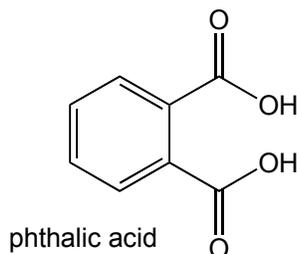


November 15, 2004

Lecture 21 Plasticizers (Phthalates) & Bisphenol A**I. Production and Use**

- A. Phthalates are used to soften plastics like PVC (polyvinyl chloride). There are a myriad of kinds of plasticizers based on esters of phthalic acid (phthalate).



- B. Historical perspective on use of plasticizers (Graham, P. R. 1973. Phthalate ester plasticizers--Why and how they are used. Environmental Health Perspectives 3:3-12)
1. "The first commercially significant plasticizers were discovered shortly after the development of cellulose nitrate in 1845."
 - a. Castor oil was patented for plasticization of cellulose nitrate circa 1856.
 - b. Celluloid plastics remained the major thermoplastics until ~1940.
 2. "The introduction of phthalate esters in the 1920's overcame the excessive volatility and undesirable odor of camphor. However, the commercial availability of poly(vinyl chloride) in 1931 and the synthesis of di-2-ethylhexy phthalate in 1933 quickly shifted emphasis away from cellulose nitrate and started the rapid growth of the flexible poly(vinyl chloride) industry."
 - a. By 1972, 1 billion pounds of 20 different phthalate esters were produced.
- C. Why plasticizers are used (Graham 1973)
1. "Plasticizers are interfused with high polymers to increase flexibility, extensibility, and workability. This is achieved by lowering the glass transition temperature to below room temperature. The polymer is changed from a hard glasslike solid to a flexible, tough elastomer.

Uses of phthalate esters in the U.S. (from Peakall 1975, Residue Reviews 54:1-41 (Although these are dated statistics on use now, the increase in use of phthalates in the intervening years suggests that the numbers are much larger, but those below illustrate the tremendous magnitude of use (which is worldwide).

Plasticizer Uses	Millions of Pounds
Building and construction	
Wire and cable	185
Flooring	150
Swimming pool liners	20
Miscellaneous	32
Subtotal	387
Home furnishings	
Upholstery	90
Wall coverings	38
Houseware	30
Miscellaneous	45

Subtotal	203
Cars	114
Wearing apparel	72
Food wrapping and closures	25
Medical tubing and intravenous bags	21
Total as Plasticizers	922
Use as Non-plasticizers	
Pesticide carriers	-
Oils	-
Insect Repellent	-
Total as non-plasticizers	50
Grand Total	
	972

- D. The phthalates are not chemically bound to the product, and thus they may leach into the surrounding medium (for example into food, or other environmental compartments if the plastic is exposed).
- E. The most heavily used phthalate is bis(2-ethylhexyl) phthalate (a.k.a. diethylhexylphthalate, DEHP), but there are many others as shown in the next table.

Phthalate Ester	European Consumption 1000's tons/year (1990's)	U.S. (1979) 1000's tons
Bis(2-ethylhexyl) phthalate (DEHP)	400-500	175,198
Diisononyl phthalate (DINP)	100-200	
Diisodecyl phthalate	100-200	65,000
Butyl benzyl phthalate (BBP)	20-50	50,000 (1978 data)
Dibutyl phthalate (DBP)	20-40	11
Diisobutyl phthalate	20-40	
Ditridecyl phthalate	3-10	8,000
Diethyl phthalate (DEP)	10-20	11,000
Dimethyl phthalate (DMP)	10-20	4,000
Diisohexyl phthalate	<2	
Diundecyl phthalate	<2	
Butyl decyl phthalate	<1	
Butyl octyl phthalate	<1	6,000
Dicyclohexyl phthalate	<1	2,000

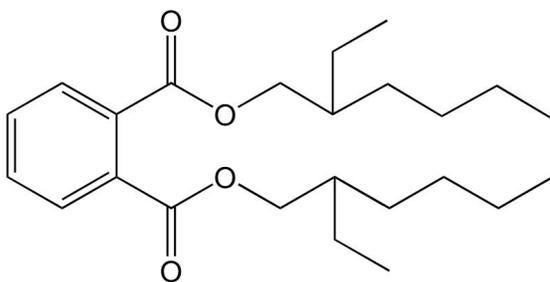
European Consumption data from Harris et al. 1997, Environ. Health Perspectives 105:802

- F. Owing to the widespread use of phthalates they have essentially been found everywhere. Emissions occur during manufacture as well as during use and disposal of the final plastic product.
1. For example, 2500 samples of industrial wastewater from 32 types of industry were analyzed, and DEHP [bis(2-ethylhexyl)] phthalate was found in 41.9% of the samples, representing 29 different industrial uses. (cited by Wams, T. J. 1987. The Science of the Total Environment 66:1-16)
 2. The loss of DEHP from modern production plants is reported to be negligible, but in the past may have been as high as ~1% of volume escaping into wastewater.

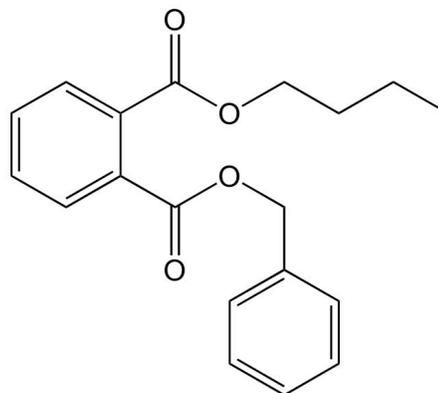
3. During distribution to the plastic producing industry, an estimated 0.05% may be lost; part of this enters sewage systems.
 4. During production of PVC, 0.03-2.0% may evaporate into the atmosphere.
 5. The average loss in injection molding and coating processes may be 0.8%.
- G. Peakall 1975 estimated that 7% of phthalates are used in direct contact with water, resulting in a migration rate of 1% per year;
1. 59% was estimated to be in direct contact with air, resulting in an annual migration rate of 0.1%, and 34% was thought to have little surface contact with a migration rate loosely estimated at 0.01% per year.
 2. Others (cited in Wams 1987) have estimated losses to the atmosphere of 0.35% of total annual consumption and a loss of 0.15% to water.
 3. 15% of DEHP in paints may evaporate into the atmosphere.

