

Development of *in vivo* guidelines for determination of endocrine disrupting effects



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Usage of test guidelines



- The guidelines are used to identify and characterize potentially dangerous new and existing chemicals and chemical mixtures
- They function as basic tool for regulatory risk assessment and chemical classification and labeling

EU Classification and labeling

GHS

- Main menu -

Nordic Council of Ministers

in collaboration with

European Chemicals Bureau

presents

The
**N-
CLASS**
Database
on
Environmental
Hazard
Classification
version
6.3

substances -
organisms

Go to
Classification strategies

Go to
Main menu

criteria:

classifying substances as dangerous for the environment are based on the elements toxicity, degradation and bioaccumulation. The database provides detailed expert advice on the interpretation of available data, including how to use non-standard data:

Degradation

Bioaccumulation

may result in any of the following categories:

H; R51-53

R52

R52-53

R53

category to display criteria)

of criteria
to:

Chronic toxicity

Category: Chronic I

Acute toxicity:

96 hr LC₅₀ (for fish) ≤1 mg/L and/or

48 hr EC₅₀ (for crustacea) ≤1 mg/L and/or

72 or 96hr ErC₅₀ (for algae or other aquatic plants) ≤1 mg/L

and the substance is not rapidly degradable and/or the log Kow ≥ 4 (unless the experimentally determined BCF <500).

Category: Chronic II

Acute toxicity:

96 hr LC₅₀ (for fish) >1 to ≤10 mg/L and/or

48 hr EC₅₀ (for crustacea) >1 to ≤10 mg/L and/or

72 or 96hr ErC₅₀ (for algae or other aquatic plants) >1 to ≤10 mg/L

and the substance is not rapidly degradable and/or the log Kow ≥4 (unless the experimentally determined BCF <500), unless the chronic toxicity NOECs are > 1 mg/L.

Category: Chronic III

Acute toxicity:

96 hr LC₅₀ (for fish) >10 to ≤100 mg/L and/or

48 hr EC₅₀ (for crustacea) >10 to ≤100 mg/L and/or

72 or 96hr ErC₅₀ (for algae or other aquatic plants) >10 to ≤100 mg/L

and the substance is not rapidly degradable and/or the log Kow ≥4 (unless the experimentally determined BCF <500) unless the chronic toxicity NOECs are >1 mg/L.

Category: Chronic IV

Poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility, and which are not rapidly degradable and have a log Kow ≥ 4, indicating a potential to bioaccumulate, will be classified in this category unless other scientific evidence exists showing classification to be unnecessary. Such evidence would include an

Link to OECD web page: <http://titania.sourceoecd.org/>

Physical Chemical Properties: TG 101 - 123

Effects on Biotic Systems: TG 201- 232

Degradation and Accumulation: TG 301 – 316

Health Effects: TG 401 – 486

- TG 105 Water solubility (physical-chemical properties)
- TG 305 Bio-concentration fish test (degradation and accumulation)
- TG 201 Algae, growth inhibition test (biotic systems)
- TG 203 Fish Acute toxicity Test (Biotic systems)
- TG 210 Fish, early life stage toxicity test (Biotic systems)
- TG 401 Acute oral toxicity (Health effects)
- TG 440 Uterotrophic Bioassay in Rodents (Health effects)

- New chemicals are not tested for specific endocrine disrupting properties
- Up to 100 000 existing chemical compounds have thus not been tested for endocrine disrupting properties
- One of the reasons are that until recently no standardized guidelines exists
- Development of test guidelines for testing of new and existing chemicals is therefore crucial and extremely necessary

Guideline proposals are based on basic scientific research

The perfect test:

- Has stable and specific biomarkers (endpoints) and in relation to endocrine disruptors – EDC specific endpoints
- Is relatively easy to perform in standard laboratories
- Is cost efficient
- Has sufficient statistical power
- Uses a minimum of experimental animals

- Estrogenic, anti-estrogenic
- Androgenic, anti-androgenic
- Aromatase inhibition
- Thyroidal
- Glycocorticoids
- Cox inhibitors

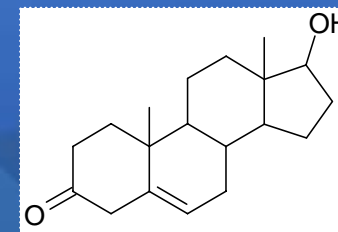
It is challenging to develop a guideline catching all mode of actions!

