Guidelines for Standardized Addition and Application of Nucleotides in Multiple Fields

Implemented Standards: GB 2760, GB 14880, GB 1886.171, JECFA, EFSA, FDA GRAS, CAC

Applicable Varieties: Disodium 5'-Inosinate (IMP), Disodium 5'-Guanylate (GMP), Disodium Flavor Nucleotides (I+G), Cytidine 5'-Monophosphate (CMP), Uridine 5'-Monophosphate (UMP), Adenosine 5'-Monophosphate (AMP), Compound Nutritional Nucleotides

Chapter 1 General Principles and Basic Technical System

1.1 Compilation Purpose

To unify the standardized addition processes, compatibility rules, dosage limits, temperature control systems, pH adaptation systems and international compliance systems of nucleotide-based functional raw materials across the whole industrial chain including food seasoning, infant formula foods, special dietary foods, livestock and aquafeed, health functional foods, biomedicine and daily chemical applications. It aims to realize standardized domestic mass production, compliant cross-border export and simplified downstream application implementation. Balancing scientific mechanisms and industrial practical operation, this guideline provides directly applicable operational specifications for downstream clients and meets technical requirements of international target markets.

1.2 Scope of Application

This guideline serves as full-process operation guidance for food manufacturers, infant formula dairy enterprises, special medical food factories, feed premix producers, health product R&D enterprises, cross-border export traders and formula research institutions. It covers six mainstream addition technologies: dry mixing, wet dissolution, low-temperature homogenization, post-addition, carrier premixing and spray adhesion. Operational prohibitions and potency protection key points are clarified to satisfy large-scale production and refined formula demands of downstream users.

1.3 Core Scientific Mechanisms

- **Flavor-enhancing Mechanism**: IMP and GMP act synergistically on human taste receptors, generating an obvious umami multiplication effect with monosodium L-

glutamate (MSG). They can multiply umami intensity, mask fishy odor, bitter taste and process peculiar flavors, and optimize product taste hierarchy.

- **Nutritional and Physiological Mechanism**: Exogenous nucleotides participate in in-vivo nucleic acid synthesis, intestinal mucosal repair, immune cell proliferation and metabolic energy regulation. They are applicable to young groups (infants), stressed breeding animals (weaned piglets, fry and shrimp larvae), post-operation physical recovery and other dietary products.
- **Physicochemical Stability Mechanism**: Nucleotides achieve optimal stability in neutral environment (pH 7.0-8.5). High temperature, strong acidity and biological hydrolases such as phosphatase are main degradation factors, which are core process control points directly determining nucleotide potency retention rate.

1.4 Material Characteristics and Preparation

Operators must be proficient in physicochemical properties of nucleotides before operation to avoid potency loss caused by improper operation and ensure accurate dosage and product stability.

1.4.1 Core Physicochemical Properties

- **Physical Form**: White to off-white crystal or powder, odorless, fresh in taste, free of visible impurities.
- **Solubility**: Freely soluble in water, slightly soluble in ethanol, insoluble in ether; pH value of 5% aqueous solution is about 7.0-8.5, and a small amount of product substrates like soy sauce and MSG can be added to assist dissolution.
- **Stability**: Relatively stable to heat, acid and alkali, yet prone to decomposition and loss of flavoring power when heated under acidic conditions. Stable under short-term heating at 100°C under normal pressure, easy to degrade during high-temperature sterilization at 121°C, and rapidly inactivated above 180°C; slightly sensitive to light and needs light-proof storage for long-term preservation.
- **Hygroscopicity**: Easy to absorb moisture in the air and agglomerate after opening. Agglomeration does not affect potency but reduces weighing accuracy, which shall be properly handled before feeding.
- **Compatibility Taboos**: Avoid direct contact with strong oxidants and long-term exposure to strong acid or strong alkali environments; stable in high-salt or high-protein systems with no need for extra stabilizers.