II. Environmental Chemistry

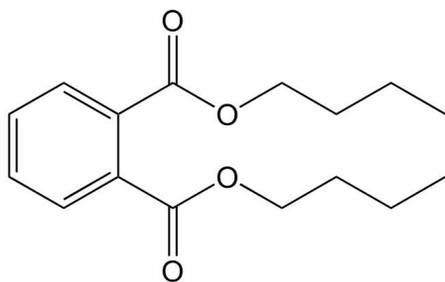
A. Examples of Structures



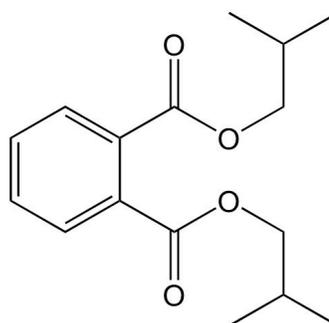
di(2-ethylhexyl) phthalate



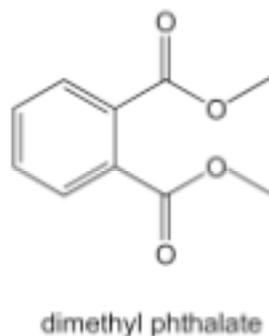
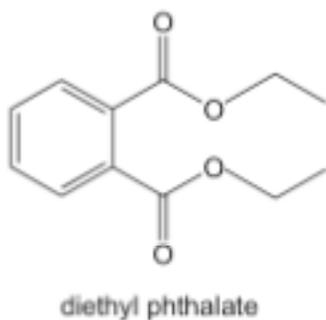
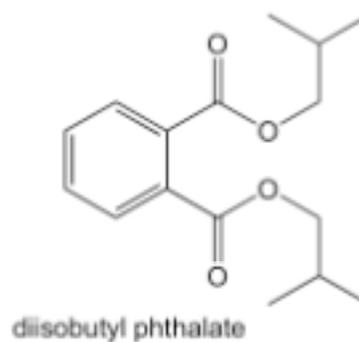
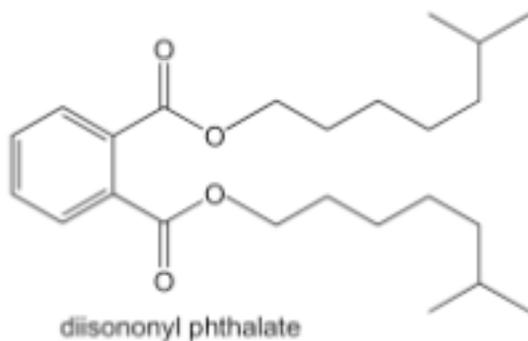
butyl benzyl phthalate



dibutyl phthalate



diisobutyl phthalate



B. Natural Occurrence

1. There is some debate about whether or not phthalates are naturally occurring substances as well as synthetic products. Samples may have been inadvertently contaminated when they were analyzed. However, the occurrence of phthalates in a wide variety of plants suggests a natural occurrence (Graham, P. R. 1973. Phthalate ester plasticizers--Why and how they are used. Environmental Health Perspectives 3:3-12).

C. Physicochemical Properties

Phthalate	Boiling Point. (°C)	Water Solub. (mg/L)	Log Kow	Vapor Pressure (mm Hg)	Henry's Law Constant atm-m ³ /mol	Koc (L/kg)
Butyl benzyl (BBP)	370	2.69	4.91	8.6×10^{-6}	1.3×10^{-6}	65-350
Diethyl (DEP)	295	1080	2.47	1.65×10^{-3}	4.8×10^{-7}	94-526
Di(2-ethylhexyl) (DEHP)	230	0.3	5.11	6.45×10^{-6}	1.1×10^{-5}	87,420
Dimethyl (DMP)	284	4000	1.56	1.65×10^{-3}	1.1×10^{-7}	44-160

Data from Howard 1989, Fate & Exposure Data for Organic Chemicals, Volume I.

D. Volatility from plastic (vinyl) film

1. DEHP was measured in an experiment cell that could flush large volumes of air (250 cm³/min across a 240 cm² film area (Graham 1973).
 - a. Concentration in air average 2.9 µg/L;
 - b. Mass flux averaged 44 µg/h

E. Environmental Fate

1. BBP; readily biodegraded;

- a. Water: 95% loss in about 7 days
- b. Activated Sludge: ~99% loss in 48 hours
- c. Abiotic degradation:
 1. Photodegradation and hydrolysis half-lives greater than 100 days
- d. Atmospheric fate: estimated half-life of 1-5 days
2. DEP; readily biodegraded
 - a. Water: half-life ranging from 2 d - >2 weeks;
 - b. Activated sludge: 94% loss in 24 h;
 - c. Atmosphere: half-life of ~22 h
3. DEHP; easily biodegraded;
 - a. Some half-lives (from Wams 1987)

Environment	Half-Life
Pure culture of <i>Penicillium lilium</i>	30 days
River water	4.5 weeks
Activated Sludge	0.8 days; 17 days
Sediments	14 days
Soil	31-98 days
Photolysis	2000 yr

F. Bioconcentration Factors

1. BBP: blue gill fish; log BCF = 2.82
2. DEP: blue gill fish; log BCF = 2.1
3. DEHP: blue gill fish; log BCF = 2.1; invertebrates, log BCF = 4
4. DMP: blue gill fish; log BCF = 1.8

G. Environmental Concentrations

1. One of the first reports of phthalates in aquatic systems was by Hites (1973) (Hites, R. A. 1973. Phthalates in the Charles and the Merimack Rivers. Environmental Health Perspectives 3:17-21.)
 - a. Hites used mass spectrometry to show that phthalate esters (identified DMP and DEHP) were present in water at concentrations of ~2 ppb near an emission source, and at concentrations near 1 ppb about 7 miles downstream.
2. Wams (1987) cataloged concentrations of phthalates in different habitats in Europe circa the late 1970's and early 1980's (see following table).

Medium	Site	Year	Concentration
Air	North Pacific	1981	0.3-2.7 ng/m ³
	Denmark	1983	22 ng/m ³
Water	Meuse	1977	0.4 – 4.0 µg/L
		1983	<0.1 – 3.5 µg/L
	Rhine	1982	ND 4 µg/L
		1983	ND – 1.2 µg/L
Groundwater	New York State	1979	Max. 170 µg/L
	Netherlands (contaminated)	1984	20-45 µg/L
Soil	Netherlands (contaminated)	1984	1.5 mg/kg
Sediment (Hydrosol)	Rhine	1978	4-36 mg/kg
	Meuse	1978	1-17 mg/kg
	Ems (F.R. G.)	1981	0.03-0.06 mg/kg
	Helgoland Bight	1981	0.02-0.22 mg/kg
	Mississippi	1981	0.14 mg/kg
	Neckar (F. R. G.)	?	2-9 mg/kg

III. Toxicology of Phthalates

A. Acute Toxicity

1. Phthalates have incredibly low toxicity (g/kg range)

Acute Toxicity of Some Phthalates (from Peakall 1975)

