Memorandum

Date March 15, 2021

From [redacted] Ph.D. (HFS-255)

Through

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Subject Regulatory status and review of available information pertaining to androsta-3,5-diene-7,17-dione: lack of general recognition of safety for its use in foods. [redacted]

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The Division of Food Ingredients' (DFI) toxicology review team was asked to review whether any food use of androsta-3,5-diene-7,17-dione meets the statutory criteria for general recognition of safety. This memorandum considers the pertinent scientific information and concludes that the use of androsta-3,5-diene-7,17-dione in food does not meet the criteria for general recognition of safety primarily because there is inadequate scientific data and information on the safety of its consumption. Secondarily, the information that is available indicates that the use of androsta-3,5-diene-7,17-dione in food may be harmful.

GRAS Provision in Defining a Food Additive

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 in its becoming a component of any food, unless the substance is the subject of a prior sanction or is generally recognized as safe (GRAS) among qualified experts under the conditions of its intended use. Furthermore, under section 201(s) of the FD&C Act, a substance is exempt from the definition of a food additive and thus, from premarket approval requirements, if its safety is generally recognized by qualified experts.

As there is no food additive regulation or prior sanction establishing safe conditions of use for

androsta-3,5-diene-7,17-dione as an ingredient in foods, this memorandum will consider the applicability of the GRAS criteria for the use of androsta-3,5-diene-7,17-dione as an ingredient in foods.

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.

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. General recognition of safety through scientific procedures must be based upon the application of generally available and accepted scientific data, information, or methods, which ordinarily are published, as well as the application of scientific principles, and may be corroborated by the application of unpublished scientific data, information, or methods. 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 in a peer-reviewed scientific journal of data on a test substance has been used to establish common knowledge throughout the expert scientific community in addition to general availability. These criteria are discussed more fully in the GRAS final rule, which took effect on October 17, 2016 (81 Federal Register (FR) 54960; August 17, 2016).
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. FDA's regulations in 21 CFR Part 170 describe the eligibility criteria for classification of a substance added to food as GRAS. 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.

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, 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.

Overview of Androsta-3,5-diene-7,17-dione

Androsta-3,5-diene-7,17-dione, also referred to as arimistane, is a synthetic anabolic-androgenic steroid which acts as an aromatase inhibitor (Numazawa et al., 1992). Androsta-3,5-diene-7,17-dione is reported to have the CAS #: 1420-49-1.

Regulatory Status of Androsta-3,5-diene-7,17-dione

Evidence Based on Common Use in Food Prior to 1958:

FDA is unaware of any evidence that androsta-3,5-diene-7,17-dione was intentionally added to food prior to 1958. In order to determine if androsta-3,5-diene-7,17-dione was used in food prior to 1958, a search was conducted in three databases– PubMed[1], Web of Science Core Collection[2], and *FDA's Scientific Terminology and Regulatory Information (STARI)*[3] database. The PubMed database has literature dating back to about 1951, and in some cases, even earlier literature is available. The Web of Science Core Collection consists of six online databases with indexing coverage from the year 1900 to the present.

All databases were searched using the search term "androsta-3,5-diene-7,17-dione", "androsta-3,5-diene-7,17-dione AND food ingredient", "arimistane", and "arimistane AND food ingredient". The searches yielded no records pertaining to the intentional addition of androsta-3,5-diene-7,17-dione to food prior to 1958. Therefore, androsta-3,5-diene-7,17-dione does not meet the "common use in food" criterion and its eligibility for classification as GRAS needs to be established on the basis of "scientific procedures." In other words, adequate technical evidence of safety must exist, and this technical evidence must be generally known and accepted by qualified food safety experts to demonstrate the safety of the intended use.

Evidence Based on Scientific Procedures (Technical Evidence of Safety):

A search of the published scientific literature was conducted between February 10, 2021 and February 24, 2021. The results from PubMed and Web of Science Core Collection database using the search terms "androsta-3,5-diene-7,17-dione", "arimistane", "androsta-3,5-diene-7,17-dione AND toxicity", and "androsta-3,5-diene-7,17-dione AND safety" are summarized in Table 1.

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[1] PubMed, https://pubmed.ncbi.nlm.nih.gov/, accessed between February 10, 2021 and February 24, 2021.
[2] Web of Science, http://www.webofknowledge.com/, accessed between February 10, 2021 and February 24, 2021.
[3] The data contained within STARI dates back 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 CFSAN. 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 between February 10, 2021 and February 24, 2021.

