

Guidance for research and development of bio-cosmetics:

**from laboratory
to market**

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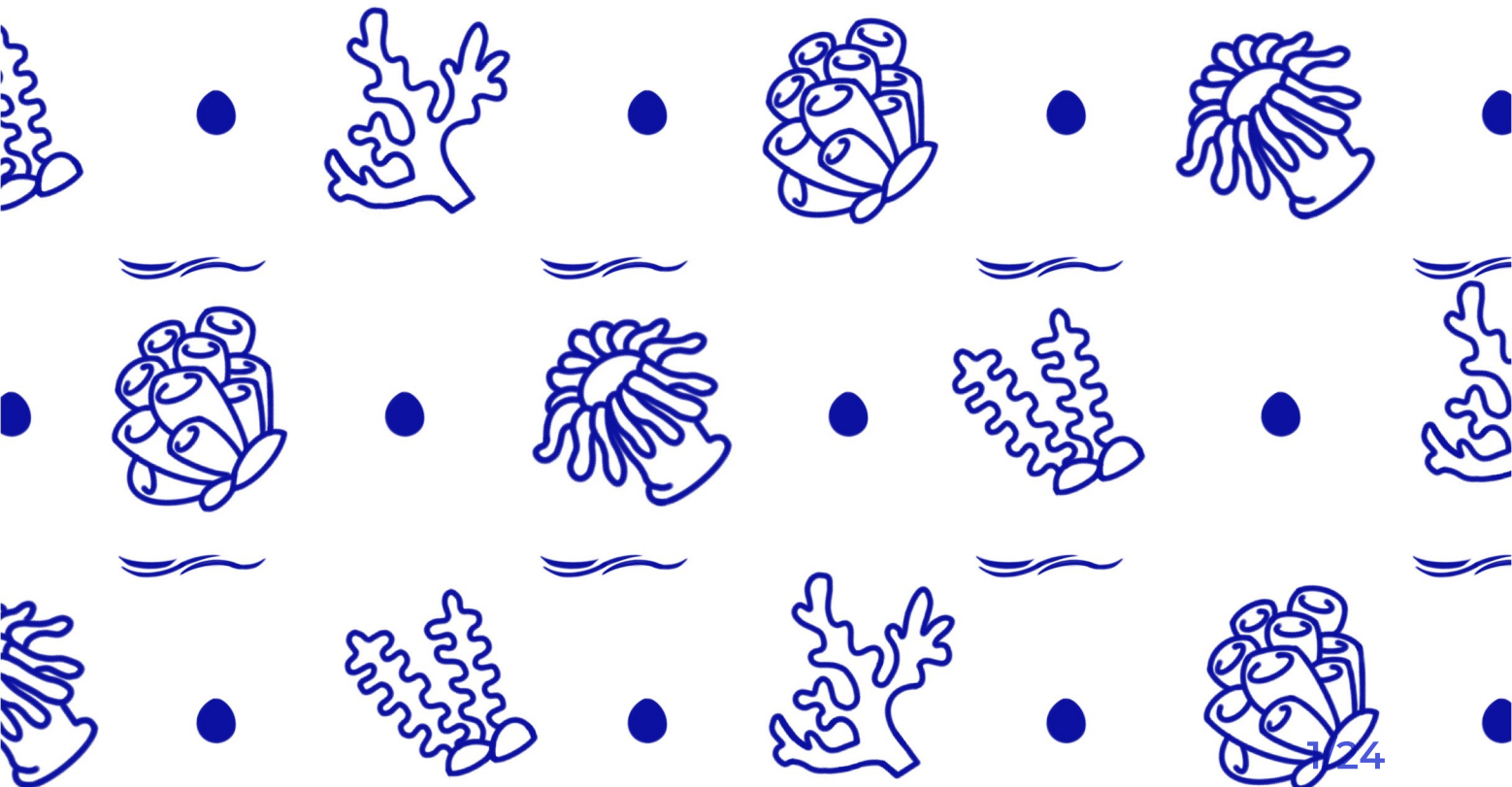
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Preface

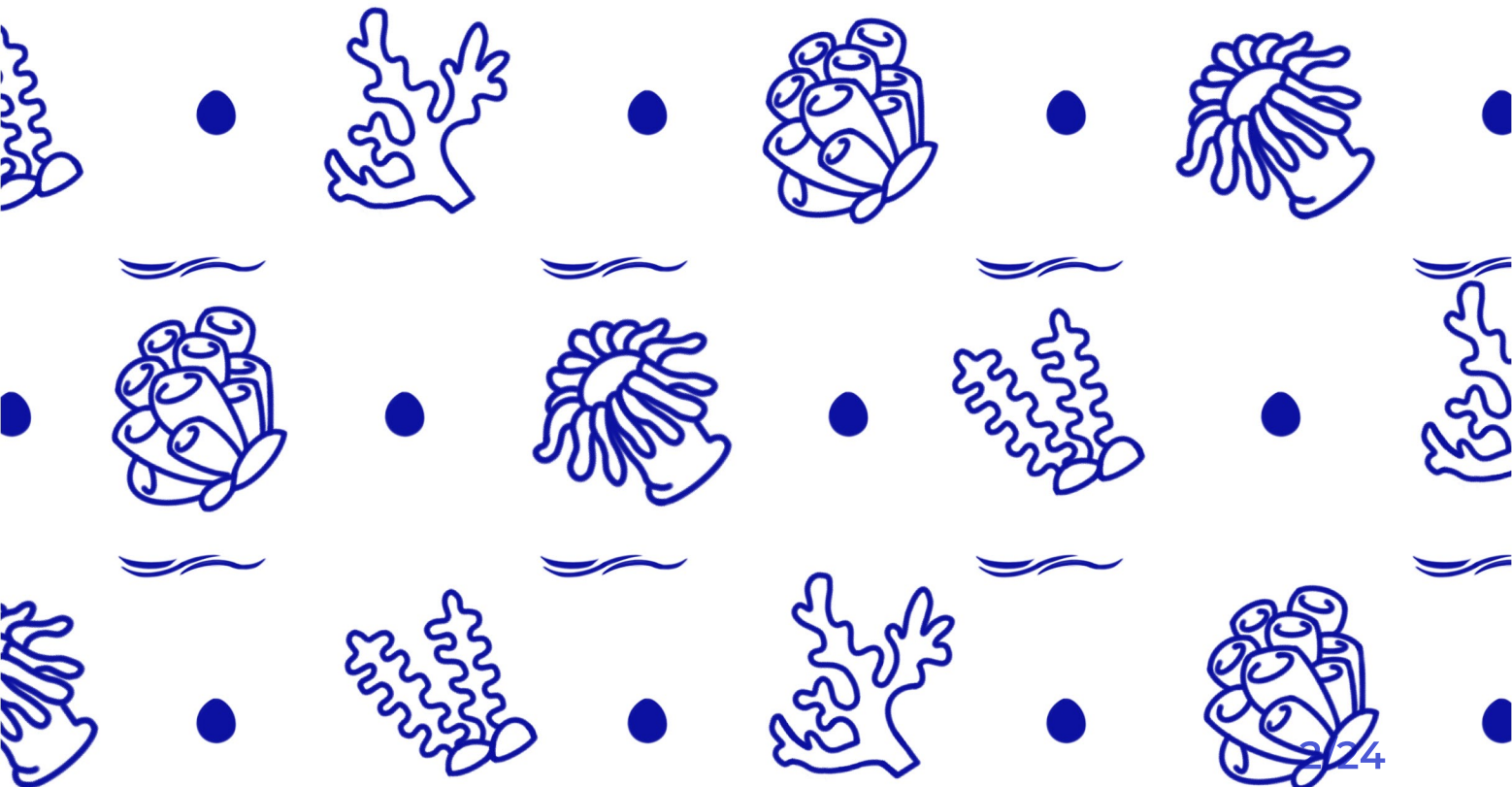
Based on the needs of the project and the relevant regulations formulated by the government in the field of cosmetics, Jiangnan_China sorted out a handbook on raw material production and cosmetics filing and listing.

