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BREAST CANCER & THE ENVIRONMENT RESEARCH CENTERS

Early Life Exposure and Breast Cancer Risk in Later Years

FACT SHEETS

Prepared by:
BCERC Community Outreach and Translation Cores

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FACT SHEETS

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Breast Cancer and the Environment Research Member Centers

The Breast Cancer and the Environment Research Centers program is a network of four collaborative research centers. The centers are comprised of teams which include scientists, clinicians, and breast cancer advocates focused on how chemical, physical, and social factors in the environment interact with genetic factors to affect mammary gland development. Jointly supported by the National Institute of Environmental Health Sciences and the National Cancer Institute, the center research program will span seven years (2003-2010).

The four centers that make up the collective Breast Cancer and the Environment Research Center are:

- Fox Chase Cancer Center, Philadelphia, PA
- Michigan State University, East Lansing, MI
- University of California San Francisco
- University of Cincinnati, Cincinnati, OH

For more information on the Breast Cancer and the Environment Research Centers, go to http://www.bcerc.org.

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FACT SHEET INTRODUCTION

The Bay Area Breast Cancer and the Environment Research Center (BABCERC) Community Outreach and Translation Core (COTC) led by Zero Breast Cancer, in collaboration with University of Cincinnati COTC, have prepared six scientific fact sheets on environmental exposures being measured and examined by the Breast Cancer and the Environment Research Centers (BCERC) epidemiology studies.

The six fact sheets and page numbers are listed below.

The objective for producing these fact sheets is to provide BCERC researchers, COTC members and community breast cancer and environmental advocates background information, based on a current review of the literature, on these environmental exposures to assist in:

- understanding the research being presented at BCERC's annual scientific symposium
- interpreting the literature as BCERC research findings are disseminated
- developing outreach materials for the lay public

The fact sheets were evaluated at the November 2006 BCERC symposium in Berkeley, California and at the June 2007 NIEHS BCERC Interim Meeting in Washington D.C., and then revised and reviewed by NIEHS for completeness and reference accuracy. All fact sheets have been reviewed for scientific content by various researchers from BCERC, NCI and the EPA.

For more information on the Breast Cancer and the Environment Research Centers, go to http://www.bcerc.org.

Breast Cancer and the Environment Research Centers Community Outreach and Translation Cores
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FACT SHEET on PERFLUORALKYL ACIDS (PFAAs)

Abstract

PFAAs are a family of synthetic, fluorinated carbon chain chemicals which are widely used in the manufacture of numerous consumer products. The most widely used and environmentally dispersed forms are the eight-carbon compounds, also known as C-8s. Within the family of C-8s, perfluorooctanoic acid (PFOA), perfluorooctanosulfonate (PFOS) and ammonium perfluorooctanoate (APFO) are the most abundant. These compounds have *surfactant* properties (reduces surface tension of liquids) and are essential *polymerization* aids (helps create chemical chains) used in very small quantities to help make *fluoropolymers* (chemical chains containing fluorine atoms). PFOS and PFOA are primarily components of larger chemical substances. Although production of PFOS has stopped in the US (37), environmental degradation of larger fluorotelomers and >8 carbon chain PFAAs, in addition to their production for use in a variety of industrial products, yield PFOA. Sources and routes of exposure are just beginning to be understood at this time. For several decades, PFAAs were thought to be environmentally and biologically inert. No regulatory standards were in place until recently. Numerous studies are underway at this time to explore and characterize biological activity of these compounds in both animal models and humans.

The appearance of various cancers, alterations in reproductive and thyroid hormones, immunotoxicity and adverse reproductive outcomes have been reported in animal studies. However, the results vary across studies, largely due to species and gender differences in how the PFAAs are metabolized and/or excreted, as well as marked differences in the half-life of these chemicals in blood (33). In humans, occupational exposures have been linked to bladder and kidney cancers and diabetes(39), while in utero exposures have been linked to reduced weight and size at birth(1,8).This fact sheet provides information about PFAAs, sources of exposures, their effects on puberty, effects in the body, and research studies associating PFAAs with breast cancer. PFAAs are one of several classes of biomarkers being measured and evaluated for health effects in the Breast Cancer and the Environment Research Centers.

What are PFAAs?

PFAAs are a family of synthetic, fluorinated carbon chain compounds which are widely used in the manufacture of numerous consumer products. Chain length may vary from 4 to 14 carbons, in which the carbon atoms are fluorinated. The terminal carbon of the chain contains a charged carboxylate, phosphonate or sulfonate *moiety* (functional group) (33). Chemical names, formulas, abbreviations and structures of some of the major PFAAs are reported in the literature (15). The most widely detected and environmentally dispersed forms of PFAAs are the eight carbon compounds, also known as C-8s. Within the family of C-8s, perfluorooctanoic acid (PFOA), perfluorooctanosulfonate (PFOS) and ammonium perfluorooctanoate (APFO) are the most prevalent. Higher order chain lengths break down in the environment to form C-8s. C-8s do not biodegrade, nor are they metabolized. EPA has classified C-8s as being environmentally persistent, bioaccumulative toxicants (6,33). PFAAs are found in fish, birds and mammals in North America, Europe, Asia, and Antarctica (33). Sera from all people tested contained some PFAAs (3). In 2002, the 3M Company phased out production of PFOS due to its widespread presence in the environment and its propensity to bioaccumulate in a wide range of wildlife and humans. The amount of this compound in the marketplace has dropped precipitously since then (3M Co., 2003). In contrast, the amount of PFOA produced increased and became one of the most popular PFAAs in commerce. There have been very few studies of the effects of PFOA on human health. Most studies have focused on occupationally exposed male workers and were cross-sectional in design (33). No consistent, confirmed associations have been reported. A large scale community health study, called the C-8 Health Project, was initiated in 2005, as part of a settlement agreement between EPA and Dupont (7). This series of related studies is following approximately 70,000 individuals exposed to PFOA in the Parkersburg, West Virginia area. There are several websites which provide Fact Sheets and updates of PFC research findings (16,17,18). This fact sheet will primarily introduce the two most abundant PFAAs in the global environment, PFOS and PFOA.

What is PFOA?

PFOA, or perfluorooctanoic acid, is a surfactant and an essential polymerization aid used in very small quantities to help make fluoropolymers, which are high performance plastic and synthetic rubber materials. Its chemical formula is C8-H1-F15-O2. The chemical form of PFOA used in fluoropolymer manufacturing is the ammonium salt, known as APFO (C8-H1-F15-O2.H3-N). Within the fluoropolymer industry, APFO is sometimes called C-8, referring to the number of carbon atoms in its molecular structure**.** In addition to being produced for use in other commercial products, PFOA is also produced as a final degradation product of a variety of precursor perfluorinated chemicals (33).

What is PFOS?

PFOS, the perfluorooctane sulfonate anion, is no longer manufactured globally (37), but is a component of larger chemical substances whose environmental degradation can yield it. Its chemical formula is C8-F17-SO2. Classified as a persistent, bioaccumulative chemical, it is found in most wildlife (33).

Which commercial products contain PFAAs?

These are almost too numerous to list. Because the physical properties of the C-8 PFAAs make them excellent surfactants, they are used in over 200 industrial and consumer applications (33). They are used in the manufacturing of 1) Water or stain-proofing agents, e.g., clothes, carpeting, upholstery, leather, mattresses, footwear, and water-proof outerwear; 2) Grease or water resistant paper packaging materials, e.g., popcorn bags, microwavable meals, and packaging on such foods as chewing gum, candy, donuts, pizza, fast-food; and 3) Miscellaneous products, e.g., fire fighting foam, photography emulsion, specialty fuels, paints, waxes, electroplating materials, and health and beauty products, such as dental floss. PFAAs are also used in the production of trademark materials such as Post It Notes, Teflon, Zonyl (paper coating material), Gor-Tex, and Tyvec. PFOS was used in the production of Scotchgard and Stainmaster. Furthermore, some industries make extensive use of PFAAs. For example, they are used by these industries: film processing, aluminum production, electronics production, refrigeration, pharmaceutical production, and printing (41).

How are humans exposed to PFAAs?

For several decades, PFAAs were thought to be environmentally and biologically inert, so no national regulatory standards were in place. More recently, a number of states, including NJ, NC, and MN, have introduced regulations to limit the level of PFAAs in water. Numerous sources of PFAA exposure have been identified (33). Ingestion and inhalation are the major routes of exposure to these compounds. Consumption of contaminated drinking water is a major source of chronic exposure in certain populations. Inhalation of air emissions around production facilities and resultant contamination of soil and house dust have also been identifies as important point sources (4,5,9,33). In fact, the entire Northern hemisphere is contaminated with PFOA and PFOS, which may be due to telomer acid distribution into the atmosphere (34). Contaminated convenience foods form the third major exposure source. Of course, occupational exposures can produce high levels of exposure in a workforce (5,6,7). There are multiple sources of quantitative exposure data available at this time on levels of serum PFAAs in US (3,10). However, few documents provide quantitative data on the levels in the actual products containing the PFAAs. Therefore, the following is largely based on a few studies and scientific inference from other exposure scenarios:

Ingestion

1. Food

PFAAs can inadvertently be added to foods during preparation or packaging for distribution. Many food packaging materials contain residual PFAA, including PFOA, from the manufacturing process. High temperatures can result in the leaching of PFAA from the packing material into the food

product. Examples where such transfer might occur include pizza boxes, french fry boxes or wrappers, microwave popcorn bags, etc (14).

2. Non-stick cookware

PFAAs are used in the application of non-stick coating to cookware. Most PFAAs are removed during the application process, but some residues may remain. Extremely high heat can release PFAAs into the air where they can be inhaled. This is very unlikely to occur during the normal cooking process, since the necessary temperatures would severely burn the food. The safety of cookware coated with fluoropolymer non-stick coatings, e.g. Teflon, has been assessed by regulatory agencies of the United States and many other countries. Non-stick cookware has been approved by the U.S. Food & Drug Administration (FDA) for conventional kitchen use. Governments in other parts of the world have also approved these coatings on cookware and housewares**.**

3. Soil and Dust

There are seven states currently dealing with environmental PFAA contamination: New Jersey, Virginia, North Carolina, West Virginia, Ohio, Alabama, and Minnesota. The Alabama and Minnesota contamination arose from 3M plant releases and landfills, while the remaining five sites are due to DuPont plant releases or landfills. Air emissions have contaminated surrounding soils and sediment, while runoff has contaminated groundwater and, in some cases, drinking water supplies (5,9,16,18). Contaminated soils, exterior dust and house dust are particularly important sources of exposure for young children due to the likely contamination of their hands and subsequent hand-to-mouth behaviors.

4. Ground Water and Drinking Water

Contaminated drinking water has been responsible for the largest episodes of community exposure (5,7,9,18; median PFOA levels in excess of 300 *ng/ml* [nanograms per mililiter of blood serum, i.e. parts per billion, or *ppb*])(5), and the greatest publicity to date (9). Levels of PFOA in drinking water can range from less than 0.01 ng/ml, i.e., <*LOD* (less than the limit of detection), in non-contaminated drinking water supplies to over 7 ppb around some plant sites (5). This contamination led to the largest penalty in the history of the EPA being imposed against DuPont, the industry known to cause the contamination event (15). Currently, several states have imposed their own MCLs for PFOA and many more are underway (18,21). PFOA is under risk assessment review by the EPA and in 2006, the EPA initiated the PFOA Stewardship Program in which the 8 major companies will reduce global emissions and product content of PFOA and related chemicals by 95% by the year 2010 (17).

5. Infant consumption

If drinking water is contaminated with PFAAs, these contaminants can potentially produce unnecessary exposure to infants drinking formula prepared from this water. This is of particular concern given the greater vulnerability of infants to absorption of toxicants and the sensitivity of developing organs to toxic insults. Furthermore, PFOA has been shown to be a developmental toxicant in mouse studies (11, 12, 33). Although there are few reports (20,36), it appears that breast milk is not a major source of PFAA exposure.

6.Toys (painted)

Some paints contain Teflon or have rubberized coatings. Therefore, they are likely to contain some residual PFAAs. Potentially, this could result in exposures if the child chews on the paint. At this time there are no exposure data available.

How do I know if I have been exposed to PFAAs?

PFAAs can be measured in blood serum samples using a technique called *mass spectrometry* (3,19). These tests are very sensitive and can detect concentrations of less than 1 ppb of PFOA. Unfortunately, there are only a few laboratories in the US that can reliably make these measurements; consequently, these tests are currently very expensive, about $300 to $400 each depending on the volume of samples (35). Because of the long *half-life* (time to eliminate half the

amount) of PFOA and PFOS in the human body, a single measurement cannot be used to determine if the measured level is due to current, ongoing exposures or a prior, episodic exposure, which might have occurred several years earlier.

How does PFOA work in the human body?

PFOA is well-absorbed by rats and mice after oral exposure. *Dermal* (skin) absorption has also been reported in the rat, but not in humans (23). In male, but not female rats there is evidence of *enterohepatic circulation* (in bile between the liver and small intestine) (24). PFOA is excreted in both the urine and feces. In animals, there are large species and gender differences in the biological half-life of PFOA, due mainly to differences in *renal* (kidney) clearance (25). Half-life in female rats is only a few hours, while it is several days in the male. There is no difference in half-life in the male and female mouse, estimated at a little over 2 weeks (32). Half-life in humans is estimated to be several years (26). The effects of PFOA in rats include increased *P-450* (hemoprotein enzyme) activity, decreased serum low density lipoproteins and cholesterol, and increased oxidation of fatty acids. These effects lower serum cholesterol and elevate lipids in the liver (22).

Does PFOA or PFOS exposure influence onset of puberty in girls?

This is unknown at this time. BCERC's biology and epidemiology studies are investigating this question. The BCERC epidemiology project entitled "Environmental and Genetic Determinants of Puberty" has measured PFCs in about 80-90 young girls. This small pilot data set will guide future expanded cohort studies. Mouse studies show that PFOA exposure during gestation produces developmental delays and growth retardation (11,12,32), and studies in children demonstrate an inverse relationship of serum PFOA and birth weight (1,8). A single mouse study (32) has assessed pubertal timing and found that low level exposures hasten puberty in males, while no effects on puberty were seen in females until high level exposures were reached. The effects of these compounds in humans are unknown.

Do PFOS and/or PFOA cross the placenta?

Yes. Recent studies reveal several different PFAAs present in cord blood, including PFOS and PFOA, in the fetus (1,8).

Are PFAAs found to be present in breast milk?

Yes. Karrman et al. studied matched serum and breast milk samples from a group of 12 Swedish women, three months post-partum. They found that the levels of PFOS in breast milk are about 1% of the level found in serum, while PFOA was only infrequently detected (20).

Are PFAAs endocrine disruptors?

Yes. According to EPA, an endocrine disrupter is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of *homeostasis* (biological stability), reproduction, development and/or behavior (31). PFAAs have been found to adversely affect both prenatal and postnatal development as well as the reproductive system in laboratory animals (11, 12, 29). In a study of adult male rats treated with PFOA, serum *estradiol* (a form of estrogen) was increased, serum testosterone was decreased, and liver *aromatase* (the enzyme responsible for conversion of testosterone to estradiol) activity increased (27).

