

ES/RP 531
Fundamentals of Environmental Toxicology

Lecture 9
Endocrine System Toxicity
Part 3: Case Studies

Widespread or Localized Effects?

- Although it is often repeated that there is widespread endocrine disruption in wildlife populations, the evidence is weak of population level effects
 - Observations have been made mostly with field-collected individuals & supplemented by lab studies
 - But comparatively little has been done to determine what proportion of a population is affected & if reproductive rates of increase have been adversely changed
- However, several species have been more intensively studied using a combination of in vitro and in vivo studies, lab specimens and field-collected individuals, and a rudimentary epidemiological approach

The Best Studied Organisms

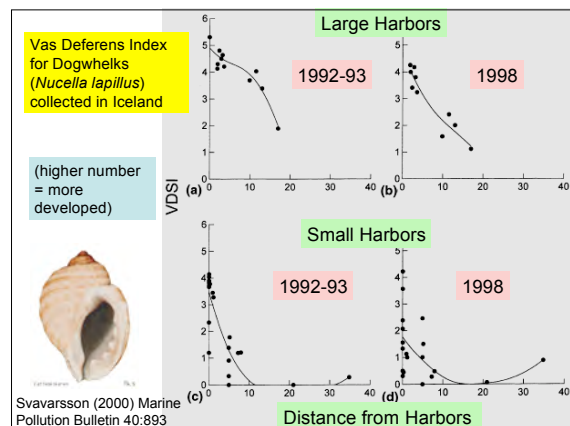
- American alligator (*Alligator mississippiensis*)
- Several fish species, but in particular the roach (*Rutilus rutilus*)
- Molluscs (Neogastropod snails)
- African clawed frog, *Xenopus laevis*

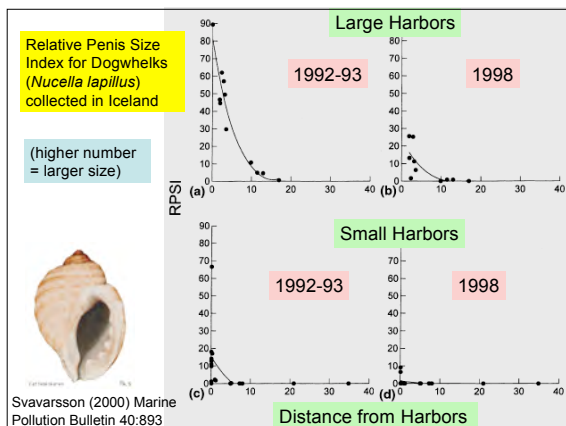
Imposex in Molluscs

- Tributyltin (active ingredient in marine antifouling paints) and triphenyltin (catalyst & stabilizer in PVC synthesis) are believed to cause a condition known as imposex in exposed gastropod molluscs (snails)
 - TBT is believed to be a competitive inhibitor of aromatase
 - Thus estradiol concentrations would be reduced compared to normal, and testosterone levels would be relatively elevated
 - Female snails become masculinized (condition is called imposex)
 - Characterized by presence of penis and vas deferens

Imposex in Molluscs
Interaction of Risk Assessment & Risk Management

- Owing to widespread findings of TBT in marine waters, and the strong association with imposex occurrence in molluscs, TBT has been essentially banned for use as an antifouling paint additive on marine vessels
- Studies suggest some alleviation of the occurrence of imposex since the ban





The Saga of the "Less than Manly" Alligators

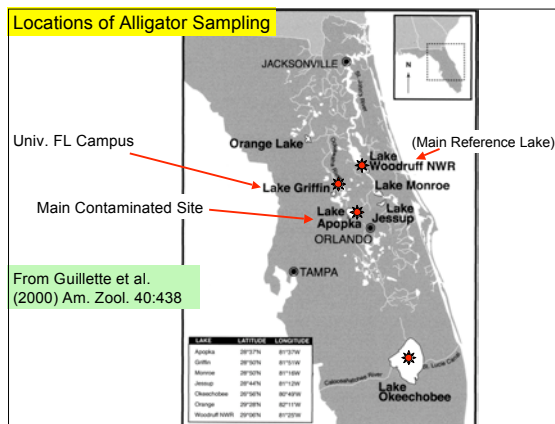
- Of all species studied for endocrine disruption, the alligator from one lake in Florida (Lake Apopka) has received careful scrutiny and study
- University of Florida lab led by Louis Guillette
- Discovered that alligators seemed to be feminized, based on morphological traits, and hormone levels in plasma



