appearance of fetal rats is abnormal, such as whether the head has encephalocele, exposed brain, small head, small ear, small eye, no eye and open eye, cleft lip and mandible, whether the trunk has abdominal wall crack, umbilical hernia, spinal curvature, whether the limbs have small limbs, short limbs, combined toes, multi toes, no toes and other deformities, whether the tail has short tail, curly tail, tailless tail and anal atresia.

5.4.4 胎鼠骨标本的制作与检查

将每窝 1/2 的活胎鼠放入 95%（V/V）乙醇中固定 2 周—3 周，取出胎仔（或可去皮、去内脏及脂肪）流水冲洗数分钟后放入 1g—2g/100mL 的氢氧化钾溶液内（至少 5 倍于胎仔体积）8h—72h，透明后放入茜素红应用液中染色 6h—48h，并轻摇 1—2 次/d，至头骨染红为宜。再放入透明液 A 中 1d—2d，放入透明液 B 中 2d—3d，待骨骼染红而软组织基本褪色后，可将标本放在甘油中保存。也可将胎鼠剥皮、去内脏及脂肪后，放入茜素红溶液染色，当天摇动玻璃瓶 2—3 次，待骨骼染成红色时为止。将胎鼠放入透明液 A 中 1—2 天，换到透明液 B 中 2—3 天。待胎鼠骨骼已染红，而软组织的紫红色基本褪色后，可将标本放在甘油中保存。（剥皮法）将标本放入小平皿中，用透射光源，在体视显微镜下作整体观察，然后逐步检查骨骼。测量囟门大小，矢状缝的宽度，头顶间骨及后头骨缺损情况，然后检查胸骨的数目，缺失或融合（胸骨为 6 个，骨化不全时首先缺第 5 胸骨、次为缺第 2 胸骨）。肋骨通常 12—13 对，常见畸形有融合肋、分叉肋、波状肋、短肋、多肋、缺肋、肋骨中断。

脊柱发育和椎体数目（颈椎 7 个，胸椎 12—13 个，腰椎 5—6 个，底椎 4 个，尾椎 3—5 个），有无融合、纵裂等。最后检查四肢骨。

5.4.4 Preparation and examination of fetal rat bone specimen

Put 1 / 2 of each litter of live fetal rats into 95% (V / V) ethanol for 2-3 weeks, take out the fetus (or remove skin, viscera and fat) and wash it with running water for several minutes, then put it into 1G-2G / 100ml potassium hydroxide solution (at least 5 times the fetal volume) for 8h-72h, put it into alizarin red application solution for 6h-48h after being transparent, and gently shake it for 1-2 times / D until the skull is dyed red. Then put it into transparent solution a for 1d-2d, and put it into transparent solution B for 2D-3D. After the bone is dyed red and the soft tissue is basically faded, the specimen can be stored in glycerin. The fetal rats can also be peeled, viscera and fat removed, and then put into alizarin red solution for staining. Shake the glass bottle for 2-3 times on the same day until the bones are dyed red. The fetal rats are put into the transparent liquid A for 1 to 2 days, and changed into the transparent liquid B for 2 to 3 days. When the skeleton of fetal mouse has been dyed red and the purplish red of soft tissue has basically faded, the specimen can be stored in glycerin.(peeling method) put the specimen into a small plate, use a transmission light source, observe the whole body under a stereomicroscope, and then gradually check the skeleton. Measure the size of the fontanelle, the width of the sagittal suture, the defect of the

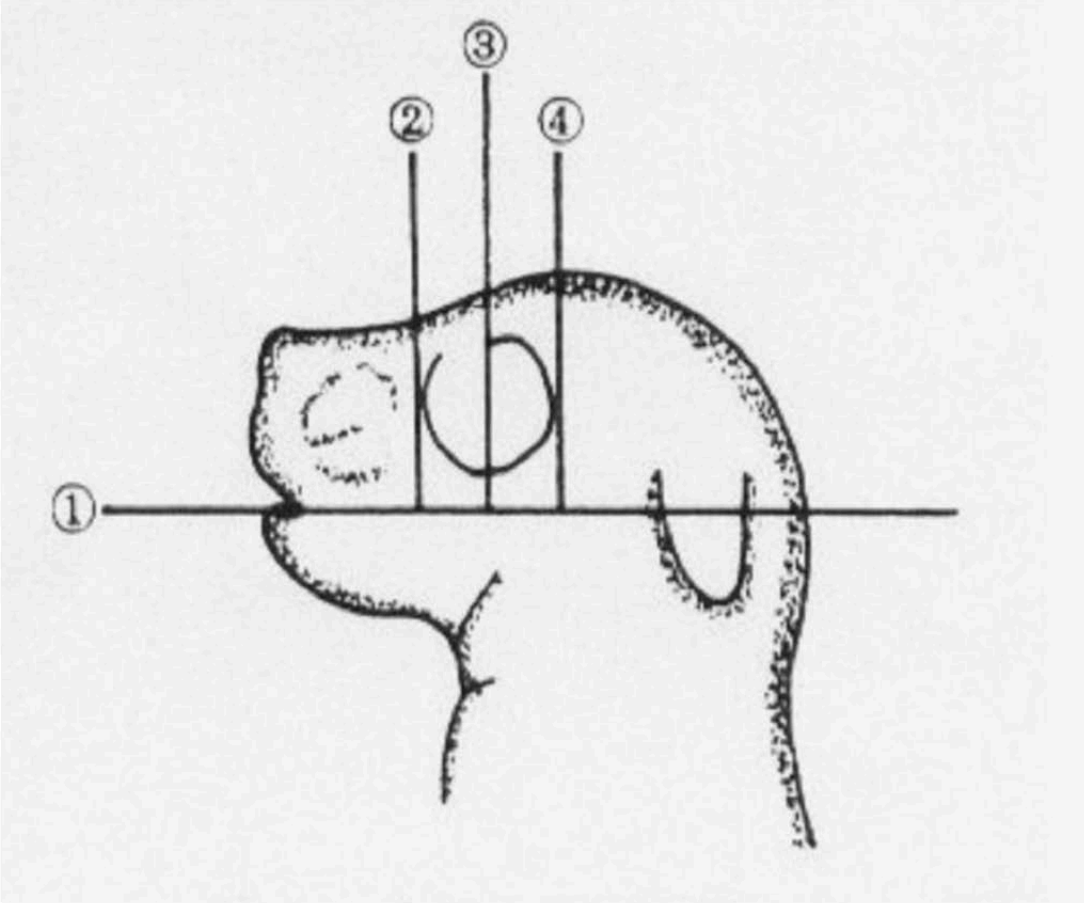
intercranial bone and the posterior skull, and then check the number of sternum, the defect or fusion (the number of sternum is 6, when the ossification is incomplete, the fifth sternum and the second sternum are missing first).rib

There are usually 12-13 pairs of bone, and the common deformities are fusion rib, bifurcate rib, undulate rib, short rib, multi rib, lack rib and rib interruption.