Chemicals with documented endocrine disrupting effects



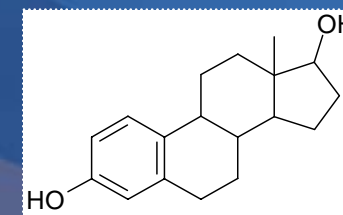
- Alkyl phenols - Estrogen
- Bisphenol A – Estrogenic, anti-androgenic
- PCBs – Estrogen/Androgen/Thyroid – Effect on reproductive systems.
- DDT - Estrogen
- TBT– Aromatase inhibition
- Arsenic – Glucocorticoid
- Prochloraz – Aromatase inhibitors

Testosterone



prochloraz

Estradiol





OECD members: 30 Industrial Countries

OECD coordinates development of test guidelines for testing of new chemicals

Great advantages of unified testing strategy

Some political interference challenges the cooperation as all countries have veto-right

Major players in the guideline development process

- OECD
- EU, European EPAs
- US EPA
- EDSTAC (*Endocrine Disruptor Screening and Testing Advisory Committee*)
- Japan
- BIAC (*Business and Industry Advisory Committee to the OECD*)
- ECETOC (*European Centre for Ecotoxicology and Toxicology of Chemicals*)

The Working group of
National Coordinators
of the Test Guidelines Programme
(WNT)

OECD secretariat

EDTA Advisory Group (EDTA AG)
Member countries,
NGO's, Industry, EU

Validation Management Groups:
VMG-mammalian
VMG-eco
VMG-NA

Expert Groups:
e. g. FDG (Fish Drafting Group)

Note: Document prepared by the Secretariat of the Test Guidelines Programme based on the agreement reached at the 6th Meeting of the EDTA Task Force

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

<p>Level 1 Sorting & prioritization based upon existing information</p>	<ul style="list-style-type: none"> - physical & chemical properties, e.g., MW, reactivity, volatility, biodegradability, - human & environmental exposure, e.g., production volume, release, use patterns - hazard, e.g., available toxicological data 	
<p>Level 2 <i>In vitro</i> assays providing mechanistic data</p>	<ul style="list-style-type: none"> - ER, AR, TR receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis <i>in vitro</i> - Aryl hydrocarbon receptor recognition/binding - QSARs 	<ul style="list-style-type: none"> -High Through Put Prescreens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)
<p>Level 3 <i>In vivo</i> assays providing data about single endocrine Mechanisms and effects</p>	<ul style="list-style-type: none"> - Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non -receptor mediated hormone function - Others (e.g. thyroid) 	<ul style="list-style-type: none"> - Fish VTG (vitellogenin) assay (estrogenic related)
<p>Level 4 <i>In vivo</i> assays providing data about multiple endocrine Mechanisms and effects</p>	<ul style="list-style-type: none"> - enhanced OECD 407 (endpoints based on endocrine mechanisms) - male and female pubertal assays - adult intact male assay 	<ul style="list-style-type: none"> - Fish gonadal histopathology assay - Frog metamorphosis assay
<p>Level 5 <i>In vivo</i> assays providing data on effects from endocrine & other mechanisms</p>	<ul style="list-style-type: none"> - 1-generation assay (TG415 enhanced)¹ - 2-generation assay (TG416 enhanced)¹ - reproductive screening test (TG421 enhanced)¹ - combined 28 day/reproduction screening test (TG 422 enhanced)¹ <p>¹ Potential enhancements will be considered by VMG mamm</p>	<ul style="list-style-type: none"> - Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)

TG 229: Fish Short-Term Reproduction Assay Adopted 2009

Mode of action	Test design	endpoints	OECD
Estrogenic, androgenic, aromatase inhibition	Reproductively active fish (fathead minnow, Japanese medaka, zebrafish) exposed under flow through conditions for 21 days. pre-exposure for 1-2 weeks.	Vitellogenin, secondary sex characteristics. Fecundity (quantified during exposure and pre-exposure)	Level 4

