U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Memorandum

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Division of Food Contact Substances (DFCS; HFS-275)
Office of Pre-market Additive Safety (OPMAS)
Office of Food Chemical Safety, Dietary Supplements & Innovation (OFCSDSI)
Human Foods Program (HFP)
United States Food and Drug Administration (FDA)

Through

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Subject Regulatory status and review of available information pertaining to the sheath of *Areca catechu* palm tree leaves in food contact articles: lack of general recognition of safety for its use as a food contact substance.

To [redacted], Ph.D.
Director, OPMAS, OFCSDSI, HFP (HFS-200) [redacted]

Keywords: *Areca (A.) catechu*; *Areca catechu Linn.*; *Areca* palm; *Areca* nut palm; betel palm; palm leaf; *A. catechu* leaf sheath; palm tree leaves; palm leaf dinnerware; palm leaf plates; food contact materials; alkaloids; arecoline; guvacoline; arecaidine; guvacine; toxicity; safety

The Division of Food Contact Substances' (DFCS) Toxicology Review Branch performed an evaluation of the currently available information for sheath of *A. catechu* palm tree leaves (*i.e.*, palm leaf or *A. catechu* dinnerware) in food contact articles to determine whether this use meets the statutory criteria for general recognition of safety. This memorandum considers the pertinent scientific information and concludes that the use of palm leaf dinnerware in contact with food does not meet the criteria for general recognition of safety primarily because there is inadequate scientific data and information demonstrating the safety of the potential resulting exposure to its known alkaloid constituents (arecoline, guvacoline, arecaidine, and guvacine).

Furthermore, the information that is available on other products manufactured from other parts of the *A. catechu* plant and resulting exposure to associated alkaloids reinforce safety concerns for the use of palm leaf dinnerware.

I. Food Contact Substances and the GRAS Provision

As defined in section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) [21 U.S.C. § 321(s)], the term "food additive" refers to any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food), unless the substance is generally recognized as safe (GRAS) among qualified experts under the conditions of its intended use. Subparagraphs (1-6) of Section 201(s) of the FD&C Act list additional exceptions to the definition of a food additive.

As specified in section 409(a) of the FD&C Act [21 U.S.C. § 348(a)], food contact substances[1] (FCSs - substances that come into contact with food through food packaging, storage, or other handling), which are reasonably expected to become a component of or otherwise affect food based on their intended use, must either be an authorized food additive (through either an effective food contact substance notification (FCN), issued Threshold of Regulation (TOR) exemption, or a promulgated food additive regulation) unless the substance is GRAS for the intended use or meets the exceptions to the definition of a food additive in section 201(s)(1-6) of the FD&C Act.

There is no effective FCN, issued TOR exemption, or promulgated food additive regulation for the use of palm leaves in contact with food. Additionally, the use of the leaf sheaths of *A. catechu* palm tree leaves as dinnerware does not meet the exceptions to the definition of a food additive in section 201(s)(1-6) of the FD&C Act. Therefore, this memorandum will discuss the applicability of the GRAS criteria to the use of palm leaf dinnerware in contact with food.

II. GRAS Criteria

A conclusion that a substance is GRAS under the conditions of its intended use requires both general recognition of safety and evidence of safety. FDA has defined “safe” (21 CFR 170.3(i)) as a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use.

General recognition of safety requires common knowledge, throughout the expert scientific community knowledgeable about the safety of substances added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use. FDA’s regulations in 21 CFR Part 170 describe the eligibility criteria for classification as GRAS of a substance directly or indirectly added to food. Under 21 CFR 170.30(a)-(c), general recognition of safety must be based on the views of qualified food safety experts. The basis of such views may be either through: (1) scientific procedures; or (2) in the case of a substance used in food prior to January 1, 1958, experience based on common use in food.

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[1] Food contact substances are defined in Section 409(h)(6) of the FD&C Act as “any substance intended for use as a component of materials used in manufacturing, packing, packaging, transporting, or holding food if such use is not intended to have any technical effect in such food.”

Pertaining to a substance used in food prior to January 1, 1958, FDA's regulations in 21 CFR Part 170 define "common use in food" and establish eligibility criteria for classification as GRAS through experience based on common use in food. Under 21 CFR 170.3(f), common use in food means "a substantial history of consumption of a substance for food use by a significant number of consumers."

Similarly, for GRAS conclusion based on scientific procedures, FDA's regulations in 21 CFR Part 170 define "scientific procedures" and establish eligibility criteria for classification as GRAS through scientific procedures. Under 21 CFR 170.3(h), scientific procedures "include the application of scientific data (including, as appropriate, data from human, animal, analytical, or other scientific studies), information, and methods, whether published or unpublished, as well as the application of scientific principles, appropriate to establish the safety of a substance under the conditions of its intended use." Under 21 CFR 170.30(b), general recognition of safety based upon scientific procedures "shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive." Section 170.30(b) further states that general recognition of safety through scientific procedures is ordinarily based upon published studies, which may be corroborated by unpublished scientific data, information, or methods.

General recognition of safety requires that the information that establishes this is both generally available and generally recognized across the scientific community that the information demonstrates that the use is safe. The usual mechanism to establish that scientific information is generally available is to show that the information is published in a peer-reviewed scientific journal. Mechanisms to establish the basis for concluding that there is common knowledge throughout the expert scientific community about the safety of a substance are more varied. Most often, publication of data in a peer-reviewed scientific journal on a test substance has been used to establish common knowledge throughout the expert scientific community in addition to general availability.

III. Overview of Palm Leaf Dinnerware

Palm leaf dinnerware is formed from the fallen leaves of the *A. catechu* palm tree *(1)*, which is a species of palm tree that is distributed and cultivated in East Africa, South Asia, and the Pacific islands *(2)*. To manufacture palm leaf dinnerware, the fallen *A. catechu* leaves undergo a palm-leaf deformation (*i.e.*, forming) process, during which the leaves are cleaned of debris, soaked in water to improve formability, and placed into molds under high heat and pressure *(3, 4)*. The manufacturing process of palm leaf dinnerware does not include the use of fillers or additives. Furthermore, these products do not contain a food contact barrier *(5)*.

Publicly-available information retrieved from web searches indicates that *Areca* palm leaf dinnerware products (*e.g.*, bowls, plates, cups, and spoons) are imported into the U.S. and marketed as single-use paper/plastic dinnerware that are eco-friendly, compostable, and biodegradable. Marketing and labeling information of some palm leaf dinnerware claim that these products can be microwaved, reused, and washed with soap and water. Some products are also alleged to withstand a range of temperatures and may be marketed for use in contact with both hot and cold foods.

It is well-documented in the scientific literature that toxic alkaloids (*i.e.*, arecoline, arecaidine, guvacoline, and guvacine) naturally occur in the nuts, fruits, leaves, shoots, and roots of the *A. catechu* palm *(6)*. The nut is chewed in some cultures as a mood enhancer. Alkaloids, phenols, and other compounds found in the nuts of the *A. catechu* palm and are suspected of contributing to the elevated risk of oral and esophageal cancers in those cultures *(7)*. FDA has previously concluded that the fruit produced by the *A. catechu* (*i.e.*, betel nuts or betel nut husks) is not GRAS for use as an ingredient in conventional foods based upon public health concerns for exposure to alkaloids present in the nut[2] and considers products composed, in whole or in part, of *A. catechu* nuts to be adulterated under sections 402(a)(1) and 402(a)(2)(C) of the Federal Food Drug and Cosmetic Act (FFDCA). Such products are subject to exclusion from U.S. commerce under section 801(a) of the FFDCA".[3] The same alkaloids found in the nut are also found in the leaves, shoots, and roots of the *A. catechu* palm *(6)*.

Specific to palm leaf dinnerware, FDA has previously refused entry of imported palm leaf dinnerware in that the product appeared to bear or contain a poisonous or deleterious substance which may render it injurious to health [Adulteration, Section 402(a)(2)(C)(i)].[4] These import refusals were based on public health concerns for potential consumer exposure to alkaloids from the use of palm leaf dinnerware.

IV. Regulatory Status of Palm Leaf Dinnerware

i. Insufficient Evidence of GRAS Status Based on Common Use in Food Prior to 1958:

Common use in food is defined as "a substantial history of consumption of a substance for food use by a significant number of consumers" [21 CFR 170.3(f)]. The fact that a substance may have been used in food prior to 1958 does not, in itself, demonstrate that such use is safe. The use prior to 1958 must be sufficiently broad to demonstrate safety to qualified experts.

FDA is unaware of any evidence that palm leaf dinnerware was manufactured, intentionally used in contact with food, or intentionally added to food in the U.S. prior to 1958. To determine whether palm leaf dinnerware was commonly used prior to 1958, a search of the scientific literature was conducted in three databases – PubMed,[5] Web of Science Core Collection,[6] and *FDA's Scientific Terminology and Regulatory Information (STARI)* database.[7] Because STARI generally contains only food-related substances, we queried STARI using the search terms

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[2] See memoranda on Betel nut (*Areca catechu*) - Original Mar 28, 2016, updated May 5, 2020. Posted on FDA's website "Post-market Determinations that the Use of a Substance is Not GRAS." Accessed January 8, 2025. https://www.fda.gov/food/generally-recognized-safe-gras/post-market-determinations-use-substance-not-gras

[3] (b) (4)

[4] *See* Compliance Management System (CMS) case #s 578970, 589256, 543764, and 580357.

[5] The PubMed database generally has scientific literature dating back to about 1951, and in some cases, even earlier literature is available. PubMed, https://pubmed.ncbi.nlm.nih.gov/.

[6] The Web of Science Core Collection consists of six online databases with indexing coverage from the year 1900 to the present. Web of Science, http://www.webofknowledge.com/.

