

Analysis of anabolic androgenic steroid in wastewater

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Wastewater-based epidemiology (WBE)

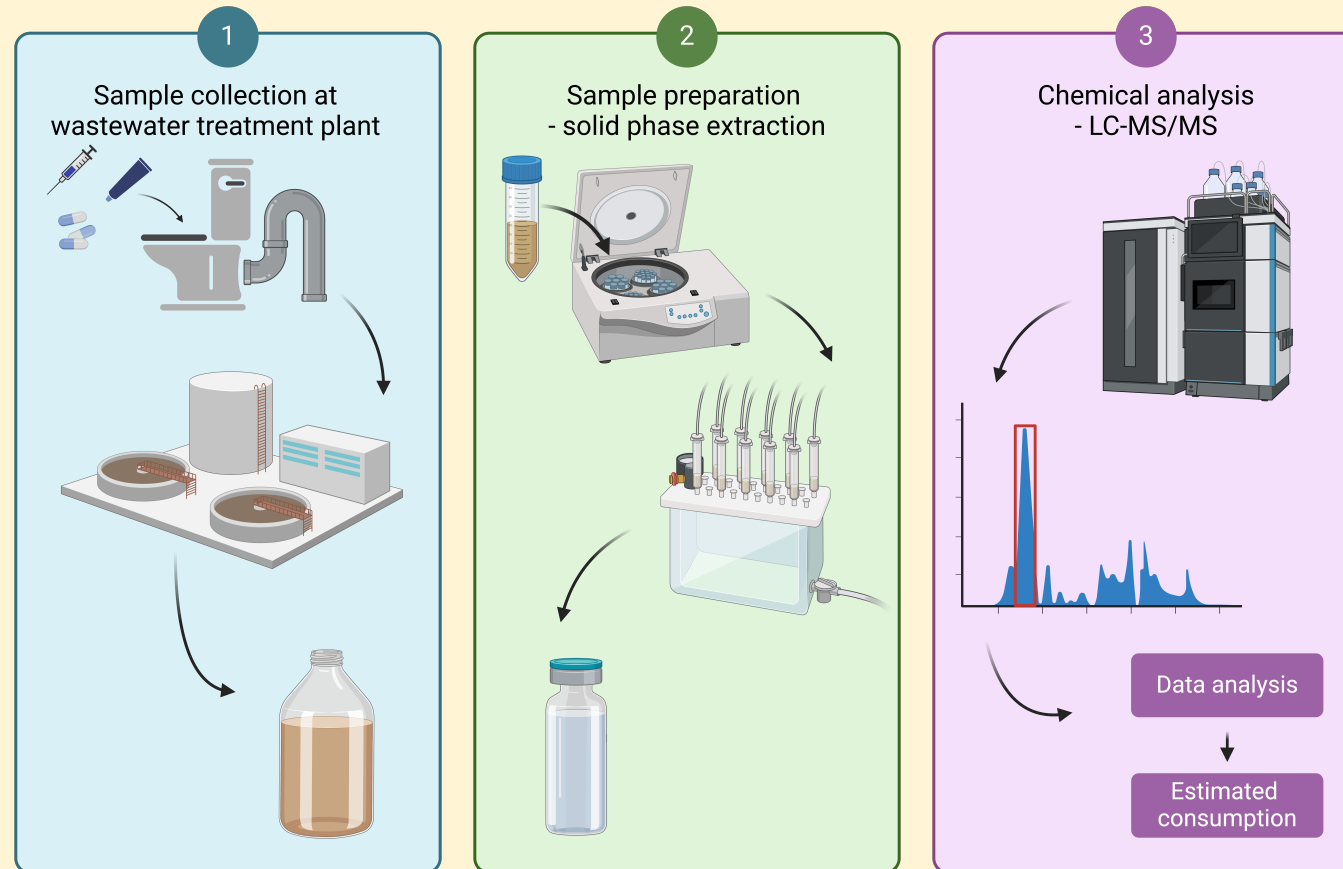


- Influent wastewater
- Measurements of excretion products
- Population-level
- **Broad application potential**
 - Drugs of abuse
 - COVID-19 pandemic
- Alternative to interviews or questionnaires
- Quantitative
- Objective and real-time data
- Low ethical risk
- High time- and cost-effectiveness

Wastewater-based epidemiology

- First suggested for monitoring illicit drug use in 2001
- Measurements can be used to estimate population normalised consumption/exposure
 - Wastewater flow
 - Population size
 - Excretion rate
- Effluent samples
 - Removal capacity
 - Detect environmental pollutants
- Few studies have used WBE to estimate AAS use
 - No studies consider endogenous production or prescribed consumption

