Memorandum

Date March 1, 2022

From [redacted], Ph.D. (HFS-255)
Toxicologist
Division of Food Ingredients (DFI)
Office of Food Additive Safety (OFAS)
Center for Food Safety and Applied Nutrition (CFSAN)

Through

[redacted], Ph.D. (HFS-255) _____
Toxicology Branch Chief, DFI, OFAS, CFSAN

[redacted], Ph.D. (HFS-255) _____
Division Director, DFI, OFAS, CFSAN

Subject Regulatory status and review of available information pertaining to 4-androstenedione: lack of general recognition of safety for its use in foods.

To [redacted], Ph.D. (HFS-200)
Deputy Director, OFAS, CFSAN, HFS-200 __

Keywords: Androstenedione; Androst-4-ene-3,17-dione; 4-Androstene-3,17-dione; Delta-4-androstenedione; 17-ketotestosterone; Androtex; Fecundin; CAS# 63-05-8

The Division of Food Ingredients' (DFI) toxicology review team was asked to review whether any food use of 4-androstenedione meets the statutory criteria for general recognition of safety. This memorandum considers the pertinent scientific information and concludes that the use of 4-androstenedione in food does not meet the criteria for general recognition of safety primarily because there is inadequate scientific data and information demonstrating the safety of its consumption. Secondarily, 4-androstenedione is a Schedule III controlled substance, and the information that is available indicates that the use of 4-androstenedione 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 it 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 4-androstenedione as an ingredient in foods, this memorandum will consider the applicability of the GRAS criteria for the use of 4-androstenedione 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 4-androstenedione

4-androstenedione is a weak androgen and androgenic prohormone that is a direct precursor of testosterone, as well as estrone and estradiol. 4-androstenedione is an anabolic steroid, classified by the Drug Enforcement Administration (DEA) as a Schedule III controlled substance.

Regulatory Status of 4-androstenedione

Evidence Based on Common Use in Food Prior to 1958:

FDA is unaware of any evidence that 4-androstenedione was intentionally added to food prior to 1958. In order to determine if 4-androstenedione 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 terms "androstenedione", "androstenedione AND food", and "androstenedione AND food ingredient". The searches yielded no records pertaining to the intentional addition of 4-androstenedione to food prior to 1958. Therefore, 4-androstenedione 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 December 1, 2021 and March 1, 2022. The results from PubMed and Web of Science Core Collection database using the search terms "androstenedione", "androstenedione AND toxicity", and "androstenedione AND safety" are summarized in Table 1. Results from Web of Science Core Collection database

[1] PubMed, https://pubmed.ncbi.nlm.nih.gov/, accessed between December 1, 2021 and March 1, 2022.
[2] Web of Science, http://www.webofknowledge.com/, accessed between December 1, 2021 and March 1, 2022.
[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 December 1, 2021 and March 1, 2022.

using the search term "androstenedione" yielded 336 and 249 records categorized into the subjects of pharmacology/pharmacy and toxicology, respectively.

Table 1: Summary of literature search terms and results.

Search Terms	Database	Search Results (Number)
Androstenedione	PubMed	12,204
	Web of Science (Core Collection)	7,667
Androstenedione AND Toxicity	PubMed	345
	Web of Science (Core Collection)	113
Androstenedione AND Safety	PubMed	119
	Web of Science (Core Collection)	129

The search criteria identified numerous studies investigating the physiological/toxicological effects of unrelated substances or treatments which included assessment of a panel of endogenous endocrine hormone levels, including 4-androstenedione, and were not considered relevant to the safety of exogenous 4-androstenedione as an ingredient in food. Several publications on the toxicological effects of exogenous 4-androstenedione in laboratory animals were identified, including a comprehensive National Toxicology Program (NTP) technical report on the toxicology and carcinogenesis studies of 4-androstenedione in rats and mice (Blystone et al., 2011; NTP, 2010). Several clinical studies also evaluated the potential ergogenic/anabolic effects of 4-androstenedione and corresponding effects on circulating androgens and estrogens. Purported efficacy or benefits of 4-androstenedione are outside the purview of DFI and such effects are not considered supportive of a GRAS conclusion for use in food. Additionally, prescribed conditions of use and/or use of 4-androstenedione as a dietary supplement are not sufficient to support the safe use of androstenedione in food for the general population.

