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ORIGINAL SUBMISSION

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January 12, 2004

04-02-03P03:20 RCVD

Robert Martin, Ph.D.
Deputy Division Director
Division of Biotechnology and GRAS Notification Review
Office of Food Additive Safety, HFS-200
Center for Food Safety and Applied Nutrition
US Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
T: 202-418-3074
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Dear Dr. Martin,

In accordance with proposed 21 CFR §170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18937-18964), I am submitting in triplicate, as the representative of the notifier, Linguagen Corp, 2005 Eastpark Boulevard, Cranbury, NJ 08512-3515, a GRAS notification of AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts) as a food and beverage flavor enhancer (flavor modifier), at specified levels that would result in a total added AMP consumption not to exceed 680 mg *per* day as AMP. A full copy of the GRAS panel report, as defined in 21CFR§170.30, setting forth the basis for the GRAS determination, and CV's of the members of the GRAS panel for review by the Agency, are also enclosed.

Sincerely

James C. Griffiths, Ph.D., DABT, CBiol FIBiol
Director of Toxicology
Burdock Group

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1. GRAS Exemption Claim

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1).

AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts) has been determined to be generally recognized as safe (GRAS) and, therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections

James C. Griffiths, Ph.D., DABT, CBiol FIBiol
Director of Toxicology
Burdock Group

1/28/04
Date

(i) Name and Address of the Notifier

James C. Griffiths, Ph.D., DABT, CBiol FIBiol
Director of Toxicology
Burdock Group
780 US Highway 1, Suite 300
Vero Beach, Florida 32962

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(ii) Common Name of the Notified Substance

AMP; AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts).

(iii) Conditions of Use

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AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts) used as a food and beverage flavor enhancer (flavor modifier), at specific use levels that would result in a total added AMP consumption not to exceed 680 mg *per* day as AMP. The specific food categories to which this material will be added, the 21 CFR regulatory citation and the use levels are: Chewing gum, including all forms (21CFR§170.3(n)(6))-173 ppm; Coffee and tea, including regular, decaffeinated, and instant types (21CFR§170.3(n)(7))-173 ppm; Snack foods, including chips, pretzels, and other novelty snacks (21CFR§170.3(n)(37))-800 ppm; Soups and soup

mixes, including commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes (21CFR§170.3(n)(40))-173 ppm; Sugar substitutes, including granulated, liquid, and tablet sugar substitutes (21CFR§170.3(n)(42))-400 ppm; Salt substitute (potassium chloride) – 400 ppm.

AMP will qualify as an ingredient exempted from the requirement for declaration on the food label/labeling under 21CFR§101.4(b)(1) as it is a flavoring under the definitions of 21CFR§101.22.

The estimated mean and 90th percentile intake of AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts) by the total population from all proposed uses at the maximum use levels as a food and beverage flavor enhancer (flavor modifier) was determined to be 149 and 307 mg/person/day, respectively. Purines, the class of compounds that includes AMP, are currently consumed in the diet at mean and 90th percentile intake levels of 560 and 1120 mg purines/person/day, respectively. Combining the current and added intakes gives a total mean purine consumption. The estimated total mean and 90th percentile consumption of purine, if AMP is added to the selected foods at the levels specified in the preceding paragraph, would be 709 mg/day and 1427 mg/day, respectively.

(iv) Basis of the GRAS Determination

Pursuant to 21CFR §170.3, AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts) has been determined GRAS by scientific procedures for its intended conditions of use. The safety of AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts) is supported by the widespread intentional use of purines in the diet, *i.e.*, purines have been used as flavoring agents in Japan since antiquity. The primary constituent in the traditional Japanese seasoning, dried bonito, is inosine 5'-monophosphate (IMP). Commercial production of IMP and guanosine 5'-monophosphate (GMP) as food flavoring agents began in Japan in 1960. They are produced either by hydrolysis of purified yeast RNA followed by purification or by chemical synthesis. The European Community has approved the nucleotide acids and sodium salts of AMP, GMP, IMP, CMP (cytidine 5'-monophosphate) and UMP (uridine 5'-monophosphate) as food additives that may be added for specific nutritional purposes in foods for particular nutritional uses. In the United States, AMP, CMP, UMP and disodium GMP are added to some infant formulas.

This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of substances used as ingredients in food. The experts (and attached curriculum vitae) for this GRAS determination were:

Joseph F. Borzelleca, Ph.D., F.A.T.S.
Walter H. Glinsman, M.D.
John A. Thomas, Ph.D., F.A.T.S.

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(v) Availability of Information

The data and information that serve as a basis for this GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at a reasonable time at the office of:

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And/or

George A. Burdock, Ph.D., DABT, FACN
President
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Alternatively, copies of data and information can be provided to FDA upon request, by contacting Dr. James Griffiths.

2. Detailed Information about the Identity of the Notified Substance

A. Identity.

AMP is an endogenous purine nucleotide found in all living organisms. AMP is composed of the purine base adenine, covalently bound to a pentose sugar, forming adenosine, which is esterified with phosphoric acid in equilibrium to salt form with normal physiological and/or biochemical buffering ions, most notably sodium (Figure 1), such that the free acid nucleotide equilibrates in normal physiological and/or biochemical buffering systems to a salt form. One could either use the free acid form or the salt form as a food ingredient, knowing that the interconversion between the free acid and the salt occurs in the mixture and final food in relation to the buffering conditions of the milieu. The exposure and safety assessment in this document are applicable to AMP irrespective of the degree of dissociation of the equilibrium at the time of safety testing and/or food addition. For the remainder of this document, the term "AMP" will refer to all normal physiological and biochemical forms of AMP from this equilibrium.

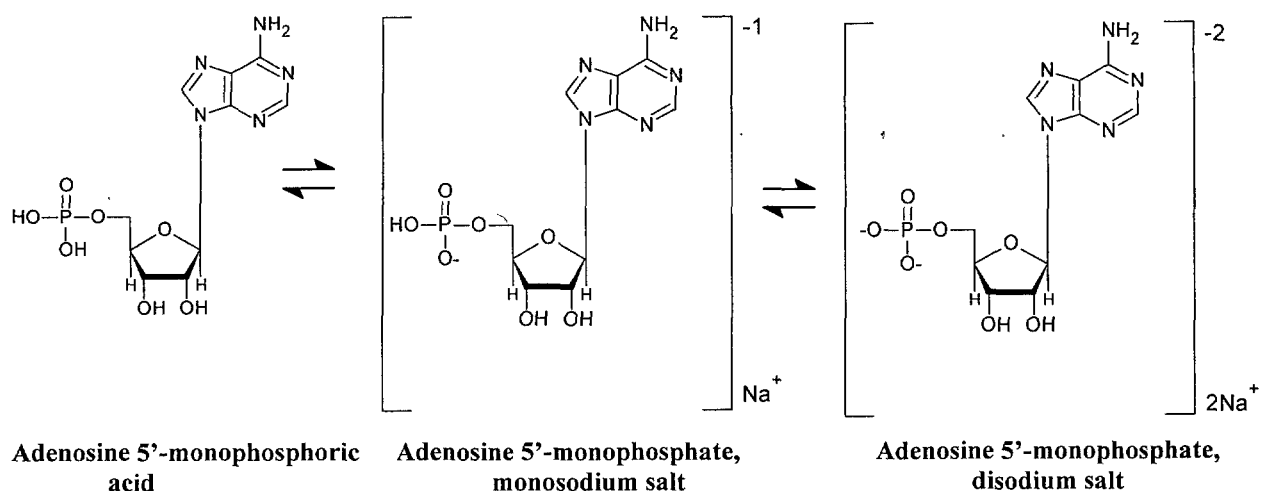


Figure 1. Chemical structure of AMP (*i.e.*, adenosine 5'-monophosphate/adenosine 5'-monophosphoric acid/adenosine 5'-monophosphate, monosodium salt/adenosine 5'-monophosphate, disodium salt)

Table 1 provides all CAS numbers identified as having been associated with AMP. As will be noted, AMP is used as a synonym for the acid form (phosphoric acid) as well as generic salt (phosphate) and specific salts (mono- and disodium salts).