Phthalate	Species	Route	LD50 (g/kg)
Dimethyl	Mouse	Intraperitoneal (i.p.)	1.6 – 3.6
Diethyl	Mouse	i.p.	2.8
	Rabbit	oral	1.0
Dibutyl	Mouse	Oral	<13
	mouse	i.p.	4-21
Diethyl hexyl	Mouse	Oral	128
	Mouse	i.p.	14.2 - 75
	Rat	oral	>26
Butyl benzyl	Mouse	i.p.	3.2
	Rat	i.p.	1.8
	rat	oral	4

B. Chronic Toxicity: Potential for Carcinogenicity

1. DEHP does not seem to be mutagenic.
2. The National Toxicology Program (NTP) reported that DEHP causes hepatic neoplasms when administered in high doses (3000-12,000 mg/kg in feed).
 - a. David et al. (2000, Toxicol. Sci.. 58:377-385) exposed mice to DEHP in the diet for a period of 104 weeks. Doses were (based on males) 0, 19, 99, 292, and 1266 mg/kg, which corresponds to a concentration (as ppm) in the feed of 100, 500, 1500, and 6000 ppm.
 1. Observed liver tumors and peroxisome proliferation at doses ≥ 99 mg/kg; they stated the NOAEL for tumors as 19 mg/kg
 - a. David et al. (2000) also observed hypospermia (low sperm counts) at doses ≥ 292 mg/kg; the NOEL for noncarcinogenic effects was 99 mg/kg.
 - b. DEHP (as well as other phthalates including DINP (see discussion below) may stimulate peroxisomal enzymes at high concentrations in the liver, resulting in the production of oxidants that could damage DNA. Thus, testing of DEHP and other phthalates at high doses seems to cause cell injury that would not be seen at lower doses.
 1. Peroxisomes are single membrane-limited, cytoplasmic organelles occurring in the cells of a wide variety of organisms ubiquitously distributed across the animal and plant kingdoms. They are characterized by their content of catalase and a number of hydrogen peroxide-generating enzyme systems. (Wilkinson, C. F. and J. C. Lamb. 1999. The potential health effects of phthalate esters in children's toys: a review and risk assessment. Regulatory Toxicology & Pharmacology 30:140-155.)
 2. Peroxisome proliferation is observed primarily in rodent liver following prolonged *in vivo* treatment of animals with a variety of chemicals including phthalate esters. It can also be demonstrated *in vitro* in primary cultures of rat and mouse hepatocytes.
 3. Ability to cause peroxisome proliferation among phthalates is probably due to their metabolism to reactive products.

- a. For example, DINP is metabolized to monoisononyl phthalate (MINP) in the intestine; it seems to be the active product but the parent compound is inactive in stimulating peroxisome proliferation in hepatocyte cultures.
- b. "It is now known that peroxisome proliferators regulate the rate of transcription of genes for increased oxidative enzyme activity and cell proliferation (Green, 1992; Ashby *et al.*, 1994; Lake, 1995a,b). The process involves interaction of the peroxisome proliferator with a nuclear receptor called the peroxisome proliferator-activated receptor (PPAR). PPAR is a member of the steroid hormone receptor family that, once activated, is able to increase the transcription rate of responsive genes by binding to specific DNA regulatory elements." (Wilkinson and Lamb 1999)
- c. The saga of carcinogenic concerns over DEHP and regulatory action (as related by Wilkinson and Lamb 1999)
 1. Until the early 1980s, DEHP had been the most common plasticizer used in children's soft PVC plastic toys (for ex., in teethers and other toys).
 2. Because of the concerns that DEHP might be carcinogenic (it was classified as a B2 carcinogen by EPA after the NTP report of high dose testing circa 1982 .. The Toy Manufacturers of America developed a voluntary standard limiting DEHP in soft PVC teethers and pacifiers to less than 3% in products designed for children less than 3 years old.
 - a. However, further analysis of DEHP carcinogenic potential concluded that phthalate esters are likely to be human carcinogens as a result of their ability to cause peroxisome proliferation in rodents (Doull, J., et al. (1999). A cancer risk assessment of di-(2-ethylhexyl)phthalate: Application of the new U.S. EPA Risk Assessment Guidelines. *Reg. Toxicol. Pharmacol.* **29**, 327–357.)
 3. In 1997, Danish regulators reported unacceptable levels of the phthalate DINP leached from various PVC teething ring imported from China.
 - a. The toys were voluntarily withdrawn from the market, and other European countries announced plans to control or limit sales of phthalate-containing children's products (Wilkinson and Lamb 1999).
 4. Greenpeace released a report in 1997 that showed many soft PVC children's products contained 40-50% by weight DINP, but they also said that DINP from these sources posed a health hazard.
 - a. The U.S. Consumer Product Safety Commission had been evaluating DINP and decided against recommending a ban on soft plastic toys containing DINP but the agency did ask toy manufacturers voluntarily to remove phthalates from teethers and rattles.
 - b. The EU circa 1999 issued a directive that proposed to ban all phthalates except DINP in soft PVC toys.
 1. Also, DINP could only be used in toys intended to be mouthed by children under 36 months of age if its rate of migration did not exceed 1.2 mg/10cm² area in 3 h.
 - (a) DINP would not be restricted in other types of toys.
 5. High doses of DINP can cause increased incidences of both benign adenomas and malignant hepatocarcinomas in rats. Other types of tumors observed included renal cell carcinomas (at and above 358 mg/kg/day and mononuclear cell leukemia.
 - a. However, there are clear NOAELs (>307 mg/kg/day, F-344 rats) and 112 mg/kg/day (female B6C3F1 mice). (Wilkinson and Lamb 1999)
 - b. ADIs (Acceptable Daily Intakes; similar in significance to EPA's Reference Dose, RfD) have been suggested to range from 1.1–3.8 mg/kg/day.

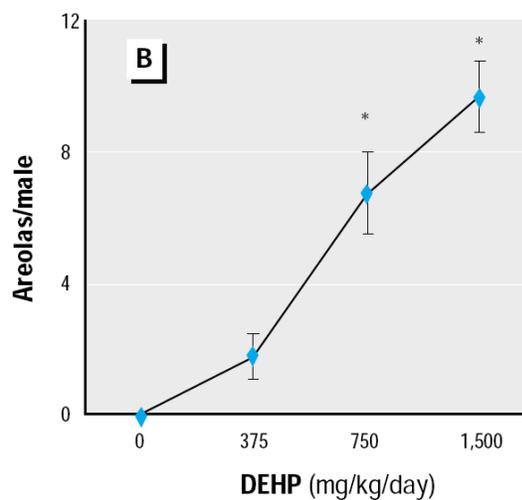
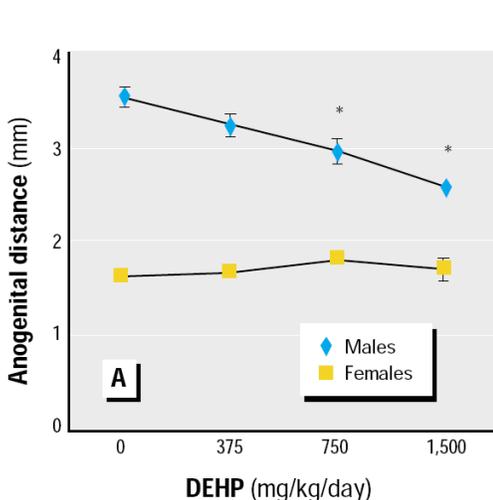
6. A scientific consensus now concludes that the mechanism of action of phthalates (peroxisome proliferation) in causing tumors in rodents is unique to rodents and should not be extrapolated to humans. (Wilkinson and Lamb 1999)
 - a. Hypolipidemic drugs are potent peroxisome proliferators but humans taking these for at least three years have no signs of altered peroxisome number or volume.
 - b. Also human epidemiology studies showed no indication of an increase in cancer associated with long-term human exposure.
7. Thus, phthalates should be considered non-genotoxic chemicals that induce peroxisome proliferation in rodents at high doses, but the effect is threshold based.

