Article title: Gossypol

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Keywords
Please provide 10-15 keywords, which will be used for indexing purposes only. These should be listed alphabetically.

Cottonseed pigment, chemotherapy (experimental), gossypol, male contraceptive,

Abbreviations/Acronyms
Please provide a list of any abbreviations or acronyms used in your chapter.
Except possibly “DNA” (deoxyribonucleic acid), “hr” (hours), “mg/kg” (milligrams per kilogram) and “ppm” (parts per million) I think no uncommon abbreviations or acronyms are used that are not defined.

Glossary
Please provide specialized terms you would like to define that may not appear in the toxicology glossaries that will be included.

Genotoxicity and other standard headings should be included. Other than that I initially said none others, but my final Word spell check program picked up the following possibilities that were evidently not yet in its glossary:
Enantiomer: either of a pair of chemical compounds whose molecular structures have a nonsuperimposable mirror-image relationship to each other
Epigenetic: a trait or change caused by an agent resulting in changes in a chromosome without altering the DNA sequence
Racemic: of, relating to, or constituting a compound or mixture that is composed of equal amounts of dextrorotatory and levorotatory forms of the same compound and is not optically active
Glioma: primary brain tumor that originates from the supportive cells of the brain called glial cells
Myoma: benign growth of smooth muscle in the wall of the uterus

Abstract
Gossypol (CAS 303-45-7) was first extracted from seeds of cotton plants of the genus Gossypium. Some of gossypol’s bioactive properties were initially recognized over a century ago as potential problems if exposure is excessive; for example, if crude cottonseed oil or flour is consumed in large quantities. Possessing the ability to interact
with many enzymes and other molecules in the body, gossypol and structurally-related derivatives have since been shown to have potential for a variety of pharmaceutical uses.

**Body of Text**

- **Name:** Gossypol
- **CAS RN**: 303-45-7
- **Synonym**: 2,2′-bis(1,6,7-trihydroxy-3-methyl-5-isopropyl-8-aldehydonaphthalene
- **Chemical formula**: C30-H30-O8

*All from ChemIDplus

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**Background**

Gossypol is a non-volatile yellow pigment first isolated in 1889 from the seeds, roots, and stems of cotton plants of the genus *Gossypium* (fam. Malvaceae). Awareness of its biological activity increased in the early 20th century after it was identified as a contaminant in cottonseed-based supplements for livestock to increase protein in the diet. Gossypol has since been called one of the “antinutritional” agents in plant-derived foods. Attempts to produce glandless cottonseeds or genetically engineer gossypol out of those plants have not yet been entirely successful. Removing gossypol from the growing plant increases vulnerability to insects and other diseases; thus, gossypol is also referred to as a naturally-occurring insecticide.

By 1958 a synthesis method had been developed to facilitate production of both large quantities of gossypol and structurally-related derivatives. Attention then shifted to beneficial uses of gossypol. During the late 1970s, Chinese researchers reported on clinical trials of gossypol as a male contraceptive that were undertaken after observing that consumption of large quantities of crude cottonseed oil causes reduced fertility. Those first clinical trials of gossypol as an oral contraceptive for men reported high efficacy, reversibility, and few side effects. Although it held great promise as a reversible male antifertility agent through the 1980s, enthusiasm has been tempered for use as a contraceptive because of concern for low potassium levels and other side effects that emerged as increasing numbers of men used gossypol.
Women also consuming excessive amounts of gossypol in China in the 1950s had experienced reduced menstruation, which was usually reversible after exposure to gossypol ceased. From 1979 through the 1980s several hundred patients were treated with gossypol to reduce post-menopausal bleeding and to treat endometriosis and uterine myoma. Follow-up studies 1-3 years after treatment ceased showed that slightly more than half the women taking gossypol for menstrual disorders did not experience a recurrence, and over 60% maintained a reduction of myoma size and endometriotic nodules. In later studies, low potassium levels of concern following gossypol use were corrected by potassium supplements, and gossypol was proclaimed to be as effective as danazol in the control of endometriosis and uterine myoma, yet safer and less expensive.

Worldwide interest in finding other uses for gossypol and structurally-related compounds escalated quickly during the decades following the announcement of its application as a male contraceptive. Early accounts discussing possible use of gossypol to treat cancer actually date back at least to the 1960s, and several relatively small scale clinical trials have been conducted since then to investigate applications of gossypol and derivatives of gossypol in cancer chemotherapy. Some of the many other proposed uses for gossypol over the years, not all of which have reached the stage of clinical trials, are also mentioned in the Uses section. At the same time some concern remains about excessive inadvertent exposure to gossypol when crude cottonseed meal is relied upon heavily as a nutritional supplement for livestock and in aquaculture.