Spine development and number of vertebrae (7 cervical vertebrae, 12-13 thoracic vertebrae, 5-6 lumbar vertebrae, 4 bottom vertebrae, 3-5 tail vertebrae), fusion, longitudinal fissure, etc. Finally, examine the bone of limbs.

5.4.5 胎鼠内脏检查

每窝的 1/2 胎鼠放入 Bouins 液中，固定两周后作内脏检查。先用自来水冲去固定液，将鼠仰放在石蜡板上，剪去四肢和尾，用刀片从头部到尾部逐段横切或纵切。按不同部位的断面观察器官的大小、形状和相对位置。正常切面见图。



(1) 经口从舌与两口角向枕部横切（切面 1），观察大脑、间脑、延髓、舌及颚裂。

(2) 在眼前面作垂直纵切（切面 2），可见鼻部。

(3) 从头部垂直通过眼球中央作纵切（切面 3）。

(4) 沿头部最大横位处穿过作横切（切面 4）。

以上切面的目的可观察舌裂、颚裂、眼球畸形、脑和脑室异常。

(5) 沿下颚水平通过颈部中部作横切，可观察气管、食管和延脑或脊髓。

以后自腹中线剪开胸、腹腔，依次检查心、肺、横膈膜、肝、胃、肠等脏器的大小、位置，查毕将其摘除，再检查肾脏、输尿管、膀胱、子宫或睾丸位置及发育情况。然后将肾脏切开，观察有无肾盂积水与扩大。

5.4.5 Visceral examination of fetal rats

Each litter of 1 / 2 fetal rats is put into bouins solution and fixed for two weeks before visceral examination. First, flush out the fixing liquid with tap water, place the rat on the paraffin plate, cut off the limbs and tail, and use the blade to cut from the head to the tail section by section or lengthwise. The size, shape and relative position of organs are observed according to different sections. See figure for normal section.

(1) The brain, diencephalon, medulla oblongata, tongue and cleft jaw are observed.

(2) The nose can be seen by making a vertical longitudinal section (Section 2) in front of the eyes.

(3) The head is cut vertically through the center of the eyeball (Section 3).

(4) Cross cut (Section 4) along the maximum transverse position of the head.

The purpose of the above sections is to observe the cleft tongue, cleft jaw, malformation of eyeball, abnormality of brain and ventricle.

(5) The trachea, esophagus and medulla oblongata or spinal cord can be observed by transverse cutting along the middle part of the neck.

After that, cut the chest and abdomen from the midline of abdomen, check the size and position of heart, lung, diaphragm, liver, stomach, intestine and other organs in turn, remove them after checking, and then check the position and development of kidney, ureter, bladder, uterus or testis. Then the kidney is cut open to observe whether there is hydronephrosis and enlargement.

5.5 统计方法及结果评定

各种率的检查用 X2 检验，孕鼠增重用方差分析或非参数统计，胎鼠身长、体重、窝平均活胎数用 T 检验。结果应能得出受试物是否有母体毒性和胚胎毒性、致畸性，最好能得出最小致畸剂量。

为比较不同有害物质的致畸强度，可计算致畸指数，以致畸指数 10 以下为不致畸，10—100 为致畸，100 以上为强致畸。为表示有害物对人体危害的大小，可计算致畸危害指数，如指数大于 300 说明受试物对人危害小，100—300 为中等，小于 100 为危害大。

5.5 Statistical method and result evaluation

X2 test is used for the examination of various rates, ANOVA or nonparametric statistics are used for the increase of pregnant rats, and t test is used for the length, weight and average number of live fetuses.Results it should be able to find out whether the test substance has maternal toxicity, embryotoxicity and teratogenicity, and it is best to get the minimum teratogenicity dose.

In order to compare the teratogenic intensity of different harmful substances, the teratogenic index can be calculated. The teratogenic index below 10 is non-teratogenic, 10-100 is teratogenic, and over 100 is strongly teratogenic. In order to indicate the harm of harmful substances to human body, teratogenic harm index can be calculated. If the

index is greater than 300, the harm of the tested substance to human body is small, 100-300 is medium, and less than 100 is great.

$$\text{致畸指数} = \frac{\text{雌鼠}LD_{50}}{\text{最小致畸剂量}}$$

$$\text{致畸危害指数} = \frac{\text{最大不致畸剂量}}{\text{最大可能摄入量}}$$

Teratogenicity index= **Female LD50/** Minimum teratogenic dose

Teratogenic hazard index = Maximum non teratogenic dose/ Maximum possible intake dose

17 慢性毒性/致癌性结合试验

Combined Chronic Toxicity / Carcinogenicity Test

1 范围

本规范规定了动物慢性毒性/致癌性结合试验的基本原则、要求和方法。本规范适用于化妆品原料的慢性毒性和致癌性的检测。

17 Combined Chronic toxicity / carcinogenicity combination test

1 Range

This specification specifies the basic principles, requirements and methods of animal chronic toxicity / carcinogenicity combined test. This specification is applicable to the detection of chronic toxicity and carcinogenicity of cosmetic raw materials.

2 定义

2.1 慢性毒性 chronic toxicity

动物在正常生命期的大部分时间内接触受试物所引起的不良反应。

2.2 未观察到有害作用的剂量水平 no observed adverse effect level(NOAEL)

在规定的试验条件下，用现有的技术手段或检测指标未观察到任何与受试物有关的毒性作用的最大剂量。

2.3 观察到有害作用的最低剂量水平 lowest observed adverse effect level(LOAEL)

在规定的试验条件下，受试物引起实验动物组织形态、功能、生长发育等有害效应的最低剂量。

2.4 靶器官 target organ

实验动物出现由受试物引起的明显毒性作用的器官。

2.5 化学致癌物 chemical carcinogen

能引起肿瘤，或使肿瘤发生率增加的化学物。

2 Definition

2.1 Chronic toxicity

Adverse reactions caused by exposure of animals to the test substance for most of their normal life.

2.2 No observed adverse effect level (NOAEL)

Under the specified test conditions, no maximum dose of toxic effect related to the test substance is observed by using the existing technical means or detection indicators.

2.3 Lowest observed adverse effect level (LOAEL)

Under the specified test conditions, the lowest dose of test substance causing harmful effects such as tissue morphology, function, growth and development of experimental animals.

2.4 Target organ

The organs of experimental animals with obvious toxic effects caused by the test substance.