Table 1. CAS Numbers, Names and Synonyms for AMP

CAS Reg. No.	Name	Synonym	Synonym
61-19-8	Adenosine monophosphate	AMP ¹	Adenosine 5'-monophosphoric acid ²
18422-05-4	Adenosine 5'-monophosphoric acid	AMP ³	
149022-20-8	Adenosine 5'-monophosphate sodium salt	AMP ⁴	Adenosine 5'-monophosphate, sodium salt from yeast ⁵ Adenosine 5'-monophosphate, disodium salt ⁶
162756-82-3	Alternate CAS	AMP ⁷	
47286-65-7	Alternate CAS	AMP ⁸	
47287-97-8	Alternate CAS	AMP ⁹	
53624-78-5	Alternate CAS	AMP ¹⁰	
67583-85-1	Alternate CAS	AMP ¹¹	

¹ = <http://chemfinder.cambridgesoft.com/result.asp>; ² = <http://chem.sis.nlm.nih.gov/chemidplus>

³ = http://www.apolloscientific.co.uk/products/lifescience/otherProducts_Lifesciences_AMP.htm;

⁴ = <http://sigma-reporter.co.uk/pdfs/eChrome/T196905.pdf>; ⁵ = <http://www.rose-hulman.edu/chemistry/000000/000473.pdf>;

⁶ = <http://www.seqchem.com/catalogue.php>; ⁷ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>;

⁸ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>; ⁹ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>;

¹⁰ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>; ¹¹ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>;

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The general chemical descriptions for AMP, and its sodium salts are listed in Table 2.

Table 2. General chemical description of AMP and its sodium salts.

Synonyms	Adenosine 5'-monophosphate/ Adenosine 5'-monophosphoric acid/ Adenosine 5'-monophosphate, monosodium salt/ Adenosine 5'-monophosphate, disodium salt (See Table 1)
Functional use	Flavor enhancer (flavor modifier)
CAS Reg. No.	See Table 1
Chemical formula	$C_{10}H_{14}N_5O_7P$; $C_{10}H_{13}N_5O_7PNa$; $C_{10}H_{12}N_5O_7PNa_2$
Molecular weight	347; 369; 391

CAS = Chemical Abstracts Service

B. Composition.

AMP is primarily isolated from hydrolyzed yeast (e.g., *Saccharomyces cerevisiae* or *Candida utilis*, or similar food approved strain) ribonucleic acid (RNA). The AMP is isolated by ion-chromatographic separation using aqueous buffers and dried into a crystallized 99% pure AMP powder.

C. Method of Manufacture.

AMP is manufactured according to current Good Manufacturing Practice (cGMP) with all the reagents used in the process conforming to FCC¹ specifications. Key elements of the primary manufacturing process are diagramed in Figure 2. AMP is isolated from hydrolyzed yeast (e.g., *Saccharomyces cerevisiae* or *Candida utilis*, or similar food approved strain) ribonucleic acid (RNA). The yeast is grown in fermentation culture, pelleted and autolysed by exposure to salt and heat. This process produces an RNA-rich yeast extract. The RNA is hydrolyzed with pancreatic ribonuclease (RNase) enzyme, which liberates the AMP. The AMP is isolated by ion-chromatographic separation using aqueous buffers and dried into a crystallized 99% pure AMP powder.

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¹ Food Chemicals Codex

Figure 2. Manufacturing process for AMP.

D. Specifications for Food Grade Material.

Batch analysis from four different lots indicates that the current manufacturing process consistently produces AMP that meets the specifications indicated in Table 3, which are equivalent to the FCC specifications for disodium inosinate, an analogous nucleotide (FCC, 1996).

Table 3. Adenosine 5'-monophosphate specifications

Test	Specification	Result (N=4)
Appearance	White powder	Conforms
Identification/Assay	HPLC	Passed
Purity	Minimum 95% based on HPLC and enzymatic assays	99%
Clarity and Color of Solution	Passes test	Passed
Other nucleotides and amino acids	Maximum 5%	Passed
Ammonia salts	Passes test	Passed
pH	5.0-6.0	Passed
Barium	Not more than 0.015%	<0.0001%
Lead	Not more than 10 mg/kg.	<0.50 ppm
Heavy Metals (as Pb)	Not more than 0.002%.	<0.50 ppm

ppm=parts per million

3. Self Limiting Levels of Use.

As AMP levels increase it contributes a strong 'savory, umami-like flavor' that can result in undesired off-flavor notes in many applications. The end result is that if AMP is added to a food above its technologically self-limiting level, the food becomes unpalatable.

4. Basis of the GRAS Determination.

The determination that AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts) is GRAS is on the basis of scientific procedures. A full copy of the dossier - "Generally Recognized As Safe (GRAS) Status of AMP (Adenosine 5'-Monophosphoric acid and its monosodium and disodium salts) as a Food Flavor Enhancer (Flavor Modifier)" - as reviewed by the expert panel members, is attached.

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**GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF
AMP (ADENOSINE 5'-MONOPHOSPHORIC ACID AND ITS
MONOSODIUM AND DISODIUM SALTS) AS A FOOD FLAVOR
ENHANCER (FLAVOR MODIFIER)**

August 29, 2003

Panel Members

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**GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF AMP
(ADENOSINE 5'-MONOPHOSPHORIC ACID AND ITS MONOSODIUM
AND DISODIUM SALTS) AS A FOOD FLAVOR ENHANCER (FLAVOR
MODIFIER)**

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GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF AMP (ADENOSINE 5'-MONOPHOSPHORIC ACID AND ITS MONOSODIUM AND DISODIUM SALTS) AS A FOOD FLAVOR ENHANCER (FLAVOR MODIFIER)

1. SUMMARY

The undersigned, independent panel of recognized experts (hereinafter referred to as the Expert Panel)¹, qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was requested by Linguagen Corp. to determine the Generally Recognized As Safe (GRAS) status of AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts) used as a food and beverage flavor enhancer (flavor modifier), at specified levels that would result in a total added AMP consumption not to exceed 680 mg *per* day as AMP. A comprehensive search of the literature for safety and toxicity information on AMP, and its sodium salts and, related purines was conducted by Burdock Group in September 2002 and is summarized in this report. This search and supporting documentation were made available to the Expert Panel. In addition, the Expert Panel independently evaluated materials submitted by Linguagen Corp. and other materials deemed appropriate and necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

2. INTRODUCTION

AMP is an endogenous purine nucleotide found in all living organisms. AMP is composed of the purine base adenine, covalently bound to a pentose sugar, forming adenosine, which is esterified with phosphoric acid in equilibrium to salt form with normal physiological and/or biochemical buffering ions, most notably sodium (Figure 1).

Table 1 provides all CAS numbers identified as having been associated with AMP. As will be noted AMP is used as a synonym for the acid form (phosphoric acid) as well as generic salt (phosphate) and specific salts (mono- and disodium salts).

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¹ Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, as amended. See also attachments (*curriculum vitae*) documenting the expertise of the Panel members.

Table 1. CAS Numbers, Names and Synonyms for AMP

CAS Reg. No.	Name	Synonym	Synonym
61-19-8	Adenosine monophosphate	AMP ¹	Adenosine 5'-monophosphoric acid ²
18422-05-4	Adenosine 5'-monophosphoric acid	AMP ³	
149022-20-8	Adenosine 5'-monophosphate sodium salt	AMP ⁴	Adenosine 5'-monophosphate, sodium salt from yeast ⁵ Adenosine 5'-monophosphate, disodium salt ⁶
162756-82-3	Alternate CAS	AMP ⁷	
47286-65-7	Alternate CAS	AMP ⁸	
47287-97-8	Alternate CAS	AMP ⁹	
53624-78-5	Alternate CAS	AMP ¹⁰	
67583-85-1	Alternate CAS	AMP ¹¹	

¹ = <http://chemfinder.cambridgesoft.com/result.asp>; ² = <http://chem.sis.nlm.nih.gov/chemidplus>

³ = http://www.apolloscientific.co.uk/products/lifescience/otherProducts_Lifesciences_AMP.htm;

⁴ = <http://sigma-reporter.co.uk/pdfs/eChrome/T196905.pdf>; ⁵ = <http://www.rose-hulman.edu/chemistry/000000/000473.pdf>;

⁶ = <http://www.seqchem.com/catalogue.php>; ⁷ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>;

⁸ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>; ⁹ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>;

¹⁰ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>; ¹¹ = <http://www.cdc.gov/niosh/rtecs/au7224b4.html>;

This unconventional nomenclature may, in part, also stem from the ease in which a free nucleotide acid equilibrates in normal physiological and/or biochemical buffering systems to a salt form. One could either use the free acid form or the salt form as a food additive, knowing that the interconversion between the free acid and the salt occurs in the mixture and final food in relation to the buffering conditions of the milieu. The exposure and safety assessment in this document are applicable to AMP irrespective of the 'side' of the equilibrium at the time of safety testing and/or food addition. For the remainder of this document, the term "AMP" will refer to all normal physiological and biochemical forms of AMP from this equilibrium.