The Problem

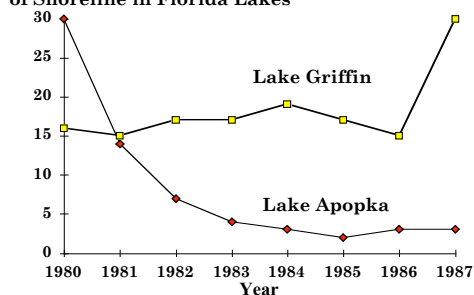
- Lake Apopka circa 1980 received the overflow waters from a waste storage area
 - Wastewater was from the Tower Chemical Co., manufacturer of dicofol (a DDT analog)
 - Lake also received agricultural drainage water as well as treated sewage water from a small city
- Investigators noted alligator populations in the 1980's were crashing

Locations of Alligator Sampling

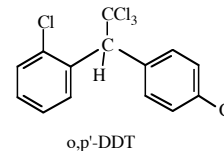
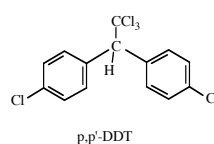
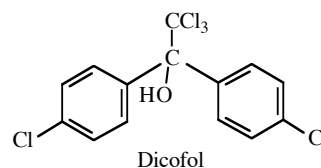


Population Abundance of Alligators at a Contaminated & Non-Contaminated Lake

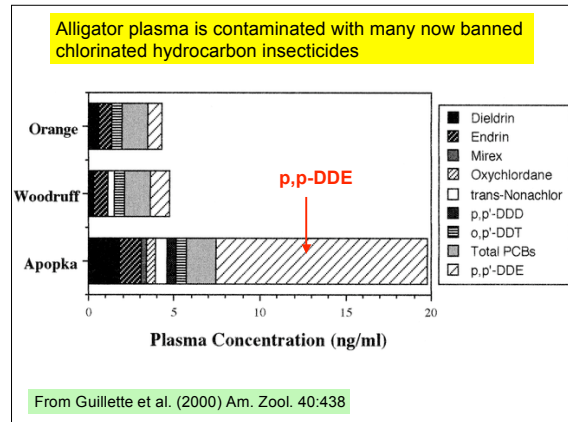
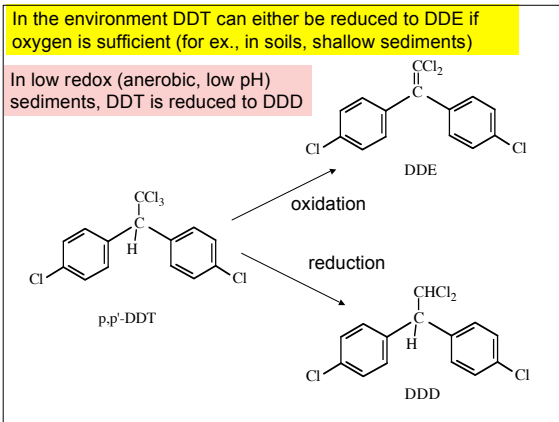
Juvenile Alligators per km of Shoreline in Florida Lakes