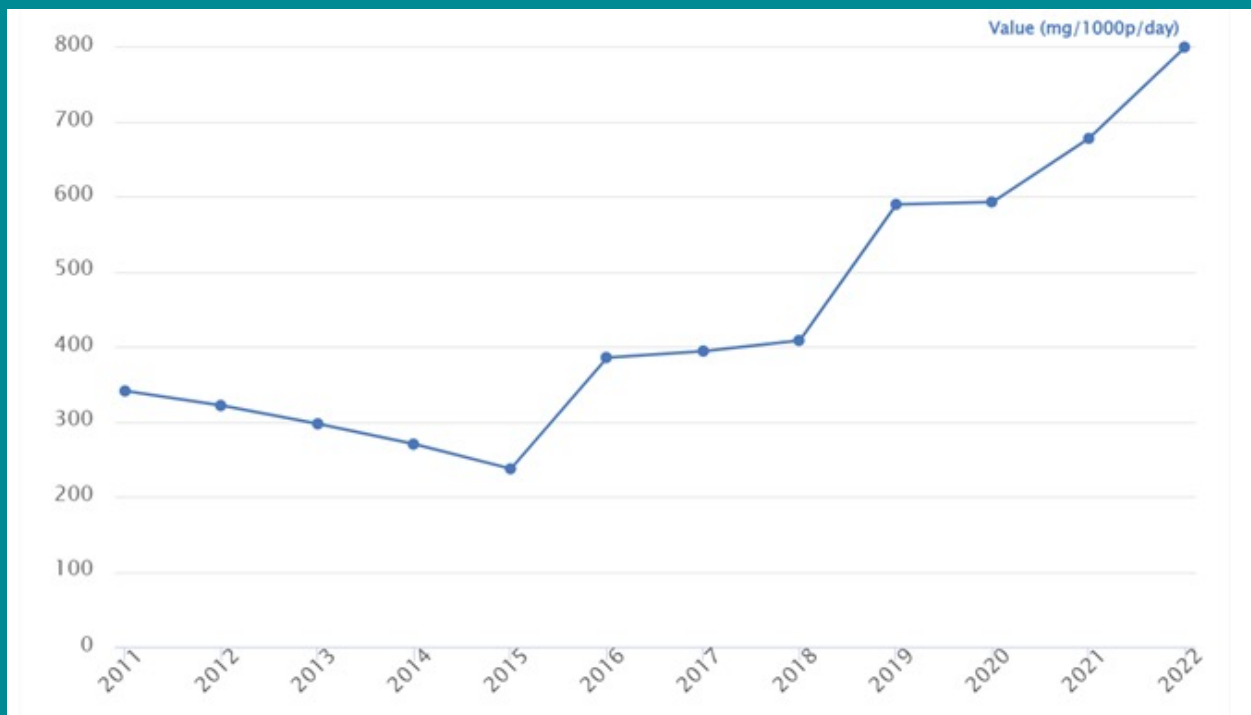
Methods



Experience from analysis of drugs

- RISE has measured drugs of abuse in wastewater since 2019
 - SCORE's annual measurement
- More than 2500 samples have been analyzed so far
- Substances
 - Cannabis
 - Cocaine
 - Benzoyllecgonine
 - Amphetamine
 - Methamphetamine
 - MDMA
 - Ketamine
 - Tramadol and Odm-tramadol
 - 6-monoacetylmorphine (6-MAM)
 - Cotinine
 - 3-CMC

Cocaine



Graph showing the development of cocaine use in 7 different EU cities, 2011 – 2022 (Antwerp, Zagreb, Milan, Eindhoven, Utrecht, Castellon, Santiago). These cities were selected since they have annual data from several years. Data from EMCDDA.

Develop an analytical method

Substance
Trenbolone
17 α -Trenbolone
Boldenone
5 β -Androst-1-en-17 β -ol-3-on
Metenolone
1-Metylenandrosteron
Metandrostenolon (= Metandienon)
Epimetendiol
Testosterone
1 α -Metylandrosterone
Drostanolone
2 α -Metyl-5 α -androstan-3 α -ol-17-on
Oxandrolone
17-Epi-Oxandrolone
Stanozolol
3-Hydroxystanozolol
Nandrolone
19-Norandrosterone
Epitestosterone
Oxymesteron e
Androstendione
Androstanolone, DHT
Oxymetholone
4-Clorodehydromethyltestosteron (CDMT)
Mesterolone
Dehydroepiandrosteron (DHEA)
Androsta-1,4-dien-3,17-dion (ADD)

