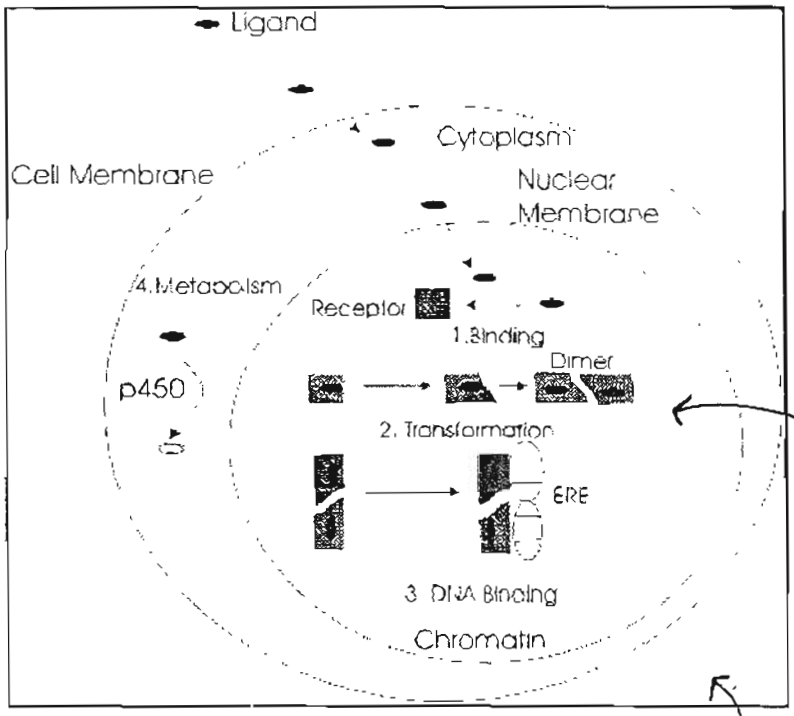
Mechanism of action of steroid hormones – estradiol

- involves binding of estradiol to a ***steroid hormone receptor*** – note similarity to mechanism of action of dioxins (my page 170)

intra-cellular receptor that performs signal transduction for steroid hormones like estradiol



Binds to specific regions of DNA in the cell nucleus .. this determines the action

B.J. Cox, MSc Thesis

- estrogen receptor found in females especially in liver and ovaries: two forms, ER-α and ER-β. (estrogen receptor, α & β)
- estrogen mimics bind to the ER, either leading to estrogenic effects (agonist) or blocking them (antagonist)
- to be an estrogen mimic, the molecule must:
  - be the correct size and shape
  - normally have polar groups (especially –OH) located to hydrogen bond with amino acids in the active site

rather "promiscuous" .. binds to a number of compounds, often structurally different to estradiol.

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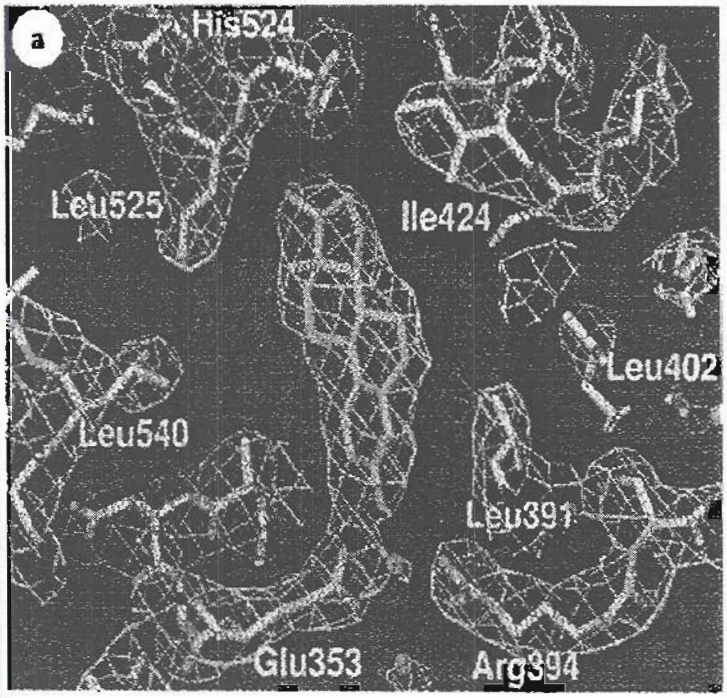
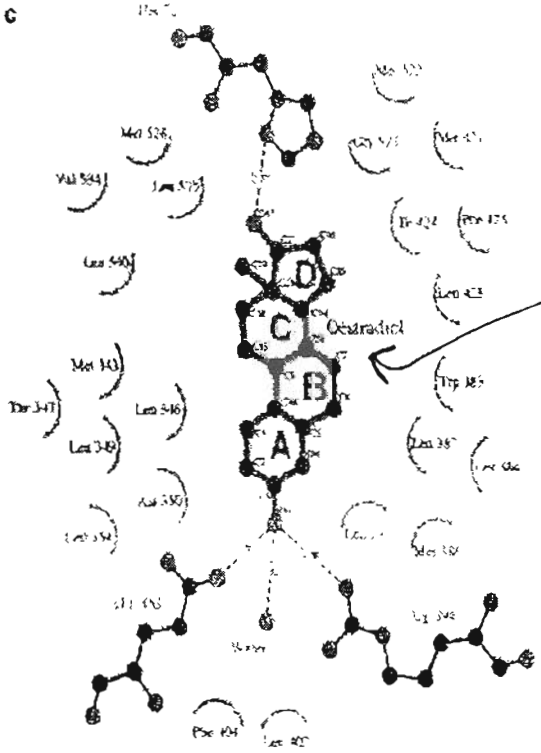
The ER Binding Domain