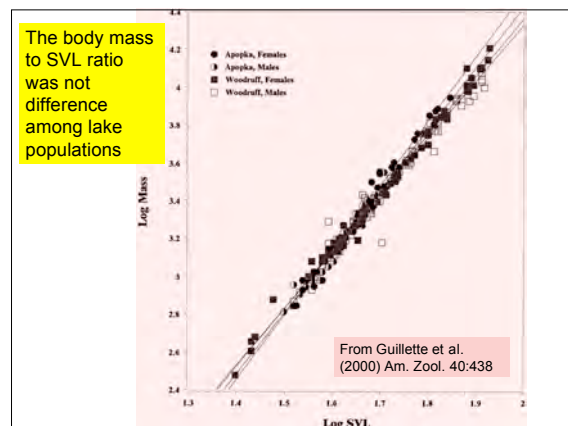
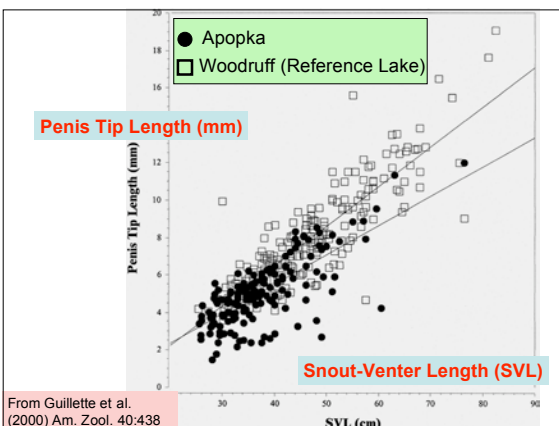
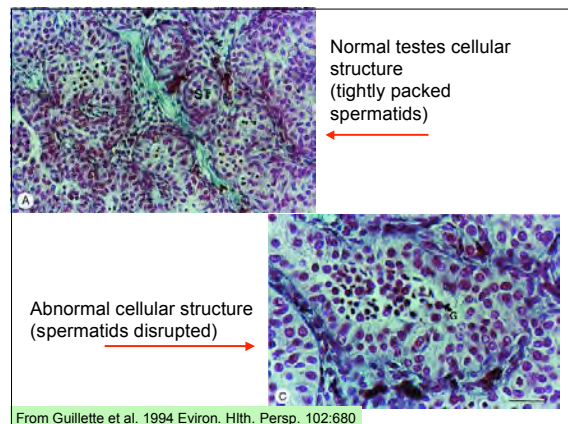
Modified from Guillette et al. 1994 Environ. Health Persp. 102:680

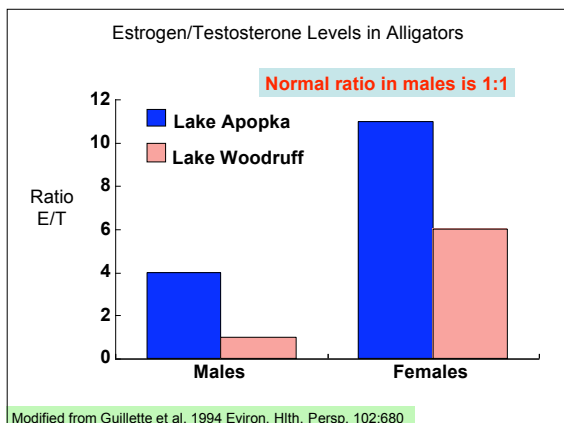


Older dicofol formulations were contaminated with DDT byproducts



- ### Benchmarks of Toxicity
- Morphological changes in male reproductive tissues that suggested feminization of male gonads
 - Comparatively reduced penis size in alligators from Lake Apopka compared to reference lakes
 - Abnormal levels of sex hormones found in alligator blood
 - Laboratory studies on sex reversal

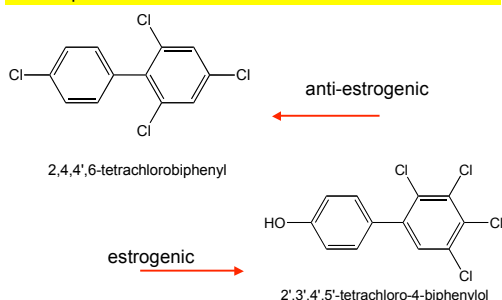




Sex Reversal

- Reptiles and some fish species can change sex when the eggs are subjected to certain temperatures
- In alligators, cool temperatures favor females and warm temperatures favor males
- When eggs incubated at male producing temperatures (32 C) are exposed to 1-10 mg/kg egg mass DDE, the proportion of female hatchlings rises to 50%
 - The expected outcome was 100% male

PCBs have also observed to cause sex reversal in treated turtle eggs; but the hydroxylated metabolites are the 'culprits'



Bergeron et al. 1994 Environ. Health Persp. 102:780

No Sex Bias!!

- Predict gender ratio bias toward females in Lake Apopka alligator population based on
 - Given the results of laboratory measurements of abnormal steroidogenesis in Lake Apopka alligators
 - the morphological abnormalities of the male genitalia
 - sex reversal experiments in incubated eggs
- But gender ratio actually favored males!!
- Furthermore, no positive correlation between plasma contaminant levels and hormone levels nor male genitalia morphology
 - However, Lake Apopka alligators did have lower testosterone levels than Lake Woodruff alligators

Guillette et al. 1999 Arch. Environ. Contam. Toxicol. 36:447

Conclusions

- Alligators from selected lakes (contaminant-affected lakes) seem to be affected by endocrine disruptors or hormonally active agents
 - Studies restricted to juvenile alligators
 - Effects on adults not known
- No transgenerational studies
- Mode of action is not precise; could be several mechanisms at work
 - Mechanism probably operating organizationally, i.e., through exposed embryo and consequent development