**Uses**
The first intentional use of gossypol appears to be industrial, specifically as an antioxidant to retard polymerization of rubber and rancidification of oils. As the brief history above and mechanisms of action discussed later both illustrate, increasing recognition of gossypol’s wide range of biological activities has prompted numerous investigations focusing on possible pharmaceutical applications. In addition to use as an oral contraceptive for men, application as a topical vaginal spermicide, potential for use as an antiviral agent against herpes and HIV, a treatment for parasitic and amoebic disorders and also for colitis and other diseases associated with chronic inflammation have all been explored. Recognition of potential side effects of consuming gossypol regularly at pharmaceutically-relevant doses has gradually shifted the focus of much of the current interest to applications in some of the more life-threatening diseases like cancer.

Relatively small scale early clinical trials have been conducted involving several dozens of patients with cancers of adrenal glands, brain gliomas, and other cancers at various stages. Some patients have responded surprisingly well, including some that had not responded to other chemotherapeutic regimens, while others have experienced little or no improvement, especially those with cancers in advanced stages. Relative lack of concurrent toxicity to healthy hematopoietic cells so common with conventional cancer treatments has been cited as a positive feature of gossypol.
Numerous non-clinical experimental studies describe how certain cell types may be more susceptible to gossypol’s effects than others, and how that knowledge might be applied to more selective cancer treatments. An newer exciting area of investigation into therapeutic applications involves use of apogossypol and other gossypol derivatives in combination with traditional chemotherapy and radiation to sensitize tumor cells selectively to be more vulnerable to cell death than normal healthy cells. This could potentially reduce the chemotherapy drug and radiation doses needed for successful treatment, and increase successful outcomes in cancer patients whose tumor cells have become resistant to conventional treatment.

Environmental Behavior, Fate, Routes, and Pathways
Gossypol is not volatile and is insoluble in water, being much more soluble in fat and other lipids. Free gossypol is unstable; therefore, it would not persist in the environment if inadvertently released from processed cottonseed products or directly from cotton roots, stems and seeds during harvesting.

Considering unintentional exposure potential, naturally-occurring gossypol is bound up in the plant pigment glands, so it is not normally considered to be easily accessible by any route through inadvertent release and distribution throughout the environment. Animal diets could contain gossypol if crude cottonseed meal was added as a source of protein. In these situations releases to the environment are not entirely impossible but can be controlled and therefore are not of great concern. Only trace amounts of gossypol would ever be dispersed in uneaten animal food in any case if allowable gossypol residue standards were being met. Animals in the wild would not likely get significant enough levels of exposure to the parts of the cotton plant that contain gossypol to cause toxicity. There is no evidence at this point that bioaccumulation or biomagnification within a food chain are significant issues for humans or any other species.

Use of pure gossypol by humans in a pharmaceutical context typically involves oral ingestion. This use is still currently largely experimental, not widespread, and otherwise of very little significance from the standpoint of potential for unintentional environmental exposures.

Exposure and Exposure Monitoring
As mentioned above in Routes and Pathways, other than intentional ingestion of gossypol as a therapeutic drug, the only appreciable exposures could be by regular consumption of crude cottonseed meal or oil. Regulatory agencies have established allowable levels in edible foods (see below).

Toxicokinetics
Gossypol distributes to most major organs of the body when administered to rats, including liver, kidney, spleen, testis and to a lesser extent brain. The half-life of a single oral dose of a racemic mixture in man is 10-11 days, with (+)gossypol retained 29 times longer than (-)gossypol. In patients consuming a single 20 mg dose of racemic (+/-) gossypol the plasma half-life of the (-) enantiomer is 4.55 hr whereas the half-life of the (+) enantiomer is 133 hours. Excretion occurs mainly in bile. Gossypol is metabolized
by microsomal enzymes, and several metabolites, including a quinone (gossypolone), have been identified in laboratory animal studies. Differences in metabolism may explain why man and other primates are more sensitive than rodents to its antifertility effects.

Mechanisms of Action
Gossypol exists normally as a racemic mixture. The (+)- and (−)- forms of gossypol manifest slightly different properties. Numerous studies of these two enantiomers alone and in combination have been conducted to understand how gossypol causes the myriad effects that it does. The presence of six phenolic hydroxyl groups and two aldehyde groups allows gossypol to react with many cell macromolecules, binding covalently to epsilon-amino acids through Schiff’s base condensation reactions. Many of the disruptive effects seen on enzymes and other proteins could be explained by this general mechanism.

Gossypol also uncouples mitochondrial oxidative phosphorylation; chelates iron, copper, aluminum and zinc; manifests both pro-oxidant and antioxidant characteristics; alters membrane potential, fluidity and permeability; binds to tubulin and inhibits microtubule assembly; and disrupts gap junctions and cell-cell communication before causing appreciable cytotoxicity. With the potential for affecting cells in so many different ways through different primary targets, gossypol can be expected to have differential effects on different cell and tissue types depending on exposure levels and the cells’ requirements for survival and replication.