C. Endocrine Activity

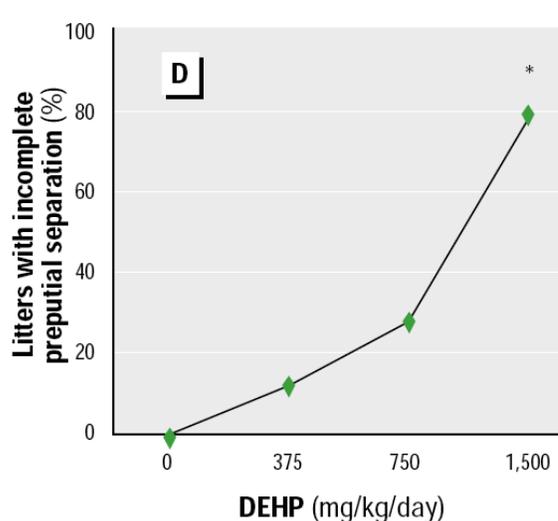
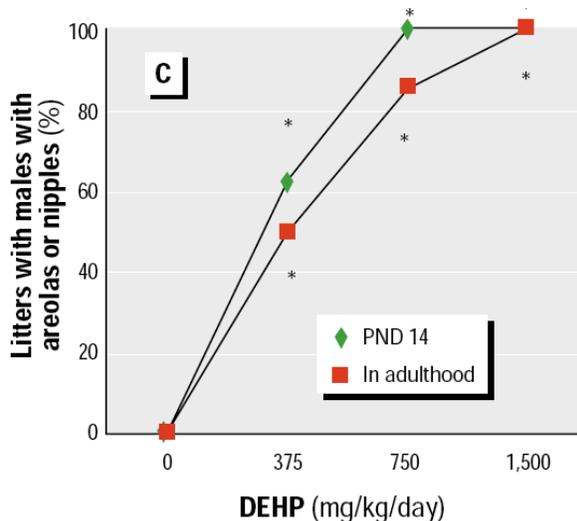
1. Plasticizers were in the news during 2000-2001 with headlines warning that leaching from medical intravenous bags could be hazardous to patients. The motivation for this hype came from a number of studies that show some of the phthalates are estrogenic.
2. Phthalates are generally weakly estrogenic, however, especially compared to estradiol.
 - a. For example, the in vitro potency of DEP, DBP, and BBP is only 0.0000005, 0.0000001, and 0.000001 of estradiol (Harris et al. 1997)
3. Phthalates have been hypothesized to have an anti-androgenic mechanism of action in vivo in a number of studies.
 - a. Maternal DEHP treatment at 750 mg/kg/day by gavage on gestational day (GD) 14 to postnatal day 3 caused a reduction in T (testosterone production) and reduced testicular and whole-body T levels in fetal and neonatal male rats from GD (gestational day)17 to PND (post natal day) 2. (Study reported by Parks et al., 2000, The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicological Sciences* 58(2):339-349.)
 1. As a consequence, anogenital distance on PND 2 was reduced by 36% in exposed male, but not female, offspring. By GD 20, DEHP treatment also reduced testis weight.
 2. Neither DEHP nor its metabolite MEHP (the monoester) displayed affinity for the human androgen receptor at concentrations up to 10 μ M in vitro.
 3. Thus, DEHP at high doses seems to inhibit testosterone synthesis rather than act as an androgen receptor antagonist.
 - b. Gray et al. 2000 (Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicological Sciences* 58[2]:350-365.) dosed pregnant rats with 750 mg/kg bw DEHP, BBP, DINP, DEP, DMP, or DOTP (GD 14 to PND 3).
 1. Male (but not female) pups from the DEHP and BBP groups displayed shortened anogenital distances (about 30%) and reduced testis weights (about 35%) compared to undosed controls.
 2. As infants, males in the DEHP, BBP, and DINP groups displayed female-like areolas/nipples (87, 70, and 22% of cohorts), respectively, vs. 0% in other groups.
 3. All three of the phthalate treatments that induced areolas also induced a significant incidence of reproductive organ malformations.
 4. Thus, DEHP, BBP, and DINP can alter sexual differentiation but not DOTP (dioctyl terephthalate), DEP, and DMP. DINP was about 10 times lower in potency than DEHP and BBP.
 5. Note that past studies showed that DBP also had antiandrogenic effects.
 - a. Results from a ten day prenatal (embryonic and fetal) exposure to DBP: The NOEL and LOEL were 50 mg/kg/day and 100 mg/kg/day, respectively. (Mylchreest, E., D. G. Wallace, R. C. Cattley, and P. M. D. Foster. 2000. Dose-dependent alterations in androgen-regulated male reproductive

development in rats exposed to di-(n-butyl) phthalate during late gestation. *Toxicological Sciences* 55(1):143-151.)

