

ENVIRONMENTAL FORMATION OF ANDROGENS AND FISH MASCULINIZATION

W. Mike Howell ¹ and Robert A. Angus ²

¹ Department of Biology, Samford University, Birmingham, AL

² Biology Department, University of Alabama at Birmingham,
Birmingham, AL

Objectives of Presentation

- Evidence of environmental androgens being formed by microbial degradation of plant sterols
- Evidence of environmental androgens masculinizing a total population of female fishes

Reasons for Referring to these Females as “Masculinized”

- They have masculine traits, e.g. gonopodium and male reproductive behavior, but remain functional females
- Many egg follicles are atretic and fecundity is reduced, but females are still capable of producing viable young
- They usually do not have ovotestes; not hermaphroditic

We Believe the Effects are Androgenic Because:

- Identical results obtainable with known androgens
- Male juvenile American eels exhibit accelerated testicular development at a time in their life history when sex determination is not normally possible
- A few masculinized females have been found which have both ovaries and testes
- Masculinized females exhibit male reproductive behavior
- Males are precociously masculinized

FACTS ABOUT TALL OIL WASTES

- Tall oil, a by-product of the kraft (sulfate) pulping of pinewood chips
- Tall oil by-products studied in 1960's and 70's by pharmaceutical companies as a commercial source for steroids
- Contains about 3% steroids which consist of about 17 compounds of which beta-sitosterol and campesterol comprise 85%
- In 1974, the U.S. produced about 800,000 tons of tall oil
- Approximately 20,000 tons of tall oil phytosterols were available as raw material for steroid drug production
- microbial removal of the aliphatic side chain of two phytosterols, beta-sitosterol and stigmasterol, transforms them into androstadienedione and androstenedione

Microbial Conversion of Tall Oil and Soybean Sterols into Androgens

Conversion of Tall Oil Phytosterols and Soybean Sitosterols by *Mycobacterium* sp.¹

<u>Sterol source</u>	<u>Incubation period (days)</u>	<u>Percent conversion to:</u>			
		<u>ADD</u>	<u>AED</u>	<u>PEO</u>	<u>PDO</u>
Soybean	2	29	1	trace	3
	6	54	1		2
	8	38	1		1
Tall Oil	2	28	1		2
	4	46	3		3
	6	52	1		4
	8	48	1	trace	3

¹ Data from Conner et al., 1976 (ADD = androsta-1,-4-diene-3,17-dione; AED = androst-4-ene-3,17-dione; PEO = 20 α -hydroxymethylpregn-4-en-3-one; PDO = 20 α -hydroxymethylpregna-1,4-dien-3-one)

What Chemicals in Paper-Mill Effluent Could Cause the Masculinization?

Conner et al., 1975. (Neutrals in southern pine tall oil. J. Amer. Oil Chem. Soc.) listed the following steroids:

•Steroids 32%

cholesterol	trace
campesterol	2.5
campestanol	0.3
sitosterol	25.1
stigmastanol	1.9
cycloartenol	0.5
24-methylene-cycloartanol	0.8
24-methyleneo-phenol	trace

3-5-campestadien-7-one	0.1
citrostadienol	0.2
obtusifoliol	0.1
cycloeucalenol	0.1
4-stigmasten-3-one	0.2
4-campesten-3-one	trace
4,6-stigmastadien-3-one	0.1
4,6-campestadien-3-one	trace
3,5-stigmastadien-7-one	0.5

What Hard Evidence Do We Have that Androgens are Responsible for Mosquitofish Masculinization?

- Steroid fractions from paper-mill effluent isolated with HPLC
- Collected peaks which showed activity with an androgen receptor (AR) developed at Laboratory for Reproductive Studies, UNC School of Medicine
- GC/MS has tentatively identified androstenedione, androstadienedione and androsterone in Fenholloway River and in microbially-transformed beta-sitosterol from soybeans
- Female mosquitofish masculinized in both river effluent and microbially-transformed beta-sitosterol from soybeans

SUMMARY

- PLANT STEROLS CAN BE MICROBIALY CONVERTED INTO ANDROGENS
- SETTLING PONDS CONTAINING PLANT PRODUCTS AND BACTERIA ARE “STEROID GENERATORS”
- ANDROGEN-LADEN EFFLUENTS FROM PAPER-MILLS CAN MASCULINIZE FEMALE MOSQUITOFISH (AND OTHER FISH SPECIES AS WELL)
- THE POSSIBLE EFFECTS OF ENVIRONMENTAL ANDROGENS ON HUMAN POPULATIONS SHOULD BE A TOPIC OF CONCERN CONSIDERING THE TISE IN ANDROGEN-DEPENDENT CANCERS