Cardiovascular Endpoints for Food-Related Substances: Scoping Review

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Abstract

Cardiovascular diseases (CVD), including heart attacks, strokes, chest pain, and other disorders of the vascular system, affect nearly half of American adults. Some of the major risk factors for CVD include hypertension and cardiometabolic syndrome. The FDA Office of Food Additive Safety (OFAS) Redbook 2000 current safety testing guidelines for food-related substances do not include recommendations for specific studies to assess the potential cardiovascular effects of food additives. The current testing guidelines were not developed to detect either cardiac or cardiovascular acute or chronic adverse effects, except for late-stage disease-related pathological changes. It is important to have corroborating early signs that may be associated with adverse cardiovascular effects. The purpose of this review is to scope out scientific publications that discuss cardiac or cardiovascular endpoints that are relevant to food-related substances, including food contact substances, food ingredients, and their impurities. In our search, we included publications on animal and human studies, as well as *in vitro* mechanistic studies, utilizing a free, publicly available web-based review tool, the Health Assessment Workspace Collaborative. The results of this scoping review may help the Agency in recommending specific endpoints for *in vitro* new approach methods (NAMs) to detect early signs of food-related cardiovascular disease-related endpoints.

Introduction

The current OFAS Redbook 2000 safety testing guidelines [1] and toxicology recommendations for food contact substance notifications [2] for food-related substances do not require that petitioners and notifiers conduct human or mechanistic studies for potential cardiovascular effects. Some petitioners elect to conduct tolerability studies for high profile food ingredients (e.g., high intensity non-nutritive sweeteners, novel fat substitutes) although with a small number of volunteer human subjects and limited toxicological endpoints for cardiovascular effects (e.g., blood pressure, self-reported adverse events). The Redbook 2000 testing guidelines for experimental rodent models are not suitable for detecting either cardiac or cardiovascular acute or chronic adverse effects, except perhaps for late-stage disease-related pathological changes. For treatment-related pathological changes, it is important to correlate the findings with earlier functional and/or physical markers, such as changes in cardiac electrophysiology, contractility, mitochondrial function, and cardiomyocyte function and injury. Many drugs have adverse effects on the cardiovascular (CV) system and the mechanisms of these effects are generally understood. Recently, scientists have published a consensus paper on the key characteristics (KCs) of chemical and non-chemical agents known to cause CV toxicity.[3] The purpose of this review is to scope out scientific publications that discuss cardiac or cardiovascular endpoints for food-related substances, including food contact substances, food ingredients, and their impurities.

Materials and Methods

In our literature search of scientific literature published until 2022, we used terms, including food, nutrition, food additive, gut microbiota, cardiovascular disease, coronary heart disease, and cardiotoxicity.

We included publications on human and experimental animal studies, as well as *in vitro* mechanistic and *in silico* studies. We systemically organized our search results utilizing a free, publicly available web-based review tool, the Health Assessment Workspace Collaborative (a.k.a. "HAWC").[4] In addition, for endpoints examined in *in vitro* studies, we tagged them based on a published consensus on the KCs for agents known to cause CV toxicity. The KCs included impaired regulation of cardiac excitability or electrophysiology, contractility, cardiomyocyte injury or death, impaired mitochondrial function, oxidative stress, and inflammation.

Results and Discussion

The figure below is the literature tree visualization of 388 publications returned during our search, and their assignment to binned categories in HAWC.

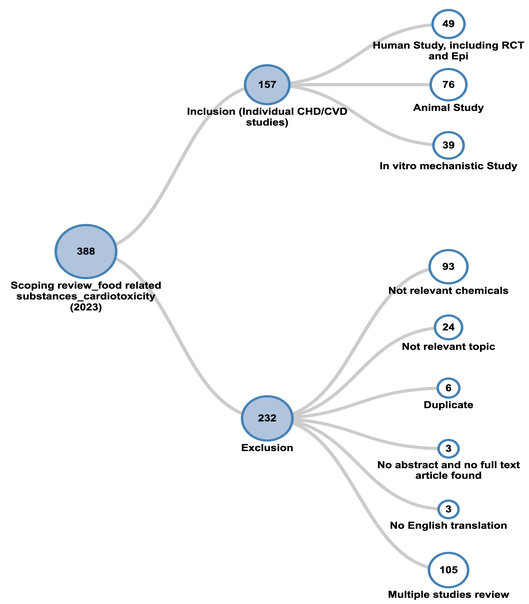

Figure 1. Visualization of the published literature and their assignment to binned categories in HAWC.