Understanding how gossypol alters DNA synthesis and cell cycle progression has received a lot of attention. An increasing number of studies suggest that gossypol has potential for use in cancer therapy, especially when used in combination with other traditional chemotherapeutic drugs and radiation. Some experimental lab studies have shown that apogossypol and other derivatives have superior efficacy with less toxicity compared with gossypol. Gossypol reduces mitotic index and decreases the rate of DNA synthesis to some extent in all types of cell tested, including tumor cells. Some studies report that protein synthesis can also be reduced in various cell lines, while others have identified various ways in which gossypol can arrest cell growth by inhibiting enzymes involved in DNA replication. Several studies show that gossypol promotes apoptosis in tumor cells.

Acute and Short Term Toxicity
The oral LD50 values of gossypol determined in male rats range from approximately 1000 to over 2300 mg/kg, indicating that acute toxicity experienced would be considered low following just a single high dose. Gossypol Material Safety Data Sheets warn that it may cause irritation of the skin, eyes and respiratory tract upon direct contact.

Several comprehensive reviews listed in Further Reading detail many studies of potential systemic acute toxic effects seen in livestock and laboratory animals. Generally speaking, acute exposures to sufficiently high doses can cause cardiac irregularities and circulatory failure, and subchronic exposures can cause pulmonary edema. Anemia, lethargy, and labored respiration are common signs and symptoms observed. Non-ruminant animals
and young ruminants are more sensitive to toxic effects of gossypol than adult ruminant animals.

Chronic Toxicity
Relatively high doses employed in early chronic animal studies produce symptoms of wasting and malnutrition. Livestock consuming cottonseed protein in feed have developed liver toxicity. Aquacultured fish fed unpurified cottonseed protein meal have manifested growth suppression. Normal processing to purify cottonseed oil fit for human consumption removes naturally-occurring gossypol to very low or nondetectable levels. Therefore, humans consuming food cooked in or containing cottonseed oil even on a regular basis are unlikely to experience any of these effects unless crude cottonseed oils are used extensively.

Low potassium level (hypokalemia) is a concern for some users of gossypol as a male contraceptive, and also irreversibility, with an estimated 10% chance of non-recovery of fertility with prolonged use. In female patients being treated for endometriosis or uterine myoma, undesirable side effects observed initially were weakness, anemia, and hypokalemia, as well as a slight elevation of cholesterol and altered liver function. Chronic effects of gossypol when consumed intentionally in these larger doses to produce the desired pharmacologic effect (e.g., reduced fertility in men) are discussed in more detail in clinical reports summarized in one of the reviews shown in Further Reading (Wang et al., 2009).

Immunotoxicity
High doses of gossypol decreased total spleen cell population and suppressed immune response in an in vitro plaque-forming cell (PFC) assay. No response was observed in a lymphocyte transformation test using mitogen induction by concanavalin A. Investigators concluded that gossypol or a metabolite appears to exert selective depression of the humoral immune response at high dose levels (50 or 75 mg/kg/day), but clinical relevance was not clear. One of the many enzymes inhibited by gossypol is calcineurin, a target of immunosuppressant drugs. Immune system impairment is not among the side effects reported in human populations after clinical use of gossypol or ingestion of cottonseed oil-containing products. This may be a function of dose, but in any case no evidence was found that this endpoint has been the main focus of any human studies thus far.

Reproductive and developmental toxicity
 Desired effects of gossypol to suppress human male reproduction when used as a contraceptive or in women treated for gynecologic disorders have been discussed. Cotton root bark extracts have also been used in some cultures to induce abortion. Gossypol is often assumed to be the active abortifacient ingredient in these preparations.

Adverse reproductive or developmental effects have also been studied in laboratory and farm animal species, using a variety of in vivo and in vitro approaches. Effects on human populations already being quite clear, selected additional information mentioned here
comes from only a couple of representative animal studies with endpoints that have not already been well characterized in humans.

In utero development was analyzed in rat pregnancies that resulted from matings between gossypol-treated male rats and untreated female rats and in pregnancies in which gossypol was administered to the pregnant rat only. Under the conditions of those experiments, gossypol administered to either the breeding male rat or the pregnant female rat had no demonstrable adverse effect on pregnancy or development in utero, including absence of increased resorption, fetal growth retardation, or rates of malformations. Doses used to treat the males apparently also did not lower fertility in males.

Gossypol was administered orally (50 or 75 mg/kg/day) to pregnant mice daily during day 1-15 of gestation, which includes the main period of organogenesis. Increased early fetal mortality and reduced body weight were seen in surviving fetuses, but no fetal malformations or other abnormalities were observed.

Readers interested in the potential negative consequences of gossypol on livestock fertility when using crude cottonseed meal containing gossypol as a protein supplement will also find many relevant studies.