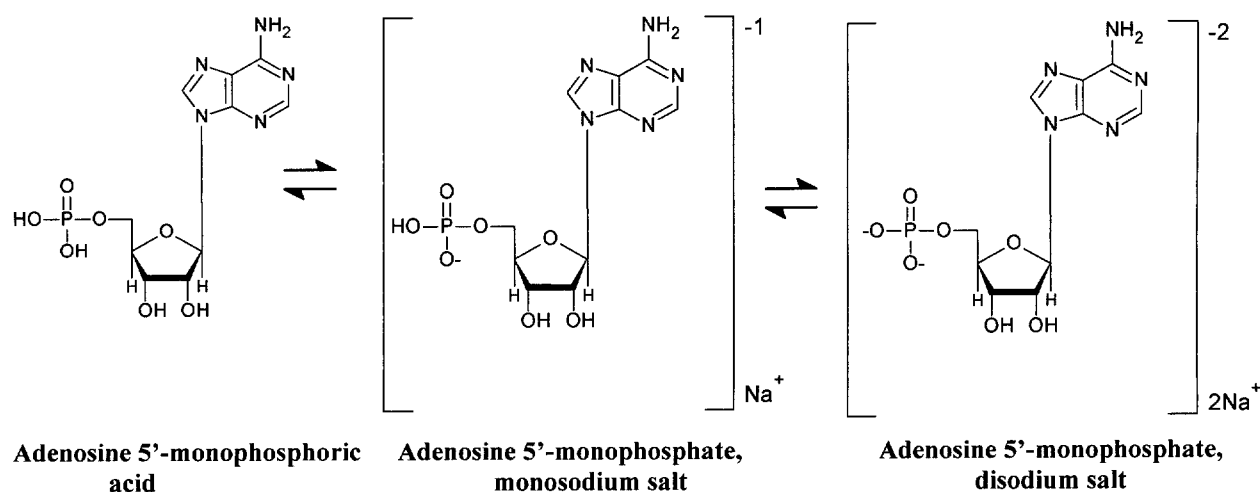


Figure 1. Chemical structure of AMP (*i.e.*, adenosine 5'-monophosphate/adenosine 5'-monophosphoric acid/adenosine 5'-monophosphate, monosodium salt/adenosine 5'-monophosphate, disodium salt)

Purines have been used as flavoring agents in Japan since ancient times. The primary constituent in the traditional Japanese seasoning, dried bonito, is inosine 5'-monophosphate (IMP) (Kojima, 1974). Commercial production of IMP and guanosine 5'-monophosphate (GMP)

as food flavoring agents began in Japan in 1960. They are produced either by hydrolysis of purified yeast RNA followed by purification or, by chemical synthesis (Kojima, 1974).

The European Community has approved the nucleotide acids and sodium salts of AMP, GMP, IMP, CMP (cytidine 5'-monophosphate) and UMP (uridine 5'-monophosphate) as food additives that may be added for specific nutritional purposes in foods for particular nutritional uses (EC, 2001). In the United States, AMP, CMP, UMP and disodium GMP are added to some infant formulas (Abbott Laboratories, 2002).

The general chemical descriptions for AMP, and its sodium salts are listed in Table 2.

Table 2. General chemical description of AMP and its sodium salts.

Systemic name	Adenosine 5'-monophosphate/ Adenosine 5'-monophosphoric acid/ Adenosine 5'-monophosphate, monosodium salt/ Adenosine 5'-monophosphate, disodium salt (See Table 1)
Synonyms	See Table 1
Functional use	Flavor enhancer (flavor modifier)
CAS Reg. No.	See Table 1
Chemical formula	$C_{10}H_{14}N_5O_7P$; $C_{10}H_{13}N_5O_7PNa$; $C_{10}H_{12}N_5O_7PNa_2$
Molecular weight	347; 369; 391
CAS = Chemical Abstracts Service	

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3. MANUFACTURING PROCESS AND SPECIFICATIONS

Figure 1. Manufacturing process for adenosine 5'-monophosphate (AMP)

Linguagen Corp. does not currently manufacture AMP. Rather, purified AMP is available as an item of commerce that may be obtained from specialty ingredient suppliers. Linguagen Corp.'s current supplier is Sigma-Aldrich, St. Louis, MO. It is currently prepared from yeast using a method similar to the one Linguagen Corp. proposes to use for future commercial production. Linguagen Corp. will manufacture AMP using current Good Manufacturing Practice with all the reagents used in the process conforming to FCC² specifications. Key elements of the manufacturing process are diagramed in Figure 1. AMP is isolated from hydrolyzed yeast (*e.g.*, *Saccharomyces cerevisiae* or *Candida utilis*, or similar food approved strain) ribonucleic acid (RNA). The yeast is grown in fermentation culture, pelleted and autolysed by exposure to salt and heat. This process produces an RNA-rich yeast extract. The RNA is hydrolyzed with pancreatic ribonuclease (RNase) enzyme, which liberates the AMP. The AMP is isolated by ion-chromatographic separation using aqueous buffers and dried into a crystallized 99% pure AMP powder. Batch analysis from four different lots (See Appendix

² Food Chemicals Codex

1; (ABC, 2003))³ indicates that the current manufacturing process consistently produces AMP that meets the specifications indicated in Table 3, which are equivalent to the FCC specifications for disodium inosinate, an analogous nucleotide (FCC, 1996).

Table 3. Adenosine 5'-monophosphate specifications (See Appendix 1; ABC, 2003; Sigma-Aldrich, 2003)*

Test	Specification	Result (N=4)
Appearance	White powder	Conforms
Identification/Assay	HPLC	Passed
Purity	Minimum 95% based on HPLC and enzymatic assays	99%
Clarity and Color of Solution	Passes test	Passed
Other nucleotides and amino acids	Maximum 5%	Passed
Ammonia salts	Passes test	Passed
pH	5.0-6.0	Passed
Barium	Not more than 0.015%	<0.0001%
Lead	Not more than 10 mg/kg.	<0.50 ppm
Heavy Metals (as Pb)	Not more than 0.002%.	<0.50 ppm

* Test methods available on request from Linguagen Corp.; ppm=parts *per* million

4. ESTIMATED DAILY INTAKE

The intake profile (amount and frequency) by individuals in USDA's Continuing Survey of Food Intakes by Individuals 1994-96, 98 (CSFII; USDA, 1998) was used to calculate the estimated daily intake (EDI) of AMP for individuals consuming the food groups selected for the addition of this AMP for this GRAS evaluation. These food groups as defined by the FDA (21 CFR 170.3(n)) are listed in Table 4.

Table 4. Maximum intended use levels of AMP

Food Category*	Intended use level (ppm)
Chewing gum, including all forms** (6)	173
Coffee and tea, including regular, decaffeinated, and instant types (7)	173
Snack foods, including chips, pretzels, and other novelty snacks (37)	800
Soups and soup mixes, including commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes (40)	173
Sugar substitutes, including granulated, liquid, and tablet sugar substitutes (42)	400
Salt substitute (potassium chloride)	400

*The food categories correspond to those listed in 21 CFR 170.3(n). **The number in parenthesis following each food category is the paragraph listing in 21 CFR 170.3(n) for that food category.

³ At the time this document was written, only four lots had been manufactured by the supplier (Sigma-Aldrich).

The means and 90th percentile EDIs were calculated for: (1) current purine intake from natural sources (current); (2) purine intake following addition of AMP to the selected food groups (added) and; (3) total estimated EDI from natural sources combined with levels from addition to the foods (total).

The mean purine consumption in the U.S., 560 mg/day, was calculated by Kojima (1974). The 90th percentile EDI of purine can be estimated by assuming two times greater consumption than the reported mean EDI (DiNovi and Kuznesof, 1995). Thus, the estimated 90th percentile EDI is 1120 mg purine *per day*.

If AMP is added to the selected foods at the levels specified in Table 4, the added mean and 90th percentile purine consumption will be 149 mg/day and 307 mg/day, respectively.

Combining the current and added intake levels gives the total mean purine consumption. The estimated total mean and 90th percentile consumption of purine, if AMP is added to the selected foods at the levels specified in Table 4, would be 709 mg/day and 1427 mg/day, respectively.

A statistical analysis of consumption of AMP from addition to potassium chloride (KCl) salt substitutes was not possible because consumption data are unavailable. Instead, consumption of AMP following addition to KCl salt substitutes was estimated from *per capita* consumption based upon average sales of salt substitutes from 2000 to 2002 (5,386,377 kg)⁴ (Linguagen, 2003). Many of the KCl salt substitute products sold are actually seasoning mixes containing only a fraction of KCl. Nevertheless, the *per capita* consumption estimate was determined with the assumption that all of the salt substitute sold was 100% KCl. Therefore, the estimated *per capita* consumption will most likely overestimate KCl consumption rather than underestimate KCl consumption. Using this conservative approach, the daily *per capita* consumption of AMP from addition to KCl salt substitutes at the level specified in Table 4 would be 0.009 mg/day⁵. Thus, the contribution of AMP from addition to salt substitutes to the total daily AMP consumption is negligible.