We excluded 232 publications that were either not relevant or review articles. We are extracting information from 157 individual published studies that examined cardiovascular endpoints for food-related substances. The following tables summarize some of the results from human studies and the *in vitro* or *in silico* mechanistic studies, in which food-related substances were examined as either positive or negative chemicals for potential cardiac or cardiovascular toxicity. (Results from *in vivo* animal studies are not shown in this poster presentation.)

Table1. *In vitro* mechanistic and *in silico* cardiotoxicity studies of food-related substances (*detailed citations not shown)

System	Food-Related substances	Endpoints	Author & Year*
Human cells: (e.g., Induced pluripotent stem cell-derived cardiomyocytes Recombinant embryonic kidney cells transfected with plasmid encoding cardiac-taste receptor, or recombinant human R-or T-type Ca^{2+} channels expressed human embryonic kidney 293 cells Tibia bone cells transfected with human ERG (hERG))	• Benzoic acid, methylparaben, ethoxyquin, t-butylhydroquinone • Arsenic trioxide • Liensinine and neferine • Polycyclic aromatic hydrocarbons • Diglycolic acid, phenylethylamine, higenamine, ephedrine, caffeine • Natural or synthetic bitter compounds (e.g. saccharin, nabenzoate, nathiocyante) • Quaternary ammonium compounds with various chemotypes (e.g., aromatic_benzene) • Cinnamaldehyde and its degradation products after heating • Bisphenol A	• Electrophysiology (e.g., hERG channel inhibition, Ca^{2+}, Na^{+}, and K^{+} channel activity, action potential) • Contractility (e.g., $[Ca^{2+}]$ flux, impedance amplitude, beating signals) • Mitochondrial function (e.g., mitochondrial membrane potential) • Cardiomyocyte function and injury/death (e.g., gene/protein/miRNA expression, metabolomic data, cytotoxicity, high content imaging for cell viability, lactate dehydrogenase (LDH) release) • Other (e.g., population variability)	Krishna Shagun 2022 Burnett Sarah D 2021 Burnett SD 2019 Nystoriak MA 2019 Palmer Jessica A 2019 Sirenko O 2017 Yu Y 2016 Calvert R 2015 Foster S.R. 2014 Deutschmann 2013 Michaela 2014 Xia M 2011
Rat or mice cells: (e.g., H9C2 cardiomyocytes H9C2 embryonic cardiomyoblast H9C2 cardiomyoblast Isolated neonatal cardiomyocytes Isolated heart cells and mitochondria GFP-LC3 transgenic cardiomyocytes)	• Arocolor 1254 • Benzimidazole fungicide • Heterocyclic amines • Bitter compounds (e.g., denatonium benzoate/Dena, diphenidol, quinine, arbutin) • Silver nanoparticles • Methylene blue and cyanide • Bisphenol A • Anthocyanins • Omega-3 fatty acid and arsenic trioxide • Docosahexaenoic acid and eicosapentaenoic acid • Extract of apple peel • Acetogenins-enriched avocado seeds • Isodunnianol • Nanoemulsion of curcumin or fresh and dry tomato extracts rich in lycopene • Sardine oil loaded vanillic acid-grated chitosan microparticles • Evodiamine	• Electrophysiology (e.g., membrane or extracellular field potential) • Contractility (e.g., $[Ca^{2+}]$ flux, beating signals) • Mitochondrial function (e.g., myocardial ATP, membrane potential, complex I activity, permeability transition, ADP/ATP exchange) • Oxidative stress (e.g., lipid oxidation products, oxidative stress enzyme induction, reactive oxygen species production) • Inflammation (e.g., TNF-α, NF-kB, interleukins, nitric oxide) • Cardiomyocyte function and injury/death (e.g., induction of genes for cellular differentiation and stress, cardiac & skeletal actin, LDH release, autophagy, apoptosis proteins and genes, ultrastructural cardiac abnormalities, DNA damage and adducts, coronary vascular tone)	Tang S et al. 2021 Mehtap K 2021 Wei X et al. 2021 Chen C 2019 Quagliariello V 2018 Vishnu KV . 2018 Ramirez-Lee MA 2018 Varghese MV 2017 Cheung JY et al. 2018 Ramadan et al. 2018 Yang W et al. 2017 Posnack et al. 2015 Vineetha VP 2014 Silva-Platas C 2012 Kawaguchi T 2012 Yan et al. 2011 Borlak J 2002 Schjøtt J et al. 1996 Davis CD et al. 1994
Hamster cells: (e.g., hERG expressed Chinese hamster ovary cells)		• Electrophysiology (e.g., hERG-related intensity) • Cardiomyocyte injury and death (e.g., annexin V (apoptosis)-related intensity, hERG protein expression)	Ferreiro SF 2014 and 2016
In silico		• Cardiomyocyte function (e.g., hazard quotient or index from consuming daily foods, binding mechanisms of serum protein-chemical complex)	Năstăsescu V 2020 Liu F et al. 2019