Genotoxicity
One of the references shown in Further Reading discusses many short-term tests that have addressed a wide variety of genetic endpoints. Gossypol does not cause chromosome aberrations at clinically relevant doses. Although no significant effects have been seen in the clear majority of other studies, several do report some activity under specific conditions. Micronucleus induction was seen in cultured meiotic mouse sperm cells at dose levels approaching toxicity, and a rat dominant lethal test was also positive, neither of which is too surprising since male germ cells are among the most sensitive cell targets. Positive results have been reported for strand breaks and DNA degradation in in vitro systems, but only in serum-free medium—not when serum proteins were present or when an in vivo exposure approach was used. Gossypol inhibits DNA synthesis, with additional evidence that this does not involve any direct negative interaction with the DNA molecule itself but rather with enzymes involved in DNA replication. A weak increase in sister chromatid exchange frequency has been seen in human lymphocyte cultures and rodents treated with gossypol, but no evidence of this yet in clinical or epidemiologic studies involving people exposed to gossypol.

Studies finding any adverse genotoxic effects observe only very weak effects. These effects are likely to be eliminated or inconsequential in vivo in the presence of serum proteins, which would always be the case in real life exposures, and at expected exposure levels, and can be explained by epigenetic mechanisms not involving direct interactions with DNA.

Carcinogenicity
No well-documented information currently exists describing any rigorous full-scale standard cancer bioassays of gossypol in animal models or any epidemiological findings
that would be cause for concern. A few sources state that gossypol has limited evidence of a carcinogenic effect. The term ‘limited’ can mean that only one experiment shows some effect, often without confirmation, or that some questions are not fully resolved, or the tumors observed are only benign tumors. ‘Limited’ in this case is based on non-standard, less-than-lifetime studies in rodents: Specifically, this designation is apparently because gossypol was reported in a conference presentation abstract to be a skin tumor initiator and promoter in SENCAR mice, the strain chosen for its known rapid response to tumor initiator/promoter combinations. The report appears in the open literature only as a brief abstract, and the type(s) of skin tumors observed were not detailed. Two other studies conducted in rats lasting 6 months or less and focusing specifically on tumors of liver and testes showed no effect of gossypol.

None of these studies is well documented in detail in the open peer-reviewed literature. None appear to have been confirmed or followed up in any way in subsequent independent experiments. Thus, any claims of a carcinogenic risk to humans following even long-term exposure to gossypol are not well supported by solid scientific evidence.

Clinical Management
Clinical experience with otherwise healthy human beings manifesting signs of toxicity known to be related to gossypol is largely restricted to controlled experimental trials when used as a contraceptive or for treatment of gynecological disorders. Low potassium levels have been successfully treated with potassium supplements. Cessation of gossypol ingestion has usually resulted in gradual disappearance of undesirable side effects. People are highly unlikely to experience toxicity from the very low levels of gossypol that might be present and consumed unintentionally in cottonseed-based products; however, if this ever occurs then the experience of high doses used in clinical trials could be helpful for medical management.

Ecotoxicology
No studies were found specifically documenting unexpected toxicity to non-human terrestrial species other than farm animals living in captivity and fed crude cottonseed meal. Toxic properties of gossypol are also unlikely to be of significant concern to many aquatic species, except in aquaculture where large amounts of crude cottonseed meal are used as nutritional supplements for fish. Channel catfish fed diets containing graded levels of gossypol-acetic acid showed reduced growth, hematocrit, red blood cell and hemoglobin levels and altered immune response. Understanding the consequences of heavy reliance on inadequately purified crude cottonseed products, these types of toxicity can be avoided.

Exposure Standards and Guidelines
The FDA-permitted concentration of gossypol in edible meal is 0.045% (450 ppm). The United Nations Food and Agriculture Organization and World Health Organization permit up to 0.6 μg/mg (600 ppm) free gossypol in edible foods.
There are no occupational or other specific standards for environmental exposures to gossypol; however, prudent safety practices are always recommended when handling large quantities of a potentially toxic compound, for example, experimentally in the lab or when formulating therapeutic drugs.

Further Reading
Three early comprehensive reviews published in the 1960s and ‘70s still serve as invaluable resources for detailed background information available up to their dates of publication. All of the subsequent genetic toxicity studies conducted on gossypol have also been reviewed in detail. A 2009 overview covers the many uses for gossypol investigated to-date, and also provides numerous other references, including some additional information about lab animal toxicity and possible side effects in humans consuming gossypol for beneficial uses.


Relevant Web Sites
N/A (none)

Cross References Recommended/Other Related Topics
Food Safety and Toxicology, Plants, Poisonous (humans).

Also if (but only if) mentioned in the following entries then these others might be worth considering: Reproductive System (check Male and Female chapters) and Cancer Chemotherapeutic Agents.