[7] The data contained within STARI dates to the 1970s. It includes primarily chemical substances (including substances/organisms used as chemicals) and associated identifying and regulatory information, but also any scientific term that may have been of interest to HFP. There are currently over 198,000 terms (preferred terms, synonyms) accessed through STARI, including over 50,000 CAS numbers, over 44,000 CERES IDs, over 17,600 UNII codes, and over 1500 Regulations (primarily 21 CFR 73-189 and 40 CFR 180-186) with over 11,000 connections to specific substances. Accessed December 23, 2024.

"*Areca catechu*" and "palm leaf" only. Our search of STARI returned one matching record of '*Areca catechu* palm leaf sheath' in the database. However, this record focused on betel nut and was not related to use in or as dinnerware and therefore, was not considered applicable to the use of leaf sheaths of the *A. catechu* in contact with food. The PubMed and Web of Science databases were searched using the search terms "*Areca catechu"* AND "plates"; "*Areca catechu"* AND "foodware"; "palm leaf" AND "food"; "palm leaf" AND "plates"; and "palm leaf" AND "foodware" (Table 1).

Table 1. Summary of literature search terms and results.

Search Terms	Database	Search Results (Number)
"Areca catechu" AND "food"	PubMed	486
	Web of Science (Core Collection)	107
"*Areca catechu"* AND "plates"	PubMed	14
	Web of Science (Core Collection)	7
"*Areca catechu"* AND "foodware"	PubMed	1
	Web of Science (Core Collection)	2
"Palm leaf" AND "food"	PubMed	14
	Web of Science (Core Collection)	41
"Palm leaf" AND "plates"	PubMed	3
	Web of Science (Core Collection)	10
"Palm leaf" AND "foodware"	PubMed	1
	Web of Science (Core Collection)	3

The PubMed and Web of Science searches yielded several literature results describing the chemical composition and pharmacological/toxicological effects of *A. catechu* extracts. Multiple PubMed and Web of Science search results also described the toxicological effects of betel nut (*i.e.*, the seed of the fruit of the *A. catechu* palm) consumption, which is the primary consumption of *A. catechu* products as a food source.[8] However, the PubMed and Web of Science searches did not yield literature results indicating that palm leaf dinnerware was intentionally used in contact with food or in food prior to 1958.

Searches using Google provide little information that palm leaf dinnerware was marketed in the U.S. prior to 2006 *(8)*.

Per 21 CFR 170.30(c)(2), a substance used in food may be generally recognized as safe through experience based on its common use in food prior to January 1, 1958, when that use occurred

[8] Due to high concentrations of *A. catechu* alkaloid constituents (*i.e.,* arecoline, guvacoline, arecaidine, and guvacine) in the betel nut, FDA determined that betel nut use in food is unsafe and does not meet the statutory criteria for classification as GRAS. *See* Post-market Determinations that the Use of a Substance is Not GRAS | FDA.

exclusively or primarily outside of the United States if the information about the experience establishes that the substance is safe under the conditions of its intended use within the meaning of section 201(u) of the Federal Food, Drug, and Cosmetic Act (see also § 170.3(i)). While there is anecdotal evidence that leaf sheaths were used in contact with food by some cultures in southeast Asian countries prior to 1958, such information is not available in a manner that meets the requirements of § 170.30(c)(2). Currently, there are no internationally recognized standards or specifications to support the safe processing and use of palm leaf dinnerware in food or in food contact applications.

Furthermore, history of use prior to 1958 is not sufficient to support GRAS status if new evidence demonstrates that there is no consensus that the ingredient is safe (see 80 FR 34650, 34653 (June 17, 2015)). FDA has already determined that other parts of the *A. catechu* plant, (*i.e.*, betel nuts or betel nut husks) are not GRAS for use as an ingredient in conventional foods based upon public health concerns for exposure to alkaloids present in the nut.[2] FDA made this determination even though there was anecdotal evidence of historical use of the betel nut by some cultures prior to 1958. The basis for FDA's "not GRAS" determination for betel nuts, that alkaloids present in the nut pose a safety concern and there are not sufficient data available to address this concern, also apply to the use of palm leaf dinnerware (see discussion under "*Insufficient Evidence of GRAS Status Based on Scientific Procedures")*.

In addition, the Technical Regulation of the Russia-Kazakhstan-Belarus Customs Union (CU) on Food Safety (TR TS 021/2011), adopted by the CU Commission in Decision No. 880, includes all parts of the *A. catechu* palm on the list of 'plants and products of their processing, containing psychotropic, narcotic, potent, or toxic substances, prohibited for use as part of biologically active food additives' *(9)*. Such prohibition from use by other regulatory bodies is inconsistent with general recognition of safety of use in food throughout the scientific community.

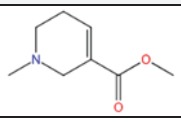
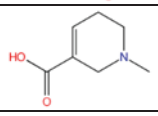
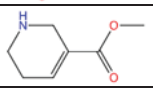
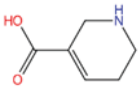
ii. Insufficient Evidence of GRAS Status Based on Scientific Procedures

a. Overview of the Alkaloid Content of the A. catechu Palm Tree

A. catechu palm trees are known to contain four structurally related alkaloids: arecoline,[9] guvacoline, arecaidine, and guvacine (Table 2; Figure 1). These alkaloids are specifically found in *A. catechu* roots, tender shoots, leaves, veins, and fruits (*i.e.* nuts) of different maturation stages *(6)*. The chemical structure for each alkaloid is provided in Table 2. Visual inspection shows a close similarity of the chemical structure of these alkaloids with each alkaloid consisting of similar functional groups attached to a tetrahydropyridine ring.

[9] Of the four alkaloids, arecoline is the most abundant in the nut (*i.e.*, fruit) and leaf of the areca palm tree.

Table 2. The four major alkaloid constituents of the Areca catechu palm.

Name	CASRN® CMS#	Structure
Arecoline	63-75-2 CMS-2490	
Arecaidine	499-04-7 CMS-53682	
Guvacoline	495-19-2 CMS-19167	
Guvacine	498-96-4 CMS-19192	

A publicly available study to determine the alkaloid content of *A. catechu* leaves detected each of the four alkaloids at varying concentrations: arecoline (0.42 ±0.26 mg/g), guvacoline (0.31 ± 0.08 mg/g), arecaidine (0.20 ± 0.07 mg/g), and guvacine (0.38 ± 0.07 mg/g) *(6)*. As the alkaloids were present in the leaves at detectable quantities, this study indicated potential for the migration of these alkaloids from the leaves to food when used as dinnerware products. For this reason, FDA recently conducted a research study to determine the migration levels of these alkaloids from dinnerware products manufactured from *A. catechu* leaves and marketed in the U.S *(5)*. The FDA researchers concluded that all four constituent alkaloids (*i.e.*, arecoline, guvacoline, arecaidine, and guvacine) migrate to a food simulant from palm leaf dinnerware products under conditions designed to mimic their marketed use. The study also determined that the migration occurred at varying levels depending on the manufacturer as well as across products from the same manufacturer (Table 2). The FDA researchers also found that the four constituent alkaloids migrate at higher concentrations over time following microwave heating of *A. catechu* bowls. For example, sampling of the food simulant after 20 minutes post-heating demonstrated a 3-fold increase in migration for both arecaidine and guvacine over the 1-minute post-heating sampling timepoint.

Table 2. Average and range of alkaloid migration values (µg/in²) into 10% ethanol solution held at 40 °C for one hour. Manufacturers were selected at random based on market availability from online retailers in the U.S. (Table sourced from Mangrum et al. (2025)).

Manufacturer	Arecaidine	Guvacine	Arecoline	Guvacoline
A	3.5 ± 2.1	1.1 ± 0.5	LLOQ	LLOQ
B	6.7 ± 4.9	0.9 ± 0.4	LLOQ	LLOQ
C	3.3 ± 1.5	0.4 ± 0.3	0.2 ± 0.1	LLOQ
D	2.6 ± 1.6	0.3 ± 0.3	LLOQ	LLOQ
E	5.2 ± 2.8	1.3 ± 0.7	LLOQ	LLOQ

**The limit of quantification LOQ) for arecoline was 0.11 µg/in² (1.71 µg/dm²) and 0.13 µg/in² (2.02 µg/dm²) for guvacoline. n = 27 plates across manufacturers. Arecoline and guvacoline were detected*

as present in the migration extracts but were below the limit of quantification for the analytical technique used.

This research indicates that all four alkaloids are present and will migrate to food from palm leaf dinnerware products when used in contact with food as marketed. As noted in the following section "*Toxicological Review of Data on Palm Leaf Dinnerware and Constituent Alkaloids*", information in the scientific literature indicates that dietary exposure to these alkaloids can induce adverse toxicological effects. This underscores that the dietary exposure to these alkaloids that occurs from the use of palm leaf dinnerware has the potential to result in adverse toxicological effects and serious harm to the general public.

FDA also conducted comparison and analysis of structural similarity of these alkaloids using two computational methods available on the ChemTunes-ToxGPS® cheminformatics database (the two computational methods used were Dice and Tanimoto similarity measures, both using RDKit Mol as the similarity descriptors). This analysis, which is visually represented below in Figure 1, show that there is a high degree of similarity (*i.e.*, similarity scores between 0.75 to 0.95) between the chemical structures for all four alkaloids *(10)*.

