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May 6, 2004

BY FEDERAL EXPRESS

Mr. Edmundo Garcia, Jr.
Consumer Safety Officer
Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
HFS-255, Room 1129
1110 Vermont Avenue, N.W.
Washington, D.C. 20005

Re: GRAS Notification for Ethanol

Dear Mr. Garcia:

On behalf of our client, Frito-Lay, Inc. ("Frito-Lay"), attached is a notification of Frito-Lay's determination that ethanol is generally recognized as safe (GRAS) when used as a preservative in the filling of shelf-stable croissants at a concentration of 3000 parts per million (ppm). This GRAS notification is submitted pursuant to section 201(s) of the Federal Food, Drug, and Cosmetic Act (FFDCA) and proposed 21 C.F.R. §§ 170.30 and 170.36 (62 Fed. Reg. 18937 (Apr. 17, 1997)). An original and two copies of the notification are attached. For convenience, a disk with an electronic copy of the notification is also attached as a portable document format (PDF) file.

If there are any questions regarding this GRAS notification, or if additional information is required, please do not hesitate to contact us.

Sincerely,

Martin J. Hahn
Counsel to Frito-Lay

Enclosures
cc: Steve Saunders, Ph.D.

GRAS EXEMPTION CLAIM

A. Name and Address of Notifier

Manufacturer: Frito-Lay, Inc.
7701 Legacy Drive
Plano, Texas 75024

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B. Common or Usual Name of Notified Substance

Ethanol (alcohol; ethyl alcohol)

C. Conditions of Use

Ethanol is intended for use as a preservative in the filling of shelf-stable croissants at a concentration of 3000 ppm.

D. Basis for GRAS Determination

Scientific procedures

E. Availability of Information Supporting GRAS Determination

The data and information that form the basis for this GRAS determination are available for FDA review and will be provided upon the agency's request.

Respectfully submitted,

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Martin J. Hahn

GRAS Notification for Ethanol in Filled Croissants

I. EXECUTIVE SUMMARY

This notification sets forth the basis upon which Frito-Lay, in consultation with experts qualified by scientific training and expertise to evaluate the safety of food ingredients, has determined that ethanol is generally recognized as safe (GRAS) for use as a preservative at a concentration of 3000 parts per million (ppm) in the filling of shelf-stable croissants. 1/ Ethanol is one of the volatiles naturally present in ripe fruits and foods produced using yeast or mold fermentation (e.g., bread, certain cheeses). Ethanol has also long been produced for direct human consumption by the fermentation of sugars produced through the milling, liquefaction, and saccharification of grains such as corn or wheat.

As described more fully below, in reaching this GRAS determination, Frito-Lay sought the opinion of qualified experts regarding recent scientific literature pertaining to the toxicity of ethanol. The experts consulted by Frito-Lay conservatively identified an appropriate endpoint (fetal alcohol syndrome), a sensitive population (women of childbearing age), and the human lowest observed adverse effect level (LOAEL) for fetal effects of greater than 180 g/day. An animal LOAEL of 1.8 g/kg in dams was also identified.

The intended use of ethanol at a concentration of 3000 ppm in the filling of shelf-stable croissants is estimated to result in an average daily intake of 0.017 to 0.023 g/day ethanol. By comparison, tangerine has been reported to contain 0.04-0.1 g ethanol and a 12-oz bottle of beer or 3.5 oz glass of wine contains about 12 g. The human LOAEL for fetal effects and the acute animal LOAEL are 7,690 to 10,500 and 4620 to 6320 times greater, respectively, than the estimated daily intake of ethanol from filled croissants—a difference of several orders of magnitude. On the basis of this information, ethanol was determined to be GRAS for use as a preservative at the intended concentration and other conditions for use.

II. DETAILED INFORMATION ON THE IDENTITY OF THE NOTIFIED SUBSTANCE

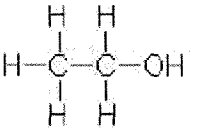
A. Chemical Abstract Service (CAS) Registry Number

The CAS Number for ethanol, which is classified chemically as a monohydric primary alcohol, is 64-17-5.