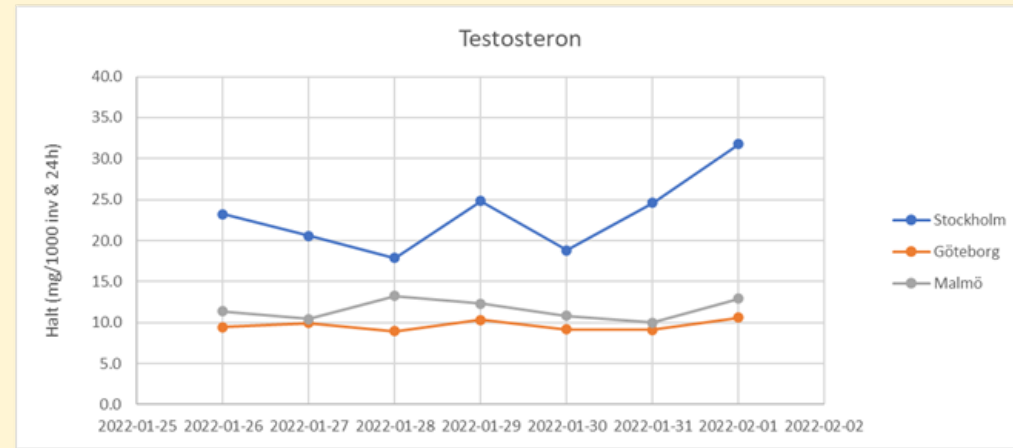
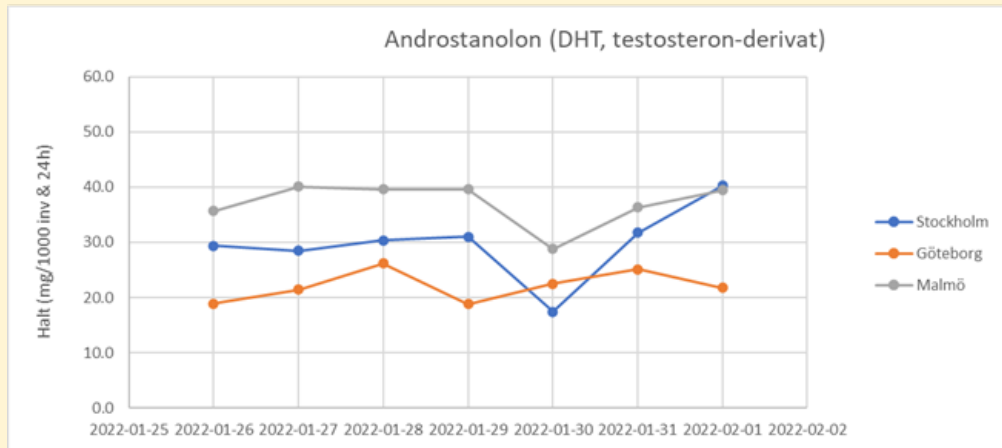
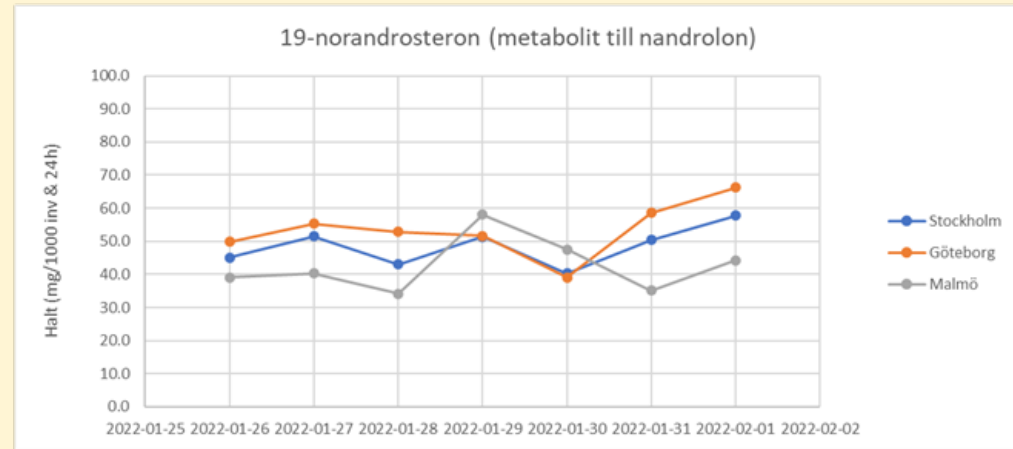
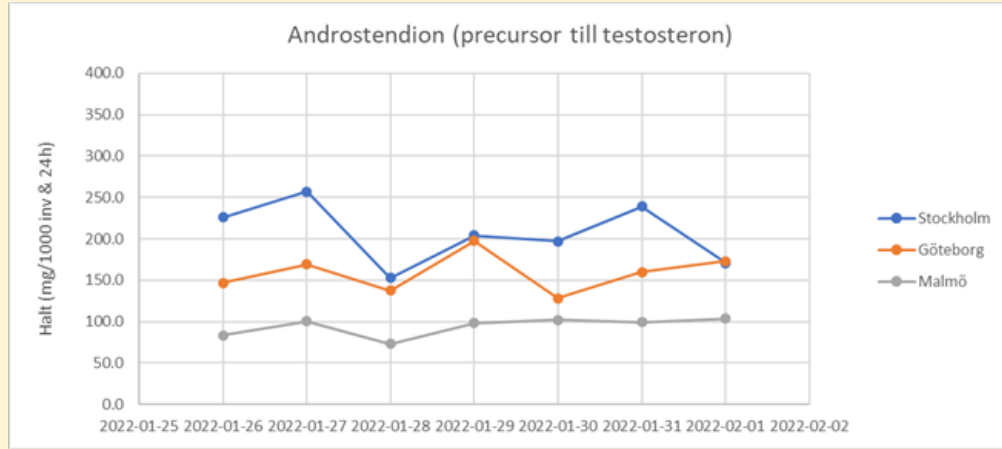
Quantification off AAS in wastewater from three Swedish cities (7-day series)