We made this handbook considering that many teams developing cosmetic raw materials by synthetic biology only have biological knowledge, but do not know how to turn experimental products into commodities. Our handbook introduces the safety experiments, registration channels and other important precautions required for the registration and filing of new cosmetic raw materials, which will be of great reference value to iGEM team and cosmetic raw material producers for such projects in the future.



catalogue

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Part .0 Overview: Cosmetic production process

As a consumer of cosmetics, we have no idea how many evaluations and examine need to be done before the skin care essence and creams can finally be put into large-scale manufacture. When we started designing our own products, we have to learn and consider these contents in advance so as to ensure that our vision of the product can be successfully realized.

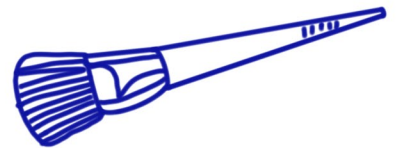
Take our project——”*Saccharomyces cerevisiae* as the chassis cell to produce sunscreen” for example. The development and marketing mainly follow the next process:



Experiments in the R&D process shall comply with the relevant provisions of the "Genetic Engineering Safety Management Measures", the efficacy of the product needs to pass the testing stipulated in the "Cosmetic efficacy claim Evaluation Specification", and issue the report on cosmetic efficacy. Before the product is put on the market, the components in the formula should be inspected according to the "Technical Guidelines for the Safety Assessment of Cosmetics" and issued a safety assessment report.

In addition, the application of cosmetics patent is in accordance with the relevant provisions of "the Patent Law of the People's Republic of China".

New cosmetics and raw materials need to be registered and put on record by the Food and Drug Administration. New chemical substances in raw materials need to be put on record by the Environmental Protection Bureau in accordance with the "Environmental Management Measures for New Chemical Substances".



PART.01 Research and development of biotechnology-derived cosmetics-chassis cell selection

In recent years, microbial cell factory is gradually becoming a highly esteemed star in the cosmetics industry because of its safety and efficiency. Among them, the popular chassis organisms include yeast, lactic acid bacteria, bacillus, algae and so on. Yeast has been given relatively great attention because of its rich active ingredients (free amino acids, small peptides, vitamins, etc.). A lot of cosmetics brands like *SK-II*, *Lancôme*, *Estee Lauder* and others have launched the "yeast essence" publicity, which mainly includes skin whitening, moisturizing, anti-aging, etc. Different kinds of yeast have their own production characteristics. When we want to use them to complete the production of cosmetic ingredients, we might as well choose them with the help of the following tables [1,2].

Name	Advantages	Disadvantages	Example
<i>Saccharomyces cerevisiae</i>	The genetic background is clear, the operation tools and regulation means are mature, and the industrialization experience is rich	The tolerance to temperature and organic solvents is narrow, the available substrate is limited (generally glucose), and the preference to produce ethanol affects the yield	<i>Saccharomyces cerevisiae</i> is widely used in cosmetics. The filtrate of the fermentation product filtrate from <i>SK-II</i> skin care essence is the fermentation liquor of sake yeast. Similarly, the Genuine <i>Namir's</i> yeast water, <i>Fresh's</i> black tea yeast extract and <i>Luerling's</i> wine meal mask are also used by <i>Saccharomyces cerevisiae</i> .

Unconventional yeast	<i>Yarrowia lipolytica</i>	The supply of acetyl CoA and NADPH in cytoplasm was sufficient, which was conducive to the synthesis of downstream products; Long chain hydrophobic fatty acids can be used as the only carbon source; Strong tolerance		Lack of research on physiological characteristics and high cost of culture medium; Not good at synthesizing more complex natural products	There is a lack of examples for cosmetics production, but in the existing reports,the biosynthesis of terpenoids and flavonoids such as carotenoids, resveratrol, astaxanthin, squalene and naringin is superior to that obtained in <i>Saccharomyces cerevisiae</i>
	<i>Pichia pastoris</i>	<i>Pichia pastoris</i> can utilize xylose	Methanol can be used as the only carbon source, pentose phosphate branch efficiency is very high, and more PPP intermediates and NADPH may be conducive to the synthesis of complex natural products;It has the ability of post-translational modification more similar to higher biological proteins	The research on it is limited and the gene operation platform is not perfect, so it is relatively difficult to construct complex natural product synthesis pathways	Like <i>Yersinia pastoris</i> , <i>Pichia pastoris</i> is also widely used for recombinant expression of terpenoids and polyketones ^[3] ; The Frapertide used in Ander F Tai Ve's mask is the <i>Pichia pastoris</i> fermentation cytosolic filtrate, which contains antibacterial peptide (thirteen peptide) and so on. The first is the use of the antibacterial peptide (PICA), and so on. It is mainly used in the fermentation of <i>Pichia pastoris</i> .
	<i>Hansenula polymorpha</i>	It can withstand high temperature above 40 °C and save cooling cost			Like <i>Pichia pastoris</i> , <i>Hansenula polymorpha</i> are methanol nutritious yeast, which have similar compound synthesis pathways, but there are few related applications
	<i>Rhodotorula</i>	It is the main strain for the production of lycopene. The fermentation process is simple and the process is easy to control			There is no cosmetics made from Rhodotorula yeast in the market, but with the development of relevant research, industrialization is expected. For example, Cai Yiwen ^[4] put forward the research and development plan of lycopene yeast extract anti-aging eye cream and mask.
	<i>Xanthophyllomyces</i>	It has the ability to synthesize natural astaxanthin			The research on <i>Phaffia rubra</i> has not yet reached the stage of