Estrogenic Activity Possible Causes

- An increase in gonadal estrogen production
- A decrease in gonadal androgen production
- An increase in the production of gonadotropin from the pituitary
- A decrease in hepatic enzyme degradation
- An increase in concentration of serum sex hormone binding proteins
- A decrease in cytosolic binding proteins
- Agonistic binding of the contaminant to the estrogen receptor

The Saga of Sexually Confused Fish

- A number of fish species have been found to have high levels of vitellogenin (Vt) in the plasma
 - OK for females because Vt is the egg yolk protein synthesized in response to estradiol signalling
 - But in males, the gene is silent unless signaled (turned on)
 - Thus, evidence suggests exposure to hormonally active substances with estrogen activity
- The fish populations affected seem to occur near wastewater (sewage) treatment outfalls (i.e., municipal waste water treatment plants)

Current Events??

MSNBC.com

Male fish becoming female?

Researchers worry about estrogen and pollutants in the water

By Tom Costello

Correspondent

NBC News

Updated: 2:54 p.m. ET Nov. 9, 2004

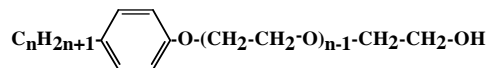
BOULDER, Colo. - Researchers in Colorado have made a startling discovery. Fish, apparently male, are developing female sexual organs. Scientists believe it's the result of too much estrogen in the water and they're finding estrogen in rivers across the country.

The Leading Candidates for Xenoestrogen Status Affecting Fish

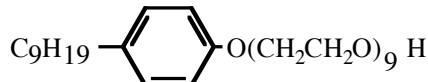
- Metabolites of detergent surfactants based on alkyl phenolic structure
- Natural estrogens and perhaps EE2, the birth control pill estrogen



Alkylphenol polyethoxylates



Have variable number of ethoxylate units in the polymers making up any surfactant formulation



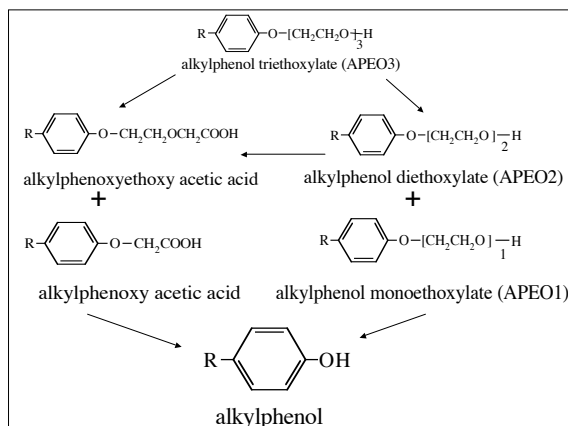
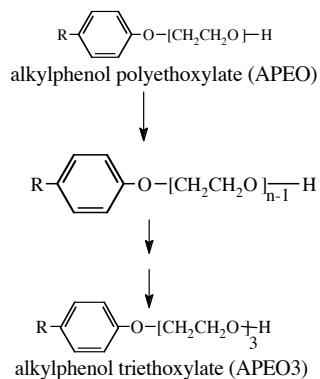
Nonylphenol ethoxylate

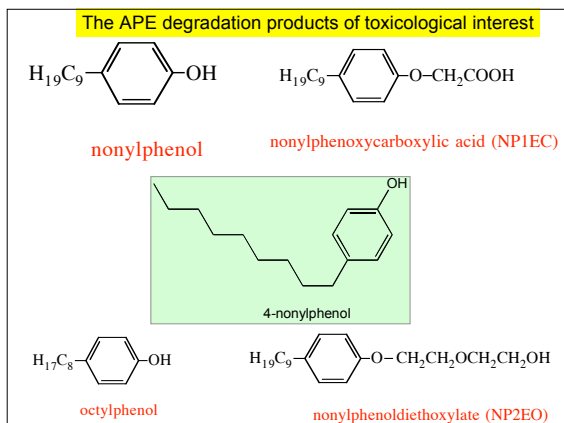
Designated NP9EO
Has 9 ethoxylate units (EO)

About 1 billion pounds produced annually;
About 80% of production are nonylphenol ethoxylate compounds
About 20% of production are octylphenol ethoxylate compounds