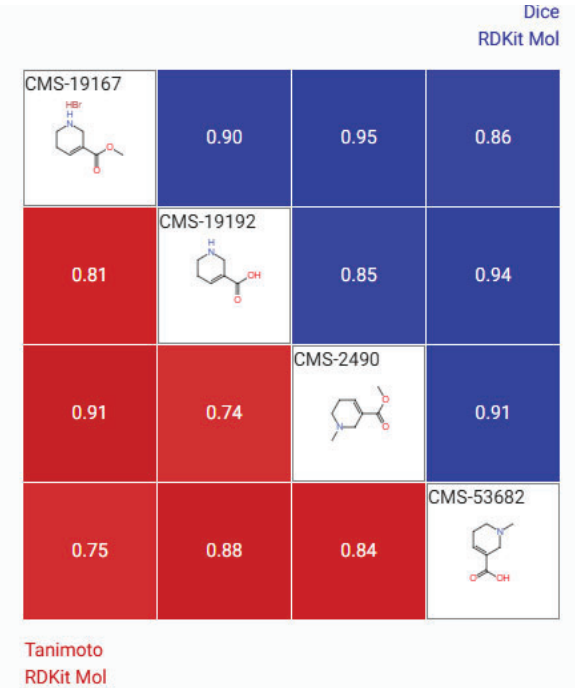


Figure 1. Similarity comparisons using two methods (Dice vs Tanimoto similarity measures, both using RDKit Mol as the similarity descriptors). The compounds, from the upper left to the lower right, are guvacoline, guvacine, arecoline, and arecaidine. Blue squares indicate similarity scores derived from Dice analysis, red squares indicate similarity scores derived from Tanimoto analysis. For both analyses a similarity of > 0.8 is considered indicative of a high degree of structural similarity.

The high degree of chemical structure similarity between all four alkaloids indicates that these alkaloids are structural analogues of each other and support the application of toxicological data from one alkaloid towards hazard identification and determination of potential safety risk from exposure to another alkaloid. Such approach to safety assessment is termed "read-across" and

is commonly used to support the safety of a data-poor substance by applying the safety data from a data-rich structural analogue to the exposure to the data-poor substance. As noted above, migration studies indicate that the alkaloids with the highest potential to migrate from palm leaf dinnerware into food are arecaidine and guvacine. However, as will be noted in the following section "*Toxicological Review of Data on Palm Leaf Dinnerware and Constituent Alkaloids"*, there is little toxicology data on arecaidine and guvacine specifically, while arecoline, which migrates at lower levels, is "data-rich". As arecoline is a structural analogue for arecaidine, guvacine, and guavacoline, data from arecoline can be applied to the exposure for all four alkaloids to determine if a potential safety concern exists for exposure to these alkaloids from the food contact use of palm leaf dinnerware.

b. Toxicological Review of Palm Leaf Dinnerware and Constituent Alkaloids

Herein, FDA Toxicology provides an updated summary of safety information associated with *A. catechu* leaves and their four major alkaloids (*i.e.*, arecoline, arecaidine, guvacoline, and guvacine). This memorandum discusses the findings of an updated search of publicly available information to evaluate whether the use of palm leaf dinnerware meets the criteria for general recognition of safety. Our review focuses on the four main alkaloids in *A. catechu* palm leaves. We report findings of cited references that raise concerns regarding the safety of these alkaloids. Only scientific papers published in English were considered.

A search of the scientific literature published through January 10, 2025 was conducted in three databases – PubMed,[5] Web of Science Core Collection,[6] and *FDA's Scientific Terminology and Regulatory Information (STARI)* database.[7]

Because STARI generally contains only food-related substances, we queried STARI using the search terms "*Areca catechu*" and "palm leaf" only. Our search of STARI returned one matching record of '*Areca catechu* palm leaf sheath' in the database. However, related information focused on betel nut and was not considered useful for this review.

The PubMed and Web of Science databases were searched using the search terms ("*Areca catechu*" OR the names of the four principal alkaloids). To narrow the scope of results to publications relevant to the safety evaluations of *A. catechu* and the four alkaloid constituents and exclude publications irrelevant to establishing safety to support a GRAS conclusion the PubMed and Web of Science databases was further limited to AND ("toxicity" OR "safety") (Table 3).

Table 3. Summary of literature search terms and results.

Search Terms	Database	Search Results (Number)
"Areca catechu"	PubMed	3008
	Web of Science (Core Collection)	720
"Areca catechu" AND "safety"	PubMed	45
	Web of Science (Core Collection)	13

"Areca catechu" AND "toxicity"	PubMed	462
	Web of Science (Core Collection)	34
"arecoline"	PubMed	1639
	Web of Science (Core Collection)	1374
"arecoline" AND "safety"	PubMed	16
	Web of Science (Core Collection)	13
"arecoline" AND "toxicity"	PubMed	232
	Web of Science (Core Collection)	86
"arecaidine"	PubMed	229
	Web of Science (Core Collection)	218
"arecaidine" AND "safety"	PubMed	6
	Web of Science (Core Collection)	3
"arecaidine" AND "toxicity"	PubMed	30
	Web of Science (Core Collection)	8
"guvacine"	PubMed	117
	Web of Science (Core Collection)	126
"guvacine" AND "safety"	PubMed	4
	Web of Science (Core Collection)	2
"guvacine" AND "toxicity"	PubMed	12
	Web of Science (Core Collection)	6
"guvacoline"	PubMed	34
	Web of Science (Core Collection)	39
"guvacoline" AND "safety"	PubMed	3
	Web of Science (Core Collection)	2
"guvacoline" AND "toxicity"	PubMed	9
	Web of Science (Core Collection)	5

Combining the results (Table 3) from the searches inclusive of "AND 'safety'" and "AND 'toxicity'" and excluding those in a language other than English yielded overall 605 unique references. Many, however, described analytical methods, betel quid (a chewable concoction, made from betel nut mixed with other materials, often used for mood enhancement), or other topics which were not considered relevant to the current safety evaluation. The relevant references have been cited below and listed in the bibliography of this memorandum.

1. A. catechu leaf sheath

There are no regulations (21 CFR 170-199) or effective authorizations for food-contact uses of products manufactured from the sheath of *A. catechu* leaves.

1.1 Acute oral toxicity:

- A lethal dose 50 (LD50) of >5000 mg/kg in mice was identified for *A. catechu (11)*. However, specific details regarding the nature of the test article (*e.g.*, sheath extracts or whole sheath) were not provided. As such, FDA Toxicology considers data based on acute exposure scenarios to be of limited relevance or this safety assessment.

1.2 Genotoxicity:

- Extracts of *A. catechu* leaves were reportedly non-mutagenic in an Ames assay with *Salmonella typhimurium* (*S. typhimurium*) strains TA100, TA1535, TA98, and TA1538 in the presence and absence of exogenous metabolic activation *(12)*.
- Extracts of *A. catechu* leaves reportedly did not increase the frequency of micronucleated polychromatic erythrocytes (PCE) in the bone marrow cells of mice administered with 2(LD50) mg of the test substance twice, i.p. *(12)*.

2. Arecoline

There are no regulations (21 CFR 170-199) or effective authorizations (FCNs, etc.) for the use of arecoline as an indirect or direct food additive. Arecoline is the most abundant alkaloid in the *A. catechu* palm and has been reported to induce several pharmacological and toxicological effects *(13)*. The earliest report in the scientific literature of an evaluation of arecoline for toxic effect dates back to 1899 when Clemensha reported that administration of arecoline causes vomiting and diarrhea and is useful in treating constipation due to its effect on the contractility of the intestine *(14)*.

2.1 Absorption, Distribution, Metabolism, and Excretion of Arecoline

Arecoline is readily absorbed, systemically distributed, and eliminated via urine and feces in dogs *(15)*. Patterson and Kosh *(16)* studied the metabolism of arecoline in mouse blood, brain, liver, and kidney homogenates prepared in saline. Arecoline was measured by GC/MS 30 min after the addition of 5 nM of the test substance. The rate of metabolism of arecoline was quantified using liver supernatant with 10-100 nM of arecoline. Six different potential inhibitors of metabolism of arecoline were tested to learn which enzymes might be involved in its metabolic processes. The researchers found that arecoline is extensively metabolized in the liver and kidney of mice. It was reported that other studies *(17)* had previously found that arecoline is quickly and completely metabolized in the rat liver homogenates. Patterson and Kosh also pointed out that there may be four different metabolites produced from three different pathways of metabolism of arecoline. Please see figures 2 and 3 below from scientific publications by Nery (1971) *(18)* and Oliveira et al. (2021) *(19)*, respectively, that illustrate the different pathways of metabolism of arecoline.