see p175 for estradiol structure

Here the OH groups H-bond

Brzozowski, Nature, 389, 753 (1997)

small 'c' so means "wide-ranging"

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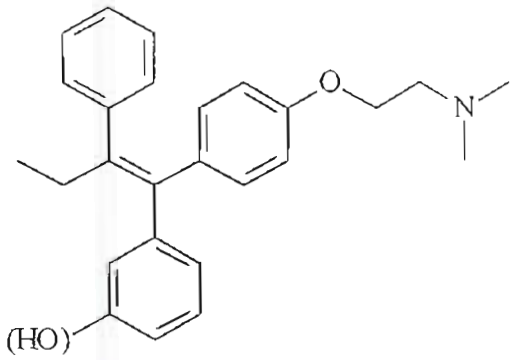
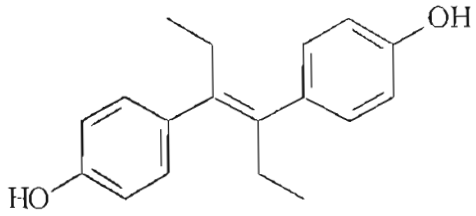
ER Binding

- ER binding site described as "catholic": other ligands make contacts with different aminoacids in the binding domain
- Presence of at least one –OH group is normally important
- Relative binding affinities (RBA), rat liver ER-α *vs* 17β-estradiol = 1

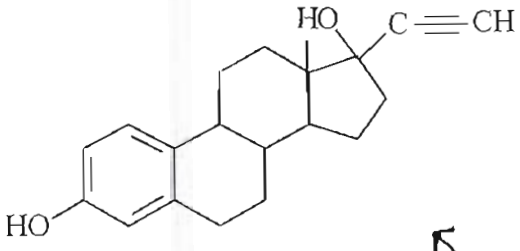
Tamoxifen	3.3	17α-Ethynylestradiol	2.0
Diethylstilbestrol	0.9	Estradiol-3-benzoate	0.4
α-Zearolenone	0.11	Estrone	0.08
Estriol	0.05	Tetrachlorobiphenylol	0.004
o,p-DDT	0.0001		



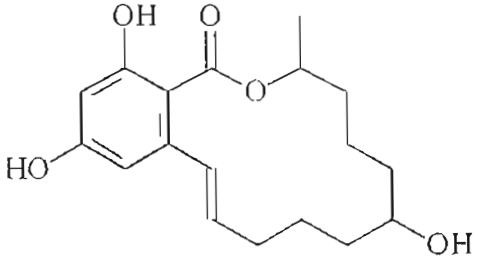
(hydroxy)-tamoxifen



diethylstilbestrol



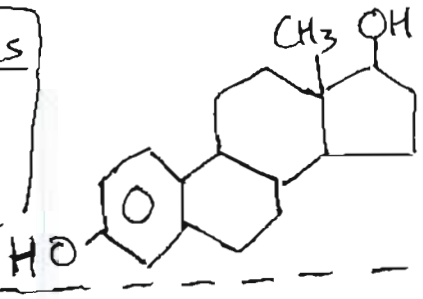
ethynylestradiol



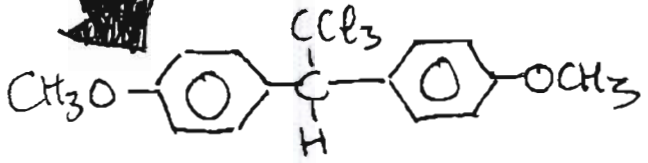
zearolenone

Note: ⇒ 17β ethynylestradiol contraceptive pill

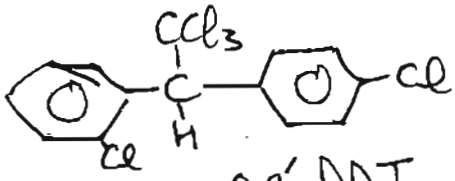
Some EDCs endocrine disruptors (environmental estrogens)



