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December 21, 2011

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Division of
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Division of Biotechnology & GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for L-Lysine monohydrochloride

Dear Sir or Madam:

In accordance with proposed 21 CFR § 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62FR 18939-18964) I am submitting in triplicate, as the agent to the notifier, The Procter & Gamble Company, a GRAS Notification for L-Lysine monohydrochloride.

L-Lysine monohydrochloride is intended for use as a food ingredient (processing aid) in fabricated snack products which is a segment of snack foods [21 CFR 170.3(n)(37)] at use levels up to 1.5% (1.2% as lysine). It will be added to fabricated snack products prior to frying, in order to reduce production of acrylamide in the finished product. Formulated snack products are typically fried in vegetable oil (temperature ~400°F). Frying time is determined by the desired textural crispness with a typical frying time being about 10 seconds. The addition of lysine in the frying process effectively competes with asparagine in the Maillard browning reaction thereby reducing the formation of acrylamide.

Please let me know if you have any questions.

Sincerely

(b) (6)

Edward A. Steele
President

Encl.

EAS Consulting Group, LLC
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(877) 327-9808 Toll Free • (703) 684-4408 Local • (703) 684-4428 Fax

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GENERALLY RECOGNIZED AS SAFE NOTIFICATION

I. Claim of GRAS Status

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

The Procter & Gamble Company (P&G) has determined that the use of L-lysine monohydrochloride is Generally Recognized As Safe, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food. Therefore, the use of L-lysine monohydrochloride is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Date 12/21/11

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B. Name and Address of Notifier:

Richard A. DePalma, Ph.D.
Global Snacks Product Safety & Regulatory Affairs
The Procter & Gamble Company
Winton Hill Business Center
6300 Center Hill Rd
Cincinnati, Ohio 45224

Phone: 513.634-7291
Fax: 513.634-2519
Email: depalma.ra@pg.com

C. Common or usual name of the notified substance:

L-Lysine monohydrochloride. The term L-lysine or lysine used in this document is for the free amino acid or the amino acid as a subunit of a protein or peptide. Typically when referring to an estimated or calculated value an attempt has been made to identify whether it represents lysine or its salt. It is assumed to be the L-isomer unless otherwise specified.

D. Conditions of use:

L-Lysine monohydrochloride is intended for use as a food ingredient (processing aid) in fabricated[1] snack products which is a segment of snack foods [21 CFR 170.3(n)(37)][2] at use levels up to 1.5% (1.2% as lysine). It will be added to fabricated snack products prior to frying, in order to reduce production of acrylamide in the finished product. Formulated snack products are typically fried in vegetable oil (temperature ~400°F). Frying time is determined by the desired textural crispness with a typical frying time being about 10 seconds. The addition of lysine in the frying process effectively competes with asparagine in the Maillard browning reaction thereby reducing the formation of acrylamide.

Based on the USDA Nutrient Database for Standard Reference (NDSR) of foods, the reference serving size for snacks is generally 28 g. Therefore, a serving of these foods containing the maximum intended level (1.5%) would contain about 420 mg of L-lysine monohydrochloride or 336 mg of lysine. According to the USDA Continuing Survey of Food Intakes by Individuals (CSFII) surveys for quantities of foods consumed daily, the mean and 90th percentile quantities of snack food (potato chips) consumption are 39 and 80 g/person/day. L-Lysine monohydrochloride at the intended use level of 1.5% (1.2% as lysine) in snack foods will result in mean and 90th percentile intakes of 465 and 960 mg lysine/person/day or 7.75 and 16.00 mg/kg body weight (bw)/day (for an individual weighing 60 kg), respectively.

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[1] The term "fabricated" refers to food products made from doughs comprising purees, flour, meal, and/or starch, such as those derived from tubers, grains, legumes, cereals, or combinations and mixtures thereof. For example, a potato chip that is prepared by frying a portion of a potato is not fabricated, but a potato chip made of potato flakes and starch made into a dough piece that is fried is a fabricated potato chip.

[2] Snack foods, including chips, pretzels, and other novelty snacks.

An additional exposure estimate was conducted using Market Research Corporation of America (MRCA) data. The MRCA database provides a realistic estimate of the acute and chronic consumption of savory snacks. This was used to determine the likely intake of lysine from the proposed uses. As compared to the CSFII survey which uses 2 days data of dietary intake, the MRCA survey relies upon the determination of the frequency of food consumption over 14 continuous days for each survey participant. The MRCA intake estimates are sufficiently conservative to be useful in safety evaluations of nutrients. The acute and chronic exposures to lysine in high snack food consuming populations were estimated using data for savory snack consumption of 13 to 17 year olds. Acute and chronic snack intakes for this group were estimated to be 48.9 and 14.8 g/day, respectively. The estimated intake of savory snacks among high consumers is taken from the work of Webb et al. (1997) for olestra, and for which MRCA data were used in determining exposure to lysine. Based on these assumptions, the intended use of L-lysine monohydrochloride at 1.5% (1.2% lysine) in snack food will result in an additional average acute and chronic consumption of 587 and 180 mg lysine/day, respectively, for the highest user group. For safety assessment purposes, the maximum daily intake value of 587 mg lysine per day is considered appropriate.

E. Basis for GRAS Determination:

In accordance with 21CFR 170.30, the intended use of L-lysine monohydrochloride has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. A comprehensive search of the scientific literature was conducted for this review. There is sufficient qualitative and quantitative scientific evidence to determine safety-in-use of L-lysine monohydrochloride. The safety determination of L-lysine monohydrochloride is based on the totality of the available evidence that includes human clinical trials and a variety of animal studies.

The available evidence from several human studies suggests that oral lysine supplements up to 3000 mg/day divided among meals for individuals consuming usual American diets would not elevate the proportion of lysine in the dietary protein beyond that in meat and would be safe for chronic use. Findings from several animal studies support the safety of lysine. In a well-designed subchronic toxicity study in rats, L-lysine monohydrochloride had no adverse effects at dose levels up to 5% (~ 3671 mg/kg bw/day), the highest dose tested (Tsubuku et al., 2005). The estimated daily intake of 587 mg L-lysine/person (9.8 mg/kg bw/day) from the intended uses in snack products is over 5-fold lower than that used in clinical trials in humans and is approximately 375-fold lower than the demonstrated safe levels of intake in animal studies. Based on the available safety-related information, the estimated daily intake of L-lysine, if ingested daily over a lifetime, is safe within the meaning of the FD&C Act, i.e., it meets the standard of reasonable certainty of no harm.

Additionally, L-lysine, including its monohydrochloride form, is approved (21CFR 172.320) as a food additive (special dietary and nutritional additive) for direct addition to human food. As per this regulation, the amount of L-lysine added for nutritive purposes plus the amount naturally present in free and combined (as protein) form must not exceed 6.4% of the total protein of the finished food. The Daily Reference Value for protein is 50 g/day (based on a 2000 calorie diet) (Federal Register, 2007). Thus, the current daily background intake of lysine from its presence in the diet is likely to be at least 3200 mg/day. Although the

proposed use of L-lysine monohydrochloride will add to the background intake, total lysine consumption (background plus added lysine as L-lysine monohydrochloride) has been estimated to be 7.0% (as percent protein) suggesting that the intended uses will be slightly higher than what is described in the regulation 21CFR 172.320. The available human and animal studies also provide support for the safety of the intended use of lysine, as exposure to lysine in these studies was in addition to the normal dietary background intake. Furthermore, available studies, especially from heat-treated feed ingredients, indicate that some amino acids, such as lysine, may be present in chemical forms, like Maillard reaction products, that may be absorbed but their utilization for protein synthesis is precluded (Stein et al., 2007). The weight of scientific evidence supports the safety of L-lysine monohydrochloride, when produced in accordance with current good manufacturing practices (cGMP) to a food-grade specification, for its intended use as a processing aid in the production of fabricated snack products.

F. Availability of Information:

The information that serves as the basis for this GRAS determination will be provided to the Food and Drug Administration upon request and is located at the offices of: Edward A. Steele, EAS Consulting Group, LLC, 1940 Duke Street, Suite 200, Alexandria, VA 22314; Phone: (703) 684-4408; Fax: (703) 684-4428; E-mail: esteele@easconsultinggroup.com.

The primary toxicologist, Dr. Madhu G. Soni, responsible for the preparation of this GRAS monograph can also be contacted for the information that forms the basis for this GRAS determination at the following address: Madhu G. Soni, Ph.D., FACN, 749 46th Square, Vero Beach FL, 32968; Phone: (772) 299-0746; E-mail: sonim@bellsouth.net.

II. Detailed Information About the Identity of the Notified Substance:

A. Chemical name

L-Lysine monohydrochloride (L-lysine added as the monohydrochloride salt)

B. Common Name:

L-Lysine HCl (added salt)

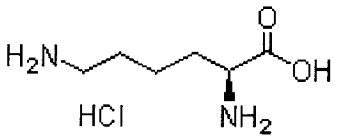
C. Chemical Abstract Service Registry Number:

CAS No.: 657-27-2

D. Chemical Formula:

Structural formula: $NH_2(CH_2)_4(NH_2)CHCOOH\text{-}HCl$

E. Structure:



F. Molecular Weight

L- Lysine HCl: 182.62 Daltons; L-lysine: 146.19 Daltons

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G. Physical Characteristics

White granular or crystalline powder.

H. Specifications

Food-grade specifications for L-lysine monohydrochloride are presented in Table 1. L-Lysine monohydrochloride is >99.5% purity and meets the Food Chemicals Codex (FCC, 2003) and United States Pharmacopoeia (USP) specifications. Stability data from multiple lots confirm the stability of both crystalline as well as granular forms of L-lysine monohydrochloride for three years. Although specifications and manufacturing details are provided below, the supplier assures that L-lysine monohydrochloride meets FCC specifications through a letter of guaranty and certificate of analysis.

Table 1. Specifications of L-lysine monohydrochloride

Parameter	Specifications	Assay method
Description	White granular or crystalline powder	Visual
Purity	99.5 – 101.5%	USP
Identification	Meets FCC and USP requirements	USP/FCC
Loss on drying	NMT 0.4%	USP
Solubility	Easily soluble in water, slightly soluble in alcohol, almost insoluble in diethylether	USP
Appearance of solution	Meets USP requirements	USP
Color of solution	Meets USP requirements	USP
Chromatographic purity	Each individual impurity NMT 0.5; total impurities NMT 2%	USP
Organic volatile impurities	Meets USP Method I <467> Reg	USP
Residue on ignition	NMT 0.1%	USP
pH (10% solution)	5.0 – 6.0	USP <791>
Chloride content	19.0 – 19.6%	USP
Transmittance (10% solution)	NLT 95%	(at 430 nm)
Specific rotation	+20.4 - +21.4	USP
Heavy metals	NMT 10 ppm	USP
Lead	NMT 0.5 ppm*	FCC
Arsenic	NMT 1 ppm	USP
Iron	NMT 0.003%	USP
Sulfate	NMT 0.03%	USP
Ammonium	NMT 200 ppm	KP
Total plate count	NMT 3000 cfu/g	USP
Mold and Yeast	NMT 300 cfu/g	USP
Salmonella species	Negative	USP
Escherichia coli	Negative	USP
Staphylococcus aureus	Negative	USP

NMT = Not more than; NLT = Not less than. *Although FCC specification for lead is set as 5 ppm, P&G proposed lead levels will be 0.5 ppm.

I. Manufacturing process

L-Lysine monohydrochloride is produced by fermentation using raw sugar and molasses as summarized in Figure 2. In a fermentation tank, sterilized raw sugar and molasses are added

along with seed culture. The fermentation microorganism is a non-pathogenic, non-toxigenic species and strain (*Corynebacterium glutamicum*) commonly[3] used in food processing. After about 40 hours, the broth is passed through an ion exchange resin. The filtrate is evaporated and hydrochloric acid is added to form L-lysine monohydrochloride. The crystallized product is centrifuged, dissolved, and decolorized. The decolorized product is purified by crystallization, centrifugation, and drying to obtain the final product. All equipment and materials used in the production process have a safe history of use in food processing. L-Lysine monohydrochloride is also manufactured in accordance with current good manufacturing practices (cGMP).