2.5 Chemical carcinogen

A chemical that causes or increases the incidence of tumors.

3 原理

化学物质在体内的蓄积作用，是发生慢性中毒的基础。慢性毒性试验是使动物长期地以一定方式接触受试物引起的毒性反应的试验。

当某种化学物质经短期筛选试验证明具有潜在致癌性，或其化学结构与某种已知致癌剂十分相近时，而此化学物质有一定实际应用价值时，就需用致癌性试验进一步验证。动物致癌性试验为人体长期接触该物质是否引起肿瘤的可能性提供资料。

3 principle

The accumulation of chemicals in the body is the basis of chronic poisoning. Chronic toxicity test is a test to make animals contact with the test substance in a certain way for a long time.

When a chemical substance is proved to have potential carcinogenicity by short-term screening test, or its chemical structure is very similar to that of a known carcinogen, and the chemical substance has certain practical application value, it needs to be further verified by carcinogenicity test. The animal carcinogenicity test provides data for the possibility of human body's long-term exposure to the substance to cause tumor.

4 试验的基本原则

在实验动物的大部分生命期间将受试化学物质以一定方式染毒，观察动物的中毒表现，并进行生化指标、血液学指标、病理组织学等检查，以阐明此化学物质的慢性毒性。

将受试化学物质以一定方式处理动物，在该动物的大部分或整个生命期间及死后检查肿瘤出现的数量、类型、发生部位及发生时间，与对照动物相比以阐明此化学物质有无致癌性。

4 Basic principles of test

During most of the life of experimental animals, the tested chemicals are poisoned in a certain way to observe the poisoning performance of animals, and the biochemical indexes, hematological indexes, histopathology and other tests are carried out to clarify the chronic toxicity of the chemicals.

The number, type, location and time of tumor occurrence are examined during most or the whole life of the animal and after death. Compared with the control animal, the carcinogenicity of the chemical substance is clarified.

5 实验动物和饲养环境

5.1 动物种类和品系的选择

为选择合适的动物（种类和品系），应该进行有关的急性、亚急性和毒物动力学试验。在评价致癌性时常用小鼠和大鼠，而进行慢性毒性试验常用大鼠和狗。

对慢性毒性/致癌性结合试验，一般均采用大鼠，但这并不排斥使用其他种类。所选用的品系应是对该类受试物的致癌和毒性作用敏感的。

5.2 性别和实验开始时的年龄

两种性别都应该使用，最常使用刚断奶或已断奶的年幼动物来进行慢性毒性和致癌性的长期生物学试验。

在啮齿类动物断奶和适应环境之后要尽快开始试验，最好在 6 周龄之前。

5 Laboratory animals and feeding environment

5.1 Selection of animal species and strains

In order to select suitable animals (species and strains), the related acute, subacute and toxicokinetic tests should be carried out. Mice and rats are commonly used in the evaluation of carcinogenicity, while rats and dogs are commonly used in the chronic toxicity test.

For chronic toxicity/carcinogenicity combination tests, rats are generally used, but this does not exclude the use of other types. The selected strain should be sensitive to carcinogenic and toxic effects of the tested substance.

5.2 Gender and age at the beginning of the experiment

The two genders should be used, and young animals just weaned or weaned are most often used for long-term biological tests of chronic toxicity and carcinogenicity.

Start the experiment as soon as possible after the rodents are weaned and acclimated, preferably before the age of 6 weeks.

5.3 实验组的动物数

应保证试验结果的可靠性并能进行统计学处理，实验组和对照组动物，应采用随机分配的方法。

每组都应有足够的动物数用来进行详细的生物学和统计学分析。

每一个剂量组和相应的对照组至少应该有 50 只雄性和 50 只雌性的动物，不包括提前剖杀的动物数。如需观察肿瘤以外的病理变化可设附加剂量组，两种性别各 20 只动物，其相应的对照组两种性别各 10 只动物。

5.4 动物的管理、饲料和饮水

必须严格的控制环境条件和合理的动物管理措施。实验动物及实验动物房应符合国家相应规定。

5.3 Number of animals in the experimental group

The reliability of the test results should be guaranteed and statistical treatment can be carried out. The animals in the experimental group and the control group should be randomly assigned.

Each group should have enough animals for detailed biological and statistical analysis.

There should be at least 50 males and 50 females in each dose group and corresponding control group, excluding the number of animals killed in advance. If pathological changes other than tumors need to be observed, additional dose groups can be set up, with 20 animals of each sex and 10 animals of each sex in the corresponding control group.

5.4 Animal management, feed and drinking water must be strictly control environmental conditions and take reasonable animal management measures. The laboratory animal and laboratory animal room shall comply with the relevant national regulations.

6 剂量组和给受试物的频率

为了评价致癌性试验，至少要设三个剂量组的实验组及一个相应的对照组。高剂量组可以出现某些较轻的毒性反应，但不能明显缩短动物寿命。这些毒性反应可能表现在血清酶水平的改变，或体重增加受到轻度抑制（低于 10%）。

低剂量不能引起任何毒性反应，应不影响动物的正常生长、发育和寿命。一般不应低于高剂量的 10%。

中剂量应界于高剂量和低剂量之间，可根据化学物的毒代动力学性质来确定。

结合慢性毒性试验，应附加一个实验组和相应的对照组。最高剂量应能产生明显的毒性。一般每天给予受试物。如果所给的化学物质是混在饮水中或饲料中，应保证连续给予。给受试物的频率也可以按其毒代动力学变化进行调整。

应设相应的对照组，除不接触受试物外，其他条件应和实验组相同。

6 Dose group and frequency of test substance administration

In order to evaluate the carcinogenicity test, at least three dose groups and a corresponding control group should be set up. In the high dose group, there are some mild toxic reactions, but the life span of animals could not be shortened obviously. These toxic reactions may be manifested in changes in serum enzyme levels or mild inhibition of weight gain (less than 10%).

Low dose can not cause any toxic reaction, and it should not affect the normal growth, development and life span of animals. Generally, it should not be less than 10% of the high dose.

The middle dose should be between high dose and low dose, which can be determined according to the toxicokinetic properties of chemicals.

Combined with chronic toxicity test, an experimental group and corresponding control group should be added. The highest dose should produce obvious toxicity. Subjects are generally given daily. If the given chemicals are mixed in drinking water or feed, continuous administration shall be ensured. The frequency of administration of the test substance can also be adjusted according to its toxicity kinetics.

A corresponding control group shall be set up, and the other conditions shall be the same as that of the experimental group except that the subjects are not contacted.