Table 5. Current purine intake, predicted purine intake following AMP addition to selected foods at the indicated levels (Table 4) and total purine intake (predicted + current) for individuals consuming foods selected for AMP addition

Purine intake from:	<i>Per User (mg/day)</i>	
	Mean	90 th Percentile
Current	560	1120
Possible maximum consumption following addition of AMP (Table 4)	149	307
Total	709	1427

⁴ This is an overestimation of KCl intake because it is based on the assumption that 100% of all salt substitutes consist of KCl, but in fact the leading brand is a seasoning blend that does not contain KCl.

⁵ Based on the 2000 US population of 281,421,906

Also considered was the addition of AMP in excipient formulations (21 CFR 170.3(o)(14)) in approved prescription drugs and approved over-the-counter (OTC) drugs. The Expert Panel believes consumption of AMP from its use in excipient formulations in prescription drugs and OTC drugs will be at levels that will not contribute to an overall AMP consumption that will exceed the acceptable daily intake as described in Section 6 (Risk Evaluation).

5. BIOLOGICAL DATA

The first step in AMP metabolism in the gastrointestinal (GI) tract is deamination of AMP to form 5'-inosine monophosphate (IMP) (Figure 3). Therefore, the studies summarized below are those on AMP, as well as its initial metabolite, IMP. In some of the studies, the amount of AMP or IMP in the diets was reported as a percentage of the diet, and in such cases, the levels were converted into mg/kg/day using comparative mammalian reference values for relative dose calculations established by the US Environmental Protection Agency (Derelanko and Hollinger, 2001; EPA, 1985). The average weight of humans was considered to be 60 kg.

5.1. Absorption, distribution, metabolism and elimination (ADME)

The normal human diet is abundant in DNA and RNA. Ribonucleic acids are degraded into nucleotides, nucleosides and free bases by the GI microflora and mucosa prior to absorption. A significant portion of AMP is degraded by intestinal flora or metabolized by GI mucosa before absorption. Up to fifty percent of radiolabeled dietary purine was degraded and lost as CO₂ within 30 minutes, with the remaining radiolabel being recovered in the urine (43%) and the feces (5%) (Simmonds, 1999). A majority of absorbed adenosine, its nucleotides and nucleosides, are rapidly degraded and converted to uric acid by the intestinal mucosal's battery of enzymes during passage and, released as such, in serosal secretions (Figure 3) (Brody, 1999; Simmonds, 1999). In non-primate mammals, hepatic uricase degrades uric acid into the extremely soluble allantoin (Simmonds, 1999). Because primates do not express uricase, uric acid is the end product of purine metabolism.

Wilson and Wilson (1965) observed complete degradation of AMP into uric acid and on to allantoin using isolated small segments of rat intestine from dams and their pups. This study demonstrated that all of the AMP degradation enzymes (nucleotide phosphorylase, adenosine deaminase, xanthine oxidase and presumably, uricase) were expressed in neonatal and adult rats. In an earlier report, these same authors noted that no detectable absorption of purines, including AMP, took place in the mucosa of segmented everted intestinal sacs of adult rats or hamsters (Wilson and Wilson, 1962). These studies indicate that purines are metabolized prior to or during intestinal absorption.

Following absorption, any intact purines and their metabolites are further rapidly metabolized and excreted. In pigs, up to 50% of radiolabelled dietary purine was degraded and expired as CO₂ within 30 min, the remaining 43% being recovered in the urine, with 5% in the feces (Simmonds, 1999). The half-life of [8¹⁴C] IMP was approximately 5 hours in male and pregnant (day 10 or 18 of gestation) rats given 25 mg/kg by gavage (Ohara *et al.*, 1973). IMP peak levels were measured at 2.5 hours with nearly complete elimination by 24 hours. About 70% of total radioactivity appeared in the urine, 6-7% in feces, none in expired air, between 0-2% remained in organs, 8-17% in the organ-free carcass and 0.77% in the fetuses.

When male rats (N=5/group; N=10/control; strain not identified) were fed 0, 500 or 2000 mg/kg/day IMP for five or ten days (Hashimoto and Ishii, 1973), there were no differences in urine or serum uric acid levels in the treated rats compared with controls. Most of the exogenously ingested IMP was rapidly excreted in the urine as allantoin. Liver hypoxanthine-guanine phosphoribosyl transferase and adenine phosphoribosyl transferase activity were increased along with the ratio of liver uricase/xanthine oxidase activity, suggesting IMP metabolism by shunt pathways.

Endogenously synthesized AMP is degraded to adenosine and hypoxanthine with the majority being reused for the synthesis of ATP in the purine salvage pathway. This is catalyzed by adenine phosphoribosyl transferase (PRPP) which converts adenine to AMP and pyrophosphate (Brody, 1999). AMP released from cells undergoes hydrolysis by ectoenzyme 5'-nucleotidase, a ubiquitous cell plasma membrane enzyme, producing adenosine (Gordon *et al.*, 1989). Any adenosine not taken back up into the cell or not used in the salvage pathway is degraded to its end product, uric acid, and excreted in the urine. AMP is also rapidly dephosphorylated to adenosine through the action of nonspecific phosphatases, such as alkaline phosphatase (Fox and Kelley, 1978).

Data on the serum uric acid levels in humans indicate that the mean values for normal men (N=969) and women (N=168) were 5.0 mg/dL and 4.04 mg/dL, respectively (Grayzel *et al.*, 1961). Several investigators have suggested an upper limit for serum uric acid that ranges from 6.8 mg/dL to 7.5 mg/dL for men and 5.7 mg/dL to 6.35 mg/dL for women (Gjörup *et al.*, 1955; Grayzel *et al.*, 1961; Zöllner, 1963). This is in agreement with uric acid's upper limit of solubility in blood of approximately 7 mg/dL (Maesaka and Fishbane, 1998). In adults, approximately 30% of the uric acid produced daily is excreted in the bile and degraded in the GI tract by bacteria through a process called uricolysis; the remaining 70% is excreted through the kidneys (Sörensen, 1960). Excretion of urinary uric acid over 24 hours was 8.1 mg/kg for men and 8.0 mg/kg for women (Cockburn, 1961).

Inhibitors of adenosine metabolism include methylxanthines, such as caffeine and the anti-asthmatic drug, theophylline, that have been reported to antagonize the metabolism of adenosine (Osswald *et al.*, 1995) and the ADP receptor antagonist, dipyridamole, reportedly slows the metabolism of adenosine by preventing cellular uptake (Crutchley *et al.*, 1980).

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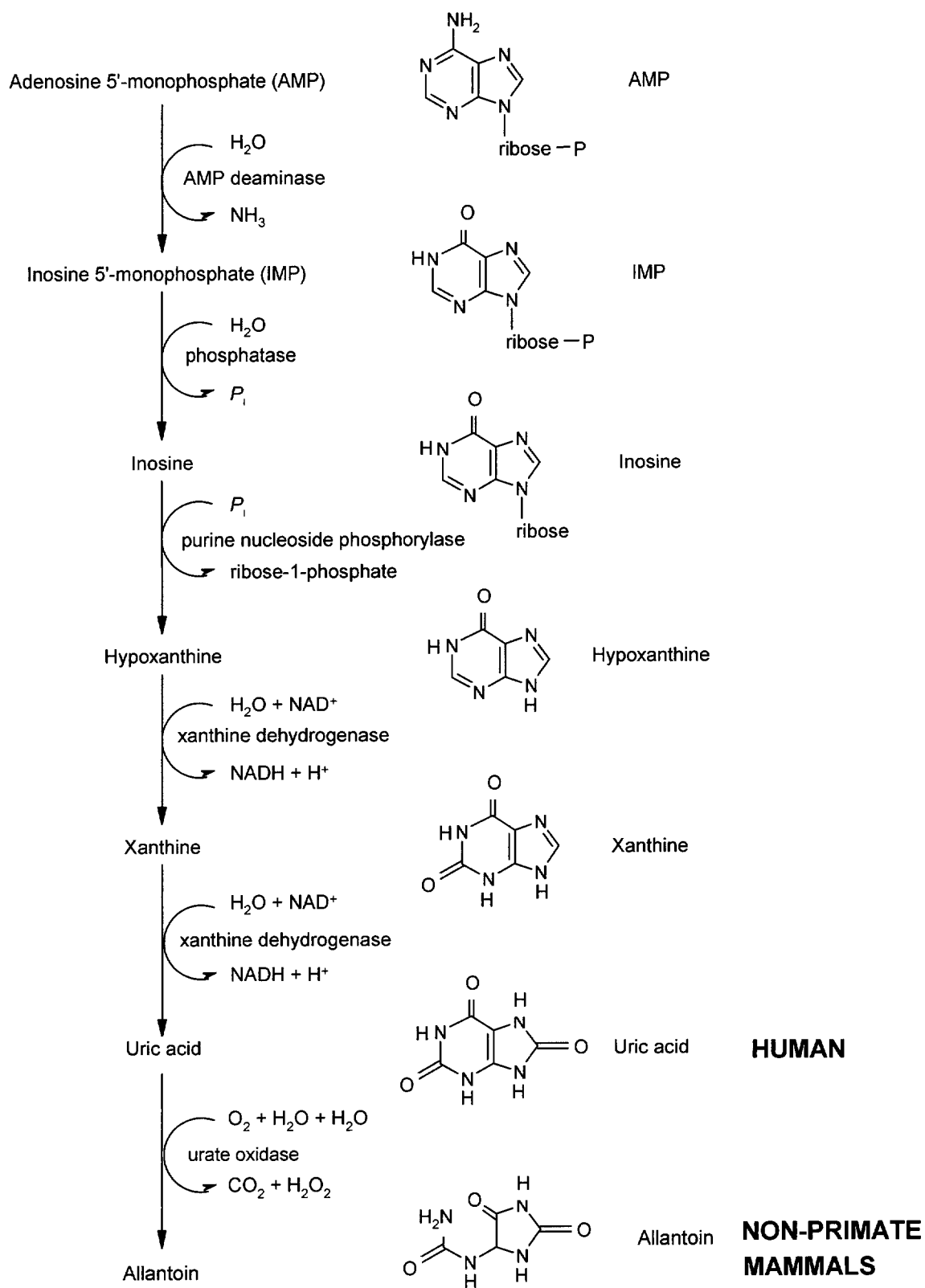