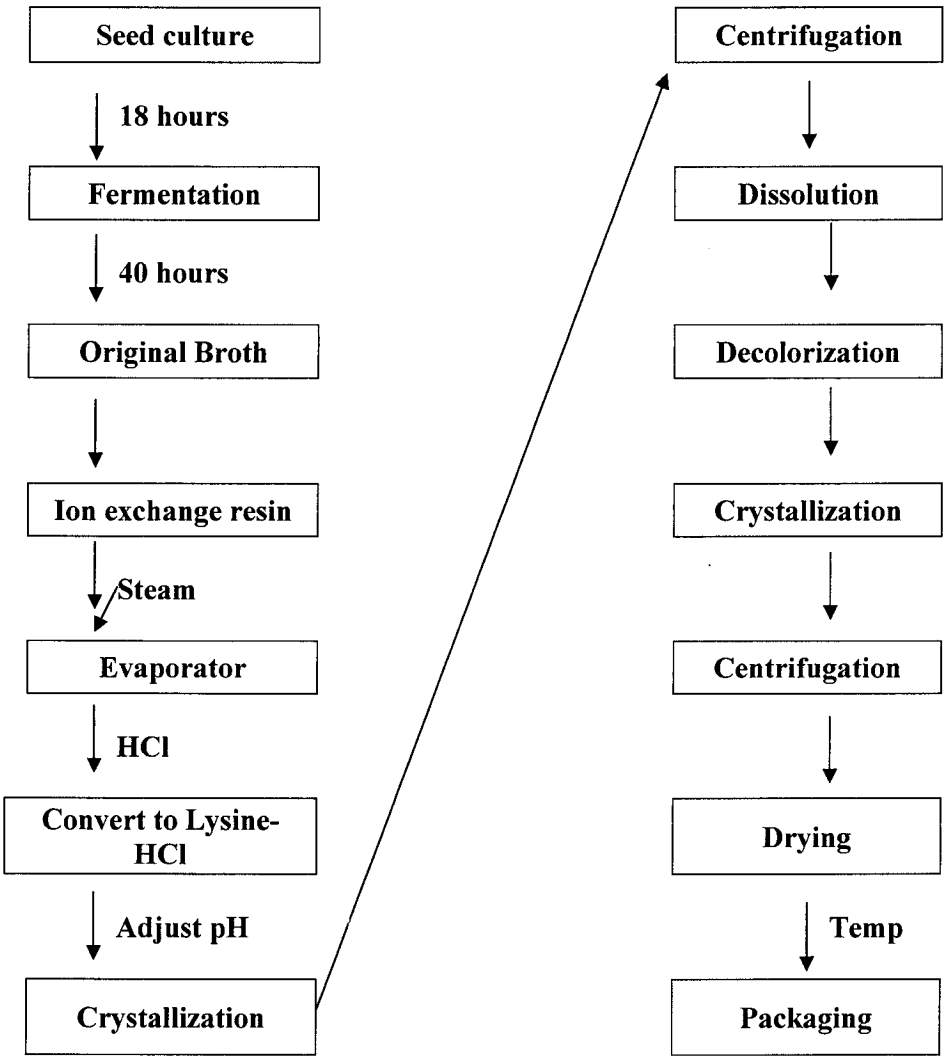

Figure 2. Manufacturing process of L-lysine monohydrochloride

[3] Proprietary information, additional information if required will be shared separately

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J. Intended Technical Effects

L-Lysine monohydrochloride is intended for addition to a limited number of conventional foods (snack foods). The intended technical effect of L-lysine monohydrochloride is as a processing aid [21 CFR 170.3(o)(24)] [4] for the reduction of acrylamide formation. Acrylamide is formed in many foods via cooking, baking, or frying. It is formed through the reaction of asparagine and a reducing sugar via the Maillard reaction. In recent years, various methods have been explored by the food industry to reduce the levels of acrylamide in food products. The addition of the amino acid L-lysine monohydrochloride has been found to substantially reduce the formation of the acrylamide in test products (Table 2) by competing with asparagine in reacting with reducing sugars.

Table 2. Acrylamide levels for a typical formulated test product (made at the P&G, R&D center)

Product	Levels in ppb
Control	1640
Control plus 0.5% L-Lysine monohydrochloride	1150
Control plus 1% L-Lysine monohydrochloride	876
Control plus 1.5% L-Lysine monohydrochloride	520

III. Summary of the Basis for the Notifier's Determination that L-Lysine monohydrochloride is GRAS

The safety of L-lysine monohydrochloride under the intended conditions of use is supported by the available human and animal safety studies. A comprehensive search of the publicly available scientific databases (PubMed, Toxline, etc.) for safety and toxicity information of L-lysine was conducted through November 2011 and was utilized for this assessment. Based on a critical evaluation of the pertinent data and information summarized herein, and employing scientific procedures, it is determined that the addition of L-lysine monohydrochloride to fabricated snack foods, meeting the specification cited above and manufactured according to current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use as specified herein.

To further support the safety of the intended use of L-lysine monohydrochloride as described in this document, we note that the FDA has classified L-lysine as an approved food additive under the use conditions defined in 21CFR 172.320. This regulation stipulates that the addition of L-lysine to food is safe under several conditions, one of which is that the amount of the L-lysine added for nutritive purposes plus the amount naturally present in free and combined (as protein) forms must not exceed 6.4% by weight of total protein (expressed as free amino acid) of the finished food.

The total intake of L-lysine (background plus intended uses) was determined using total recommended dietary consumption of protein (for 19-to-30 year olds- background intake of

[4] *Processing aids*: Substances used as manufacturing aids to enhance the appeal or utility of a food or food component, including clarifying agents, clouding agents, catalysts, flocculents, filter aids, and crystallization inhibitors, etc.

3400 mg lysine/day) (IOM, 2005) and the acute (587 mg/day) and chronic (180 mg/day) intakes of lysine from the intended uses (Webb et al., 1997). Based on these data, the total daily lysine consumption values would be 3987 mg/day and 3580 mg/day for acute and chronic consumption, respectively. Based on the recommended protein consumption of 56 g for the age group of 19-to-30 year old males (IOM, 2005), the total lysine consumption (background plus added L-lysine monohydrochloride) would be 7.0% and 6.6% (as percent protein) for acute and chronic consumption, respectively. This indicates that the intended uses of L-lysine monohydrochloride will be slightly higher than the level currently authorized for its use under 21CFR 172.320. However, the assessment of changes in amino acid profile suggest that the addition of L-lysine monohydrochloride to snack formulations is unlikely to significantly impact the amino acid quality of the protein consumed or to result in excessive lysine levels relative to other amino acids.

In arriving at this decision that L-lysine monohydrochloride is GRAS for the proposed use, we relied upon the conclusions that neither L-lysine nor any of its degradation products pose any toxicological hazards or safety concerns at the intended use levels. This conclusion is based on published toxicology studies and other articles relating to the safety of the L-lysine product. It is also our opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

IV. Basis for a Conclusion that L-Lysine Monohydrochloride is GRAS for its Intended Use.

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF L-LYSINE MONOHYDROCHLORIDE AS A PROCESSING AID

1. EXECUTIVE SUMMARY

At the request of Procter & Gamble (P&G), a comprehensive search of the scientific literature for safety and toxicity information on lysine was conducted through November 2011 by EAS Consulting Group LLC, to determine the Generally Recognized As Safe (GRAS) status of L-lysine monohydrochloride as a processing aid [21 CFR 170.3(o)(24)][5]. P&G intends to use L-lysine monohydrochloride (>99% purity) as a processing aid at levels up to 1.5% in fabricated[6] snack foods. The exposure from added L-lysine in the proposed food uses in the total U.S. population is estimated as 587 mg/day. The total intake of L-lysine (background plus intended uses) is determined as 3987 mg/day. Available information indicate that the addition of L-lysine monohydrochloride to snack formulations is unlikely to significantly impact the amino acid quality of the protein consumed or to result in providing excessive dietary lysine levels relative to other amino acids. As described below, the weight of evidence clearly supports the safety and GRAS status of L-lysine monohydrochloride, when produced in accordance with cGMP to a food-grade specification, for its intended use in fabricated snack foods at levels up to 1.5% in these snack foods. No studies were identified showing any adverse effects when this amount of lysine is added to the diet. Additionally, there are no studies suggesting any adverse effects from the minor changes in the dietary amino acid ratios when this amount of lysine is added to the diet.

1.1. Background

In 2002, news reports of high levels of acrylamide in many fried and baked foods surfaced following publication of research conducted in Sweden (Tareke et al., 2000; 2002). Because these foods are widely consumed and since acrylamide is known to be toxic and can cause cancer in experimental animals, these reports raised concerns among consumers, the scientific community, and regulatory agencies. The available information shows that acrylamide is a natural byproduct that forms when certain carbohydrate-rich foods are fried, baked, or roasted at temperatures above 120°C. Becalski et al. (2003) reported high concentrations of acrylamide in potato chips, breakfast cereals, and crisp bread. The formation of acrylamide in food results from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of a reducing sugar (Stadler et al., 2002). While the mechanism of acrylamide formation in foods is well understood, the asparagine level within a certain food category (e.g., potatoes) is known to vary greatly resulting in a large variability in acrylamide content even within one food category. This appears to be the result of the asparagine-dependent reaction.

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[5] *Processing aids* : Substances used as manufacturing aids to enhance the appeal or utility of a food or food component, including clarifying agents, clouding agents, catalysts, flocculants, filter aids, and crystallization inhibitors, etc.

[6] The term "fabricated" refers to food products made from doughs comprising purees, flour, meal, and/or starch, such as those derived from tubers, grains, legumes, cereals, or combinations and mixtures thereof. For example, a potato chip that is prepared by frying a portion of a potato is not fabricated, but a potato chip made of potato flakes and starch made into a dough piece that is fried is a fabricated potato chip.

Since the earliest reports of high levels of acrylamide in foods surfaced, a number of reports have been identified that focused on ways to mitigate the formation of acrylamide in foods (Kotsiou et al., 2011; Taeymans et al., 2005; CCFAC, 2006; CIAA, 2006). Techniques such as reducing the availability of free asparagine or reducing sugars, changing the pH, adding ingredients that compete with asparaginase, and changing cooking time or temperature have been explored to reduce acrylamide formation. Different techniques appear to be useful for different types of products, and frequently, several techniques are needed to effectively reduce acrylamide. For potato products, selecting potatoes low in reducing sugar, controlling storage conditions, and reducing the time or temperature of cooking are frequently cited methods. Additionally, the use of ingredients (e.g., amino acids, calcium salts, citric acid) that interfere with acrylamide formation, and the use of asparaginase to reduce asparagine levels prior to cooking have also been suggested.

One method that has demonstrated a consistent and significant reduction of acrylamide in fabricated snack products is through the addition of the amino acid lysine in the frying medium. P&G intends to use food-grade L-lysine monohydrochloride, an essential amino acid found naturally in animal and plant-based food (proteins), as one of several techniques to reduce the formation of acrylamide in fabricated snack products.

1.2. Description, Specifications and Manufacturing Process

L-Lysine is a member of α-amino acids. It is classified as an essential amino acid for humans and therefore must be supplied in the diet (Handler and Rorvik, 2001). The subject of this GRAS determination, L-lysine monohydrochloride is a white or nearly white, practically odorless, free flowing granular or crystalline powder. It is freely soluble in water, but is almost insoluble in alcohol and ether. It melts at about 260°C with decomposition. Food grade specifications of L-lysine monohydrochloride are presented in Table 1. The manufacturing process is presented in Figure 2.

1.3. Food and Other Uses

L-Lysine occurs in the diet mainly as a component of protein, and to a minor extent as a free amino acid. In 1955, L-lysine monohydrochloride was introduced in the US market (Flodin, 1997). Soon, a marked interest developed for its application as a protein-enhancing supplement to bread and other cereal foods. In 1961, FDA refused to modify the standards of identity for white bread to permit the use of L-lysine monohydrochloride. Since the reports of the effectiveness of oral L-lysine monohydrochloride supplements in suppressing recurrent herpes simplex infection in the 1970s, this amino acid again received some attention. Current usage of lysine as a supplement is estimated at >100,000 lb annually, sold mostly as 500 mg tablets (Flodin, 1997). As described earlier, L-lysine (21CFR 172.320) in the free, hydrated or anhydrous form or as the hydrochloride, sodium, or potassium salt and meeting the specifications described in the Food Chemicals Codex has been permitted by the FDA for addition as a nutrient to foods for human consumption.

