U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

Memorandum

Date	May 5, 2020
From	Toxicologist, Division of Food Ingredients (DFI), Office of Food Additive Safety (OFAS) (HFS-255) Through: Toxicology Review Branch Chief, DFI, OFAS (HFS-255) Director DFI, OFAS (HFS-255) Office Director, OFAS (HFS-200)
Subject	Update on the review of the safety of betel nut use as an ingredient in conventional foods
To	the file

Betel nut (*Areca catechu* L.,) has not been approved for use as a food additive, and we are not aware of a basis for its use in food to be considered GRAS. In fact, in a 2004 memorandum (August 17, 2004), OFAS concluded that the use of betel nut as an ingredient in conventional foods is not GRAS. This conclusion was not altered when OFAS in 2016 conducted an updated review of the scientific literature pertaining to the safety evaluation of betel nut's use as an ingredient in conventional foods (March 28, 2016).

The purpose of this memorandum is to provide an update on the previous review of safety information pertaining to betel nut use as an ingredient in food (March 28, 2016). In the previous memorandum, OFAS laid out concerns related to betel nut's use and its association with genotoxicity, carcinogenicity, as well as reproductive and developmental toxicity.

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

For the purpose of updating the Agency's knowledge base on toxicological effects of betel nut consumption, PubMed was searched from April 2015 to March 2020 for new scientific articles, utilizing the search term "betel nut." The updated literature search retrieved approximately 600 publications. The search was narrowed by refining and limiting the search terms to "betel nut," "adverse effects," and "toxicity," which resulted in approximately 40 articles (English language only). These articles, in general, relate to possible toxic endpoints, mechanistic studies, biomarker determination, and scientific reviews of betel nut. No new toxicological studies or other information were identified that contradict the Agency's position regarding betel nut, which is that there is no basis for concluding that food safety experts would recognize the use of betel nut in food as safe.

The literature search found four betel nut review articles; these review articles discuss the close relationship between betel quid chewers and the prevalence of oral and pharyngeal cancers, various factors and pathways involved in the molecular pathogenesis of carcinogenicity of added ingredients in betel quid, and the pharmacology and toxicology of arecoline, the most abundant and active constituent of areca nut[1-4]. To expand further on some of the publications which link betel nut exposure to adverse effects since the last OFAS literature review (March 28, 2016) we will highlight some of the negative effects reported in publications from our current literature search. There are three articles published on the repeat dose oral toxicity studies of either areca nut extract or arecoline administered to rats for 14 days [5], 30 days [6] and 13-weeks [7]. In the 14-day study, Wistar rats were administered arecoline hydrobromide daily via gastric lavage. The results demonstrated significant dose related toxicity in the liver and kidney with associated histopathological changes and alterations in the hematopoietic system. The authors concluded that no adverse effects were observed at 100 mg/kg/day. In the 30-day study, rats treated with the aqueous extract of dried betel nuts showed no toxicity to the extract in 750 mg/kg group (arecoline 3.8 mg/kg) but deaths occurred in the 1500 mg/kg group (arecoline 7.6 mg/kg) in which the content of arecoline was far below 100 mg/kg as observed in the 14-day study. The authors suggested that the other toxic compounds such as arecaidine, guavacine, guvacoline, and/or tannins present in the extract may be responsible for the mortality. The 13-week repeated oral administration of *Areca catechu* water extract (arecoline 1%) caused adverse effects at ≥ 500 mg/kg/day in both sexes of rats and the target organs were determined to be the liver, kidney, and female reproductive system. The NOEL for both male and female rats was considered to be 166.7 mg/kg/day.