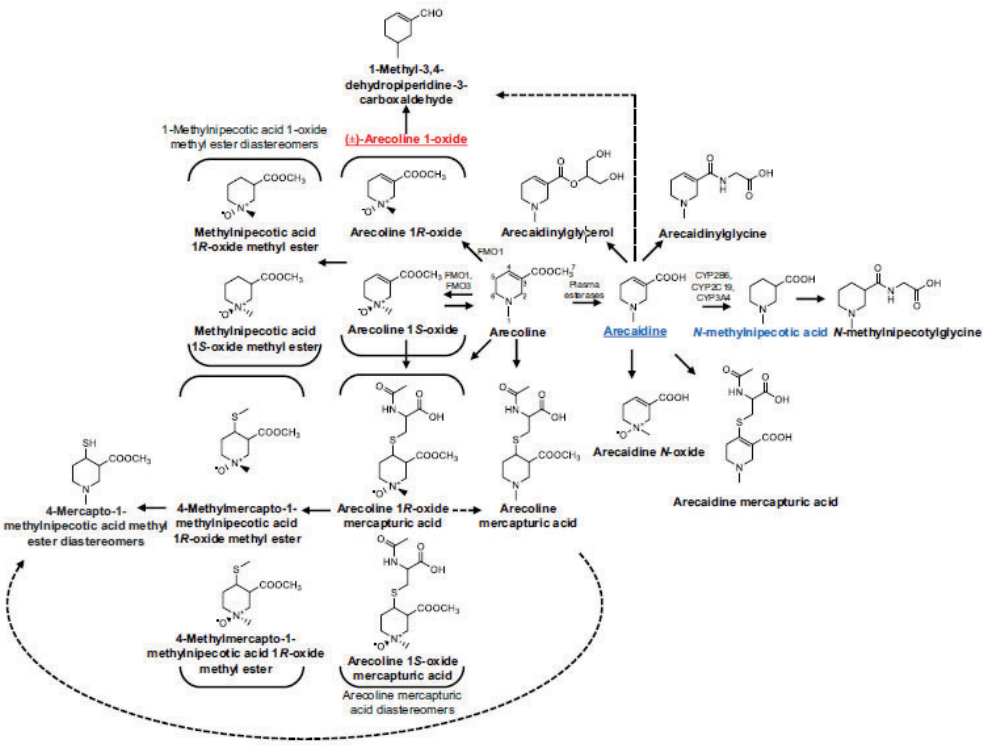
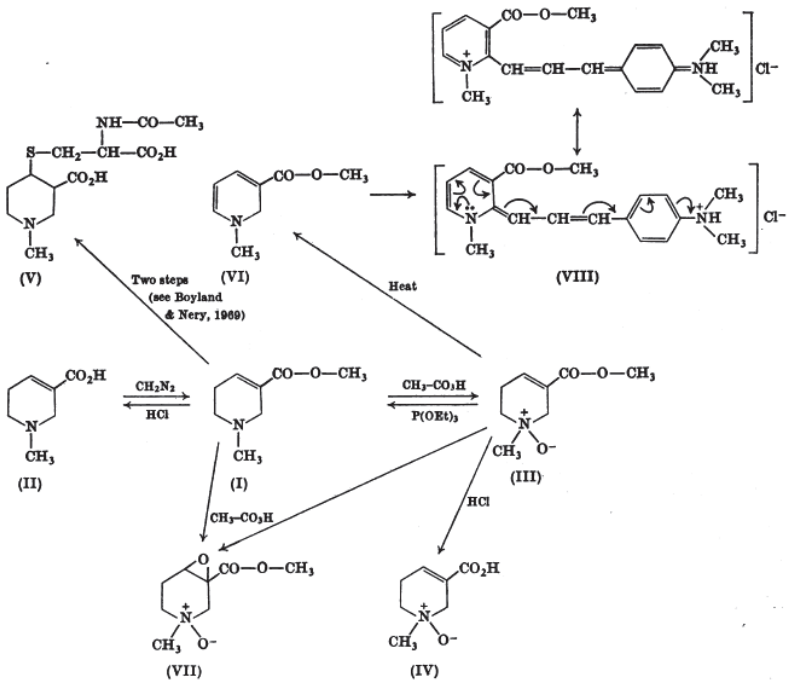


Figure 2. Proposed metabolism of arecoline in rats (Nery, 1971).



Scheme 1. Summary of the reactions of arecoline and arecoline 1-oxide described in the text. Arrows indicate chemical reactions. Compounds (I) to (V) were excreted in the urine after administration of arecoline or arecoline 1-oxide to rats. Other details are given in the text.

Figure 3. The metabolism of arecoline and arecaidine as later developed by Oliveira et al., 2021.

It has been reported that orally administered arecoline could be detected in rat blood *(20)*, human urine *(21),* human saliva *(22),* human placenta and meconium *(23),* and human breast milk *(24)*. Arecoline could also increase membrane permeability of rat brain neurons *(25)*. Orally administered arecoline could be detected in many tissues, such as human hair *(26)* and mouse kidney, brain, and liver *(27)*. Those authors also suggested carboxylesterase (Enzyme Commission no. 3.1.1.1) as being the enzyme that is primarily responsible for the metabolism of arecoline in mice. Further, Pan et al. *(28)* suggested that the differences in the effects of arecoline between rodents and dogs might be due to species-related carboxylesterase activity.

2.2 Genotoxicity:

Arecoline is an alkylating agent *(29),* a classification of substances which are known to induce DNA damage, and arecoline itself has been reported to show genotoxic effects in some studies, as summarized below:

- Mutagenic effects were reported in an Ames assay with *S. typhimurium* strains TA 100, TA 1535, TA 98, and TA 1538 treated with arecoline in the absence and presence of exogenous metabolic activation *(12)*.
- An increase in the frequency of sister-chromatid exchanges and chromosomal aberrations was reported in Chinese Hamster Ovary (CHO) cells *(30)*. The study authors also reported that chromosomal damage was more severe in cells treated with low concentrations of arecoline for a longer duration, which the authors believe mimics the reported effects from chronic *Areca* nut consumption.
- An increase in the frequency of chromatid breaks, chromosome breaks, ring structure, multiple breaks, and cells with pulverized chromosomal complements was reported in bone marrow cells of mice administered (via intraperitoneal injection) with 0.25, 0.5, 1.0, and 2.0 mg of arecoline for 10, 20, and 30 days *(29)*.
- An increase in the frequency of micronucleated PCE was reported in the bone marrow cells of mice administered (via intraperitoneal injection) with 2.0 mg of arecoline *(12)*.
- An increase in the mutation frequency was reported in V79 Chinese hamster cells treated with 5 and 10 µg/mL of arecoline in the presence of exogenous metabolic activation *(12)*.
- Mixed results were reported in an Ames assay with *S. typhimurium* strains TA 98 and TA 100 treated with arecoline in the absence (6.5-26 µmol/plate) and presence (6.5-39 µmol/plate) of exogenous metabolic activation. Negative results were reported in the TA 98 strain (in the presence and absence of exogenous metabolic activation). Positive results were reported in the TA 100 strain (in the absence and presence of exogenous metabolic activation) *(31)*.

2.3 Carcinogenicity:

A median toxic dose (TD50) of 39.5 mg/kg bw/d for arecoline hydrochloride has been reported in the Carcinogenic Potency Database (CPDB) *(32)*. This value is based on a published oral carcinogenicity study conducted in Swiss mice *(33)*. In this study, male and female mice were administered a dose of 1 mg/day/mouse of arecoline via gavage five times a week for a duration of 25 weeks. Upon reviewing the study, some design limitations were identified. For example, the duration of the study was shorter than the recommended 24 months for carcinogenicity studies conducted in rodents *(34)*. In addition, only one dose was tested and this study included fewer numbers of animals per sex per group (*e.g.*, 20 male and female mice each in the control group and 18 female mice in the arecoline-treated group) than the recommended minimum

number of animals per sex per group (*i.e.*, 25 animals per sex per group) remaining at study termination *(34)*.

Nonetheless, the study reported increased incidences of tumors in several vital organs, including liver, lungs, and stomach, in 43% (15) of 35 male mice exposed to arecoline over a shorter study duration and was therefore used to estimate a unit cancer risk (UCR) for arecoline to conduct a conservative safety assessment. Upon reviewing the results of this study, FDA Toxicology concluded that arecoline is potentially carcinogenic and calculated a UCR of 0.0126 (mg/kg bw/d)$^{-1}$ for arecoline based on the TD50 value from this study.[10] In the context of carcinogenicity, a TD50 value represents the measure of carcinogenic potency of a substance *(35, 36)*. It is defined as the daily dose of a substance which would induce tumors in 50% of animals in a carcinogenicity study. A cancer risk assessment based on a TD50 value from a positive carcinogenicity study of a substance is considered a conservative approach to evaluate its safety *(37, 38)*.

FDA toxicology noted that the UCR of 0.0126 (mg/kg bw/d)$^{-1}$ calculated for arecoline is higher than FDA's current UCR values for several potent carcinogens, including some vinyl-containing compounds (*e.g.*, divinyl benzene,[11] vinyl acetate,[12] and 4-vinylcyclohexene[13]). Nitrosation of arecoline and other alkaloids *in vivo*, which leads to the formation of nitrosamines, has been hypothesized as a potential mechanism of action of carcinogenicity reported in mice *(39)*.

2.4 Non-cancer Systemic Toxicity Endpoints:

Gao et al. recently reviewed the toxicity of arecoline hydrobromide (AH), for which the active component is understood to be arecoline *(40)*. The study authors summarized a 14-day study conducted by Wei et al. *(41)* to evaluate sub-chronic toxicity of AH. The study authors randomly divided eighty rats into four groups, including a high-dose group (1000 mg/kg bw), medium-dose group (200 mg/kg bw), low-dose group (100mg/kg bw), and a vehicle control group. Each group consisted of 10 male and 10 female rats, which were administered arecoline or the vehicle control daily via gastric lavage for 14 days. During treatment, the animals were inspected daily for any clinical abnormalities. After treatment, the animals were euthanized and hematological, clinical biochemistry, visceral index, and histopathological parameters were analyzed. Arecoline-treated rats exhibited a significant reduction in body weight compared to controls at all dose levels ($p < 0.01$). Hematocrit and HGB levels and leukocyte, lymphocyte, and erythrocyte counts were significantly decreased in female rats treated with arecoline hydrobromide at all doses compared to control animals ($p < 0.01$). Some blood biochemical parameters (alanine aminotransferase [NS], total protein [$p < 0.01$], and blood urea nitrogen [p, 0.05]) in treated groups were also affected versus control. Male and female rats in the high-dose group exhibited significantly decreased cholesterol and aspartate aminotransferase ($p < 0.05$). In female rats, liver, kidney, and brain weights were significantly increased following treatment with low, medium, and high doses of arecoline hydrobromide ($p < 0.05$). Liver and spleen weights were increased in male rats from the high-dose group compared to the control group ($p < 0.05$). Treatment with arecoline hydrobromide did not result in histopathological findings in the low-

[10] The Unit Cancer Risk (UCR) for arecoline is: 0.5/39.5 mg/kg bw/d = 0.0126 (mg/kg bw/d)$^{-1}$.
[11] The UCR for divinyl benzene (CAS Reg. No. 1321-74-0) is 0.0014 (mg/kg bw/day)$^{-1}$.
[12] The UCR for vinyl acetate (CAS Reg. No. 108-05-4) is 0.0012 (mg/kg bw/day)$^{-1}$.
[13] The UCR for 4-vinylcyclohexene (CAS Reg. No. 100-40-3) is 0.00355 (mg/kg bw/day)$^{-1}$.

dose animals. However, treatment-related pathological findings were observed in the colon, spleen, heart, liver, kidney, and brain of rats treated with medium and high doses of arecoline hydrobromide and these results were statistically significant between the high-dose and control groups ($p < 0.05$).