In 2004, the Joint FAO/WHO Expert Committee on Food Additives (JECFA[7]) evaluated L-lysine as a flavoring agent and concluded that there is no safety concern at current levels of intake when used as a flavoring agent. Lysine was not evaluated using the Procedure for the

[7] Available at: http://www.inchem.org/documents/jecfa/jeceval/jec_1291.htm (website visited on October 20, 2011)

Safety Evaluation of Flavoring Agents as its is a macronutrient and a normal component of protein and, as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as a flavoring agent.

1.4. Intake from Natural Presence in Food

As an indispensable (essential) amino acid, lysine is not synthesized in the human body. It is one of the metabolically indispensable amino acids. Of all the essential amino acids, lysine is the most strongly conserved. This is crucial, as in cereal protein sources, lysine is the limiting amino acid. It also appears to be the first limiting amino acid in human milk (Reed and Beckett, 1996). Certain proteins, such as those found in meat, poultry, and milk are rich in L-lysine (Hendler and Rorvik, 2001). The lysine content of navy beans, milk, and eggs is reported as 72, 79, and 70 mg/g protein, respectively (IOM, 2005). Using the Dietary Reference Intakes from the IOM (2005), the recommended protein intake for 19 to 30 year olds is 56 g/day and assuming that this protein contain 6% lysine (as percent of protein), then this recommended diet would contain 3400 mg lysine/day. Additionally, for food labeling purposes, the Daily Reference Value for protein (50 g/day) is set at 10% of 2000 calories based on an adjusted average of the 1989 Recommended Daily Allowance (Federal Register, 2007). Therefore, the current daily background intake of lysine from its presence in the diet is likely to be at least 3200 mg/day. The IOM (2005) reported the mean lysine requirement of 31 mg/kg/day and the Recommended Dietary Allowance (RDA) to be 38 mg/kg/day. While the IOM (2005) concludes that the protein RDA is 0.82 g/kg/day, Humayun et al. (2007) recently reported that the protein RDA is around 1.0 g/kg/day. In a more recent publication, Elango et al. (2011) reported the revised children protein requirement as about 1.5 g/kg/day. The IOM (2005) report also provides estimates for the background levels of lysine. The median dietary intake of lysine by adult men during 1988–1994 ranged from 4650 to 7500 mg/day for various ages and by adult women from 3590 to 4560 mg/day. The mean usual daily intake of lysine for all individuals from foods and supplements is estimated as 5270 mg/day (IOM, 2005). Based on above discussion and estimates, the 3400 mg/day is a good conservative level of the background levels of lysine and will be used in this document for comparisons.

1.5. Intended Use Levels and Food Categories

As described earlier, P&G intends to use L-lysine monohydrochloride as a processing aid in the manufacture of fabricated snack foods at use levels up to 1.5%. L-Lysine monohydrochloride (1.2% L-lysine) will be added to formulated snack products, prior to frying, to reduce acrylamide formation in the finished product.

1.6. Amino Acid Profile of Formulated Product

Lysine has been reported to compete with asparagine in the Maillard browning reaction during the frying process resulting in the reduction of acrylamide formation. The amino acid profile of the formulated product, without (control) and with the addition of L-lysine monohydrochloride at levels of 0.5, 1.0 and 1.5% as a processing aid (0.4, 0.8 and 1.2% lysine, respectively), is summarized in Table 3. These results show that the total lysine concentration of the control product was 0.17%, while with the addition of L-lysine monohydrochloride (the amount added plus the amount naturally present) at levels of 0.5, 1.0 and 1.5%, the final product (background) was found to contain 0.28, 0.88 and 1.28% of lysine, respectively. The total amino acids level of all these products was 4.15, 4.56, 4.79 and 5.23%, respectively.

Table 3. Lysine and total amino acid profile for the formulated snack products

	Control	Control plus 0.5% L-Lysine HCl	Control plus 1% L-Lysine HCl	Control plus 1.5% L-Lysine HCl
Lysine (% w/w)	0.17%	0.28%	0.88%	1.28
Total amino acids (% w/w)	4.15%	4.56%	4.79%	5.23
Note: Values are for free and constituent amino acids after hydrolysis				

1.6.1. Estimated Daily Intake of Lysine from the Intended Uses

The reference serving size and dietary intake surveys can be used to estimate the impact on total lysine exposure from salty snacks with added L-lysine monohydrochloride to reduce acrylamide. The reference serving sizes for snacks in the USDA Nutrient Database for Standard Reference (NDSR) is 28 g. Hence a serving of snack food containing the maximum intended level of 1.5% L-lysine monohydrochloride would contain about 420 mg added lysine or 336 mg total (added plus background) lysine per serving. The USDA Continuing Survey of Food Intakes by Individuals (CSFII) for quantities of foods consumed daily also surveyed the consumption of snack foods. This survey reported the mean and 90th percentile snack food (potato chips) intake of 39 and 80 g/person/day, respectively. If all snack food consumption was converted to added lysine snack food at the intended use of L-lysine monohydrochloride at maximum levels of 1.5% (1.2% lysine), the resulting mean and 90th percentile intake levels would be 465 and 960 mg/person/day, respectively, of added lysine. For an individual weighing 60 kg, the intended use would result in mean and 90th percentile intakes levels of 7.75 and 16 mg/kg bw/day, respectively, of added lysine. However, there are some limitations to the use of CSFII survey data. Among the limitations, the individual food consumption data were collected for only two days, a brief period which may not necessarily depict 'usual intake.' Usual dietary intake is defined as 'the long-run average of daily intakes by an individual.' Upper percentile estimates may differ for short-term and long-term data because short-term food consumption data tend to be inherently more variable. Hence, relatively long-term surveys such as Market Research Corporation of America (MRCA) may be more appropriate for the exposure assessment.

The impact of the intended use of L-lysine monohydrochloride on the total dietary acute and chronic exposure of lysine (background plus lysine from snacks) of high snack food consuming populations was estimated using savory snack consumption of 13-to-17 year olds and total recommended dietary consumption of protein for (19-to-30 year olds). The estimate of the daily intake of lysine from savory snacks among high consumers is derived from the work of Webb et al. (1997) for olestra. This estimated consumption and eating frequency for Olestra from snack foods, used MRCA data which provides a realistic estimate of the acute and chronic consumption of savory snacks. The intake estimates from the MRCA database are sufficiently conservative such that they can be useful in nutrient safety evaluation. The method is realistic because the survey relies upon the determination of the frequency of food consumption over 14 continuous days for each survey participant. The average acute intake of snacks for the highest user (13-to-17 year old males) was reported as 48.9 g/day, while the chronic intake was 14.8 g/day. These values were converted using the estimated daily intake values reported by Webb et al. (1997) for olestra and using a typical fat content of savory snacks of 33.7%. Based

on these consumption estimates, the intended use of L-lysine monohydrochloride at 1.5% (1.2% lysine) in snack food will result in an additional average acute and chronic consumption of 587 and 180 mg L-lysine/day, respectively, for the highest user group (13-to-17 year old males). For safety assessment purposes, the value for the maximum estimated daily intake of 587 mg lysine/day was used.

The total daily lysine consumption (background of 3400 mg lysine/day) plus the lysine values (587 and 180 mg L-lysine/day) from the above estimate would be 3987 mg/day and 3580 mg/day for acute and chronic consumption, respectively. Based on the recommended protein consumption of 56 g for the age group of 19-to-30 year old males (IOM, 2005), the total lysine consumption (background plus added L-lysine monohydrochloride) would be 7.0% and 6.6% (as percent protein) for acute and chronic consumption, respectively. The estimated intake of lysine based on the amino acid profile in the diet of snack food consuming populations where L-lysine monohydrochloride has been added at the 1.5% level is slightly higher than the level authorized under the regulation 21CFR 172.320 [i.e., the amount of L-lysine added plus the amount naturally present in free and combined (as protein) form is not to exceed 6.4% by weight of the total protein of the finished food].

1.6.2. Effect on Amino Acid Ratio from the Intended Uses

In studies examining amino acid levels, the ratio of two amino acids (e.g., lysine/tryptophan ratio) are often used to evaluate the quality of the protein or to define excess amino acids in the diet and any concern for imbalance. Using the dietary reference values for proteins, amino acid levels, and for the estimated salty snack product, the ratio of lysine to tryptophan was calculated as follows: 5.7 without the added lysine, and 36 after the addition L-lysine monohydrochloride (1.5%); as compared to14.1 and 13.2 for the total dietary intake of amino acids from both acute and chronic salty snack consumption. The background dietary values assume adequate high quality protein. If the background protein was of a lesser quality, the ratios could in fact, be significantly less. Since only the levels of lysine are changing in the snack formulations and because the snack formulations represent only one part of the consumed diet, other lysine/ amino acid ratios would be similarly affected. Based on these estimates, the addition of lysine to snack formulations would not be expected to significantly impact the amino acid quality of the protein consumed or to provide excessive lysine levels relative to other amino acids.

1.7. Summary of Consumption

The available information suggests that a realistic and conservative background intake level of lysine will be ~3400 mg/day. The estimated daily intake of lysine from its intended uses in snack foods was determined using the CSFII and MRCA data. Based on CSFII data on snack food consumption, the intended use of L-lysine monohydrochloride at a maximum level of 1.5% (1.2% lysine) will result in mean and 90th percentile intakes of 465 and 960 mg/person/day, or 7.75 and 16 mg/kg bw/day, respectively, of added lysine. However, there are some limitations in this exposure assessment in terms of duration of exposure (2 days for CSFII versus using the MRCA database (14 days). Based on MRCA data, the intended use will result in an additional average acute and chronic consumption of 587 and 180 mg L-lysine/day, respectively. As the MRCA data provides a realistic estimate of the consumption of savory snacks, and is sufficiently conservative to be useful in nutrient safety evaluation, the high intake of 587 mg L-lysine/day is considered as appropriate for the safety assessment purposes.

2. DATA PERTAINING TO SAFETY

As an essential amino acid, there has been considerable effort to elucidate the biological importance and role of lysine in the human body. As a result, the literature is full of information on lysine. In the published literature, thousands of preclinical *in vitro* and *in vivo* studies with lysine have appeared. Additionally, as lysine supplementation has been claimed to benefit subjects with herpes simplex virus and in other conditions, several efficacy-related trials can be found in the published literature. The safety data from animal studies is extensive and sometimes confusing, as these studies were designed to assess the biological and nutritive role of lysine. Efforts have been made to present both the data supporting the safety as well as any data on the adverse effects of lysine. In several studies, the metabolic role of individual amino acids and protein has been investigated by varying the ratio or content of an amino acid (lysine) or protein. An attempt has been made to interpret the findings from such studies and their relevance to the current GRAS assessment. Relevant biological and toxicological studies on lysine are included in the following section in the order of their importance and in support of the conclusions drawn in this determination. An attempt has also been made to identify the form of lysine in the studies described below. It should be noted that in several studies reported, the L-lysine monohydrochloride is used on a weight basis 1.5% of this form (equivalent to ~1.2% lysine).

2.1. Human Studies

In an earlier review of the human requirements for lysine, Jansen (1962) concluded that neither toxicity nor imbalance of lysine poses a significant hazard in the proper use of lysine as a dietary supplement. Similarly, Harper et al (1974) reported that effect of excess lysine is moderate relative to that of methionine and tryptophan. In another extensive and critical review article on the metabolic roles, pharmacology, and toxicology of lysine, Flodin (1997) suggested that with respect to the administration of a high-dose L-lysine monohydrochloride with food to humans, it is necessary first to define what dosage may be considered excessive as it relates to various protein sources. With high biological value proteins such as meat, fish, and certain legumes, the lysine/tryptophan ratio may be as high as 8.7. Flodin (1997) stated that whether mixed with food or taken with food as a dietary supplement in pill form, if the amount of lysine so supplied does not increase the lysine/tryptophan ratio of the dietary protein beyond 8.7, then the supplement must be considered for its nutritional contributions, rather than pharmacological effects, and this amount is not an excess from a potential safety perspective. In an earlier article, Flodin (1993) reported that American diets of usual protein level and amino acid composition have lysine/tryptophan ratios of 4.4 to 4.8. Such diets can receive additions of up to 3 g L-lysine/day, distributed among meals, without exceeding the lysine/tryptophan ratio of meat or fish. By this criterion, there are only a few clinical studies reported that used excessive lysine supplementation when taken with food. Based on the measured amino acid profiles in formulated product with 1% addition of L-lysine monohydrochloride, the lysine/tryptophan ratios do not change significantly with chronic snack consumption (8.6 vs 8.8). These levels are very close to a high quality dietary source of protein. If the quality of the background diet is not from a high quality protein source as assumed in these calculations with the addition of L-lysine monohydrochloride, then the ratio would still be below those defined as high quality protein sources.