Out of the 26 AAS included in the doping panel, 7 AAS were detected in all 21 investigated samples, with concentrations between 1 and 690 $\mu\text{g}/\text{m}^3$ (mass flow 0.4 – 290 mg/1000 inhabitants & 24h).

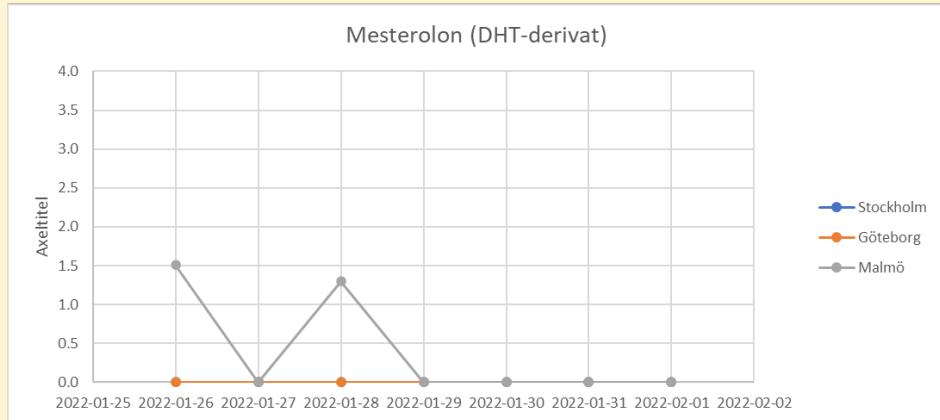
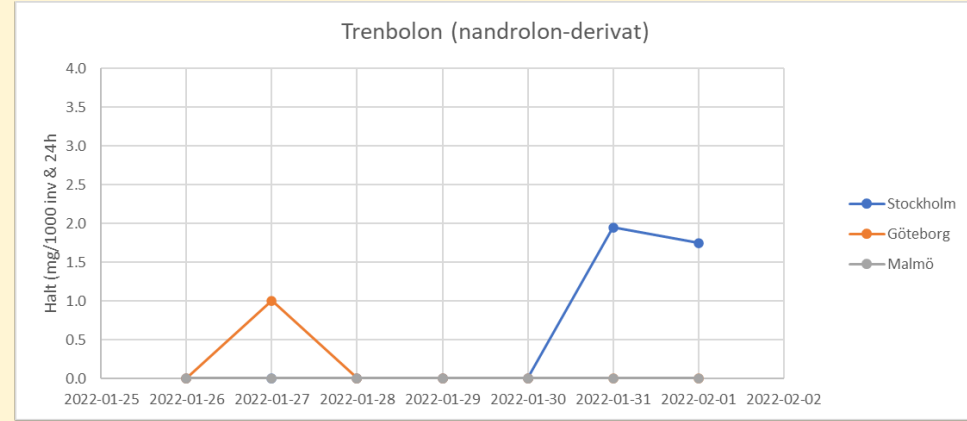
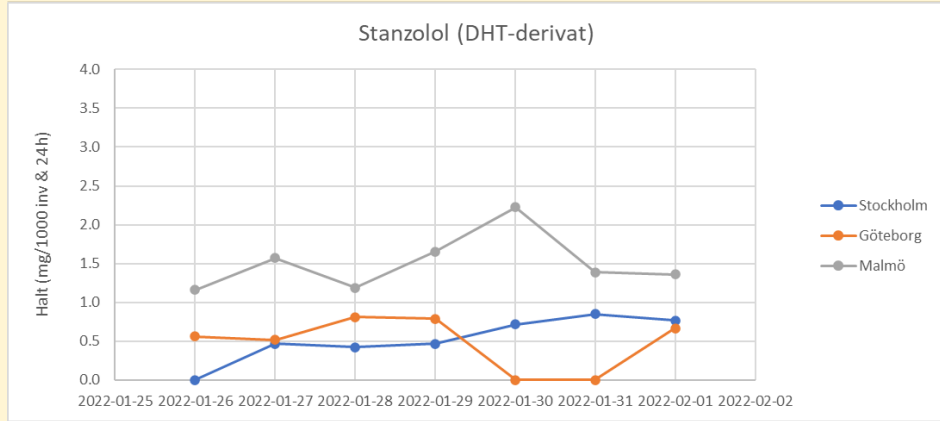
In addition, some of the AAS were detected sporadic.