Forbes et al. also conducted a study to assess the effect of AH on oral, esophageal, gastric, and intestinal absorption in dogs *(15)*. The authors reported that oral administration of AH had an effect on the intestinal wall, resulting in vomiting.

2.4.1 Gastrointestinal Toxicity

Xu et al. examined the effects of acute arecoline exposure on gastrointestinal toxicity in C57BL/6 mice *(42)*. Experimental animals were divided into three groups, including control, low-dose arecoline, and high-dose arecoline groups. The control group was administered saline, and the low-dose and high-dose groups were administered 6 mg/kg bw and 30 mg/kg bw arecoline, respectively, *via* gavage once daily for 23 days. The study authors found that arecoline intake induced a significant increase in intestinal inflammatory factor levels, such as a significant increase in IL-1β levels and significantly increased secretion of IL- and IL-10 following administration of high-dose arecoline ($p < 0.05$). Histopathological sections of intestinal tissue from arecoline-treated mice revealed loose arrangement of local intestinal glands, a decrease in the number of goblet cells, and lymphocyte infiltration when compared to control animals. Arecoline intake also led to changes in the composition of gut microbes in both the low-dose and high-dose groups ($p < 0.05$). The authors concluded that arecoline intake caused intestinal injury under the conditions of the study.

2.4.2 Nephrotoxicity

Lin et al. studied the chronic effects of arecoline on nephrotoxicity in male ICR mice administered a control solution or arecoline (6.11 mg/kg bw/day in drinking water) for 24 weeks *(43)*. Kidney morphology and renal pathology were assessed for control and arecoline-treated mice. The authors identified increased tubulointerstitial fibrosis in arecoline-treated mice compared to controls (Masson's trichrome stain, 7.7 ± 1% versus 2.2 ± 0.3% in 10 randomly chosen fields in each mouse, $p < 0.01$). Immunoblotting also demonstrated significantly increased renal cortical fibronectin and PAI1 protein expression in mice treated with arecoline for 24 weeks. Concurrent experiments in LLC-PK1 cells (a cell line analogous to proximal tubule cells of the kidney) *in vitro* demonstrated that arecoline induces cytotoxicity and increased TGF-β and pro-fibrotic protein levels in proximal tubular cells. The authors concluded that increased cytotoxicity, fibronectin, and PAI1 protein expression induces chronic tubulointerstitial fibrosis in arecoline-treated mice under the conditions of the study.

2.4.3 Oral Submucosal Fibrosis

Tang et al. studied the effects of arecoline administration on the development and severity of oral submucosal fibrosis in water drinking and injection mouse models *(44)*. For the water drinking mouse model, sixty-six female C57BL/6 mice were offered water containing 2 mg/mL arecoline for 4, 8, 12, 16, and 20 weeks; the control group was provided regular drinking water. For the injection mouse model, sixty-six female C57BL/6 mice were injected with a 45 μL of 4 mg/mL arecoline-saline solution every other day for 4, 8, 12, 16, and 20 weeks; the control group

received a saline solution under the same conditions. Starting at week 16, the authors found that the mice administered arecoline in drinking water or *via* injection exhibited a significantly lower degree of mouth opening when compared to the control groups ($p < 0.05$). The authors also noted increased fibrosis in the buccal mucosa of mice treated with arecoline. The status of the buccal mucosa at each stage of the fibrotic process was evaluated by histopathological analyses. The epithelial thickness of the buccal mucosa was significantly thickened at 16 weeks in mice administered arecoline in drinking water and at 20 weeks in mice injected with arecoline ($p < 0.001$). Collagen thickness of the lamina propria of the buccal mucosa was significantly increased after arecoline induction at 8 weeks in mice administered arecoline in drinking water ($p < 0.05$) and at 16 weeks in mice injected with arecoline ($p < 0.001$). Evaluation of collagen accumulation in the buccal mucosa also demonstrated that the relative area of collagen I and III was significantly increased in mice administered arecoline in drinking water or injection after 12 weeks of treatment ($p < 0.01$). The number of α-smooth muscle actin (SMA)-positive myofibroblasts in the lamina propria were also evaluated as an indicator of the degree of fibrosis in the arecoline-treated mouse models. After treatment with arecoline injection, the authors observed a significant increase in the number of α-SMA-positive myofibroblasts following 20 weeks of induction compared to the control group ($p < 0.001$). Similarly, in the water-drinking model, a significant increase in the number of α-SMA-positive myofibroblasts in the lamina propria was observed after 12 and 20 weeks of induction ($p < 0.01$). Finally, in the water-drinking model, the authors found a significant increase in sublingual collagen and in collagen accumulation in the lungs and small intestine ($p < 0.001$). The authors concluded that arecoline induced fibrosis not only in the buccal mucosa but also in other organs such as the tongue, lungs, and small intestine in mice under the conditions of the study.

2.4.4 Developmental and Reproductive Toxicity

Mice

Liu et al. conducted a preliminary reproductive toxicity study of arecoline in mice *(45)*. This study evaluated the effects of arecoline on embryos during the peri-implantation stages in female mice. Mice consuming varying dosages (0.0067, 0.067, 0.67, 6.7 mg/kg bw) of arecoline were assessed for their ability to successfully produce viable embryos. In addition, trophoblast outgrowth from blastocysts was evaluated to assess the survival status of the embryos. The doses were 0, 0.67, 0.0067, 0.067, 6.7 mg/kg or 0.0, 0.2, 2, 20, or 200 μg/mouse. The authors reported that arecoline exposure > 0.2 μg/mouse. Therefore, the authors concluded that arecoline induced toxic effects on mouse embryos as early as peri-implantation under the conditions of this study.

Li et al. investigated the effects of arecoline on mouse oocyte development *(46)*. In this study, oocytes were first cultured for 12 hours with 0, 160, 180, and 200 μg/mL of arecoline. The authors found that arecoline exposure at 160 μg/mL: 66.67 ± 6.73%, n = 115, $p < .05$, 180 μg/mL: 50.00 ± 3.09%, n = 317, $p < .01$, 200 μg/mL: 38.22 ± 5.79%, n = 117, $p < .01$ inhibited meiotic maturation of mouse oocytes in a statistically significant dose-dependent manner. Furthermore, the frequency of morphologically aberrant spindles with misaligned chromosomes was significantly increased in oocytes treated with arecoline compared to the control group ($p < 0.05$). Similarly, the rates of defective kinetochore-microtubule attachments and chromosomal aneuploidy were significantly increased in oocytes exposed to arecoline ($p < 0.05$). Arecoline treatment also altered the distribution of mitochondria and reduced ATP production in developing oocytes ($p < 0.05$). Finally, the authors observed significantly increased levels of

reactive oxygen species and rates of apoptosis in oocytes treated with arecoline compared to controls ($p < 0.05$). In summary, the authors concluded that arecoline exposure disrupted actin filament dynamics, spindle assembly, and kinetochore-microtubule attachment stability in mouse oocytes, leading to aneuploidy and oocyte meiotic arrest under the conditions of this study.

Chickens

Paul et al. conducted a study of AH in chickens *(47)* in which AH was aseptically dissolved in distilled water and injected into the air-sac of the fertilized egg at doses of 0.25, 0.50, 0.75 and 1 mg/egg at 2, 3 and 4 days of incubation. The authors monitored the development of these chick embryos for up to 14 days. Edema, exencephaly, and fetotoxicity were observed in all AH-treated groups, among which arthrogryposis or clubfoot was one of the most consistent deformities. Although the toxicological mechanisms behind the teratogenic effects of AH were not explored in this study, the authors inferred that arecoline may disrupt multiple biochemical pathways, leading to teratogenesis.

Humans

Yuan et al. evaluated the effects of arecoline on human sperm motility *in vitro (48)*. Sperm motility parameters (*i.e.*, motility, average path velocity, straight-line velocity, curvilinear velocity, linearity, and amplitude of lateral head displacement) were compared between groups treated with varying concentrations of arecoline (0, 50, 100, 200, and 300 μg/mL) under the same incubation periods. The authors reported a significant decrease in all six sperm motility parameters following treatment with arecoline for 1, 2, and 3 hours compared to human sperm samples treated under control conditions ($p < 0.05$). The authors concluded that arecoline reduced human sperm motility in a statistically significant dose-dependent manner ($p < 0.05$) and stated that arecoline may alter gonadal functions in human males.

2.4.5 Immunotoxicity

Selvan et al. studied immune system responses to arecoline using a murine model system *(49)*. The purpose of the study was to evaluate the modulatory influence of arecoline on B cell-mediated immune response. The *in vivo* and *in vitro* effects were assessed at sub-toxic i.p. doses of arecoline: 5, 10 and 20 mg/kg bw. The number of primary antibody-forming cells (AFC) and hemagglutinating (HA) and hemolysis (HL) antibody titers to Sheep Red Blood Cells (SRBC) were evaluated in male mice. Arecoline exposure for a week invoked a dose-dependent effect on primary antibody forming cells to SRBC with no effect at 5 mg/kg bw, a moderate reduction at 10 mg/kg bw, and a maximum reduction at the dosage of 20 mg/kg bw dose level. HA and HL antibody titers to SRBC were suppressed significantly *($p < 0.001$)* at arecoline dosage of 20 mg/kg bw and moderately at a dose of 10 mg/kg bw, given daily for 1, 2 or 3 weeks. The difference from controls was significant for the 10 mg/kg bw dose at 3 weeks ($p < 0.01$). The inhibitory effect of arecoline was not dependent on the duration of treatment. Like the primary antibody response, the secondary HA and HL antibody titers were also decreased after arecoline exposure 10 mg/kg bw *($p < 0.01$)* and 20 mg/kg bw *($p < 0.001$)* Concomitant exposure of arecoline at the concentrations of 10^{-5} – 10^{-4} M with PWM (pokeweed mitogen, *sic*) suppressed 3H-thymidine incorporation of splenic cells in vitro. The study authors concluded that the intensity of arecoline-mediated suppression of antibody response to SRBC and PWM-induced splenic cell

proliferation is dependent upon the dosage and the mode of administration of arecoline.