Infants, as the most sensitive population, also appear not to be adversely affected by excessive amounts of lysine added to milk, at least acutely. Dubow et al. (1958) investigated the tolerance of infants to lysine, added as lysine monohydrochloride (isomer not specified in the

article) to whole cow's milk. In this study, six infants, aged 4 to 11 months participated, and a lysine monohydrochloride-supplemented milk batch was fed to each infant for 3 to 4 days. Lysine supplement levels studied were 60, 120, 240, 480, 720, 960, and 1080 mg lysine monohydrochloride (isomer not identified) per 8 ounces of milk. At the highest level, daily added lysine consumed was 5.18 g (6.5 g lysine monohydrochloride). As a percentage of milk protein, the maximum supplementation level increased the total amount of L-lysine to 17.7% vs. 7.4% for unsupplemented milk, an excess of about 140%. The lysine monohydrochloride supplements were well tolerated in this study, even at the highest level. Plasma lysine levels varied but were within normal limits, while urinary lysine levels were roughly proportional to the amount of supplementation. None of the subjects showed cystinuria.

In a randomized, blinded, 16 week parallel feeding trial, 65 healthy infants were fed either an experimental partially hydrolyzed rice protein-based infant formula fortified with lysine [4769 μmol (~0.7 g) lysine/L] and threonine (2518 μmol threonine/L) (n=32), or a standard intact cow's milk protein-based formula (n=33) as a control (Lasekan et al., 2006). Assessments of growth, tolerance, and plasma biochemistries in infants were performed at enrollment, and at 2, 4, 8, and 16 weeks of age. Growth was not different between the two formulas. Plasma biochemistries (phosphorus, alkaline phosphatase, total protein, albumin, blood urea nitrogen, and prealbumin) for both groups were within the reference normal range. However, the rice protein formula group had lower phosphorus and urea nitrogen, lower essential amino acids except threonine, which was higher, and a lower ratio of essential (including semi-essential) to non-essential amino acids. Differences in the concentrations and ratios of amino acids diminished as feeding progressed with age. Plasma total protein, albumin, prealbumin, calcium, magnesium, and alkaline phosphatase were not different between groups. The experimental rice formula fortified with lysine and threonine was well tolerated (based on no differences in gastrointestinal- spit-up or vomit, and descriptions of the number, color, and consistency of these infants' stools). No adverse events were reported. The results of this study suggest that rice protein-based formula fortified with lysine and threonine was adequate in supporting normal growth and biochemistries in healthy term infants during the first 4 months after birth.

Mack et al. (1962) investigated the effects of lysine-supplemented diets on growth and skeletal density of pre-adolescent children. In this study, 84 children (48 boys and 36 girls) from an American orphanage participated. The children were aged 6-to-12 years, 42 of whom received L-lysine monohydrochloride added to their diet, while the other 42 did not. Supplementation was at the rate of approximately 70 mg L-lysine monohydrochloride/kg bw, averaging 2.5 g/day, for 5.5 months. This was calculated to raise the lysine/tryptophan ratio of the diet from 5.0 to 7.9. Compared to the control, significant increases in growth and gains in bone density in two anatomic sites (radius and os calcis) were noted at the end of the study in the treatment group. The positive effects on growth do not appear to be related to any pharmacological action of the lysine supplement, as the dietary lysine/tryptophan ratio did not exceed that of meat or fish, but may suggest that a dietary lysine/tryptophan ratio higher than 5 could be advantageous for enhancing growth and bone mineral deposition during the pre-adolescent period.

In three separate publications, Sackler and Sophian (1957a, 1957b, 1958) reported findings from the administration of a single-dose of 5 g L-lysine hydrochloride with a meal comprised of bread and water to 21 adult volunteers (7 male and 14 female), using a stomach tube for direct delivery of both lysine and food. The amounts of free hydrochloric acid and pepsin were determined in samples taken every 15 minutes for 1 hour. In the majority of the

subjects, variable increases in hydrochloric acid and pepsin occurred. Arginine monohydrochloride administered in the same way did not produce these effects (Sackler and Sophian, 1958). Both amino acids were well tolerated.

Spencer and Samachson (1963) investigated the effect of a high-dose of L-lysine monohydrochloride added to a diet and providing 60 g protein/day (2/3 was in the form of meat), on calcium and phosphorus metabolism in two postmenopausal osteoporotic women and one man with hypoparathyroidism. One woman, age 77 years, received 10 g L-lysine monohydrochloride/day for 22 days in one study, and 24 days in another. The other woman, age 67 years, received 20 g L-lysine monohydrochloride/day for 12 days in one experiment and 10 g/day for 12 days in a second experiment. The man, age 50 years, received 20 g lysine monohydrochloride/day for 30 days. There were no significant changes in calcium balances and no adverse effects were reported.

In addition to the above described studies, the effects of L-lysine monohydrochloride on the treatment of recurrent herpes simplex infection have been investigated in multiple studies. In a multicenter trial, 45 herpes simplex patients received an additional 312 to 1200 mg of L-lysine daily (400-1500 mg L-lysine monohydrochloride) as single or multiple doses for periods ranging from 2 months to 3 years (Griffith et al., 1978). No adverse effects were reported.

In a subsequent double-blind placebo-controlled trial by these investigators (Griffith et al., 1987), a total of 27 (6 male and 21 female; mean age 31 years) subjects on L-lysine monohydrochloride and 25 (6 male and 19 female; mean age 32 years) subjects on placebo completed the trial. The treatment group was given L-lysine monohydrochloride tablets (1000 mg L-lysine per dose) 3 times a day for 6 months. During the trial, no significant adverse effects were reported but three subjects in the lysine group and one in the placebo group reported 'upset stomach' and one subject in the placebo group reported nervousness. No other adverse effects were reported.

In a prospective, randomized, double-blind, placebo-controlled, cross-over trial, McCune et al. (1984) investigated the effects of L-lysine monohydrochloride in 41 patients with herpes simplex infection. All patients had at least bimonthly recurrence during the six months immediately before the study. Oral ingestion of 1248 mg/day of lysine monohydrochloride was found to be an effective treatment (decreasing severity of symptoms associated with recurrence). No subject dropped out of the study due to side effects. In a double-blind study, L-lysine monohydrochloride or mannitol capsules (250 mg each) were randomly given to 31 patients suffering from herpes simplex infection (Simon et al., 1985). During the first three months, patients were given two capsules (1000 mg/day) twice a day. Eighteen of the patients then continued taking one capsule every morning and two every evening for a total of 750 mg per day for three more months; the other 13 subjects withdrew for unexplained reasons. No adverse effects were reported. In a double-blind placebo controlled, crossover study, Milman et al. (1980) evaluated the effect of L-lysine monohydrochloride (1000 mg/day) on recurrent herpes simplex labialis in 65 patients. After 12 weeks of lysine treatment, the patients were shifted to placebo treatment for a similar period. On the whole, lysine prophylaxis had no effect on recurrence rate. No adverse effects of lysine treatment were reported.

In summary, the available evidence from human studies suggests that consumption of supplemental lysine at levels up to 3000 mg/person/day for up to six months did not reveal any adverse effects. Additionally, lysine supplementation was well tolerated by both infants and

children. In a critical review article on the safety of lysine (Flodin, 1997), it was concluded that oral lysine supplements of 1000 to 3000 mg/day (1250 to 3750 mg/day as L-lysine monohydrochloride) divided among meals for individuals consuming usual American diets would not elevate the proportion of lysine in the dietary protein beyond that already found in meat and would be safe for chronic use. The levels of lysine used in these clinical studies were over 7-fold higher than those estimated from the intended uses of L-lysine monohydrochloride in snacks.

In a recent, trial, Wass et al. (2011) investigated the benefit of L-lysine as an add-on treatment for schizophrenia. In this study, L-lysine (6 g/day) was administered to 10 patients with schizophrenia as an adjunct to their conventional antipsychotic medication. The study was designed as a single-blinded, cross-over trail where patients were randomly assigned to initial treatment with either L-lysine or placebo and screened at baseline, after four weeks when treatment was crossed over, and after eight weeks. L-Lysine treatment caused a significant increase in blood concentration of L-lysine and was well tolerated. Treatment with L-lysine did not reveal any adverse side effects including extrapyramidal effects. Furthermore, L-lysine treatment did not significantly alter blood concentration of any of the other amino acids analyzed, such as citrulline, arginine, proline, glutamate and alanine.

2.2. Long-term Animal Toxicity Studies

In a well-designed subchronic toxicity study conducted in compliance with Good Laboratory Practices (GLP), Tsubuku et al. (2004) evaluated the toxicological and behavioral effects of L-lysine hydrochloride in male and female Sprague-Dawley rats. Six week-old rats (12/sex/group) were divided into four groups and were fed a diet containing 0, 1.25, 2.5 and 5% (w/w) L-lysine hydrochloride (added lysine) for 13 consecutive weeks. The average intake of lysine for these four groups in males was reported as 841.2 ±29.3, 1677.4 ± 69.4, and 3356.6 ± 115.1 and for females it was 967.9 ± 46.5, 1917.2 ±86.5, and 3986.3 ± 282.9, respectively. The administration period was followed by a 5-week recovery period, during which time only the standard diet was provided to all animals. Rats were observed twice daily during the 13 weeks of feeding, and twice a week body weights and feed consumption were measured. Ophthalmologic examinations were performed prior to initiation of the study and during the 13 weeks. Urine analysis was conducted during week 5 and 13. At the end of study, rats were euthanized and blood was collected to measure the following hematology parameters: mean corpuscular volume, hemoglobin, platelet, white blood cell count, differential leukocyte count, prothrombin, activated partial thromboplastin time, and fibrinogen, hematocrit and mean corpuscular hemoglobin. Additional plasma parameters such as alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase and serum parameters such as total cholesterol, triglycerides, phospholipids, total bilirubin, blood glucose, urea nitrogen, creatine, uric acid, sodium, potassium, chloride, calcium, inorganic phosphorus, and total protein were measured. Over 45 organs and tissues were collected and processed for histopathological examinations.

No treatment-related changes in clinical signs, body weights, diet consumption, water intake, ophthalmology, gross pathology, organ weights, or histology were noted in any of the groups. Among the serum chemistry and urinalysis parameters in males, a lysine-related drop in serum concentration chloride (controls, 111.9±0.7 mEq/L; 1.25% group, 109.9±1.1 mEq/L; 2.5% group, 110.3±1.5 mEq/L; 5.0% group, 109.8±1.3 mEq/L), and in females of the 5.0% concentration group (controls, 112.7±1.2 mEq/L; 5.0% group, 110.1±1.2 mEq/L) an increase in urinary excretion of chloride were noted. No other significant changes in hematology, clinical

chemistry or urinalysis parameters were noted. The changes in chloride concentrations were considered by the investigators as a compensatory response to the ingested hydrochloride. Additionally, no functional, biochemical, or histological changes in renal function were noted. Histological examinations did not reveal any significant treatment-related pathology. The minor changes noted were few and not dose-related. Based on the results of this study, the investigator determined a no-observed-adverse-effect level (NOAEL) for L-lysine monohydrochloride of 5% (~ 3670 mg/kg bw/day) for both genders (male, 3360 ± 120 mg/kg/day; female, 3990 ± 280 mg/kg/day), the highest dose tested (Tsubuku et al., 2004).

A secondary citation from a 1992 FASEB special publication on the safety of amino acids referenced a two year *ad libitum* feeding study, rats were fed a diet containing lysine monohydrochloride (95% L- and 5% D-isomer) (Anderson and Raiten, 1992). In this study, a commercial diet containing 25% protein with a lysine content of 5.6% was used. For the first 15 weeks of the study, rats (weaning) were fed the commercial diet with 1% lysine monohydrochloride added. The addition of lysine monohydrochloride by 1% increased the already adequate lysine level by about 50%. After the first 15 weeks, the concentration of supplemental lysine monohydrochloride was increased to 3%, providing about 170% excess lysine over the levels in unsupplemented ration. The results of this two year feeding study with lysine monohydrochloride did not reveal any adverse clinical, hematological, or pathological effects. Additional details of the study were not available and not provided in the report.