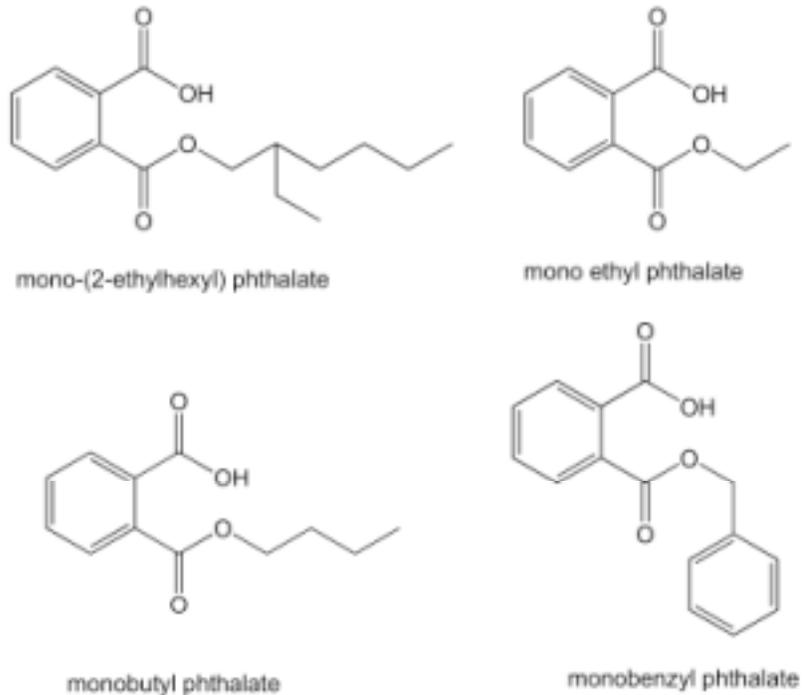
4. Note that the in vivo doses given to pregnant dams in the studies showing anti-androgenic effects are quite high. One study has used its results to calculate a reference dose (RfD)
 - a. Rats were dosed with either corn oil or DEHP at 0, 375, 750, or 1500 mg/kg/day from gestational day 3 through post natal day 21 (Moore, R. W. et al. 2001. Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer di-(2-ethylhexyl) phthalate. *Environmental Health Perspectives* 109(3):229-237.)
 - b. Measurements included examining male rats for signs of feminization, including anogenital distance (the closer the distance than the more “female” the morphology), number of areolas per male, testis weight, sexual behavior. The following graphs show clear dose-response relationships for a couple of these parameters.



Evidence for feminizing effects of high dose exposures of rats exposed to DEHP through their pregnant mothers. Although the two “effects” shown above show a NOAEL at 750 mg/kg/day, another parameter showed no NOAEL (percent of litters with males exhibiting areolas or nipples; see next graph on right)



- c. Moore et al. (2001) concluded that the NOAEL was not reached (see graph C above).
- d. Thus, Moore et al. estimated a reference dose (RfD) for anti-androgenic effects by applying a 1000-fold safety factor to the LOAEL of 375 mg/kg/day; the resulting RfD was designed as 375 $\mu\text{g}/\text{kg}/\text{day}$.
5. The endocrine system effects of phthalates may be due to the monoester metabolites.



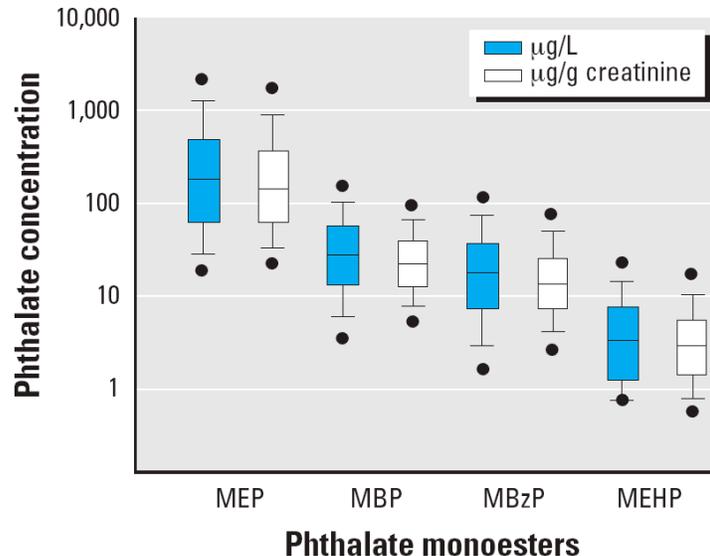
D. Toxicokinetics

1. DEHP is readily metabolized; thus biomagnification through food webs is not significant.
 - a. In mammals, 60-90% of an administered dose is excreted within 24 hours.
2. Other plasticizers are also quickly metabolized and excreted.
 - a. For example, using modeling based on empirically measured pharmacodynamic/kinetic measurements, it was estimated that the gut metabolism rate of DBP was 0.61 per hour (first-order kinetics). [Keys, D. A., D. G. Wallace, T. B. Kepler, and R. B. Conolly. 2000. Quantitative evaluation of alternative mechanisms of blood disposition of di(n-butyl) phthalate and mono(n-butyl) phthalate in rats. *Toxicological Sciences* 53(2):173-184.]
 - b. The gut absorption rate of MBP (monobutyl phthalate, the monoester metabolite of DBP) was 9.9 per hour.
 - c. The maximum rate of MBP metabolism in the liver was 4.3 mg/h (not first-order).

E. Exposure Assessment

1. Recently, the Center for Disease Control released a study in which the researchers analyzed phthalate ester metabolites (as opposed to the parent compounds) excreted in the urine. (*Environ. Health Perspectives*, 2000, 108 [10]:979-982).
 - a. Using such a biomarker, one could estimate the daily dose of exposure and reduce the confounding factor of ubiquitous contamination of samples with parent phthalates (in other words, phthalates are everywhere, including in the lab; thus, it is difficult to avoid contamination of samples).

- b. Exposure to DEHP and DINP was low compared to exposure to DEP, DBP, and BBP.
 1. Ten women in the study had urinary concentrations $>300 \mu\text{g/g}$ (creatinine adjusted basis; this is equivalent to $\sim 200 \mu\text{g/L}$ urine).
- c. The estimated exposure of DBP to a woman (20-40 years) at the 95th percentile was $32 \mu\text{g/kg/day}$ (Kohn et al. 2000, Environ. Health Perspective 108:A442).
2. The comparatively lower excretion of mono-DEHP metabolite compared to the other mono-phthalate esters was confirmed by a more recent study (Silva, M. J et al. 2004. Urinary levels of seven phthalate metabolites in the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Environmental Health Perspectives 112(3):331-338.)



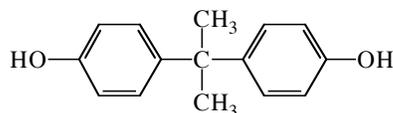
Distribution of mono phthalate ester concentrations in urine (all age group analysis). Note that the oxidative metabolite of DEHP is MEHP, and it has the lowest median of all phthalates studies (Silva et al. 2004)

- a. The distribution of MEP concentrations among age groups showed the lowest creatinine adjusted concentrations among 6-11 year olds (95th percentile excretion concentration was $625 \mu\text{g MEP/g creatinine}$), and the highest excretion concentration was among 20-39 year olds (95th percentile excretion concentration was $2,661 \mu\text{g/g}$)
3. A recent paper by Kato et al. (2004) (Mono(2-Ethyl-5-Hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate. Environmental Health Perspectives 112(3):327-330) suggests that there are other metabolites of DEHP that mask the true exposure in comparison to just using the excreted mono-EHP (i.e., MEHP) as a surrogate biomarker.
 - a. Kato et al. found the DEHP metabolites mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) in urine and sera were 10 times higher than the MEHP metabolite.
 - b. Kato et al. (2004) also noted that many of the metabolites in the urine were glucuronide conjugates.
 1. An earlier review reported that rats do not conjugate the mono phthalate metabolites, but rather this pathway is uniquely characteristic of humans. (Kluwe, W. M. 1982. Overview of phthalate ester pharmacokinetics in mammalian species. Environmental Health Perspectives 45:3-10.)