estradiol; the main human estrogen



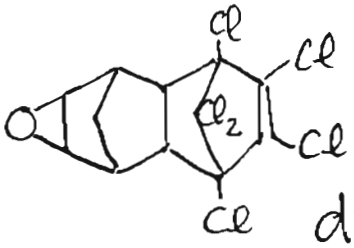
methoxychlor



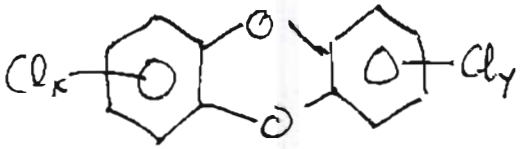
o,p'-DDT

pesticides

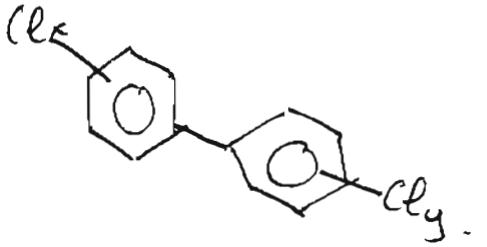
kepone



dieldrin

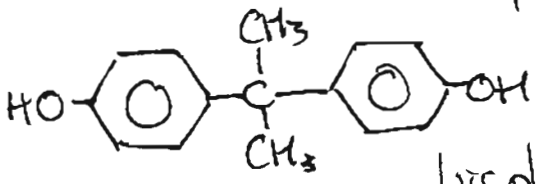


dioxins



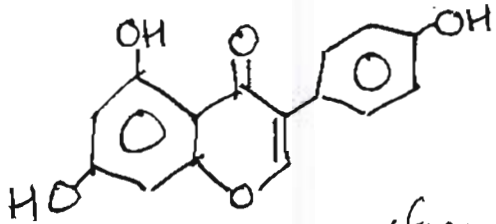
PCBs.

nonylphenol (octylphenol, C_8, more active).

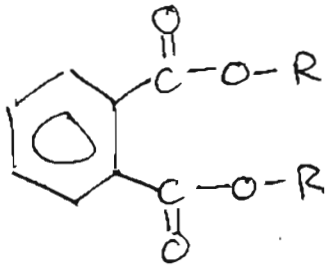


bisphenol-A

polymerised into plastics and ↑ expoxy resins



genistein (from pulp-mill effluent)



phthalate esters (R = ethyl, n-butyl, n-hexyl, n-octyl .....)

used as plasticizers (inc. in PVC intravenous bags.

Note: overall structures are different, but many have –OH groups (like estradiol)

or e.g. dioxins/PCBs/some pesticides, get metabolised ⇒ OH groups

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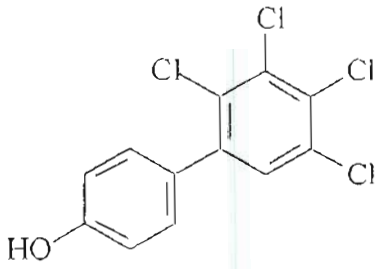
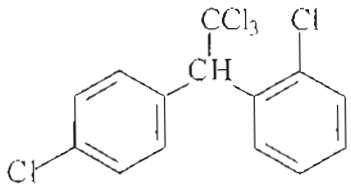
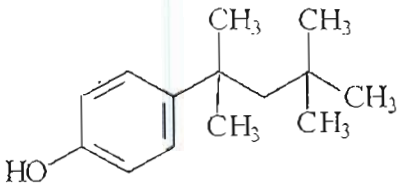
Other estrogenic compounds