McClure and Folk (1955) studied the effects of L-lysine monohydrochloride on the incidence and severity of dental caries. In these investigations, weanling rats were fed diets based on skim-milk powder that had been reduced in bioavailable lysine by dry autoclaving, which promotes the Maillard reaction. A total of 721 weanling rats were assigned in groups of 16 to 44 to control or lysine-supplemented diets and fed for 60 or 90 days. Lysine was added to diets at levels ranging from 0.25 to 2.5% (250 to 2500 mg/kg bw/day). L-Lysine monohydrochloride supplementation reduced caries approximately 59% in incidence, 76% in number of carious teeth, and 83% in severity score. Supplementation with L-lysine monohydrochloride at 0.25% or 0.5% (250 or 500 mg/kg bw/day) in the diet was as effective as 2.5% (2500 mg/kg bw/day), indicating a nutritional rather than a pharmacological action. The article did not provide any information concerning effects on safety related parameters. In a review article on the importance of nutrition in vascular diseases, Yamori (1994) stated that there were no adverse effects of increasing lysine intake by about 150% from the requirement in stroke-prone, spontaneously hypertensive rats over a 25-week period. Compared to the control group, the stroke incidence was markedly decreased by lysine supplementation.

In summary, the results of a 13-week GLP compliant animal study demonstrate that feeding of rats with L-lysine monohydrochloride in diet at dose levels up to 5% (~ 3670 mg/kg bw/day) did not cause any adverse effects as evaluated by a series of safety-related parameters. In a two year study, feeding of lysine monohydrochloride did not cause any adverse effects as evaluated by clinical, hematological, or pathological changes. The available evidence from animal studies suggests that levels of lysine well above background levels in the diet do not cause adverse effects.

2.3. Absorption and Metabolism

Following ingestion, L-lysine is absorbed by the active transport process from the lumen of the small intestine into the enterocytes (Handler and Rorvik, 2001). Once, inside the

enterocyte, some of the lysine is metabolized. The unmetabolized lysine is transported to the liver via the portal circulation and once inside the liver, lysine along with other amino acids participates in protein biosynthesis. Some of the lysine in the liver is metabolized to acetoacetyl CoA via formation of L-alpha-aminoadipic acid and saccharopine. L-Lysine does not participate in transamination. Unmetabolized lysine from the liver is transported to various tissues in the body where it is involved in reactions similar to those described in the liver. L-Hydroxylysine, found in collagen and elastin, is formed post-translationally.

In a study in mice, [^{14}C]-lysine at a dose of 1.46 mg was given via intravenous injection. The majority of the radioactivity was rapidly removed from the blood. At 10 min and at 4 hours after administration, total lysine (cumulative from blood, viscera, and expired CO_2) was found to be 46 and 58%, respectively. In the 4 hours following administration, 27% of lysine was metabolized and exhaled as $^{14}CO_2$ (Borsook et al., 1950). Based on the results of a dog study, Longenecker and Hause (1958; 1959) reported that the rate of absorption of free lysine added to wheat gluten was similar to that of amino acids liberated from the digestion of proteins. Several human studies have also shown that lysine monohydrochloride supplements are absorbed at rates similar to those from digestion of proteins suggesting that supplementation is an effective and efficient means of correcting lysine deficiency (Flodin, 1993).

Pawlak and Pion (1968) reported the affinity of muscle tissue for free lysine in growing rats fed wheat gluten diets containing increasing levels of lysine monohydrochloride (also see section 2.2.2). The results of this study revealed that as the total lysine level increased from the unsupplemented level of 2.9% of the diet to 6-7%, where maximum growth rate was achieved, the plasma and muscle lysine concentrations increased in parallel (muscle concentration to about twice that in plasma). Further increases in dietary lysine levels significantly increased muscle free-lysine content relative to the plasma levels such that the diet richest in lysine (18%) increased the free lysine concentration in muscle by 27-fold and in the plasma free-lysine increased by 7-fold versus the respective concentrations observed with the unsupplemented diet.

Available evidence from studies in adult (Said et al., 1974) and growing (Gahl et al., 1991) rats suggest that of all the indispensible amino acids, lysine is one of the most strongly conserved. In a study examining the response of adult rats to deficiencies in different essential amino acids, the smallest weight loss (30 g) in rats occurred following feeding a lysine-deficient diet over a 14-week period. Feeding a diet deficient in threonine produced a weight loss (~100 g) that was same as that of animals fed a protein- free diet (Said et al., 1974). Similarly, in growing rats the least nitrogen loss caused by lack of a single essential amino acid-free diet fed over a 21 day period was noted in animals fed a lysine diet (Gahl et al., 1991).

In a review article on amino acid bioavailability and digestibility in pig feed ingredients, Stein et al. (2007) suggested that a careful assessment of the bioavailability of each of the dietary indispensable amino acid is critical for evaluating the nutritional value of feed ingredients and for estimating amino acid requirements of pigs and other species. Lysine is considered as first limiting amino acid in pig feed (Henry et al., 1992). For heat-treated feed ingredients, some amino acids, such as lysine, may be present in chemical forms, like Maillard reaction products, that may be absorbed but preclude utilization for protein synthesis. In this case, the calculated digestibility overestimates amino acid bioavailability. Moughan and Rutherfurd (1996) reported that the true ileal lysine digestibility in casein was 98.8%, while the corresponding value in the heated lactose/casein was 70.5%. In another study, Moehn et al. (2007) also reported that heating or cooking peas reduces lysine bioavailability (75.8% to 68.3%). These observations indicate

that, given the use of lysine as a processing aid in snack foods that are fried, not all added lysine will be bioavailable or utilized for protein synthesis. The reductions in acrylamide levels from 520 to 1640 ppb (Table 2) following the lysine treatment also indicate that some of the added lysine is not bioavailable.

In summary, orally administered L-lysine is absorbed by active transport into the gut enterocytes, where part of it is metabolized. Lysine is transported via the portal circulation to the liver, where it participates in protein biosynthesis. Unmetabolized lysine from the liver is transported to various tissues. The rate of absorption of free lysine added to food has been reported to be similar to that of amino acids liberated from the digestion of proteins. Lysine has been reported to be one of the most strongly conserved amino acids. It is primarily catabolized via the saccharopine pathway by 2 linked enzymes, reductase and dehydrogenase. The proposed use of L-lysine monohydrochloride is unlikely to saturate or overwhelm the metabolic system. Additionally, the metabolic products of lysine, saccharopine and α-aminoadipic acid are measurable components of consumed foods, and their safety is supported by history of dietary exposure and safe consumption of these two metabolites by humans.

2.4. Nutritional Role

It has been well established that all proteins with high biological value have high lysine content. The importance of lysine has been demonstrated both for animal growth (Osborne and Mendel, 1914) and adult human maintenance (Hawley et al., 1948) early in the 19th century. The protein digestibility-corrected amino-acid score is considered the most suitable regulatory method for evaluating protein quality of foods and infant formulas. Because this method is based on human amino acid requirements, it is inherently more appropriate than animal assays used for predicting protein quality of foods (Eggum, 1991). Proteins may be considered to be of high biological value if they have 5-8 times as much lysine as tryptophan and three or more times as much threonine as tryptophan (Flodin, 1997). Protein foods derived from poultry, cows, fish and plants, (i.e., eggs, milk, beef chicken, fish, and legumes such as bean (*Phaseolus vulgaris*), lentil, Lima bean and soybean with high ratio of lysine to tryptophan) have a high biological value. Low lysine diets produce lower growth rates and nitrogen retention in both animals and humans than diets in which the lysine/tryptophan ratio is 5 or higher. At normal protein intakes for omnivorous species (10-20% of total calories), several growth studies conducted in rats, pigs, and poultry have demonstrated that doubling lysine intake by doubling the dietary levels of a low lysine protein is far less effective for nitrogen retention than raising the nitrogen to tryptophan ratio by supplementation of lower protein levels with lysine monohydrochloride (Flodin, 1997).

In a study examining the effects of amino acid supplementation in young children, Bressani (1971) also observed a similar phenomenon while studying the effect of lysine supplementation on nitrogen retention from wheat gluten diets fed at different protein levels. It was noted that in the utilization of wheat protein, lysine was the most limiting amino acid. Addition of lysine to the basal diet resulted in sustained nitrogen retention similar to that obtained with milk feeding, and when the basal diet was supplemented with all of the limiting amino acids. In another study with adult cebus monkeys, Ausman and Hegsted (1980) found that the lysine requirement for weight maintenance was the same per gram of protein at 16.2% protein calories as at 9.34% protein calories. Notably, weight loss was observed at the unsupplemented higher-protein levels, in spite of the additional protein bound lysine present in this diet, until enough lysine monohydrochloride was added to make the ratio of total lysine to protein the same as that found effective for weight maintenance at the lower protein levels.

Commonly consumed diets in the US have lysine to tryptophan ratio close to 5. However, factors such as inadequate income to purchase sufficient animal protein foods, poor appetite, and dentition problems in the elderly, vegetarian diets without adequate consumption of high lysine legumes, and generally poor dietary habits can influence the quality of protein in the diet, or reduce the ratio of lysine to tryptophan (Flodin, 1997). Approximately 10 to 25% of American women >55 years of age have been reported to consume <30 g protein/day (NCHS, 1980). Elderly women consuming a low protein diet experienced a loss of lean body mass and a decline in immune response and muscle function (Castenada et al., 1995). To address this concern, a modest (amount not mentioned by the authors) mealtime supplement of lysine has been suggested to improve the biological value of dietary protein in elderly women with low protein intake (Flodin, 1997).

In summary, proteins with high biological values have high lysine content. The available evidence from growth studies in animals suggest that lysine monohydrochloride supplementation of a low lysine protein diet was more effective for nitrogen retention when compared to increasing the amount of low lysine protein in the diet. Studies in children and monkeys suggest the important role of lysine in weight maintenance. A lysine supplement has been suggested to improve the biological value of dietary protein in elderly women with low protein intake.

2.5. Acute and Short-term Animal Studies

In acute toxicity studies, the LD_{50} of L-lysine monohydrochloride following oral administration to male and female rats was reported as 10,130 mg/kg bw (Breglia et al., 1973). In another study, the LD_{50} of lysine monohydrochloride following intravenous administration to young adult male rats was determined as 22 mmol/kg bw (~ 4000 mg/kg) (Anderson and Raiten, 1992).

Pawlak and Pion (1968) fed male Wistar rats (weight 100 to 110 g) a 12% wheat protein diet with additions of lysine monohydrochloride at levels up to 18%. The L-lysine content of the unsupplemented diet was 2.9 g/kg. Groups of eight animals each received single supplementary levels and their weight gain and food intake were measured for two weeks. Maximum growth rate was attained with the addition of 6 to 7 g lysine monohydrochloride/kg diet, corresponding to an average of 8.1 g total lysine/kg of diet or 6.15% L-lysine based on weight of protein. Groups receiving higher supplements, up to 12% lysine monohydrochloride, grew at the maximum rate and consumed about the same amount of food. With the 12% supplement, there was a 54% excess of lysine over the 8.1 g total L-lysine/kg diet sufficient for maximum growth. In the group receiving the largest supplement [18 g/kg (a 214% excess of lysine)], daily food intake dropped from 18 to 16 g, and daily weight gain from 7 to 6 g. No other adverse effects were reported.

2.6. Reproductive and Teratogenicity Studies

Funk et al. (1991) examined the impact of supplemental lysine on pregnancy course and outcome in female Sprague-Dawley rats. From day 0 until day 20 of pregnancy, rats were fed a complete 20% casein diet containing lysine at 50, 100, or 500% excess over controls. The addition of lysine was 0.78, 1.56, and 7.79 g/100 g of the diet. One group of rats received a control diet *ad libitum* and each treatment group had a matched pair-fed group receiving a control diet. The control diet content of lysine was 7.78 g/100 g. Based on daily consumption of feed and body weight, the resulting dose of lysine was estimated as 140, 280, or 1400 mg/day (1120, 2240, or 11200 mg/kg bw/day), respectively. The groups receiving 50 and 100% excess

lysine showed no significant differences from controls in maternal weight gain or fetal condition. Compared to control, the treatment group receiving a 500% excess of lysine had lower maternal weight gain and smaller fetuses. The investigators concluded that variable levels of lysine supplementation did not cause recognizable fetal malformations. However, diets highly supplemented with lysine may affect pregnancy through a decrease in maternal weight gain and fetal size. The 500% excess lysine relates to human supplementation with lysine of approximately 35 g/day. The results of this study suggest that feeding of supplemental lysine in diet to rats during pregnancy had no adverse effects on pregnancy course and outcome at levels up to 2.24 g/kg bw. These dose levels are over 30-fold higher than total daily lysine consumption (background of 3400 mg lysine/person/day plus the maximum lysine values after the addition of L-lysine monohydrochloride) of 3987 mg/day and 3580 mg/day for acute and chronic consumption, respectively. These values equate to 66.45 and 59.67 mg/kg/day for acute and chronic snack consumption, respectively, for a 60 kg person.