1/ The consulting experts included Marcia Van Gemert, Ph.D., Senior Managing Toxicologist of Exponent, Inc., and Nancy J. Rachman, Ph.D., Senior Managing Scientist of Exponent, Inc.

B. Empirical and Structural Formulas

The empirical formula for ethanol is C_2H_6O. The structural formula for ethanol is represented by the following:



C. Manufacturing Process

Food grade ethanol is produced using traditional methods of milling, fermentation, and distillation. In a dry milling process, grain such as corn is ground into meal, mixed with water and enzymes, and heated. The liquefied starch mash is treated enzymatically to produce sugars, which are fermented by yeast to yield ethanol and carbon dioxide. When the mash is fully fermented, it is distilled and concentrated (and may be dehydrated) to produce ethanol.

In wet milling, a similar process is used, except that the grain (typically corn) is first steeped in water and dilute acid to facilitate separation of the grain into its component parts. The starch component may be fermented to produce ethanol as described previously.

Following is a flow chart depicting the dry mill process:

CORN

⇩

GRINDER

⇩

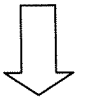
COOKER

(starch mash, water, and enzymes)

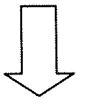
⇩

FERMENTER

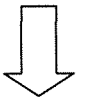
(including CO_2 scrubber)



DISTILLATION COLUMN



MOLECULAR SIEVE



ETHANOL

D. Properties

Appearance	Clear, colorless, mobile liquid
Miscibility	Water, ether, and chloroform
Formula weight	46.07
Boiling point	Approximately 78° C
Refractive index at 20° C	Approximately 1.364

E. Specifications and Methods

Ethanol used as a preservative will conform to the specifications set forth in the Food Chemicals Codex (5th ed. 2003), as follows:

Assay	Not less than 94.9% by volume (92.3% by weight) of C_2H_6O.
Acidity (as acetic acid)	Not more than 0.003%
Alkalinity (as NH_3)	Not more than 3 mg/kg
Fusel Oil	Passes test
Ketones, isopropyl alcohol	Passes test
Heavy metals (as Lead)	Not more than 0.5 mg/kg
Methanol	Passes test
Nonvolatile residue	Not more than 0.003%
Solubility in water	Passes test
Substances darkened by sulphuric acid	Passes test
Substances reducing permanganate	Passes test

III. SELF-LIMITING LEVELS OF USE

Frito-Lay is unaware of a specific self-limiting level of use for ethanol when used as a preservative in the filling of shelf-stable croissants. Alcohol will, however, have an adverse effect on flavor and odor when present in food at sufficient levels. As noted previously, Frito-Lay's intended use of ethanol will result in a maximum concentration of 3000 ppm (0.3%) in the filling.

IV. BASIS FOR THE GRAS DETERMINATION

A. Brief Review of Toxicological Effects of Ethanol on Human Health

Ethanol (commonly referred to as "alcohol") has a long history of use, in the neighborhood of many thousands of years, in food products and in various forms of alcoholic beverages. There are many effects, both beneficial and deleterious, to consider when examining alcohol consumption. The amount consumed is a critical variable in evaluating the various effects.

Although moderate consumption of alcohol contributes to feelings of well-being and happiness, and may reduce the risk of some diseases, heavy drinking leads to increase of trauma and exerts deleterious effects on various body organs. Adverse effects associated with excessive alcohol consumption include increased obesity, cardiovascular diseases including hypertension and stroke, coronary heart disease, alcoholic cardiomyopathy, liver diseases, cancer, diseases of the nervous system, and fetal alcohol syndrome (Ahmed, 1995).

The Fourth Special Report of the U.S. Congress on Alcohol and Health (NIAAA report, 1987) classified drinkers into moderate drinkers (i.e., those who consumed 0.22 to 0.99 oz of alcohol per day) and light drinkers (i.e., those who consumed 0.01 to 0.21 oz/day). In the NIAAA report, it was proposed that moderate alcohol intake should not exceed 0.8 g/kg body weight per day, or an average of 0.7 g/kg body weight over a 3-day period.