7 给受试物的途径

经口，经皮，吸入是三种主要给受试物途径。选择何种途径要根据受试物的理化特性和对人有代表性的接触方式。

给受试物的频率按所选择的给予途径和方式可以有所不同，如有可能，应按照受试物的毒代动力学变化进行调整。

7.1 经口染毒

如果受试物是通过胃肠道吸收的则最好选用经口途径。按试验期限（9）中指明的试验期限，把受试物混入饲料中、溶于饮水中，或用管饲法连续给予动物。每周 7 天均给予受试物，中断染毒可使动物得到恢复或毒性缓解，从而影响结果及以后的评价。

7.2 经皮染毒

选择皮肤接触方式是用于模仿人接触有关物质的一个主要途径，并作为诱发皮肤病变的试验模型。有关诱导皮肤肿瘤的特殊试验在本方法中不作介绍。

7.3 吸入染毒

吸入方式不是化妆品主要接触途径，因此吸入染毒本方法不作介绍。

7 Approach to test substance

Oral, dermal and inhalation are the three main ways to give the test substance. Which way to choose should be based on the physical and chemical characteristics of the test object and the representative contact way to people.

The frequency of administration of the test substance can be different according to the chosen administration route and method, and if possible, it should be adjusted according to the toxicity dynamics of the test substance.

7.1 Oral poisoning

If the test substance is absorbed through gastrointestinal tract, oral route is the best choice. According to the test period specified in test period (9), the test substance shall be mixed into feed, dissolved in drinking water, or continuously given to animals by tube feeding method. The test substance is given 7 days a week. Discontinuation of exposure could restore or alleviate the toxicity of the animal, thus affecting the results and subsequent evaluation.

7.2 Transdermal poisoning

The choice of skin contact mode is a main way to imitate human contact with related substances, and it is used as the experimental model to induce skin lesions. Special tests for the induction of skin tumors are not described in this method.

7.3 Inhalation exposure

Inhalation is not the main contact way of cosmetics, so this method is not introduced.

8 试验期限

在附加组中 20 只实验动物/每性别和 10 只相应对照组动物/每性别至少应该维持到 12 个月。这些动物的剖杀，应是用于评价和受试物有关的，但并非老年性改变所导致的病理变化。致癌性试验的期限必须包括受试物正常生命期的大部分时间。确定试验期限的几条准则：

（1）一般情况下，试验结束时间对小鼠和仓鼠应在 18 个月，大鼠在 24 个月；然而对某些生命期较长的或自发肿瘤率低的动物品系，小鼠和仓鼠可在 24 个月，大鼠可在 30 个月。

（2）当最低剂量和对照组存活动物只有 25%时，也可以结束试验。对于有明显性别差异的试验，则其结束的时间对不同的性别应有所不同。在某种情况下因明显的毒性作用只造成高剂量组动物过早死亡，此时不应结束试验。

8 Test period

In the additional group, 20 experimental animals/each sex and 10 corresponding control animals/each sex should be maintained for at least 12 months. The necropsy of these animals should be used to evaluate the pathological changes related to the test object, but not caused by senile changes. The duration of carcinogenicity test must include most of the normal life of the subject. Several Criteria for Determining Test Duration:

(1) In general, the end time of the test should be 18 months for mice and hamsters and 24 months for rats; However, for some animal strains with long life span or low spontaneous tumor rate, mice and hamsters can be within 24 months and rats can be within 30 months.

(2) When only 25% of the animals in the lowest dose and control group survived, the experiment could also be ended. For experiments with significant gender differences, the end time should be different for different genders. In some cases, the high dose group animals died prematurely due to the obvious toxic effect. At this time, the test should not be ended.

9 临床观察和检查

9.1 观察

至少每天进行一次动物情况的检查。每天还应有数次有目的的观察，如剖检死亡动物或存入冰箱，将有病或垂死的动物分开或处死。及时发现所有的毒性作用的开始及其变化，并能减少因疾病、自溶或被同类所食造成的动物损失。

详细记录动物的症状包括神经系统和眼睛的改变，可疑肿瘤在内的所有毒性作用出现和变化的时间，以及死亡情况。

在试验的前 13 周内，每周称量体重一次，以后每 4 周称量一次。在试验的前 13 周内，每周检查一次动物的食物摄取情况，以后如动物健康状况或体重无异常改变，则每 3 个月检查一次。

9 Clinical observation and examination

9.1 observation

Check the condition of animals at least once a day. There should be several times of purposeful observation every day, such as dissecting dead animals or storing them in the refrigerator, separating or killing sick or dying animals. To detect the beginning and change of all toxic effects in time, and reduce the loss of animals caused by diseases, autolysis or being eaten by the same kind.

Detailed records of animal symptoms including changes in the nervous system and eyes, the time of occurrence and change of all toxic effects including suspicious tumors, and death are made.

During the first 13 weeks of the test, the body weight was weighed once a week and every 4 weeks thereafter. During the first 13 weeks of the experiment, the food intake of the animals was checked once a week, and thereafter every 3 months if there was no abnormal change in the animal's health or body weight.

9.2 血液学检查

血液学检查（血红蛋白含量，血球压积，红血球计数，白血球计数，血小板，或其他血凝试验）应在 3 个月，6 个月，以后每隔 6 个月及实验结束时进行，各组每个性别要检查 20 只大鼠。每次采集的血标本应来自相同的大鼠。最高剂量组和对照组大鼠应在同样的时间间隔内进行白血球分类计数，中等剂量组大鼠只是在必要时才做。

在试验期间，如果大体观察表明动物健康恶化，应对有关动物进行血球分类计数检查。高剂量和对照组动物要进行血球分类计数。如两组间有很大差异时，应对较低剂量组的动物进行血球分类计数。

9.2 Hematology examination

Hematological examination (hemoglobin content, hematocrit, red blood cell count, white blood cell count, platelet, or other hemagglutination test) should be carried out in 3 months, 6 months, and then every 6 months and at the end of the experiment. 20 rats of each sex in each group should be examined. Each blood sample collected should be from the same rat. The rats in the highest dose group and the control group should be counted in the same time interval, and the rats in the middle dose group should only do it when necessary.

During the test, if the general observation shows that the health of the animals has deteriorated, the relevant animals shall be checked for blood cell classification and counting. The blood cells of high dose and control group should be classified and counted. If there is a big difference between the two groups, the blood cells in the lower dose group should be classified and counted.

9.3 尿分析

收集各组每性别 10 只大鼠尿样进行分析，最好是在做血液检查的同时并取自同一大鼠。应测下列指标，可单个进行，也可每组相同性别的尿标本混在一起测定。

分析指标：外观；每个动物的尿量和比重；蛋白，糖，酮体，潜血（半定量）；沉淀物镜检（半定量）。

9.3 Urinalysis

The urine samples of 10 rats of each sex in each group are collected for analysis, preferably at the same time of blood examination and taken from the same rat. The following indicators should be measured, either individually or by mixing urine samples of the same sex in each group.

