BASIS OF NOMINATION TO THE NTP

Androstenedione is presented to the CSWG for review because of its potential for abuse by athletes and bodybuilders as a steroidal precursor to testosterone. This use came to the attention of the National Cancer Institute (NCI) during preparation of the Summary Sheet on DHEA (dehydroepiandrosterone). Like DHEA, androstenedione is being marketed as a dietary supplement. Unlike DHEA, which is being sold primarily for anti-aging, androstenedione sales target mostly young men. The supplements are often taken in large amounts along with other testosterone boosters including DHEA. Although the number of persons taking androstenedione as a dietary supplement is probably much smaller than the number of persons taking DHEA, their relatively young age and the high doses being consumed suggest a population at exceptionally high risk.

A search of the available literature revealed very little information relevant to the carcinogenicity of androstenedione. Androstenedione is converted ultimately to estradiol through a P450 enzyme, aromatase. Several compounds structurally related to androstenedione inhibit aromatase, and are used to treat estrogen-dependent breast cancer in postmenopausal women. The relevance of this information to potential carcinogenicity of androstenedione in young men is unknown.

SELECTION STATUS

ACTION BY THE CSWG: 9/16/98

Studies requested:
- Carcinogenicity

Priority: High

Rationale/Remarks:
- Used by young adults at very high doses
- Lack of information on chronic toxicity
CHEMICAL IDENTIFICATION

CAS Registry Number: 63-05-8

Chemical Abstracts Service Name: Androst-4-ene-3,17-dione

Synonyms and Tradenames: Androtex; 4-androstene-3,17-dione; Δ4-androstene-3,17-dione; Δ-4-androstenedione; SKF 2170

Structural Class: Steroid

Structure, Molecular Formula, and Molecular Weight:

\[ \text{C}_{19}\text{H}_{26}\text{O}_{2} \quad \text{Mol. wt.: 286.41} \]

Chemical and Physical Properties:

Description: Dimorphous (Budavari, 1996)

Melting Point: 143°C (needles from acetone (a)); 173°C (crystals from hexane (b)) (Budavari, 1996)

Boiling Point: 671°C (Lide, 1997)

Technical Products and Impurities: 4-Androstene-3,17-dione is available at 98% purity from Aldrich and Sigma (Aldrich, 1997; Sigma, 1997).


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Androstenedione is sold in health food stores and vitamin shops as a dietary supplement. Because dietary supplements are not held to the same standards of purity and efficacy as drugs in the United States, tremendous variability of the same product can occur between manufacturers, and it is suspected that some products may even contain none of the active ingredient. Some androstenedione products being advertised on the Internet are listed below in Table 1.

Table 1. Some dietary supplements containing androstenedione

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Company</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen</td>
<td>Discount Natural Foods</td>
<td>Capsules, 50 or 100 mg</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>Power Shack Fitness</td>
<td>Capsules, 50 mg</td>
</tr>
<tr>
<td>(Androgen®)</td>
<td>Products</td>
<td></td>
</tr>
<tr>
<td>Androstenedione 100</td>
<td>AST Research</td>
<td>Capsules, 100 mg</td>
</tr>
<tr>
<td>Andro-6</td>
<td>EAS</td>
<td>Capsules, 100 mg androstenedione, 50 mg DHEA, 250 mg <em>Tribulus terrestris</em> (herbal hormone booster) &amp; chrysin, saw palmetto, indole-3-carbinol &amp; zinc glycinate</td>
</tr>
<tr>
<td>Andro-XS</td>
<td>Power Shack Fitness</td>
<td>Capsules, 100 mg androstenedione &amp; 500 mg <em>Tribulus terrestris</em></td>
</tr>
<tr>
<td>AndroPlex 700</td>
<td>AST Research</td>
<td>Capsules, 50 mg androstenedione, 250 mg <em>Tribulus terrestris</em> &amp; 50 mg DHEA</td>
</tr>
<tr>
<td>Andros-LH</td>
<td>Nutritional Technologies</td>
<td>Capsules, androstenedione, <em>Tribulus terrestris</em>, DHEA, Bioperine (to enhance absorption), zinc &amp; copper</td>
</tr>
<tr>
<td>Androstene 50</td>
<td>OSMO Therapy</td>
<td>Capsules, 50 mg androstenedione, zinc &amp; lysophosphatidyl choline for enhanced uptake</td>
</tr>
<tr>
<td>Andro-forté100</td>
<td>Essentials, Inc.</td>
<td>Capsules, 100 mg androstenedione, 5 mg zinc gluconate &amp; 40 mg nicotinic acid</td>
</tr>
</tbody>
</table>
**EXPOSURE INFORMATION**

**Production and Producers:** Androstenedione is an important raw material for the commercial synthesis of testosterone. It is made commercially by direct microbiological oxidation of cholesterol or phytosterols (Petrow, 1980). A new procedure for producing androstenedione from tall oil soap through a proprietary process is under development (Forbes Medi-Tech Inc., 1998).