Figure 3. Metabolism of adenosine 5'-monophosphate (AMP) (Brody, 1999)

5.2. Biochemical/ Pharmacological effects

Purines are well-established cell signaling molecules that act through three families of purinergic receptors. These receptors are divided into the P1 receptors, mainly binding adenosine, and the ligand-gated ion-channel P2X receptors and P2Y G-protein-coupled receptors that have a greater affinity for ATP, ADP and UTP (reviewed in Burnstock, 2002). In the cardiovascular system, adenosine activation of P1 receptors affects local control of vessel tone and causes vasodilation. Intravenous infusion of 6 to 12 mg adenosine is currently used to reverse supraventricular tachycardia (PDR, 2002). In the kidney, binding of adenosine to P2 purinoceptors following *i.v.* infusion can cause vasoconstriction (Osswald *et al.*, 1995). Adenosine diphosphate (ADP) binding to P2 receptors induces platelet aggregation (Gachet, 2001).

Inhalation of adenosine reportedly elicits a concentration-dependent airflow limitation in asthmatics possibly through activation of P1 receptors on intermediary inflammatory cells such as mast cells and afferent nerve endings (Van Schoor *et al.*, 2000). Antagonism of P1 receptors is currently being pursued for novel anti-asthma medications and is the underlying mechanism for the activation of theophylline (Burnstock, 2002). Consumption of AMP has not induced bronchiolar constriction in asthmatics, thus AMP's asthma-inducing effects following inhalation are not relevant to assessing the safety of AMP as a food ingredient.

5'-Inosine monophosphate (IMP)-induced hypotension in the rabbit and dog following intravenous administration (Flossner, 1934), but had no effect on heart rate and ECG of the rabbit (Hara *et al.*, 1966; Yabo, 1964). Versprille (1966) reported no effect from IMP on the S-A or A-V nodes in the Langendorff preparation in the isolated rat heart. Isolated guinea pig intestine motility was decreased upon exposure to a 1% IMP solution (10,000 ppm) (Hara *et al.*, 1966). Isolated guinea pig uterus showed a biphasic response to the application of IMP (Flossner, 1934). In rats and cats, topical IMP enhanced the electrical response of the chordotympani to topical monosodium glutamate (MSG) (Adachi, 1964; Sato M *et al.*, 1965). Intravenous IMP had no significant effect on blood electrolytes in the rabbit (Hara *et al.*, 1966).

Kojima (1974) reported the following observations from studies on disodium 5'-inosinate (IDP). In mice, 500 mg/kg bolus *i.v.* dose caused (1) behavioral excitement, (2) increased reflex response, (3) lack of muscular relaxation, (4) depressed rotating activity during the first hour, (5) inability to modify electroshock convulsions and (6) dose-dependently decreased the metrazol convulsive dosage. Doses of 50-500 mg/kg *i.v.* prolonged loss of righting reflex. One hundred (100) mg/kg *s.c.* depressed salivary secretion, but had no effect on intestinal transport as measured by charcoal transportation. In rats, 100 mg/kg *s.c.* had no effect on gastric juice volume, but slightly increased pH. Rats given 100 mg/kg intragastric IDP showed no diuresis. Oral 500 mg/kg IDP had no effect on analgesic response of mice or carrageenan edema in rats. In cats, 10 and 50 mg/kg *i.v.* had no effect on blood pressure, heart rate, ECG or blood flow of hind limbs. IDP (10^{-4} g/ml) did not affect the contractile response of guinea-pig ileum to acetylcholine, histamine or barium chloride, but 10^{-2} IDP decreased motility.

5.3. Safety studies

5.3.1. Acute studies

Acute lethal toxicity studies on inosine monophosphate indicated that it was practically non-toxic by the oral route with $LD_{50} \geq 12$ g/kg in rats and mice (Table 6). Regardless of the route, species or sex, near-lethal doses produced depression, clonic convulsion and dyspnea. Near-lethal doses by oral administration or intraperitoneal injection induced diarrhea and writhing. Surviving animals recovered from the clinical signs by 16 hours (Kojima, 1974).

Table 6. Acute lethal toxicity studies on inosine monophosphate (IMP)

Animal	Route*	LD ₅₀ (mg/kg)	Reference
Mouse	<i>p.o.</i>	12 000-14 000	Hara <i>et al.</i> (1966)
	<i>p.o.</i> (male)	17 600	Ichimura & Muroi (1973)
	<i>p.o.</i> (female)	19 800	Ichimura & Muroi (1973)
	<i>s.c.</i>	6 200-7 000	Hara <i>et al.</i> (1966)
	<i>s.c.</i> (male)	5 480	Ichimura & Muroi (1973)
	<i>s.c.</i> (female)	5 630	Ichimura & Muroi (1973)
	<i>i.p.</i>	5 400-5 600	Hara <i>et al.</i> (1966)
	<i>i.p.</i> (male)	6 300	Ichimura & Muroi (1973)
	<i>i.p.</i> (female)	6 200	Ichimura & Muroi (1973)
	<i>i.v.</i>	3 300-3 900	Hara <i>et al.</i> (1966)
	<i>i.v.</i> (male)	3 950	Ichimura & Muroi (1973)
	<i>i.v.</i> (female)	4 600	Ichimura & Muroi (1973)
Rat	<i>p.o.</i>	16 000	Usui <i>et al.</i> (1971)
	<i>p.o.</i> (male)	17 100	Ichimura & Muroi (1973)
	<i>p.o.</i> (female)	15 900	Ichimura & Muroi (1973)
	<i>s.c.</i> (male)	3 900	Ichimura & Muroi (1973)
	<i>s.c.</i> (female)	4 340	Ichimura & Muroi (1973)
	<i>i.p.</i> (male)	5 400	Ichimura & Muroi (1973)
	<i>i.p.</i> (female)	4 850	Ichimura & Muroi (1973)
	<i>i.v.</i> (male)	2 730	Ichimura & Muroi (1973)
	<i>i.v.</i> (female)	2 870	Ichimura & Muroi (1973)

* *s.c.*, subcutaneously; *i.p.*, intraperitoneally; *i.v.*, intravenously; *p.o.*, perorally

Pregnant ICR-JCL mice (N=20) and SD-JCL rats (N=20-25) were treated with 0, 5, 50, 100, 300 or 500 mg/kg (mice) or 0, 5, 50, 100, 200 or 400 mg/kg (rats) AMP from gestation day 7 to gestation day 13 by intraperitoneal injections (Hashimoto *et al.*, 1970). In mice, no adverse effects were observed, at any dose, on erythrocytes, leucocytes or hematocrits. Hemoglobin was slightly elevated only at 500 mg/kg AMP with no effects at lower doses. In rats, no adverse effects were observed, at any dose, on erythrocytes, hemoglobins or hematocrits. Leukocyte numbers were elevated at 400 mg/kg AMP with no effects at lower doses.

5.3.2. Subchronic studies

Male rats (N=10/group; strain not specified) were fed 0, 10, 100 or 1000 mg/kg/day naturally or synthetically-derived IMP for 90 days (Hara *et al.*, 1966). There were no adverse effects on weight gain, weight or volume of cerebrum, cerebellum, thyroid, heart, stomach, liver, spleen, kidney, adrenal, testis, epididymis or urinary bladder, weight of lung or 'length of tail' in comparison with control group. No histological changes in internal organs were found by

macroscopic and microscopic examination. The No Observed Adverse Effect Level (NOAEL) in this rat study was 1000 mg/kg/day, the highest dose tested.