Are concentration levels of PFAAs the same in men and women?

No. Studies have shown that, on average, men have higher serum levels of PFOA than women. The median level in adult men in the US is 6.0 ppb, while it is 4.6 ppb in women (3). There are no significant differences across age groups within in the 12 to 60 year range. Currently, there are no national data for children less than 12 years of age.

Are there in vitro or in vivo studies that have found an association with PFAA exposure and breast cancer risk?

Yes. A recent study investigated estrogen-like properties of five perfluorinated compounds (30). Using an E-screen assay, they found that several fluorotelomer alcohols have proliferation promotion capacity. However, PFOS and PFOA did not share this hormone-dependent proliferation capacity. Employing the standard MCF-7 human Caucasian breast adenocarcinoma cell assay, they also undertook gene expression analysis of selected estrogen-responsive genes, e.g., TFF1, PRG, ESR1, PDZK1, and ERBB2. Numerous changes were found. White and coworkers also demonstrated in mice a profound effect of prenatal PFOA exposure on the development of the mammary tissue of the offspring (11). The effect was seen with just a 6 day exposure. PFOS is linked with mammary, pancreatic, thyroid, and liver tumors in rats, as well as hyperthyroidism and altered lung development (40)**.** Because of tumor development in a variety of other tissues following lifetime exposure to PFOA in rats, EPA's Science Advisory Board has classified PFOA as a "likely human carcinogen" (6).

Are there epidemiological studies that have found an association with PFAA exposure and breast cancer risk?

Not at this time. The studies to determine the health effects of PFAA exposures in developing children and adults are in their infancy. However, there is a large study currently underway which might provide such information within the next several years. Nearly 70,000 highly-exposed residents of Parkersburg, WV are participating in a long term health study to answer such questions (7).

What is known about PFAAs from biomonitoring measurements in the National Health and Nutrition Examination Survey (NHANES) or other study Reports?

Serum samples collected during the NHANES survey were subsequently analyzed for eight different PFAAs, including PFOA and PFOS (3). This study found higher levels in men than women; higher levels in non-Hispanic whites and non-Hispanic blacks than in Mexican-Americans; and no age-related trends between 12-19 year olds and those over age 60. Measurement of PFAAs in sera collected from a separate cohort of 598 children, ages 2 to 12 years old, found a median PFOA level of 5.1 ppb and a 90th percentile of 8.5 ppb (10). Children aged 2-5 years were reported to have higher levels than those aged 6 -10 or 11–15 in Parkersburg WV (5). Lau et al. (33) have summarized available data regarding PFAA serum levels in adult men and women and children in both the US and other countries.

Has the federal government made recommendations for PFOA or PFOS exposure limits to protect human health?

EPA

EPA has yet to set a national maximum contaminant level (MCL) for PFOA or PFOS in drinking water. However, EPA has set an interim drinking water MCL in Parkersburg, WV, which was initially 150 ppb, but reduced to 0.5 ppb in 2006 (7). In March 2007, the Minnesota Department of Health established maximum safe drinking water limits of 0.5 ppb and 0.3 ppb for PFOA and PFOS, respectively (18). In April 2007, New Jersey set a preliminary health-based lifetime exposure limit which translates to an MCL of 0.04ppb (21). In January 2006, the EPA initiated a PFOA stewardship program with eight major producers to reduce emissions and product content by 95% no later than 2010 and to eliminate these chemicals from emissions and products by 2015 (38).

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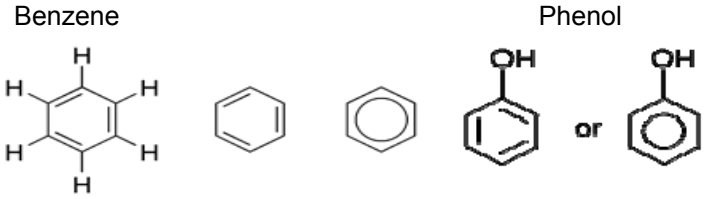
Abstract
Phenols or phenolics are a manufactured class of weakly acidic water-soluble chemical compounds related to the organic chemical compound phenol naturally present in most foods. Phenol is used as a slimicide, a disinfectant, in medical products, and as a reagent in research laboratories and as a precursor or intermediate during the manufacture of phenolic resins, bisphenol A, caprolactam, adipic acid, alkylphenols, aniline, and chlorinated phenols. Phenol ranks in the top 50 chemical volumes produced in the United States. Phenols are readily absorbed following inhalation, ingestion or skin contact, and are widely distributed in the body, can cross the placenta, and have been found in human breast milk. Some phenols are weak endocrine disrupters. Epidemiological studies are needed to determine the association between phenol exposure and human breast cancer risk. Research studies investigating the association of phenols with breast cancer risk, sources of exposures, effects on puberty, and general effects in the body are ongoing. The International Agency for Research on Cancer (IARC) classification for phenols is Group 3, not classifiable with regard to its carcinogenicity to humans (1,2,3).

This fact sheet provides information about nine phenolic compounds being measured and examined by the Breast Cancer and the Environment Research Centers (BCERC) epidemiology studies, sources of exposures, effects on puberty, effects in the body, and research studies looking at phenols as being associated with breast cancer risk. Phenols are nonessential chemical compounds.

What are phenols?

Phenols, sometimes called **phenolics**, are a class of aromatic organic compounds consisting of one or more hydroxyl groups attached to an aromatic hydrocarbon group (4). Phenol is a benzene derivative and is the simplest member of the phenolic chemical. Its chemical formula is C_6H_5OH and its structure is that of a hydroxyl group (-OH) bonded to a phenyl ring (Fig. 1). Synonyms for phenol include carbolic acid, benzophenol, and hydroxybenzene.

Figure 1:



Phenol is produced naturally and synthesized as a manufactured chemical. Naturally, it is a constituent of coal tar and creosote, decomposing organic material, human and animal wastes, and as a compound found in many non-foods and foods. For example, salicylic acid is a natural phenolic compound found in willow bark. Salicylic acid is also synthesized from phenol as an intermediate in the industrial production of aspirin. Phenol is also formed during forest fires, and by atmospheric degradation of benzene in the presence of light. In addition, phenol is produced by the body and excreted as a metabolic product independent of external exposure or intake.

Phenol is a high volume chemical with production exceeding 3-billion pounds annually in the United States and 6-billion pounds worldwide. It ranks in the top 50 in production volumes for chemicals produced in the United States with the housing and construction industries accounting for about half of the phenol used (8). Manufacture of phenolic resins is the largest single use of phenol, reported to be 1.188 billion pounds in 1988 (33). Phenol is usually sold commercially as a thick liquid.

The three major uses of manufactured phenol are as chemical intermediates to produce:

1. phenolic resins (human made polymers consisting of phenol) used in plywood adhesive, construction, automotive, and appliance industries
2. bisphenol A which is used primarily in the manufacture polycarbonate plastics, epoxy resins and non-polymer additives to other synthetic polymers
3. caprolactam which is used in the manufacture of nylon 6 and other synthetic fibers

Phenol is also used as an antiseptic, a general disinfectant, and a slimicide (chemicals that kill bacteria and fungi in slimes), in medical preparations including lotions, ointments, mouthwashes, salves. Phenol is also the active ingredient in some over-the-counter oral anesthetics sprays used as a treatment for sore throats. Minor uses of phenol include the manufacture of paint and varnish removers, lacquers, paints, rubber, ink, illuminating gases, tanning dyes, perfumes, soaps and toys (6,7).

How are humans exposed to phenols?

Exposures to phenol can occur in the workplace, from environmental media, from contaminated drinking water or foodstuffs, or from use of consumer products containing phenol (ATSDR). Phenol is readily absorbed following inhalation, ingestion, and skin contact.

Very small amounts of phenol is produced endogenously as a breakdown product of protein metabolism by the action of bacteria on normal constituents of the diet in the gut and excreted independent of external exposure to the compound.

Inhalation

- **Indoor air and house dust**

Exposure to phenol through inhalation is a less probable route than oral and dermal. Phenol can be released during the combustion of wood, fuel emissions and tobacco. It has been found that the smoke of 1 nonfilter cigarette contains 60–140 µg of phenol, 19–35 µg for a filter-tipped cigarette, and 24–107 µg in cigars (IARC 1986; NCI 1998), and smoking these products indoors produces a measurable amount of phenol (Guerin et al. 1992). If children are present in indoor environments polluted with tobacco smoke, they may be exposed to low levels of phenol.

Ingestion

- **Water**

Ingestion of contaminated water

- **Food**

Free and bound phenol compounds are found naturally in foods. High phenol foods include tomatoes, apples, peanuts, bananas, oranges, cocoa, red grapes, colored fruits (e.g., cranberries), and milk. These compounds may also be a contaminant in packaged foods, as these compounds are used in can liners and foil wraps. The "phenol" category contains quite a few subgroups, both food and non-food. For example, a non-food is salicylate a natural chemical made by many plants is a subgroup of phenol.

Skin Contact

Phenol compounds are found in dental sealants, sunscreen, lotions, hand soap, and toothpaste. Topically applied these compounds are a skin irritant.

The nine phenolic compounds and exposure sources examined by the BCERC epidemiology studies are listed in Table 1. Below the table each compound exposure source is further described.

Table 1. Phenolic compounds, their parent compounds, and examples of environmental sources. (9)

	Chemical name or common synonym	Abbreviation	Parent Compound, if applicable	Additives, commercial and personal product exposure sources
1	Bisphenol A	BPA		Polycarbonate containers and coatings (cans, cups), dental sealant
2	Benzophenone-3(2-hydroxy-4-methoxy-benzophenone), (oxybenzone)	BP3		Sunscreen agent, photostabilizer for synthetic resins
3, 4, 5	2,4-Dichlorophenol and trichlorophenols (chlorinated phenols)	24DCP, 245TCP, 246TCP	Phenoxy- and other derivatives (245, 246 TCP are metabolites of Hexachlorobenzene and Hexachlorocyclohexane.)	Herbicides (organochlorine pesticides)
6	2,5-Dichlorophenol	25DCP	4-dichlorobenzene (metabolite of p-DCB)	Mothballs
7	ortho-Phenylphenol	o-PP		Fungicide
8	4-tert-Octylphenol	4-t-OP		Detergent surfactant
9	Triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol]	TRCS		Microbicide in home cleaning and personal care products

1. Bisphenol A (BPA)

Bisphenol A belongs to the phenol class of aromatic organic compounds and is a chemical compound containing two phenol functional groups. It was first synthesized over 100 years ago and during the 1930's BPA was investigated as an estrogen drug. Beginning in the 1950's BPA has primarily been used to manufacture polycarbonate plastic. It is also found in epoxy resins used to line metal food and drink cans, as a polymer additive to polyvinyl chloride plastic (e.g. plastic cling wraps and plastic pipes), and some dental sealants. Bisphenol A is also used during the manufacture of specialty resins and flame retardants, such as tetrabromobisphenol A. The recycling code 7 on the bottom of some plastic containers, such as large water bottles used in water dispensers, often indicates that the plastic is made of polycarbonate. There are many synonyms for bisphenol A (10).

Human exposure is primarily through ingestion.

Ingestion sources of BPA include:

- **Contaminated Foods and Beverages**
  - BPA can migrate from **polycarbonate plastic bottles or food storage containers** into foods or beverages especially once the container has been heated to high temperatures (e.g. boiling water)
  - BPA can migrate from the **epoxy resin inner lining of some metal food and drink cans** into the food or liquid containing the food
  - BPA may also migrate from **polycarbonate plastic in some clear plastic spill-proof cups and cutlery** (forks, knives, and spoons) into hot or fatty foods

- **Oral exposure from dental procedures**
  - Several studies have shown that some dental sealants and composite materials used to fill cavities can release BPA

The US EPA has set a safe human intake dose of 50 micrograms per kilogram of body weight per day for **bisphenol A.**

2. Benzophenone-3(2-hydroxy-4-methoxy-benzophenone)[oxybenzone] (BP3)
BP3 (oxybenzone) is an ultraviolet (UV) filter and is used in the manufacture of sunscreens, as a photostabilizer for synthetic resins, many personal care, and household products. A listing of products containing BP3 can be found at: http://householdproducts.nlm.nih.gov/cgi-bin/household/brands?tbl=chem&id=240. Use of the term "sunscreen" or similar sun protection terminology in a product's labeling generally causes the product to be subject to regulation as a drug (11). There are many synonyms for BP3 (25).

Humans can be exposed through skin absorption, inhalation, and ingestion.

3-5. 2,4-Dichlorophenol and trichlorophenols (24DCP, 245TCP, 246TCP)
2,4-Dichlorophenol and trichlorophenols (24DCP, 245TCP, 246TCP) are chlorinated phenols and are primarily used to manufacture herbicides.

245TCP and 246TCP are metabolites of several organochlorine chemicals, including hexachlorobenzene and hexachlorocyclohexane. Trichlorophenols are no longer intentionally manufactured, but they may be produced as byproducts of the manufacture of other chlorinated aromatic compounds. Small amounts of trichlorophenols can be produced during combustion of natural materials and from the chlorination of waste water that contains phenols. IARC classifies polychlorophenols (including trichlorophenols) as possibly carcinogenic to humans, and NTP classifies 246TCP as reasonably anticipated to be a human carcinogen.

The general population may be exposed to 246TCP through ingestion of contaminated food or water and inhalation of contaminated air (12).

Exposure is primarily through ingestion of contaminated water, inhalation, and skin contact.

6. 2,5-Dichlorophenol (25DCP)
2,5-Dichlorophenol (25DCP), an aromatic chemical compound, is a metabolite of paradichlorobenzene. It is primarily used to manufacture mothballs. 25DCP replaced the more traditional naphthalene. P-dichlorobenzene is the parent compound(13). Trade names for p-DCB include *Paramoth*, *Para crystals*, and *Paracide* reflecting its widespread use as a pesticide to kill moths, molds, and mildew. p-DCB is also used as a precursor in the production of the polymer poly(p-phenylene sulfide) used in urinal deodorant blocks to deodorize restrooms and waste containers.

Exposure is primarily through inhalation and skin contact.

7. ortho-Phenylphenol (o-PP) Fungicide
Ortho-Phenylphenol (o-PP) is primarily used to manufacture fungicides.

Exposure is primarily through inhalation and skin contact.

8. 4-tert-Octylphenol (4-t-OP)
4- tert octylphenol (4-t-OP) is a chemical used primarily to manufacture phenolic resins (98%), with the remainder converted into ethoxylates to produce detergent surfactants. Octylphenol belongs to a larger family of chemicals called alkylphenols (APs). The most commercially important alkylphenols are nonylphenol (NP) and octylphenol (OP). They exist in different forms, or “isomers”, and are used to make nonylphenol ethoxylates (NPEs) and octylphenol

ethoxylates (OPEs). APs are high production volume man–made chemicals that are reacted with ethylene oxide primarily to manufacture surfactant products called alkylphenol ethoxylates (APEs). APEs are made from and break down into alkylphenols, which are used as antioxidants in plastics and rubber products. The most common APEs are nonylphenol ethoxylates (NPEs).