Table 1: Summary of literature search terms and results.

Search Terms	Database	Search Results (Number)
androsta-3,5-diene-7,17-dione	PubMed	3
	Web of Science (Core Collection)	3
arimistane	PubMed	4
	Web of Science (Core Collection)	5
androsta-3,5-diene-7,17-dione AND toxicity	PubMed	0
	Web of Science (Core Collection)	0
androsta-3,5-diene-7,17-dione AND safety	PubMed	0
	Web of Science (Core Collection)	0

The available literature consisted primarily of publications describing analytical methods to detect androsta-3,5-diene-7,17-dione and/or its metabolites in products marketed as dietary supplements or human biological samples (Brito et al., 2019; Lorenz et al., 2019; Martinez-Brito et al., 2020; Schubert et al., 1971). Due to its purported aromatase inhibiting pharmacologic activity, androsta-3,5-diene-7,17-dione was added to the World Anti-Doping Agency (WADA) prohibited substances list in 2017 (World Anti-Doping Agency, 2017). FDA notes that such determinations raise safety concerns and are considered inconsistent with general recognition of safety of use as a food ingredient.

Lack of Sufficient Data to Establish Safety in Food Use

The scientific literature available in the public domain presented no evidence that androsta-3,5-diene-7,17-dione has been used in food. Findings in the publicly available literature raise concerns regarding the safety of androsta-3,5-diene-7,17-dione; relevant records retrieved from our literature search and additional information relevant to our review of androsta-3,5-diene-7,17-dione are discussed.

Cramer Toxicity Classification:

Based on the toxic hazard decision tree criteria set forth by Cramer et al. (1978), androsta-3,5-diene-7,17-dione would be classified as a Class III substance (Cramer et al., 1976). This classification indicates high toxicological potential based on the chemical structure and available metabolism data. Additional cumulative human exposure data are necessary for assignment of an overall toxicity risk concern level (i.e., low (I), intermediate (II) or high (III)).

Background and Purported Biological Activity/Mode of Action:

Androsta-3,5-diene-7,17-dione is reported to be an irreversible inhibitor of aromatase (Lorenz et al., 2019; Numazawa et al., 1992). Aromatase is a cytochrome p-450 enzyme complex that catalyzes the conversion of androgens; androstenedione and testosterone, to estrogens; estrone

and estradiol. Biosynthesis of estrogens by aromatase is an important factor in numerous biological processes including, sexual development, menstrual cycling, fertility, spermatogenesis, and bone health. Potential adverse effects related to the use or presence of aromatase inhibitors in foods have not been adequately evaluated.

Perturbation of estrogenic signaling by pharmacological inhibition of aromatase could cause potential adverse health effects. Aromatase inhibitors are a class of drugs used to treat breast cancer in postmenopausal women and gynecomastia in men. Additionally, aromatase inhibitors may be administered to individuals undergoing exogenous androgen therapy to reduce subsequent conversion to estrogen. Adverse events associated with the use of FDA-approved aromatase inhibitor drugs include decreased rate of bone maturation and growth, decreased sperm production, infertility, aggressive behavior, adrenal insufficiency, kidney failure, and liver dysfunction. Reductions in bone mineral density and increased fracture risk were observed in breast cancer patients undergoing aromatase inhibitor therapy (Chen et al., 2005; Coombes et al., 2004; Goss et al., 2003; Lonning et al., 2004; Mincey et al., 2006; Pandya & Morris, 2006). Joint pain (arthralgia) and altered blood lipid profiles serum cholesterol have also been reported with aromatase inhibitor treatment (Bundred, 2005; Burstein, 2007).

Inhibition of aromatase activity by androsta-3,5-diene-7,17-dione and disruption of endocrine signaling may have adverse effects on male and female reproductive health. In females, regulation of the menstrual and ovarian cycles are dependent upon coordinated signaling of the hypothalamus-pituitary-gonadal (HPG) axis (Findlay et al., 2010). Biosynthesis of estradiol by ovarian granulosa cells are necessary for normal development of ovulatory follicles, cervical mucus production, and preparation of the endometrial lining for implantation (Findlay et al., 2010). Perturbations to the production or action of estrogen can disrupt these processes. In premenopausal women, increased gonadotropin secretion secondary to the reduced negative feedback of estrogen to the pituitary can occur, resulting in ovarian stimulation. Estrogens also play a critical physiological roles in spermatogenesis (gonocyte and spermatogonia proliferation, meiosis, Sertoli cell function), spermiation, sperm transport, and epididymal sperm cell maturation (Carreau et al., 2012; Hess et al., 1997; Lucas et al., 2011). Altered testosterone:estradiol [T/E] ratios may influence sperm production and quality parameters with corresponding effects on fertility.