Unconventional yeast	<i>Xanthophyllomyces dendrorhous</i>	It has the ability to synthesize natural astaxanthin		The research on <i>Phaffia rubra</i> has not yet reached the stage of industrialization
<i>Bifida yeast</i> ^[5] (<i>Bifidobacterium</i>)		It is rich in glucose metabolizing enzymes; Some oligosaccharide "bifidus factor" can promote its growth	Strictly anaerobic; High nutritional requirements, amino acids, peptides and other substances need to be added; The tolerance temperature window is narrow	In recent years, <i>Bifida bifida</i> has become the hot spot of net red. <i>Estee Lauder's</i> small brown bottle, <i>Lancome's</i> small black bottle and <i>L'OREAL's</i> youth cryptogram enzyme essence are used in the two fold yeast cell lysate or fermentation filtrate.

Reference

- [1]YE Min, GAO Jiao-qi, ZHOU Yong-jin. Engineering Non-conventional Yeast Cell Factory for the Biosynthesis of Natural Products[J].Biotechnology Bulletin, 2021, 37(08): 12-24.
- [2]LIU Ting, YAO Lingyun, WANG Caixia, PAN Xianhua, MA Laiji, SHI Yanqin, WANG Yue.Research Progress on Application of Biological Fermentation Technology in Cosmetics[J].Flavour Fragrance Cosmetics,2021(03):114-119.
- [3]Xingpeng Duan,Jiaoqi Gao,Yongjin J.Zhou.Advances in engineering methylotrophic yeast for biosynthesis of valuable chemicals from methanol[J]. Chinese Chemical Letters, 2018, 29(05): 681-686.
- [4]CAI Yiwen. Lycopene rich yeast extract anti aging eye cream and mask development[D].South China University of Technology, 2018.
- [5]XU Zhen-guo, CAI Yu-hua, LIU Xiu-shu, FAN Gao-fu, DAI Yin .Research progress and application prospect of bifidobacterium [J]. Chinese Journal of Biologicals, 2017, 30(02): 215-220.

PART.02 Product Positioning—Our product efficacy

1.Classification

The "Cosmetics Classification Rules and Classification Catalogue" classifies cosmetics according to efficacy claim, action part, product dosage form, user group and use method.

The effect claims → 28 categories, including whitening, hydrating, and cleaning.

The position of action → 13 kinds including hair, head skin, whole body skin.

Dosage forms → 14 categories including creams, lotions, liquids, gels

Users → general population, infants (born to 3 years old) and children (before 3 to 12 years old)

method of use → leaching class and resident class

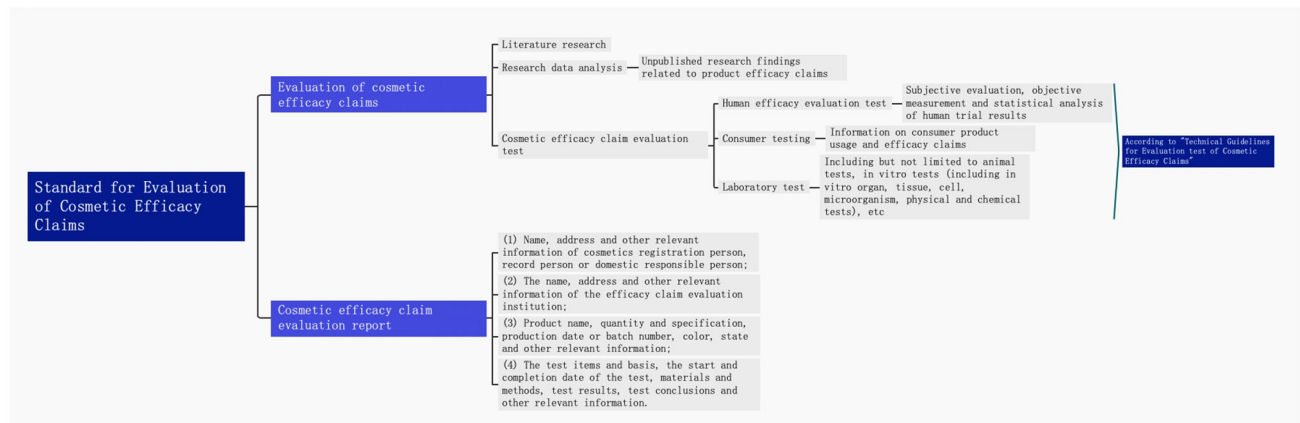
Besides, cosmetics which cannot be classified according to this method should be placed in the special cosmetics management.

Cosmetic research and development can refer to the "Cosmetic Classification Rules and Classification Catalogue" to understand the category of cosmetics, and then targeted data query and experimental preparation.