Alkylphenol ethoxylates (APEs) are synthetic surfactants used in some detergents and cleaning products. APES and/or other alkyphenol derivatives are also used in pesticides, lube oil, hair dyes and other hair care products, and as nonoxynol-9 in spermicides. APs and APEs have been in use for over 50 years and are important to a number of industrial processes, including pulp and paper, textiles, coatings, agricultural pesticides, lube oils and fuels, metals and plastics used in food storage.

Exposure is primarily through skin contact.

9. Triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol] (TRCS)

Tricolosan **(TRCS)** is an anti-bacterial (microbicide) ingredient that can be found in a wide variety of home care products such as detergents and dish soaps, personal care products such as anti-acne cleansers, deodorants, hand soaps, cosmetics, lotions, creams, toothpastes, mouthwashes, and first aid creams. Microban is another trade name for this compound.

Exposure is primarily through ingestion and skin contact. Oral exposure is primarily through consumer medical products, such as mouthwashes, throat lozenges, and toothpastes.

How does phenol work in the human body?

Phenol is well absorbed from the gastrointestinal tract and through the skin of both animals and humans. It is metabolized principally by conjugation (by sulfation and glucuronidation) with a minor oxidation pathway leading to quinone-related reactive intermediates which bind covalently to protein and are detoxified by conjugation with glutathione. Most of the absorbed phenol and its metabolites are excreted in the urine, with trace amounts of excreted in expired air and the feces.

In addition, very small amounts of phenol is produced endogenously as a breakdown product of protein metabolism by the action of bacteria on normal constituents of the diet in the gut and excreted independent of external exposure to the compound. Some of this internally-produced phenol may be eliminated in the feces and some may pass to the blood.

[To be added: specifics for how each of the nine phenol compounds work in the human body.]

Are phenols endocrine disruptors?

Some. It is certain that some phenols are endocrine disruptors, and it is likely that some phenolic compounds will not have endocrine disrupting activity.

The two phenols that have been characterized as acting as endocrine disruptors are bisphenol A and alkylphenol (octylphenol and nonylphenol isomers). Bisphenol A (BPA) is an estrogenic compound and may also act as a disruptor of androgen action. Two research labs have shown on multiple occasions that BPA causes altered mammary gland development in animal models following early life exposure (26). Nonylphenol exposure during pregnancy has also been shown to disrupt normal mammary gland development in rats (Moon, Kim, Fenton et al., 2007).

According to EPA, an endocrine disrupter is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of *homeostasis* (biological stability), reproduction, development and/or behavior (27).

Does phenol exposure influence onset of puberty in girls?
Unknown. BCERC's biology and epidemiology studies are investigating this question.

Several animal studies reveal low-dose exposure to BPA (2.4 and 50 ppb per day) can affect the timing of the onset of sexual maturation in females (14,15,16). A multi-generation reproductive toxicity study conducted by the drinking-water route in rats reported delayed puberty, as evidenced by increased age at vaginal opening, and decreases in absolute and relative uterine weights (29).

There is a lack of studies concerning the developmental or reproductive effects of phenol in humans. The National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) expert panel in August 2007 expressed some concern that exposure to Bisphenol A causes neural and behavioral effect, and minimal concern that exposure potentially causes accelerations in puberty (24).

The BCERC epidemiology study entitled "Environmental and Genetic Determinants of Puberty" completed a small pilot study in November 2006 and measured phenols in young girls urine. The pilot study completed in November 2006 examined urinary biomarkers in ninety peripubertal Asian, Black, Hispanic and White girls to determine exposures to three chemical families known or likely to possess hormonal activity that may be estrogen agonistic or antagonistic (phytoestrogens, phthalate acids, and phenolic compounds). Nine phenols were sampled. Phenols had the lowest concentrations of the three chemical families, and only 3 (BPA, BP3, and 25DCP) of the 9 were detected in > 94% of the samples collected. The highest individual measurement was for benzophenone-3 (BP3; 26,700 µg/L). BP3 was higher in whites and 2,5-dichlorophenol (25DCP) was higher in blacks. BP3 was higher in samples collected in summer (9). *O*-Desmethylangolensin (*O*-DMA), 25DCP, and 2,4-dichlorophenol (24DCP) levels differed across the three study sites included in this study. The highest median concentrations for individual analytes in each chemical family were for the phytoestrogen enterolactone (298 µg/L), phthalate acid monoethylphthalate (MEP; 83.2 µg/L), and phenolic compound benzophenone-3 (BP3; 14.7 µg/L) (9). This small pilot data set will guide future expanded cohort studies.

Do phenols cross the placenta?
Yes.

Are phenols found to be present in breast milk?
Yes.

In addition to a number of previous studies, a recent study of 20 breast milk samples eight phenolic compounds: bisphenol A (BPA), 4-tert-octylphenol (4-tOP), ortho-phenylphenol (OPP), 2,4-dichlorophenol, 2,5-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and 2-hydroxy-4-metoxybenzophenone (BP-3), BPA, OPP, and BP-3 were detected in more than 60% of the samples tested (17).

Chemical components (PAHs, cresol, phenols) of cal tar creosote may be stored in body fat, they may be found in breast milk and could pass to nursing infants (18).

Are concentration levels of phenols the same in men and women?
No. The concentration level depends on the exposure compound. Males have higher occupational exposure to phenols manufactured for use in construction industries.

Are there medical tests for phenol exposure?
Yes.

Phenol can be detected in urine. This test can be used to determine whether a person has recently been exposed to phenol or to substances that are changed to phenol in the body. However, no test will tell whether a person has been exposed only to phenol, because many substances are changed to phenol in the body. Most of the phenol that enters the body is excreted in the urine within 24 hours. There are tests that measure presence of substances converted to phenol in the body in blood, however, they are less common.

Urine Tests
Urine can be tested for the presence of substances converted to phenol in the body recently, usually within one or two days of exposure (19). For example, the most common urine test measures a breakdown product of p-DCB called 2,5-dichlorophenol(25DCP) to measure for exposure to p-DCB.

Measurement of phenol in urine requires special laboratory equipment and techniques that are not routinely available in most hospitals or clinics. However, urine samples can be taken at a doctor's office and can be sent to specialized laboratories for analysis.

The normal range of phenol in the urine of unexposed individuals is 0.5–80 milligrams of phenol per liter of urine (mg/L); normal concentrations in urine generally do not exceed 20 mg/L (ACGIH 2001).This test can be used to determine if the urine has a higher than normal concentration of phenol, thus suggesting recent exposure to phenol or to substances that are converted to phenol in the body (e.g., benzene). However, health effects associated with any level of phenolic exposures are not known.

In in vitro studies, what is the association between phenol exposure and breast cancer risk? [An experiment in a test tube or cell culture system is an in vitro experiment.]
Unknown.

In in vivo studies, what is the association between phenol exposure and breast cancer risk? [An experiment in an animal model is referred to as an in vivo experiment.]
BCERC's laboratory-based biology research project entitled, "Environmental Effects on the Molecular Architecture and Function of the Mammary Gland across the Lifespan," is investigating this question. However, it is apparent that BPA has significant effects on both mammary gland development and tumor susceptibility in rodent models. Female offspring of timed-pregnant Wistar rats exposed to 25 pg/kg body weight/day demonstrated precocious mammary epithelial development, and chemical carcinogen exposure further induced increased ductal hyperplasia and development of neoplastic lesions compared to controls (30). Offspring of pregnant BPA-exposed mice demonstrate similar morphological changes (31)..

In epidemiological studies, what is the association between phenol exposure and breast cancer risk? [Studies of diseases in populations of humans or other animals.]

There have been "virtually no" studies of direct effects of phenols in humans. Epidemiological studies are needed to determine the association between phenol exposure and human breast cancer risk.

Bisphenol A is known to mimic the endogenous hormone estradiol and there is a growing body of scientific research that is raising concerns about exposures to low doses of BPA during development resulting to adverse effects on health later in life. Specific examples include breast and prostate cancers and obesity.

Were phenols included in the National Report on Human Exposure to Environmental Chemicals biomonitoring measurements from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) Third Report?

Yes. There were seven phenols followed and reported in selected participants. Four environmental phenols, Bisphenol A, 2-Hydroxy-4-methoxybenzophenone (Benzophenone-3), 4-tert-Octyl phenol and 2,4,4'-Trichloro-2'-hydroxyphenyl ether (Triclosan), and three organchlorine pesticides, Pentachlorophenol, 2,4,5-Trichlorophenol, 2,4,6-Trichlorophenol. Reported for the first time in the Third Report are the four environmental phenols.

Below is a summary of three of the phenols monitored in children aged 6-11 that are also being biomonitored in the BCERC pilot epidemiology study. No data is available in children for certain phenols, including bisphenol A (BPA), a chemical with hormonal activity relevant to pubertal development (28), however, CDC has analyzed urine samples from a nationally representative group of people for the presence of bisphenol A in the NHANES **2003-2004 survey.** Calfat, Ye, Wong, Reidy, and Needham in October 2007 has evaluated this data in a recently published scientific journal (33).

3-5. 2,4-Dichlorophenol and trichlorophenols (24DCP, 245TCP, 246TCP)

Geometric mean levels of urinary 2,4,6-TCP were slightly higher for children aged 6-11 years than for either groups aged 12-19 or 20-59 years, and the group aged 12-19 had higher levels than the group aged 20-59 years. It is unknown whether these differences associated with age represent differences in exposure, pharmacokinetics, or the relationship of dose per body weight.

The *Third Report* released in July 2005 by the US Centers for Disease Control (CDC) presents first-time exposure data for 38 of the 148 chemical compounds and their breakdown products found in consumer goods and manufacturing byproducts in a representative cross section of 2,400 Americans. The *Report* also includes the data from the *Second Report*; that is, data for 1999-2000. The *National Report on Human Exposure to Environmental Chemicals* provides an ongoing assessment of the U.S. population's exposure to environmental chemicals using biomonitoring. Biomonitoring is the assessment of human exposure to chemicals by measuring the chemicals or their metabolites in human specimens such as blood or urine (20). Since 1999, the US Centers for Disease Control and Prevention (CDC) has conducted the National Health and Nutrition Examination Survey (NHANES) to assess the health and nutritional status of adults and children in the United States. The survey, which currently examines about 5,000 people each year, includes a detailed interview and a range of physical examinations. The survey is designed to produce information that is representative of the US population aged 2 months and older (32).

CDC as yet has no optimal biomarker for nonylphenol (21).

What has the IARC determined about phenols and carcinogenesis?

The International Agency for Research on Cancer (IARC) classification for phenols is Group 3, not classifiable with regard to its carcinogenicity to humans. The IARC is part of the World Health Organization.

Has the federal government made recommendations to protect human health?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. **Recommendations** provide valuable guidelines to protect public health, **but *cannot* be enforced by law.** The Agency for Toxic

Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Environmental Protection Agency (EPA)
EPA has classified phenol as a Group D, not classifiable as to human carcinogenicity, based on a lack of data concerning carcinogenic effects in humans and animals (22,23).

EPA has determined that the level of phenol in ambient water (lakes, streams) should be limited to 21 mg/L in order to protect human health from the potential toxic effects of exposure to phenol through ingestion of water and contaminated aquatic organisms. EPA requires that spills of 1,000 pounds of phenol or more to the environment be reported to the Agency. The EPA lifetime health advisory for phenol in water is 2 mg/L.

FDA
Phenol is listed on the FDA's Everything Added to Foods in the United States (EAFUS) List and is approved as a component of food packaging materials.

National Toxicology Program (NTP) (NTP a division of the Department of Human Health Services within the National Institutes of Environmental Health Sciences (NIEHS); National Institute of Health (NIH))
The NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) convened an expert panel of 12 independent scientists in August 2007 to review and assess scientific studies on the potential reproductive and developmental hazards of bisphenol A (BPA). From scientific studies on rats and mice, the report determined that for pregnant woman and fetuses, infants and children exposure to BPA in utero potentially causes neural and behavioral effects and accelerations in puberty, and for the general population, exposure to BPA was categorized as negligible concern for adverse reproductive effects. For highly exposed subgroups, such as occupationally exposed populations, the level of concern was elevated to minimal (24). There is no data in women and fetuses, infants and children.

Breast Cancer and the Environment Research Centers Community Outreach and Translation Cores http://www.bcerc.org/cotc.htm

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For more information on the Breast Cancer and the Environment Research Centers, go to http://www.bcerc.org.

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Abstract

Phthalates are a family of man-made compounds used in the manufacture of plastics, including polyvinyl chloride plastics (PVC), and solvents. Phthalates can leach from these products into the environment. The ubiquitous use of phthalate esters in plastics, personal care products, medical devices used in patient care, and food packaging materials results in widespread general population exposure. Ingestion, inhalation, intravenous injection tubing and solutions, and skin absorption are all potential pathways of exposure. Phthalates can cross the placenta and have been found in human breast milk. Phthalate metabolites have also been found in the urine of average Americans and people worldwide. In general, females have higher levels of phthalates than males. It is possible that phthalates influence early onset of puberty in girls, but more research needs to be conducted. Phthalates are endocrine disrupters and have been linked to adverse reproductive effects in male rodents. Several studies have shown that phthalate exposure increases the growth of breast cancer cells *in vitro*, however, studies of phthalate exposure *in vivo* are limited. Epidemiological studies are needed to determine the association between phthalate exposure and human breast cancer risk. Phthalates are one of the most intensely studied class of compounds due to their extensive use and high production levels - not necessarily their toxicity. The International Agency for Research on Cancer (IARC) has not determined whether phthalates are carcinogenic to humans.

This fact sheet provides information about the ten phthalate biomarkers being measured and examined by the Breast Cancer and the Environment Research Centers (BCERC) epidemiology studies, sources of exposures, effects on puberty, effects in the body, and research studies looking at phthalates as being associated with breast cancer risk.

What are phthalates?

Phthalates are a family of compounds made from alcohols and phthalic anhydride. They are oily, colorless, odorless liquids that do not evaporate readily. Often called plasticizers, phthalates are used in the manufacture of plastics, including polyvinyl chloride plastics (PVC). Phthalates can prolong the lifespan or durability of plastics and increase the flexibility of some plastics. They can be found in hundreds of products such as toys, vinyl flooring, herbal pill coating, and plastic shower curtains. In addition, phthalates are also used as solvents. Phthalates are used in a variety of cosmetic products, such as nail polishes, perfumes, skin moisturizers and shampoos to enhance penetration and hold scent and/or color (1). Phthalates are ubiquitous in the environment.