Many effects seen from alcohol consumption are seen only after chronic ingestion (Becker, et al., 1996). To assess the safety of ethanol used as a preservative in a filled croissant, a toxicological endpoint for ethanol had to be chosen that (1) was a deleterious effect for the consumer, (2) could occur with or without chronic exposure, and (3) had sufficiently reliable experimental animal models to verify the doses and results. Fetal Alcohol Syndrome (FAS) was chosen as the toxicological endpoint of concern.

The teratogenic effects of ethanol have been widely studied both in humans and experimental models. FAS causes a distinct pattern of physical and behavioral anomalies in human fetuses characterized by craniofacial, limb, central nervous system, and cardiovascular effects, in addition to growth delay and mental retardation (Ahmed, 1995). In humans, ethanol teratogenicity results from both direct and indirect effects (Shibley, et al., 1999). Direct effects of ethanol are caused by ethanol interacting directly with the fetal cells, and indirect effects include ethanol-induced maternal undernutrition, ethanol-induced placental dysfunction, and acetaldehyde teratogenicity. It has been shown that women who drink excessively, defined as greater than 180 g of alcohol/day (3 g/kg for a 60 kg woman) deliver babies with mild FAS (Ahmed, 1995). In experimental animal models, there is great variability in the doses of ethanol capable of causing fetal damage, depending on the route of administration (intraperitoneal,

oral exposure via drinking water). Acute prenatal doses of 1.8 g/kg or greater to dams were capable of affecting acute morphological development in experimental animal models (Clarren, et al., 1992, Shibley, et al., 1999).

B. Dietary Intake: Ethanol in Foods

Food sources of ethanol include alcoholic beverages, fruits, baked goods and fermented foods. A preliminary literature search provides quantitative information on the levels of ethanol in alcoholic beverages and ranges for some other foods.

1. Ethanol in alcoholic beverages

Ethanol levels in select alcoholic beverages are presented in Table 1.

Table 1. Ethanol in alcoholic beverages (Baum-Baicker, C., 1995)

Beverage type	Ethanol content (%)	Unit of measure	Ethanol in a drink Oz (g)
Whiskey (80 proof)	40	1-oz shot (30 ml)	0.4 (11.83)
Table wine	12.1[a]	3.5-oz glass (104 ml)	0.42 (12.42)
U.S. beer	3.5[b]	12-oz bottle (355 ml)	0.42 (12.42)

[a] Most table wines contain 11 to 13% ethanol. Fortified wines, such as sherry and port, contain approximately 20% ethanol.
[b] Most brands contain 3.2 to 4.0% ethanol.

2. Ethanol in ripening fruit and fruit juice

Ethanol is one of the aroma and flavor volatiles associated with ripening fruit, and is a sensitive marker for abuses in postharvest storage and handling. For example, Bender et al. (no date) reported that the most sensitive indicator of atmospheric stress in mango was found to be elevated ethanol production. Hagenmaier (2001a, personal communication) investigated effects of different fruit wax coatings on the ethanol content of tangerine. Ethanol was used as an indicator of how fast off-flavors (over-ripeness) develop. Fruit with the same coating but harvested at different times had approximately the same ethanol content.

The type of coating has been shown to have a large effect on ethanol content. Mean ethanol concentration in juice was 800 ppm when prepared from fruit with polyethylene coatings, which permit normal gas exchange and respiration. Mean ethanol concentration was about 1300 ppm for juice prepared from fruit with a carnauba wax coating, which tends to block gas exchange somewhat, and about 1900 ppm for high-gloss, shellac-resin coatings, similar to those used by many citrus packinghouses.

Hagenmaier (2001b) also investigated the "flavor life" and acceptability of mandarin orange varieties (tangelos and tangerines) treated with different coatings before storage, as a function of different chemical parameters of the fruit. Juice made from freshly harvested fruit contained, on average, 117 ppm ethanol. Results of a sensory panel indicated that juice flavor was judged unacceptable when ethanol concentration was greater than 1500 ppm; the tasters did not detect off flavors when ethanol was below this level. This suggests that consumers may well be drinking juice containing up to 1500 ppm ethanol, at least on occasion.