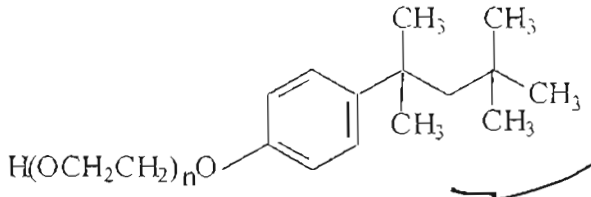
tetrachlorobiphenylol



o,p-DDT



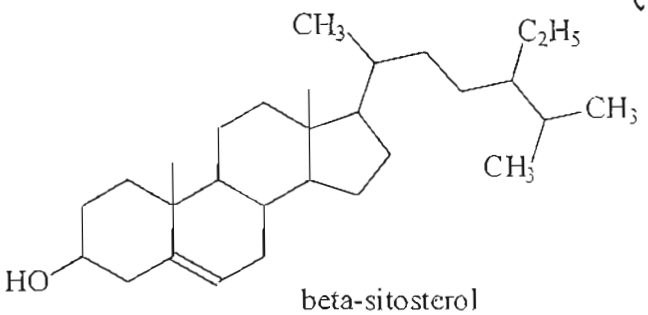
octylphenol



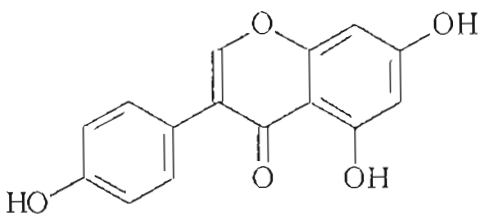
an octylphenolethoxylate [detergent]

EU banning ethoxylates (detergents, spermicides, paints, plastics

(breakdown leads to octylphenol (prev. page).



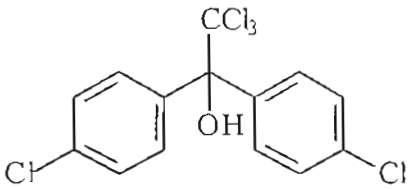
beta-sitosterol



genistein (in clover and soy)

(a flavonoid)

↓ some worries about babies fed with soy milk



difocol

↔ Toxic equivalency factors

- application of the TEF concept to xenoestrogens

  $\text{TEQ} = \Sigma(c_i \times \text{TEF}_i)$ where the TEF of estradiol = 1

- relative binding affinities (RBAs) sometimes taken as a surrogate for TEF: *why are they not the same?*

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Effects possibly linked to endocrine disruptors

ex. Kate Stuttaford notes.

- reproductive effects
  - decreased fertility
  - genital deformities
  - early puberty
  - endometriosis
  - low sperm count
  - breast, uterine, prostate and testicular cancers
- thyroid malfunction - lasting effects on growth and development
  - short-term memory loss
  - planning ability
  - attention deficit disorder
  - problems with metabolism and blood pressure
- neurological disorders
  - abnormalities in behavior
  - difficulty in learning
  - distorted sensory functions
- immunological disorders
  - susceptibility to disease
  - hypersensitivity
  - allergies

IMP. point? many of the EDC effects don't necessarily show up in the adult mammals, but they (EDCs) do get transferred to the fetus (or egg). Disrupts the hormone balance in the young or when they get older.

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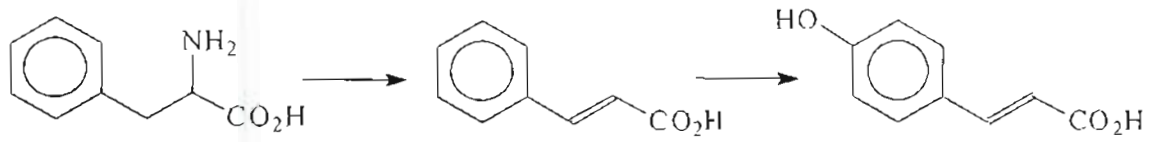
ex. Kate Stuttaford notes

Vitellogenin Production in Fish

- vitellogenin is an egg yolk protein normally produced in mature female fish by estrogenic stimulation
- vitellogenin production in male or immature female fish is taken as an indicator of estrogenic exposure
- this effect has been observed in fish exposed to domestic and industrial effluents
- European study of various effluents found many induced vitellogenin production
    - in some domestic sewage most estrogenic effects were attributed to estrogenic steroids from human excretion
    - a significant portion was due to ethinylestradiol (active ingredient in birth control pills)
    - in industrial effluent there were no estrogenic steroids, but found other estrogenic species:
        - nonyl phenol and related compounds derived from industrial surfactants
        - bisphenol A (BPA) used in manufacturing of plastics and epoxy resins
        - hydroxy phenyl hexanoic acid found in effluent from chemical manufacturer
    - study found that most raw sewage influent was highly estrogenic, but effluent varied
        - some sewage treatments may be better than others at removing estrogenic compounds
        - secondary (biological) treatment and residence time found to be major factors

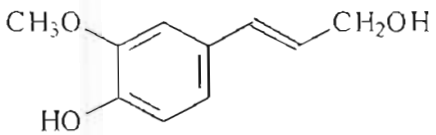
The pulp and paper industry

- wood pulp contains both cellulose fibers (—> paper) and lignin (extracted and discarded). Lignin is polymeric – biosynthesized from phenylalanine, CYP 73, a cinnamate-4-hydroxylase, catalyzes a key step in the conversion of phenylalanine into lignin.