Some of the most widely used phthalates and their human metabolites are:

♦ BBzP: butyl benzyl phthalate	—►	MBzP:	mono benzyl phthalate
♦ DnBP: di-*n*-butyl phthalate	—►	MnBP:	mono-n-butyl phthalate
♦ DEHP: di-(2-ethylhexyl)phthalate	—►	MEHP:	mono-(2-ethylhexyl) phthalate
♦ DEP: diethyl phthalate	—►	MEP:	monoethyl phthalate
♦ DiBP: di-isobutyl phthalate	—►		
♦ DiDP: di-isodecyl phthalate	—►		
♦ DiNP: di-isononyl phthalate	—►		
♦ DMP: di-methyl phthalate	—►		
♦ DnHP: di-*n*- hexyl phthalate	—►		
♦ DnOP: di-*n*-octylphthalate	—►		

Uses of the various phthalates depend in part on their molecular weight:

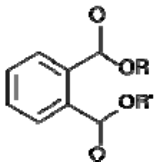
- Higher molecular weight phthalates, DEHP, DiDP, and DiNP, are the phthalates produced in highest volume for use in construction material, clothing, children's toys, and household furnishings.

- Relatively low molecular weight phthalates, DBP, DEP, DMP, tend to be used as solvents and in adhesives, waxes, inks, cosmetics, insecticides, and pharmaceuticals.

The physiochemical characteristics of phthalates vary with the chemical structure and may include a vapor phase, although vapor pressures are generally low. Phthalates are generally lipophilic, which influences their leaching and environmental partitioning characteristics. Phthalates are not chemically bound in the polymers. Therefore, migration or emission of phthalates from the products into the environment is likely to occur (2).

The general chemical structure of phthalates (R and R' = C_nH_{2n+1}) is shown in figure 1.

Figure 1:



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How are humans exposed to phthalates?

The ubiquitous use of phthalate esters in plastics, personal care products and food packaging materials results in widespread general population exposure. All populations of people, domestic animals, and wildlife regularly encounter opportunities for exposure to phthalates because of their widespread use.

Ingestion, inhalation, intravenous injection tubing and solutions, and skin absorption are potential pathways of exposure. Human exposure to phthalates can occur as a result of direct contact or use of a product containing phthalates, through the leaching of phthalates from one product into another, as may occur with food packaging or intravenous fluids, or by general contamination of the ambient environment.

Ingestion

When ingested, phthalates are often converted to other forms, called metabolites. Human metabolism of di-(2-ethylhexyl) phthalate (DEHP) is complex and yields mono (2-ethylhexyl) phthalate (MEHP) and numerous oxidative metabolites. Diethyl phthalate (DEP) yields phthalate monoester mono-ethyl phthalate (MEP) and di-n-butyl phthalate (DBP) yields monobutyl phthalate (MBP).

- **Food**
  Phthalates can be released into aqueous solution foods during microwaving in plastic containers (3). Phthalates may also enter food by environmental uptake during crop cultivation or by migration from processing equipment or packaging materials (4, 5)

- **Water**
  Phthalates are found in ground water and drinking water. From 1987 to 1993, according to EPA's Toxic Chemical Release Inventory, DEHP releases to land and water totaled over 500,000 lbs., of which about 5 percent was to water (6).

- **Infant formula and milk**
  Some phthalates occur as contaminants in consumer milk and ready-to-use baby formulas based on cow's milk (7-8). One study analyzed seven samples of consumer milk and ten samples of infant formula (7). Only MBP and MEHP were detected in these samples, in the ranges 0.6–3.9 ug L(–1) (MBP) and 5.6–9.9 ug L(–1) (MEHP).

- **Medications and nutritional supplements**
  Pharmaceutical preparations intended to treat diseases of the gastrointestinal tract, such as ulcerative colitis and colorectal cancer, are often coated with a polymer that allows the drug to be delivered directly to the colon or small intestine. This polymer may contain plasticizer phthalates such as DBP and DEP (9, 10). Other pharmaceutical products may also have phthalate plasticizers in their coatings, including some antibiotics, antihistamines and laxatives. Patented herbal preparations and nutritional supplements may also contain phthalates (2).

- **Toys**
  Polymer toys softened with phthalates are a source of potential oral exposure in children (2). In 1999, the European Union temporarily banned marketing of all children's toys and child-care articles containing DEHP, DBP, and BBP as well as toys containing DiNP, DnOP, and DiDP intended for children <3 years old. DiNP is the primary phthalate used in toys in the US. The estimated mean DiNP exposure resulting from children's mouthing activities range from 5.7 to 44 ug/kg/day depending on the assumptions and statistical techniques used in several different studies (11).

Inhalation

- **Indoor air and house dust**
  Vapors emitted from building materials, furniture and household fragrances are potential indoor sources of phthalate exposures (12, 13). Phthalates have been found in house dust in different countries, including the US, Germany, Japan and Norway (14-18). One study in Norway found a mean of 960 µg total phthalates/g dust in 38 homes (range 130–2920 µg/g dust) (14). Of the individual phthalates tested, DEHP was present in the highest levels (mean 640µg/g dust; range 100–1610 µg/g dust). The researchers estimated mean adult inhalation exposure to DEHP from this source to be 0.76 µg/day. A German study of 254 children, found that the levels of DEHP in house dust were not correlated with urinary levels of DEHP metabolites (15). However, another study found a significant correlation between urinary levels and house dust levels of DEP, DBP and BBP (16). This suggests that inhalation of house dust may be an important source of exposure for the lower molecular weight phthalates, but not the higher weight phthalates (2).

- **Medical devices**
  Some phthalate esters, such as DEHP, may be transferred into respiratory gases passing through PVC tubing (2, 19).

- **Baking modeling clay**
  Polymer modeling clay contains a complex mixture of phthalates that give the clay a soft consistency at room temperature. When the clay is baked, phthalates are released into the air and can be inhaled (20).

Intravenous

- **Medical devices**
  A variety of medical devices used to deliver medical care such as bags and tubing for intravenous fluids, nutritional formulas, blood transfusions, and dialysis are made of PVC plastics softened with phthalates, usually DEHP. DEHP can leach out from these products (20). DEHP has been found in newborns treated in neonatal intensive care units with medical devices made with polyvinyl chloride plastic containing DEHP (22-24).

Estimated Upper-Bound Dose of Intravenous Exposure to DEHP from Select Medical Procedures

Procedure	DEHP dose (mg/kg/day) Adult (70 kg)	Neonate (4kg)
Infusion of crystalloid IV solutions	0.005	0.03
Total parenteral nutrition with added lipid	0.13	2.5
Blood transfusion in a trauma patient	8.5	
Exchange transfusion in a neonate		22.6
Coronary artery bypass graft	1.0	
Artificial heart transplant	2.4	
Hemodialysis	0.36	
Enteral nutrition	0.14	0.14
Extracorporeal membrane oxygenation (ECMO)		14.0

Source: Adapted from http://www.fda.gov/cdrh/ost/dehp-pvc.pdf (20).

Skin Absorption

- **Clothing**
  Skin absorption can occur through direct contact with phthalate-containing clothing products, such as DEHP-containing gloves (artificial leather) and waterproof clothing.

- **Cosmetics and personal care products**
  Phthalates are used in a variety of cosmetic and personal care products, such as nail polishes, perfumes, hairsprays, skin moisturizers and shampoos. In one study, the levels of selected phthalates were measured in 102 branded hair sprays, perfumes, deodorants, and nail polishes (25). The median exposure levels to phthalates in cosmetics by skin absorption were estimated to be 0.0006 g/kg body weight /d for DEHP, 0.6 g/kg body weight /d for DEP, and 0.103 g/kg body weight/d for DBP. Skin absorption of chemicals from the face may be up to 10-fold higher than the arm (2).

- **Modeling clay**
  Skin absorption may occur through direct contact with polymer modeling clay containing phthalates (20).

- **Denture materials**
  Phthalates can be found in temporary denture soft lining materials. One study tested four brands of plasticizer-based soft lining materials (26). For two of the brands, the average amount of leached DBP within the first day exceeded the proposed tolerable daily intake for an average adult person by about 11 and 32 times, respectively. The cumulative amount leached over 30 days for each of the four materials was 128-253 mg plasticizer /g(-1).

How do phthalates work in the human body?

Diester phthalates are hydrolyzed into monoester phthalates in the intestine and parenchyma, i.e., phthalates are converted in the body to a metabolite, a break-down substance produced by metabolism (27). For example, metabolism of the phthalate diester DEP yields the phthalate monoester MEP. Short-branched phthalates (e.g. DEP and DMP) are mainly excreted in urine as monoester phthalates, while the more long-branched phthalates (e.g. DEHP) undergo several biotransformations, including further hydroxylation and oxidation before they are excreted in urine and feces (27). Metabolism of DEHP is complex and yields MEHP and numerous oxidative metabolites, such as diacids and ketoacids.

In vitro and in vivo studies have shown that diester phthalates have a greater effect when they are hydrolyzed to monoester phthalates (28).

Phthalate metabolites are routinely found in the urine of average Americans and people worldwide (1, 29-32). Phthalate metabolites can activate a nuclear receptor PPAR-alpha (peroxisome proliferator-activated receptor) in the liver, which may be linked to the development of liver cancer in animals (33). In addition*, in vitro* phthalate treatment of breast cancer cells leads to increased cell proliferation and PPAR-alpha activation (34, 35).

Are phthalates endocrine disruptors?

Yes. According to EPA, an endocrine disrupter is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of *homeostasis* (biological stability), reproduction, development and/or behavior ().

Phthalates are capable of binding to the estrogen receptor. In breast cancer cells, some phthalates have weak estrogenic effects and some have weak anti-estrogenic effects in the presence of 17beta-estradiol (35, 37, 38). In animal studies, several phthalates show antiandrogenic activity (39). Phthalates have been linked to adverse reproductive effects in male pubertal and adult rodents exposed in utero and during lactation, such as reduction in the weights of reproductive organs and a reduction in sperm count (40-42). There is also some evidence of

reproductive toxicity in adult female rodents exposed to DEHP, such as prolonged estrous cycles and lowered circulating estradiol levels (43).

In one human study, infant boys born to mothers with high phthalate urine levels were more likely to have smaller penises and scrotums and incomplete testicular descent (44). Boys born to mothers with the highest levels of phthalates were four to ten times more likely to have reduced genital development. The odds ratios for MBP, MEP, MBzP, and MiBP were 10.2, 4.7, 3.8, and 9.1, respectively (all p-values < 0.05).

Does phthalate exposure influence onset of puberty in girls?

Unknown. BCERC's biology and epidemiology studies are investigating this question.

Some evidence indicates that *in utero* and prepubertal exposure to DEHP, including dose levels relevant for human exposure, delays the onset of puberty in rats (45, 46).

Human studies on pubertal female development and phthalate exposures are limited. One study in Puerto Rico found that girls with premature breast development (younger than 8 years) had higher blood levels of several phthalates than a control group of girls without premature breast development (47).

The BCERC epidemiology study entitled "Environmental and Genetic Determinants of Puberty" completed a small pilot study in November 2006 and measured phthalates in young girls urine. The pilot study examined urinary biomarkers in ninety peripubertal Asian, Black, Hispanic and White girls to determine exposures to three chemical families known or likely to possess hormonal activity that may be estrogen agnostic or antagonistic (phytoestrogens, phthalate acids, and phenolic compounds). Phytoestrogens as a group had the highest concentrations (48). The study found detectable and variable amounts of three phthalate metabolites (MBP, MBzP, MEP). The exposures varied by characteristics that may be relevant to hormonal activity during developmental years. The highest median concentrations for individual analytes in each chemical family were for the phytoestrogen enterolactone (298 µg/L), phthalate acid monoethylphthalate (MEP; 83.2 µg/L), and phenolic compound benzophenone-3 (BP3; 14.7 µg/L) (48). This small pilot data set will guide future expanded cohort studies.

Do phthalates cross the placenta?

Yes.

Phthalates are found in young children and in human amniotic fluid (49-51). There is evidence in rodents and humans that *in utero* exposure to phthalates adversely affects reproductive development (40-42, 44).

Are phthalates found to be present in breast milk?

Yes.

Results from several studies have shown significant levels of phthalates in breast milk (7, 52, 53). In a study of Danish and Finnish women, phthalate monoesters were found in breast milk with large variations [medians (minimum-maximum)]: MMP 0.10 (< 0.01-5.53 µg/L), MEP 0.95 (0.07-41.4 µg//L), MBP 9.6 (0.6-10,900 µg//L), mBzP 1.2 (0.2-26 µg//L), mEHP 11 (1.5-1,410 µg//L), miNP 95 (27-469 µg//L) (51). Interestingly, levels of some phthalate esters in German women were higher than in Canadian mothers, indicating a regional exposure to specific phthalates (53). Despite the potential for phthalate exposure, breast milk remains the best and most complete nutritional source for young infants.

Are concentration levels of phthalates the same in men and women?

No. In the National Health and Nutrition Examination Survey (NHANES) 2001-2002, females had higher urine levels of several phthalate metabolites than males (39). The table below shows the urine concentrations of MIBP, MnBP, MBP, and MEHP, adjusted for creatinine, for males and females in NHANES 2001-2002.

Phthalate Metabolite	Mean Concentration in µg/g of creatinine	
	Males	Females
MnBP	14.4(13.5-15.4)	21.7(19.6-23.9)
MiBP	2.21(2.08-2.35)	2.87(2.59-3.17)
MBP	12.7(11.4-14.2)	15.7(14.2-17.3)
MEHP	3.49(3.06-3.98)	4.53(4.01-5.11)

Source: Adapted from http://www.cdc.gov/exposurereport/ (39).

Are there medical tests for phthalate exposure?

Yes.

There are no routine medical tests for phthalate exposure currently offered to patients by physicians. However, phthalates can be measured in both urine and blood. Because phthalates are metabolized before being excreted, urine and blood tests typically measure the monoester phthalate metabolites instead of the diester phthalates. In addition, because the diester phthalates are ubiquitous, they often contaminate samples and cause high background levels. This problem is eliminated by measuring the monoester phthalates as a biomarker for exposure (27).

Short-branched phthalates (e.g. DEP and DMP) are mainly excreted in urine as monoester phthalates, while the more long-branched phthalates (e.g. DEHP) undergo several biotransformations before they are excreted (27). Recent metabolism studies of DEHP indicate that the secondary metabolites such as MECPP in urine and MCMHP in serum are much stronger biomarkers for DEHP exposure than the previously used biomarker MEHP (54-56).

One study showed that the content of phthalate metabolites in serum is generally lower compared with the excretion of metabolites in urine (57). Phthalates are excreted in even lower amounts in semen, meconium and saliva (27).

In in vitro studies, what is the association between phthalate exposure and breast cancer risk? [An experiment in a test tube or cell culture system is an in vitro experiment.]

Several studies have shown that phthalate exposure increases the growth of breast cancer cells *in vitro* (35, 37, 38, 65). This effect may be mediated through phthalate activation of the estrogen receptor as well as activation of the PPARalpha receptor (34). In addition, phthalates have low binding affinity for the estrogen receptor (ER), thus possibly affecting breast cancer cell growth in the absence of estrogen, such as under hormone therapy conditions (58). In rodents, however, levels of phthalate exposure required to elicit an effect is at a high dose level (~ 1g/kg/day) which is significantly greater than levels humans are normally exposed to (~ 113 ug/kg bodyweight/day).