2.Cosmetics efficacy claim



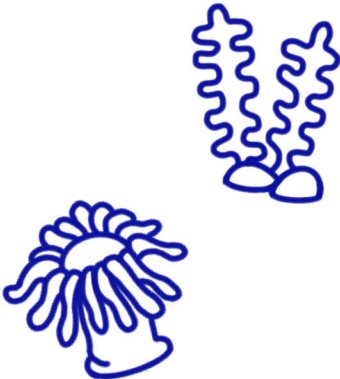
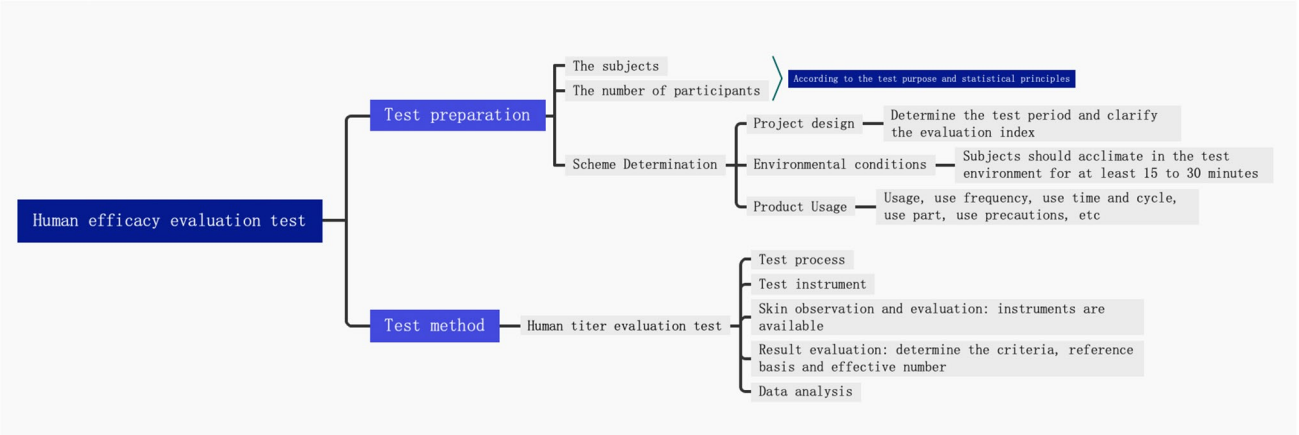
Cosmetics efficacy claim should be in accordance with the "Cosmetics efficacy claim evaluation criteria" collated literature and research data, and according to the requirements of cosmetics efficacy claim evaluation test, and issued according to the requirements of the report.



Cosmetics efficacy claim evaluation test includes human efficacy evaluation test, consumer test, laboratory test, and all test should be carried out according to the "cosmetics efficacy claim evaluation test technical guidelines".

When conducting the test, researchers shall indicate the test purpose and the principle and basis according to which the human body efficacy evaluation test and consumer test shall determine the number of subjects and subjects according to the test purpose and statistical principles. When determining the formal test plan, it is necessary to determine the test cycle, clarify the evaluation index, indicate the product use method, and prepare the reference product; Consumer test, laboratory test should also consider the test place, environment. Finally, conclusions are drawn according to the test results, and the adverse reactions/application and limitations of the product are clarified.

Generally, cosmetics that claim to have the efficacy of sun-proof, anti-hair loss, acne and other functions must be tested for human efficacy evaluation when issuing efficacy claim reports.



PART.03 Safety Assessment—Preparation before market

The safety testing of cosmetics and raw materials involved in the registration and filing process shall be evaluated and reported under the guidance of "the Technical Guidelines for Safety of Cosmetics" and in accordance with the test testing methods in "the Technical Specifications for Safety of Cosmetics".