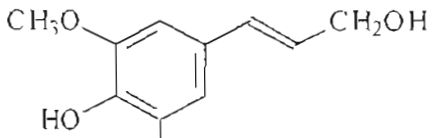


- hardwoods contain ~25% lignin; softwoods ~30% lignin

- lignin building blocks



coniferyl alcohol



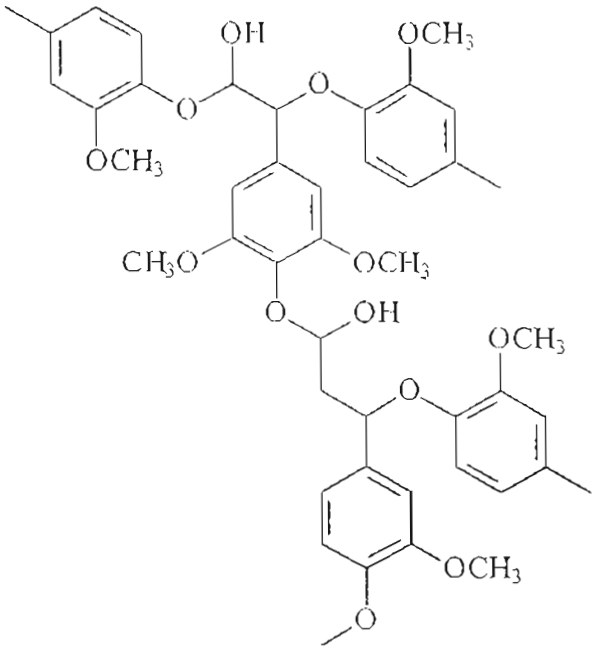
sinapyl alcohol

- lignin formed by polymerization catalyzed by peroxidases and laccases in the plant —> free radical processes

- controversy over whether lignin is a directed structure or a random structure

- paper-making requires removal of the lignin: deligninification: Kraft pulping; sulfite pulping; thermomechanical pulping

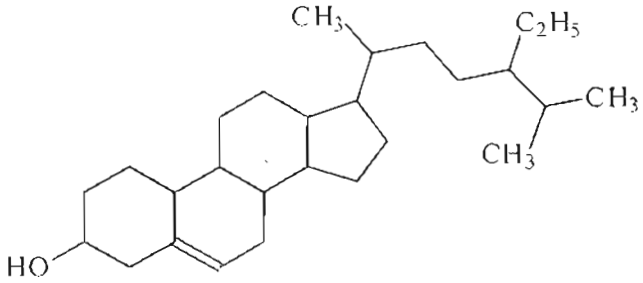
Partial lignin structure



Separated pulp is **bleached** to make fine paper

- bleaching with chlorine —> traces of dioxins (a concern in the late 1980s)
- bleaching with chlorine dioxide
- bleaching with hydrogen peroxide

- endocrine effects down stream of pulp mills are similar for bleached and unbleached pulp —> a natural component of the wood. Most likely candidate is β-sitosterol



Metals in the Environment Chapter 10

- Unlike organic contaminants, metallic components cannot be "destroyed"
- The speciation of the metal may significantly affect its toxicity, and hence its environmental impact.

Issue #1: Mercury

- Mercury occurs as the element or as the sulfide (HgS, two forms: cinnabar and vermillion). Roasting in air gives the element directly

$$HgS(s) + O_2(g) \longrightarrow Hg(l) + SO_2(g)$$

- Hg(*l*), b.p. 357°C, has a significant vapour pressure at ambient temperatures, 2.4×10^{-6} atm

Calculate this in mg/m³ at 298 K and compare with the TLV of 0.05 mg/m³

↗ LD_{50} Hg^{2+} 5000 mg kg^{-1}
C_6H_5HgOAc 1000 mg kg^{-1}
EtHgCl 20 mg kg^{-1}.