In in vivo studies, what is the association between phthalate exposure and breast cancer risk? [An experiment in an animal model is referred to as an in vivo experiment.]

BCERC's laboratory-based biology research project entitled, "Environmental Effects on the Molecular Architecture and Function of the Mammary Gland across the Lifespan," is investigating this question.

To date, only one published study has been conducted on phthalate exposure and mammary tumors in animals (59). This study only examined BBP, and it found that phthalate exposure actually decreased the incidence of mammary tumors in rats exposed to the polycyclic aromatic hydrocarbon DMBA. More studies are needed to determine the association between phthalates and breast cancer *in vivo*.

In epidemiological studies, what is the association between phthalate exposure and breast cancer risk? [Studies of diseases in populations of humans or other animals.]

To date, only one epidemiological study on the association between phthalate exposure and breast cancer risk has been published (60). This small, limited occupational study examined one phthalate (BBP) and did not find an association with breast cancer risk. More epidemiological studies are needed to determine the association between phthalate exposure and human breast cancer risk.

Were phthalates included in biomonitoring measurements from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) Third Report?

Yes.

Urinary levels of phthalate metabolites were measured in a subsample of NHANES participants aged 6 years and older (39). Participants were randomly selected with the specified age range to be a representative sample of the U.S. population. For most of the phthalate metabolites tested, levels were higher in children aged 6 to 11 than they were in teens and adults. Eleven out of 12 phthalate metabolites measured higher in children aged 6 years and older than adults. The CDC study did not test children under 6.

What has the IARC determined about phthalates and carcinogenesis?

There are no phthalates classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC). The IARC in January 2000 downgraded its former classification of the phthalate di(2-ethylhexyl) phthalate (DEHP) to a Group 3 agent – meaning the substance cannot be classified as causing cancer in humans. DEHP was initially classified as a potential carcinogen following rodent studies, in which it was found to cause liver tumors through a mechanism called peroxisome proliferation when administered at high doses. IARC has now ruled that the mechanism by which DEHP increases the incidence of liver tumors in rats and mice is not relevant to humans. The IARC is part of the World Health Organization.

Has the federal government made recommendations to protect human health?

Yes.

NIEHS

In 2000, the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) Expert Panel selected seven phthalate chemicals to evaluate because of high production volume, extent of human exposures, use in children's products, and/or published evidence of reproductive or developmental toxicity. The seven phthalates selected were:

- Butyl Benzyl Phthalate
- Di-n-Butyl Phthalate
- Di-(2-Ethylhexyl) Phthalate
- Diisodecyl Phthalate
- Diisononyl Phthalate
- Di-n-Hexyl Phthalate
- Di-n-Octyl Phthalate

Expert panel reports were published on all seven phthalates and links to the reports can be found at http://cerhr.niehs.nih.gov/reports/index.html. Many of the reports cite lack of data on the reproductive and developmental effects of phthalates in human. After reviewing the expert panel report on DEHP, the NTP concluded that there is serious concern that human development or reproduction might be adversely affected by exposure to DEHP in critically ill male infants (60). There is also concern for male infants younger than one year, and male offspring of women undergoing certain medical treatments during pregnancy. There is some concern for male offspring exposed during pregnancy and male children older than one year.

EPA

The US Environmental Protection Agency (EPA) reference doses (RfDs) for phthalates (DBP, DEP, and DEHP) were formulated in the early 1990s using older animal studies. The RfDs, as defined by the US EPA, are intended to be a dose for which daily oral exposure to the human population is likely to be without an appreciable risk of deleterious effects during a lifetime. According to the US EPA, the lowest tested dose of a substance (LOEAL) that has been reported to cause harmful (adverse) health effects on people or animals for DEHP is 19 mg/kg/day (61). However, adverse effects have been seen in male newborns of mothers treated with DEHP at much lower levels (i.e.1.32 to 9.32 ug/kg/day) (44). The EPA has set the Maximum Contaminant

Level (MCL) for DEHP in drinking water at 6 parts per billion (ppb). (6)

FDA

The FDA allows the use of phthalates in food contact items, and in the past has found that exposures are very low. Food contact items include packaging materials (adhesives and compounds of coatings, paper, and paperboard products, polymers, adjuvants, and productions aids) as well as a wide array of other materials. However, there has not been a recent review of their toxicities and the potential for exposures via this use (63).

In September 2001, the Food and Drug Administration (FDA) completed its safety assessment of DEHP released from medical devices made with PVC (21). It found that, for several medical procedures, the dose of DEHP that patients might receive exceeds the "Tolerable Intake" (TI) value for DEHP. However, the FDA advises that "the risk of not doing a needed procedure is far greater than the risk associated with exposure to DEHP (64)." In addition, it recommends considering "alternatives when these high-risk procedures are to be performed on male neonates, pregnant women who are carrying male fetuses, and peripubertal males. One source for identifying alternative devices that do not contain DEHP-plasticized PVC is http://www.sustainablehospitals.org, associated with the University of Massachusetts Lowell."

Breast Cancer and the Environment Research Centers Community Outreach and Translation Cores
http://www.bcerc.org/cotc.htm

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Abstract

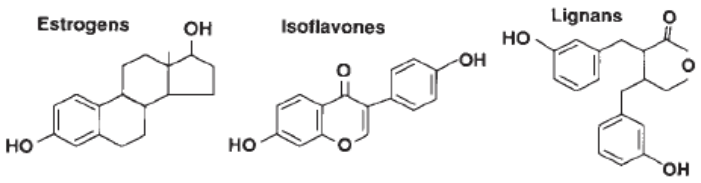
Enterolactone (ENL) is a phytoestrogen (estrogen-like chemical compound present in plants) that binds to estrogen receptors and has both weak estrogenic and weak anti-estrogenic effects. There are three major classes of phytoestrogens that have estrogen-like actions in the human body. They are lignans, isoflavones, and coumestans. Enterolactone is a lignan. Enterolactone is produced when the plant lignan matairesinol is acted upon by the action of bacterial flora in the colon of humans or animals. Exposure to enterolactone primarily occurs through ingesting whole grain products, seeds, and some fruits and vegetables. No studies were found showing that enterolactone crosses the placenta or that it is found in breast milk. It is unknown whether enterolactone influences early onset of puberty in girls. Exposure to enterolactone can be measured using a blood or urine test; however levels vary widely in each person due to considerable variability in the metabolism of enterolactone. *In vitro*, *in vivo* and epidemiologic studies are limited and inconclusive. A few *in vivo* studies have found high consumption of flax seeds to reduce breast tumors. The few epidemiologic studies looking at enterolactone have found little evidence that dietary intake of plant lignans is significantly associated with breast cancer risk. More research needs to be conducted on the association between breast cancer risk and enterolactone specifically before conclusions can be drawn. The International Agency for Research on Cancer (IARC) has not determined whether phytoestrogens are carcinogenic to humans.

This fact sheet provides information about enterolactone, one of three phytoestrogens being measured and examined by the Breast Cancer and the Environment Research Centers (BCERC) epidemiology studies, sources of exposures, effects on puberty, effects in the body, and research studies looking at enterololactone as being associated with breast cancer risk.

What is enterolactone?

Enterolactone is a phytoestrogen (estrogen-like chemical compound present in plants) that is derived from certain plant precursors by the action of human colonic bacteria. Phytoestrogens are naturally occurring chemical constituents that may interact with estrogen receptors to produce estrogenic or anti-estrogenic effects and are composed of a wide group of nonsteroidal compounds similar in structure and function to human estrogens (1,2,3). A conspicuous feature of the chemical structure of phytoestrogens is the presence of a phenolic ring that, with few exceptions, is a prerequisite for binding to the estrogen receptor (Fig. 1). For this reason, phytoestrogens can act as weak estrogen agonists, partial agonists, or as antagonists to endogenous estrogens and xenoestrogens with estrogen receptors in both animals and humans (4,5). Therefore, working as estrogen mimics, phytoestrogens may either have the same effects as estrogen or block estrogen's effects (1). There are three major classes of plant chemical compounds that have estrogen-like actions in the body. They are lignans (enterolactone, enterodiol), isoflavones (genistein, daidzein, biochanin A), and coumestans. The two major chemical classes of phytoestrogens found in people's diets are lignans (enterodiol and **enterolactone**) and isoflavones (daidzein, genistein, and glycitein). Lignans are the main class of phytoestrogens present in Western diets. Enterolactone is a lignan.

Figure 1:



A plant lignan, referred to as a mammalian precursor, is acted upon (metabolized) by human intestinal microflora (bacteria) in the colon to produce the mammalian lignans enterolactone and enterodiol. The lignan precursors that have been identified in the human diet include pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, and others. Matairesinol and secoisolariciresinol were among the first lignan precursors identified in the human diet and are therefore, the most extensively studied. Matairesinol is metabolized into the biologically active mammalian lignan enterolactone; secoisolariciresinol is metabolized into the biologically active mammalian lignan enterodiol. Enterodiol can be converted to enterolactone, but not the reverse.

The common biological roles of phytoestrogens are to protect plants from stress and to act as part of a plant's defense mechanism. Some ecologists postulate that phytoestrogens may have evolved to protect the plants by interfering with the reproductive ability of grazing animals (5).

How are humans exposed to enterolactone?

Ingestion is the source of human exposure to the mammalian lignan enterolactone. Exposure to enterolactone primarily occurs through ingesting whole grain products, seeds, and some fruits and vegetables. Ground flaxseed is the richest known dietary source of enterolactone. The principal lignan precursor found in flaxseed is secoisolariciresinol diglucoside (SDG).

Ingestion

- **Food**
  When you eat lignans, bacteria in the digestive tract convert them to enterodiol and enterolactone. Seeds (ground flax, sesame, pumpkin, and sunflower), cereals and grains (oatmeal, rye meal), cereal bran (rye and oats), legumes (peanut and soybean), fruits (apricots, blackberry, cranberry, strawberry, red current), and vegetables (asparagus, brussell sprouts, broccoli, cabbage, curly kale) all contain lignans.

  Ground flaxseed is the richest known dietary source. Whole flaxseeds that are consumed pass through the digestive system undigested, and do not produce a significant amount of lignans. Flaxseed oil lacks lignans, but some processors add them to their oil.

Total Lignan Content of Selected Foods		
Food	**Serving**	**Total Lignans (mg)**
Flaxseeds (ground)	1 oz	85.5
Sesame seeds	1 oz	11.2
Curly kale	½ cup, chopped	0.8
Broccoli	½ cup, chopped	0.6
Apricots	½ cup, sliced	0.4
Cabbage	½ cup, chopped	0.3
Brussels sprouts	½ cup, chopped	0.3
Strawberries	½ cup	0.2
Tofu	¼ block (4 oz)	0.2
Dark rye bread	1 slice	0.1

Source: http://lpi.oregonstate.edu/infocenter/phytochemicals/lignans/

- **Infant Formulas**
  None known to contain enterolactone.

- **Dietary Supplements**
  Dietary supplements containing lignans derived from flaxseed are available in the U.S. without a prescription. One supplement can provide 50 mg of secoisolariciresinol diglycoside (SDG) per capsule. The appropriate lignan dosage has yet to be determined, but a range of 10 mg to 30 mg daily dose of SDG may be sufficient to deliver the health benefits associated with flax lignans.

- **Water**
  Enterolactone is a solid substance that is practically insoluble in water.

Inhalation
Not a significant route of exposure. Lignans are present in high concentration in Norwegian spruce bark (Picea abies).

Intravenous
Not a significant route of exposure.

Skin absorption
Not a significant route of exposure.

How does enterolactone work in the human body?
Enterolactone is produced in the human body from plant lignans. When plant lignans are ingested, they can be metabolized by gut flora (intestinal bacteria) in the large intestine into the mammalian lignans, enterodiol and enterolactone. Enterodiol can also be converted to enterolactone by intestinal bacteria (6). Enterodiol and enterolactone have two metabolic fates. One, they can be excreted directly in the feces; or two, after being absorbed from the gut, they can be secreted into the bile and be reabsorbed from the intestine, and eventually excreted in the urine in conjugated form.

The metabolism of lignans in animals and humans is complex and involves both mammalian and gut microbial processes. There is considerable individual variation in the absorption and metabolism of ingested lignans. Mammalian lignans differ from plant lignans. Once mammalian lignans are produced in the colon, they are absorbed, transported to the liver, and secreted in bile. A portion reaches the kidney and eventually is excreted in the urine (7).

Is enterolactone an endocrine disruptor?
Yes.

Endocrine disruptors are exogenous synthetic or natural chemicals that can mimic or modify the action of endogenous hormones. Enterolactone binds to estrogen receptors found on sex hormone-binding globulin (SHBG). When enterolactone attaches to these receptors, estrogen (and testosterone) cannot, and as such can compete with estrogen for binding sites may help to reduce the growth of certain types of cancers.

Phytoestrogens eaten at sufficiently high concentrations can cause them to be active as estrogens (8).

Does enterolactone exposure influence onset of puberty in girls?
Unknown. BCERC's biology and epidemiology studies are investigating this question.

The BCERC epidemiology study entitled "Environmental and Genetic Determinants of Puberty" completed a small pilot study in November 2006 and measured enterolactone in young girls urine. The pilot study examined urinary biomarkers in ninety peripubertal Asian, Black, Hispanic and White girls to determine exposures to three chemical families known or likely to possess hormonal activity that may be estrogen agonistic or antagonistic (phytoestrogens, phthalate acids, and phenolic compounds). Phytoestrogens as a group had the highest concentrations (9). All six phytoestrogens (Enterolactone, Genistein, Daidzein, Equol, Enterodiol, *O*-DMA) were detected in > 98% of the samples collected. The highest median concentration of the six phytoestrogens was for enterolactone. Enterolactone was higher among girls with body mass index < 85th reference percentile than those at or above the 85th percentile (9). The levels of phytoestrogen metabolites were similar to those reported in the NHANES 2001–2002 children(9). The highest median concentrations for individual analytes in each chemical family were for the phytoestrogen enterolactone (298 µg/L), phthalate acid monoethylphthalate (MEP; 83.2 µg/L), and phenolic compound benzophenone-3 (BP3; 14.7 µg/L) (9). This small pilot data set will guide future expanded cohort studies.

Does enterolactone cross the placenta?
Unknown.

Is enterolactone found to be present in breast milk?
Unknown.

Despite the potential for enterolactone exposure, breast milk remains the best and most complete nutritional source for young infants.

Are concentration levels of enterolactone the same in men and women?
No.

Levels of enterolactone are diet dependent. Some studies have shown men to have higher levels.

Are there medical tests for enterolactone exposure?
Yes. Concentration levels of enterolactone can be detected by blood and urine tests. Enterolactone levels measured in either **blood** or **urine** reflect an increase in a dose dependent manner related to dietary intake of plant lignans and to the activity of intestinal bacteria. Vegetarians have high plasma and urinary concentrations of lignans.