4-androstenedione was previously marketed as an ingredient in dietary supplements with purported anabolic and ergogenic effects. Following the signing into law of the Anabolic Steroid Act (2004), the definition of an anabolic steroid was amended, and under 21 CFR 1300.01, 4-androstenedione was reclassified as an anabolic steroid. Anabolic steroids are classified as Schedule III controlled substances by the DEA and are subject to the regulatory control provision of the Controlled Substances Act. Schedule III substances are defined as drugs with moderate to low potential for physical and psychological dependence. FDA notes classification of 4-androstenedione as a Schedule III controlled substance is cause for serious safety concerns and is contradictory with general recognition of safety of use as a food ingredient

Due to its purported physiological activity and classification as an anabolic steroid, 4-androstenedione is on the U.S. Anti-Doping Agency (USADA) and World Anti-Doping Agency (WADA) prohibited substances lists. 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

Findings in the publicly available literature raise concerns regarding the safety of 4-androstenedione; relevant records retrieved from our literature search and additional information relevant to our review of 4-androstenedione are discussed.

Cramer Toxicity Classification:

Based on the toxic hazard decision tree criteria set forth by Cramer et al. (1978), 4-androstenedione 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.

Background and Purported Biological Activity/Mode of Action:

4-androstenedione is an endogenous steroid hormone produced in the adrenal glands and gonads. 4-androstenedione is a prohormone and intermediate in the biosynthesis of estrogens (i.e., estradiol and estrone) and testosterone from dehydroepiandrosterone (DHEA). Prior to the 2004 Anabolic Steroid Act, 4-androstenedione was marketed as a performance enhancing dietary supplement. Notably, ergogenic and anabolic effects related to 4-androstenedione intake have not been substantiated (Powers, 2002). However, short-term administration of 4-androstenedione has been associated with increased serum testosterone and estrogen levels in men and women (Kicman et al., 2003; King et al., 1999; Leder et al., 2002; Leder et al., 2000). Use of exogenous 4-androstenedione and purported increases in androgenic and/or estrogenic signaling have been shown to induce adverse effects related to cardiac function, sexual development, fertility, and bone growth/development. Potential adverse effects related to the use or presence of androstenedione in foods have not been adequately evaluated.

Aberrant effects in the developmental androgenic signaling milieu can lead to detrimental effects on sexual differentiation and maturation. Excessive androgen exposure at any point during gestation can result in virilization of developing females (Cabrera & Rogol, 2013). The degree of virilization and observable phenotype will likely depend on the stage, duration, and level of androgen exposure. Virilization of female offspring is quantified by Prader staging, and may present as genital ambiguity, clitoral enlargement, and if exposure occurs prior to the period of sexual determination, persistence of urogenital sinus, labial fusion, and phallic enlargement (Ogilvy-Stuart & Brain, 2004). Case reports of unintentional secondary topical testosterone exposure in children under the age of six have been associated with androgenic clinical signs such as clitoromegaly, acne, and pubic hair growth in girls, and penile enlargement and pubic hair growth in boys (Cabrera & Rogol, 2013). Additionally, supraphysiological androgen levels in children may be associated with contra-sexual and isosexual precocity, in young girls and boys, respectively.

Available studies report that exogenous exposure to 4-androstenedione is associated with increased levels in circulating estrogens (Broeder et al., 2000). An increased magnitude of effect on estrogen was noted in males, possibly related to basally decreased estrogen levels, relative to females (King et al., 1999; Leder et al., 2000). Exposure to supraphysiological levels of estrogens is associated with adverse effects on sexual development and maturation. In

adolescent females, increased exposure to estrogens may be associated with premature menarche, thelarche, and other indications of isosexual precocity. In males, increased estrogen exposure is associated with clinical observations of feminization, such as gynecomastia and testicular atrophy. 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). Premature estrogenic signaling could induce premature closure of long bones in adolescents leading to compromised adult stature. Notably, periods of sexual differentiation and development are recognized as critical windows of susceptibility. Perturbation of appropriate hormonal signaling milieus in the developing fetus, children, and adolescents related to intake of 4-androstenedione would present serious safety concerns and could lead to irreversible adverse effects.