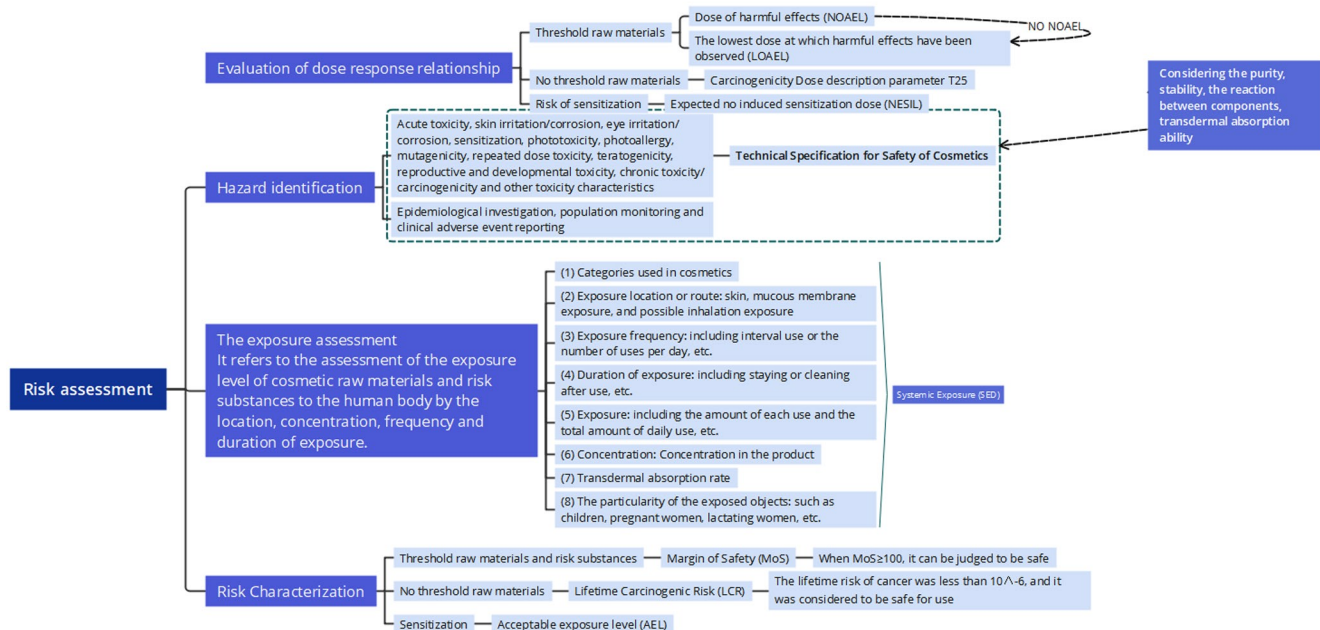


1. Safety assessment test

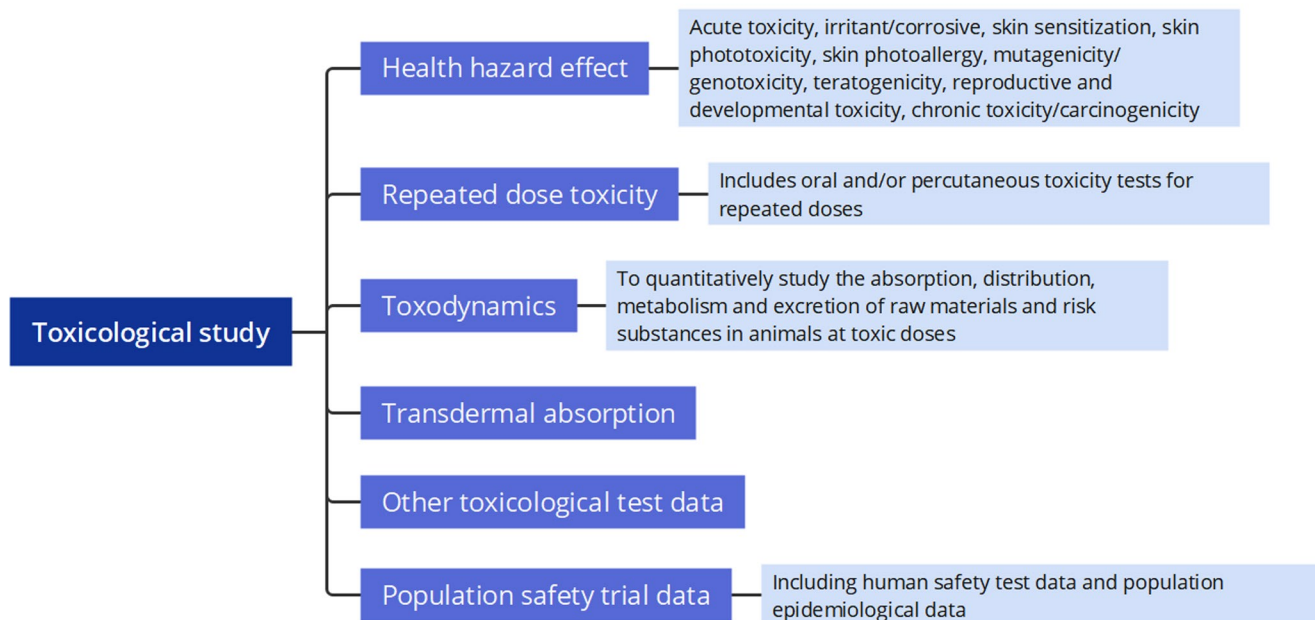
The safety assessment is for both cosmetic ingredients and cosmetic products, examining the ingredients themselves and the risk substances that may be introduced into the preparation process, and considering chemical and/or biological interactions between different ingredients in the product.

The safety assessment is divided into risk assessment and toxicology study. Risk assessment includes hazard identification, dose-response relationship assessment, exposure assessment and risk characteristic description. The relationship between toxicity of components, dose and exposure level must be obtained. Among them, the health hazard effect of the hazard identification part also belongs to the category of toxicological research, which is tested according to the "Cosmetic Safety Technical Specification".





Toxicological studies include various health hazard effects, repeated dose toxicity, toxokinetics, transdermal absorption and other required information.



In accordance with the principles of "risk assessment" and "toxicological study" in the Technical Guidelines for The Safety of Cosmetics, the risk assessment report of cosmetics raw materials and the safety assessment report of cosmetics shall be issued.

2. Risk assessment of cosmetic raw materials

The risk assessment of cosmetic raw materials first needs to follow the "risk assessment" and "toxicological study" for risk assessment.

Secondly, physical and chemical properties are tested to predict specific toxicological properties, including:

Name of raw material (including general name, commodity name, INCI name, standard Chinese name, CAS Number, EINECS Number, etc.)

Physical state

Molecular formula and relative molecular weight

Chemical properties and purity

Impurity/residue (concentration or content)

solubility

The distribution coefficient

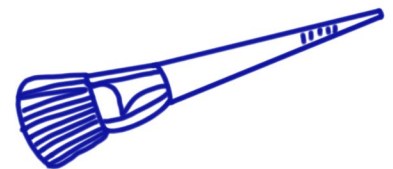
Homogeneity and stability

Uv-vis absorption spectrum

Isomer composition

Functions and Uses

Other relevant physical and chemical indicators (e.g. uv absorption wavelength and uv absorption spectrum of compounds for uV-absorbing components)



According to the sources, cosmetics raw materials can be divided into mineral sources, plant sources, animal sources and biotechnology sources.

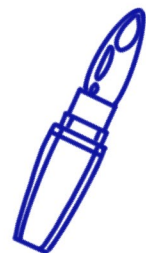
Raw materials created by biotechnology, including raw materials from genetic engineering, cell engineering, fermentation engineering, enzyme engineering and protein engineering, should all be issued in the risk assessment report:

The preparation process

- Description of organisms used: including donor organisms, recipient organisms and modified microorganisms

(genetic engineering, cell engineering and fermentation engineering). Raw materials from the source of the gene used in the production, vector construction, engineering bacteria (strains, cells) and other details. If the species should provide the genus name, species name and species number of the species, and provide the corresponding Latin scientific name; Provide the origin of the strain, identification reports (including phenotypic characteristics and genotype-based strain level identification results) and biological, genetic, and ergonomic characteristics of the strain (strain), as well as stability study data.)

- Host pathogenicity
- Toxic components: including biometabolites, toxins produced
- Physical and chemical properties
- Microbiological quality control measures
- Preservatives and other additives



3. Safety assessment of cosmetic products

For cosmetic products, the risk assessment of the raw materials and risk substances must also be carried out in accordance with the risk assessment procedure.

Also, the physical and chemical stability of the product should be evaluated. Related physical and chemical indicators should be evaluated based on the specific situation of the product, generally including the following parameters:

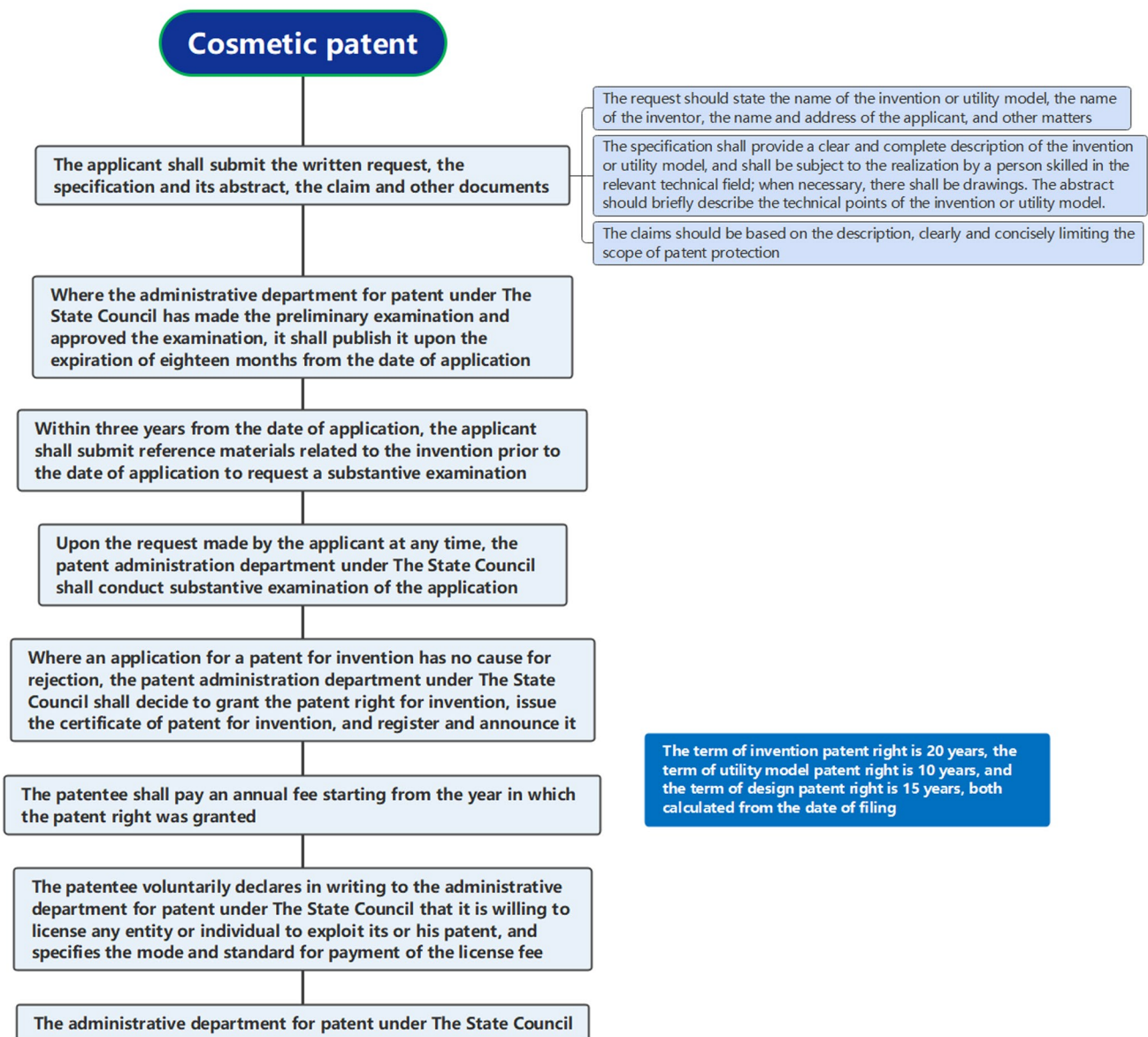
- Host pathogenicity
- Toxic components: including biometabolites, toxins produced
- Physical and chemical properties
- Microbiological quality control measures
- Preservatives and other additives

The product also needs to be evaluated by product microbiology, and the harmful microbial content should be detected by referring to the microbiology test method in the "Cosmetic Safety Technical Specification".



PART.04 Patent registration-protect our intellectual property

For self-developed cosmetic products, patent applications can also be made in accordance with the "Patent Law of the People's Republic of China".



PART.05 Registration and filing

Newly-developed raw materials and cosmetics products need to be registered with the FOOD and Drug Administration before being marketed.

1. New cosmetics raw materials

The registration and filing of new cosmetic raw materials shall follow the Provisions on the Management of Registration and Filing Materials of New Cosmetic raw materials.

The document refines the requirements for registration and filing materials of new raw materials, subdivides the situation of new raw materials based on risk management principles, and standardizes the compilation of technical materials related to new raw materials, with the following key points:

Provisions on the management of registration and filing materials of new cosmetics raw materials

Registrants and record holders of new cosmetic raw materials shall register user information through the information service platform before applying for registration of new raw materials or putting new raw materials on record.

- Information of registrant and record holder of new cosmetics raw materials
- Overview of safety risk monitoring and evaluation system for registrants and record holders of new cosmetics raw materials
- If the registrant or record holder of new cosmetic raw materials is from abroad, the domestic responsible person shall fill in the information and submit the authorization letter and the original notarial certificate of the domestic responsible person

In case of any change in the following information information of the registrant, record holder or domestic responsible person of new cosmetic raw materials, it shall be updated to ensure the authenticity and accuracy of relevant information information in the information service platform.

The letter of authorization of the domestic responsible person shall contain at least four categories of contents and information.

- Name of registrant, record holder and domestic responsible person of new cosmetics raw materials
- Authorization and authorized relationship
- Scope of authorization
- Mandate

The registrant or record holder of new cosmetic raw materials shall submit four kinds of materials when applying for the registration of new cosmetic raw materials or handling the record of new cosmetic raw materials.

- Name, address and contact information of the registrant, the record holder and the responsible person within China
- New raw material development report
- Research data on preparation technology, stability and quality control standard of new raw materials
- New raw material safety assessment data

The development report of new cosmetics raw materials should generally include five types of content.

- Raw materials research and development background
- Basic information of raw materials
- Raw material usage information
- Functional reference data
- Other materials related to the development of new raw materials

The preparation process of new raw materials from different sources has different requirements.

- Chemical synthetic raw material
- Natural ingredients
- Biotechnology comes from raw materials
- For raw materials from other sources, submit relevant materials according to the characteristics of raw materials and specific preparation process

The quality control standard of new cosmetics raw materials should generally include three types of content.

- Stability test data
- Quality specifications and inspection methods thereof
- Possible safety risk substances and their control standards

The safety evaluation data of new cosmetic materials should generally include two types of content.

- Toxicological safety assessment data
- Safety risk assessment materials

The samples of new cosmetic raw materials shall have complete packaging and labels, which shall include the name of the registrant, record holder or domestic responsible person of the new raw materials, the Chinese name of the raw materials, the name of INCI, the date of production and the term of use, storage conditions and other information.