Male rats (N=10/group; strain not specified) were fed 0, 50 or 500 mg/kg/day IMP for 12 weeks or 24 weeks (Usui *et al.*, 1971). There were no adverse effects in treated rats compared with control on food intake, weight gain, organ weights, hematological parameters (erythrocyte/leukocyte counts, hemoglobin, hematocrit), clinical chemistry (total protein, urea nitrogen), urine parameters (pH, protein, glucose) or macroscopic and microscopic examination of visceral organs. The NOAEL in this rat study was 500 mg/kg/day, the highest dose tested.

Yonetani *et al.* (1973) reported the results from a 25 week feeding study in which Sprague-Dawley rats (N=8/group/sex) were fed 0, 250, 500, 1000 or 2000 mg/kg/day IMP for the first 12 weeks followed by 0, 400, 750, 1500 or 3000 mg/kg/day IMP for the remaining 13 weeks. There were no effects on behavior, body weight gain, food intake, hematology and urinalysis. One rat in the highest dose group died of a spontaneous nephroblastoma. Some animals in higher dosage groups showed renal medullary calcification. However, glomerulonephritis was observed in some animals from all of the groups with no significant differences noted. Organ weights were normal, but the relative mean weight of kidney and spleen in the 3000 mg/kg/day group were significantly higher than the other dose groups. The NOAEL from this rat study was 1500 mg/kg/day.

There were no adverse effects reported in male and female Beagle dogs fed IMP at ~1000 mg/kg/day (3.6-3.9% of diet) or ~2000 mg/kg/day (8% of diet) for four to six weeks (no other details provided) (Rivett *et al.*, 1973).

5.3.3. Chronic studies

The effects of dietary IMP were studied in Sprague-Dawley rats (N=6/sex/group) fed 0, 500, 1000, 2000 or 4000 mg/kg/day for 52 weeks (Yonetani *et al.*, 1973). There was a slight (non-statistically significant) decrease in body weight gain in the 4000 mg/kg dose group starting at week 42, but not at the lower dose groups. No clinical signs of adverse effects were observed and food consumption was the same in all groups throughout the study. At study termination, no significant abnormalities were observed in hematology (hematocrit, hemoglobin, red cell count, white cell count, differential white cell count), blood chemistry (plasma urea nitrogen, glucose, serum proteins, serum alkaline phosphatase, serum glutamic-pyruvic transaminase, serum glutamic-oxaloacetic transaminase, total cholesterol, whole blood specific gravity, serum electrolytes) or urinalysis (urinary volume, pH, specific gravity, protein, glucose, ketones, bile pigment, urobilinogen, blood pigments, electrolytes (Na, K, Mg, Ca)). There were no differences in the absolute mean organ weights including heart, liver, spleen, kidneys, adrenals and gonads. However, the relative mean kidney weight in the 4000 mg/kg/day groups was slightly heavier (non-statistically significant) than in the other dose groups. A statistically significant greater number of renal calcifications noted in female rats fed 2000 or 4000 mg/kg/day and the male rats fed 4000 mg/kg/day. This was considered a non-specific effect from changes in urine osmolarity rather than a direct effect of IMP. Nephrosis was noted in nearly all the rats from all the treated groups, but appeared to be more severe in the female rats fed 2000 or 4000 mg/kg/day and the male rats fed 4000 mg/kg/day (data not provided). Therefore, the authors report that the NOAEL in this rat study was 1000 mg/kg/day.

Yonetani *et al.* (1973) also studied the effects of IMP on Sprague-Dawley rats (N=14/group/sex) fed 0, 420, 880, 1790 or 3765 mg/kg/day IMP for 95 weeks. No significant changes were seen in mortality, behavior, body weight gain, food intake, hematology, blood chemistry or urinalysis. Histopathology revealed changes in several organs from all of the groups including controls, but the degree of changes were not treatment or dose related. Progressive glomerulonephritis was found in every rat in every group, including the control, with some differences in severity in the rats fed 3765 mg/kg/day IMP compared with the other treatment groups and control group. Thus, the NOAEL in this rat study was 1790 mg/kg/day.

Beagles (N=4/group/sex) were fed 0, 25, 250 or 500 mg/kg/day IMP for two years (Rivett *et al.*, 1972). No significant abnormalities were found clinically in body weight gain, food consumption or ophthalmoscopy. Hematology (erythrocyte sedimentation rate, packed cell volume, hemoglobin, reticulocyte count, mean corpuscular hemoglobin concentration, mean corpuscular volume, red cell count, white cell count, differential white cell count, platelet count, prothrombin time), biochemistry (plasma urea, plasma glucose, serum proteins, serum protein electrophoresis, albumin/globin ratio, serum alkaline phosphatase, serum glutamic-pyruvic transaminase, bilirubin, Na, K, allantoin, uric acid) and urinalysis (pH, volume, specific gravity, protein, glucose, ketones, bile pigments, bile salts, urobilinogen, microscopy of sediment) were normal. Macroscopic and microscopic examination of major organs (brain, heart, lungs, liver, pancreas, kidneys, spleen, gonads, prostate/uterus, thymus, thyroids, adrenals, pituitary) were normal. In addition, normal histopathology was noted for aorta (arch, abdominal), trachea, lymph nodes (cervical, mesenteric), gall bladder, urinary bladder, salivary gland, tongue, esophagus, stomach, duodenum, jejunum, ileum, colon, skin, mammary gland, skeletal muscle, bone marrow, peripheral nerve, eye and optic nerve. Small differences in serum allantoin levels were noted in dogs fed IMP, but this was not persistent nor dose related. The NOAEL in this dog study was 500 mg/kg/day, the highest dose tested.

5.3.4. Studies on reproduction/teratogenicity

Hashimoto *et al.* (1970) reported AMP (0, 5, 200, or 400 mg/kg/day) was not a teratogen when injected intraperitoneally during gestation days 7 – 13 (GD7-GD13) in ICR-JCL mice (N=20 litters) and SD-JCL rats (N=20-25 litters). There was slight (non-statistically significant) growth retardation in fetuses, but no growth retardation was noted in the pups. The NOAEL in this mouse study was 400 mg/kg/day, the highest dose tested.

Palmer *et al.* (1971) conducted a three-generation reproduction study in rats (N=10M + 20F/group) fed 0, 250, 500 or 1000 mg/kg/day IMP. The rats were started on their test diets 60 days before mating. There were no effects on mating performance, pregnancy rate or duration of gestation. In male rats in all generations, body weight gain was greater in treated rats compared with controls. Litter size, pup weight, pup mortality and incidence of abnormalities were unaffected by treatment. Organ weight analysis, histopathology and skeletal staining of F_{3B} pups revealed no consistent pattern related to treatment. The NOAEL in this rat study was 1000 mg/kg/day exposed during GD6-GD18, the highest dose tested.

Female Japanese white rabbits (N=13-18/group) were given 0, 200 or 2000 mg/kg/day IMP by gavage during gestation days 6-18 (GD6-GD18) (Jojima *et al.*, 1973). Four to five females of each group were delivered spontaneously and pups observed to day 30. All other dams were

killed at day 29 of gestation. No significant effects were noted on implantation sites, number of live or dead fetuses, body weight of live fetuses and external abnormalities. There was lower fetal mortality in the 200 mg/kg/day group compared to the control and high dose groups. All groups showed some delay in ossification, but no specific skeletal abnormalities were found that appeared to be due to IMP. The NOAEL in this rabbit study was 2000 mg/kg/day exposed during GD6-GD18.

5.3.5. Gene/DNA effects

AMP and adenine, with and without pre-incubation with liver extracts, tested negative for DNA-binding activity in Ehrlich ascites cells and *Escherichia coli* Q13 (Kubinski *et al.*, 1981). AMP (≤ 15 mM) prevented the mutation of *Salmonella typhimurium* (strain DG2670) and *E. coli* (strain DG1669) DNA when co-incubated with the mutagen, 9-aminoacridine (10 μ g/ml) (Kopsidas and MacPhee, 1996).

5.3.6. Cell effects

Mitosis in human keratinocytes measured *in vitro* was reduced up to 59% compared with controls by treatment with 1 mM AMP (Flaxman and Harper, 1975; Harper *et al.*, 1974). There was no effect on the numbers of mitotic cells in the basal layer of mouse epidermis in adrenalectomized mice treated with 30 or 100 mg/kg AMP (no other details provided) (Vincent, 1973).