No information on the amount of androstenedione manufactured in the United States was identified in the available literature. Androstenedione is not listed in the EPA’s Toxic Substances Control Act (TSCA) Inventory. Between September 4, 1996, and February 26, 1998, the Piers Imports database listed 337,314 lb. of androstenedione imports. Nearly all were shipped to Charleston as the port of discharge from Bergkamen, Germany, as the point of origin (Dialog, 1998).

**Use Pattern:** Androstenedione is a starting material used in the manufacture of various pharmaceutical steroids such as oral contraceptives, hormones, and anti-inflammatories (Forbes Medi-Tech Inc., 1998).

Androstenedione is also a dietary supplement being used by body builders and other athletes as a male hormone booster to “peak out” testosterone levels before and during work outs (Discount Natural Foods, 1998). According to a German patent application, oral
administration of androstenedione at 50 mg and 100 mg increased testosterone levels in men 140 to 183% and 211 to 237%, respectively (Nutritional Technologies, 1998). There are also some indications of older men using androstenedione to increase sexual drive (Anon., 1998). Some products contain warnings, for example, that the product is not for use by women, by pregnant or lactating women or by men with benign prostatic hyperplasia, or by men with prostate problems and women who may have a predisposition towards breast cancer. One company notes that women should watch for signs of masculinility, such as increased facial hair growth and deepening of the voice (Netrition, 1998a).

Other ingredients may also be added to androstenedione supplements. These include zinc “needed for enzyme activity in the biosynthesis of testosterone,” nicotinic acid to “know when the androstenedione hits the bloodstream,” lysophosphatidyl choline and Bioperine “to increase bioavailability absorption of androstenedione,” the Chinese herb *Tribulus terrestris* “to maintain or increase luteinizing hormone production,” indole-3-carbinol “to remove excess estrogen from the body via a benign pathway, thereby decreasing the likelihood of estrogen-related side effects like gynecomastia,” and chrysin which “may be useful in helping to prevent the aromatization to estrogen” (Netrition, 1998a,b).

Some body builders combine different steroids to make a testosterone boosting stack. Other steroids taken with androsterone include androstene-diol, DHEA, and 19-nor-androstenedione (Netrition, 1998b). According to one company’s promotional literature, androstenedione has been effective at increasing musculosity, strength, endurance, and vascularity, but “many people believe that [19-norandrostenedione] is at least 5 times stronger than the strongest legal supplement on the market.” According to this company, 19-nor-androstenedione is so new to the market that no official human studies exist (Pinson’s Fitness Products, 1998).
**Human Exposure:** Endogenous androstenedione is present in human tissue, including fat. For bodybuilding, androstenedione is taken orally just before or during work-out sessions to enhance peak performance. Various recommended dosages are 100 mg androstenedione with 100 mg DHEA and 500 mg *Tribulus terrestris* taken twice a day; 250 mg androstenedione, 200 mg DHEA, and 1,000 mg *Tribulus terrestris* taken once at midday; 100-300 mg androstenedione with zinc gluconate and nicotinic acid to be taken 30 minutes into the workout; and 50-100 mg androstenedione to be taken 30 minutes prior to physical activity (Netrition, 1998a).

According to the *American Hospital Formulary Service 95 Drug Information* (McEvoy, 1995), the extent of abuse of androgens has not been fully determined, but nonmedical use is believed to be widespread. Estimates of misuse by weight lifters and body builders have ranged up to 50-80%. Most abuse of androgens appears to occur in individuals who never compete in sports. Evidence from one study indicates that about 7% of male high school seniors use or have used such drugs. In studies of college students, androgen use among athletes ranged up to about 20%.

**Environmental Status:** No information on environmental contamination with androstenedione from its manufacture or from dietary supplement use was identified in the available literature.

**Regulatory Status:** No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace maximum allowable levels of androstenedione. Androstenedione was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made.
Androstenedione is regulated by FDA under the Dietary Supplement Health and Education Act (DSHEA) of 1994. For dietary supplements marketed before October 15, 1994, DSHEA requires no proof of safety in order for them to remain on the market. The labeling requirements for supplements allow warnings and dosage recommendations as well as substantiated "structure or function" claims. All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement, "This product is not intended to diagnose, treat, cure, or prevent any disease" (Croom & Walker, 1995).