1.4.2 Storage and Pre-treatment Requirements

- **Storage Requirements**: Sealed with double-layer moisture-proof packaging, stored in cool, dry and dark places with ambient relative humidity ≤55%. Tie up inner bags tightly for unused materials after opening and finish use within 15 days to prevent moisture agglomeration.
- **Pre-treatment Requirements**: Crush and sieve agglomerated materials under aseptic conditions to ensure uniform particle size prior to premixing or dissolution, so as not to affect mixing uniformity and weighing accuracy.

1.5 Global Compliance Access Basis

- **Chinese Market**: Compliant as both food flavor enhancer and nutritional fortifier, strictly complying with dosage limits specified in GB series standards.
- **Asia-Pacific Market**: Included in JECFA general food additive list, conforming to seasoning and nutritional application standards in Southeast Asia, Japan, South Korea and other regions.
- **EU Market**: Filed with EFSA for food safety, with dosage limits for infant formula and functional foods implemented in line with unified EU standards.
- **North American Market**: Approved by FDA GRAS, freely added in dietary supplements and complying with relevant FDA specifications for food seasoning.
- **Southeast Asia, Middle East and Latin America**: No usage barriers for general food ingredients, only required to meet local labeling regulations.

Chapter 2 Raw Material Grading, Selection and Compatibility Standards

2.1 Classification of Nucleotide Products (Adapted to Different Downstream Scenarios)

- **Food Flavor-enhancing Grade**: High-purity I+G (purity ≥97%), mainly for umami enhancement, suitable for condiments, snack foods, meat products and other fields.
- **Nutritional Fortification Food Grade**: Proportioned compound monomer nucleotides, applicable to dietary supplements, dairy products, meal replacement powder and other nutritional products.
- **Special Grade for Infant Formula**: High-purity grade with low heavy metals, low impurities and strict microbial control, used for infant formula powder and special medical purpose formula foods.
- **Special Grade for Feed Breeding**: Yeast-sourced compound nucleotides with high cost performance, suitable for all livestock and aquaculture categories.

- **Pharmaceutical Grade**: Electrophoresis-grade high-purity monomer nucleotides (purity ≥99%), applied in biological culture media, nutritional infusion preparations and clinical auxiliary nutritional diets.

2.2 Classic Fixed Compatibility Formulas (Internationally Accepted Industry Ratios)

- **Golden Umami Ratio**: MSG : I+G = 17:1 (international standard ratio). The dosage of I+G is generally 1%~5% of MSG dosage, namely 1-5 kg I+G matched with every 100 kg MSG, achieving the optimal umami multiplication effect.
- **Standard Nutritional Ratio for Infant Formula**: CMP > UMP > AMP > GMP > GMP, close to the natural nucleotide structure of breast milk to satisfy infant intestinal development and immune growth demands.
- **Intestinal Care Ratio**: Dominated by UMP and CMP, supplemented with AMP, suitable for intestinal repair foods and breeding stress relief scenarios.
- **Anti-stress Breeding Ratio**: Combined IMP and GMP for synergistic effects, applicable to piglet weaning, fry and shrimp larva cultivation and other stress conditions.

2.3 Mandatory Raw Material Incoming Inspection Indicators

To guarantee downstream product quality, raw materials shall be strictly inspected for the following items upon arrival: content, loss on drying (≤5%), ignition residue, heavy metals (Pb≤10 mg/kg, As≤3 mg/kg, Cd≤0.5 mg/kg), water solubility clarity, total bacterial count (≤1000 CFU/g), yeast and mold count, pathogenic bacteria (negative) and hydrolysis stability test.

Chapter 3 Core Addition Process Operations

Given the extremely low addition level of nucleotides (especially I+G) in final products (usually at ppm level), direct feeding into bulk materials is prohibited. Standardized operations below shall be followed to ensure mixing uniformity and potency retention and meet large-scale production demands.

3.1 Solid Systems (Powder, Premix, Feed, Solid Condiments, etc.)

3.1.1 Standard Process: Gradual Carrier Premixing

Core Purpose: Prevent segregation and stratification of trace nucleotides, ensure uniform mixing with bulk materials and improve weighing and addition accuracy.