Degradation Pathway

The environmental concern over APE use is the metabolism to nonylphenol or octylphenol that occurs by successive removal of EO units



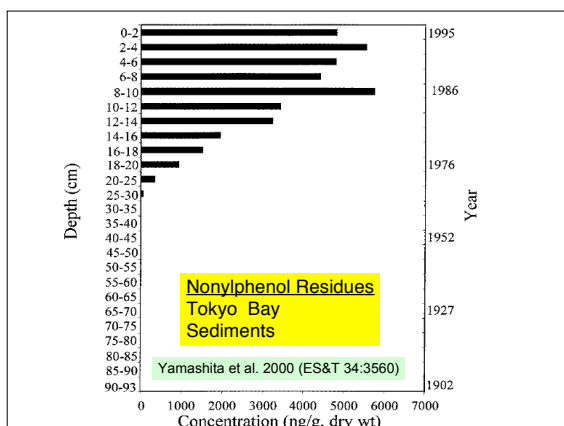


Concentration ($\mu\text{g/L}$) of Various LAS Homologs, Total NPEs, & NP in Sewage

Surfactant	Raw Sewage		Treated Sewage	
	Liquid	Solid	Liquid	Solid
C₉ LAS	239	13	0.8	—
C₁₀ LAS	959	271	3.3	0.1
C₁₁ LAS	1139	907	8.1	1.3
C₁₂ LAS	684	1676	5.9	2.2
C₁₃ LAS	138	1100	1.9	1.8
Total NPEO	154	49	6.9	3.0
NP (nonylphenol)	1	11	0.3	0.7

LAS = linear alkyl benzene sulfonate surfactant

Di Corcia et al. 1994 (ES&T 28:850)

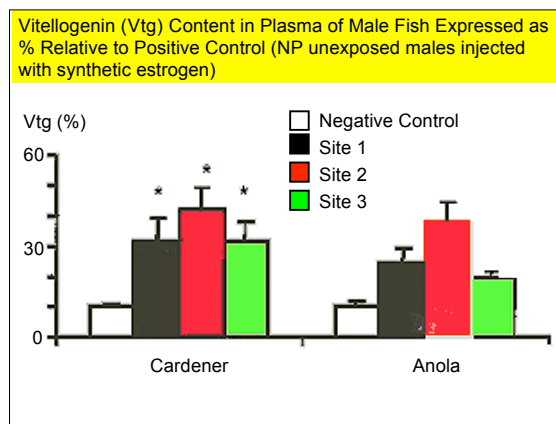


- Acute Toxicity**
- APEs (Alkyl phenol polyethoxylates)
 - LD50 rat oral: 1,420 - >28,000 mg/kg
 - LD50 rat oral: 2,000 mg/kg - >10,000 mg/kg
 - Freshwater fish: LC50 ~1.3 - 1000 mg/L
 - Crustaceans: LC50 ~ 2.9 - 10,000 mg/L
 - LAS (linear alkylbenzene sulfonates)
 - Various aquatic organisms: ~0.1 mg/L - 1,000 mg/L
 - Field test, NOEC on aquatic populations: 0.36 mg/L

Nonylphenol & Metabolites in Spanish Tributaries with Sewage Treatment Plant Discharges

Site	Distance to STP (km)	NP ($\mu\text{g/L}$)	NPEO ($\mu\text{g/L}$)	NPEC ($\mu\text{g/L}$)
Anola				
1 (upstream)	5	18	<0.2	<0.8
2 (downstream)	23	644	100	70
3 (downstream)	27	<0.15		<0.8
Cardener				
1 (upstream)	1.5	51	<0.2	<0.8
2 (downstream)	4	398	20	40
3 (downstream)	8	42	<0.2	<0.8

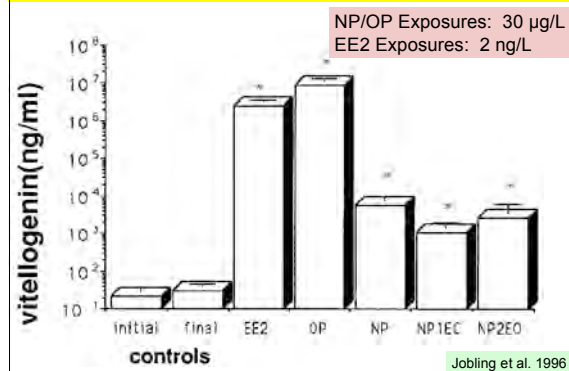
Sole et al. (2000) Environ. Sci. Technol. 34:5076