Analysis indicators: appearance; urine volume and specific gravity of each animal; protein, sugar, ketone body, occult blood (semi quantitative); precipitation objective microscopy (semi quantitative).

9.4 临床化学

每 6 个月及实验结束时，收集各组每性别的 10 只大鼠的血液标本进行临床化学检查，尽可能在各个时间间隔内采取相同的大鼠血标本。分离血浆，进行下列指标测定：

总蛋白浓度；白蛋白浓度；肝功能试验（如碱性磷酸酶，谷丙转氨酶，谷草转氨酶，γ 谷氨酰转肽酶，鸟氨酸脱羧酶）；糖代谢，如糖耐量；肾功能，如血尿素氮。

9.4 Clinical chemistry

Every 6 months and at the end of the experiment, blood samples of 10 rats of each sex in each group are collected for clinical chemical examination, and the same blood samples of rats are taken as far as possible in each time interval. Plasma is separated and the following indexes are determined:

Total protein concentration; albumin concentration; liver function test (such as alkaline phosphatase, glutamic pyruvic transaminase, glutamic oxaloacetylase, gamma glutamyltranspeptidase, ornithine decarboxylase); glucose metabolism, such as glucose tolerance; renal function, such as blood urea nitrogen.

9.5 病理检查

肉眼和病理检查常常是慢性/致癌性结合试验的基础。

9.5.1 肉眼剖检

所有的动物包括那些在实验过程中死亡或因处于垂死状态而被处死的，应进行肉眼检查。在所有动物被处死前，应收集血样品进行血球分类计数。保存所有肉眼可见的肿瘤或可疑为肿瘤的。所有的器官或组织都应保留以进行镜下检查。一般包括下列器官和组织：

脑* （髓/脑桥，小脑皮质，大脑皮质），垂体，甲状腺（包括甲状旁腺），胸腺，肺（包括气管），心脏，唾液腺，肝*，脾，肾*，肾上腺*，食管，胃，十二指

肠，空肠，回肠，盲肠，结肠，直肠，膀胱，淋巴结，胰腺，性腺*，生殖附属器官，乳腺，皮肤，肌肉，外周神经，脊髓（颈，胸，腰），胸骨或股骨（包括关节）和眼。肺和膀胱用固定剂填充能更好地保存组织。

9.5 Pathological examination

Macroscopic and pathological examination are often the basis of the chronic / carcinogenic combination test.

9.5.1 Visual sectioning

All animals, including those who died in the course of the experiment or are put to death due to being in a dying state, should be examined with naked eyes. Blood samples should be collected for blood cell count before all animals are killed. Preserve all visible or suspected tumors. All organs or tissues should be preserved for microscopic examination. It generally includes the following organs and tissues:

Brain * (medulla/pontine, cerebellar cortex, cerebral cortex), pituitary gland, thyroid gland (including parathyroid gland), thymus gland, lung (including trachea), heart, salivary gland, liver *, spleen, kidney *, adrenal gland *, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, bladder, lymph node, pancreas, gonad *, reproductive accessory organs, breast, skin, muscle, peripheral nerve, spinal cord (neck, chest, waist), sternum or femur (including joint) and eye. Filling lung and bladder with fixative can better preserve tissue.

9.5.2 组织病理检查

所有肉眼可见的肿瘤和其他病变都应进行病理检查。此外还要注意下列方面：

（1）对所有保存的器官和组织进行镜下检查，详细描述发现的所有病变。

①包括实验过程中死亡或处死的动物。

②所有最高剂量组和对照组动物。

（2）在较低剂量组，由受试物引起或可能由受试物引起异常的器官或组织也应进行检查。

*啮齿动物每组每性别 10 只，非啮齿动物全部标有*号的器官包括甲状腺及甲状旁腺都应称重。

9.5.2 Histopathological examination

All visible tumors and other lesions should be examined by pathology. In addition, pay attention to the following aspects:

(1) All preserved organs and tissues are examined under microscope, and all lesions found are described in detail.

① Including animals that died or are executed during the experiment.

② All animals in the highest dose group and the control group.

(2) In the lower dose group, organs or tissues caused by or possibly caused by the test substance should also be examined.

*There are 10 rodents of each sex in each group. All organs marked with * in non rodents, including thyroid gland and parathyroid gland, should be weighed.

10 数据处理和结果评价

可通过表格形式总结试验结果，显示试验各时段各组动物数、出现病变的动物数、病变类型等。对所有数据应采用适当、合理的统计学方法进行评价，统计学方法应在试验设计时确定。

慢性毒性与致癌合并试验应结合前期试验结果，并考虑到毒性效应指标和解剖及组织病理学检查结果进行综合评价。结果评价应包括受试物慢性毒性的表现、剂量-反应关系、靶器官、可逆性，得出慢性毒性相应的 NOAEL 和（或）LOAEL。

10.1 肿瘤发生率

肿瘤的发生率是整个实验终了时患瘤动物总数在有效动物总数中所占的百分率。有效动物总数指最早出现肿瘤时的存活动物总数。必要时根据试验中动物死亡率来调整计算致癌率，计算方法可参考有关文献。

10 Data processing and result evaluation

The results of the test can be summarized in the form of tables, showing the number of animals in each group, the number of animals with pathological changes and the types of pathological changes in each period of the test.All data should be evaluated with appropriate and reasonable statistical methods, which should be determined at the time of test design.

The combined test of chronic toxicity and carcinogenesis should be combined with the results of previous tests, and the toxicity index, anatomical and histopathological examination results should be taken into account for comprehensive evaluation. Results the evaluation should include the performance of chronic toxicity, dose-response relationship, target organ, reversibility, and the NOAEL and / or LOAEL corresponding to chronic toxicity.