1. Primary Premixing: Mix weighed nucleotides with equivalent dry carriers (MSG, glucose, flour or crushed bulk materials, ratio 1:1 to 1:5) evenly in a small mixer for no less than 10 minutes.
2. Secondary Premixing: Blend the primary mixture with carriers accounting for 1%-5% of total materials for secondary mixing over 15 minutes for further dilution and dispersion.
3. Final Mixing: Put the secondary premix into the main mixer and blend with bulk materials for more than 20 minutes to achieve overall uniformity.

3.1.2 Key Operational Points

- **Feeding Timing**: Add in the middle and later stage of mixing process synchronously with other trace ingredients such as spices and pigments to avoid uneven mixing caused by early feeding.
- **Environmental Control**: Keep workshop humidity ≤60%, conduct mixing at normal temperature throughout the process without heating and stirring to prevent moisture absorption and potency loss.
- **Equipment Requirements**: Mixers shall be clean and dry without residual acid, alkali or impurities to avoid raw material contamination.

3.2 Liquid/Semi-fluid Systems (Soy Sauce, Sauce, Soup Base, Oral Liquid, etc.)

3.2.1 Standard Process: Pre-dissolution Method

● **Pre-dissolution:**

Fully dissolve nucleotides in warm water at 40℃-60℃ (avoid boiling water or acidic liquid to prevent degradation). Add a small amount of product substrates for auxiliary dissolution and stir until completely clear.

● **Mother Liquor Dilution:**

Prepare high-concentration mother liquor, then add it into bulk liquid materials via spraying or pumping while stirring continuously for 10-15 minutes to ensure uniform dispersion.

● **Post-terminal Addition:**

Add nucleotides after liquid materials are sterilized and cooled if high-temperature sterilization is required. Never conduct high-temperature sterilization together with raw materials to avoid potency degradation.

3.2.2 Enzymolysis Prevention Measures

Fresh food substrates such as unsterilized raw soy sauce and meat paste may contain phosphatase which decomposes nucleotides and causes potency loss. It is recommended to conduct heat treatment (enzyme inactivation at ≥85℃ for no less than 5 minutes) on substrates in advance or add nucleotides in the final procedure after sterilization and enzyme inactivation.

3.3 Addition Processes for Special Systems

- **Low-temperature Systems (Wet process of infant formula powder, aquafeed)**: Control dissolution temperature at 50~60℃, conduct low-temperature concentration and spray drying after homogenization for infant formula powder; dissolve and spray then air-dry for aquafeed with temperature strictly controlled within limits.
- **High-temperature Processed Products (Puffed foods, fried foods)**: Forbid addition before high-temperature procedures including frying and baking above 180℃. Only add via late-stage low-temperature processes such as external sprinkling and spraying to retain potency.

Chapter 4 Standardized Addition Process Systems for Different Fields

4.1 Food Industry (Mainly for Seasoning)

4.1.1 Solid Compound Condiments (Chicken essence, chicken powder, halogen seasoning, hot pot base)

- **Addition Process**: Adopt three-stage carrier premixing process to eliminate segregation and stratification.
- **Process Control Points**: Workshop humidity ≤60%, normal-temperature mixing without heating; pre-treat agglomerated raw materials before feeding.
- **Internationally Accepted Dosage**: Chicken essence & chicken powder: 1.0~2.0 g/kg (0.1%~0.2%); solid compound seasoning powder: 0.1%~0.6%; hot pot base: 0.1%~0.3%.

4.1.2 Liquid Condiments (Soy sauce, oyster sauce, soup stock, compound seasoning liquid)

- **Addition Process**: Pre-dissolution process: warm water dissolution → fine filtration → post-addition after sterilization and cooling → low-speed homogenization → aseptic filling.
- **Core Control**: No co-sterilization with raw materials; only add at the final cooking stage for acidic seasoning liquid (pH < 5.0) to prevent degradation.
- **Reference Dosage**: Brewed soy sauce: 0.3~0.5 g/kg; oyster sauce: 0.3~0.5 g/kg; compound concentrated soup: 0.10%~0.25%.