2.4.6 Cardiotoxicity

Lin et al. evaluated the cardiotoxic effects of arecoline in Sprague-Dawley rats following intraperitoneal injections of a low dosage (5 mg/kg/day) or a high dosage (50 mg/kg/day) of arecoline for 21 days *(50)*. The study authors examined the effects of arecoline treatment on heart architecture and found that arecoline induces irregulated arrangement of myocardial fibers, aberrant cell shape of cardiomyocytes, and increased small interspaces in both arecoline treatment groups compared to controls. Finally, the study found that treatment with arecoline damaged cardiomyocytes in rats and resulted in increased cardiomyocyte apoptosis under the conditions of the study.

Ho et al. studied the acute cardiotoxic effects, including pathologic heart hypertrophy, of intraperitoneal arecoline exposure in Sprague-Dawley rats *(51)*. The authors induced heart injury in rats by administering arecoline at either a low dosage (5 mg/kg/day) or a high dosage (50 mg/kg/day) via intraperitoneal injections for 21 days. Echocardiography demonstrated impaired cardiac contractility in both arecoline-treated groups compared to the control group ($p < 0.05$). Furthermore, the authors identified significantly increased levels of the cardiac hypertrophy biomarker BNP in the heart of rats treated with 50 mg/kg/day arecoline. The authors concluded that exposure to arecoline induced cardiac hypertrophy, alters heart function, and caused heart damage in rats under the conditions of the study.

2.4.7 Hepatotoxicity

Dasgupta et al. researched the hepatotoxic effects of arecoline administration in albino Swiss mice *(52)*. Arecoline hydrobromide was injected intraperitoneally at three different doses (5 mg/kg bw, 10 mg/kg bw, and 20 mg/kg bw) for 14 days. The authors found that arecoline induced disruption of hepatocytes at the ultrastructural level in mice treated with arecoline. For example, the authors reported decreased nuclear size with indented nuclear membranes, rough endoplasmic reticulum with profusely inflated cisternae and dilated mitochondrial cristae, and increased accumulation of vacuoles in arecoline-treated mice compared to controls. The authors also reported a significant increase in clinical markers of liver toxicity (*i.e.*, SGOT, SGPT, and ALP) in the serum of arecoline-treated mice in a dose-dependent manner ($p < 0.05$). As such, the authors concluded that arecoline administration induced liver injury in mice under the conditions of the study.

2.4.8 *Endocrine Toxicity*

Dasgupta et al. investigated the effects of arecoline administration on endocrine function in male albino Swiss mice *(53)*. In this study, mice were injected intraperitoneally with 10 mg/kg bw arecoline hydrobromide and untreated control or vehicle control solutions. The authors found that serum corticosterone was significantly increased in mice following arecoline treatment ($p < 0.001$). Adrenal epinephrine and norepinephrine levels were significantly reduced in mice treated with arecoline compared to the control groups ($p < 0.001$). Blood glucose and liver glycogen were significantly decreased and increased, respectively, in mice treated with arecoline compared to control animals ($p < 0.05$). These data indicate that arecoline treatment caused hypoglycemia with an elevation of glycogen levels in mice treated with arecoline hydrobromide.

Taken together, the authors concluded that arecoline inhibits adrenomedullary function and disturbs glucose-glycogen homeostasis in mice.

3. Arecaidine

There are no regulations (21 CFR 170-199) or effective authorizations for the use of arecaidine as an indirect or direct food additive.

Arecaidine is reported to be a metabolite of arecoline with 2-9% of arecaidine formed when arecoline was administered to rats in drinking water *(54)*.

3.1 Genotoxicity:

Arecaidine has been reported to show genotoxic effects in some assays, as summarized below:

- Mutagenic effects were reported in an Ames assay with *S. typhimurium* strains TA 100, TA 1535, TA 98, and TA 1538 treated with arecaidine in the absence and presence of exogenous metabolic activation *(12)*.
- An increase in the frequency of chromatid breaks, chromosome breaks, ring structure, multiple breaks, and number of cells with pulverized chromosomal complements was reported in bone marrow cells of mice administered (via intraperitoneal injection) 0.25, 0.5, 1.0, and 2.0 mg of arecaidine for 10, 20, and 30 days *(29)*.
- An increase in the frequency of micronucleated PCE was reported in the bone marrow cells of mice administered (via intraperitoneal injection) 14 mg of arecaidine *(12)*.
- A slight increase in the mutation frequency was reported in V79 Chinese hamster cells treated with 10 μg/mL of the arecaidine in the presence of exogenous metabolic activation; however, the study authors concluded that arecaidine was negative under the study conditions *(12)*.

3.2 Carcinogenicity:

In our literature search, we did not identify any studies that have evaluated the carcinogenic potential of arecaidine. However, arecaidine is a metabolite and structural analog of arecoline[14] (Figure 1). As arecoline has been shown to be carcinogenic in a mouse study, arecaidine was also considered to be potentially carcinogenic as a conservative approach to perform its risk assessment. As such, FDA Toxicology applied the UCR value of 0.0126 $(mg/kg\ bw/d)^{-1}$ for arecoline[10] to the safety assessment of arecaidine.

3.3 Non-cancer Systemic Toxicities:

3.3.1 Developmental and Reproductive Toxicity

Humans

Yuan et al. evaluated the effects of arecaidine on human sperm motility *in vitro (48)*. Sperm motility parameters[15] were compared between groups treated with varying concentrations of

[14] Similarity scores of 0.84 or 0.91 based on Tanimoto or Dice models, respectively. *See* Figure 1.

[15] Sperm motility parameters measured by Yuan et al., 2012 included motility, average path velocity, straight-line velocity, curvilinear velocity, linearity, and amplitude of lateral head displacement.

arecaidine (0, 50, 100, 200, and 300 μg/mL). The authors reported that arecaidine induced a statistically significant reduction in sperm motility at the highest concentration tested (300 μg/mL) ($p < 0.05$). Correlation and regression analyses demonstrated that arecaidine induced a reduction in the sperm motility parameters tested, except for amplitude of lateral head displacement, in a dose-dependent manner ($p < 0.05$).

Zebrafish
Yan et al. reported that arecaidine and arecoline N-oxide showed no changes in mortality and hatchability rates in zebrafish, but the malformation rate of zebrafish larvae was significantly increased in a dose-dependent manner and accompanied by changes in body length *(55)*.

4. Guvacoline

There are no regulations (21 CFR 170-199) or effective authorizations for the use of guvacoline as an indirect or direct food additive.

4.1 Genotoxicity and Carcinogenicity:

In our literature search, we did not identify any studies that have evaluated either the genotoxic or carcinogenic potential of guvacoline. However, guvacoline is a structural analog for arecoline[16] (Figure 1). As arecoline has been shown to be carcinogenic in a mouse study, guvacoline was also considered to be potentially carcinogenic as a conservative approach to perform its risk assessment. As such, FDA Toxicology applied the UCR value of 0.0126 (mg/kg bw/d)$^{-1}$ for arecoline[10] to the safety assessment of guvacoline.

5. Guvacine

There are no regulations (21 CFR 170-199) or effective authorizations for the use of guvacine as an indirect or direct food additive. Guvacine is reported to be a hydrolysis product of guvacoline.

5.1 Genotoxicity and Carcinogenicity:

In our literature search, we did not identify any studies that have evaluated either the genotoxic or carcinogenic potential of guvacine. However, guvacine is a structural analog of arecoline[17] (Figure 1). As arecoline has been shown to be carcinogenic in a mouse study, guvacine was also considered to be potentially carcinogenic as a conservative approach to its risk assessment. As such, FDA Toxicology applied the UCR value of 0.0126 (mg/kg bw/d)$^{-1}$ for arecoline[10] to the safety assessment of guvacine.

5.2 Non-cancer Systemic Toxicities:

5.2.1 Developmental and Reproductive Toxicity

Humans
Yuan et al. evaluated the effects of guvacine on human sperm motility *in vitro (48)*. Sperm

[16] Similarity scores of 0.91 or 0.95 based on Tanimoto or Dice models, respectively. *See* Figure 1.
[17] Similarity scores of 0.74 or 0.85 based on Tanimoto or Dice models, respectively. *See* Figure 1.

motility parameters were compared between groups treated with varying concentrations of guvacine (0, 50, 100, 200, and 300 μg/mL). The authors reported that guvacine induced a statistically significant reduction in sperm motility at the highest concentration tested (300 μg/mL) ($p < 0.05$). Correlation and regression analyses demonstrated that guvacine induced a reduction in the sperm motility parameters, linearity and amplitude of lateral head displacement, in a dose-dependent manner ($p < 0.05$).

6. Cramer Classification:

Based on the toxic hazard decision tree criteria set forth by Cramer, et al. (1976), the four alkaloids are classified as **Cramer Class III substances** (*56*), which has an associated threshold of toxicological concern (TTC) of 1.5 μg/kg bw/d. Cramer classification was determined using the Cramer Decision Tree (CDT) within ChemTunes-ToxGPS® database *(57)*. CDT uses chemical structure and predicts chemical reactivity of a substance to categorize substances into three classifications with associated Threshold of Toxicological Concern (TTC) values. Cramer Class III substances have complex chemical structures with reactive functional groups or exhibit a high potential for toxicity *(58)*.

c. *Conclusions of Review of Evidence of GRAS Status Based on Scientific Procedures (Technical Evidence of Safety):*

As discussed above, there are numerous scientific publications of multiple toxicology studies reporting cancer and non-cancer effects from exposure to the four alkaloid constituents found in palm leaves. This review notes that such determinations raise safety concerns regarding *A. catechu* leaves, and therefore, palm leaf dinnerware does not meet the criteria for general recognition of safety. As such, the data that is generally available does not support a conclusion of safety throughout the scientific community for the food contact use of palm leaf dinnerware.