3. Other Ethanol Uses in Food

Ethyl alcohol meeting the specifications of the Food Chemicals Codex, 4th ed. (1996) is affirmed as GRAS when used as an antimicrobial agent in pizza dough pursuant to 21 C.F.R. § 184.1293. Because ethanol is expected to volatilize during baking, dietary intake associated with this use would be negligible.

C. Dietary Intake Associated with Use in Filling in Shelf Stable Croissants

Intake associated with the use of ethanol in a croissant filling was estimated in two ways: (1) using data from the Continuing Survey of Food Intakes by Individuals (CSFII 1994-96, 1998) and the USDA reference weight for croissants, and (2) using product-specific data for the filled croissants for which the ethanol is intended for use.

Using food consumption data from the USDA CSFII (1994-96, 98), Exponent/Novigen's FARE™ software, USDA reference data for croissant size and filling content, and a concentration of 3000 ppm ethanol in croissant filling, the anticipated intake of ethanol by women of childbearing age was estimated. Results are presented in Table 2.

Table 2. Ethanol Consumption from Filled Croissants (3000 ppm)

Population	Per Capita (mg/day)	Per User (mg/day)	% Of Population Consuming Filling
Females 13 to 55			0.14 %[1]
Mean	0.024526	17.04548	
90th Percentile	NA[2]	NA[2]	

[1] According to USDA's CSFII, there are approximately 80,613,275 women of childbearing age in the US population; therefore, approximately 112,858 women 13-55 consume croissants.
[2] Too few respondents to allow calculation of meaningful distributions

These results are consistent with the interpretation that women who eat croissants tend to eat one per day on average.

The USDA Nutrient Database for Standard reference lists a medium croissant weight as 57 grams, and Exponent/Novigen's proprietary recipes indicate that a croissant is 10% filling by weight.

A daily intake of ethanol was estimated as follows:

(57 g croissant/day) x (0.10 g filling/g croissant) x (3,000 μg EtOH/g filling) = 17,100 μg EtOH/day

(17,100 μg EtOH/day) x (1 mg/1,000 μg)= 17.1 mg EtOH/day

Using this estimation approach, the human LOAEL for fetal effects (180 g/day) is more than 10,000 times greater than the estimated intake, by the following calculation:

(180 g EtOH/day) / (0.0171 g EtOH/day) = 10,500.

The acute animal LOAEL (1.8 g/kg/day) is more than 6,000 times greater than estimated intake of ethanol from filled croissants (the following calculation assumes a woman's average weight is 60 kg):

(1.8 g EtOH/kg/day) / [(0.0171 g EtOH/day) / 60 kg]] = 6,320.

The specific croissant product for which ethanol is intended is smaller than the standard US croissant and weighs, on average, 15 g. The croissants are packaged in bags averaging 2.75 oz by weight. If it is assumed that a woman consumes an entire bag of croissants per day, and that the proportional content of filling is the same as in the USDA reference filled croissant, daily ethanol intake is estimated as follows:

(2.75 oz croissant/day) x (453.6 g/16 oz) = 78.0 g croissant/day

(78.0 g croissant/day) x (0.10 g filling/g croissant) x (3,000 μg EtOH/g filling) = 23,400 μg EtOH/day

(23,400 μg EtOH/day) x (1 mg/1,000 μg)= 23.4 mg EtOH/day

Using this approach, the human LOAEL for fetal effects (180 g/day) exceeds the intake of ethanol from filled croissants by a factor of more than 7,000:

(180 g EtOH/day) / (0.0234 g EtOH/day) = 7,690

The acute animal LOAEL (1.8 g/kg/day) is more than 4,000 times greater than intake of ethanol from filled croissants (the following calculation assumes a woman's average weight is 60 kg.)

(1.8 g EtOH/kg/day) / [(0.0234 g EtOH/day) / 60 kg]] = 4,620.

On the basis of this information and data, particularly the considerable margin of safety demonstrated under the intended conditions of use, ethanol is determined to be GRAS for use as a preservative in the filling of shelf-stable croissants at a concentration of 3000 ppm.

D. Data Inconsistent with GRAS Position

Frito-Lay is unaware of any data that would be inconsistent with a finding that ethanol is GRAS when used as a preservative at a concentration of 3000 ppm in the filling of shelf-stable croissants.