Proper androgenic and estrogenic signaling are also required for many physiological functions following periods of sexual differentiation and puberty. Perturbation of sex steroid signaling can cause deleterious effects on fertility related to abnormal menstrual cycling and spermatogenesis, as well as virilization of females and feminization of males. Furthermore, increased and/or sustained estrogenic signaling in females may confer increased lifetime risk of certain types of cancer, such as uterine and breast cancer. The International Agency for Research on Cancer (IARC) classifies androgenic (anabolic) steroids as Group 2A carcinogens (Probably carcinogenic to humans), and estrogens as Group 1 carcinogens (Carcinogenic to humans) (IARC, 1987). Moreover, there was clear evidence of carcinogenic activity of 4-androstenedione in male and female B6C3F1 mice in 2-year gavage studies conducted by the NTP (NTP, 2010).

Cardiotoxic outcomes have been associated with use of anabolic steroid substances (Dhar et al., 2005; Hurley et al., 1984). While the precise mechanism in which use of androgenic anabolic steroids leads to cardiovascular disease has not been elucidated, numerous studies report significant alterations in lipid metabolism, such as decreased levels of high-density lipoprotein (HDL) and increased low-density lipoprotein (LDL) levels (Feller et al., 2002; Melchert & Welder, 1995). Such lipid alterations may contribute to atherogenesis and subsequent cardiac disease (Rosenson et al., 2016). Reductions in serum HDL levels have also been observed in individuals following 4-androstenedione supplementation (Broeder et al., 2000; King et al., 1999). Abuse of anabolic androgenic steroids has also been associated with adverse neuropsychiatric effects, often manic-like states defined by irritability and aggression (Clark & Henderson, 2003; van Amsterdam et al., 2010). Due to its reported androgenic effects, 4-androstenedione may be expected to produce adverse effects similar to what has been observed following use of androgenic anabolic steroids.

The FDA summarized health effects and safety concerns related to use of 4-androstenedione in a White Paper dated March 11, 2004 (FDA, 2004). The FDA noted increased vulnerability and concern regarding 4- androstenedione use in children and adolescents and the potential for differential effects in men relative to women due sex-specific differences in hormonal signaling milieus and potential for increased androgenic or estrogenic signaling. Importantly, sex steroid signaling pathways and their role in normal human development and physiology are well understood and documented in the generally available literature, including standard reference materials and textbooks. The potential for endocrine disruption related to use of 4-androstenedione in food is cause for serious safety concerns.

Absorption, Distribution, Metabolism, and Excretion (ADME)

4-androstenedione is an endogenously synthesized prohormone with limited intrinsic androgenic activity. 4-androstenedione is produced primarily in the adrenal glands and gonads and can be converted to testosterone or estrogens (estrone and estradiol) by the enzymes 17β-hydroxysteroid dehydrogenase (17β-HSD) and aromatase (CYP19), respectively.

Results from NTP-sponsored experimental animal studies investigating the ADME profile of exogenously administered 4-androstenedione were described in the NTP Androstenedione Technical Report (NTP, 2010). The report denotes that ^{14}C-labeled 4-androstenedione ADME studies were conducted in F344/N rats, B6C3F1 mice, and beagle dogs. Summarized results from these studies report that 4-androstenedione was well absorbed following oral administration in rodents. However, overall bioavailability of 4-androstenedione was low in rats due to extensive first-pass metabolism. Further studies in rat, mouse, and dog primary hepatocytes suggested sex-specific and interspecies variability in 4-androstenedione hepatic metabolism.

The ADME profile of exogenous 4-androstenedione is not well characterized. Variable effects on serum testosterone and estrogen levels in male and female subjects were reported following intake of 4-androstenedione (Broeder et al., 2000; Brown et al., 2004; Brown et al., 2000; King et al., 1999; Leder et al., 2000). Notable interindividual variability in serum testosterone concentrations were observed in male subjects administered 4-androstenedione and suggest possible influence by basal testosterone levels (Brown et al., 2006). Administration of 4-androstenedione (1500 mg/day) to hypogonadal men for 12 weeks was associated with increased circulating levels of 4-androstenedione and testosterone (Jasuja et al., 2005). Higher fold increases of serum testosterone following 4- androstenedione administration to female subjects has been reported (Brown et al., 2004; Kicman et al., 2003; Leder et al., 2002). In another study, 4-androstenedione administration (200 mg/day) in healthy adult males (age 35-60) for 12 weeks was associated with increased estrone and estradiol levels (Broeder et al., 2000). Human studies suggest that numerous factors may influence the observed hormonal response to exogenous 4-androstenedione and the exact mechanisms of observed effects are currently unknown.