Blood Tests
Phytoestrogens persist in **blood plasma** for about 24 hours. The concentration of enterodiol and enterolactone can be detected in blood serum.

Urine Tests
The concentration of enterodiol and **enterolactone** can be detected in urine. The relationship between the dose and urinary excretion is linear for many phytoestrogens.

Lignans have short have-lives. Because excretory half-lives are reported to be in the range of 3-10 hours, urinary concentrations reflect recent consumption.

In in vitro studies, what is the association between enterolactone exposure and breast cancer risk? [An experiment in a test tube or cell culture system is an in vitro experiment.]
Unknown.

In in vivo studies, what is the association between enterolactone exposure and breast cancer risk? [An experiment in an animal model is referred to as an in vivo experiment.]

BCERC's laboratory-based biology research project entitled, **"**Environmental Effects on the Molecular Architecture and Function of the Mammary Gland across the Lifespan," is investigating the association between enterolactone exposure and breast cancer risk.

Some animal studies, when fed high amounts of flaxseeds, have been shown to cause developmental abnormalities as well as a decrease in breast and lung tumors. However, there have been no studies showing a direct effect. Flaxseed supplies alpha-linolenic acid. Alpha-linolenic acid (omega 3 fat) has been shown in animal studies to be protective for cancer, while omega 6 fats (linoleic acid, arachidonic acid) have been found to be cancer promoting. Lignans can act as antioxidants in the test tube, but the significance of such antioxidant activity in humans is not clear because lignans are rapidly and extensively metabolized (2,15).

In epidemiological studies, what is the association between enterolactone exposure and breast cancer risk? [Studies of diseases in populations of humans or other animals.]

There is no evidence that dietary intake of plant lignans is associated with breast cancer risk. Two prospective cohort studies and two case-control studies that examined plant lignan intake and breast cancer risk found them not to be related (10,11,12,13). An inverse association between a

high enterolactone concentration in both urine and serum, and the risk of breast cancer suggests a chemopreventive action for enterolactone.

Was enterolactone included in biomonitoring measurements from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) Third Report?

Yes.

Urinary levels of phytoestrogens were measured in a subsample of NHANES participants aged 6 years and older (14). Participants were selected within the specified age range to be a representative sample of the U.S. population. In general, the concentrations observed in the NHANES 1999-2000 and 2000-2001 subsamples reflect a diet higher in lignans and lower in isoflavones, consistent with consumption of a Western diet in which whole grains and cereals rather than soybean products contribute the bulk of phytoestrogens. For lignans, enterolactone levels were highest. Vegetarian women in Boston, men and women consuming experimental cruciferous diet, and Boston women consuming a macrobiotic diet excreted significantly higher levels of these lignans (14).

In NHANES 2001-2002, urinary enterolactone levels were higher in the group aged 6-11 years than in the group aged 12-19 years. Levels of the lignans previously have been reported to differ by race and in an NHANES III statistical analysis, to differ by income, gender, and age. Males had higher urinary levels of enterolactone in the both the1999-2000 and 2001-2002 subsamples (14).

The *Third Report* released in July 2005 by the US Centers for Disease Control (CDC) presents first-time exposure data for 38 of the 148 chemical compounds and their breakdown products found in consumer goods and manufacturing byproducts in a representative cross section of 2,400 Americans. The *Report* also includes the data from the *Second Report*; that is, data for 1999-2000. The *National Report on Human Exposure to Environmental Chemicals* provides an ongoing assessment of the U.S. population's exposure to environmental chemicals using biomonitoring. Biomonitoring is the assessment of human exposure to chemicals by measuring the chemicals or their metabolites in human specimens such as blood or urine.

What has the IARC determined about enterolactone and carcinogenesis?

The International Agency for Research on Cancer (IARC) has not determined phytoestrogens to be carcinogenic to humans. The IARC is part of the World Health Organization (WHO).

Has the federal government made recommendations to protect human health?

Unknown.

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http://www.bcerc.org/cotc.htm

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Abstract

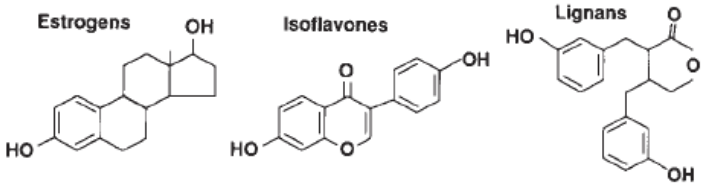
Daidzein is a phytoestrogen (estrogen-like chemical compound present in plants) that binds to estrogen receptors and has both weak estrogenic and weak anti-estrogenic effects. There are three major classes of phytoestrogens that have estrogen-like actions in the human body. They are lignans, isoflavones, and coumestans. Daidzein is an isoflavone. Exposure to daidzein occurs principally through foods made with soybeans and soy protein. In a proportion of the population, daidzein is metabolized by intestinal bacteria to produce equol and O-DMA, metabolites that are more estrogenic than daidzein. Daidzein can cross the placenta and has been found in breast milk. It is unknown whether daidzein influences early onset of puberty in girls. Exposure to daidzein can be measured using a blood or urine test; however levels vary widely in each person due to considerable variability in the metabolism of daidzein. *In vitro* and *in vivo* studies have found that daidzein stimulates the growth of estrogen-sensitive breast cancer cells. Epidemiologic studies have found conflicting evidence; some studies have found an association between soy exposure and decreased breast cancer risk while others have found no association. Some epidemiological evidence indicates that soy intake may be more protective when the exposure occurs prior to puberty. More research needs to be conducted on the association between breast cancer risk and daidzein specifically before conclusions can be drawn. The International Agency for Research on Cancer (IARC) has not determined whether phytoestrogens are carcinogenic to humans.

This fact sheet provides information about daidzein, one of three phytoestrogens being measured and examined by the Breast Cancer and the Environment Research Centers (BCERC) epidemiology studies, sources of exposures, effects on puberty, effects in the body, and research studies looking at daidzein as being associated with breast cancer risk.

What is daidzein?

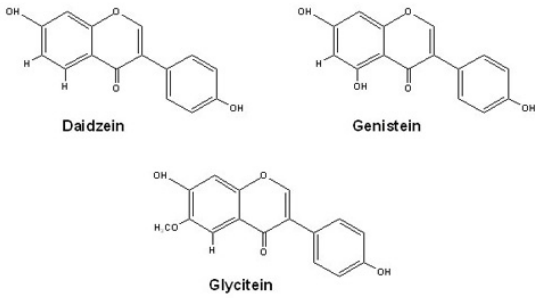
Daidzein is a phytoestrogen (estrogen-like chemical compound present in plants) that is derived from certain plant precursors by human metabolism. Phytoestrogens are naturally occurring chemical constituents that may interact with estrogen receptors in humans to produce weak estrogenic or anti-estrogenic effects. They are composed of a wide group of nonsteroidal compounds similar in structure and function to human estrogens (1). A conspicuous feature of the chemical structure of phytoestrogens is the presence of a phenolic ring that, with few exceptions, is a prerequisite for binding to the estrogen receptor (Fig. 1). For this reason, phytoestrogens can act as weak estrogen agonists, partial agonists, or as antagonists to endogenous estrogens (such as estradiol) and xenoestrogens (including phytoestrogens) at estrogen receptors in both animals and humans. Therefore, working as estrogen mimics, phytoestrogens may either have the same effects as estrogen or block estrogen's effects (24, 25, 26). There are three major classes of plant chemical compounds that have estrogen-like actions in the body. They are lignans (enterolactone, enterodiol), isoflavones (genistein, daidzein, biochanin A), and coumestans. The two major chemical classes of phytoestrogens found in people's diets are lignans (enterodiol and enterolactone) and isoflavones (daidzein, genistein, and glycitein). Lignans are the main class of phytoestrogens present in Western diets. Daidzein is an isoflavone.

Figure 1:



Isoflavones are a subgroup of flavinoids. Among commonly consumed foods, isoflavones are found in dietary-relevant amounts only in the soybean. The two primary isoflavones in soybeans are daidzein and genistein and their respective glucosides genistin and daidzin (Fig. 2). Soy foods typically contain more genistein than daidzein, although this ratio varies among different soy products.

Figure 2:



The terms “soy” and “soybean” are commonly used for the leguminous Asian plant *Glycine max*. Soybean is also used to designate the edible seed of this plant. In this fact sheet, the term “soy” is used as an adjective to denote products derived from the edible seed (e.g., soy milk, soy formula, soy meal) and soybean is used to refer to the edible seed itself.

The common biological roles of phytoestrogens are to protect plants from stress and to act as part of a plant’s defense mechanism. Some ecologists postulate that phytoestrogens may have evolved to protect the plants by interfering with the reproductive ability of grazing animals (2).

How are humans exposed to daidzein?

Ingestion is the source of human exposure to daidzein. Exposure occurs principally through food, infant formulas, and/or dietary supplements made with soybeans and soy protein, but not soy oils. All soybean foods and proteins currently available for human consumption contain significant amounts of the isoflavones genistein and daidzein, either as the aglycone (unconjugated form) or as different types of glycoside conjugates.

Ingestion

- **Food**
  Leguminous plant foods contain daidzein. Soybeans, a cholesterol-free, high protein legume, contain the most daidzein. Daidzein can be found in many food products containing soy such as soy-based infant formulas, tofu, soymilk, soy flour, textured soy protein, soy protein isolates, tempeh, and miso, as well as over-the-counter dietary supplements. Often, soy flour is used for fortification of other flours, including wheat, rice, and corn. The daidzein content of these products is quite variable.

  Soy flour contains 53% soy protein. Textured Soy Protein (TSP), a meat substitute made from defatted soy found in hamburgers, sausages, hot dogs, meatballs, meat loafs, can contain 50% to 70% soy protein, depending on the starting soy material used. Soy Protein Isolates (SPI), used in the preparation of specialty nutrition foods such as infant formulas, sports drinks, bodybuilding beverages, energy bars, and special diets for the very sick, contain 90% soy protein. Soy oil and soy sauce contain little to zero daidzein.

Total Isoflavone, Daidzein and Genistein Aglycone Content of Selected Foods				
Food	**Serving**	**Total Isoflavones (mg)**	**Daidzein (mg)**	**Genistein (mg)**
Soy protein concentrate, aqueous washed	3.5 oz	102	43	56
Soy protein concentrate, alcohol washed	3.5 oz	12	7	5
Miso	½ cup	59	22	34
Soybeans, boiled	½ cup	47	23	24
Tempeh	3 ounces	37	15	21
Soybeans, dry roasted	1 ounce	37	15	19
Soy milk	1 cup	30	12	17
Tofu yogurt	½ cup	21	7	12
Tofu	3 ounces	20	8	12
Soybeans, green, boiled (Edamame)	½ cup	12	6	6
Meatless (soy) hot dog	1 hot dog	11	3	6
Meatless (soy) sausage	3 links	3	0.6	2
Soy cheese, mozzarella	1 oz	2	0.3	1

Source: http://lpi.oregonstate.edu/infocenter/phytochemicals/soyiso/index.html#source

Daidzein and daidzin are also found in Radix puerariae (RP), an herbal medicine prepared from the root of the legume Pueraria labata (also known as kudzu). RP has been used for centuries in traditional Chinese medicine to treat a variety of disorders, including alcohol-dependency in people who abuse alcohol. It is thought that the “anti-drinking” effect of RP is due to daidzein and daidzin (3).

- **Infant Formulas**
  Soy-based infant formulas have been commercially available since the mid 1960s (4). The formulas are made from soy protein isolate (SPI) and contain significant amounts of soy isoflavones. In 1997, the total isoflavone content of soy-based infant formulas commercially available in the US ranged from 32-47 mg/liter (~ 34 fluid ounces) (5).

Total Isoflavone, Daidzein and Genistein Aglycone Content of Selected Soy-based Infant Formulas				
Soy-based Formula	**Serving**	**Total isoflavones (mg)**	**Daidzein (mg)**	**Genistein (mg)**
Mead Johnson Prosobee, ready to feed	8 fl oz	9.4	4.1	5.3
Ross Isomil, ready to feed	8 fl oz	10.2	4.7	5.5
Wyeth-Ayerst Nursoy, ready to feed	8 fl oz	6.4	1.8	3.9

Source: http://lpi.oregonstate.edu/infocenter/phytochemicals/soyiso/index.html#sources

Infants are able to absorb isoflavones, and infants fed soy formula were demonstrated to have plasma isoflavone blood levels exceeding those of Japanese adults several-fold (6). Soy-based infant formula can result in plasma concentrations of isoflavones in infants that are 13,000 - 22,000 times higher than endogenous estrogen concentrations in infants (7).

- **Dietary Supplements**
  Dietary supplements containing daidzein are available in the US without a prescription. These products are not standardized, and the amounts of soy isoflavones they provide may vary considerably. For example, in an analysis of soy supplements purchased at a local health food store containing daidzein, the daidzein content measured was 8.9 mg/tablet; the value represented 84% of the daidzein level listed on the product label (8).

  Daidzein is mainly present in the form of its beta-glucoside, daidzin in supplements. A standard soy isoflavone supplement is usually comprised of approximately 38% daidzin, 50% genistin, and 12% glycitin (3). A typical daily dose (50 mg) of soy isoflavone supplement delivers approximately 19 mg of daidzin, 25 mg of genistin and about 6 mg of

glycitin. Smaller amounts of daidzein are also contained in some red clover supplement preparations.

Women with estrogen receptor-positive tumors are advised to exercise caution in the use of daidzein/daidzin supplements and should only use them if they are recommended and monitored by a physician (3).

- **Water**
  Not a significant route of exposure. Daidzein is a solid substance that is practically insoluble in water.

Inhalation
Not a significant route of exposure.

Intravenous
Not a significant route of exposure.

Skin Absorption
Not a significant route of exposure.

How does daidzein work in the human body?

Daidzein is an isoflavone aglycone and is produced in the body from plant isoflavones. Isoflavones are contained in soybean or soy foods in two chemical forms, i.e., aglycones (uncongugated form) and glucosides (bound to a sugar molecule). The main dietary source of daidzein is the biologically active glucoside daidzin. Fermentation or digestion of soybeans or soy products results in the release of the sugar molecule from the isoflavone glycoside, daidzin, leaving the isoflavone aglycone, daidzein (9). Before daidzein can act it first needs to be released from daidzin. This normally happens in the stomach (acid hydrolysis) and intestine (action of bacterial enzymes).

After daidzein is released from daidzein, it may be absorbed into the blood or it may be further metabolized by intestinal bacteria into the metabolites equol and O-desmethylangolensin (O-DMA) (3, 10). The extent of this metabolism appears to be highly variable among individuals and is influenced by the specific bacteria present in the intestine and other components of the diet. After consuming soy or daidzein, approximately 30%-50% of the population produces equol, and approximately 80%-90% produces O-DMA (10).