V. Overall Conclusions

General recognition of safety requires common knowledge, throughout the expert scientific community knowledgeable about the safety of substances added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use. Under 21 CFR 170.30(a)-(c), general recognition of safety must be based on the views of qualified food safety experts. The basis of such views may be either through: (1) scientific procedures; or (2) in the case of a substance used in food prior to January 1, 1958, experience based on common use in food.

We were unable to find any evidence that palm leaf dinnerware was used in contact with food in the U.S. by a significant number of consumers prior to 1958. While there is anecdotal evidence that leaf sheaths were used in contact with food by some cultures outside of the U.S. prior to 1958, such information is not available in a manner that meets the requirements of § 170.30(c)(2). Furthermore, history of use prior to 1958 is not sufficient to support GRAS status if new evidence demonstrates that there is no consensus that the ingredient is safe (See 80 FR 34650, 34653 (June 17, 2015)).

Generally available scientific data demonstrates that palm leaf sheaths contain alkaloid constituents, and those alkaloids migrate from palm leaf dinnerware into food simulants under

testing protocols designed to mimic the marketed food contact use of these products. Information in the scientific literature indicates that dietary exposure to the alkaloid constituents of the leaf sheaths of the *A. catechu* palm can induce adverse toxicological effects (*e.g.*, genetic toxicity, carcinogenicity, immunotoxicity, cardiotoxicity, etc.) in multiple animal species. FDA has concluded that the generally available, relevant scientific data are insufficient to support the safety of the exposure to alkaloid constituents (arecoline, guvacoline, arecaidine, and guvacine) resulting from the use of palm leaf dinnerware in contact with food.

Moreover, the available scientific information on the alkaloid constituents of palm leaf dinnerware underscores that dietary exposure to these alkaloids may result in adverse toxicological effects and serious harm to the general public. FDA reiterates that the reports of adverse health events associated with these alkaloids, such as increased incidences of liver hemangiomas, lung adenocarcinomas, and squamous cell carcinomas of the stomach in male mice, as well as scientific evidence of their genotoxicity, immunotoxicity, and neurotoxicity, in animals and/or humans are cause for safety concerns. Therefore, there is no basis to conclude that there is general recognition across the expert scientific community knowledgeable about the safety of substances added to food that available data demonstrates that there is reasonable certainty that the use of dinnerware products manufactured from the leaf sheaths of the *A. catechu* palm are not harmful under the intended conditions of use.

As the available data in the scientific literature do not support consensus among qualified experts that palm leaf dinnerware is safe for use in contact with food based on either common use in food (prior to 1958) or technical evidence of safety, such use does not meet the statutory criteria for GRAS. Furthermore, based on a review of the available information, the use or intended use of the leaf sheaths of the *A. catechu* palm in contact with food is not eligible for a listed exception to regulation as a food additive [Section 201(s)(1)-(6) of the FD&C Act]. In addition, there is no promulgated food additive regulation, effective FCN, or issued TOR exemption establishing safe conditions of use of palm leaf dinnerware.

Accordingly, when palm leaf dinnerware is used in contact with food, it is an unapproved food additive and is deemed an unsafe food additive within the meaning of Section 409(a) of the FD&C Act. Food contact products, such as dinnerware, that bear or contain an unsafe food additive are adulterated under 21 U.S.C. 342(a)(2)(C)(i). Offering an adulterated product for sale in interstate commerce is a prohibited act under the FD&C Act.

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References

1. Mohanty, D. P., Mann, J. B., Udupa, A., Anil Chandra, A. R., & Chandrasekar, S. (2022). Improving formability of palm leaf materials for foodware manufacturing using sodium hydroxide treatment. *MRS Communications*, 12(6), 1235-1243.
2. Elevitch, C. (2006). Species profiles for Pacific Island agroforestry. *Permanent Agriculture Resources series. Western Region Sustainable Agriculture Research and Education, Holualoa, Hawaii.*
3. Mohanty, D. P., Udupa, A., Chandra AR, A., Viswanathan, K., Mann, J. B., Trumble, K. P., & Chandrasekar, S. (2021). Mechanical behavior and high formability of palm leaf materials. *Advanced Energy and Sustainability Research*, *2*(4), 2000080.
4. EcoSoul. How Palm Leaf Plates and Bowls are Made: A Step-by-Step Process. (https://www.ecosoulhome.com/blogs/sustainable-living/how-palm-leaf-plates-and-bowls-are-made-a-step-by-step-process; accessed December 17, 2024).
5. Mangrum, J. B., DeJager, L., and Begley, T. (2025). Investigation into the presence of alkaloids in Areca catechu based single use food-contact articles (FCA). *Food Additives & Contaminants: Part A",* 1-13
6. Wang, C. K., Lee, W. H., & Peng, C. H. (1997). Contents of phenolics and alkaloids in Areca catechu Linn. during maturation. *Journal of Agricultural and Food Chemistry*, 45(4), 1185-1188.
7. Ashby, J., Styles, J. A., and Boyland, E. 1979. Betel nuts, arecaidine, and oral cancer. *Lancet* 1979 Vol. 1 Issue 8107 Pages 112. DOI: 10.1016/s0140-6736(79)90109-0.
8. Searches for marketing or company history data related to the use or marketing of palm leaf dinnerware in the U.S. returned a result from one company stating they are the "creator and originator of The Palm Leaf Plate" and that the company was created in 2006. (*see* "https://www.verterra.com/pages/our", accessed March 13, 2025), however, other references to the same company state the company was founded in 2007 (*see* https://www.smithsonianmag.com/arts-culture/turning-fallen-leaves-into-dinner-plates-114764049/#:~:text=VerTerra%2C%20a%20company%20founded%20in,single%2Duse%20plates%20and%20bowls, accessed March 13, 2025). We were unable to find other relevant marketing or company history data for the use or marketing of palm leaf dinnerware in the U.S.
9. USDA Foreign Agricultural Service. (2012). *Customs Union Technical Regulation on Food Safety*. Global Agricultural Information Network. Retrieved from https://bcglobal.bryantchristie.com/marketinfo/reports/peanut%20aflatoxin%20limits/Eurasian%20Economic%20Union%20TR%20TS%20021%202011%20-%20USDA%20FAS%20Translation.pdf
10. A similarity cutoff of >0.8 is considered to indicate a high degree of structural similarity, but matches of the structure bitstring fingerprint can increase or decrease the confidence of the matches based on the method used. (Available on: "https://s3.amazonaws.com/dmap-ccte-ccd/current/manual/similar-compounds.html#similar-compounds" CCD Help Pages | Similar Compounds | US EPA; accessed January 17, 2025 by [redacted]).
11. Food Hygiene & Health Laboratory, "Acute Oral Toxicity of Food Contact Substance," Laboratory Report No. FHHL11 2004/25CM (Dated 11/20/2004) ("Kshitij", 20, Sadhana Society, 222/1, Hadapsar, Solapur Road, Pune -411028, India).
12. Shirname, L. P., et al. (1983) Correlation of mutagenicity and tumorigenicity of betel quid and its ingredients. *Nutr. Cancer*, 5(2):87-91.
13. Liu, Y., et al. (2016) The pharmacology, toxicology and potential applications of arecoline: a review. *Pharmaceutical Biology*, 54(11):2753-2760.
14. Clemesha, J. C. A Note on Arecoline. *Buffalo Med J.* 1899 Sep;39(2):103. PMID: 36884915; PMCID: PMC8723513.
15. Forbes, L. S. (1964). The relation between method of administration, route of absorption, inhibitory actions and acute toxicity of arecoline hydrobromide in dogs. *Annals of Tropical Medicine & Parasitology*, 58(2), 119–131. https://doi.org/10.1080/00034983.1964.11686222.

16. Patterson, T. A., Kosh, J. W. Elucidation of the rapid in vivo metabolism of arecoline, *General Pharmacology: The Vascular System*, Volume 24, Issue 3, 1993, Pages 641-647, ISSN 0306-3623, https://doi.org/10.1016/0306-3623(93)90224-L. (https://www.sciencedirect.com/science/article/pii/030636239390224L).

17. Nieschulz, O. and Schmersahl, P. (1968) Zur Pharmakologie tier Wirkstoffe des Betels. Arzneim.-Forsch. 18, 222-225.

18. Nery, R. The metabolic interconversion of arecoline and arecoline 1-oxide in the rat. *Biochem J*. 1971 May;122(4):503-8. doi: 10.1042/bj1220503. PMID: 5123883; PMCID: PMC1176807.

19. Oliveira, N. G., Ramos, D. L., Dinis-Oliveira, R. J. Genetic toxicology and toxicokinetics of arecoline and related areca nut compounds: an updated review. *Arch Toxicol*. 2021 Feb;95(2):375-393. doi: 10.1007/s00204-020-02926-9. Epub 2020 Oct 24. PMID: 33097969.

20. Giri, S., Idle, J. R., Chen, C., Zabriskie, T.M., Krausz, K.W., Gonzalez, F.J. A metabolomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. *Chem Res Toxicol*. 2006 Jun;19(6):818-27. doi: 10.1021/tx0600402. PMID: 16780361; PMCID: PMC1482804.

21. Pichini, S., Pellegrini, M., Pacifici, R., Marchei, E., Murillo, J., Puig, C., Vall, O., García-Algar, O. Quantification of arecoline (areca nut alkaloid) in neonatal biological matrices by high-performance liquid chromatography/electrospray quadrupole mass spectrometry. *Rapid Commun Mass Spectrom*. 2003;17(17):1958-64. doi: 10.1002/rcm.1140. PMID: 12913859.

22. Cox, S., Piatkov, I., Vickers, E. R., Ma, G. High-performance liquid chromatographic determination of arecoline in human saliva. *J Chromatogr A*. 2004 Apr 2;1032(1-2):93-5. doi: 10.1016/j.chroma.2003.11.076. PMID: 15065782.