Toxicity Studies:

Multiple animal toxicity studies relevant to evaluating the safety of 4-androstenedione were identified in our review, including comprehensive toxicity and carcinogenicity studies conducted by the U.S. National Toxicology Program. The available studies identify numerous toxicological findings associated with oral administration of 4-androstenedione, including androgenic effects, induction of non-neoplastic lesions, and liver and pancreatic islet cancer.

Due to concern of adverse health effects related to prolonged use of 4-androstenedione, the NTP conducted subchronic toxicity (14-week) studies with 4-androstenedione administered by oral gavage, followed by an evaluation of its chronic toxicity and carcinogenicity (2-year) in both F344/N rats and B6C3F1 mice (Blystone et al., 2011; NTP, 2010). Increased incidence of adrenal gland X-zone atrophy and X-zone cytoplasmic vacuolization was reported in female mice following 14-weeks of 4-androstenedione administration and is indicative of an androgenic effect. Additionally, induction of non-neoplastic lesions characteristic of

masculinization in the kidney, submandibular salivary gland, and clitoral gland of female mice chronically exposed to exogenous 4-androstenedione were observed (Blystone et al., 2011). Increased bodyweights in 4-androstenedione-treated female rats in both 14-week and chronic studies may have corresponded to an anabolic effect. However, muscle and adipose mass were not specifically evaluated. 4-androstenedione administration was also associated with reduced epididymal sperm concentrations and reduced sperm motility in male rats and mice, respectively. Such effects indicate a potential for exogenous 4-androstenedione to produce adverse effects on fertility and reproduction. Under the conditions of the 2-year cancer bioassay, the NTP concluded that there was "*clear evidence of carcinogenic activity*" of 4-androstenedione in male and female mice, based on increased incidences of multiple hepatocellular adenoma and hepatocellular carcinoma and increased incidence of hepatoblastoma (NTP, 2010). Notably, other androgenic substances have been characterized as hepatocellular carcinogens (IARC, 1987). Increased incidence of pancreatic islet adenoma in male and female mice was also considered related to 4-androstenedione administration.

Additional studies investigating the effects of exogenous 4-androstenedione administered to female rats prior to, and during pregnancy were identified in the available literature (Flynn et al., 2005; Kim et al., 2007; Sahu et al., 2005; Sprando et al., 2004; Sprando et al., 2005; Wiesenfeld et al., 2006). These studies reported effects on hepatic xenobiotic metabolism, altered tissue free fatty acid profiles, and possible effects on estrous cyclicity associated with daily 4-androstenedione administration via oral gavage. Effects on reproductive physiology and development in experimental, zebrafish (*Danio rerio*), mosquitofish (*Gambusia affinis*) and fathead minnow (*Pimephales promelas*) experimental models were observed following developmental exposure to 4-androstenedione (DeQuattro et al., 2015; Hou et al., 2018; Ma et al., 2022). The evidence of endocrine disruption from exogenous 4-androstenedione exposure in experimental fish models further increases concerns related to potential perturbation of hormonal signaling in humans.

The observed adverse effects of 4-androstenedione on sex-specific traits, development, and carcinogenic effects in experimental animal models present serious safety concerns that are contrary to general recognition of safety for use in food.

Human Studies and Case Reports:

Identified human studies were of limited utility in supporting the safe use of 4-androstenedione in food. Available studies were principally designed to evaluate the purported ergogenic and anabolic properties of 4-androstenedione intake, and not parameters useful for evaluation of the safety of long-term use in food, especially considering vulnerable pediatric and adolescent populations (Powers, 2002; Smurawa & Congeni, 2007). The weight-of-evidence from human studies indicates that exogenous 4-androstenedione administration is associated with increased circulating estrogen and androgen levels in healthy men and women, respectively (Broeder et al., 2000; Brown et al., 2004; Brown et al., 2000; King et al., 1999; Leder et al., 2002; Leder et al., 2000; Powers, 2002). Slight increases in testosterone levels have been reported in male subjects, however effects were inconsistent and vary significantly with dose, duration of treatment, and basal levels of circulating testosterone (Leder et al., 2000). Determinants of overall hormonal response following ingestion of 4-androstenedione have not been fully elucidated and are likely multifactorial in nature. Evidence that 4-androstenedione ingestion induces contrasexual effects on circulating sex-steroid levels is

cause for serious safety concerns.

