REACTIVATION OF CONJUGATED ENVIRONMENTAL HORMONES IN WATER

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Outlines

- Endocrine disruption & environmental hormones
- Behavior of estrogens from sources to the environment
- Activity of arylsulfatase and the deactivation of conjugated estrogens
- Conclusions

Endocrine Disruption & Environmental Hormones

Endocrine disruption observed in wild life

- Compromised immunity and reproduction in

- If *animals* are affected by endocrine disrupters……

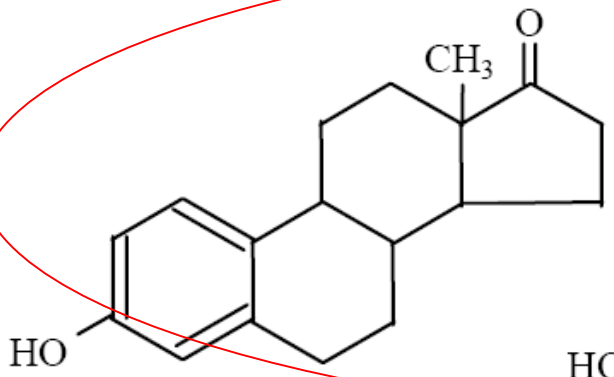
what about *humans*?

Environmental Hormones/Endocrine Disruptors

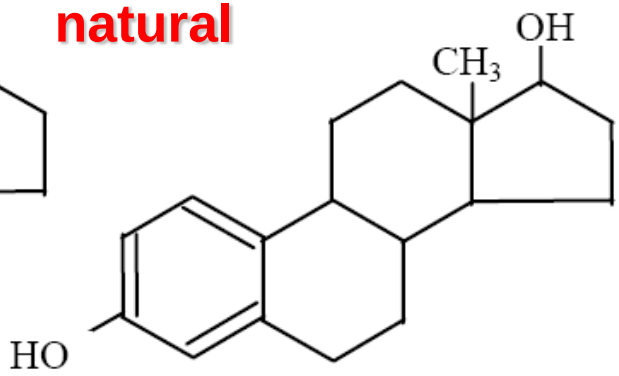
- Steroid estrogens
- Phytoestrogens
- Organic oxygen compounds
  (bisphenols, dioxin, phthalate……)
- Surfactant
  (alkylphenolic compounds)
- Polyaromatic compounds
  (PolyChlorinated Biphenyls, brominated flame retardants, Polycyclic Aromatic Hydrocarbons)
- Pesticides

Chemical structure of free estrogens

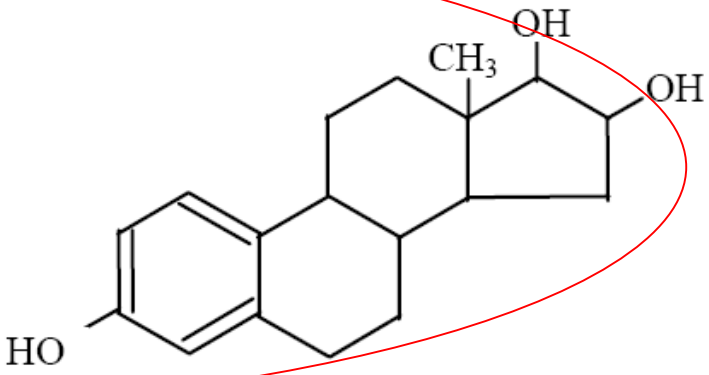
natural



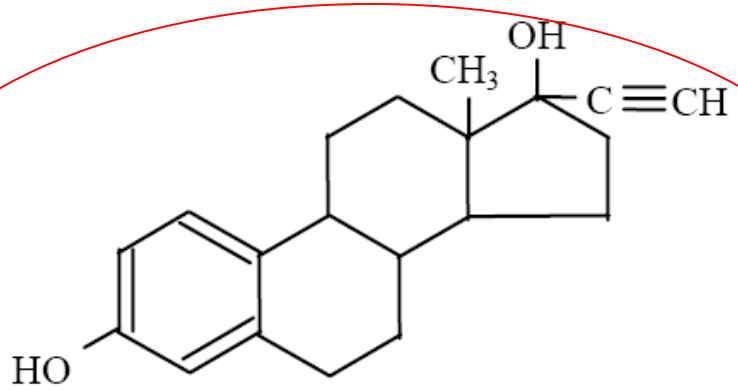
Oestrone (E1)