4.1.3 Meat Product Processing (Fresh meat products, cooked braised meat, sausages, canned food)

- **Preferred Process**: Dissolve nucleotides in ice water to prepare pickling solution for low-temperature tumbling penetration without extra addition in cooking stage; conduct enzyme inactivation for fresh meat paste in advance.
- **Temperature Control**: Control tumbling temperature at 2~8℃ to avoid nucleotide decomposition by endogenous meat hydrolase; limit cooking temperature below 100℃ to retain potency.
- **Reference Dosage**: Ham & sausage: 0.06~0.10 g/kg; canned meat: 0.03~0.06 g/kg; braised meat products: 0.1~0.2%.
- **Application Advantages**: Remove fishy smell, enrich meat flavor, improve meat texture and reduce processing peculiar odor.

4.1.4 Convenience and Snack Foods (Puffed foods, instant noodles, freeze-dried foods)

- **Addition Process**: Premix and attach with external seasoning powder via three-stage premixing; prohibit addition before instant noodle embryo frying.
- **Reference Dosage**: External seasoning powder: 0.04%~0.15%; instant noodle seasoning: 3.0~6.0 g/kg.

4.1.5 Protein Drinks and Plant-based Beverages

Add at low temperature after sterilization for neutral protein drinks (pH 6.0~7.2); addition is not recommended for strongly acidic fruit drinks (pH < 4.0) due to poor stability, easy degradation and bitter taste formation.

4.2 Special Addition Specifications for Infant Formula Foods and Special Diets

4.2.1 National and international mandatory dosage limits:

In accordance with GB 14880 and international general principles for infant formula foods, the total addition amount of nucleotides in formula foods shall be 0.12~0.58 g/kg, and over-dosage is strictly forbidden.

4.2.2 Addition methods for two mainstream production processes

- Wet spray process: Fully dissolve in liquid materials at 50~60℃ → low-pressure homogenization → low-temperature concentration → temperature-controlled spray drying with inlet air temperature controlled below 175℃ to avoid high-temperature degradation.
- Dry blending process: Feed high-uniformity premix after base powder is fully cooled to normal temperature in clean workshops with low humidity (≤55%).

4.2.3 Functional Orientation:

Promote infant intestinal development, enhance immunity and optimize nutrient absorption and metabolism, conforming to breast milk nutritional structure.

4.2.4 Differentiated ratios for cross-border export:

Adopt relatively low dosage for EU market and breast-milk-simulated standard ratio for Southeast Asia and domestic markets.

4.3 Professional Application Solutions for Livestock and Aquafeed Industry

4.3.1 Core Application Values:

Alleviate weaning stress, repair intestinal damage, increase feed intake, reduce breeding mortality rate, shorten marketing cycle and improve meat and egg quality.

4.3.2 Standardized Dosage by Category

- Piglet feed: High dosage addition during weaning period: 0.01%~0.05% by dry weight (0.1~0.5 g/kg complete feed).
- Poultry feed: 0.08~0.30 g/kg for growth promotion of meat poultry and egg quality improvement of laying poultry.
- Aquaculture: Prioritized for fry and shrimp larvae at 0.10%-0.20%, with feed mixing and spraying as the optimal process.
- Ruminant feed: Targeted addition at 0.2~0.4 g/kg in milk replacer for young calves and lambs.

4.3.3 Feed Processing Taboos:

Control feed conditioning and pelleting temperature ≤85℃; adopt post-spraying process for high-temperature expanded feed to maintain activity; dissolve nucleotides in pure water and evenly spray on aquafeed surface then air-dry before feeding for maximum utilization rate.

4.4 Application in Functional Health Foods and Dietary Supplements

- **Solid Dosages (Tablets, granules, meal replacement powder)**:

  Conduct three-stage premixing with vitamins, minerals, probiotics and dietary fiber to form stable compound systems with dosage of 0.10%-0.40%.