A study by Broeder and colleagues evaluated the effects of daily androstenedione administration in healthy male subjects (age 35-65) for up to 12 weeks, in combination with a high-intensity fitness regimen (Broeder et al., 2000). No ergogenic or anabolic effects were reported and circulating estrone and estradiol levels were significantly increased in individuals administered 4-androstenedione. Additionally, 4-androstendione supplementation was associated with significantly decreased serum HDL cholesterol levels and corresponding increased cardiovascular disease risk.

Two case reports were identified in the available literature which describe serious adverse reproductive effects associated with 4-androstenedione intake. A report by Kachhi and Henderson (2000), described a case of priapism in a 30-year-old male associated with the use of a product containing 4-androstenedione (Kachhi & Henderson, 2000). Onset of symptoms occurred following daily 4-androstenedione intake for seven days, and the patient reported a previous episode of priapism that also coincided with use of 4-androstenedione supplements. Ritter et al. (2005) described a case report in which a 29-year old recreational bodybuilder presented with impotence and severe oligospermia corresponding to suppression of the hypothalamic-pituitary axis following oral 4-androstenedione supplementation (Ritter et al., 2005). Cessation of 4-androstenedione use, and testosterone replacement therapy resulted in resolution of symptoms and normalization of semen parameters.

Overall Conclusions

Overall, the available data do not support the safety of 4-androstenedione for use as a food ingredient that will be consumed by the general public. Moreover, the available reports underscore its potential for serious reproductive, developmental, and cardiovascular toxicity, as well as carcinogenic potential. 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. Classification of 4-androstenedione as a Class III controlled substance suggests serious risk of adverse effects related to its use and is contradictory to a GRAS conclusion for use in food.

Due to the lack of adequate data and information in the scientific literature to support the safe use of 4-androstenedione in food, DFI is unable to conclude that the addition of 4-androstenedione to food meets the statutory criteria for classification as GRAS. Indeed, the available data raise serious safety concerns as there are potential adverse effects of 4-androstenedione on the reproductive system, cardiovascular system, and possible carcinogenic effects. Additionally, it seems plausible that perturbation of endocrine signaling related to 4-androstenedione use would raise safety concerns related to effects on the developing reproductive system, pubertal onset, and fertility. As such, there is an absence of consensus among qualified experts regarding the safety of 4-androstenedione use as a food ingredient. Therefore, based on the current status of data and information, 4-androstenedione 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 4-androstenedione 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 4-androstenedione is added adulterated within the meaning of Section 402(a)(2)(C) of the FD&C Act.

References

Blystone, C. R., Elmore, S. A., Witt, K. L., Malarkey, D. E., & Foster, P. M. (2011). Toxicity and carcinogenicity of androstenedione in F344/N rats and B6C3F1 mice. *Food Chem Toxicol, 49*(9), 2116-2124. doi:10.1016/j.fct.2011.05.026

Broeder, C. E., Quindry, J., Brittingham, K., Panton, L., Thomson, J., Appakondu, S., . . . Yarlagadda, C. (2000). The Andro Project: physiological and hormonal influences of androstenedione supplementation in men 35 to 65 years old participating in a high-intensity resistance training program. *Arch Intern Med, 160*(20), 3093-3104. doi:10.1001/archinte.160.20.3093

Brown, G. A., Dewey, J. C., Brunkhorst, J. A., Vukovich, M. D., & King, D. S. (2004). Changes in serum testosterone and estradiol concentrations following acute androstenedione ingestion in young women. *Horm Metab Res, 36*(1), 62-66. doi:10.1055/s-2004-814201

Brown, G. A., Vukovich, M., & King, D. S. (2006). Testosterone prohormone supplements. *Med Sci Sports Exerc, 38*(8), 1451-1461. doi:10.1249/01.mss.0000228928.69512.2e

Brown, G. A., Vukovich, M. D., Reifenrath, T. A., Uhl, N. L., Parsons, K. A., Sharp, R. L., & King, D. S. (2000). Effects of anabolic precursors on serum testosterone concentrations and adaptations to resistance training in young men. *Int J Sport Nutr Exerc Metab, 10*(3), 340-359. doi:10.1123/ijsnem.10.3.340

Cabrera, S. M., & Rogol, A. D. (2013). Testosterone exposure in childhood: discerning pathology from physiology. *Expert Opin Drug Saf, 12*(3), 375-388. doi:10.1517/14740338.2013.782000