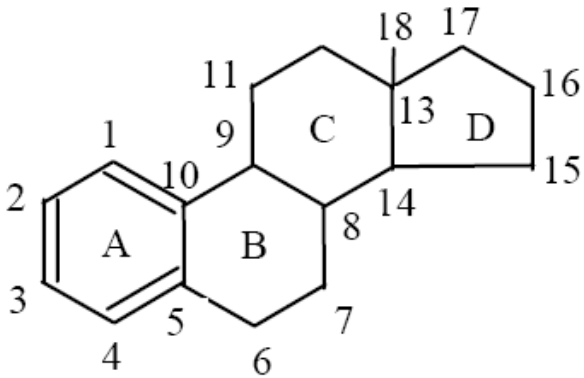
Oestradiol (E2)



Oestriol (E3)



Ethinyl oestradiol (EE2)



Cydopentanophenathrene ring

synthetic

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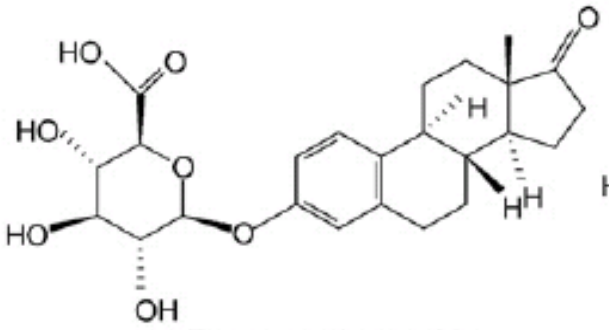
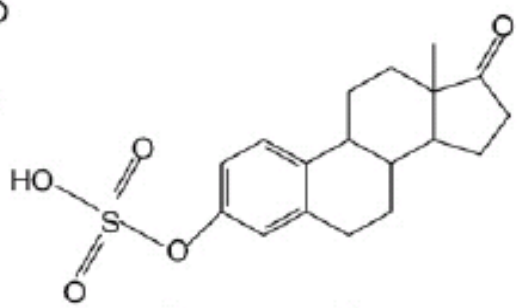
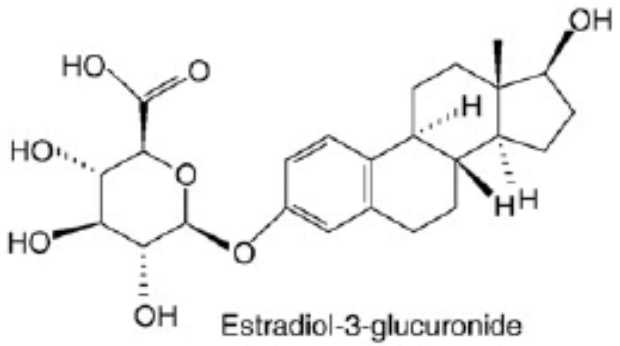