10.1 Tumor incidence

The incidence of tumor is the percentage of the total number of animals with tumor in the total number of effective animals at the end of the whole experiment. The total number of effective animals refers to the total number of living animals at the time of the earliest occurrence of tumor. If necessary, the carcinogenic rate should be adjusted according to the animal mortality in the experiment. The calculation method can refer to the relevant literature.

$$\text{肿瘤发生率} = \frac{\text{试验结束时患瘤动物总数}}{\text{有效动物总数}} \times 100\%$$

Tumor incidence rate= The total number of animals suffering from tumor at the end of the experiment/ Total number of animals valid x 100%

10.2 致癌试验阳性的判断标准

采用世界卫生组织提出的四条判断诱癌试验阳性的标准：

（1）肿瘤只发生在染毒组动物中，对照组无该类型肿瘤；

（2）染毒组与对照组动物均发生肿瘤，但剂量组发生率明显增高；

（3）染毒组动物中多发性肿瘤明显，对照组中无多发性肿瘤或只少数动物有多发性肿瘤；

（4）染毒组与对照组动物肿瘤的发生率无显著性差异，但染毒组中肿瘤发现的时间较早。

上述四条中，试验组与对照组之间的数据经统计学处理后任何一条有显著性差异即可认为该受试物的致癌试验为阳性结果。染毒组和对照组肿瘤发生率差别不明显，但癌前病变差别显著时，不能轻易否定受试物的致癌性。

10.3 致癌试验阴性结果的确立

假如动物实验的规模为两种种属、两种性别，至少 3 个剂量水平，其中一个接近最大耐受剂量，每组动物数至少 50 只，实验组肿瘤发生率与对照组无差异，才算阴性结果。

10.2 Criteria for positive carcinogenesis test

Four criteria proposed by the World Health Organization are used to judge the positive results of cancer induction test：

(1) The tumor only occurred in the animals in the exposure group, but not in the control group;

(2) The incidence of tumor in the dose group is significantly higher than that in the control group;

(3) In the control group, there are no multiple tumors or only a few animals had multiple tumors;

(4) There is no significant difference in the incidence of tumor between the treated group and the control group, but the tumor is found earlier in the treated group.

Among the above four items, if there is significant difference between the data of the test group and the control group after statistical processing, it can be considered that the carcinogenic test of the test substance is a positive result. The difference of tumor incidence between the exposure group and the control group is not obvious, but when the difference of precancerous lesions is significant, the carcinogenicity of the test substance could not be easily denied.

10.3 Establishment of negative result of carcinogenic test

If the scale of animal experiment is two genera, two genders, at least three dose levels, one of which is close to the maximum tolerance

The number of animals in each group is at least 50, and the incidence of tumor in the experimental group is no different from that in the control group, so the negative result is calculated.

EXHIBIT 4

No.	Product Name	Manufacturing Company	Registered Agent	Approval Date	NMPA Approval Number
1	Paula's Choice 2% Beta Hydroxy Acid Gel, All Skin Types	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	12/22/2009	J20098895
2	Paula's Choice Hydralight One Step Face Cleanser, Normal to Oily/ Combination Skin	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	01/05/2010	J20100065
3	Paula's Choice Skin Balancing Toner, Normal to Oily/Combination skin	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	01/05/2010	J20100068
4	Paula's Choice Skin Recovery Hydrating Treatment Mask, Normal to Very Dry Skin	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	01/05/2010	J20100067
5	Paula's Choice Hydralight Moisture-Infusing Lotion	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	12/26/2011	J20114917
6	Paula's Choice Clear Anti-Redness Exfoliating Solution	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	01/19/2012	J20120594
7	Paula's Choice Skin Balancing Invisible Finish Moisture Gel	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	01/19/2012	J20120590
8	Paula's Choice Resist Daily Smoothing Treatment	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	03/29/2012	J20122091
9	Paula's Choice Skin Balancing Oil-Absorbing Mask	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	03/29/2012	J20122088
10	Paula's Choice Clear Pore Normalizing Cleanser	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	06/07/2012	J20124133
11	Paula's Choice Skin Balancing Oil-Reducing Cleanser	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	06/07/2012	J20123858
12	Paula's Choice Skin Balancing Super Antioxidant Concentrate Serum	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	06/07/2012	J20124167
13	Paula's Choice Skin Perfecting 2% BHA Liquid	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	06/07/2012	J20124128

No.	Product Name	Manufacturing Company	Registered Agent	Approval Date	NMPA Approval Number
14	Paula's Choice Resist Anti-Aging Clear Skin Hydrator	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	07/04/2012	J20124587
15	Paula's Choice Resist Optimal Results Hydrating Cleanser	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	07/04/2012	J20124515
16	Paula's Choice Resist Weightless Body Treatment 2% BHA	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	07/04/2012	J20124626
17	Paula's Choice Shine Stopper Instant Matte Finish	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	07/04/2012	J20124593
18	Paula's Choice Resist Advanced Replenishing Toner	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	06/17/2013	J20135360
19	Paula's Choice Moisture Boost Hydrating Treatment Cream	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	09/17/2013	J20139348
20	Paula's Choice Resist Barrier Repair Moisturizer	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	09/17/2013	J20139368
21	Paula's Choice Resist Super Antioxidant Concentrate Serum	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	09/17/2013	J20139362
22	Paula's Choice Skin Recovery Enriched Calming Toner	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	09/17/2013	J20139342
23	Paula's Choice Skin Recovery Replenishing Moisturizer	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	09/17/2013	J20139343
24	Paula's Choice Hydralight One Step Face Cleanser, Normal to Oily/Combination Skin	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	01/04/2014	J20100065
25	Paula's Choice Skin Balancing Toner, Normal to Oily/ Combination Skin	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	01/04/2014	J20100068
26	Paula's Choice 2% Beta Hydroxy Acid Gel, All Skin Types	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/21/2014	J20098895

No.	Product Name	Manufacturing Company	Registered Agent	Approval Date	NMPA Approval Number
27	Paula's Choice Skin Recovery Hydrating Treatment Mask, Normal to Very Dry Skin	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/21/2014	J20100067
28	Paula's Choice Earth Sourced Antioxidant-Enriched Natural Moisturizer	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	08/26/2015	J20156735
29	Paula's Choice Earth Sourced Perfectly Natural Cleansing Gel	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	08/26/2015	J20156734
30	Paula's Choice Earth Sourced Purely Natural Refreshing Toner	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	08/26/2015	J20156736
31	Paula's Choice Resist Intensive Wrinkle-Repair Retinol Serum	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	08/26/2015	J20156733
32	Paula's Choice Barely There Sheer Matte Tint SPF 30 (special use)	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	07/18/2016	J20160836
33	Paula's Choice Clear Anti-Redness Exfoliating Solution	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	10/27/2016	J201610526
34	Paula's Choice Clear Pore Normalizing Cleanser	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	10/27/2016	J201610528
35	Paula's Choice Skin Balancing Oil-Reducing Cleanser	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	10/27/2016	J201610520
36	Paula's Choice Skin Perfecting 2% BHA Liquid	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	10/27/2016	J201610527
37	Paula's Choice Resist Daily Smoothing Treatment	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	04/27/2017	J20174514
38	Paula's Choice Resist Anti-Aging Clear Skin Hydrator	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	06/14/2017	J20124587
9	Paula's Choice Shine Stopper Instant Matte Finish	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	06/14/2017	J20124593