Clark, A. S., & Henderson, L. P. (2003). Behavioral and physiological responses to anabolic-androgenic steroids. *Neurosci Biobehav Rev, 27*(5), 413-436. doi:10.1016/s0149-7634(03)00064-2

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. doi:https://doi.org/10.1016/S0015-6264(76)80522-6

DeQuattro, Z. A., Hemming, J. D., & Barry, T. P. (2015). Effects of androstenedione exposure on fathead minnow (Pimephales promelas) reproduction and embryonic development. *Environ Toxicol Chem, 34*(11), 2549-2554. doi:10.1002/etc.3092

Dhar, R., Stout, C. W., Link, M. S., Homoud, M. K., Weinstock, J., & Estes, N. A. M. (2005). Cardiovascular Toxicities of Performance-Enhancing Substances in Sports. *Mayo Clinic Proceedings, 80*(10), 1307-1315. doi:https://doi.org/10.4065/80.10.1307

FDA, W. P. (2004). *Health Effects of Androstenedione*. Retrieved from Rockville, MD: https://web.archive.org/web/20090117053926/http:/www.fda.gov/oc/whitepapers/andro.html

Feller, A. A., Mylonakis, E., & Rich, J. D. (2002). Medical complications of anabolic steroids. *Med Health R I, 85*(11), 338-340.

Flynn, T. J., Sapienza, P. P., Wiesenfeld, P. W., Ross, I. A., Sahu, S., Kim, C. S., . . . Sprando, R. L. (2005). Effects of oral androstenedione on steroid metabolism in liver of pregnant and non-pregnant female rats. *Food Chem Toxicol, 43*(4), 537-542. doi:10.1016/j.fct.2004.12.007

Hou, L. P., Yang, Y., Shu, H., Ying, G. G., Zhao, J. L., Fang, G. Z., . . . Cheng, X. M. (2018). Masculinization and reproductive effects in western mosquitofish (Gambusia affinis) after long-term exposure to androstenedione. *Ecotoxicol Environ Saf, 147*, 509-515. doi:10.1016/j.ecoenv.2017.08.004

Hurley, B. F., Seals, D. R., Hagberg, J. M., Goldberg, A. C., Ostrove, S. M., Holloszy, J. O., . . . Goldberg, A. P. (1984). High-density-lipoprotein cholesterol in bodybuilders v powerlifters. Negative effects of androgen use. *Jama, 252*(4), 507-513.

IARC. (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl, 7*, 1-440.

Jasuja, R., Ramaraj, P., Mac, R. P., Singh, A. B., Storer, T. W., Artaza, J., . . . Bhasin, S. (2005). Delta-4-androstene-3,17-dione binds androgen receptor, promotes myogenesis in vitro, and increases serum testosterone levels, fat-free mass, and muscle strength in hypogonadal men. *J Clin Endocrinol Metab, 90*(2), 855-863. doi:10.1210/jc.2004-1577

Kachhi, P. N., & Henderson, S. O. (2000). Priapism after androstenedione intake for athletic performance enhancement. *Ann Emerg Med, 35*(4), 391-393. doi:10.1016/s0196-0644(00)70059-x

Kicman, A. T., Bassindale, T., Cowan, D. A., Dale, S., Hutt, A. J., & Leeds, A. R. (2003). Effect of androstenedione ingestion on plasma testosterone in young women; a dietary supplement with potential health risks. *Clin Chem, 49*(1), 167-169. doi:10.1373/49.1.167

Kim, C. S., Ross, I. A., Sprando, R. L., Johnson, W. D., Sahu, S. C., Flynn, T. J., . . . Sapienza, P. (2007). Distribution of androstenedione and its effects on total free fatty acids in pregnant rats. *Toxicology and Industrial Health, 23*(2), 65-74. doi:10.1177/0748233707076774

King, D. S., Sharp, R. L., Vukovich, M. D., Brown, G. A., Reifenrath, T. A., Uhl, N. L., & Parsons, K. A. (1999). Effect of oral androstenedione on serum testosterone and adaptations to resistance training in young men: a randomized controlled trial. *Jama, 281*(21), 2020-2028. doi:10.1001/jama.281.21.2020

Leder, B. Z., Leblanc, K. M., Longcope, C., Lee, H., Catlin, D. H., & Finkelstein, J. S. (2002). Effects of oral androstenedione administration on serum testosterone and estradiol levels in postmenopausal women. *J Clin Endocrinol Metab, 87*(12), 5449-5454. doi:10.1210/jc.2002-021035