4. Typical human exposure to DEHP has been estimated to be 4-30 $\mu\text{g}/\text{kg}/\text{day}$ (cited in Moore et al. 2001)
 - a. However, some individuals have substantially greater exposure resulting from DEHP-plasticized medical devices such as blood bags, hemodialysis tubing and membranes, autophoresis equipment and nasogastric feeding tubes.
 - b. The average long-term dialysis patient has been reported to receive approximately 12 g of DEHP over the course of a year.
5. Given the estimated exposure to DEHP, and allowing for even higher exposures assuming additive effects of other phthalate esters (for example add multiply the 4-30 $\mu\text{g}/\text{kg}/\text{day}$ by 100 because MEP (shown in the excretion concentration distribution graph above) may have an approximately 100 times greater exposure (based on ~100 times greater excretion concentration).
 - a. Thus, exposure may range from 400 $\mu\text{g}/\text{kg}/\text{day}$ -3000 $\mu\text{g}/\text{kg}/\text{day}$.
 1. Although this very conservative exposure estimate is higher than the Moore et al. calculated RfD, note that Moore et al. used a 1000 fold safety parameter on an aggregated observation (i.e., percent of litters with males showing areolas and nipples, not on a direct observation of number of males in any one litter with areolas or nipples). In other words, just a single male with areolas or nipples in a litter would qualify it as an "adverse effect".
 - a. Note that in the Moore et al. study, gonadal organ weights and spermatogenesis were not significantly affected by maternal doses of 375 mg/kg/day.
 2. Thus, the exaggerated exposure calculation assuming additivity of all phthalate exposures is still less than concentrations that show no effects on all the studied parameters if the RfD was 3750 $\mu\text{g}/\text{kg}/\text{day}$ (to account for truly observed effects in individuals).

IV. The Saga of Bisphenol A

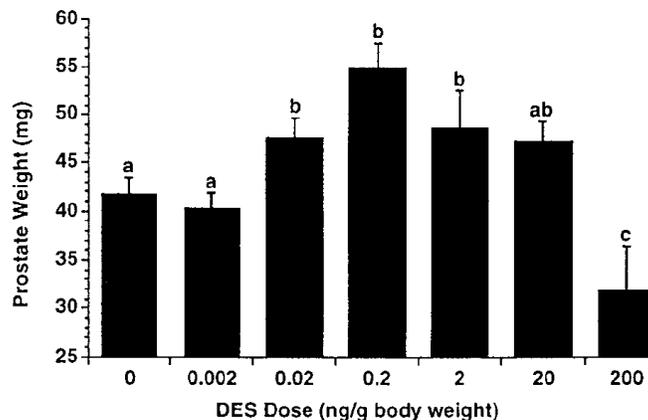
- A. Bisphenol A (BPA) is a monomer used in the manufacture of polycarbonates and epoxy resins from which many products are generated, including food and beverage containers, dental sealants, and babies' bottles.
 1. Polycarbonate is used in eyeglass lenses, medical equipment, water bottles, digital media (e.g. CDs and DVDs), cell phones, consumer electronics, computers and other business equipment, electrical equipment, household appliances, safety shields, construction glazing, sports safety equipment, and automobiles¹. Among the many uses for epoxy resins are industrial floorings, adhesives, industrial protective coatings, powder coatings, automotive primers, can coatings and printed circuit boards.



bisphenol A

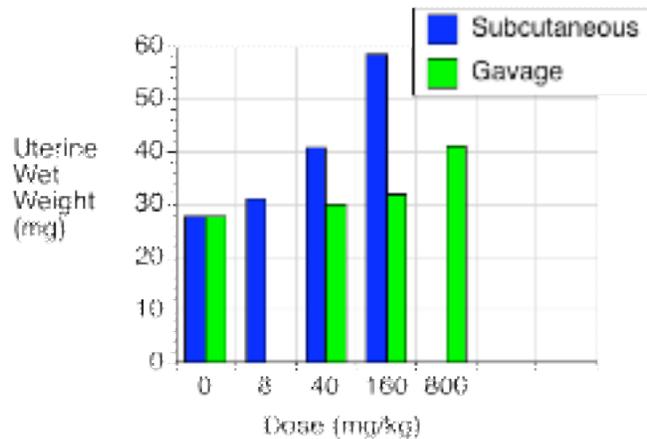
- B. Physicochemical Properties: (Howard 1989, Handbook of Environmental Fate & Exposure Data, vol. 1, Large Production and Priority Pollutants, Lewis Publ.)
 1. Water solubility: 120 mg/L;
 2. Vapor Pressure: 4×10^{-8} mm Hg
 3. Henry's Law Constant: 1×10^{-10} atm-m³/mole
 4. Bioconcentration Factor < 100
- C. Half-life in natural waters: 2.5 – 4 days (based on discharged effluent, ~3 mg/L) (Howard 1989)
- D. Research has shown that bisphenol A leaches out of its polymerized products.

1. For example, tin cans are coated with a lacquer-like liner containing that contains bisphenol A.
 2. A study of polycarbonate plastics indicated concentrations in the plastic ranging from 7 – 58 $\mu\text{g/g}$. (Biles et al. 1997, J. Agric. Food Chem. 45:3541.
 - a. Migration (leaching) tests into water (from a polycarbonate water carboy) showed a concentration of 5 ppb in water.
 - b. About 76% of available bisphenol migrated from plastic into a food oil simulant material;
 - c. With agitation, about 43.7% of available migrated into water (note: 1 $\mu\text{g}/\text{cm}^2$ had migrated)
 - d. Under sterilization conditions (100 °C), final concentrations in leachate medium were about 2 ng/g (10% ethanol in water) or 2 ppb.
- E. Bisphenol A tests positive in in vitro estrogenicity assays, but it was thought to be a weak xenoestrogen.
1. Recent in vivo studies (immature female exposures; using an implant to deliver doses), show that at high doses of BPA can have in vivo activity; however, some researchers now claim it has activity at low doses and shows an inverted dose-response relationship (i.e., a non-monotonic dose-response relationship (Markey, C. M, C. L. Michaelson, e. C. Veson, C. Sonnenschein, and A. M. Soto. 2001. The mouse uterotrophic assay: a reevaluation of its validity in assessing the estrogenicity of bisphenol A. Environ. Health Perspectives 109(1):55-60.)
 - a. The putative effects include levels of 100 mg/kg bw increasing uterine weight
 - b. Increase in uterine epithelial cells (definitive at 75 mg/kg)
 - c. Early onset of vaginal opening (claimed at 0.1mg/kg but not seen at higher doses until 100 mg/kg was administered).
 2. The study by Markey et al., as well as others that claim an inverted or U-shaped non-monotonic dose response usually cite in support of their conclusions an earlier 1997 study by vom Saal et al. (Saal, F. S. V. and et.al. 1997. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc. Natl. Acad. Sci. USA 94:2056-2061.)
 - a. The hypothesis is that at very low doses one effect is seen, at moderate doses the effect disappears or is different, and at high doses the effect (or a different effect) may be seen again.
 - b. In the above cited vom Saal paper, the low dose, non-monotonic effect was observed when gestational mice were given either estradiol or DES (diethylstilbestrol), a synthetic estrogen mimic that is as potent (in vitro) as the natural estrogen.
 1. Note that the dose was delivered in one bolus to the mouth via pipette.