In the review report prepared by the Life Sciences Research Office (LSRO) of the FASEB to the FDA on the safety of amino acids used as dietary supplements, Anderson and Raiten (1992) reported that feeding of pregnant rats with lysine monohydrochloride supplemented diets providing lysine excesses relative to tryptophan, arginine, and other essential amino acids (10 or 25% L-lysine) failed to produce congenital malformations, although fetal growth was reduced. Maternal weight gain and the total number of surviving fetuses were decreased in the highest levels of lysine supplementation (25%). The animals were fed 5% L-lysine in a 6% casein diet or 10 or 25% L-lysine in 9 or 27% casein diets. As indicated earlier, the objective of this and other similar studies was to investigate the nutritional role of the amino acid and protein by varying the ratio of amino acid (lysine) to protein. Hence it is difficult to interpret the findings and relevance of such studies from the current GRAS assessment perspective.

Cohlan and Stone (1961) investigated the effects of excess dietary L-lysine on rat pregnancy and offspring viability. Pregnant CF Wistar strain rats (240-260 g) were fed (days 5 to 15 of pregnancy) diets containing 10 or 25% excess L-lysine prepared either in a low-protein (9% casein) or optimum-protein (27% casein) powdered diet. A slight decrease (statistical analysis not performed by the authors) in litter survival and expected maternal weight gain was noted in rats fed the 27% casein diet containing 10% excess L-lysine. When the amino acid excess was increased to 25%, however, a more pronounced decrease in litter survival and maternal weight gain was observed. Litter survival was reduced to 40% and a maternal weight gain of 7.0 g as compared with the control gain of 60.2 g. Average fetal weights in the groups receiving a 10% excess of L-lysine were not significantly affected. In rats fed the 9% casein diet, the decreases in litter survival and maternal weight gain were more marked but followed the same pattern observed in the 27% casein-based diet experiments. Interestingly, the average fetal weights were remarkably constant with both the 10 and 25% L-lysine-excess diets. Moreover, though litter survival and maternal weight gain were severely reduced, the average fetal weight was only 5 to 10% less than that of the control. No gross fetal malformations were observed in any of the surviving litters. As feed consumption was not constant in these experiments and decreased in direct proportion to the increase in amino acid distortion of the diet, an attempt was made to circumvent this variable by administering the amino acid intraperitoneally. In these experiments, the average fetal weights of surviving fetuses were unaffected by maternal challenge of lysine. The results of this study suggest that although maternal weight gain and fetal resorption may be influenced by the effect of amino acid levels on maternal metabolism, the fetuses that persevere attain a "relatively" normal growth pattern consistent with the maternal

total protein intake. This is further supported by intraperitoneal administration of the amino acid. Contrary to the observations from this study, in the above described study, feeding of 100% excess lysine did not affect fetal condition (Funk et al., 1991).

Matsueda and Niiyama (1982) investigated the effects of a dietary excess of one essential amino acid on the maintenance of pregnancy and fetal growth in rats. For these experiments, pregnant Sprague-Dawley rats were fed a 6% casein diet supplemented with 5% lysine from days 1-to-14 or to day 21 of pregnancy. Urine and feces were collected at 3- day intervals during pregnancy to determine nitrogen balance. Food intake and body weight were measured daily. On day 22, blood samples were collected and the animals were euthanized. The weight gains in the lysine fed group were comparable to the control. Although the nitrogen intake of the lysine supplemented group was considerably larger than the control, the nitrogen retention was comparable to the control. Lysine supplementation did not affect weights of conception products (i.e., uterus, placenta, fetus, and amniotic fluid). The weights of fetuses were significantly lower. The litter size was unaffected by lysine supplementation. The results of brain weight revealed that lysine supplementation decreased prenatal brain growth. Total DNA content of the fetus brain was reduced. Ingestion of a diet containing excess lysine did not result in appreciable alteration in maternal plasma and fetal brain amino acid concentration. The resulting daily dose of lysine in this study was approximately 2.5 g/kg bw. It is apparent that the adverse effects noted in this study are related to the very high doses of lysine. In this study, the effects of lysine at lower levels were not studied. Secondly, the protein content of the diets was low and addition of amino acid (lysine) at high levels appears to have caused imbalance in the amino acid profile.

In a multigenerational study in rats, Culik and Rosenberg (1958) investigated the effects of lysine deficiency and supplementation on reproduction and lactation. Each group of rats (strain not specified) consisted of 10 females and 5 males. The 45 animals used at the start of the experiment were selected at weaning from 10 litters. Litter mates were assigned to one of three groups. The study involved the feeding of lysine-supplemented bread diets containing 6% non-fat dry milk to rats through six generations while monitoring breeding performance. The lysine hydrochloride was added to the flour before baking. The lysine content of the prepared commercial bread diet was 0.30%, while that of lysine supplemented commercial diet was 0.46%. This addition increased the lysine content of the diet by 0.16%. In addition to these diets, a group of rats was also included that received stock diet containing 25% milk solids. The lysine content of the stock diet was 1.2%. Supplementation of commercial bread containing 0.16% lysine resulted in marked improvements in reproduction and lactation compared to rats receiving the unsupplemented bread diet. The overall performance between the lysine supplemented bread diet group and the group receiving the stock diet was similar. No adverse effects were noted.

Chase and Jansen (1976) studied brain development in the first and second litters of offspring from a limited study following consumption of bread, bread plus lysine, or bread plus lysine and threonine by rats during pregnancy and lactation. In this study, the following diets were fed: Diet 1: white bread; Diet 2: bread plus 0.35% L-lysine-HCl; Diet 3: bread plus 0.6% L-lysine-HCl plus 0.35% DL-threonine; Diet 4: bread plus 13% casein; Diet 5: bread plus 26% casein; and Diet 6: a stock diet. Each diet was fed to three adult female rats during two pregnancies and lactations. Nursing litters consisted of eight pups, so that 16 offspring were studied from each mother. Brains from each offspring were analyzed individually. Brain weight, protein, and cellularity progressively increased as lysine and lysine plus threonine were added to the maternal bread diets. The results of this study demonstrate improved brain growth in both the

first and second litter offspring when lysine or lysine plus threonine were added to a bread diet fed during pregnancy and lactation.

In an interim report on ten cases with familial hyperlysinemia, Dancis et al. (1983) reported that a 24-year old woman with hyperlysinemia gave birth to a normal infant. Although serum lysine levels were not measured at birth, it is a reasonable presumption that intrauterine fetal lysine levels were at least as high as the maternal levels. This observation suggests that the human fetus may develop normally despite constant exposure to high maternal lysine levels. In this genetic disease, serum lysine levels usually run 5-10 times higher than normal despite greatly enhanced urinary lysine excretion.

Schneider et al. (1979) investigated the transfer of amino acids using an *in vitro* perfusion technique of human placenta in which independent maternal and fetal circulations were established. When the fetal perfusate was recirculated, a concentration gradient was established with fetal concentrations of alanine, glycine, and lysine about 1.5 times higher than the maternal concentration. In this system, the placenta could actively transfer 4.1 mM/day of each of these amino acids.

In summary, feeding lysine at levels up to 100% excess (~2240 mg/kg bw/day) did not cause adverse teratogenic effects. In one study, administration of 10 or 25% excess lysine in optimum protein (25% casein) diet resulted in decreases in maternal weight gain and fetal survival. The maternal decrease in body weight gain appears to be related to fetal survival. In another study, lysine supplementation (6% casein diet containing an additional 5% lysine) was reported to affect prenatal brain growth, while in yet another limited study, first and second litter offspring brain development was improved following lysine supplementation of a bread diet fed during pregnancy and lactation. In several of the teratogenicity studies reported, different ratios of amino acids in proportion to protein content or to other amino acids were used, thus complicating the interpretation of the findings. In a limited sample size multigenerational study, supplementation of lysine to rats for six generations did not cause adverse effects on reproduction and lactation. Based on the findings from a case report, a 24-year old woman with hyperlysinemia gave birth to a normal infant. Overall, the available evidence suggests that the intended use of L-lysine hydrochloride as a processing aid is unlikely to cause any teratogenic effects.

2.7. Lysine-Arginine Antagonism

Several feeding studies have been conducted in laboratory animals on casein-based diets with high supplemental lysine levels. Like other milk proteins, casein is marginal in arginine content relative to lysine, compared to other high-quality proteins. As lysine and arginine share a common transport system, casein-based diets are logical to investigate the effect of excess lysine on arginine metabolism, growth, and other biological variables, as well as the opposing effect of arginine supplements (Flodin, 1997). With 18% casein as the basal protein source, a small increase of 1.5% added lysine monohydrochloride reduced the growth rate of young rats by 9%. The addition of lysine monohydrochloride raised the lysine/tryptophan ratio from 5 to 9.1 and the lysine/arginine ratio from 2.2 to 3.9. The use of a commercial stock diet with 24.4% protein that would lower the lysine/arginine ratio of lysine monohydrochloride-supplemented diet overcame most of the growth depression. Supplementation of diet with 1% arginine could also overcome the growth depression (Anderson and Raiten, 1992). These studies were designed to

investigate the effects of lysine on arginine metabolism, and supplementation of diet (24.4% protein) with lysine did not cause adverse effects.

Hevia et al. (1980) investigated changes in liver and serum lipid levels in rats fed 7.5, 15, or 30% casein diet supplemented with 0, 2.5, or 5% lysine. Increasing dietary casein from 7.5 to 15% increased serum total lipids, triglycerides and cholesterol. A further increase in casein from 15 to 30% revealed an additional increase in plasma cholesterol and a decrease in triglycerides. Rats fed 7.5% casein alone developed fatty livers. The addition of 5% lysine to 15% casein caused fatty livers, while addition of 5% lysine to 7.5% casein diet did not cause fatty liver. Fatty liver was almost completely prevented by arginine supplementation. Lysine had no effect on total liver lipids with casein at 30%. The lipids of the fatty livers with 7.5% casein or with lysine were similar in composition. In both cases, liver triglycerides increased four-fold and cholesterol two-fold. The results of this study show that excess lysine causes changes in lipid metabolism that depend on the quantity of casein fed. These investigators also suggested that the hypercholesterolemia observed in rats fed casein by others is probably unrelated to lysine. It appears that lysine supplementation of an optimum protein diet is unlikely to cause any adverse changes in liver lipids. In the above described subchronic study (Tsubuku et al., 2004), lysine at levels up to 5% in diet did not cause any adverse effects.

Compared to young rats, the growing dog and pig appear less sensitive to an elevated lysine/arginine ratio. Czarnecki et al. (1985) fed puppies a purified crystalline amino acid basal diet containing 0.40% L-arginine or 0.91% L-lysine (Lys/Arg = 2.3, L/T = 5.7). An increase in dietary lysine/arginine ratio to 4.8 and 7.3 by adding 15 and 25% L-lysine (acetate salt), respectively, did not reduce weight gain or the gain/feed ratio. The addition of 4% of lysine as the acetate depressed growth and food intake, with the classic signs of arginine deficiency: orotic aciduria, depressed urea formation, hyperammonemia, and emesis. Supplemental arginine alleviated most of these adverse effects. The results of these investigations have shown that excess dietary lysine (4.91%- basal diet 0.91% + 4% from a supplement) decreased weight gain in puppies but not the 2.91%- basal diet + 2% from a supplement). These results show that the highest no-effect-level of lysine for puppies was 2.91%.

In another study, Edmonds and Baker (1987) fed young pigs a diet based on casein, whey, and soybean meal as the protein source. The diet was formulated to supply 14.41% crude protein, 1.15% lysine, and 0.53% arginine. Feeding pigs a diet containing twice the recommended level of lysine (i.e., 2.3%) added as lysine monohydrochloride did not affect weight gain or gain/feed ratio. Lysine levels at 3 or 4 times the basal level lowered both weight gain and feed intake without lowering feed efficiency. The level of lysine ingested did not affect the tissue levels of arginase and ornithine transcarbamylase. The results of this study suggest that excess dietary lysine does not antagonize arginine in the pig. It appears that pigs have a remarkable capacity to excrete lysine in the urine when a great excess is fed.