- Toxicity of mercury: the metal and inorganic salts are much less toxic than alkylmercury compounds; arylmercury compounds (antifungal seed dressings) are not as toxic

i.e. toxicity depends on speciation

Typical drinking water standards are ~1.0 μg L^{-1} (1 ppb)

- Methylmercury compounds are formed in the environment by microbial alkylation, involving cobalamin (Vitamin B_{12}) which has a Co–CH_3 bond

i.e. biological methylation to CH_3Hg^+ and $(CH_3)_2Hg$

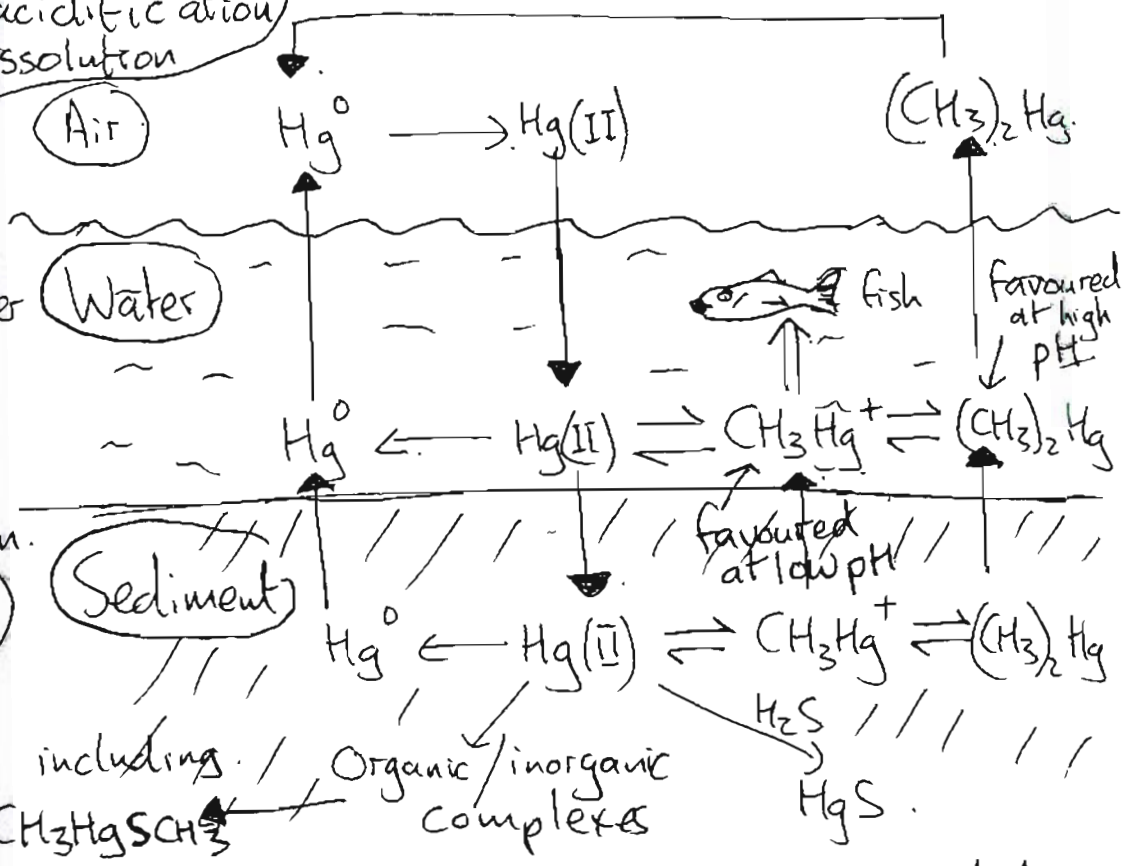
→ i.e. $L_5Co-CH_3 + Hg^{2+} \longrightarrow L_5Co^+ + CH_3Hg^+$

Process of ~~di~~ $(CH_3)_2Hg$ formation occurs in the muddy sediments of rivers and lakes. The cobalmin (vitamin B_{12} derivative) is common in microorganisms

Remember also... acidification of lakes ⇒ dissolution of Hg^{2+} myp124

Recycling of mercury in fresh-water lakes.

(From. Env. Tox. & Chem. 1990, 9, 853)



- In natural waters Hg^{2+} is attached to suspended particulates & eventually ends up in the sediments.
- Most of the mercury in humans is methylmercury, most of which originates from fish (mercury in fish is ~80% methylmercury)

~95% of this is absorbed when we eat fish.