Daidzein inhibits the class I isoenzymes of human alcohol dehydrogenase (ADH) and the human mitochondrial aldehyde dehydrogenase (ALDH-2). Inhibition of both class I ADH and ALDH-2 may suppress alcohol consumption in humans (3). Both daidzein and daidzin significantly reduced free-choice alcohol intake by more than 50% in hamsters (11).

Daidzein is also an antioxidant. It is thought that daidzein is a less potent antioxidant than genistein; however, there are few studies comparing the antioxidant activity of the two isoflavones (12, 13). Equol is a more potent antioxidant than daidzein (10).

Is daidzein an endocrine disruptor?

Perhaps.

Endocrine disruptors are exogenous synthetic or natural chemicals that can mimic or modify the action of endogenous hormones. Isoflavones bind to both estrogen receptors (ER α and ER β), however, they preferentially bind to and activate ER β (14). For this reason, they are sometimes classified as selective estrogen receptor modulators (SERMs). Daidzein has been found to have both weak estrogenic and weak anti-estrogenic effects (3, 24). In vivo, daidzein's estrogenic activity is one-fourth that of genistein (15). The metabolites of **daidzein**, equol and O-DMA, have been shown to bind to human estrogen receptors with a greater affinity than daidzein (10).

Does daidzein exposure influence onset of puberty in girls?
Unknown. BCERC's biology and epidemiology studies are investigating this question.

In vivo research investigating the association of daidzein exposure with mammary tissue development and onset of menarche is ongoing.

The BCERC epidemiology study entitled "Environmental and Genetic Determinants of Puberty" completed a small pilot study in November 2006 and measured daidzein in young girls urine. The pilot study examined urinary biomarkers in ninety peripubertal Asian, Black, Hispanic and White girls to determine exposures to three chemical families known or likely to possess hormonal activity that may be estrogen agonistic or antagonistic (phytoestrogens, phthalate acids, and phenolic compounds). Phytoestrogens as a group had the highest concentrations (16). All six phytoestrogens (Enterolactone, Genistein, Daidzein, Equol, Enterodiol, *O*-DMA) were detected in >98% of samples collected. The levels of phytoestrogen metabolites were similar to those reported in the NHANES 2001–2002 children (7). The exposures varied by characteristics that may be relevant to development (7). The highest median concentrations for individual analytes in each chemical family were for the phytoestrogen enterolactone (298 µg/L), phthalate acid monoethylphthalate (MEP; 83.2 µg/L), and phenolic compound benzophenone-3 (BP3; 14.7 µg/L) (16). This small pilot data set will guide future expanded cohort studies.

Does daidzein cross the placenta?
Yes.

By measuring the levels of daidzein at birth in human newborns and umbilical cords, studies have shown that daidzein can be transferred from mother to fetus (17, 18). In the US, typical diets are low in soy products, and the fetus is thus hypothesized to be exposed to low levels of daidzein. In Asian cultures consuming soy products, the fetus is exposed to daidzein as a result of maternal soy product intake.

Pregnant women are advised to avoid the use of daidzein/daidzin-containing supplements pending long-term safety studies (3).

Is daidzein found to be present in breast milk?
Yes.

Despite the potential for daidzein exposure, breast milk remains the best and most complete nutritional source for young infants. Nursing mothers are advised to avoid the use of daidzein/daidzin-containing supplements pending long-term safety studies (3).

Are concentration levels of daidzein the same in men and women?
Yes.

A recent study of 1414 adults from 9 European countries found that plasma concentrations of daidzein did not differ significantly in men and women; the mean concentration for men was 0.89 mg/L and the mean concentration for women was 0.80 mg/L (19). In the National Health and Nutrition Examination Survey (NHANES) 2001-2002, females and males also had similar urinary levels of daidzein; the mean concentration for males was 49.8 µg/L and the mean concentration for females was 53.6 µg/L (7).

Are there medical tests for daidzein exposure?
Yes.

Blood Tests
Phytoestrogens persist in plasma for about 24 hours. The plasma half-life of genistein and daidzein, measured from their plasma appearance and disappearance curves to be 7.9 hours in adults; peak concentrations occur 6-8 hours after ingestion. Consequently, adherence to a soy-containing diet will ultimately lead to high steady-state plasma concentrations. Plasma concentrations of 50-800 ng/mL are achieved for daidzein, genistein and equol in adults

consuming modest quantities of soy-foods containing in the region of 50 mg/day of total isoflavones. These values are similar to those of Japanese consuming their traditional diet (20).

Urine Tests

Most studies of the metabolism of isoflavones have focused on urinary excretion. This is partly because of the high concentrations found in urine after soy intake and the methodologic difficulties encountered in measuring the lower concentrations in other biological fluids. Few studies have measured circulating concentrations of isoflavones; this reflects the greater difficulty of measurement in plasma compared with urine.

Isoflavones have short-half lives (approximately 8 hours), and nearly all ingested isoflavones are excreted within 24 hours in both urine and feces (21). There is considerable interindividual variation in gut bacterial metabolism of daidzein which leads to markedly different urinary concentrations of daidzein and its metabolites in different individuals (14). In NHANES 2001-2002, the mean urine concentration for daidzein in the total population age 6 and older was 48.6 µg/L. The range from the 50th percentile to the 95th percentile was 48.5-957.0 µg/L (7).

In in vitro studies, what is the association between daidzein exposure and breast cancer risk? [An experiment in a test tube or cell culture system is an in vitro experiment.]

In vitro studies of daidzein and breast cancer risk are limited. One recent study found that both daidzein and equol stimulated the growth of estrogen-dependent breast cancer cells at concentrations between 0.001 and 50 µM (22, 26).

In in vivo studies, what is the association between daidzein exposure and breast cancer risk? [An experiment in an animal model is referred to as an in vivo experiment.]

In vivo studies of daidzein and breast cancer risk are limited. One recent study found that dietary daidzein stimulated the growth of estrogen-dependent mammary tumors in mice, but dietary equol did not (22). Another study demonstrated that daidzein in the diet had no effect on chemically-induced mammary cancer in rats (23).

In epidemiological studies, what is the association between daidzein exposure and breast cancer risk? [Studies of diseases in populations of humans or other animals.]

There is no evidence that dietary intake of plant isoflavones is associated with breast cancer risk. Evidence from epidemiological studies is conflicting for soy and total phytoestrogen intake. Some case-control and cohort studies have found a protective effect and some have not found any effect. One recent meta-analysis of 18 epidemiologic studies concluded that soy intake may be associated with a small reduction in breast cancer risk (27). In the studies that stratified by menopausal status, the reduction in breast cancer risk was somewhat stronger among premenopausal women. However, the authors also noted that there were methodological problems with many of the studies included in the meta-analysis.

Some epidemiological evidence indicates that soy intake may be more protective when the exposure occurs prior to puberty. One study of Chinese women found that intake of soyfood during adolescence reduced breast cancer risk in a dose-dependent manner (28). The highest quintile of intake reduced risk by 49%.

There are a limited number of epidemiological studies that have examined the relationship between daidzein specifically and breast cancer. One 2007 Dutch study found high plasma levels of daidzein, O-DMA, and equol were associated with a 17%, 17% and 23% reduction in risk, respectively; however, none of these associations were statistically significant (13). The same study also found that high plasma levels of genistein were associated with a 32% reduction in breast cancer risk, and this association was statistically significant.

Was daidzein included in biomonitoring measurements from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) Third Report?

Yes.

Urinary levels of phytoestrogens were measured in a subsample of NHANES participants aged 6 years and older (7). Participants were selected within the specified age range to be a representative sample of the U.S. population. In general, the concentrations observed in the NHANES 1999-2000 and 2000-2001 subsamples reflect a diet lower in isoflavones than lignans, consistent with consumption of a Western diet in which whole grains and cereals rather than soybean products contribute the bulk of phytoestrogens. Enterolactone levels were highest followed by daidzein, genistein, enterodiol, equol, and O-desmethylangolensin. Isoflavone levels at the higher percentiles may reflect dietary supplementation with soy products.

In NHANES 2001-2002, both urinary genistein and daidzein levels were higher in the group aged 6 – 11 years than in either of the groups aged 12-19 years or 20 years and older. Females and males had similar urinary levels of daidzein.

The *Third Report* released in July 2005 by the US Centers for Disease Control (CDC) presents first-time exposure data for 38 of the 148 chemical compounds and their breakdown products found in consumer goods and manufacturing byproducts in a representative cross section of 2,400 Americans. The *Report* also includes the data from the *Second Report*; that is, data for 1999-2000. The *National Report on Human Exposure to Environmental Chemicals* provides an ongoing assessment of the U.S. population's exposure to environmental chemicals using biomonitoring. Biomonitoring is the assessment of human exposure to chemicals by measuring the chemicals or their metabolites in human specimens such as blood or urine.

What has the IARC determined about daidzein and carcinogenesis?

The International Agency for Research on Cancer (IARC) has not determined phytoestrogens to be carcinogenic to humans. The IARC is part of the World Health Organization (WHO).

Has the federal government made recommendations to protect human health?

Yes.

FDA

In October 1999, FDA approved a health claim that can be used on labels of soy-based foods to tout their heart-healthy benefits. The agency reviewed research from 27 studies that showed soy protein's value in lowering levels of total cholesterol and low-density lipoprotein (LDL, or "bad" cholesterol).

Since 1999, food marketers can now use the following claim, or a reasonable variation, on their products: "Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease. One serving of (name of food) provides __ grams of soy protein."

To qualify for the claim foods must contain per serving:

- 6.25 grams of soy protein
- low fat (less than 3 grams)
- low saturated fat (less than 1 gram)
- low cholesterol (less than 20 milligrams)
- sodium value of less than 480 milligrams for individual foods, less than 720 milligrams if considered a main dish, and less than 960 milligrams if considered a meal.

Foods made with the whole soybean, such as tofu, may qualify for the claim if they have no fat other than that naturally present in the whole bean.

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Abstract

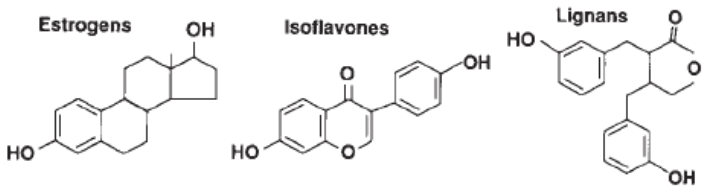
Genistein is a phytoestrogen (estrogen-like chemical compound present in plants) that binds to estrogen receptors and has both weak estrogenic and weak anti-estrogenic effects. There are three major classes of phytoestrogens that have estrogen-like actions in the human body. They are lignans, isoflavones, and coumestans. Genistein is an isoflavone. Exposure to genistein occurs principally through foods made with soybeans and soy protein. Genistein has been found in breast milk and is available via soy milk. It is possible that genistein influences early onset of puberty in girls, but more research needs to be conducted. Exposure to genistein can be measured using a blood or urine test; however, levels vary widely in each person due to considerable variability in the metabolism of genistein. *In vitro* studies with high concentrations of genistein demonstrate inhibition of cell proliferation while under some conditions low concentrations stimulate cell proliferation. *In vivo* studies demonstrate that genistein inhibits chemically-induced mammary cancer rats while others report that it stimulates growth of cancer cells in immune deficient rodent models. Epidemiologic studies have found conflicting evidence; some studies have found an association between soy exposure and decreased breast cancer risk while others have found no association. Some epidemiological evidence indicates that soy intake may be more protective when the exposure occurs prior to puberty. More research needs to be conducted on the association between breast cancer risk and genistein specifically before conclusions can be drawn. The International Agency for Research on Cancer (IARC) has not determined whether phytoestrogens are carcinogenic to humans.

This fact sheet provides information about genistein, one of three phytoestrogens being measured and examined by the Breast Cancer and the Environment Research Centers (BCERC) epidemiology studies, sources of exposures, effects on puberty, effects in the body, and research studies looking at genistein as being associated with breast cancer risk.

What is genistein?

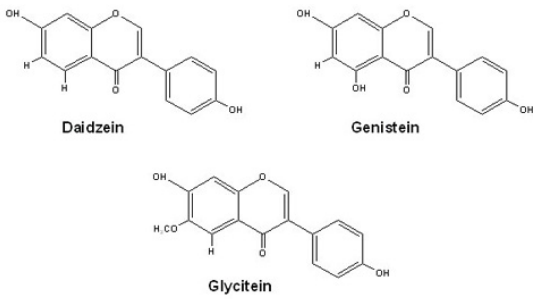
Genistein is a phytoestrogen (estrogen-like chemical compound present in plants) that is derived from certain plant precursors by human metabolism. They are naturally occurring chemical constituents that may interact with estrogen receptors to produce weak estrogenic or anti-estrogenic effects. They are composed of a wide group of nonsteroidal compounds similar in structure and function to human estrogens (1). A conspicuous feature of the chemical structure of phytoestrogens is the presence of a phenolic ring that, with few exceptions, is a prerequisite for binding to the estrogen receptor (Fig. 1). For this reason, phytoestrogens can act as weak estrogen agonists, partial agonists, or as antagonists to endogenous estrogens (such as estradiol) and xenoestrogens (including phytoestrogens) with estrogen receptors in both animals and humans. Therefore, working as estrogen mimics, phytoestrogens may either have the same effects as estrogen or block estrogen's effects. There are three major classes of plant chemical compounds that have estrogen-like actions in the body. They are lignans (enterolactone, enterodiol), isoflavones (genistein, daidzein, biochanin A), and coumestans. The two major chemical classes of phytoestrogens found in people's diets are lignans (enterodiol and enterolactone) and isoflavones (daidzein, genistein, and glycitein). Lignans are the main class of phytoestrogens present in Western diets. Genistein is an isoflavone.

Figure 1:



Isoflavones are a subgroup of flavinoids. Among commonly consumed foods, isoflavones are found in dietary-relevant amounts only in the soybean. The two primary isoflavones in soybeans are daidzein and genistein and their respective glucosides genistin and daidzin (Fig. 2). Soy foods typically contain more genistein than daidzein, although this ratio varies among different soy products.

Figure 2:



The terms “soy” and “soybean” are commonly used for the leguminous Asian plant *Glycine max*. Soybean is also used to designate the edible seed of this plant. In this fact sheet, the term “soy” is used as an adjective to denote products derived from the edible seed (e.g., soy milk, soy formula, soy meal) and soybean is used to refer to the edible seed itself.

The common biological roles of phytoestrogens are to protect plants from stress and to act as part of a plant’s defense mechanism. Some ecologists postulate that phytoestrogens may have evolved to protect the plants by interfering with the reproductive ability of grazing animals (2).

How are humans exposed to genistein?

Ingestion is the source of human exposure to genistein. Exposure occurs principally through food, infant formulas, and/or dietary supplements made with soybeans and soy protein, but not soy oils. All soybean foods and proteins currently available for human consumption contain significant amounts of the isoflavones genistein and daidzein, either as the aglycone (unconjugated form) or as different types of glycoside conjugates.