2.8. Lysine-Potassium Relationship

Available evidence indicates that potassium depletion leads to the accumulation of lysine and other cationic amino acids in tissues. Eckel et al. (1958a) reported that feeding potassium-deficient, casein-based diets to young male rats (100-140 g) resulted in increased lysine content in the muscle and kidney. These investigators concluded that lysine contributes significantly to the total cations of muscle in potassium deficiency. In a subsequent study, Eckel et al. (1958b) studied the effect on muscle potassium of feeding control and potassium-deficient casein-based

diets in both of which the total lysine content was increased 5-fold by addition of lysine monohydrochloride. In this study, both control and potassium-deficient diets were fed to rats for 2 or 3 weeks and tissue and urine electrolytes were measured. Feeding of lysine in the control diet resulted in its accumulation in muscle without depletion of muscle potassium. The lysine accumulation in muscle was accentuated in the potassium deficient animals but without further potassium depletion. The investigators concluded that lysine does not compete with potassium either for entrance into the muscle cell or at the renal level.

In a study described by Anderson and Raiten (1992), rats fed 6% lysine monohydrochloride in an 18% casein diet resulted in a significant reduction in muscle potassium concentration along with an increase in lysine. The investigators suggested that the very high (10%) lysine monohydrochloride supplement used by Eckel et al. (1958b) could have resulted in too low a food consumption to raise the plasma lysine concentration sufficiently to interfere with potassium transport. The secondary arginine deficiency induced in rats fed excessive supplemental lysine in casein-based diets would also tend to reduce feed consumption.

In another study, Dickerman and Walker (1964) reported that infusion of dogs with high dosages of each of the cationic amino acids lysine, arginine, and ornithine (e.g., 80 μmol/kg/minute for lysine) increased urinary potassium excretion to excessive levels. Hyperkalemia occurred during amino acid infusion due to displacement of cellular potassium by basic amino acids. Infused arginine and ornithine were as effective or more effective, compared to lysine, in increasing potassium excretion. Eckel et al (1958b) found that oral administration to rats of 10% lysine monohydrochloride in a casein-based diet modestly increased urinary potassium excretion, but no more than an equimolar amount of supplemental NH_4Cl. This increase in urinary potassium may have been due to muscle wastage, as animals of both groups lost weight. The mechanism of the effect of cationic amino acids, including lysine on potassium equilibrium in muscle is not clear. However, it seems unlikely that these amino acids compete with potassium at the cell surface for entry.

2.9. By-Product Safety

All amino acids can form Schiff bases when aldehydes (from sugars) are present. The structure of asparagine indicates that it is capable of eliminating a Schiff's base leading to the formation of acrylamide. The mechanism of formation is well documented by Zyzak et al. (2003). If lysine would undergo a similar mechanism, then 4-penten-1-amine (CAS 22537-07-1) would be the by-product. However, the formation of this by-product is only postulated and there is no evidence for its presence.

The safety of this potential by-product was assessed by reviewing the literature and performing a Deductive Estimation of Risk from Existing Knowledge (DEREK) evaluation. The literature search for toxicity, genotoxicity and carcinogenicity references for this compound did not find any references. The DEREK evaluation did not find any alerts for genotoxicity, carcinogenicity, or many other toxicity endpoints.

Given the absence of DEREK alerts for genotoxicity and carcinogenicity as well as lack of reported safety references, 4-penten-1-amine is unlikely to cause adverse effects. Additionally, the Maillard reaction products are commonly consumed from food products that are subjected to thermal treatment, as these products are generated during the browning reaction. Thus there is a long history of consumption of these products.

In a recent isotope labeling experiments using lysine/glucose model systems, Nikolov and Yaylayan (2010) reported that lysine can generate piperidine, a reactive amine capable of undergoing Maillard-type interactions. These investigators identified two possible mechanisms for the formation of piperidine: one arising through decarboxylation of lysine alone to generate cadaverine (1,5-diaminopentane) followed by deamination to form pent-4-en-1-amine which in turn can cyclize into piperidine. On the other hand, in the presence of sugars, lysine, similarly to asparagine and phenylalanine, can undergo a carbonyl-assisted decarboxylative deamination reaction to generate pent-4-en-1-amine, the counterpart of acrylamide. The pent-4-en-1-amine thus formed can then cyclize to form piperidine. Given the current approved uses of piperidine as a flavoring agent (21 CFR 172.515); it is unlikely to cause adverse effects.

3. SUMMARY AND DISCUSSION

L-Lysine monohydrochloride is intended for use as a processing aid in the manufacture of fabricated snack foods at use levels up to 1.5%. It is manufactured in accordance with cGMP and meets appropriate food-grade specifications cited in the Food Chemicals Codex. The addition of L-lysine monohydrochloride to formulated snack products, prior to frying, reduces the formation of acrylamide during frying by competing with asparagine in the Maillard browning reaction. L-Lysine is permitted for use as a special dietary and nutritional additive (21 CFR 172.320) in foods for human consumption. The regulation stipulates that the amount of L-lysine added for nutritive purposes plus the amount naturally present in free and combined (as protein) form must not exceed 6.4% by weight of the total protein of the finished food.

L-Lysine is an essential amino acid that occurs in the diet as a component of protein. Food proteins with a high biological value are always rich in lysine content. These proteins generally have a lysine/tryptophan ratio of 5 - 8 by weight. Muscle proteins of meat, poultry, and fish, as well as those of certain legumes have high lysine/tryptophan ratios. The mean conservative daily intake of L-lysine for all individuals from foods is estimated as 3400 mg/person/day. Based on MRCA data, the intended use of L-lysine monohydrochloride as a processing aid in snack food is estimated to result in an additional average acute and chronic consumption of 587 and 180 mg L-lysine/day, respectively. From a nutrient safety evaluation perspective, the high intake of 587 mg L-lysine/day is considered appropriate and acceptable. The total daily lysine consumption plus the maximum lysine values would be 3987 mg/day and 3580 mg/day for acute and chronic consumption, respectively. The intended uses of L-lysine monohydrochloride will add to the background intake (the total acute and chronic lysine consumption (background plus added) has been estimated to be 7.0% and 6.6% (as percent protein), respectively), which is slightly higher than that described in the regulation 21CFR 172.320 for L-lysine. The addition of L-lysine monohydrochloride to snack formulations is unlikely to significantly impact the amino acid quality of the protein consumed or provide excessive lysine levels relative to other amino acids.

Given its essential role in human nutrition, the literature is full of information on lysine, particularly on its biological role. The oral LD_{50} of 10 g/kg bw for lysine suggests a very low order of toxicity. In a 13-week rat toxicity study, the highest level studied, 5% L-lysine monohydrochloride mixed into the diet was established as the NOAEL. This level corresponded to a daily intake of ~3670 mg/kg bw/day for both genders (males- 3360 mg/kg/day; female- 3990 mg/kg/day), in addition to the amount provided by the basal diet. In a two year study, feeding rats a diet containing 1% lysine monohydrochloride did not reveal any adverse effects. In several

animal studies, the potential reproductive, developmental and teratogenic effects of lysine were investigated. It is important to note that in several of these studies, different ratios of amino acids in proportion to protein content or to other amino acids were used. Overall, based on the findings from these studies, the intended use of L-lysine monohydrochloride as a processing aid is unlikely to cause any teratogenic effects.

The Health Canada Compendium of Monograph[8] on lysine (regarding use as a drug to treat herpes infections) suggests a caution or warning statement as: "Consult a health care practitioner prior to use if you are pregnant or breastfeeding. Note: This statement is not required at a daily dose below or equal to 300 mg per day." Health Canada also clarified that the cautionary statement is for pharmaceutical dosage form, not for food-like dosage forms such as bars, chewing gums or beverages. The basis for the warning statement was not provided in the monograph; however articles reviewed for the monograph were listed. These studies are described earlier in the Reproductive and Teratogenicity Studies section. Overall, these studies do not show a basis for concern for the intended uses of L-lysine monohydrochloride in formulated snack products. Secondly, the proposed intake of lysine is in formulated foods. The available information from the proposed uses suggest that any potential effects indicated in the Health Canada monograph are unlikely to result from the proposed uses of L-lysine monohydrochloride in formulated snack food.

Metabolism studies suggest that L-lysine monohydrochloride supplementation of a low lysine protein diet was more effective for nitrogen retention than increasing the amount of lysine protein in the diet. A lysine supplement has been suggested to improve the biological value of dietary protein in elderly women with a low protein intake. The available metabolism-related information indicates that additional intake of lysine from the intended uses is unlikely to have any adverse effects. The information from the human studies further supports the safety data provided from animal studies. The body of evidence on oral administration of L-lysine in humans indicates that it can be consumed in considerable amounts without adverse effects. In healthy subjects, no adverse effects were reported in subjects consuming supplemental lysine at levels up to 3000 mg/day for up to six months. Additionally, lysine supplementation was well tolerated by infants and children. In a review article, Flodin (1997) concluded that oral lysine supplements of 1000 to 3000 g/day (1250 to 3750 mg/day as L-lysine monohydrochloride) divided among meals for individuals consuming usual American diets would not elevate the proportion of lysine in the dietary protein beyond that in meat and would be safe for chronic use. The estimated daily intake of 587 mg L-lysine/person (9.8 mg/kg bw/day) from the intended uses in snack products is over 7-fold lower than that used in clinical trials in humans and is over 350-fold lower than the demonstrated safe levels of intake in animal studies.

The GRAS status of L-lysine monohydrochloride for its intended use is supported by:

- It is a high-purity, well-characterized ingredient, that is manufactured in accordance with cGMP and meets appropriate food-grade specifications;
- Its safety for the intended use in snack food is supported by published studies that include (i) human clinical studies in which no adverse effects were noted at levels up to 3000 mg/day for up to six months, (ii) a 90-day rat study, in which a NOAEL of 5% in the diet

[8]Accessible at: http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/prodnatur/mono_lysine-eng.pdf

was determined (equivalent to 3670 mg/kg bw/day, in addition to the amount present in the basal diet) for L-lysine monohydrochloride;

- Additional published animal and human studies with L-lysine, including nutritional and metabolism studies further corroborate the safety;
- Regular dietary consumption of L-lysine as a component of protein, and the permitted uses of L-lysine in food in the U.S. further supports its safe uses;
- The data supporting the safety of L-lysine are available in the published literature

The totality of available evidence clearly supports the safety of L-lysine monohydrochloride for its intended use in snack food. On the basis of scientific procedures[9] and history of exposure from natural sources, the consumption of L-lysine monohydrochloride as a processing aid resulting in an estimated daily exposure of 587 mg L-lysine/person, in addition to the background intake, is considered safe. The intended uses are compatible with current regulations, *i.e.,* L-lysine monohydrochloride will be used in fabricated snack foods at use levels up to 1.5% and it is produced according to current good manufacturing practices (cGMP) to a food-grade specification.

[9] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized above, it is concluded that L-lysine monohydrochloride, meeting the specifications cited in the Food Chemicals Codex, and when used as a food ingredient in fabricated snack products at levels of up to 1.5%, when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in an estimated exposure of 587 mg L-lysine/person/day is Generally Recognized As Safe (GRAS). It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that L-lysine monohydrochloride, when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

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5. REFERENCES

Anderson, S.A., Raiten, D.J., 1992. Safety of amino acids used as dietary supplements. Life Sciences Research Office, Bethesda, MD. FASEB Special Publications Office. pp 22, 59.

Ausman, L.M., Hegsted, D.M., 1980. Protein requirements of adult cebus monkeys (*Cebus albifrons*). Am. J. Clin. Nutr. 33:2551-2558.

Becalski, A., Lau B.P.Y., Lewis D., Seaman, S.W., 2003. Acrylamide in food: Occurrence, sources, and modeling. J. Agric. Food Chem. 51: 802–808.

Borsook, H., Deasy, C.L., Haagen-Smit, A.J., Keighley, G. & Lowy, P.H., 1950. Metabolism of ^{14}C-labeled glycine, l-histidine, l-leucine and l-lysine. J. Biol. Chem., 187: 839-848.