A retrospective study of patients with esophageal squamous cell carcinoma (ESCC) revealed that areca nut chewing history is significantly associated with younger age of onset, poor response to chemoradiotherapy, and shorter overall survival. Further, in the 4-nitroquinoline 1-oxide (4-NQO)-induced murine ESCC model, it was demonstrated that arecoline accelerates esophageal tumorigenesis [8]. Other published articles focus on the *in vitro* studies using human cell lines and animal studies aimed at delineating arecoline or its oxidative metabolite, arecoline N-oxide (ARNO)-induced molecular mechanisms involved in hepatotoxicity [9], neurotoxicity [10], nephrotoxicity [11], effects on thyroid function [23], and oral carcinogenesis [12]. There are several publications that focus on the molecular basis of areca nut mediated oral submucous fibrosis and oral cancer including mechanisms involved in oral epithelial hyperplasia [13], role of reactive oxygen species [14-15], changes in signaling pathways (Transforming growth factor β1, Epidermal growth factor, Janus kinase (JAK)) [14-15], aberrations of cell cycle and differentiation [15]. Furthermore, published articles demonstrate the

acquisition of cancer stemness [16-17], epithelial to mesenchymal transition [16,18], atrophied epithelium [22], chemoresistance properties [16,19], effects on protein expression [20], cytotoxicity and transformation [18] and enhancement of genotoxicity by betel quid components and tobacco on DNA repair [21]. These articles clearly demonstrate that betel nut is involved in multistep oral carcinogenesis and plays a role in the initiation and promotion stages of oral cancer. Finally, some authors are trying to develop methods and elucidate prognostic markers for betel nut toxicity [24-25].

Conclusions

In reviewing the recent literature, there is no evidence to contradict the conclusions made in the previous betel nut memorandum (March 28, 2016); in fact, the updated scientific information reinforces the Agency's concerns and position that betel nut use in food is unsafe. As such, OFAS would continue to support a 402(a)(2)(C)(i) charge in cases where applicable.

References

1. Baig S, et al. J Coll Physicians Surg Pak, 28 (5): 381-385, 2018 Review.
2. Chen PH, et al. Biomed Res Int Article ID 3904098, 25 pages, 2017 Review.
3. Liu YJ, et al. Pharm Biol 54 (11), 2753-2760, 2016 Review.
4. Garg A, et al. Indian J Cancer 52 (4), 663-6, 2015 Review.
5. Wei et al. PLoS ONE 10(4): e0120165, 2015.
6. Lin Q, et al. J Ethnopharmacol 215, 176-183, 2018.
7. Kim HJ, et al. Food Chem Toxicol 114: 41-51, 2018.
8. Chen CH, et al. PLoS One 12 (2), e0172752, 2017.
9. Wang TS, et al. Environ Toxicol 33 (10): 1029-1038, 2018.
10. Jiang JM, et al. Neurochem Res 41 (8), 2140-2148, 2016
11. Lin SH, et al. Toxicology 344-346, 53-60, 2016.
12. Rehman A, et al. J Oral Pathol Med 45 (5), 365-372, 2016.
13. Chang PY, et al. J Agric Food Chem 65 (47), 10197-10205, 2017.
14. Hsieh YP, et al. J Formos Med Assoc 117 (6), 527-534, 2018.
15. Chang MC, et al. Oncotarget 7 (13), 16879-16894, 2016.
16. Wang TY, et al. Oncotarget 7 (51), 84072-84081, 2016.
17. Li YC, et al. Mol Carcinog 55 (5), 1012-1023, 2016.
18. Yeh CY, et al. J Formos Med Assoc 115 (2), 108-112, 2016
19. Wu SY, et al. Oncotarget 7 (32), 51482-51493, 2016.
20. Chiang CH, et al. J Proteome Res 15 (9), 2981-2997, 2016.
21. Huang JL, et al. Toxicol In Vitro 33, 80-87, 2016.

22. Khan I, et al. J Cell Mol Med 19 (10), 2397-2412, 2015.
23. Dasgupta R, et al. Arch Physiol Biochem 123 (2), 105-111, 2017.
24. Peng W, et al. Sci Rep 7, 40278, 2017.
25. Liu YC, et al. Environ Toxicol 32 (3), 799-812, 2017.