V. LABELING

As required by sections 403(i) and (k) of the Federal Food, Drug, and Cosmetic Act (FFDCA) and FDA's implementing regulations (21 C.F.R. §§ 101.4 and 101.22), ethanol will be declared on the labels of croissant products in which it is present. The declaration will appear in the list of ingredients for the food and will identify ethanol as a preservative (e.g., "ethanol (preservative)"). Because there are no health consequences reasonably expected to result from the use of ethanol at the low levels at which it will be used, no warnings or disclosures beyond the listing of ethanol as an ingredient are necessary as a condition of GRAS status. Moreover, no additional claims or statements, other than the listing of ethyl alcohol as a preservative in the ingredient statement, are necessary to satisfy sections 403(a)(1) and 403(n) of the FFDCA, which govern the circumstances under which material information may be required to be disclosed.

Frito-Lay is aware that there may be certain consumers that may want to avoid an alcohol-containing product, regardless of the low level of alcohol in the product, for religious or cultural reasons. The declaration of ethanol or ethyl alcohol in the list of ingredients will provide sufficient notice to these consumers that ethanol is present in the product. In addition, our review of applicable federal and state laws did not reveal any specific laws or regulations that would limit this proposed use of ethanol. Please let us know if you would like to discuss labeling issues or if additional information would be useful.

VI. CONCLUSION

The daily consumption of filled croissants containing ethanol at a concentration of 3000 ppm results in an average daily intake of 0.017-0.023 g/day ethanol. By comparison, fresh citrus (tangerine) juice may contain 0.2-0.4 g ethanol per 8 oz serving, depending on the coating applied to the fruit and the conditions of storage. A 12-oz bottle of beer or 3.5 oz glass of wine contains about 12 g. Using a conservative estimation approach, the human LOAEL for fetal effects is 7,690 -10,500 times greater than the intake of ethanol from filled croissants, and the acute animal LOAEL is more than 4,000-6,000 times greater. That is, there

is a margin of error (MOE) of at least 7690, and more likely greater than 10,000, for this effect. Accordingly, the data summarized in this GRAS notification establish that there is general recognition, among experts qualified by scientific training and experience to evaluate the safety of substances added to food, that there is reasonable certainty that ethanol is not harmful under the intended conditions of use described herein.

Respectfully submitted,

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Martin J. Hahn

References

Ahmed, F.E. Toxicological effects of ethanol on human health. *Critical Reviews in Toxicology,* 25(4): 347-367, 1995.

Baum-Baicker, C., The health benefits of moderate alcohol consumption: a review of the literature, *Drug Alcohol Depend*. **15**: 207, 1995.

NIAAA Report: National Institute on Alcohol Abuse and Alcoholism, The Sixth Special Report to the U.S. Congress on Alcohol and Health from the Secretary of Health and Human Services, DHHS Publ. No. (ADM) 7-1519. National Institute of Health, Public Health Service, US.S. Department of Health and Human Services, Washington, D.C., U.S. Government Printing Office, 1987.

Becker, H.C., Diaz-Granados, J.L., Randall, C.L., Teratogenic action of ethanol in the mouse: a minireview. *Pharmacology Biochemistry and Behavior* **55**(4): 501-513, 1996.

Clarren, S.K., Astley, S., Pregnancy outcomes after weekly oral administration of ethanol during gestation in the pig-tailed macaque: comparing early gestational exposure to full gestational exposure. *Teratology* **45**: 1-9, 1992.

Clarren, S. K., et al., Cognitive and behavioral deficits in nonhuman primates associated with very early embryonic exposures to ethanol. *Journal of Pediatrics*: **121**: 789-796, 1992.

Hagenmaier, R. Ethanol content of 'Murcott' tangerines harvested at different times and treated with coatings of different o2 permeability. http://www.nal.usda.gov/ttic/tektran/data/000012/20/0000122027.html, 2001a.

Hagenmaier, R. the flavor of mandarin hybrids with different coatings. *Postharvest Biol. and Technol.* **24**:79-87. 2001b.

Shibley, I.A., et al., Experimental models used to measure direct and indirect ethanol teratogenicity. *Alcohol & Alcoholism*. **34**(2): 125-140, 1999.