TG 230: Fish Screening Assay:

Adopted 2009

Mode of action	Test design	endpoints	OECD
Estrogenic, androgenic, aromatase inhibition	Reproductively active fish (fathead minnow, Japanese medaka, zebrafish) exposed under flow through conditions for 21 days.	Vitellogenin, secondary sex characteristics.	Level 3



TG 440 : Uterotrophic Assay Adopted 2007

Mode of action	Test design	Endpoints	OECD
Estrogenic	Female rats Immature or ovariectomised adult administered chemical for 3 d.	uterine weight (wet weight and blotted weight) compared to control	Level 3



TG 441 : Hersberger Assay in rats –Adopted 2009

Mode of action	Test design	Endpoints	OECD
(Anti)androgen effect	The test substance is administered to castrate-peripubertal male rat by gavage or subcutaneous injection daily for 10 consecutive days.	Males without testes Decreased or increased weight og reproductive organs	Level 3

OECD adopted ED- guidelines Mammalian



TG 407*: Repeated Dose 28-day study – updated in 2008
EDC endpoints: Organ weights (e.g. testes) and histopathology

TG 415: One-Generation Reproduction Toxicity Study – Adopted
1984 – Extended one-gen rep. Study under development

TG 416**: Two-Generation Reproduction Toxicity - updated 2001
EDC endpoints: E.g. Estrus cyclicity, sexual maturity, Anogenital distance

TG 421*: Reproduction/Developmental Toxicity Screening Test –
Adopted **1995**

TG 422*: Combined Repeated Dose Toxicity Study with the
Reproduction/Developmental Toxicity Screening Test - Adopted
1996