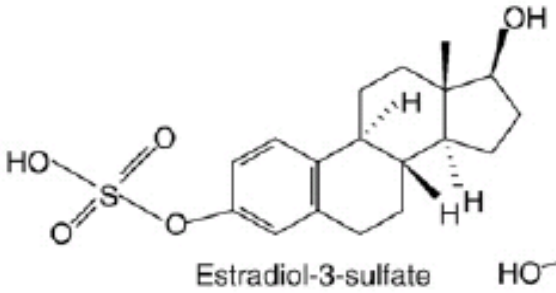
Estrone-3-glucuronide



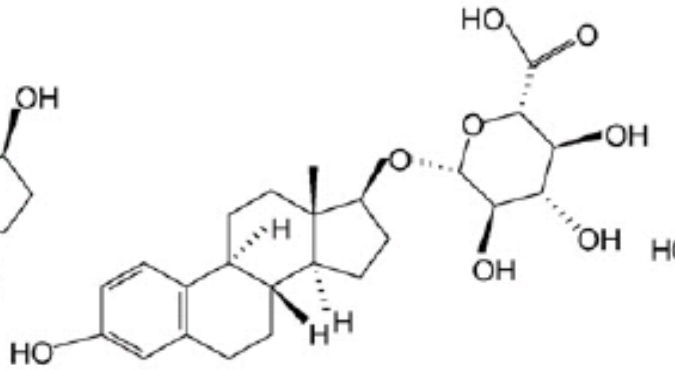
Estrone-3-sulfate



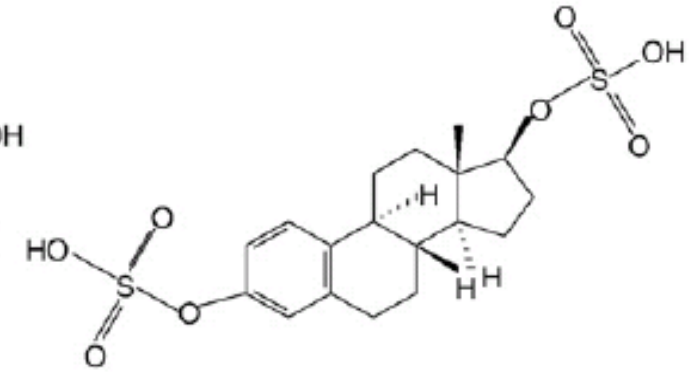
Estradiol-3-glucuronide



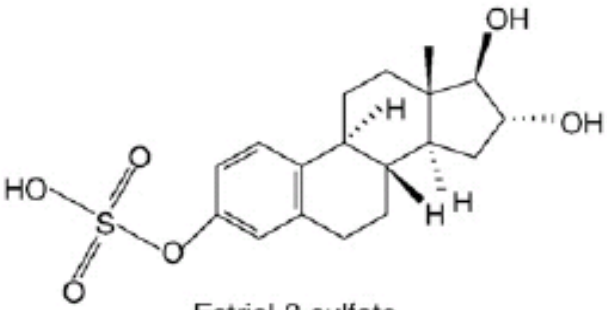
Estradiol-3-sulfate



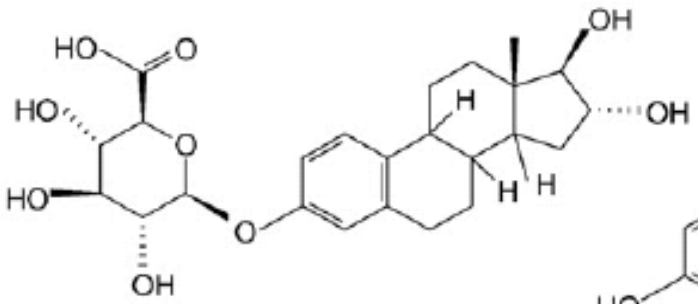
Estradiol-17-glucuronide



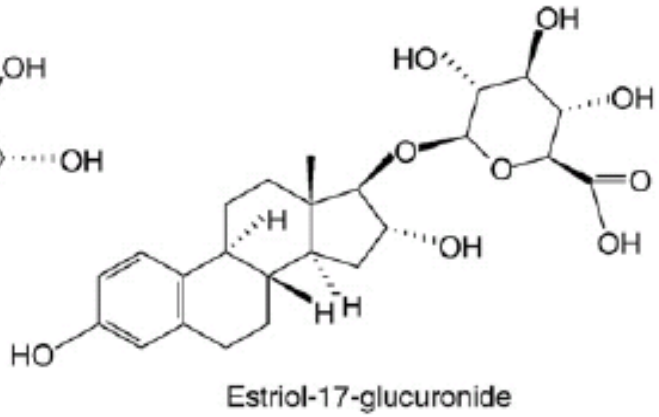
Estradiol-3,17-disulfate



Estriol-3-sulfate



Estriol-3-glucuronide



Estriol-17-glucuronide

Chemical structure of conjugated estrogen

- To protect the safety of eco-environment and water resources, the threat of steroid estrogens need to be concerned.
- E1, E2 & EE2 have been restricted to discharge in the UK
- E2 & EE2 have been listed in the new drinking water standard in Japan
- European Union and America have listed E1, E2, and EE2 in the first watch list and contaminant candidate list

Behavior of estrogens from sources to the environment

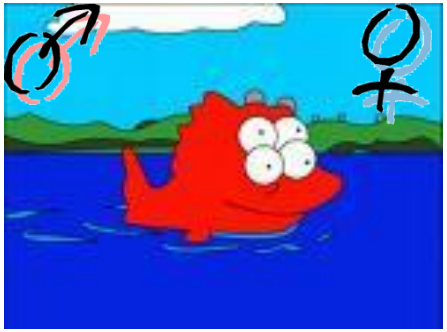
How does the intersex occur?

live stock

human urine & feces

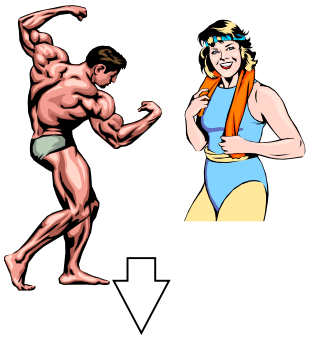
Sewage treatment

oestrogenic STW effluent



river fish intersex

Transformation of estrogens



Human body excretion: free estrogens (E1, E2, E3, EE2)