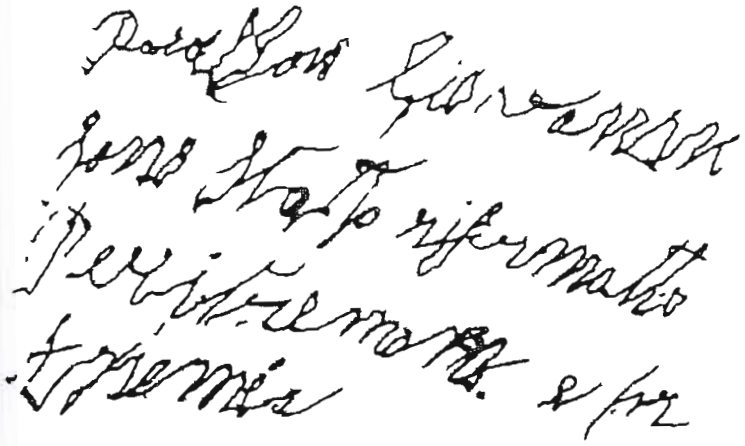
And.... methylmercury is distributed throughout the fish (unlike organoCl's which predominate in the fatty tissue)

Ratio of methylmercury in fish to that in the water can exceed 10^6 (shark, king mackerel, tilefish, swordfish, tuna are especially bad)

→ Famous 1997 case: Karen Wetterhahn (Dartmouth College) died from mercury poisoning some months after having $(CH_3)_2Hg$ seep through latex gloves in the lab. (187)

- C-Hg bonds are non-polar —> organomercury compounds are lipophilic and bioaccumulative and able to cross the blood-brain barrier. Hence give neurological symptoms
- Mercury and its compounds: neurotoxins and renal toxins

Fig 10.1 text.

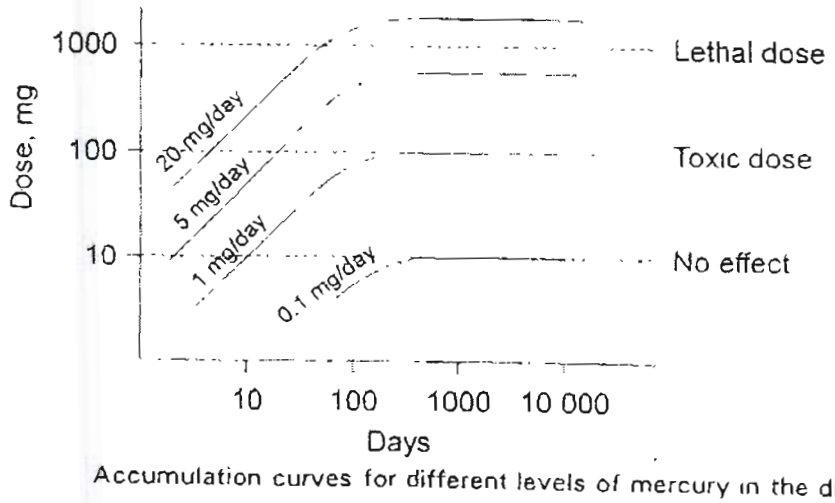


Handwriting of an Italian who worked in a mercury mine

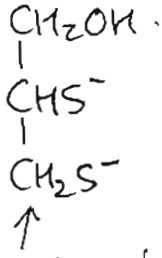
Renal toxicity due to strong $Hg^{(II)}$–S bonds: metallothioneins small proteins with a high percentage of –SH groups, Hg–metallothioneins deposited in kidney

Fig 10.2 text

- Mercury is a cumulative poison:



Accumulation curves for different levels of mercury in the diet



Acute poisoning treated with 2,3-dimercaptoethanol ...binds mercury.

Known as BAL. (British Antilewisite) ← used as antidote to gases in WWI

Exposure to mercury compounds:

- Historically, gilding mirrors, alchemy (Newton), hat-making (the Mad Hatter) --- exposure to Hg(II) nitrate used for felt for hats (roughening)
- Mining and refining mercury
- Gold mining (Amazon basin) ← Hg forms an amalgam with the small gold particles. Then roast to drive off Hg
- Dental amalgams (dental offices a major source of mercury in Toronto's sewage)
- Scientific equipment and old science labs (Dr. Nick used to play with it at school!)