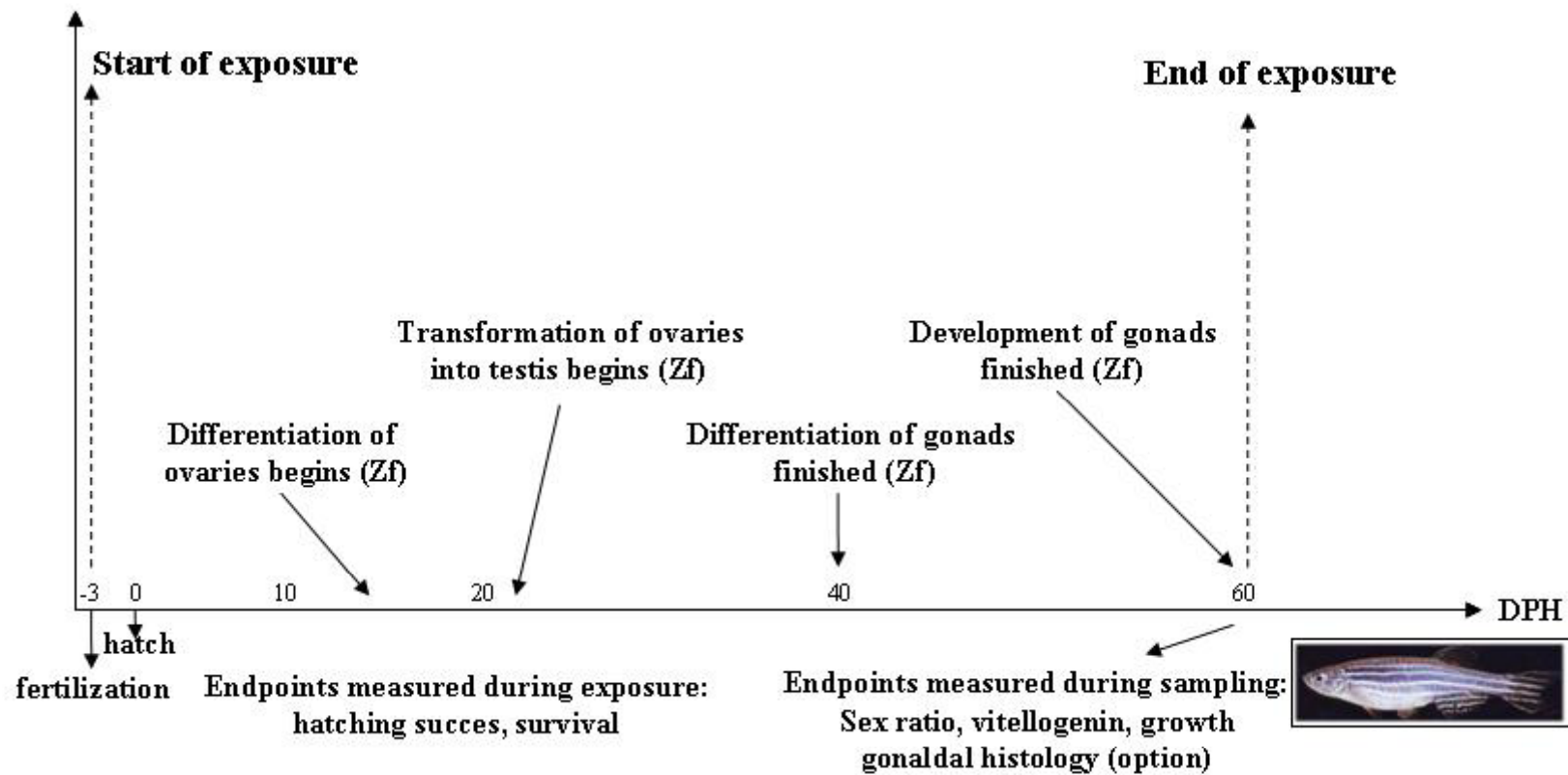
Draft TG: Fish Sexual Development Test

Mode of action	Test design	endpoints	OECD
Estrogenic, androgenic, aromatase inhibition	From fertilisation – sexual differentiation has finished (fathead minnow, Japanese medaka, zebrafish, three spined stickleback) exposed under flow through conditions	Vitellogenin, sex-ratio. Histopathology (option)	Level 4/5

Exampel: Fish Sexual Development Test

- 2001-2003: Development of a FSDT proposal for zebrafish (DK, N, SE)
2003: SPSF (Standard Project Submission Form) submitted to the OECD - zebrafish
- 2004: SPSF including Fathead minnow, Japanese medaka and Stickleback submitted to the OECD and accepted for validation
- 2006: A phase 1 validation was initiated with zebrafish and fathead minnow (10 laboratories)
- 2008: Termination of the phase 1 validation
- 2009: Initiation of phase 2 validation (10 laboratories)
- 2011: Adopted Guideline!!