Results from 3 cities in Sweden



Results from 3 cities in Sweden



Substances in RISE panel (16)

Substans

19-Norandrosterone (Nandrolone metabolite)

Trenbolone

Androstendione

Boldenone

Testosterone

Oxandrolone

Epitestosterone

4-Dihydroboldenon (metabolite)

Androstanolone (Dihydrotestosterone, DHT)

Stanozolol

Oxymesterone

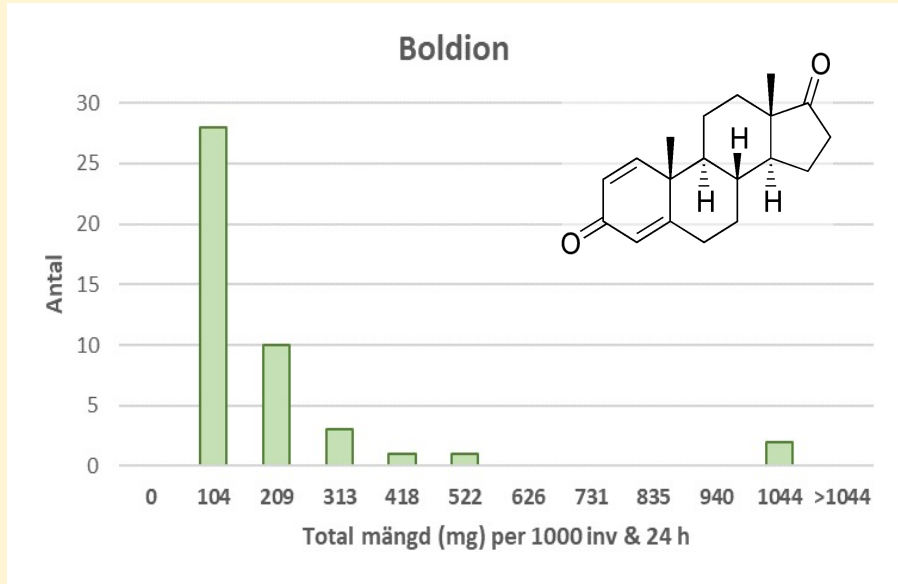
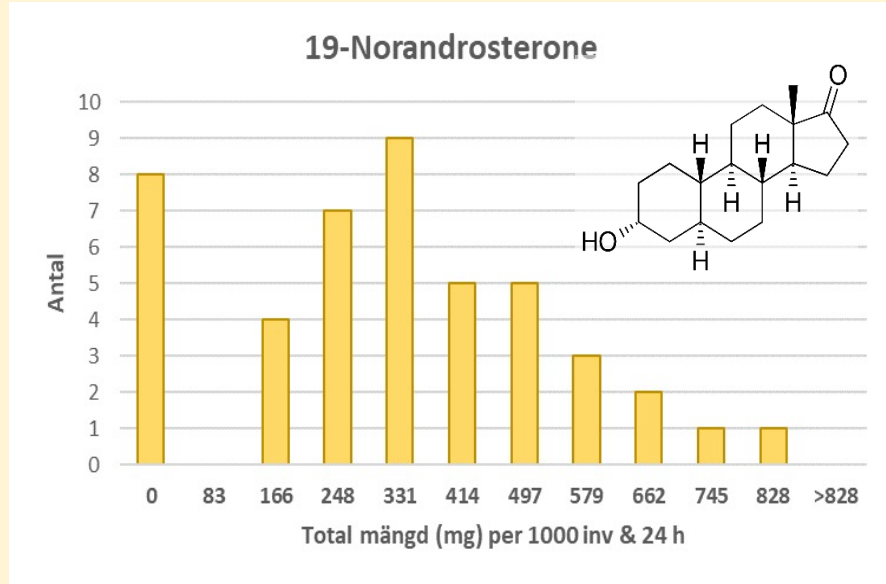
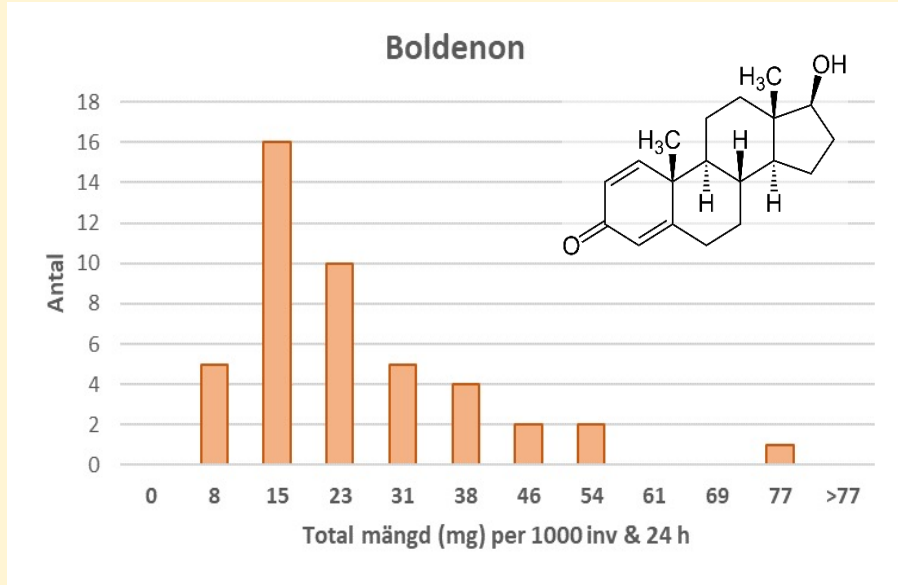
Oxymetholone