The registrant, record holder or domestic responsible person of new cosmetic raw materials shall collect and sort out information and materials in accordance with the relevant requirements of the safety monitoring period of new cosmetic raw materials, and compile an annual report on the safety monitoring of new cosmetic raw materials based on the collected information and materials.

- Information about registrants, record holders or entrusted manufacturers of cosmetics produced with new raw materials
- Information of cosmetics produced with new raw materials, including product name, product registration certificate or filing certificate number, product production or import, sales quantity, etc
- Supervise spot check, investigation and recall of cosmetics produced with new raw materials
- The adverse reaction monitoring system, statistical analysis and measures taken by cosmetic manufacturers for cosmetics produced with new raw materials
- The risk monitoring and evaluation management system and measures of cosmetics production enterprises using new raw materials

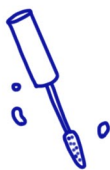
Where the registrant, record holder or domestic responsible person of new cosmetic raw materials finds any situation that should be reported to the technical evaluation institution in accordance with the Measures for the Administration of Cosmetics Registration and Record, or other circumstances that it considers necessary to report, It shall immediately collect and organize the basic information of new raw materials, production and use, cause analysis of safety problems or emergencies, disposal measures and treatment results, etc., prepare safety risk control reports of new cosmetics raw materials, and submit them to the technical evaluation institutions through the information service platform.

The preparation of archival materials includes 9 parts: application form, development report, preparation process, stability, quality control standard, safety standard, functional basis, technical requirements and sample preparation.

Take the new cosmetics raw materials with the functions of sunscreen, freckle whitening, hair loss prevention as examples. In terms of test and detection:

Stability:

- Destructive test
- Accelerated test
- Long-term storage test



Safety Standards -- Review of toxicological safety Evaluation:

- Acute oral or acute percutaneous toxicity test;
- Skin and acute eye irritation/erosion test
- Skin allergy test
- Skin phototoxicity test (including photoirritation and photoallergy, raw materials with uv absorption characteristics need to do this test)
- Photoallergy test (this test should be done when raw materials have uv absorption characteristics)
- Mutagenic test (should include at least one gene mutation test and one chromosome aberration test)
- Oral or percutaneous toxicity test of repeated dose (oral toxicity test of repeated dose is provided if oral ingestion of the material is likely to be used in cosmetics)

Function basis:

Scientific literature/regulatory material

In vitro studies

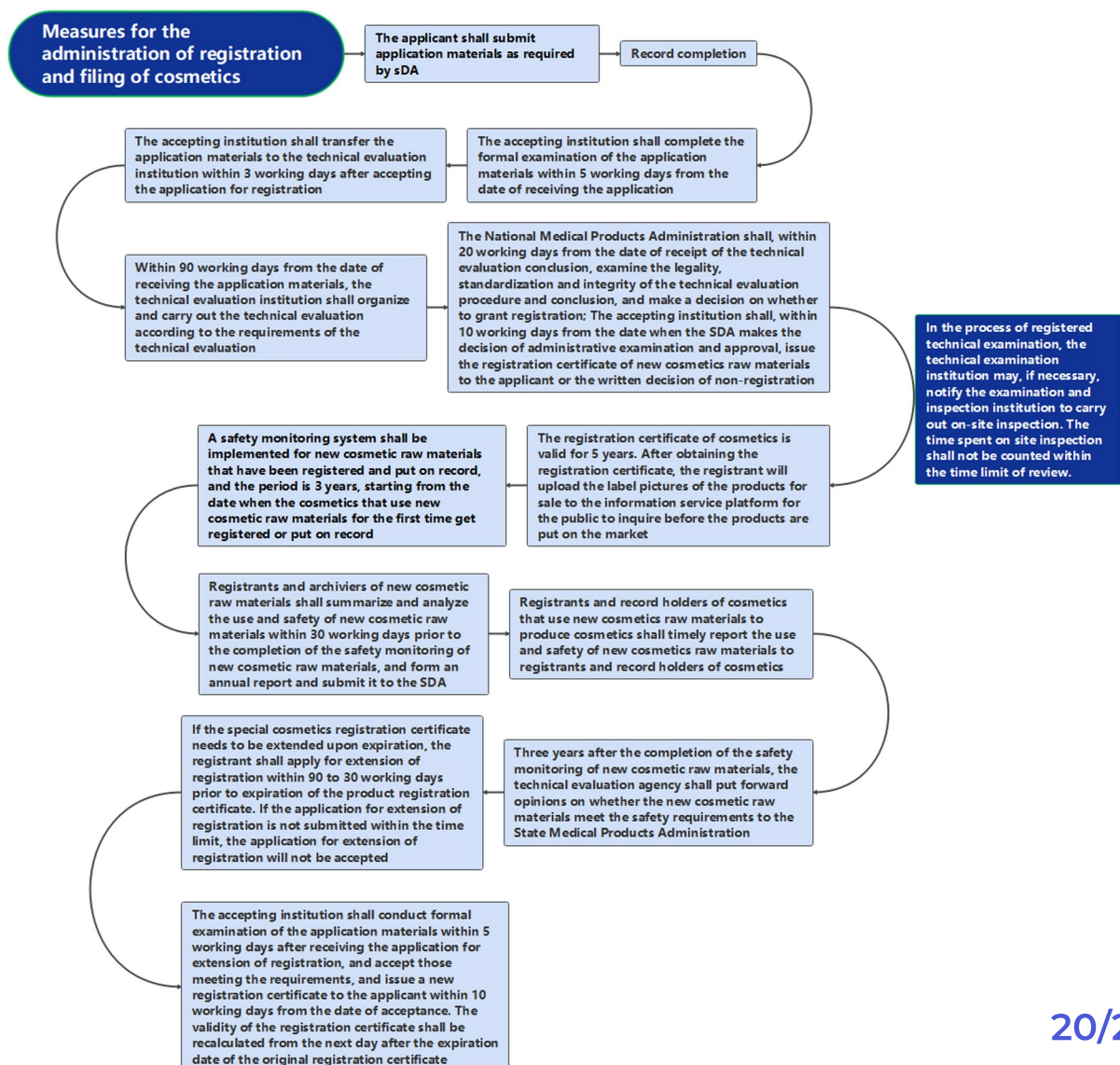
(Animal test and human test should be carried out selectively according to specific needs)