No.	Product Name	Manufacturing Company	Registered Agent	Approval Date	NMPA Approval Number
40	Paula's Choice Resist Optimal Results Hydrating Cleanser	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	09/30/2017	J20124515
41	Paula's Choice Resist Weightless Body Treatment 2% BHA	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	09/30/2017	J20124626
42	Paula's Choice Skin Balancing Super Antioxidant Concentrate Serum	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	11/28/2017	J201712149
43	Paula's Choice Moisture Boost Hydrating Treatment Cream	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	12/18/2017	J20139348
44	Paula's Choice Resist Barrier Repair Moisturizer	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	12/18/2017	J20139368
45	Paula's Choice Skin Recovery Enriched Calming Toner	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	12/18/2017	J20139342
46	Paula's Choice Resist Advanced Replenishing Toner	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	12/20/2018	J201816357
47	Paula's Choice Resist Super Antioxidant Concentrate Serum	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	12/20/2018	J201816358
48	Paula's Choice Skin Recovery Replenishing Moisturizer	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	12/20/2018	J201815963
49	Paula's Choice Resist Pure Radiance Skin Brightening Treatment	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	03/08/2019	J20190706
50	Paula's Choice 2% Beta Hydroxy Acid Gel, All Skin Types	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	03/19/2019	J20098895
51	Paula's Choice Skin Recovery Hydrating Treatment Mask, Normal to Very Dry Skin	Paula's Choice, Inc.	Shanghai Yingwen Economic and Trade Co. Ltd.	04/04/2019	J20100067
52	Paula's Choice Perfectly Natural Cleansing Gel	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	04/26/2020	2020002901

No.	Product Name	Manufacturing Company	Registered Agent	Approval Date	NMPA Approval Number
53	Paula's Choice 10% Azelaic Acid Booster	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/13/2020	2020003505
54	Paula's Choice Antioxidant-Enriched Natural Moisturizer	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/13/2020	2020003508
55	Paula's Choice Clinical 1% Retinol Treatment	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/13/2020	2020003506
56	Paula's Choice Earth Sourced Power Berry Serum	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/13/2020	2020003503
57	Paula's Choice Intensive Wrinkle-Repair Retinol Serum	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/13/2020	2020003509
58	Paula's Choice Invisible Finish Moisture Gel	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/13/2020	2020003504
59	Paula's Choice Purely Natural Refreshing Toner	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/13/2020	2020003507
60	Paula's Choice Skin Balancing Pore-Reducing Toner	Shanghai Yingwen Economic and Trade Co. Ltd.	Shanghai Yingwen Economic and Trade Co. Ltd.	05/13/2020	2020003511

JS 44 (Rev. 04/21)

CIVIL COVER SHEET

The JS 44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. *(SEE INSTRUCTIONS ON NEXT PAGE OF THIS FORM.)*

I. (a) PLAINTIFFS

JESSE VARGISON and RACHAEL FORBIS

(b) County of Residence of First Listed Plaintiff King County
(EXCEPT IN U.S. PLAINTIFF CASES)

(c) Attorneys *(Firm Name, Address, and Telephone Number)*

Hagens Berman Sobol Shapiro LLP, 1301 Second Ave., Suite 2000, Seattle, WA 98101; (206) 623-7292

DEFENDANTS

PAULA'S CHOICE, LLC, a Washington Limited Liability Company

County of Residence of First Listed Defendant
(IN U.S. PLAINTIFF CASES ONLY)

NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE TRACT OF LAND INVOLVED.

Attorneys *(If Known)*

II. BASIS OF JURISDICTION *(Place an "X" in One Box Only)*

- ☐ 1 U.S. Government Plaintiff
- ☐ 3 Federal Question *(U.S. Government Not a Party)*
- ☐ 2 U.S. Government Defendant
- ☒ 4 Diversity *(Indicate Citizenship of Parties in Item III)*

III. CITIZENSHIP OF PRINCIPAL PARTIES *(Place an "X" in One Box for Plaintiff and One Box for Defendant)*

(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	☐ 1	☐ 1	Incorporated *or* Principal Place of Business In This State	☐ 4	☒ 4
Citizen of Another State	☒ 2	☐ 2	Incorporated *and* Principal Place of Business In Another State	☐ 5	☐ 5
Citizen or Subject of a Foreign Country	☐ 3	☐ 3	Foreign Nation	☐ 6	☐ 6

IV. NATURE OF SUIT *(Place an "X" in One Box Only)*

Click here for: Nature of Suit Code Descriptions.

CONTRACT
- ☐ 110 Insurance
- ☐ 120 Marine
- ☐ 130 Miller Act
- ☐ 140 Negotiable Instrument
- ☐ 150 Recovery of Overpayment & Enforcement of Judgment
- ☐ 151 Medicare Act
- ☐ 152 Recovery of Defaulted Student Loans (Excludes Veterans)
- ☐ 153 Recovery of Overpayment of Veteran's Benefits
- ☐ 160 Stockholders' Suits
- ☒ 190 Other Contract
- ☐ 195 Contract Product Liability
- ☐ 196 Franchise

TORTS

PERSONAL INJURY
- ☐ 310 Airplane
- ☐ 315 Airplane Product Liability
- ☐ 320 Assault, Libel & Slander
- ☐ 330 Federal Employers' Liability
- ☐ 340 Marine
- ☐ 345 Marine Product Liability
- ☐ 350 Motor Vehicle
- ☐ 355 Motor Vehicle Product Liability
- ☐ 360 Other Personal Injury
- ☐ 362 Personal Injury - Medical Malpractice

PERSONAL INJURY
- ☐ 365 Personal Injury - Product Liability
- ☐ 367 Health Care/ Pharmaceutical Personal Injury Product Liability
- ☐ 368 Asbestos Personal Injury Product Liability

PERSONAL PROPERTY
- ☐ 370 Other Fraud
- ☐ 371 Truth in Lending
- ☐ 380 Other Personal Property Damage
- ☐ 385 Property Damage Product Liability

FORFEITURE/PENALTY
- ☐ 625 Drug Related Seizure of Property 21 USC 881
- ☐ 690 Other

LABOR
- ☐ 710 Fair Labor Standards Act
- ☐ 720 Labor/Management Relations
- ☐ 740 Railway Labor Act
- ☐ 751 Family and Medical Leave Act
- ☐ 790 Other Labor Litigation
- ☐ 791 Employee Retirement Income Security Act