23. García-Algar, O., Vall, O., Alameda, F., Puig, C., Pellegrini, M., Pacifici, R., Pichini, S. Prenatal exposure to arecoline (areca nut alkaloid) and birth outcomes. *Arch Dis Child Fetal Neonatal Ed*. 2005 May;90(3):F276-7. doi: 10.1136/adc.2004.061325. PMID: 15846024; PMCID: PMC1721892.

24. Pellegrini, M., Marchei, E., Rossi, S., Vagnarelli, F., Durgbanshi, A., García-Algar, O., Vall, O., Pichini, S. Liquid chromatography/electrospray ionization tandem mass spectrometry assay for determination of nicotine and metabolites, caffeine and arecoline in breast milk. *Rapid Commun Mass Spectrom*. 2007;21(16):2693-703. doi: 10.1002/rcm.3137. PMID: 17640086.

25. Semenov, EV. Vliianie arekolina, M- i N-kholinolitikov na vkliuchenie 22Na v neĭrony raznykh otdelov mozga krys [Effect of arecoline and m- and n-cholinolytics on 22Na incorporation into the neurons of various sections of the rat brain]. *Biull Eksp Biol Med*. 1982 May;93(5):66-8. Russian. PMID: 7093512.

26. Marchei, E., Durgbanshi, A., Rossi, S., Garcia-Algar, O., Zuccaro, P., Pichini, S. Determination of arecoline (areca nut alkaloid) and nicotine in hair by high-performance liquid chromatography/electrospray quadrupole mass spectrometry. *Rapid Commun Mass Spectrom*. 2005;19(22):3416-8. doi: 10.1002/rcm.2183. PMID: 16259042.

27. Patterson TA, Kosh JW. Elucidation of the rapid in vivo metabolism of arecoline. *Gen Pharmacol*. 1993 May;24(3):641-7. doi: 10.1016/0306-3623(93)90224-l. PMID: 8365645.

28. Pan H., Li, Y., Huang, L., Zhou, X., Lu, Y., Shi, F. Development and validation of a rapid LC-MS/MS method for simultaneous quantification of arecoline and its two active metabolites in rat plasma and its application to a pharmacokinetic study. *J Pharm Biomed Anal*. 2018 May 30;154:397-403. doi: 10.1016/j.jpba.2018.03.033. Epub 2018 Mar 16. PMID: 29573735

29. Panigrahi, G. B. and Rao, A. R. (1982) Chromosome-breaking ability of arecoline, a major betel-nut alkaloid, in mouse bone-marrow cells in vivo. *Mutation Research*, 103:197-204.

30. Dave, B. J., et al. (1992) In vitro genotoxic effects of areca nut extract and arecoline. *J. Cancer Res. Clin. Oncol*., 118(4):283-288.

31. Wang, C. K. and Peng, C. H. (1996) The mutagenicities of alkaloids and n-nitrosoguvacoline from betel quid. *Mutat. Res*. 60(3):165-171.

32. The TD50 is available at https://healthdata.gov/dataset/Carcinogenic-Potency-Database-CPDB-/sqjy-rr5s/about_data. (Accessed December 20, 2024).
33. Bhide, S. V., et al. (1984) Arecoline tumorigenicity in Swiss strain mice on normal and vitamin B deficient diet. *J Cancer Res Clin Oncol*. 107:169-171.
34. USA FDA (Food and Drug Administration). (2006). Redbook 2000: IV. C. 6 carcinogenicity studies with rodents [Redbook 2000: IV.C.6. Carcinogenicity Studies with Rodents | FDA].
35. Thresher, A., Gosling, J. P., & Williams, R. (2019). Generation of TD50 values for carcinogenicity study data. *Toxicology research*, *8*(5), 696-703.
36. Peto, R., Pike, M. C., Bernstein, L., Gold, L. S., & Ames, B. N. (1984). The TD50: a proposed general convention for the numerical description of the carcinogenic potency of chemicals in chronic-exposure animal experiments. *Environmental Health Perspectives*, *58*, 1-8.
37. Guidance for Industry: Preparation of Food Contact Notifications (Toxicology Recommendations). October 2021.
38. Zang, Y.J. and Kabadi, S.V. Food Additives. Patty's Toxicology (pages 1-22), Sept 2023.
39. IARC. Tobacco habits other than smoking; betel-quid and areca-nut chewing; and some related nitrosamines. IARC Working Group: Lyon, 23–30 October 1984. *IARC Monogr. Eval. Carcinog. Risk Chem. Hum*. 37:1–268; 1985.
40. Gao, W., He, Y., Zhang, Y., Sun, M., and Sun, Y. Comprehensive Insights into Arecoline Hydrobromide: Pharmacology, Toxicity, and Pharmacokinetics. *Med Sci Monit* 2024 Vol. 30 Pages e945582. DOI: 10.12659/msm.945582.
41. Wei, X. J., Zhang, J. Y., Niu, J. R., et al. Evaluation of arecoline hydrobromide toxicity after a 14-day repeated oral administration in Wistar rats. *PLoS One*. 2015;10(4):e0120165.
42. Xu, M., Su, S., Jiang, S., Li, W., Zhang, Z., Zhang, J., & Hu, X. (2023). Short-term arecoline exposure affected the systemic health state of mice, in which gut microbes played an important role. *Ecotoxicology and Environmental Safety*, *259*, 115055.
43. Lin, S. H., Chiou, S. J., Ho, W. T., Chuang, C. T., Chuang, L. Y., & Guh, J. Y. (2016). Arecoline-induced pro-fibrotic proteins in LLC-PK1 cells are dependent on c-Jun N-terminal kinase. *Toxicology*, *344*, 53-60.
44. Tang, S., Jiang, L., Zhou, Y., Zhou, T., Peng, Y., Zhou, S., ... & Feng, X. (2024). Comparative analysis of two arecoline-induced oral submucous fibrosis models. *Oral Diseases*, *30*(6), 3897-3911.
45. Liu, S. T., Young, G. C., Lee ,Y. C., Chang, Y. F. A preliminary report on the toxicity of arecoline on early pregnancy in mice. *Food Chem Toxicol*. 2011 Jan;49(1):144-8. doi: 10.1016/j.fct.2010.10.009. Epub 2010 Oct 19. PMID: 20940028.
46. Li, W. D., Zang, C. J., Yin, S., Shen, W., Sun, Q. Y., & Zhao, M. (2020). Metformin protects against mouse oocyte apoptosis defects induced by arecoline. *Cell Proliferation*, *53*(7), e12809.
47. Paul K, Moitra PK, Mukherjee I, et al. Teratogenicity of arecoline hydrobromide on developing chick embryos: A preliminary report. *Bull Environ Contam Toxicol*. 1999;62(3):356-62.
48. Yuan, J., Yang, D., Liang, Y., Gao, W., Ren, Z., Zeng, W., ... & Guo, D. (2012). Alkaloids from areca (betel) nuts and their effects on human sperm motility in vitro. *Journal of Food Science*, *77*(4), T70-T78.
49. Selvan, R. S., & Rao, A. R. (1993). Influence of Arecoline on Immune System: III. Suppression of B Cell-Mediated Immune Response in Mice After Short-Term Exposure. *Immunopharmacology and Immunotoxicology*, 15(2–3), 291–305. https://doi.org/10.3109/08923979309026000.
50. Hung, C. R., Cheng, J. T., & Shih, C. S. (2000). Gastric mucosal damage induced by arecoline seizure in rats. *Life Sciences*, 66(24), 2337-2349.

51. Lin, W. Y., Tsai, B. C. K., Day, C. H., Chiu, P. L., Chen, R. J., Chen, M. Y. C., ... & Huang, C. Y. (2021). Arecoline induces heart injure via Fas/Fas ligand apoptotic pathway in heart of Sprague–Dawley rat. *Environmental toxicology*, 36(8), 1567-1575.

52. Ho, T. J., Tsai, B. C. K., Kuo, C. H., Luk, H. N., Day, C. H., Hsieh, D. J. Y., ... & Huang, C. Y. (2022). Arecoline induces cardiotoxicity by upregulating and activating cardiac hypertrophy-related pathways in Sprague–Dawley rats. *Chemico-biological interactions*, 354, 109810.

53. Dasgupta, R., Saha, I., Pal, S., Bhattacharyya, A., Sa, G., Nag, T. C., ... & Maiti, B. R. (2006). Immunosuppression, hepatotoxicity and depression of antioxidant status by arecoline in albino mice. *Toxicology*, *227*(1-2), 94-104.

54. Dasgupta, R., Saha, I., Ray, P. P., Maity, A., Pradhan, D., Sarkar, H. P., & Maiti, B. R. (2020). Arecoline plays dual role on adrenal function and glucose-glycogen homeostasis under thermal stress in mice. *Archives of Physiology and Biochemistry*, *126*(3), 214-224.

55. Boyland, E. and Nery, R. (1969) Mercapturic acid formation during metabolism of arecoline and arecaidine in the rat. *Biochem. J.* 113:123-130.

56. Yan W, Zhang T, Li S, Wang Y, Zhu L, Cao Y, Lai X, Huang H. Oxidative stress and endoplasmic reticulum stress contributes to arecoline and its secondary metabolites-induced dyskinesia in zebrafish embryos. *Int J Mol Sci*. 2023 Mar 28;24(7):6327. doi: 10.3390/ijms24076327. PMID: 37047326; PMCID: PMC10094114.

57. Cramer, G. M., Ford, R. A., & Hall, R. L. (1976). Estimation of toxic hazard—a decision tree approach. *Food and cosmetics toxicology*, *16*(3), 255-276.

58. ChemTunes Database and ToxGPS Predictions are available at https://www.ceres.chemtunes.com/ and provided by ChemTunes•ToxGPS® v3.2020, MN-AM (Molecular Networks Altamira), Nuremberg, Germany, and Columbus, OH, USA. www.mn-am.com. Accessed December 27, 2024.

59. ToxTree User Manual. Accessed at https://sourceforge.net/projects/toxtree/. Accessed December 27, 2024.