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

**Human Data:** No epidemiological studies or case reports investigating the association of exposure to androstenedione and cancer risks in humans were identified in the available literature.

**Animal Data:** No 2-year carcinogenicity studies of androstenedione in animals were identified in the available literature. A limited study reported tumors at the site of application when 750 mg/kg/9 wk was given by subcutaneous injection (sc) to mice (NLRI, 1998).

At dosages meant to mimic plasma levels in post-menopausal women, androstenedione stimulated the growth of dimethylbenz[a]anthracene (DMBA)-induced mammary carcinomas in female Sprague-Dawley rats. Three months after the mammary tumors were induced with DMBA, animals bearing tumors ≥1 cm were divided into groups. In the first experiment, intact and ovariectomized animals received 200 or 500 μg/day androstenedione by infusion from an osmotic pump. In a second experiment, additional rats were assigned to the following groups: ovariectomized; ovariectomized and implanted with androstenedione; or ovariectomized and implanted with androstenedione and treated for 18 days with an aromatase inhibitor, aminoglutethimide (AG), or an antiandrogen, flutamide (FLU). During 12 days of observation, total tumor area increased from 100 to...
165% in intact animals; in ovariectomized animals, a marked reduction to 20% of the original size occurred. Release of 500 µg/day androstenedione maintained a total tumor area 85% of the original value. In the second group, total tumor area decreased to 38% of pretreatment value 18 days after ovariectomy. In animals given androstenedione implants, total tumor area was maintained at 92% of control values. FLU was ineffective; however, treatment with AG decreased total tumor area from 92% to 27%. The authors concluded that conversion into estrogens by aromatase was the predominant effect of androstenedione in their system (Dauvois & Labrie, 1989).

**Short-Term Tests:** Very little information on the mutagenic activity of androstenedione was found in the available literature perhaps because hormones are thought to exert a carcinogenic effect through complex epigenetic mechanisms. Androstenedione at 500 µg per disc was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with or without S9 using the Ames test (McKillop *et al*., 1983).

**Metabolism:** Androstenedione is an intermediate in the production of testosterone from cholesterol, as shown in Figure 1 below. In postmenopausal women, the conversion of androstenedione to estrone and of testosterone to estradiol by the P450 enzyme, aromatase, in peripheral tissues, including adipose tissue, skin, muscle, liver and breast tissue is the major source of endogenous estrogen (Wiseman & Goa, 1996).
Other Biological Effects: Experience with medical uses of androgens and case reports in athletes indicate that potential adverse effects in either men or women include increased aggression and antisocial behavior, psychotic manifestations and affective disorders, changes in libido, adverse alterations in lipoprotein profiles and increased risk of cardiovascular disease, hepatotoxicity, liver tumors, premature bone maturation, acne, and possible increased risk of ruptured tendons and ligaments and of tendinitis. Other potential adverse effects of androgens in males include gynecomastia, hair loss, testicular atrophy and sperm abnormalities, impotence, and prostatic enlargement. In females, other
potential adverse effects include clitoral enlargement, menstrual irregularities, hirsutism, alopecia, deepened voice, and breast atrophy (McEvoy, 1995).

Increased levels of androstenedione can affect fertility in laboratory rodents. Androstenedione administration during pregnancy produces maternal effects and specific developmental abnormalities in offspring (NLM, 1998).

According to Juchau (1997), androgenic steroids elicit teratogenic effects in all species that have been investigated, and do so in a highly predictive and consistent fashion. In humans, frank effects on the morphology of the external genitalia (clitoromegaly, labial enlargement, labioscrotal fusion, etc.) may be observed in fetal or infant females after maternal exposure to relatively high doses of potent androgens. The morphologic defects elicited by androgens are selective for the developing external genitalia with few other abnormalities. Exposure in humans between the 8th and 13th weeks of gestation is regarded as the most critical period. The masculinizing effects described for humans are similar to those obtained in experimental animals. Androgen deficiencies are also known to cause birth defects, including pseudohermaphroditic feminization of male conceptuses.