Leder, B. Z., Longcope, C., Catlin, D. H., Ahrens, B., Schoenfeld, D. A., & Finkelstein, J. S. (2000). Oral androstenedione administration and serum testosterone concentrations in young men. *Jama, 283*(6), 779-782. doi:10.1001/jama.283.6.779

Ma, D. D., Jiang, Y. X., Zhang, J. G., Fang, G. Z., Huang, G. Y., Shi, W. J., & Ying, G. G. (2022). Transgenerational effects of androstadienedione and androstenedione at environmentally relevant concentrations in zebrafish (Danio rerio). *J Hazard Mater, 423*(Pt B), 127261. doi:10.1016/j.jhazmat.2021.127261

Melchert, R. B., & Welder, A. A. (1995). Cardiovascular effects of androgenic-anabolic steroids. *Med Sci Sports Exerc, 27*(9), 1252-1262.

NTP. (2010). Toxicology and carcinogenesis studies of androstenedione (CAS No. 63-05-8) in F344/N rats and B6C3F1 mice (gavage studies). *Natl Toxicol Program Tech Rep Ser*(560), 1, 7-31,33-171 passim.

Ogilvy-Stuart, A. L., & Brain, C. E. (2004). Early assessment of ambiguous genitalia. *Arch Dis Child, 89*(5), 401-407. doi:10.1136/adc.2002.011312

Powers, M. E. (2002). The Safety and Efficacy of Anabolic Steroid Precursors: What is the Scientific Evidence? *J Athl Train, 37*(3), 300-305.

Ritter, R. H., Cryar, A. K., & Hermans, M. R. (2005). Oral androstenedione-induced impotence and severe oligospermia. *Fertil Steril, 84*(1), 217. doi:10.1016/j.fertnstert.2005.01.125

Rosenson, R. S., Brewer, H. B., Jr., Ansell, B. J., Barter, P., Chapman, M. J., Heinecke, J. W., . . . Webb, N. R. (2016). Dysfunctional HDL and atherosclerotic cardiovascular disease. *Nature reviews. Cardiology, 13*(1), 48-60. doi:10.1038/nrcardio.2015.124

Sahu, S. C., Sapienza, P. P., Sprando, R. L., Collins, T. F., Ross, I. A., Flynn, T. J., . . . Kim, C. S. (2005). Hepatotoxicity of androstenedione in pregnant rats. *Food Chem Toxicol, 43*(2), 341-344. doi:10.1016/j.fct.2004.11.005

Smurawa, T. M., & Congeni, J. A. (2007). Testosterone precursors: use and abuse in pediatric athletes. *Pediatr Clin North Am, 54*(4), 787-796, xii. doi:10.1016/j.pcl.2007.05.002

Sprando, R. L., Collins, T. F., Black, T. N., Olejnik, N., Grundel, E., & Ruggles, D. I. (2004). Effects of androstenedione on in utero development in rats. *Food Chem Toxicol, 42*(6), 917-924. doi:10.1016/j.fct.2004.01.015

Sprando, R. L., Collins, T. F., Black, T. N., Olejnik, N., Sapienza, P., Ramos-Valle, M., & Ruggles, D. I. (2005). Maternal exposure to androstenedione does not induce developmental toxicity in the rat. *Food Chem Toxicol, 43*(4), 505-513. doi:10.1016/j.fct.2004.11.020

van Amsterdam, J., Opperhuizen, A., & Hartgens, F. (2010). Adverse health effects of anabolic–androgenic steroids. *Regulatory Toxicology and Pharmacology, 57*(1), 117-123. doi:https://doi.org/10.1016/j.yrtph.2010.02.001

Wiesenfeld, P. W., Sapienza, P. P., Flynn, T. J., Ford, C. E., Ross, I. A., Sahu, S., . . . Sprando, R. L. (2006). Effects of oral androstenedione on phospholipid fatty acids, ATP, caspase-3, prostaglandin E(2) and C-reactive protein in serum and livers of pregnant and non-pregnant female rats. *Food Chem Toxicol, 44*(4), 579-587. doi:10.1016/j.fct.2005.09.005

Wit, J. M., Hero, M., & Nunez, S. B. (2011). Aromatase inhibitors in pediatrics. *Nat Rev Endocrinol, 8*(3), 135-147. doi:10.1038/nrendo.2011.161