Liver deactivation: conjugation—sulfated and glucuronided estrogens

Urine & feces:

Sewers:

STWs:

Water bodies

Deconjugation: enzyme catalysis—arylsulfatase(AryS), β-glucuronidase (β-GluD)

Reported investigations of free estrogens

Counties/cities	Water samples	E1（ng/L）	E2（ng/L）	EE2（ng/L）
UK	Severn Trent River	<7.1	<2.5	-
Italy	Tiber River	1.5	0.22	0.11
Germany	rivers	0.10-4.1	0.15-3.6	0.10-5.1
Netherlands	Rivers and sea	<3.4	<5.5	<4.3
Japan	Over 100 rivers	<107.6	<27	-
US	River	<27.0	-	-
France	Rivers	-	-	1.0-4.0
Span	Liobregat River	0.75-0.95	-	-
Korea	Han River	1.7-5.0	-	-
CHINA Cities	**Water samples**	**E1（ng/L）**	**E2（ng/L）**	**EE2（ng/L）**
Harbin	Songhua River	26.0-44.0	7.0-29.0	<13.0
Hangzhou	River	-	<0.32	1.17-3.35
Wuhan	River, lake	28.1	-	-
Qingdao	Haibo River	97.0	31.0	70.0
Guangzhou	Pear River	<50.0	<2.0	-
Chongqing	Jialing River	2.1-12.8	0.9-4.8	ND-5.4

The concern on conjugated estrogens

- CE-G can be deconjugated by β-GluD in hours and the CE-S are more stable to survive the sewage treatment.
- **UK**: E1-3-S and E2-3-S were 10-17 ng/L and 5-11ng/L in STWs final effluent. E2-3-S can be converted to E1-3-S.
- **Italy**: **E1--**44 ng/L to 17ng/L, **E1-3-S**--25 ng/L to 9 ng/L, E1-3-S is **40-50%** of E1 in STWs final eff.
- **US:** CE-S concentrations were 1/3-1/2 of the free ones in the farm wastewater.

CE-S may be the 'reservoir' of free estrogens in the environment!

Pressure brought by rapid urbanization

- centralized sewage treatment in towns and cities — non-pointed pollution to pointed pollution
- Rapid population increase — soared estrogen loads in a specific catchment

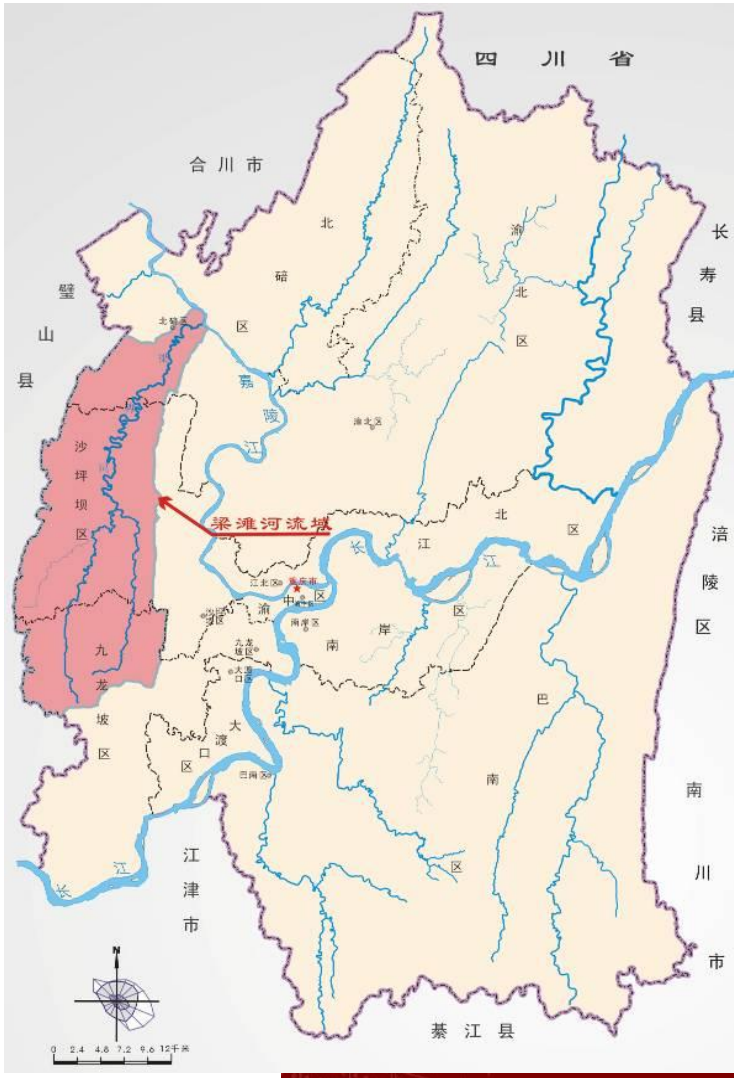
Activity of Arylsulfatase and the reactivation of conjugated estrogens