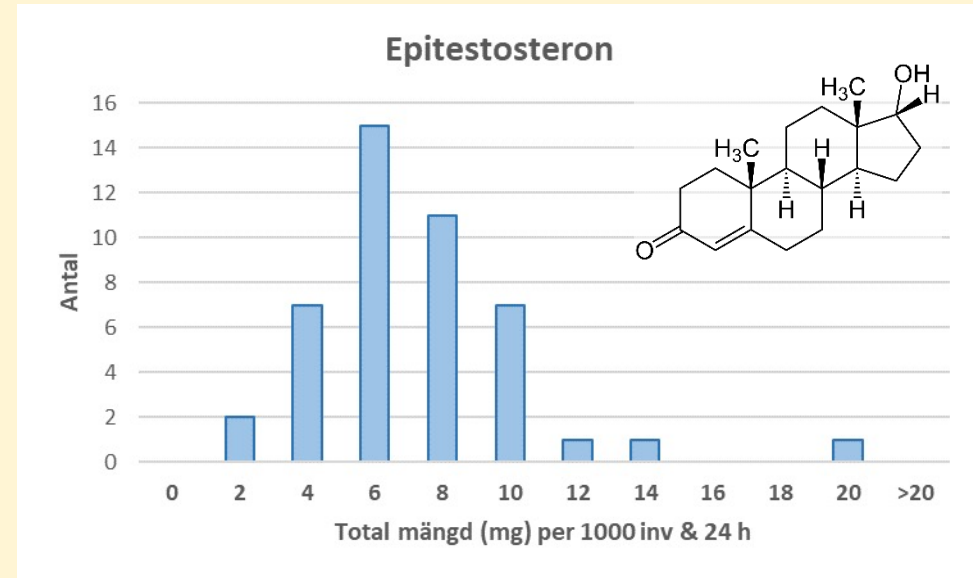
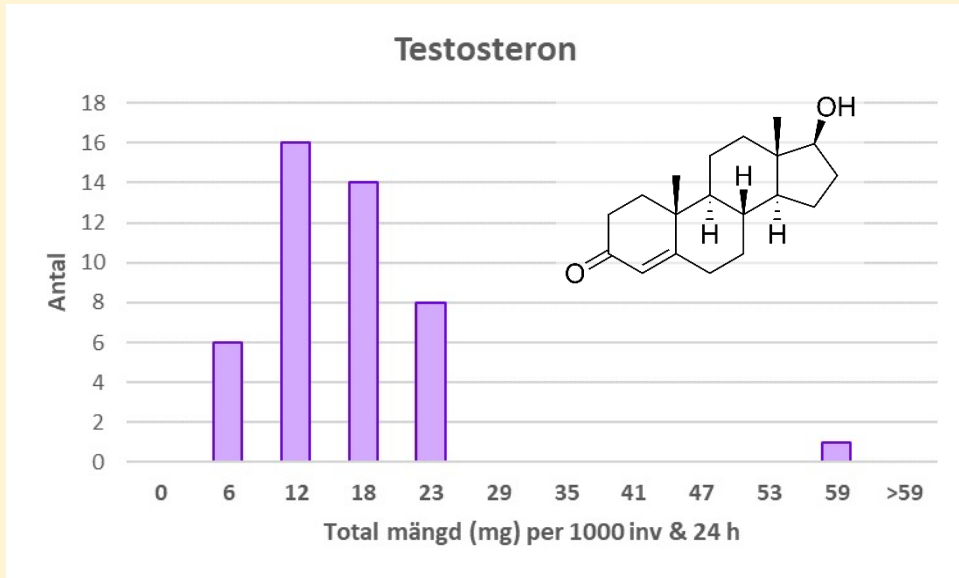
Chlorodehydromethyltestosterone (4-CDMT)