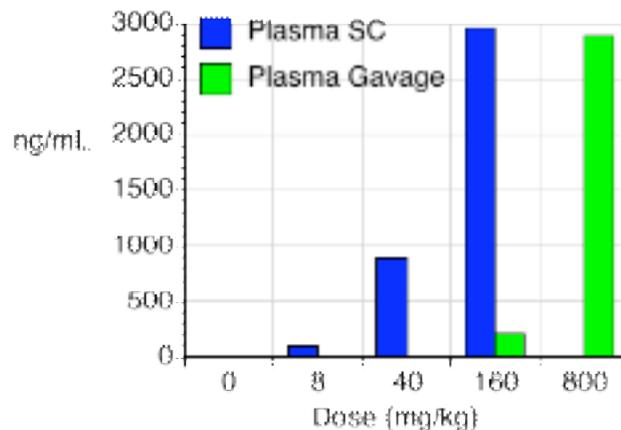


3. Later studies by vom Saal et al. concluded low dose effects of BPA

- a. For example, Howdeshell & vom Saal concluded that a oral dose (directly administered, not dietary ad lib) of 2.5 $\mu\text{g}/\text{kg}/\text{day}$ to dams during GD 11-17 increased postnatal growth in males and females and advanced the timing of puberty. (Howdeshell, K. L. and F. S. vom Saal. 2000. Developmental exposure to bisphenol A: Interaction with endogenous estradiol during pregnancy in mice. *American Zoologist* 40(3):429-437.)
 1. The effect was most pronounced if fetus was in an intrauterine position that exposed it to comparatively more estradiol, suggesting an interaction between an endogenous hormone and an exogenous chemical with estrogenic like activity.
4. Another example of a “low-dose effect was reported by Palanza, P., K. L. Howdeshell, S. Parmigiani, and F. S. vom Saal. 2002. Exposure to a Low Dose of Bisphenol A during Fetal Life or in Adulthood Alters Maternal Behavior in Mice . *Environmental Health Perspectives Supplements* 110(3):415-422.
 - a. Pregnant mice were orally dosed with the equivalent of 10 $\mu\text{g}/\text{kg}/\text{day}$ BPA. The female offspring were raised to adulthood and then exposed again (or not as a control) to BPA.
 - b. Results: Dams exposed to BPA either as fetuses or in adulthood spent less time nursing their pups and more time out of the nest compared with the control group. Females exposed to BPA both as fetuses and in adulthood did not significantly differ from controls.
5. vom Saal et al. have propounded a hypothesis to explain the mechanism of low dose, non-monotonic effects of several hormonally active agents (i.e., those with estrogenic activity), including BPA (Welshons, W. V., K. A. Thayer, B. M. Judy, J. A. Taylor, E. M. Curran, and F. S. vom Saal. 2003. Large Effects from Small Exposures. I. Mechanisms for Endocrine-Disrupting Chemicals with Estrogenic Activity . *Environ. Health Perspectives* 111:994-1006).
- F. The existence of low dose, nonmonotonic (inverted or U-shaped dose-response) effects have been disputed in other studies.
 1. Cagen, S. Z. et al. 1999. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. *Toxicological Sciences* 50:36-44.
 - a. Rats were administered bisphenol A subcutaneously or orally in sesame oil. Response to bisphenol significantly greater when administered subcutaneously than when administered by gavage. (Yamasaki, K., M. Sawaki, and M. Takatsuki. 2000. Immature rat uterotrophic assay of bisphenol A. *Environ. Health Perspectives* 108(12):1147-1150.)
 - b. For example, NOEL and LOEL for uterine weight after subcutaneous administration (3 day administration to immature female rats) was 8 and 40 mg/kg, respectively.
 - c. For oral exposure, the NOEL and LOEL was 160 and 800 mg/kg, respectively.
 2. In another study, pre- and postnatal exposure of rats to BPA at 3.2, 32, or 320 mg/kg/day from GD 11 through PND 20 did not have any apparent adverse effects on female rat pubertal development and reproduction functions. (Kwon, S., D. B. Stedman, and B. A. Elswick. 2000. Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. *Toxicological Sciences* 55(2):399-406.)



1. Kwon also examined the levels of bisphenol A in the plasma of dosed rats.



3. Bisphenol A was fed to pregnant rats in a single oral dose of 1 g/kg. (Takahashi and Oishi 2000, Environ. Health Perspectives 108:931)
 - a. The maximal concentration of BPA in the fetuses was 9 $\mu\text{g/g}$ at 20 min.
 1. Indicates rapid absorption of BPA across the placenta.
4. Two studies published during 2002 further dispute evidence of either a low dose effect or an in vivo developmental/reproductive effect over three generations
 - a. Tinwell, H., J. Haseman, P. A. Lefevre, N. Wallis, and J. Ashby. 2002. Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. Toxicological Sciences 68:339-348.
 1. Doses of 20 $\mu\text{g/kg}$, 100 $\mu\text{g/kg}$, or 50 mg/kg body weight of BPA were given to rats by gavage during gestation days 6-21
 - a. Only 50 mg/kg decreased daily sperm production and increased age of vaginal opening
 - b. No other effects at any dose
 - c. Synthetic estrogen at 200 $\mu\text{g/kg}$ was toxic to dams (although severe endocrine disruption did occur)
 - b. Tyl, R. W. and et al. 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicological Sciences 68:121-146.
 1. Administered BPA in diet to three generations of rat offspring
 2. Doses were 0, 0.001, 0.02, 0.3, 5, 50, and 500 mg/kg/day BPA
 3. Adult systemic toxicity at 50 and 500 mg/kg/day
 - a. NOAEL for adult systemic toxicity was 5 mg/kg/day