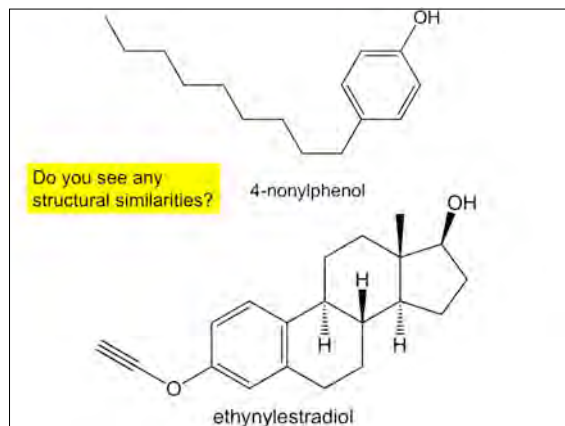
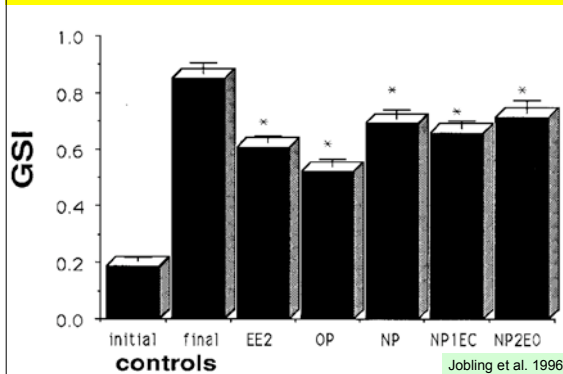
Lab "Feminization" Experiments

- One biomarker of feminizing effects in male fish is the production of vitellogenin and measurement of its concentration in plasma.
 - Vitellogenin is an egg storage protein made by females
 - Estradiol is used as a positive control
- Exposure of male rainbow trout to OP and NP metabolites induced vitellogenin synthesis (Jobling et al. 1996, ETAC 15:194)
 - Nonylphenol: NOAEC ~5 µg/L (LOAEC ~20 µg/L)
 - Octylphenol: NOAEC ~1.6 µg/L (LOAEC ~4.8 µg/L)

Exposure of Rainbow Trout to APE Metabolites Found in Sewage Effluents: Induction of Vitellogenin Synthesis in Males

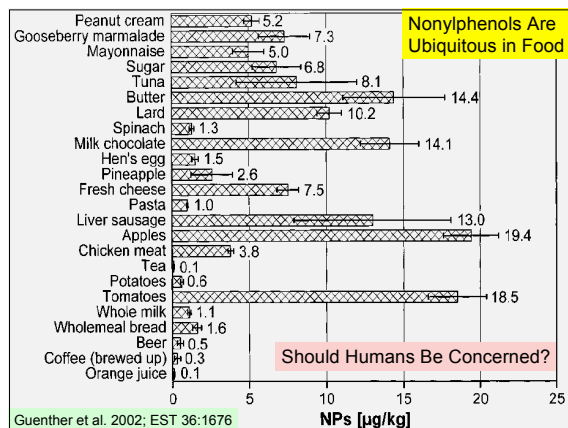


Exposure of Rainbow Trout to APE Metabolites Found in Sewage Effluents: Effect on Gonadal-Somatic Index in Males



The Big Concern? APE Degradation Products-- Especially Nonylphenol & Octylphenol

- In vitro studies show that NP & OP are estradiol agonists and thus may be in vivo hormonally active agents
- Studies of fish physiological responses around sewage outfalls suggest that "feminizing" effects on male fish may be occurring
- Rat oral exposure studies (usually acute doses or gestational exposures) suggest morphological changes (gland weight loss, feminizing morphologies) in male pups and adults



Intakes and Hypothetical Sources in Food

- NPs found in all German food items (many were imports)
 - Concentration range: 0.1 - 19.4 $\mu\text{g/g}$
- Estimated daily intake at 7.5 $\mu\text{g/day}$
 - Infants on breast milk: 0.2 $\mu\text{g/day}$
 - ✓ Whole breast milk contained on average 0.3 $\mu\text{g/kg}$ NP
 - Infants on formula: 1.4 $\mu\text{g/day}$
- Sources:
 - Pesticide formulation residues on fruit;
 - Antioxidant tris(nonylphenol)phosphite for plastics;
 - Surfactant used in disinfectants