Boldione

3-Hydroxystanozolol (metabolite)

Mesterolone



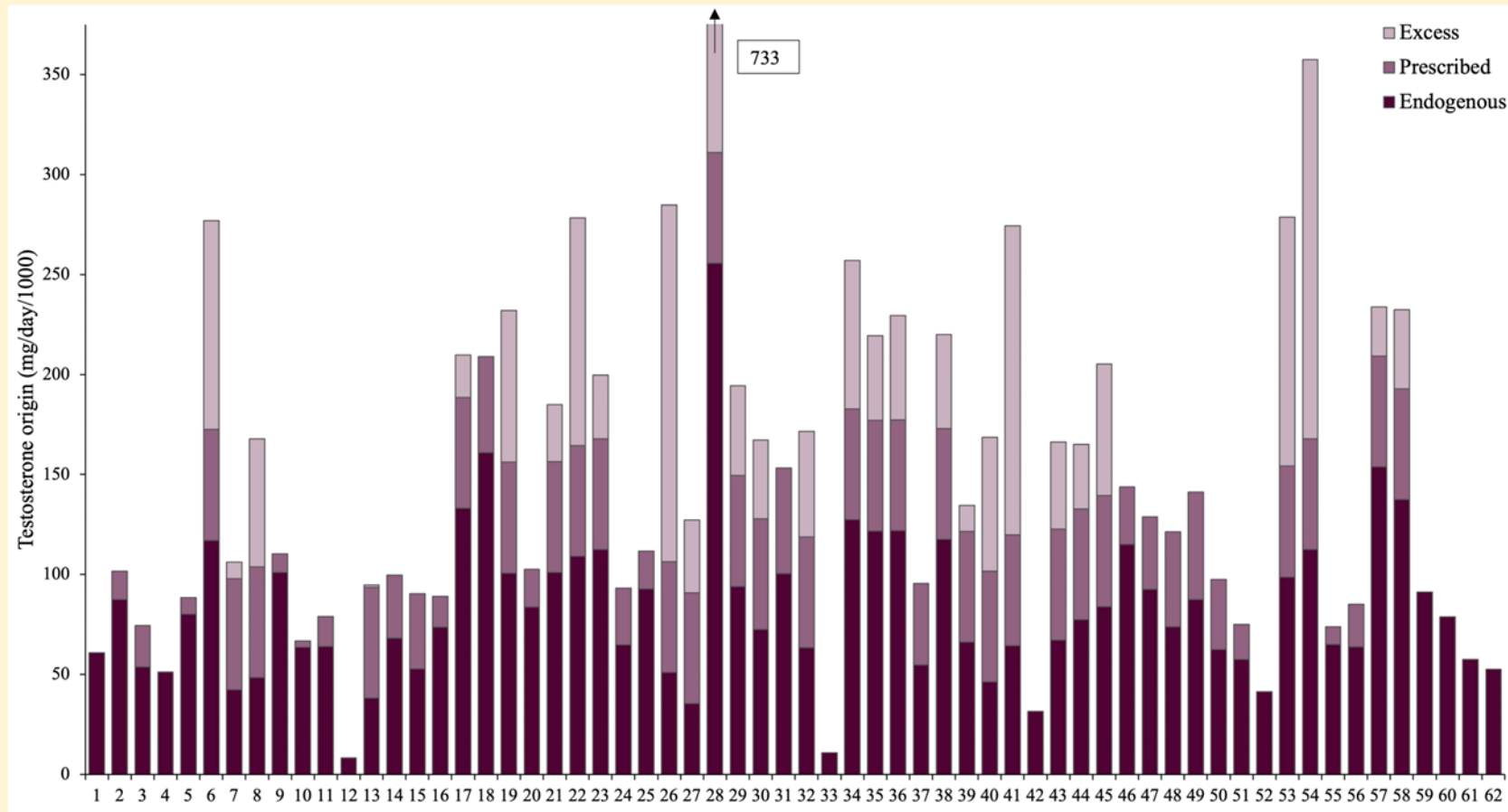


Strictly synthetic AAS

Findings

- Trenbolone (24.2%)
 - Stanozolol (4.8%)
 - 3-Hydroxystanozolol (1.6%)
 - Oxandrolone (1.6%)
 - Oxymetholone (8.1%)
- Strongly indicative of illicit activities
 - All handling and use prohibited
 - No known natural sources

Testosterone origin



- 29 samples had concentrations exceeding the expected, can be seen as an indicator of illicit use