5.3.7. Observations in humans

Thirty-two adult patients were enrolled in a 28 day randomized, placebo controlled double-blind trial of AMP for the treatment of acute *Herpes zoster* (Sklar *et al.*, 1985). Treated patients (N=8M + 9F; 20 – 82 years) received by intramuscular injections 100 mg AMP in an aqueous gelatin base three times *per week* (~43 mg/day or 0.7 mg/kg/day for a 60 kg individual). Control patients (N=9M + 6F) received only aqueous gelatin base. Patients were evaluated at two-week intervals. There were no clinical signs of toxicity and cardiorespiratory function and blood pressures were normal. Clinical tests for complete blood cell counts, blood chemistry and urine evaluations were all normal. Gajdos (1974) reported the successful use of 160 – 200 mg/day oral AMP for greater than or equal to four weeks for the treatment of porphyria cutanea tarda.

The effects on uric acid levels from exposure to IMP was studied in healthy volunteers (N=3/group) given 0, 1.0, 1.5, 2.0 or 2.5 g/day IMP for 7 days (route not identified) (Copper *et al.*, 1972). Basal diet purine levels were maintained at 400 ± 40 mg/day throughout the experiment. Serum uric acid levels increased from 3.6 mg/dL in control group to 6.9 mg/dL in subjects receiving 2.5 g/day IMP (no other data provided). Uric acid 24-hour urinary excretion rate increased from 506 mg to 1100 mg in the control and 2.5 g/day groups, respectively (no other data provided). No side effects or toxic effects were reported observed.

Clifford *et al.* (1976) reported the acute effects from purine consumption, including AMP, on serum and urine uric acid levels in normouricemic (6.3 mg/dL; N=6; 45 years mean), hyperuricemic (8.5 mg/dL; N=11; 45 years mean) and gouty (8.3 mg/dL; N=8; 49 years mean) human male volunteers. Subjects were fed a low purine diet for 5 days prior to receiving a single

0.1 mmol/kg body weight oral dose of a single purine, AMP (2.1 g/person)⁶ dissolved in fruit juice. Blood was drawn at 0, 2, 4, 6 and 8 hours and urine was collected 2 days prior to and 24 hours following treatment. Changes in uric acid were reported as the greatest difference observed between 0 hours and the time point (2, 4, 6 or 8 hours) with the highest measured uric acid level. Consumption of 2.1 g of AMP significantly elevated serum uric acid levels with a significantly greater increase observed in subjects with gout (+4.2 mg/dL) compared with hyperuricemic subjects (+1.9 mg/dL) and normouricemic controls (+2.2 mg/dL). Urinary uric acid output significantly increased 31%, 35% and 71% in the normouricemic, hyperuricemic and gouty subjects, respectively, but no significant differences were measured between the groups.

Waslien *et al.* (1968) fed healthy men (N=5/group; 21 - 38 years) 0, 2, 4 or 8 g/day yeast RNA equally distributed among four meals for 5 consecutive days. The basal diets contained egg albumin as the sole protein source and were therefore "purine free." Plasma and urinary uric acid increased linearly. At the end of the study, serum uric acid levels in subjects fed 0, 2, 4 or 8 g/day of yeast RNA (~0, 1, 2 or 4 g/day purine) were 4.9, 6.0, 7.7 and 9.4 mg/dL, respectively.

The effect of dietary protein level and yeast RNA on uric acid metabolism was studied in healthy men (N=6; mean age, 25 years) using a randomized block study protocol (Bowering *et al.*, 1970). Subjects lived in a metabolic unit for 60 days and were fed liquid diets providing daily nitrogen levels of 0.9 g (low-protein), 13 g (control), 62 g (high-protein) or 13 g nitrogen plus 4 g RNA (~2 g/day purine). There was a 1.7-fold increase in urine uric acid levels (1043 mg/day) in the group fed RNA compared to the control group (388 mg/day). Serum uric acid levels in the group fed RNA (9.2 mg/dL) were elevated by 0.8-fold compared to the control group (5.1 mg/dL). There was a ~25% increase in uric acid turnover time in the RNA group (1.3 days) compared to the control group (1.7 days).

The intravenous infusion of ATP for the treatment of advanced non-small cell lung cancer was studied in Phase I and Phase II clinical trials (Rapaport, 1993). Patients received ATP levels below those that affected arterial blood pressure (no other details provided). Toxicity and side effect profiles were characterized by minor cardiopulmonary events that were short lived and thought to be due to adenosine from ATP metabolism. Following consumption, AMP is thoroughly metabolized and would not be expected to cause any adverse cardiovascular responses. This is supported by the lack of any reported cardiovascular toxicity following oral AMP or other purine consumption.

6. RISK EVALUATION

AMP is ubiquitous to all living matter and the human diet. Purines have been continuously used as food additives in Japan prior to 1960. Purines and pyrimidines are approved food additives in Europe and are currently added to some infant formulas in the United States.

⁶ Based on a 60 kg individual.
Adenosine 5'-monophosphate.final
August 29, 2003

JECFA has approved IMP as a food additive with the ADI not specified⁷ and FEMA (No. 3669) has determined IMP as GRAS as a food ingredient.

AMP is rapidly and completely metabolized into uric acid in humans and into allantoin in all other non-primate mammals. The majority of evidence indicates that metabolism of AMP is complete prior to intestinal absorption. A study with AMP's initial metabolite, IMP, revealed a half-life of 5 hours and complete elimination, mainly in the urine, by 24 hours.

Adenosine induces physiological effects through binding to cell surface purinergic receptors. Intravenous adenosine administration causes vasodilation in the cardiovascular system, vasoconstriction in the kidneys and platelet aggregation. Inhalation of adenosine can induce asthma in asthmatics. These physiological responses have not been reported to occur following oral consumption of AMP. This is not surprising given that complete or near complete metabolism of AMP occurs following ingestion. Therefore, none of these non-oral adenosine-induced effects are considered relevant to assessing the risk from adding AMP to food.

Studies have reported that the free purine base, adenine, is nephrotoxic (reviewed in Warner, 1977). In rats, nephrotoxicity was observed after 14 days consumption of 420 mg/kg/day adenine (Story *et al.*, 1977). Mechanistic studies have identified that the kidney damage occurs from precipitation of the adenine metabolite, 2,8-dihydroxyadenine (2,8-DHA), in the renal tubules (reviewed in Bartlett, 1977). AMP and its initial metabolite, IMP, are not metabolized to 2,8-DHA, thus they would not cause similar renal toxicity. This fact is borne out by the lack of treatment-dependent renal toxicity observed in multiple animal feeding studies using comparable or greater doses of nucleotide, including rats fed 1790 mg/kg/day of IMP for 95 weeks.

Subchronic animal studies with IMP have identified NOAELs ranging from 500 to 2000 mg/kg/day with an average of 1250 mg/kg/day. NOAELs for IMP from chronic studies ranged from 500 mg/kg/day (the highest dose tested) to a maximum of 1790 mg/kg/day in a 95-week study conducted in rats. AMP and IMP were not teratogenic or reproductive toxins in mice and rats (see Section 5.3.4). ✓

Prolonged excessive purine consumption may lead to chronically elevated plasma uric acid levels (*i.e.*, hyperuricemia), which is one known risk factor for the development of gout. Gout is one of a group of disorders of purine metabolism resulting from deposition of uric acid crystals in the joints (Emmerson, 1996). Although the development of gout is thought to be related to hyperuricemia, the incidence of acute gout is only about 5 percent *per year* among patients with serum uric acid concentrations of 9.0 mg/dL and higher (Emmerson, 1996). The upper level of solubility of uric acid⁸ in blood is considered to be ~7.0 mg/dL and normal serum

⁷The statement "ADI not specified" means that, on the basis of the available data (toxicological, biochemical, and other), the total daily intake of the substance, arising from its use or uses at the levels necessary to achieve the desired effect and from its acceptable background in food, does not in the opinion of the Committee, represent a hazard to health. For this reason, and for the reasons stated in individual evaluations, the establishment of an acceptable daily intake (ADI) in mg/kg bw is not deemed necessary.

⁸Uric acid is the commonly used term, however, at physiological pH, 99% of uric acid is in the form of urate *i.e.*, the salt of uric acid. At pH less than 5.7, most of the molecules are in the form of uric acid (Emmerson, 1996).

uric acid levels in the U.S. have been reported to range between 4.0 to 7.5 mg/dL. The estimated average contribution to the serum uric acid levels in normal individuals from consumption of purines is approximately 1.4 mg/dL *per* gram purine consumed (Clifford *et al.*, 1976). In gouty individuals, serum uric acid levels increased twice as much *per* gram purine consumed compared with normal individuals giving an expected increase in serum uric acid levels of approximately 2.8 mg/dL *per* gram purine consumed. Thus, a conservative estimated change in serum uric acid levels from mean consumption of selected foods with added AMP (179 mg/day) would be an increase of 0.25 and 0.50 mg/dL in normal and gouty individuals, respectively. An increase of 0.50 mg/dL represents a possible maximum increase of 6.7% in individuals with gout at the high end of the normal range of serum uric acid (7.5 mg/dL). These small increases in serum uric acid levels from consumption of AMP are well within the standard deviation of the normal range in the U.S. population as well as the normal fluctuations in serum uric acid levels in individuals.