Breglia, R.J., Ward, C.O., Jarowski, C.I., 1973. Effect of selected amino acids on ethanol toxicity in rats. J. Pharm. Sci., 62:49–55.

Bressani, R., 1971. Amino acid supplementation of cereal grain flours tested in children. In Amino Acid Fortification of Protein Foods. Scrimshaw, N.S., Altschul, A.M. (eds). Cambridge, MA: MIT Press, pp 184-204.

Castenada, C., Charnley, J.M., Evans, W. J., Crim, M.C., 1995. Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. Am. J. Clin. Nutr. 62:30-39.

CCFAC, 2006. Codex Committee on Food Additives and Contaminants. Discussion paper on acrylamide and Annex I, Outline draft code of practice for the reduction of acrylamide in food. ftp://ftp.fao.org/codex/ccfac38/fa38_35e.pdf.

Chase, H.P., Jansen, G.R., 1976. Effect of feeding lysine and threonine fortified bread during gestation and lactation on growth of the brain in rats. J. Nutr. 106: 41-47.

CIAA, 2006. Confederation of Food and Drink Industries of the EU. The CIAA acrylamide toolbox. www.ciaa.be/documents/brochures/CIAA_Acrylamide_Toolbox_Oct2006.pdf

Cohlan, S.Q., Stone, S.M., 1961. Effects of dietary and intraperitoneal excess of L-lysine and L-leucine on rat pregnancy and offspring. J. Nutr. 74:93-95.

Culik, R., Rosenberg, H.R., 1958. The fortification of bread with lysine. IV. The nutritive value of lysine-supplemented bread in reproduction and lactation studies with rats. Food Tech. 12:169.

Czarnecki, G.L., Hirakawa, D.A., Baker, D.H., 1985. Antagonism of arginine by excess dietary lysine in the growing dog. J. Nutr. 115: 743-752.

Dancis, J., Hutzler, J., Ampola, M.G., Shih, V.E., van Gelderen, H.H., Kirby, L.T., Woody, N.C., 1983. The prognosis of hyperlysinemia: an interim report. Am. J. Hum. Genet. 35:438-442.

Dickerman, H.W., Walker, W.G., 1964. Effect of cationic amino acid infusion on potassium metabolism in vivo. J. Am. Physiol. 206: 403-408.

Dubow, E., Maher, A., Gish, D., Erk, V., 1958. Lysine tolerance in infants. J. Pediatr. 52: 30-37.

Eckel, R.E., Norris, J.E.C., Pope, C.E.II., 1958a. Basic amino acids as intracellular cations in K deficiency. Am. J. Physiol, 193:644-652.

Eckel, R.E., Pope, C.E.II., Norris, J.E.C., 1958b. Influence of lysine and NH_4Cl feeding on the electrolytes of normal and K-deficient rats. Am. J. Physiol. 193:652-656.

Edmonds, M.S., Baker, D.H., 1987. Failure of excess dietary lysine to antagonize arginine in young pigs. J. Nutr. 117: 1396-1401.

Eggum, B., 1991. Comments on Report of a joint FAO/WHO expert consultation on protein quality evaluation, Rome 1990. Z. Emahrungswiss 30: 8-88.

Elango, R., Humayun, M.A., Ball, R.O., Pencharz, P.B., 2011. Protein requirement of healthy school-age children determined by the indicator amino acid oxidation method. Am. J. Clin. Nutr. 94:1545–1552.

FCC., 2003. L-Lysine Monohydrochloride. Food Chemical Codex 5th Edition, p. 257.

Federal Register, 2007. Federal Register Advance Notice of Proposed Rulemaking (Food Labeling) 72 FR 62149 November 2, 2007: Revision of Reference Values and Mandatory Nutrients Federal Register: Volume 72, Number 212 Page 62149-62175.

Flodin, N.W., 1997. The metabolic roles, pharmacology, and toxicology of lysine. J. Amer. Coll. Nutr. 16:7-21.

Flodin, N.W., 1993. Lysine supplementation of cereal foods: a retrospective. J. Am. Coll. Nutr. 12:486-500.

Funk, M.S.D.N., Worthington-Roberts, B., Fantela, A., 1991. Impact of supplemental lysine or tryptophan on pregnancy course and outcome in rats. Nutr. Res. 11:501-512.

Gahl, J., Finke, M.D., Crenshaw, T.D., Benevenga, N.J., 1991. Use of a four-parameter logistic equation to evaluate the response of growing rats to ten levels of each indispensable amino acid. J. Nutr. 121: 1720-1729.

Griffith, R.S., Norins, A.L., Kagen, C., 1978. A multicentered study of lysine therapy in herpes simplex infection. Dermatologica 156:257-267.

Griffith, R.S., Walsh, D.E., Myrmel, K.H., Thompson, R.W., Behforooz, A., 1987. Success of L-lysine therapy in frequently recurrent herpes simplex infection. Dermatologica 175:183-190.

Harper, A.E., 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiol. Rev. 50:428-558.

Hawley, E.E., Murlin, J.R., Nasset, E.S., Szymanski, T.A., 1948. Biological values of six partially purified proteins. J. Nutr. 36:153-169.

Hendler, S.S., Rorvik, D., 2001. Lysine. In: PDR for Nutritional Supplements. Medical Economics™ Thomson Healthcare, Montvale, NJ, pp. 270-271.

Henry, Y., Colleaux, Y., Seve, B., 1992. Effects of dietary level of lysine and of level and source of protein on feed intake, growth performance, and plasma amino acid pattern in the finishing pig. J. Anim. Sci. 70:188-195.

Hevia, P., Kari, F.W., Ulman, E.A., Visek, W.J., 1980. Serum and liver lipids in growing rats fed casein with L-lysine. J. Nutr. 110: 1224-1230.

Humayun, M.A., Elango, R., Ball, R.O., Pencharz, P.B., 2007. A re-evaluation of protein requirement in young men using the indicator amino acid oxidation technique. Am. J. Clin. Nutr. 86:995-1002.

IOM, 2005. Protein and Amino Acids. In: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, DC: Institute of Medicine (IOM), National Academy Press (NAP), pp. 589-768. Available at: http://www.nap.edu/catalog.php?record_id=10490

Jansen, G.R., 1962. Lysine in human nutrition. J. Nutr. 76:1-35.

Kotsiou, K., Tasioula-Margari, M., Capuano, E., Fogliano, V., 2011. Effect of standard phenolic compounds and olive oil phenolic extracts on acrylamide formation in an emulsion system. Food Chem. 124: 242-247. Published online ahead of print.

Lasekan, J.B., Koo, W.W., Walters, J., Neylan, M., Luebbers, S., 2006. Growth, tolerance and biochemical measures in healthy infants fed a partially hydrolyzed rice protein-based formula: a randomized, blinded, prospective trial. J. Amer. Coll. Nutr. 25:12-19.

Longenecker, J.B., Hause, N.L., 1958. Rate of absorption of supplementary free amino acids during digestion. Nature 182:1739-1740.

Longenecker, J.B., Hause, N.L., 1959. Relationship between plasma amino acids and composition of the ingested protein. Arch. Biochem. Biophys. 84:46-59.

Mack, P.B., Vose, G.P., Kinard, C.L., Campbell, H.B., 1962. Effects of lysine supplemented diets on growth and skeletal density of preadolescent children. Am. J. Clin. Nutr. 11:255-262.

Matsueda, S., Niiyama, Y., 1982. The effects of excess amino acids on maintenance of pregnancy and fetal growth in rats. J. Nutr. Sci. Vitaminol. 28:557-573.

McClure, F.J., Folk, J.E., 1955. Lysine and cariogenicity of two experimental rat diets. Science 122:557-558.

McCune, M.A., Perry, H.O., Muller, S.A., O'Fallon, W.M., 1984. Treatment of recurrent herpes simplex virus infection with L-lysine monohydrochloride. Cutis 34:366-373.

Milman, N., Scheibel, J., Jessen, O., 1980. Lysine prophylaxis in recurrent herpes simplex labialis: a double-blind, controlled crossover study. Acta Dermato-Venereologica 60:85-87.

Moehn, S., Martinazzo-Dallagnol, E., Bertolo, R.F.P., Pencharz, P.B., Balle, R.O., 2007. Metabolic availability of lysine in feedstuffs determined using oral isotope delivery. Livestock Science 109:24-26.

NCHS, 1980. National Center for Health Statistics. NCHS dietary intake source data: United States 1976-1980. Washington, DC: US Department of Health 231:149-151.

Nikolov, P.Y., Yaylayan, V.A., 2010. Formation of Pent-4-en-1-amine, the counterpart of acrylamide from lysine and its conversion into piperidine in lysine/glucose reaction mixtures. J. Agric. Food Chem. 58: 4456-4462.

Osborne, T.B., Mendel, L.B., 1914. Amino acids in nutrition and growth. J. Biol. Chem. 17:325-349.

Pawlak, M., Pion, R., 1968. Influence of supplementing wheat proteins with increasing amounts of lysine on the free amino acid content of blood and muscle in growing rats. Ann. Biol. Anim. Bioch. Biophys. 8:517-530.

Reed, P.J., Beckett, P.R., 1996. Protein and Amino Acids. Chapter 8; In: Present Knowledge in Nutrition. Eds.: Zigler, E.E. Filer, L.J., Jr. pp. 67-86.

Sackler, A.M., Sophian, L.H., 1957a. Gastric secretagogue effect of lysine monohydrochloride. Science 126:255-256.

Sackler, A.M., Sophian, L.H., 1957b. The effect of the ingestion of amino acid on gastric secretion with particular reference to L-lysine monohydrochloride. J. Clin. Pathol. 28:258-263.

Sackler, A.M., Sophian, L.H., 1958. The effects of amino acid ingestion on gastric secretion: comparison of lysine and arginine. J. Lab. Clin. Med. 52:43-47.

Said, A.K., Hegsted, D.M., Hayes, K.C., 1974. Response of adult rats to deficiencies of different essential amino acids. Br. J/ Nutr. 31:47-57.

Schneider, H., Mohlen, K.H., Dancis, J., 1979. Transfer of amino acids across the in vitro perfused human placenta. Pediatr. Res. 13:236-240.

Simon, C.A., Van Melle, G.D., Ramelet, A.A., 1985. Reply. (Failure of lysine in frequently recurrent herpes simplex infection.) Arch. Dermatol. 121:167-168. Letter to Editor.

Spencer, H., Samachson, J., 1963. Effect of lysine on calcium metabolism in man. J. Nutr. 81:301-306.

Stadler, R.H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P.A., Robert, M.C., Riediker, S., 2002. Acrylamide from Maillard reaction products. Nature 419:449-450.

Stein, H.H., Seve, B., Fuller, M.F., Moughan, P.J., de Lange, C.F.M., 2007. Invited Review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180.

Taeymans, D., Andersen, A., Ashby, P., et al., 2005. Acrylamide: update on selected research activities conducted by the European food and drink industry. J. AOAC Intl. 88:234-241.

Tareke, E., Rydberg, P., Karlsson. P., Eriksson, S., Tornqvist, M., 2000. Acrylamide: a cooking carcinogen? Chem. Res. Toxicol. 13:517–522.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Tornqvist, M., 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J. Agric. Food. Chem. 50:4998–5006.

Tsubuku, S., Mochizuki, M., Mawatari, K., Smriga, M., Kimura, T., 2004. Thirteen-week oral toxicity study of L-lysine hydrochloride in rats. Inter. J. Toxicol. 23:113-118.

Wass, C., Klamer, D., Katsarogiannis, E., Pålsson, E., Svensson, L., Fejgin, K., Bogren, I.B., Engel, J.A., Rembeck, B., 2011. L-lysine as adjunctive treatment in patients with schizophrenia: a single-blinded, randomized, cross-over pilot study. BMC Med. 9:40.

Yamori, Y., Nara, Y., Mizushima, S., Sawamura, M., Horie, R., 1994. Nutritional factors for stroke and major cardiovascular diseases: international epidemiological comparison of dietary prevention. Health Rep. 6:22-27.

Zyzak, D. V., Sanders, R. A., Stojanovic, M., Tallmadge, D. H., Eberhart, B. L., Ewald, D. K., Gruber, D. C., Morsch, T. R., Strothers, M. A., Rizzi, G. P., Villagran, M. D., 2003, Acrylamide formation mechanism in heated foods. J Agric. Food Chem. 51: 4782-4787.

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