- **Liquid Dosages (Oral liquid, energy drinks, sports nutritional drinks)**:

  Prepare at low temperature in neutral systems (pH 5.5~7.0), conduct low-speed homogenization after pre-dissolution and fill away from light to extend shelf stability with dosage of 0.05%~0.1%.

- **Core Efficacy Orientation**:

  Immune regulation, intestinal care, post-operation physical recovery, sub-health metabolism conditioning and nutritional supplementation for middle-aged and elderly people.

4.5 Extended Application in Biomedicine and Daily Chemicals

- **Pharmaceutical Field**:

  Pharmaceutical-grade monomer nucleotides are used in biological culture media, nutritional infusion preparations and clinical auxiliary nutritional diets with daily adult dosage of 0.1~1 g subject to medical advice and prescriptions, adopting aseptic dissolution, sterile filtration and low-temperature filling processes.

- **Daily Chemical Skin and Hair Care Field**:

  Add in low dosage (0.05%~0.5%) to repair skin barriers and improve damaged hair conditions, compatible with mild non-strong acid formulas and incompatible with strong reducing agents.

Chapter 5 Formula Synergy and Dosage Control

5.1 Synergistic Effect

I+G presents strong umami synergism with MSG. Combined use can multiply umami intensity, greatly cut down MSG dosage, enrich taste mellow sense and eliminate bitter aftertaste caused by single MSG application.

Recommended Ratio: The dosage of I+G is 1% ~ 5% of MSG, namely 1-5 kg I+G per 100 kg MSG, and the optimal ratio of MSG to I+G is 17:1 suitable for all seasoning scenarios.

5.2 Reference Typical Dosage by Field (International Version)

Application Category	Subdivided Products	Preferred Nucleotide Type	Standard Dosage Range	Core Addition Process
Seasoning Food	Chicken essence & powder	I+G	1.0~2.0 g/kg	Three-stage dry premixing
Seasoning Food	Soy sauce & oyster sauce	I+G	0.3~0.5 g/kg	Pre-dissolution after sterilization
Meat Products	Sausages & braised meat	I+G	0.06~0.12 g/kg	Dissolve in low-temperature pickling solution (enzyme inactivation first)
Infant Formula Food	Infant formula powder	Compound monomer nucleotides	0.12-0.58 g/kg	Low-temperature wet/dry three-stage premixing
Piglet Feed	Weaned piglet feed	Yeast compound nucleotides	0.1~0.5 g/kg	Premix compatibility
Aquafeed	Adult fish & shrimp feed	Compound nucleotides	0.10%-0.20%	Post-spray adhesion & air-drying
Functional Food	Solid nutritional powder	Nutritional nucleotides	0.10%-0.40%	Carrier three-stage premixing
Convenience Food	Instant noodle seasoning	I+G	3.0~6.0 g/kg	Premix then blend with seasonings

5.3 Key Dosage Control Points

- Strictly abide by industry dosage limits; excessive addition leads to bitter taste, peculiar flavor and increased compliance risks.
- Adopt three-stage premixing process for trace addition (< 0.5%) to ensure uniform mixing and avoid local over-concentration affecting taste and potency.
- Adjust dosage for cross-border exported products in accordance with local regulations to meet regional limit standards.

5.4 Compatibility Taboos and Notes

- Avoid direct contact with strong oxidants and long-term exposure to environments with pH < 4.0 or pH > 8.5.
- Stable in high-salt and high-protein systems without extra stabilizers required; shorten storage time after addition in acidic systems to prevent degradation.
- Avoid direct contact with raw meat and plant crude extracts containing phosphatase; conduct enzyme inactivation treatment in advance to prevent nucleotide decomposition.

Chapter 6 Industry-leading Universal Operation Control System

6.1 Graded Temperature Control Criteria (Core Potency Protection)

- Optimal Dissolution Temperature: 40~60℃ for fast dissolution without degradation.
- Safe Processing Temperature: ≤100℃ for short-term cooking with potency retention rate ≥90%.
- Dangerous Inactivation Temperature: Long-term heating above 120℃ causing rapid potency degradation with retention rate below 60%.
- Absolutely Prohibited Process: Direct addition before high-temperature frying and baking above 180℃ leading to complete potency loss.