Humans and animals can consume large quantities of purines with little or no toxicity. The principal concern for purine consumption is the development of prolonged hyperuricemia, >7.0 mg/dL serum uric acid, which may lead to precipitation of uric acid in tissues. Based on regression equations established in human intervention studies, serum uric acid levels of 7.0 mg/dL would result from consumption of 1.83 g/day added purine in addition to the low levels (<11 mg/day) found in the study diets. Therefore, an acceptable daily intake (ADI) for total purine consumption in humans is 1800 mg/day. The current intake of purines in the human diet at the 90th percentile is 1120 mg/day, leaving an ADI for AMP of 680 mg/day (*i.e.*, 1800 mg/day minus 1120 mg/day).

The mean and 90th percentile consumption of purines from all sources in the U.S. is estimated at 560 mg/day and 1120 mg/day, respectively. Addition of AMP to these foods at the levels indicated in Table 4 increases the estimated mean purine consumption to 709 mg/day and the 90th percentile consumption to 1427 mg/day. This theoretical intake level at the 90th percentile represents a conservative estimate and indicates that total purine consumption is within the ADI for total purines of 1800 mg/day and is therefore safe.

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7. CONCLUSION

After critically evaluating the information available, the Expert Panel has determined that, based on common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food, there is reasonable certainty that AMP (Adenosine 5'-monophosphoric acid and its monosodium and disodium salts), produced and used in accordance with current Good Manufacturing Practice (cGMP), is safe under the intended conditions of use, and therefore is Generally Recognized As Safe (GRAS), by scientific procedures, when used as a food and beverage flavor enhancer (flavor modifier), so that total daily consumption of AMP, and its sodium salts, does not exceed 680 mg *per* day.

8. SIGNATURES

Joseph F. Borzelleca, Ph.D., F.A.T.S.
Medical College of Virginia

27 August 2003
Date

Walter H. Glinsmann, M.D.
Glinsmann Incorporated

8/26/03
Date

John A. Thomas, Ph.D., F.A.T.S.
Consultant

8/26/03
Date

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10. APPENDIX 1 - Individual lots of AMP

AMP, lot # B01P01N007

AMP, lot # B01P01N008

AMP, lot # B01P01N009

AMP, lot # B01P01N010

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Sample #: 03002422
Received: Mar 3 2003
Description: AMP

Finalized: Mar 10 2003
Print Date: Mar 10 2003

DR. RICHARD BARNDT
LINGUAGEN CORP.
2005 EASTPARK BLVD
CRANBURY, NJ 08512-3515

Client #: 14660
Phone: 609-860-1500
Fax: 609-860-5900

ANALYTICAL RESULTS

Results are representative of the sample(s) as submitted

ANALYSIS	RESULT	UNIT	METHOD REFERENCE
Sample 1	AMP, lot # B01P01N007		
ESSENTIAL AMINO ACIDS	Passed		AAP
BARIUM	<0.0001	%	SW 6010 (FOODS)
COLOR	Passed		NONE
LEAD	<0.50	ppm	AOAC 986.15
HEAVY METAL TOTAL	<0.50	ppm (as Pb)	AOAC 986.15
AMMONIA	Passed	as ammonium salts	EPA 350.3
	5.83		ION ACTIVITY
SPECIAL TESTING	Passed	Other nucleotides	NONE

Tests were performed using the Food Chemicals Codex IV procedures for Disodium Inosinate.

Deviations from standardized methods: None unless otherwise noted.

Respectfully submitted for ABC Research

Kathy Barry
Manager, General Chemistry

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Unless notified, sample disposed of within 30 days of final report.

000036

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Sample #: 03002830
Received: Mar 12 2003
Description: AMP

Finalized: Mar 18 2003
Print Date: Mar 18 2003

DR. RICHARD BARNDT
LINGUAGEN CORP.
2005 EASTPARK BLVD
CRANBURY, NJ 08512-3515

Client #: 14660
Phone: 609-860-1500
Fax: 609-860-5900

ANALYTICAL RESULTS

Results are representative of the sample(s) as submitted

ANALYSIS	RESULT	UNIT	METHOD REFERENCE
----------	--------	------	------------------

Sample 1: B01P01N008

ESSENTIAL AMINO ACIDS	Passed		AAP
BARIUM	<0.0001	%	SW 6010 (FOODS)
COLOR	Passed		NONE
LEAD	<0.50	ppm	AOAC 986.15
HEAVY METAL TOTAL	<0.50	ppm (as Pb)	AOAC 986.15
AMMONIA	Passed	as ammonium salts	EPA 350.3
	5.49		ION ACTIVITY
SPECIAL TESTING	Passed	Other nucleotides	NONE

Sample 2: B01P01N009

ESSENTIAL AMINO ACIDS	Passed		AAP
BARIUM	<0.0001	%	SW 6010 (FOODS)
COLOR	Passed		NONE
LEAD	<0.50	ppm	AOAC 986.15
HEAVY METAL TOTAL	<0.50	ppm (as Pb)	AOAC 986.15
AMMONIA	Passed	as ammonium salts	EPA 350.3
PH	5.72		ION ACTIVITY
SPECIAL TESTING	Passed	Other nucleotides	NONE

000037

Sample 3: B01P01N010

ESSENTIAL AMINO ACIDS	Passed		AAP
BARIUM	<0.0001	%	SW 6010 (FOODS)
COLOR	Passed		NONE

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A Better Company For Your Professional Analytical Needs

Sample #: 03002830
Received: Mar 12 2003
Description: AMP

Finalized: Mar 18 2003
Print Date: Mar 18 2003

DR. RICHARD BARNDT
LINGUAGEN CORP.
2005 EASTPARK BLVD
CRANBURY, NJ 08512-3515

Client #: 14660
Phone: 609-860-1500
Fax: 609-860-5900

ANALYTICAL RESULTS

Results are representative of the sample(s) as submitted

ANALYSIS	RESULT	UNIT	METHOD REFERENCE
Sample 3	B01P01N010		
LEAD	<0.50	ppm	AOAC 986.15
HEAVY METAL TOTAL	<0.50	ppm (as Pb)	AOAC 986.15
AMMONIA	Passed	as ammonium salts	EPA 350.3
PH	5.65		ION ACTIVITY
SPECIAL TESTING	Passed	Other nucleotides	NONE

Tests were performed using the Food Chemicals Codex IV procedures for Disodium Inosinate.

Deviations from standardized methods: None unless otherwise noted.

Respectfully submitted for ABC Research

Kathy Barry /
Manager, General Chemistry

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Pages 000038-000110 removed under Freedom of Information Act
Exemption 6.

SUBMISSION END

000111



Lubin, Lisa

From: Jim Griffiths
Sent: Friday, May 07, 2004 5:12 PM
To: Lubin, Lisa
Cc: Alan Hood; DP; GB; Mihalov, Jeremy J.
Subject: RE: GRAS Notice No. GRN 000144 (AMP)

Dear Ms. Lubin,

No, the pyrimidines were not part of the calculation as a re-examination of the Kojima (1974) paper indicates that only purines were part of the description and evaluation:

AMP
GMP
IMP
XMP

and their bases, nucleosides, nucleotides and nucleic acids.

Please let me know if this is a sufficient response at this juncture. For any further info, can you also copy Dr. Alan Hood (ahood@burdockgroup.com) as I will be out of the office next week and he is quite proficient in consumption analyses.

Warm regards,

Jim

-----Original Message-----

From: Lubin, Lisa
Sent: Thursday, May 06, 2004 10:42 AM
To:
Cc: Mihalov, Jeremy J.
Subject: GRAS Notice No. GRN 000144 (AMP)

Dear Dr. Griffiths:

In reviewing GRAS Notice No. GRN 000144, Agency scientists had a few questions regarding the section on estimated daily intake of AMP in Linguagen's GRAS Panel Report. In your calculation of estimated daily intake of AMP, you include a current daily intake from the diet for purines. Can you elaborate further on how you define "purines"?

We note that you cite the publication Kojima, 1974 in this section, which describes purines (p. 189) as "purine bases, nucleosides, nucleotides, and nucleic acids." Since this definition of purines includes nucleic acids, we also ask if pyrimidines are, therefore, part of your current daily intake estimate?

We look forward to your reply.

Sincerely,

Lisa F. Lubin, MS, RD
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway, HFS-255
College Park, MD 20704

000117