IMMIGRATION
- ☐ 462 Naturalization Application
- ☐ 465 Other Immigration Actions

BANKRUPTCY
- ☐ 422 Appeal 28 USC 158
- ☐ 423 Withdrawal 28 USC 157

INTELLECTUAL PROPERTY RIGHTS
- ☐ 820 Copyrights
- ☐ 830 Patent
- ☐ 835 Patent - Abbreviated New Drug Application
- ☐ 840 Trademark
- ☐ 880 Defend Trade Secrets Act of 2016

SOCIAL SECURITY
- ☐ 861 HIA (1395ff)
- ☐ 862 Black Lung (923)
- ☐ 863 DIWC/DIWW (405(g))
- ☐ 864 SSID Title XVI
- ☐ 865 RSI (405(g))

FEDERAL TAX SUITS
- ☐ 870 Taxes (U.S. Plaintiff or Defendant)
- ☐ 871 IRS—Third Party 26 USC 7609

OTHER STATUTES
- ☐ 375 False Claims Act
- ☐ 376 Qui Tam (31 USC 3729(a))
- ☐ 400 State Reapportionment
- ☐ 410 Antitrust
- ☐ 430 Banks and Banking
- ☐ 450 Commerce
- ☐ 460 Deportation
- ☐ 470 Racketeer Influenced and Corrupt Organizations
- ☐ 480 Consumer Credit (15 USC 1681 or 1692)
- ☐ 485 Telephone Consumer Protection Act
- ☐ 490 Cable/Sat TV
- ☐ 850 Securities/Commodities/ Exchange
- ☐ 890 Other Statutory Actions
- ☐ 891 Agricultural Acts
- ☐ 893 Environmental Matters
- ☐ 895 Freedom of Information Act
- ☐ 896 Arbitration
- ☐ 899 Administrative Procedure Act/Review or Appeal of Agency Decision
- ☐ 950 Constitutionality of State Statutes

REAL PROPERTY
- ☐ 210 Land Condemnation
- ☐ 220 Foreclosure
- ☐ 230 Rent Lease & Ejectment
- ☐ 240 Torts to Land
- ☐ 245 Tort Product Liability
- ☐ 290 All Other Real Property

CIVIL RIGHTS
- ☐ 440 Other Civil Rights
- ☐ 441 Voting
- ☐ 442 Employment
- ☐ 443 Housing/ Accommodations
- ☐ 445 Amer. w/Disabilities - Employment
- ☐ 446 Amer. w/Disabilities - Other
- ☐ 448 Education

PRISONER PETITIONS

Habeas Corpus:
- ☐ 463 Alien Detainee
- ☐ 510 Motions to Vacate Sentence
- ☐ 530 General
- ☐ 535 Death Penalty

Other:
- ☐ 540 Mandamus & Other
- ☐ 550 Civil Rights
- ☐ 555 Prison Condition
- ☐ 560 Civil Detainee - Conditions of Confinement

V. ORIGIN *(Place an "X" in One Box Only)*

☒ 1 Original Proceeding ☐ 2 Removed from State Court ☐ 3 Remanded from Appellate Court ☐ 4 Reinstated or Reopened ☐ 5 Transferred from Another District *(specify)* ☐ 6 Multidistrict Litigation - Transfer ☐ 8 Multidistrict Litigation - Direct File

VI. CAUSE OF ACTION

Cite the U.S. Civil Statute under which you are filing ***(Do not cite jurisdictional statutes unless diversity)***:
28 U.S.C. § 1332(d)

Brief description of cause:
Breach of warranty and breach of the Washington Consumer Protection Act.

VII. REQUESTED IN COMPLAINT:

☒ CHECK IF THIS IS A **CLASS ACTION** UNDER RULE 23, F.R.Cv.P.

DEMAND $

CHECK YES only if demanded in complaint:

JURY DEMAND: ☒ Yes ☐ No

VIII. RELATED CASE(S) IF ANY

(See instructions): JUDGE _____ DOCKET NUMBER _____

DATE
Mar 14, 2024

SIGNATURE OF ATTORNEY OF RECORD
/s/ Sean R. Matt

FOR OFFICE USE ONLY

RECEIPT # _____ AMOUNT _____ APPLYING IFP _____ JUDGE _____ MAG. JUDGE _____

AO 440 (Rev. 06/12) Summons in a Civil Action

UNITED STATES DISTRICT COURT

for the

Western District of Washington

JESSE VARGISON and RACHAEL FORBIS, individually and on behalf of themselves and all others similarly situated,

Plaintiff(s)

v.

PAULA'S CHOICE, LLC,

Defendant(s)

Civil Action No.

SUMMONS IN A CIVIL ACTION

To: *(Defendant's name and address)* PAULA'S CHOICE, LLC
C/O C T CORPORATION SYSTEM
711 CAPITOL WAY SOUTH, SUITE 204,
OLYMPIA, WA, 98501-1267

A lawsuit has been filed against you.

Within 21 days after service of this summons on you (not counting the day you received it) — or 60 days if you are the United States or a United States agency, or an officer or employee of the United States described in Fed. R. Civ. P. 12 (a)(2) or (3) — you must serve on the plaintiff an answer to the attached complaint or a motion under Rule 12 of the Federal Rules of Civil Procedure. The answer or motion must be served on the plaintiff or plaintiff's attorney, whose name and address are: Sean R. Matt (WSBA No. 21972)
HAGENS BERMAN SOBOL SHAPIRO LLP
1301 Second Avenue, Suite 2000
Seattle, Washington 98101
Telephone: (206) 623-7292; Facsimile: (206) 623-0594
Email: sean@hbsslaw.com

If you fail to respond, judgment by default will be entered against you for the relief demanded in the complaint. You also must file your answer or motion with the court.

CLERK OF COURT

Date: _____

_____
Signature of Clerk or Deputy Clerk

Civil Action No.

PROOF OF SERVICE

(This section should not be filed with the court unless required by Fed. R. Civ. P. 4 (l))

This summons for *(name of individual and title, if any)* _____ was received by me on *(date)* _____ .

❒ I personally served the summons on the individual at *(place)* _____ on *(date)* _____ ; or

❒ I left the summons at the individual's residence or usual place of abode with *(name)* _____ , a person of suitable age and discretion who resides there, on *(date)* _____ , and mailed a copy to the individual's last known address; or

❒ I served the summons on *(name of individual)* _____ , who is designated by law to accept service of process on behalf of *(name of organization)* _____ on *(date)* _____ ; or

❒ I returned the summons unexecuted because _____ ; or

❒ Other *(specify):*

My fees are $ _____ for travel and $ _____ for services, for a total of $ 0.00 .

I declare under penalty of perjury that this information is true.

Date: _____

_____
Server's signature

_____
Printed name and title

_____
Server's address

Additional information regarding attempted service, etc:

Print Save As... Reset

ClassAction.org