Investigtion in Liangtan River

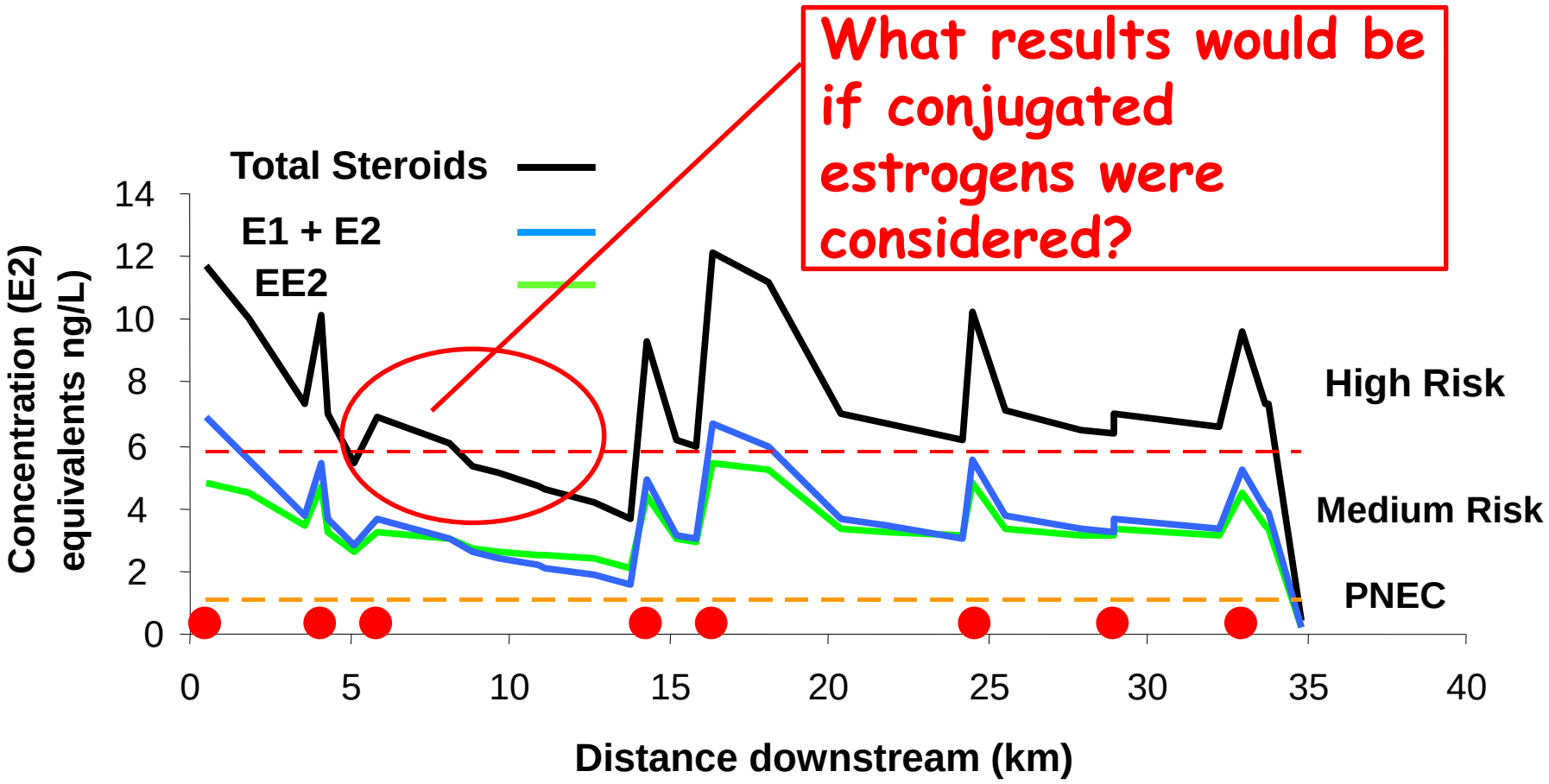
CSO

Final effluent

Sampling sites in STW



Concentrations of free steroid estrogens along a river receiving multiple STW inputs