**Structure/Activity Relationships:** Androstenedione is converted to testosterone, and ultimately, to estrogen through a pathway mediated by the P450 enzyme aromatase. Aromatase inhibition provides a theoretical basis for the development of chemotherapies for the treatment of estrogen-sensitive breast cancers in postmenopausal women. Several aromatase inhibitors in clinical use strongly resemble androstenedione, indicating the small changes in structure needed to profoundly influence response (Brueggemeier, 1994; Wiseman & Goa, 1996).
Aromatase inhibitors reduce the biosynthesis of estrone and, through that mechanism, inhibit the stimulatory effects of estrogens. Two general classes of aromatase inhibitors exist, suicide inhibitors and competitive inhibitors. Suicide inhibitors are acted upon specifically by the aromatase enzyme to open up high-affinity sites which bind to the enzyme irreversibly. Competitive inhibitors may be steroidal or non-steroidal compounds. An example of a suicide inhibitor is 4-hydroxyandrostenedione. An example of a competitive inhibitor is 1-methyl-1,4-androstene-3,17-dione (Santen, 1990). No information on the carcinogenicity or mutagenicity of these aromatase inhibitors was found in the available literature.

Information on carcinogenicity and genotoxicity of DHEA and testosterone is also relevant to an analysis of androstenedione. This information is summarized in Table 2.
Table 2. Summary of Information on Androstenedione and Related Steroids

<table>
<thead>
<tr>
<th>Carcinogenicity Information</th>
<th>Genotoxicity Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Androstene-3,17-dione [CAS No. 63-05-8]</td>
<td><img src="" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>No data found</td>
<td>(-) in <em>S. typhimurium</em> TA98, TA100, TA1535, TA1537 &amp; TA1538 (+/-S9) (McKillop <em>et al.</em>, 1983)</td>
</tr>
<tr>
<td>DHEA [CAS No. 53-43-0]</td>
<td><img src="" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Carcinogenicity Information</td>
<td>Genotoxicity Information</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>(+) for hepatocellular carcinomas in rats (Rao et al., 1992; Metzger et al., 1995)</td>
<td>(-) in <em>S. typhimurium</em> TA98, TA100, TA1535, TA1537 &amp; TA1538 (McKillop et al., 1983)</td>
</tr>
<tr>
<td>promoted development of ovarian granulosa cell tumors in susceptible mice (Beamer et al., 1988)</td>
<td>(+) for sister chromatid exchange (SCE) induction in Chinese hamster lung fibroblasts (Bynum et al., 1980)</td>
</tr>
<tr>
<td>increased incidence of lung lesions in rats initiated by DHPN injections (Moore et al., 1988) and of pancreatic lesions in rats initiated by azaserine injections (Tagliaferro et al., 1992)</td>
<td>(-) for unscheduled DNA synthesis (UDS) in male rat hepatocytes (Oshiro et al., 1986); (+) for replicative DNA synthesis in rat hepatocytes (Uno et al., 1994)</td>
</tr>
<tr>
<td>(+) for liver tumors in rainbow trout, (+) for liver tumors and kidney tumors in rainbow trout initiated with MNNG (Orner et al., 1996)</td>
<td></td>
</tr>
</tbody>
</table>

Testosterone [CAS No. 58-22-0]
<table>
<thead>
<tr>
<th>Carcinogenicity Information</th>
<th>Genotoxicity Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>rats:</td>
<td>(-) in <em>S. typhimurium</em> TA1535, TA1537 &amp; TA1538 (+/- S9) (Ingerowski <em>et al.</em>, 1981)</td>
</tr>
<tr>
<td>prostatic adenocarcinomas in male rats given sc implants of testosterone propionate (IARC, 1987)</td>
<td>induced morphological transformation of SHE cells; (-) for chromosome aberrations &amp; aneuploidy (Tsutsui <em>et al.</em>, 1995)</td>
</tr>
<tr>
<td>mice:</td>
<td>(-) for increased SCE induction in Chinese hamster lung fibroblasts (Bynum <em>et al.</em>, 1980)</td>
</tr>
<tr>
<td>cervical-uterine tumors in female mice given sc implants of testosterone propionate (IARC, 1987)</td>
<td>(-) for aneuploidy in adult male human synovial cells (Galloway &amp; Ivett, 1986)</td>
</tr>
</tbody>
</table>

(+)=positive; (-)=negative; DHPN = dihydroxy-di-n-propyl nitrosamine; MNNG = N-methyl-N'-nitro-N-nitrosoguanidine
References


Prepared by Technical Resources International, Inc. under contract No. NOI-CB-50511 (8/98)


NLM (1998) *RTECS (Registry of Toxic Effects of Chemical Substances)*, Bethesda, MD, searched April 1998 [Record No. 8116]


Prepared by Technical Resources International, Inc. under contract No. NO2-CB-50511 (8/98)


Sigma (1997) *Biochemicals and Reagents for Life Science Research*, St. Louis, MO, p. 135


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