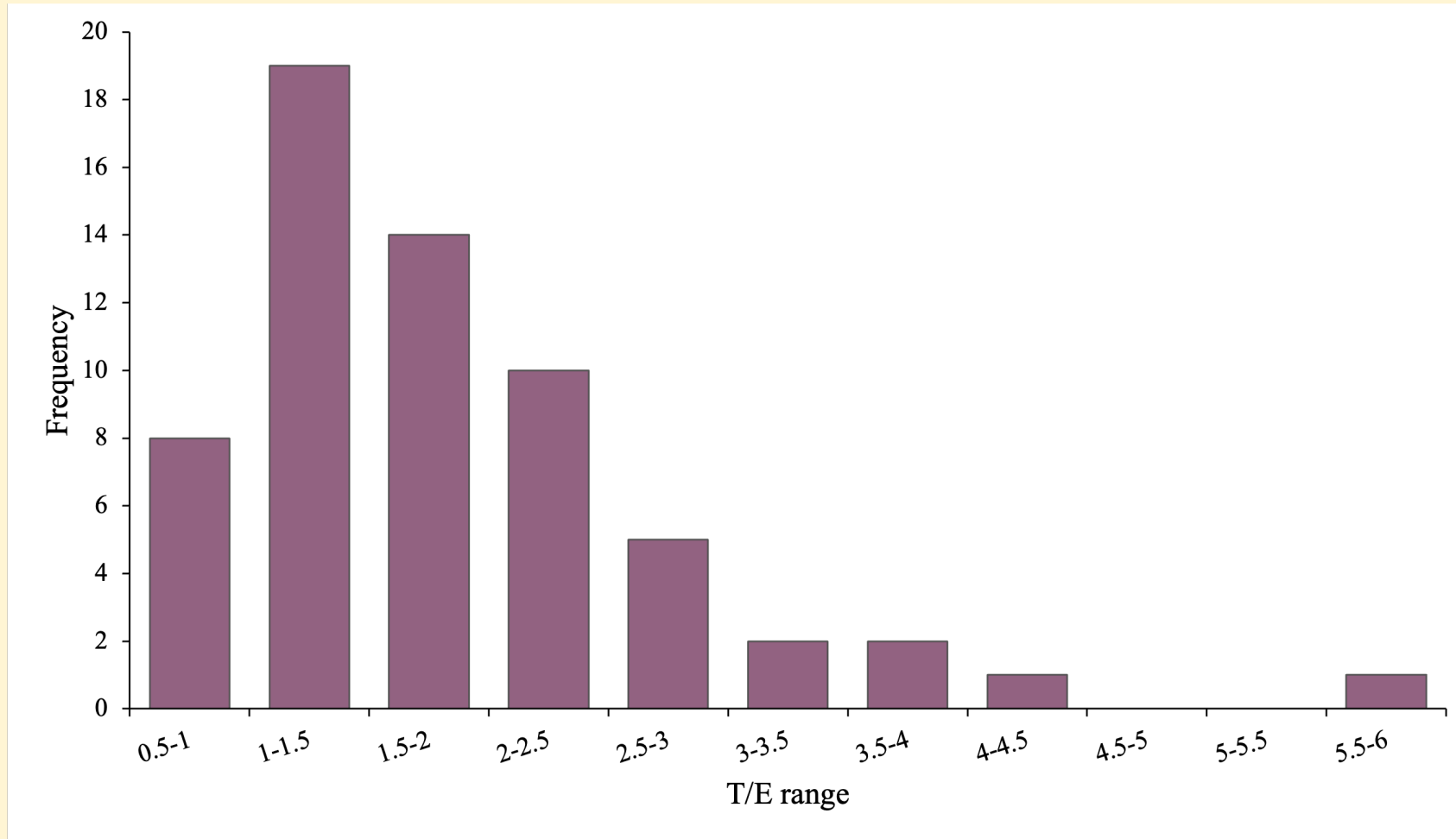
Testosterone- to-epitestosterone ratio

- T/E traditionally used for doping-control of individuals
- T/E > 2.0 indicative of illicit use
- T/E can be affected by
 - AAS use (other than Testosterone)
 - High alcohol consumption
 - Masking agents
 - To increase Epitestosterone
 - Demographics

Wanted to evaluate if T/E could be applied to WBE to predict if illicit use has occurred in a population

T/E	<i>n</i> (%)	Indicates illicit use <i>n</i> (%)
< 1.5	27 (43)	0 (0)
> 1.5 < 2.0	14 (23)	8 (57)
> 2.0	21 (34)	21 (100)

Testosterone- to-epitestosterone ratio



Examples of application



- Total use in a population
- Changes in consumption patterns over time – following trends
- Evaluation of measures for prevention
- Upstream measurements: part of a city, sports facilities, gym
- Monitoring during police operations (eg Operation Hagelstorm)

Conclusion

- First known attempt of using WBE to estimate community AAS abuse
- Strong evidence of trenbolone, stanozolol, oxandrolone, and oxymetholone use
- Indicative of testosterone use
- No data on whether AAS were used by humans or directly disposed into sewer

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