Estrogen is a critical signaling hormone in perinatal development. Exposure to endocrine-disrupting substances during critical windows of susceptibility could produce adverse effects on placental function and sexual development. During pregnancy, the placental syncytiotrophoblast becomes a major source of estrogens that are transferred between the placental and fetal compartments and subjected to transformation (Chatuphonprasert et al., 2018; Pasqualini & Chetrite, 2016). Impaired developmental aromatase activity could lead to increased androgen exposures resulting in maternal virilization and masculinization of female offspring (Ito et al., 1993; Morishima et al., 1995). Female mice lacking a functional aromatase enzyme displayed underdeveloped external genitalia, uteri, and mammary glands, and arrested ovulation; whereas male mice presented with enlarged accessory sex glands and reduced sperm quality (Fisher et al., 1998; Robertson et al., 2001). Moreover, normative endocrine signaling is required for the onset of puberty and the development of secondary sexual characteristics in adolescents.

Therefore, maternal exposure to an aromatase inhibitor, such as androsta-3,5-diene-7,17-dione may cause aberrations in developing fetuses, and also present a safety concern related to accelerated onset of puberty in male children, and delayed menarche and virilization in female children (Ito et al., 1993; Morishima et al., 1995). Estrogen is also a key regulator of longitudinal growth due to signaling effects on the closure of the epiphyseal growth plates (Wit et al., 2011). Notably, aromatase inhibitor drugs have been prescribed in children and adolescents for treatment of a variety of disorders related to perturbations in endocrine signaling including congenital virilizing adrenal hyperplasia, aromatase excess syndrome, familial male-limited precocious puberty, pubertal gynecomastia, growth hormone deficiency, idiopathic short stature, and constitutional delay of growth and puberty (Matti Hero et al., 2020).

Aromatase is expressed in numerous organs/tissues including, muscle, liver, blood, heart, hair follicles, adipose tissue, bone and brain, suggesting that estrogen signaling may contribute to an array physiological processes other than reproduction. Notably, estrogen and androgen DNA response elements are observed throughout the genome suggesting widespread effects of steroid hormone signaling on gene expression (Beato & Klug, 2000; Björnström & Sjöberg, 2005). However, direct effects of androsta-3,5-diene-7,17-dione on tissue specific gene expression, and corresponding physiological effects have not been adequately characterized.

Absorption, Distribution, Metabolism, and Excretion (ADME)

The available literature did not provide sufficient details to evaluate the ADME profile of androsta-3,5-diene-7,17-dione. Several available publications describe analytical methods to detect androsta-3,5-diene-7,17-dione in human biological samples and possible metabolites due to its prohibited use status by WADA. Brito et al. (2019), reported urinary metabolite profiles in three healthy volunteers following a single administration of androsta-3,5-diene-7,17-dione (25 mg). The principal urinary metabolite androst-3,5-diene-7β-ol-17-one was identified, with up to 15 distinct metabolites detected in post-administration samples (Brito et al., 2019).

Toxicity Studies:

No traditional toxicology studies (acute, subacute, subchronic, or chronic) conducted in laboratory animals that are relevant to the safe use of androsta-3,5-diene-7,17-dione as an ingredient in food were identified in our review.

However, reproductive and developmental toxicity studies in animal models evaluating the effects of aromatase inhibitors were identified in the available literature. Gestational exposure to the aromatase inhibitor, letrozole, at levels lower than the daily recommended human dose, resulted in dose-related increases in post implantation loss and vertebral anomalies in Sprague-Dawley rats (Tiboni et al., 2008). Delayed attainment of pubertal endpoints, such as vaginal opening and preputial separation, were reported in in juvenile Sprague-Dawley rats orally treated with letrozole (Pouliot et al., 2013). Adverse effects on spermatozoa counts, sperm motility, sperm morphology, testicular histopathology, decreased fertility and mating indices, and delayed bone maturation were also reported following oral letrozole treatment (Pouliot et al., 2013). Sustained reduction in bone strength and alteration in skeletal geometry, lowering of IGF1 levels, inhibition of growth resulting in significantly lower weight and length

of treated animals and development of focal prostatic hyperplasia was reported in male Wistar rats treated with Letrozole (Bajpai et al., 2010). Impaired skeletal modeling and decreased bone mineral density were observed in juvenile male Wistar rats treated with the aromatase inhibitor, Vorozole (Vanderschueren et al., 1997). Similar effects were reported in rats following treatment with the steroidal aromatase inhibitor, exemestane (Cappon et al., 2011; Van Gool et al., 2010a, 2010b). The consistency of toxic effects noted following oral exposure to different classes of aromatase inhibitors raise safety concerns related to the possibility of similar adverse effects related to consumption of androsta-3,5-diene-7,17-dione in food.