The enzyme hydrolysis of E1-3-S

$$R\text{-}O\text{-}SO_3H + H_2O \xrightarrow{\text{Arylsulfatase}} R\text{-}OH + 2H^+ + SO_4^{2-}$$

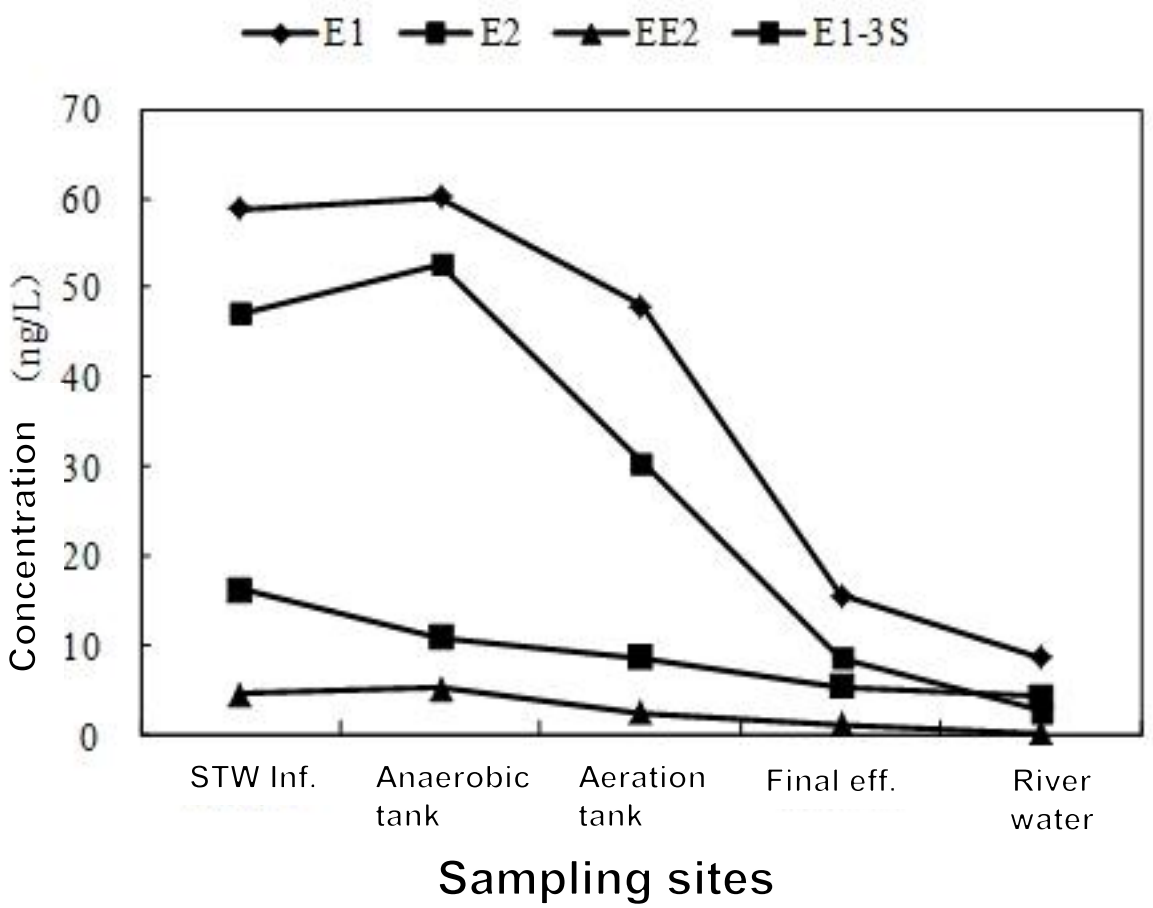
Estrone-3-sulfate(E1-3-S)