FSDT experimental design

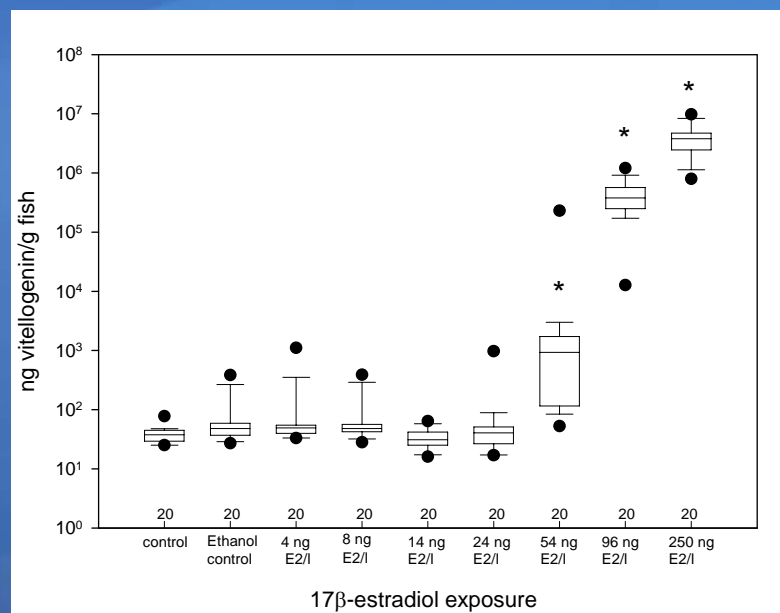


Examples of results

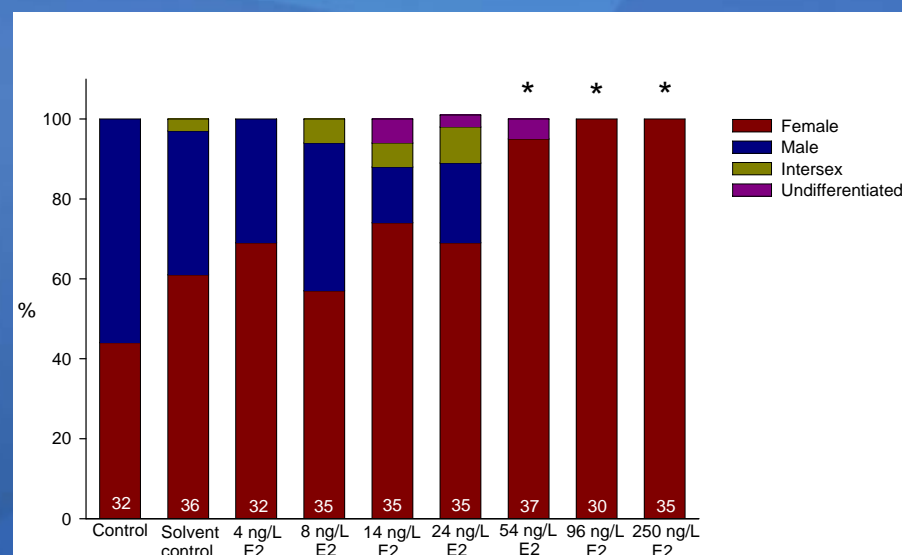


17 β -estradiol

VTG (males)



Sex ratio



Examples of FSDT results



Mode of action	Model compound	Endpoint	
		VTG	Sex ratio
Strong estrogen	17 β -Estradiol	↑ (32 ng/l)	↑ F (54 ng/l)
Weak estrogen	4-tert pentylphenol	↑ (274 μ g/l)	↑F (68 μ g/l)
Androgen	Trenbolone	↓ (117 ng/l)	↓F (9.7 ng/l)
Anti androgen	Flutamide	Not metabolised	Not metabolised
Aromatase inhibitor	Prochloraz	↓F (202 μ g/l)	↓F (202 μ g/l)
Anti estrogen	ZM189,156	↑ (100 μ g/l)	↑ undif.(100 μ g/l) ↓F (100 μ g/l)

Trenbolone, a growth hormone used in beef cattle



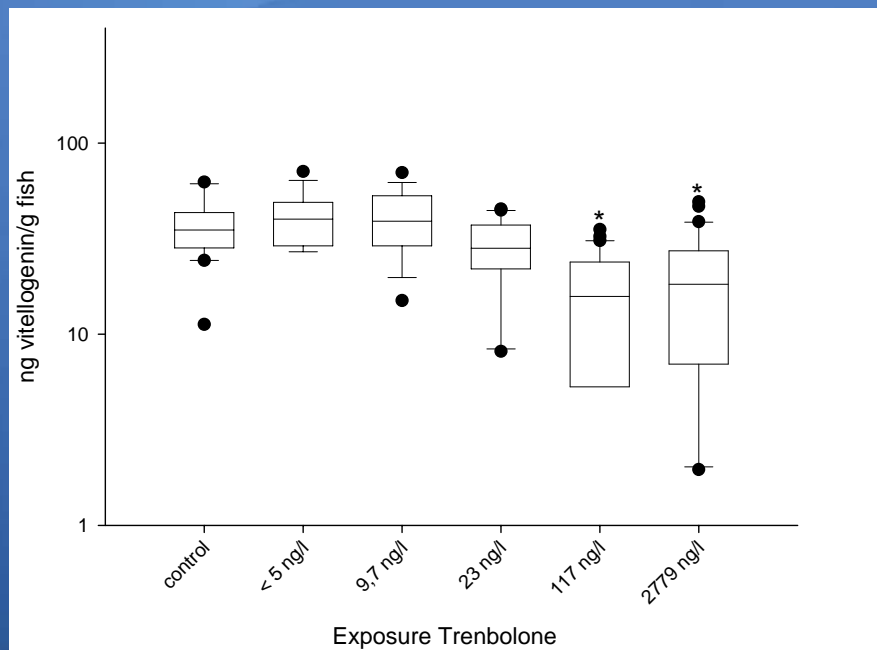
In e.g U.S.A. Beef cattle are implanted with capsules containing 140 mg trenbolone