Human Studies and Case Reports:

No clinical studies or case reports relevant to a safe use of androsta-3,5-diene-7,17-dione as an ingredient in food were identified in our literature review.

Clinical studies and reviews evaluating the efficacy and side effects of prescribed aromatase inhibitor therapies in various patient populations were identified in the literature. The most common patient population included post-menopausal women diagnosed with hormone-receptor-positive breast cancer. Adverse effects of aromatase inhibitor therapy on bone health and athralgia are well documented (Bundred, 2005; Henry et al., 2008). Supraphysiological circulating testosterone levels induced by aromatase inhibitor therapy has also been associated with hematological effects and diagnosis of erythrocytosis (Diaz-Thomas & Shulman, 2010; Iyengar & Sheppard, 2013; Yeruva et al., 2015).

In young men diagnosed with idiopathic short stature, letrozole treatment (2 years) was associated with significantly increased risk of vertebral body deformities (M. Hero et al., 2010). Such effects may reflect perturbation of vertebral body growth and suggest adverse effects related to aromatase inhibitor during critical windows of bone growth/development.

Additionally, several case reports describing individuals diagnosed with congenital aromatase deficiency (AD) were identified in the literature (Jones et al., 2007). Male AD subjects are often tall, and present with elevated testosterone levels, bone abnormalities (low bone mass and delayed bone age), metabolic syndrome, and impaired fertility (Baykan et al., 2013; Jones et al., 2006; Miedlich et al., 2016; Rochira & Carani, 2009). Female AD subjects often present with severe terata at birth as well as adverse effects during later postnatal development. Some examples include ambiguous genitalia related to antenatal androgen excess associated with estrogen deficiency, and pubertal hypergonadotropic hypogonadism with corresponding delays or absence of menarche and thelarche (Alsaleem et al., 2019; Marino et al., 2015; Morishima et al., 1995; Shozu et al., 1991). While aromatase activity and estrogen conversion are completely abated in these individuals, observed physiological effects may in part correspond with estrogen deficiencies during critical perinatal windows of development. FDA notes that such reports of serious adverse events associated with aromatase inhibition are cause for safety concerns regarding androsta-3,5-diene-7,17-dione, and are inconsistent with general recognition of safety of use as a food ingredient.

Overall Conclusions

Overall, the available data are insufficient to support the safety of androsta-3,5-diene-7,17-dione for use as a food ingredient that will be consumed by the general public. Moreover, the

available reports underscore its potential for serious reproductive and developmental toxicity. It should be emphasized that because a substance added to food may be consumed by the entire population over a lifetime, assurance of safety requires an evaluation of potential effects of long-term use within various segments of the population, with consideration for vulnerable subpopulations such as pregnant women/conceptus/fetus, infants, and young children, if appropriate.

Due to the lack of adequate data and information in the scientific literature to support the safe use of androsta-3,5-diene-7,17-dione in food, DFI is unable to conclude that the addition of androsta-3,5-diene-7,17-dione to food meets the statutory criteria for classification as GRAS. Indeed, the available data raise safety concerns as there are potential adverse effects of androsta-3,5-diene-7,17-dione on the reproductive system, bone health and possibly other organ-systems. Additionally, it seems plausible that perturbation of endocrine signaling related to androsta-3,5-diene-7,17-dione use would raise safety concerns related to effects on the developing reproductive system, placenta function, and pubertal onset. As such, there is an absence of consensus among qualified experts regarding the safety of androsta-3,5-diene-7,17-dione use as a food ingredient. Therefore, based on the current status of data and information, androsta-3,5-diene-7,17-dione does not meet the experience based on common use in food (prior to 1958) criterion or the technical evidence of safety and the general recognition of safety necessary for it to be GRAS for use in food. Accordingly, the use of androsta-3,5-diene-7,17-dione in food constitutes use of an unsafe food additive within the meaning of Section 409 of the FD&C Act, rendering the food product to which androsta-3,5-diene-7,17-dione is added adulterated within the meaning of Section 402(a)(2)(C) of the FD&C Act.

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