6.2 Graded pH Adaptation Criteria

- Optimal Stability Range: pH 5.0~7.2 with the highest potency retention rate applicable to all scenarios.
- Weak Acid Adaptation Range: pH 4.0~5.0, only for short-term terminal addition without long-term storage.
- Prohibited Range: Strong acid systems with pH < 4.0 prone to degradation, not recommended for addition.

6.3 Mixing Uniformity Control Principles

- Mandatory three-stage carrier gradual premixing for addition dosage below 0.5% to avoid segregation and stratification.
- Prioritize V-type mixers and three-dimensional motion mixers for powder mixing with mixing duration over 20 minutes; adopt low-speed stirring homogenization for liquid systems to prevent degradation caused by high-speed shearing.
- Conduct random sampling uniformity inspection after mixing to meet downstream production quality standards.

6.4 Scientific Solutions to Common Production Abnormalities

- Weak finished product umami: Verify MSG-I+G ratio, check processing temperature and confirm addition sequence.
- Slight bitter taste: Caused by excessive nucleotide degradation, over-dosage or long-term storage in acidic environment; solve by reducing dosage, adjusting pH value and implementing terminal addition.
- Powder agglomeration and moisture absorption: Resulted from high workshop humidity and unsealed raw material storage; improve environmental humidity control,

pre-treat agglomerated materials and strengthen sealed storage.

- Declined nutritional activity: Caused by early high-temperature feeding and direct compatibility with enzymatic raw materials; adjust feeding timing to low-temperature terminal addition and inactivate enzymes for enzymatic substrates.
- Uneven mixing: Due to unimplemented three-stage premixing and insufficient mixing time; strictly execute standardized premixing process, extend mixing time and conduct uniformity sampling test.

6.5 Globally Accepted Labeling Specifications

- **Chinese Market Labeling**: Disodium flavor nucleotides, Disodium 5'-Inosinate, Disodium 5'-Guanylate, Nucleotides labeled according to product categories.
- **English Export Labeling**: Disodium 5'-Inosinate (IMP), Disodium 5'-Guanylate (GMP), Nucleotides Compound.
- Mark specific monomer nucleotide names and total addition amount on infant formula food labels complying with local relevant laws and regulations.
- Label feed, pharmaceutical and daily chemical products in line with international industry standards with clear indication of raw material grade and dosage.

6.6 International Warehousing and Logistics Standards (Raw Material Quality Assurance)

- **Packaging Requirements**: Double-layer moisture-proof sealed packages (aluminum foil bag + fiber drum) marked with moisture-proof, light-proof and sealed storage signs.
- **Warehousing Requirements**: Constant-temperature cool storage at 15~25℃ with ambient relative humidity ≤55%, away from light, dust and peculiar odor; prohibit co-storage with strong acid and strong oxidant raw materials.
- **Transportation Requirements**: Prevent container condensation and moisture during sea export; avoid direct sunlight and rain exposure in land transportation; no mixed transportation with toxic and harmful substances and keep full sealing throughout transit.
- **Shelf Life**: 24 months for unopened products; finish use within 15 days after opening with detailed opening records kept.

Chapter 7 Supplementary Provisions

This guideline serves as a classic universal professional industry template integrating internationally accepted processes and downstream practical operation demands, which can be directly applied as enterprise internal SOP documents, technical disclosure materials for clients, foreign trade technical documents and cross-border market formula filing references.

Enterprises may appropriately adjust parameters within the specified standard range according to self-owned equipment processes, target market regulations and product positioning to ensure compliance and practical operability.

Combining oriental catering seasoning theories, European and American nutritional dietary standards and global feed breeding application systems, this guideline possesses universal professionalism and authority covering all channels to satisfy demands of domestic and overseas downstream clients.

This guideline will be revised and updated timely in accordance with global regulatory updates and industrial technological upgrades to guarantee scientificity and timeliness of all contents.