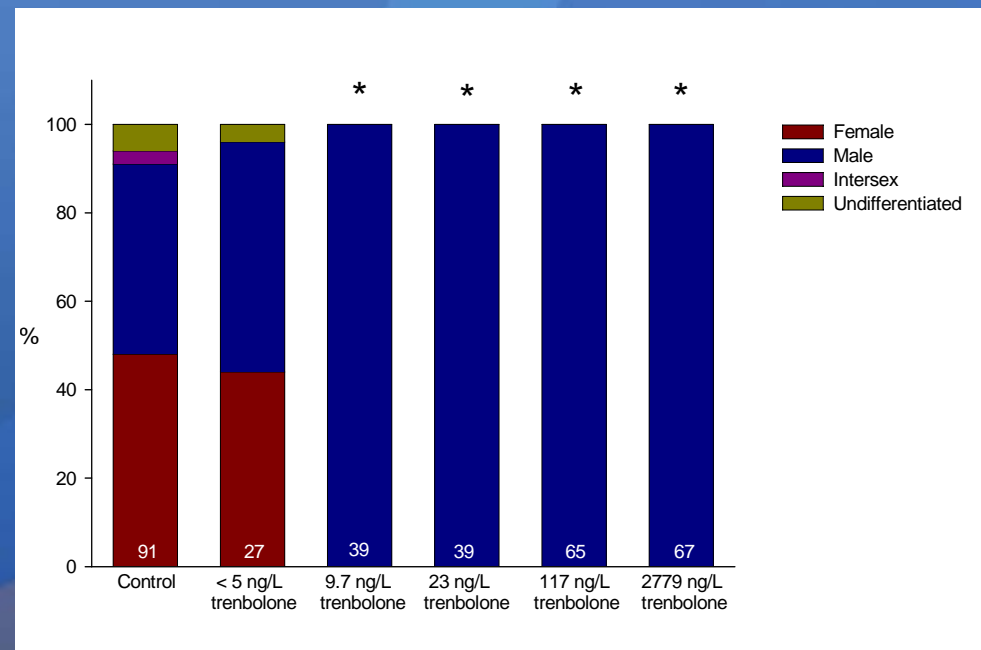
Trenbolone, FSDT results



VTG



Sex ratio



Trenbolone, reasons for concern

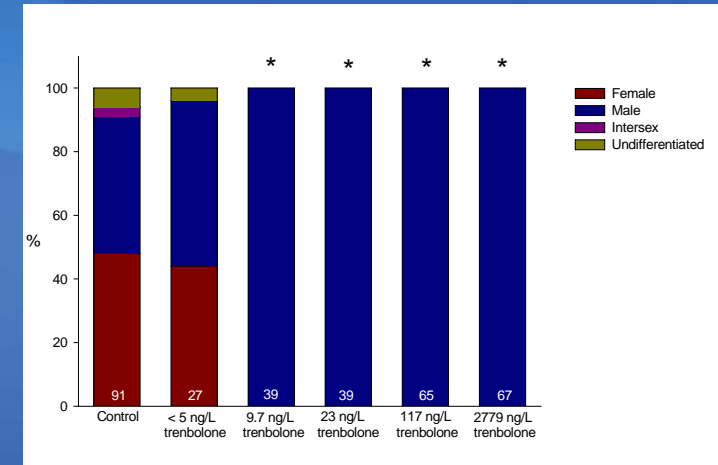


Beef cattle are implanted with capsules containing 140 mg trenbolone

FSDT - All mail population at 10 ng/L

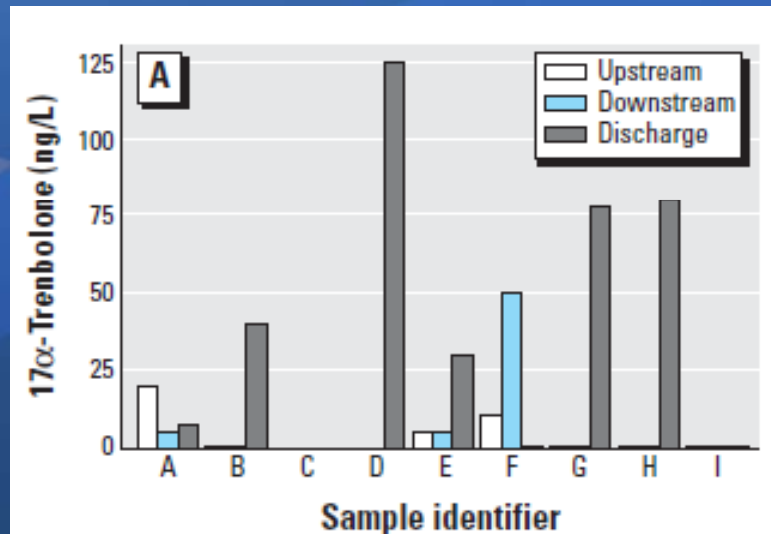
A swimming pool contains approx. 500 m³

1 capsule of trenbolone => 280 ng/L!!



Measured Concentrations in the environment:

South West central Ohio:



Durhan et al. 2005

Trenbolone and humans



Trenbolone - Google-søgning - Mi

File Rediger Vis Foretrukne Fur

Tilbage

Adresse http://www.google.dk/search

Google Trenbolone

Google Søg: på ne

Nettet

Tip: Søg efter resultater på Dansk alene.

[Pumping Iron 2001-2007](#)
Trenbolone acetate er en meget stærk a
Da esteren i Trenbolone er acetate, virke
www.bodyhouse.dk/idealbb/view.asp?topic

[Pumping Iron 2001-2007](#)
"Trenbolone er forholdsvis nyregift
myth that trenbolone was "hard or
www.bodyhouse.dk/idealbb/view.as

[Why2K - Trenbolone Acetate](#)
I bodybuilding og styrkeløft er trenbolone
bestemt ikke uden grund da trenbolone u
members.tripod.com/why2k_info/tren.htm

[Trenbolone - Wikipedia, the free e](#)
Trenbolone is a steroid used by veterinar
appetite. To increase its effective half-life,
en.wikipedia.org/wiki/Trenbolone - 30k -

[Trenbolone Acetate \(Finaplix\) Pro](#)
Trenbolone is a steroid having the advant