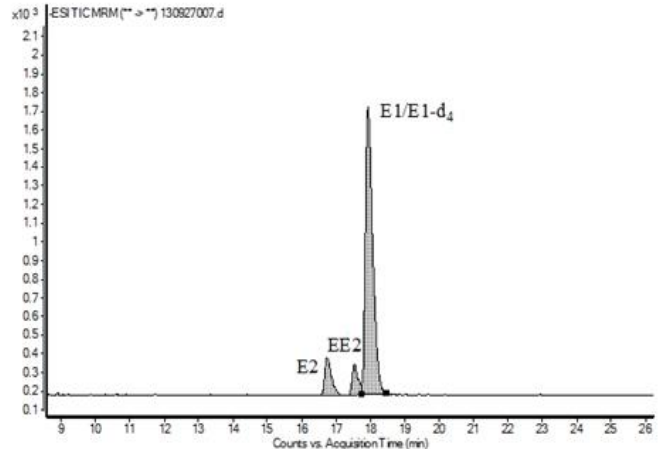
inactive

Estrone (E1)

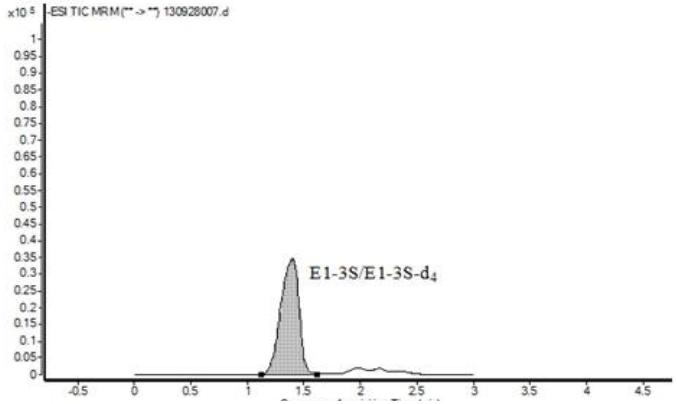
active

Concentrations of free and conjugated estrogens



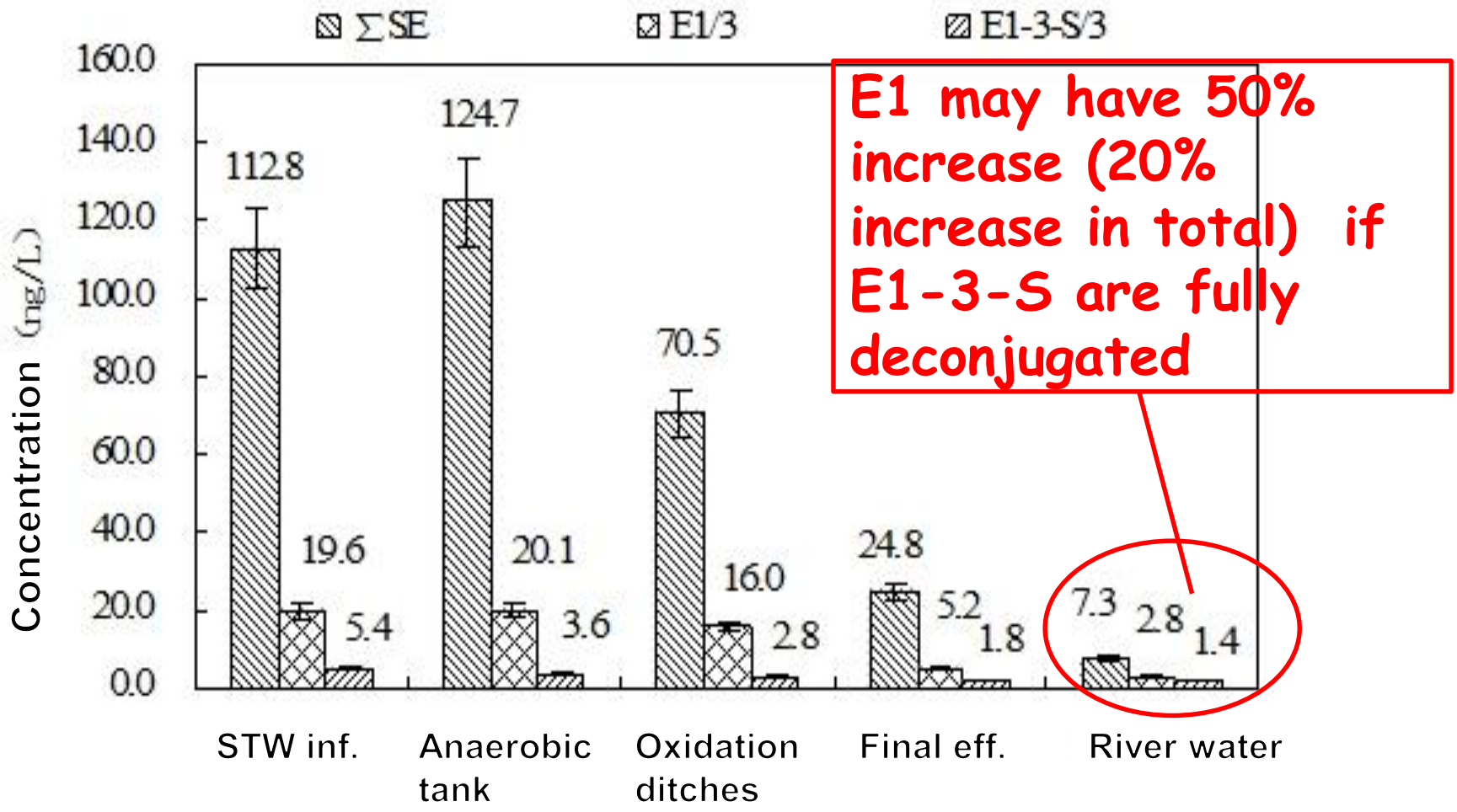


Chromatography chart of E1, E2, & EE2, E1-d4



Chromatography chart of E1-3S & E1-3S-d4

Concentrations of total estrogen, E1 and E1-3-S in E2 equivalent



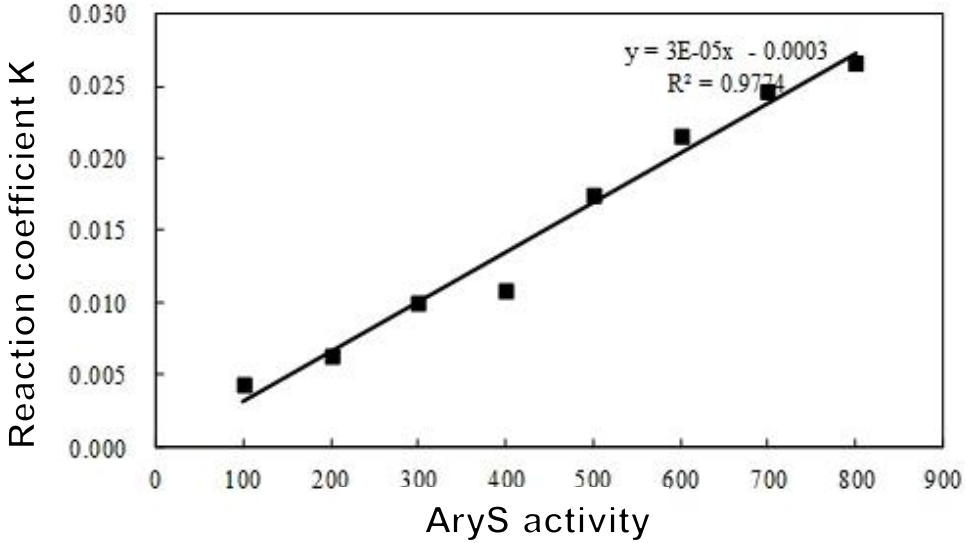
$\sum SE=[E1]/3 + [E2] + 10\,[EE2]$

Activity of AryS in different sampling sites

Sampling sites	River water	CSO	Aerobic section	Anoxic section	Anaerobic section
Activity of AryS (μg *p*-nitrophenol/g·h)	40.35	122.77	586.25	592.44	600.26
Activity of AryS (U)	0.143	0.422	1.992	2.013	2.040

- The activities of AryS in STW are higher than in sewers and river water.
- Activated sludge may have plenty of microorganims which excrete AryS to maintain a higher activity.

- The deconjugation of E1-3-S follows the **first order reaction**, and the reaction rate is positively linearly correlated with AryS activity
- The **half life time** of E1-3-S at the AryS activities similar to **STW (2.04U), CSO (0.42U)** and **river water (0.14U)** at normal conditions would be**39**, **204** and **770 hours**, respctively.



Conclusions

Conclusions

- Sulfated estrogens can survive the sewage treatment with the E1-3-S most stable, which may contribute to 20% increase of total estrogen (in E2) if thoroughly deconjugated.

- Ambient environmental conditions may affect the deconjugation of E1-3-S by affecting the activity of AryS, which is positively correlated with the enzymatic hydrolysis reaction rate.

- Based on the investigated AryS activities and labscale experiment on E1-3-S enzymatic hydrolysis, The half life time of E1-3-S at STW, CSO and river water would be 39, 204 and 770 hours, respctively


Many thanks!
Any questions?