Mercury in the wider environment:

- Burning coal (mercury is volatile and not trapped as a flue dust) ... also volcanoes
- Garbage incineration: especially the use of Hg batteries for cameras, hearing aids (also landfills for the same reason)

Mercury battery:

Steel/Zn | ZnO/aq. KOH | HgO | Hg/Steel

for which (overall rx. $Zn(s) + HgO(s) \rightarrow ZnO(s) + Hg(\ell)$.

$$\Delta G = \Delta G^\circ + RT \ln Q$$

$Q = 1$ because all components are pure (s) or (ℓ), hence E_{cell} does not change with time i.e. remains at 1.35 V

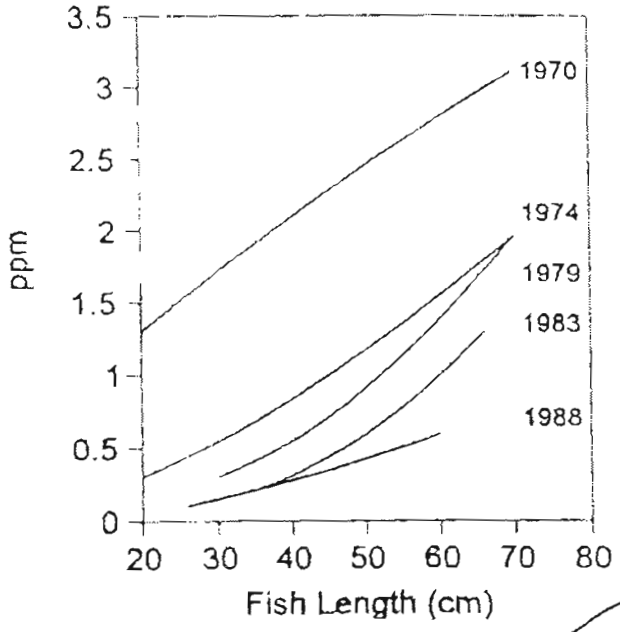
$$Q = \left[\frac{a(Hg,\ell)\cdot a(ZnO,s)}{a(Zn,s)\cdot a(HgO,s)}\right] \quad \| \quad E^\circ - \frac{RT}{nF}\ln Q$$

- Use of organomercury fungicides (minor)

- The old flowing mercury cells for chloralkali process (1960s, losses of 200 g Hg per tonne of Cl_2 produced: reduced to 0.15 g/tonne by 1980): Canadian example: Reed Paper Co in Northern Ontario: White Dog and Grassy Narrows Indian Reserves ← These aboriginal lands were downstream. Fish became contam. with Hg .... eaten by Indian bands ... hi levels ↑ up to 600 ppb
  Compensation $8 million (1985)
  The Hg sits in pools in river sediments!
  ↓ used chlor-alkali process to supply chemicals to bleach pulp.

my notes p151

- Mercury in fish: the limit of 0.5 ppm was set in the 1970s when Hg pollution was more of a problem ***but*** analytical methods were less advanced



- Use of mercury as an industrial catalyst: Minamata Disease (Japan): 1500 cases in the 1950s, 200 deaths; neurological symptoms; easily associated with shellfish but originally not clear whether this was a disease condition or a chemical poisoning. Eventually traced to chem. plant making CH_3CHO

anorexia irritable psych. / symptom

→ in prepn of CH_3CHO (eqn. below) ↓

$$HC{\equiv}CH + H_2O \xrightarrow{Hg^{2+}/H^+} CH_3{-}CH{=}O$$

Note the preceding two examples as historical only; these problems have been largely resolved

methylmercury compounds, including $CH_3Hg{-}SCH_3$ bioaccum. in fish to ca. 100ppm. Eaten by the locals.

Interestingly first symptoms observed in cats (who really like discarded fish!) ... jumped around, twitched, ran in circles, and finally, threw themselves in the water and drowned.

Principal problem remaining today:

- air pollution due to coal burning and incineration

US EPA documents (1997) on mercury releases (1994/95 data):

http://www.epa.gov/~~ttn/oarpg/t3/reports/~~ /ttncaaa1/t3/reports/volume5.pdf ← Health effects of mercury

Volumes 1 → 8

Total of combustion sources ~140 t/yr: includes (major)

- Coal-fired electric power plants ~ 50 t/yr
- Coal and oil fired industrial boilers ~ 30 t/yr
- MSW incinerators ~30 t/yr (municipal solid waste)
- Medical and hazardous waste incinerators ~ 30 t/yr

Clean Air Act Amendments expected to reduce emissions from incinerators

Total non-combustion sources ~ 20 t/yr

- Chloralkali now ~ 5 t/yr
- Cement production ~ 5 t/yr

Natural and anthropogenic releases of Hg are of similar orders of magnitude

- volcanoes (Hawaii volcanoes national park, ~ 20 $\mu g\, m^{-3}$)
- flooding (including hydroelectric dams)

Ontario Env. Protection Acts sets 2.0 $\mu g\, m^{-3}$ (24 h)
Clean air ~ 10 $ng\, m^{-3}$

Recent article on Hg and cerebral palsy in Ontario: *Globe & Mail*, Saturday June 12, 2004.