being affected by aromatase or 5alpha-red
www.mesomorphosis.com/steroid-profiles/

[Bodybuilding.com - Big Cat - In De](#)
Now only finaplix remains as the original s
acetate is rather short-acting but well liked
bodybuilding.com/fun/catfinaplix.htm - 30k

[Finaplix or Finaplex trenbolone ac](#)
Finaplix, also known as Finaplex is made
Finaplex are both well used by bodybuilde
www.steroid.com/Trenbolone-Acetate.ph

[Trenbolone](#)
Cycles, uses and side-effects of trenbolo
www.muscle-talk.co.uk/article-trenbolone

[Trenbolone](#)

Internet Explorer

http://www.gearprofiles.com

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Gear Profiles.com
Free information about Steroids and sources

Trenbolone Acetate (Finaplix)
Home >> Steroid Profiles >> Trenbolone Acetate

advertisement

by Bill Roberts - While Finject itself is no longer available, in some cases injectable preparations from Finaplix have been made. The substance is the same: trenbolone acetate.

Deca Durabolin

... You trust

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Anabolic Steroid Book

Anabolics 2007 by William Llewellyn - Regarded as the undisputed mother of all steroid reference manuals! Pro bodybuilders, world-class powerlifters, Olympic competitors, and professional athletes alike have relied on Anabolics for the better part of a decade.

Internet

**Recommended' dose:
100 mg every second day!**