2. Cosmetics

Cosmetics registration and filing mainly follow the following laws and regulations:

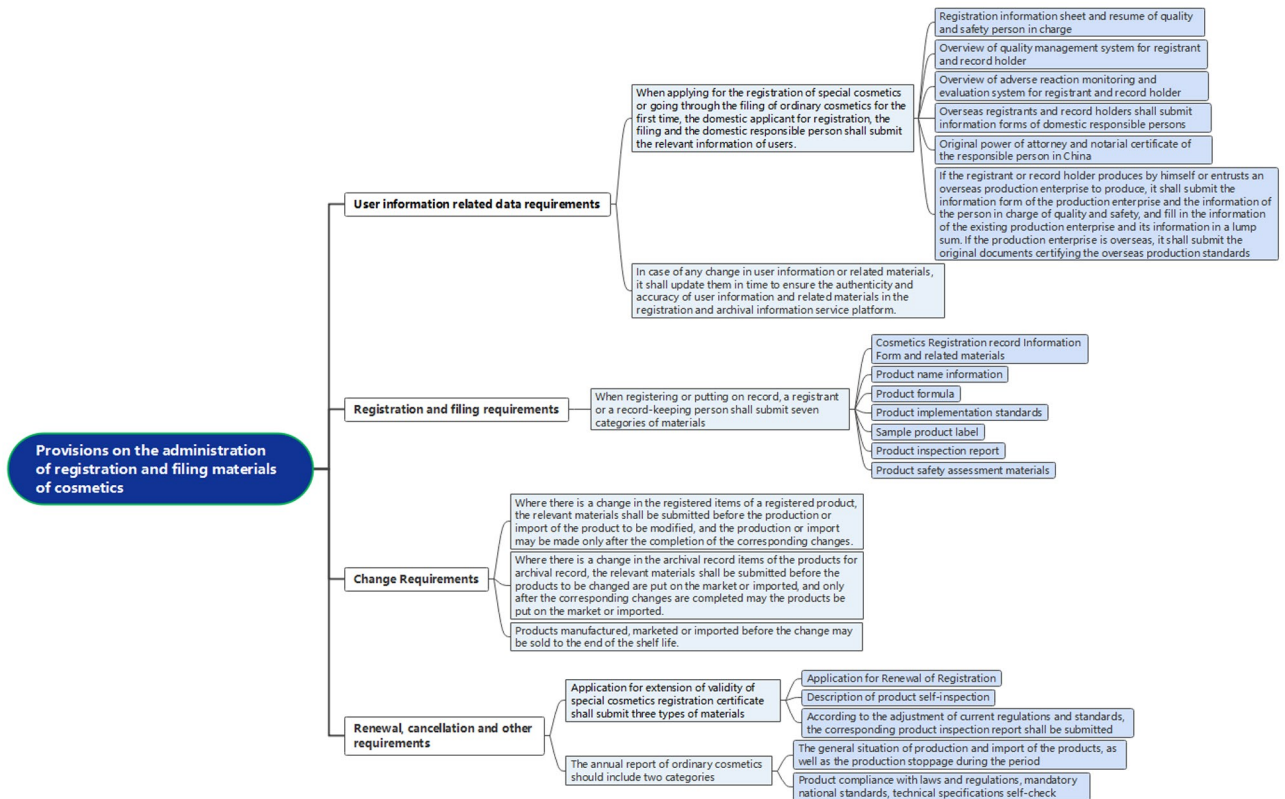
1) Administrative Measures for Registration and Filing of Cosmetics

The registration and filing process of cosmetics is as follows:



2) Provisions on The Management of Cosmetics Registration and Filing Materials

The document provides detailed provisions on "requirements for user information related materials, requirements for registration and filing materials, requirements for changes, requirements for continued cancellation and other matters", with the following key points:



In addition, after the completion of registration, listing and circulation of cosmetics, routine testing should also be carried out according to the "Technical Guidelines for Cosmetic Safety Assessment". Production and business activities should be standardized in accordance with the "Regulations on The Supervision and Management of Cosmetics".

Resources

《化妆品功效宣称评价规范》

Cosmetic efficacy claim Evaluation Standard

《化妆品分类规则和分类目录》

Classification rules and catalogue of cosmetics

《化妆品安全评估技术导则》

Technical guide for safety assessment of cosmetics

《化妆品安全技术规范》

Technical specification for safety of cosmetics

《化妆品监督管理条例》

Regulations on the Supervision and Administration of Cosmetics

《中华人民共和国专利法》

Patent Law of the people's Republic of China

《化妆品新原料注册备案资料管理规定》

Provisions on the Administration of Registration and filing of New Cosmetic Raw Materials

《化妆品注册备案管理办法》

Measures for the Administration of Cosmetics Registration and filing

《化妆品注册备案资料管理规定》

Provisions on the Administration of the Registration and filing of Cosmetics

《基因工程管理办法》

Measures for the Management of genetic Engineering

《新化学物质环境管理办法》

Measures for Environmental Management of New Chemical Substances

*All regulations mentioned are Chinese legislation

iGEM Cosmetic Projects



Technion_Israel

Technion, Haifa, Israel 2015

By designing *Bacillus subtilis*, a bacterium found naturally on scalp, we can provide hair growth and hair care products for baldness patients.

SCAU-China

South China Agricultural University,
China 2016

Using rice (*Oryza sativa* L.) as chassis organism to produce a genetically modified organism (GMO) named aSTARice, which enables biosynthesis of astaxanthin in the rice endosperm. Our products consist of purified astaxanthin powder and some kinds of preliminarily-processed products (e.g. astaxanthin mask).



SSTI-SZGD

SHENZHEN INSTITUTE OF TECHNOLOGY,
China 2018

By introducing a hydrolase gene, we constructed a *B. Subtilis* strain that is able to biosynthesize LMW-HA and developed a novel cosmetic product: HA Micro-Needle Patch.



XH-China

High school, China 2020

Our team expressed keratinase through genetically edited plasmid, and designed a mild skin care product to solve a series of skin problems, such as dryness, itching and redness.

Jiangnan_China

Jiangnan University, China 2021

14000 tons of sunscreens are deposited into the ocean annually and then worsen the coral bleaching. We aim to engineer *S.cerevisiae* to produce an eco-friendly bio-sunscreen made of gadusol to balance the use of sunscreen and protection of corals.





**THANK
YOU**

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