4. Reproductive and postnatal developmental NOAEL was 50 mg/kg/day
 5. There were no treatment-related effects in the low-dose region (0.001 – 5 mg/kg/day)
- G. At the request of the U.S. Environmental Protection Agency (EPA), the National Toxicology Program (NTP)/National Institute of Environmental Health Sciences (NIEHS) organized and conducted an independent and open peer review aimed at evaluating the scientific evidence on reported low-dose effects and dose-response relationships for endocrine disrupting chemicals in mammalian species that pertain to assessments of effects on human health. The peer review took place in Research Triangle Park, North Carolina, on October 10-12, 2000.
1. One of the chapters of the subpanel review committee dealt with the BPA studies and low doses. The following conclusions show the uncertainty surrounding the current knowledge of low dose, non-monotonic effects.
 - a. The Subpanel concluded that “there is credible evidence that low doses of BPA [bisphenol A] can cause effects on specific endpoints. However, due to the inability of other credible studies in several different laboratories to observe low dose effects of BPA, and the consistency of these negative studies, the Subpanel is not persuaded that a low dose effect of BPA has been conclusively established as a general or reproducible finding.”
 - b. “Data are insufficient to establish the shape of the dose-response curve for bisphenol A in the low dose region, and the mechanism and biological relevance of reported low dose effects are unclear.”
- H. Estimated human exposure to BPA
1. Milman et al. 2002 reported human exposure to BPA from use of polycarbonate plastics, epoxy resins, and other products to be <2.5 ppb on a dietary basis, and less than 0.1 $\mu\text{g}/\text{kg}/\text{day}$ for a 60 kg person consuming 3 kg of food and beverage per day. (Milman, H. A., M. C. Bosland, P. D. Walden, and J. E. Heinze. 2002. Evaluation of the adequacy of published studies of low-dose effects of bisphenol A on the rodent prostate for use in human risk assessment. *Regulatory Toxicology and Pharmacology* 35:338-346.)
- I. Levels in fish and potential effects
1. Belfroid, A., M. van Velzen, B. van der Horst, and D. Vethaak. 2002. Occurrence of bisphenol A in surface water and uptake in fish: evaluation of field measurements. *Chemosphere* 49:97-103.
 - a. Monitored fish in water systems in the Netherlands
 - b. BPA present in Dutch surface water at levels up to 330 ng/L; one sample contained 21 $\mu\text{g}/\text{L}$
 - c. BPA was detected in fish livers, 2-75 ng/g, and in muscle, 1-11 ng/g
 1. The predicted no effect concentration (PNEC) is 64 $\mu\text{g}/\text{L}$
 2. Concluded therefore that ecorisk was nil
- J. A report on ecological hazards of BPA by the Bisphenol A Global Industry Group (available from the internet; report dated October 2002); the report is well referenced using peer-reviewed literature. Some of the conclusions are quoted below.
1. Aquatic Concentrations:
 - a. Numerous publications have reported measured concentrations of BPA in streams and rivers in Japan, Europe and the United States. The median reported water concentrations from 21 European and 13 United States studies are 0.016 and 0.5 $\mu\text{g}/\text{L}$ respectively (Cousins *et al*, 2002). In cases where individual concentration data are reported, many samples have no detectable level of BPA.
 - b. A recent report from the U.S. Geological Survey provides data on the occurrence of BPA (and numerous other substances) in a large number of U.S. streams, most of which were characterized as streams susceptible to contamination (Kolpin *et al*, 2002). Approximately 60% of the streams contained no detectable level of BPA

(detection limit 0.09 $\mu\text{g/L}$), the median detected concentration was 0.14 $\mu\text{g/L}$, and only 2 streams were reported to contain BPA at levels above 1 $\mu\text{g/L}$.

2. Ecotoxicological Effects:
 - a. The No-Observed-Effect-Concentration (NOEC) of BPA in a 21-day chronic reproduction test in *Daphnia* was 3160 microgram/L (Caspers, 1998). Effect concentrations at the 10% level (EC10) were determined for both freshwater and marine algae to be 1360 to 1680 micrograms/L and 400 to 690 micrograms/L, respectively (Alexander *et al*, 1988).
 - b. The results of a multi- generation study on fathead minnows showed that survival, growth and reproductive fitness for three generations were affected only at concentrations of 640 micrograms/L and higher, with hatchability of F2 (second generation) eggs slightly reduced at 160 micrograms/L. The NOEC measured in this study was 16 micrograms/L. (Sohoni *et al*, 2001; Caunter, 2000).
 - c. A weight-of-evidence analysis of the aquatic hazards posed by BPA was conducted with a focus on validated studies and the ecologically relevant endpoints of survival, growth and reproductive fitness. This analysis included the use of statistical extrapolation techniques to assess the full database of reported effect concentrations. The study concluded that no adverse aquatic effects are expected at concentrations below 100 $\mu\text{g/L}$ of BPA (Staples *et al*, 2002).
 - d. Cited references:

Alexander, H. C., D. C. Dill, L. W. Smith, P. D. Guiney, and P. B. Dorn, 1988, "Bisphenol A: Acute Aquatic Toxicity", *Environmental Toxicology and Chemistry*, vol. 7, pages 19-26.

Caspers. N., 1998, "No Estrogenic Effects of Bisphenol A in *Daphnia magna* STRAUS", *Bulletin of Environmental Contamination and Toxicology*, vol. 61, pages 143-148.

Caunter, J. E., 2000, Bisphenol A: Multigeneration Study with Fathead Minnow (*Pimephales promelas*), Study No. BL6878/B. 91 pp.

Cousins, I. T., C. A. Staples, G. M. Klecka, and D. Mackay, 2002, "A Multimedia Assessment of the Environmental Fate of Bisphenol A", *Human and Ecological Risk Assessment*, vol. 8, pages 1107-1135.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton, 2002, "Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance," *Environmental Science and Technology*, vol. 36, pages 1202-1211. Full report with individual stream data is available on the Internet at http://toxics.usgs.gov/regional/emc_surfacewater.html.

Sohoni P., C. R. Tyler, K. Hurd, J. Caunter, M. Hethridge, T. Williams, C. Woods, M. Evans, R. Toy, M. Gargas, and J. P. Sumpter, 2001, "Reproductive effects of long-term exposure to bisphenol A in the fathead minnow (*Pimephales promelas*)", *Environmental Science and Technology*, vol. 35, pages 2917-2925.

Staples, C. A., P. B. Dorn, G. M. Klecka, D. R. Branson, S. T. O'Block, and L. R. Harris, 1998, "A Review of the Environmental Fate, Effects and Exposures of Bisphenol A," *Chemosphere*, vol. 36, pages 2149-2173.

Staples, C. A., P. B. Dorn, G. M. Klecka, S. T. O'Block, D. R. Branson, and L. R. Harris, 2000, Bisphenol A Concentrations in Receiving Waters Near U.S. Manufacturing and Processing Facilities. *Chemosphere*, vol. 40, pages 521-525.

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