Table 2. Cardiac or cardiovascular toxicity studies of food-related substances in humans (*detailed citations not shown)

Population	Study Design	Food-Related Chemicals	Route	Effect or Endpoint	Author Year*
Hong Kong birth cohort	Cohort, Prospective	•Methylmercury	*in utero* & oral	• Blood pressure • Heart rate variability	Chan et al., 2021
General population		• Trans-fatty acid	oral	• Hospitalized for myocardial infarction • Hospitalized for stroke	Brandt et al., 2017
French NutriNet-Santé cohort		• Sugary drinks and artificially sweetened beverages	oral	• Cardiovascular disease	Chazelas et al., 2020
General population		• Sucrose, fructose, high fructose corn syrup, or glucose		• Body mass index • C-reactive protein • Blood pressure • Low density lipoprotein cholesterol, triglyceride, and total cholesterol • Plasma glucose • Waist circumference	Angelopoulos et al., 2016
PREDIMED cohort		• Sugar- or artificially-sweetened beverages		• Cardiometabolic syndrome	
Trials of hypertension prevention	Randomized controlled trial	• Sodium			
Multiethnic adiposity phenotype study cohort	Cross-sectional	• Trimethylamine N-oxide (from dairy, red meat, carnitine, choline, etc.)	unknown route		Fu et al., 2020
General population					Steffensen et al. 2020; Wang et al. 2019; Kataria et al. 2017; Yang et al. 2009

As wider international acceptance and adoption of the 3Rs continues, the safety testing of food-related substances using rodent and non-rodent animal species will decline. It will become important to develop appropriate *in vitro* NAMs for the assessment of potential cardiac and cardiovascular toxicity of food-related substances that can be related to human responses.[5] We have identified a body of literature that could be used to confirm the relevance of specific cardiac or cardiovascular endpoints.

Conclusion

The results of this scoping review may help the Agency in recommending specific human-relevant endpoints for *in vitro* cardiotoxicity NAMs for safety testing for early signs of cardiovascular disease-related endpoints for food-related substances.

References

[1] FDA, *Redbook 2000: Guidance for Industry and Other Stakeholders*, Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-and-other-stakeholders-redbook-2000 (Accessed: March 25, 2023).

[2] FDA, *Guidance for Industry: Preparation of Food Contact Substance Notifications (Toxicology Recommendations)*. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-preparation-food-contact-substance-notifications-toxicology-recommendations, October 2021 (Accessed: March 25, 2023).

[3]Lind L, Araujo JA, Barchowsky A, Belcher S, Berridge BR, Chiamvimonvat N, Chiu WA, Cogliano VJ, Elmore S, Farraj AK, Gomes AV, McHale CM, Meyer-Tamaki KB, Posnack NG, Vargas HM, Yang X, Zeise L, Zhou C, Smith MT. *Key Characteristics of Cardiovascular Toxicants*. Environ Health Perspect. 2021 Sep;129(9):95001. https://pubmed.ncbi.nlm.nih.gov/34558968/.

[4] Shapiro, A.J. *et al. Software Tools to Facilitate Systematic Review Used for Cancer Hazard Identification*, Environmental Health Perspectives, 2018, 126(10), p. 104501. Available at: https://doi.org/10.1289/EHP4224.

[5] FDA, *Advancing New Alternative Methodologies at FDA*. 2021 Available at: https://www.fda.gov/media/144891/download (Accessed: 25 March 2023)