Ingestion

- **Food**
  Leguminous plant foods contain genistein. Soybeans, a cholesterol-free, high protein legume, contain the most genistein. Other legumes, such as chickpeas (garbanzo beans), contain small amounts of genistein. Genistein can be found in many food products containing soy such as soy-based infant formulas, tofu, soymilk, soy flour, textured soy protein, soy protein isolates, tempeh, and miso, as well as over-the-counter dietary supplements. Often, soy flour is used for fortification of other flours, including wheat, rice, and corn. The genistein content of these products is quite variable.

  Soy flour contains 53% soy protein. Textured Soy Protein (TSP), a meat substitute made from defatted soy found in hamburgers, sausages, hot dogs, meatballs, meat loafs, can contain 50% to 70% soy protein, depending on the starting soy material used. Soy Protein Isolates (SPI), used in the preparation of specialty nutrition foods such as infant formulas, sports drinks, bodybuilding beverages, energy bars, and special diets for the very sick, contain 90% soy protein. Soy oil and soy sauce contain little to zero genistein.

  Other plant foods that have been shown to contain genistein include alfalfa and clover sprouts, barley meal, broccoli, cauliflower, and sunflower, caraway, and clover seeds.

Total Isoflavone, Daidzein and Genistein Aglycone Content of Selected Foods				
Food	**Serving**	**Total Isoflavones (mg)**	**Daidzein (mg)**	**Genistein (mg)**
Soy protein concentrate, aqueous washed	3.5 oz	102	43	56
Soy protein concentrate, alcohol washed	3.5 oz	12	7	5
Miso	½ cup	59	22	34
Soybeans, boiled	½ cup	47	23	24
Tempeh	3 ounces	37	15	21
Soybeans, dry roasted	1 ounce	37	15	19
Soy milk	1 cup	30	12	17
Tofu yogurt	½ cup	21	7	12
Tofu	3 ounces	20	8	12
Soybeans, green, boiled (Edamame)	½ cup	12	6	6
Meatless (soy) hot dog	1 hot dog	11	3	6
Meatless (soy) sausage	3 links	3	0.6	2
Soy cheese, mozzarella	1 oz	2	0.3	1

Source: http://lpi.oregonstate.edu/infocenter/phytochemicals/soyiso/index.html#source

- **Infant Formulas**

  Soy-based infant formulas have been commercially available since the mid 1960s (3). The formulas are made from soy protein isolate (SPI) and contain significant amounts of soy isoflavones. In 1997, the total isoflavone content of soy-based infant formulas commercially available in the US ranged from 32-47 mg/liter (~ 34 fluid ounces) (4).

  Infants are able to absorb isoflavones, and infants fed soy formula were demonstrated to have plasma isoflavone blood levels exceeding those of Japanese adults several-fold (5). Soy-based infant formula can result in plasma concentrations of isoflavones in infants that are 13,000 - 22,000 times higher than endogenous estrogen concentrations in infants (6). Infants consuming soy-based formula are exposed to 6-11 mg/kg per day of isoflavones (4-7 mg/kg per day of total genistein) that result in circulating levels of approximately 1-5 µM of total genistein. In contrast, adults consuming a moderate to large amount of soy in the diet are exposed to ~1 mg/kg per day of total genistein resulting in circulating levels of approximately 0.5 µM of total genistein (7). Even though infants ingesting soy milk are exposed to high concentrations of genistein, little toxicity has been reported. The most noted consequence is hypothyroidism in infants with compromised thyroid function.

Total Isoflavone, Daidzein and Genistein Aglycone Content of Selected Soy-based Infant Formulas				
Soy-based Formula	**Serving**	**Total isoflavones (mg)**	**Daidzein (mg)**	**Genistein (mg)**
Mead Johnson Prosobee, ready to feed	8 fl oz	9.4	4.1	5.3
Ross Isomil, ready to feed	8 fl oz	10.2	4.7	5.5
Wyeth-Ayerst Nursoy, ready to feed	8 fl oz	6.4	1.8	3.9

Source: http://lpi.oregonstate.edu/infocenter/phytochemicals/soyiso/index.html#sources

- **Dietary Supplements**

  Dietary supplements containing genistein are available in the US without a prescription. These products are not standardized, and the amounts of soy isoflavones they provide may vary considerably. For example, in an analysis of a soy supplement purchased at a local health food store containing genistein, the genistein content measured was 1.4 mg/tablet; the value represented 48% of the genistein level listed on the product label (8).

  Genistein is mainly present in the form of its glycoside genistin in supplements. It is found in capsules, powder, and tablets. Some genistein supplements contain genistein which has been

hydrolyzed in a chemical process. Tablets contain up to 20 mg genistein (9). Supplements that are labeled as 1000 mg relate to soybean content.

Women with estrogen receptor-positive tumors are advised to exercise caution in the use of genistein/genistin supplements and should only use them if they are recommended and monitored by a physician (20).

- **Water**
  Not a significant route of exposure. Genistein is a solid substance that is practically insoluble in water.

Inhalation
Not a significant route of exposure.

Intravenous
Not a significant route of exposure.

Skin Absorption
Not a significant route of exposure.

How does genistein work in the human body?

Genistein is an isoflavone aglycone and is produced in the body from plant isoflavones. Isoflavones are contained in soybean or soy foods in two chemical forms, i.e., aglycones (uncongugated form) and glucosides (bound to a sugar molecule). The main dietary source of genistein is the biologically active glucoside genistin. Fermentation or digestion of soybeans or soy products results in the release of the sugar molecule from the isoflavone glycoside, genistin, leaving the isoflavone aglycone, genistein (10). Before genistein can act it first needs to be released from genistin. This normally happens in the stomach (acid hydrolysis) and intestine (action of bacterial enzymes).

There is considerable individual variation in the absorption and metabolism of ingested genistin and genistein. There are some data suggesting that genistein may be more bioavailable than genistin. However, other data suggest that the extent of absorption of genistein is similar for the aglycone and the glucoside forms. There is little data available on the tissue distribution of genistein. The pharmacokinetics of genistein in humans is complex and not well understood.

Genistein affects the process by which signals at the cell surface are transferred to the interior of the cell and inhibits the activity of several enzymes intimately involved in controlling cell growth and regulation (11). The complete metabolic fate of exposure to genistein is not known.

Genistein is the most studied of the soy isoflavones with regard to antioxidant activity. It is thought that genistein may be a more potent antioxidant than daidzein (12). There are few studies comparing the antioxidant activity of the two isoflavones (13).

Is genistein an endocrine disruptor?

Perhaps.

Endocrine disruptors are exogenous synthetic or natural chemicals that can mimic or modify the action of endogenous hormones. Isoflavones bind to both estrogen receptors (ER α and ER β), however, they preferentially bind to and activate ER β (14). For this reason, they are sometimes classified as selective estrogen receptor modulators (SERMs). At concentrations that are achieved from dietary soy exposures, genistein has been found to have both weak estrogenic and weak anti-estrogenic effects (15). *In vivo*, genistein's estrogenic activity is one-third that of glycitein and four times greater than that of daidzein (16).

Does genistein exposure influence onset of puberty in girls?

Unknown. BCERC's biology and epidemiology studies are investigating this question.

Some evidence indicates that *in utero* and prepubertal exposure to genistein accelerates puberty onset in rodents (17, 18). One study also found that neonatal injections of genistein exposure influenced subsequent mammary gland development in mice, depending on the dose used. Mice

injected with 50 mg/kg.d of genistein had stunted mammary gland development, whereas mice injected with 0.5 mg/kg.d of genistein exhibited advanced mammary gland development (19). On the other hand, genistein fed in the diet to rats at concentrations that yield similar blood levels as humans eating a diet high in soy enhances mammary gland differentiation and suppresses chemically-induced mammary cancer and results in no toxicity (20). Ingesting genistein, as opposed to injecting it, alters bioavailability and mechanism of action.

The BCERC epidemiology study entitled "Environmental and Genetic Determinants of Puberty" completed a small pilot study in November 2006 and measured genistein in young girls urine. The pilot study completed in November 2006 examined urinary biomarkers in ninety peripubertal Asian, Black, Hispanic and White girls to determine exposures to three chemical families known or likely to possess hormonal activity that may be estrogen agonistic or antagonistic (phytoestrogens, phthalate acids, and phenolic compounds). Phytoestrogens as a group had the highest concentrations (21). All six phytoestrogens (Enterolactone, Genistein, Daidzein, Equol, Enterodiol, *O*-DMA) were detected in > 98% of samples collected. The levels of phytoestrogen metabolites were similar to those reported in the NHANES 2001–2002 children (6). The exposures varied by characteristics that may be relevant to development (6). The highest median concentrations for individual analytes in each chemical family were for the phytoestrogen enterolactone (298 µg/L), phthalate acid monoethylphthalate (MEP; 83.2 µg/L), and phenolic compound benzophenone-3 (BP3; 14.7 µg/L) (21). This small pilot data set will guide future expanded cohort studies.

Does genistein cross the placenta?
Yes.

By measuring the levels of genistein at birth in human newborns and umbilical cords, studies have shown that genistein can be transferred from mother to fetus (22, 23). In the US, typical diets are low in soy products, and the fetus is thus hypothesized to be exposed to low levels of genistein. In Asian cultures consuming soy products, the fetus is exposed to genistein as a result of maternal soy product intake, yet little or no toxicity is reported.

Pregnant women are advised to avoid the use of genistein/genistin-containing supplements pending long-term safety studies (24).

Is genistein found to be present in breast milk?
Yes.

The highest concentrations of isoflavones were reported in breast milk from women eating vegan and vegetarian diets (25). Despite the potential for genistein exposure, breast milk remains the best and most complete nutritional source for young infants. Nursing mothers are advised to avoid the use of genistein/genistin-containing supplements pending long-term safety studies (24).

Are concentration levels of genistein the same in men and women?
Yes.

A recent study of 1414 adults from 9 European countries found that plasma concentrations of genistein did not differ significantly in men and women; the mean concentration for men was 1.77 mg/L and the mean concentration for women was 1.70 mg/L (26). In the National Health and Nutrition Examination Survey (NHANES) 2001-2002, females and males also had similar urinary levels of genistein; the mean concentration for males was 32.2 µg/L and the mean concentration for females was 33.7 µg/L (6).

Are there medical tests for genistein exposure?
Yes.

Blood Tests
Phytoestrogens persist in plasma for about 24 hours. The plasma half-life of genistein and daidzein, measured from their plasma appearance and disappearance curves to be 7.9 hours in adults; peak

concentrations occur 6-8 hours after ingestion. Consequently, adherence to a soy-containing diet will ultimately lead to high steady-state plasma concentrations.

Plasma concentrations of 50-800 ng/mL are achieved for daidzein, genistein and equol in adults consuming modest quantities of soy-foods containing in the region of 50 mg/day of total isoflavones. These values are similar to those of Japanese consuming their traditional diet (27).

Urine Tests

Most studies of the metabolism of isoflavones have focused on urinary excretion. This is partly because of the high concentrations found in urine after soy intake and the methodologic difficulties encountered in measuring the lower concentrations in other biological fluids. Few studies have measured circulating concentrations of isoflavones; this reflects the greater difficulty of measurement in plasma compared with urine.

Isoflavones have short-half lives (approximately 8 hours), and nearly all ingested isoflavones are excreted within 24 hours in both urine and feces (28). There is considerable interindividual variation in gut bacterial metabolism of genistein which leads to markedly different urinary concentrations of genistein and its metabolites in different individuals (14). In NHANES 2001-2002, the mean urine concentration for genistein in the total population age 6 and older was 33.0 µg/L. The range from the 50th percentile to the 95th percentile was 28.9-613.0 µg/L (6).

In in vitro studies, what is the association between genistein exposure and breast cancer risk? [An experiment in a test tube or cell culture system is an in vitro experiment.]

Genistein is the most studied of the phytoestrogens. *In vitro* studies have shown that the growth of both estrogen receptor-positive breast cancer cells and estrogen receptor-negative breast cancer cells is inhibited when high levels of genistein (>10µM) are added to the culture medium; however, the growth of estrogen receptor-positive breast cancer cells is actually stimulated when low and physiologically relevant concentrations of genistein are added (14,32). The association between genistein and breast cancer risk *in vitro* is complex and depends on both the concentration of genistein and the concentration of estrogen.

In in vivo studies, what is the association between genistein exposure and breast cancer risk? [An experiment in an animal model is referred to as an in vivo experiment.]

BCERC's laboratory-based biology research project entitled, **"**Environmental Effects on the Molecular Architecture and Function of the Mammary Gland across the Lifespan," is investigating the association between genistein exposure and breast cancer risk. Genistein is the most studied of the phytoestrogens in *in vivo* studies and thus far, rodent studies of genistein and breast cancer risk have had conflicting results. Some studies have shown that genistein inhibits the development and growth of mammary tumors, while other studies have found that genistein stimulates the growth of existing estrogen-sensitive mammary tumors (16).

Evidence indicates that the timing of exposure may be important. Genistein has consistently been shown to inhibit the development of estrogen-sensitive mammary tumors when given to prepubertal rats (15). Genistein has also been shown to accelerate mammary gland development as well as alter mammary gland development following early life exposure (exposures that took place before or around the time of birth or around the time of puberty) in rat models. Effects depend on time and route of exposure (19, 20). A study in 1999 by Hilakivi-Clarke showed an increase in carcinogen-induced mammary cancer in female rat offspring after maternal genistein injection, suggesting that an elevated estrogenic environment *in utero* could increase subsequent breast cancer risk (29). However, genistein administered prenatally in the diet to rats did not increase predisposition for mammary cancer (20)

In epidemiological studies, what is the association between genistein exposure and breast cancer risk? [Studies of diseases in populations of humans or other animals.]

There is no evidence that dietary intake of plant isoflavones is associated with breast cancer risk. There are a limited number of epidemiological studies that have examined the relationship between genistein specifically and breast cancer risk. One 2007 Dutch study found that high plasma levels of genistein were associated with a 32% reduction in breast cancer risk (13).

Evidence from epidemiological studies is conflicting for soy and total phytoestrogen intake. Some case-control and cohort studies have found a protective effect and some have not found any effect. One recent meta-analysis of 18 epidemiologic studies concluded that soy intake may be associated with a small reduction in breast cancer risk (15). In the studies that stratified by menopausal status, the reduction in breast cancer risk was somewhat stronger among premenopausal women. However, the authors also noted that there were methodological problems with many of the studies included in the meta-analysis.

Some epidemiological evidence indicates that soy intake may be more protective when the exposure occurs prior to puberty. One study of Chinese women found that intake of soyfood during adolescence reduced breast cancer risk in a dose-dependent manner (30). The highest quintile of intake reduced risk by 49%.

Was genistein included in biomonitoring measurements from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) Third Report?

Yes.

Urinary levels of phytoestrogens were measured in a subsample of NHANES participants aged 6 years and older (6). Participants were selected within the specified age range to be a representative sample of the U.S. population. In general, the concentrations observed in the NHANES 1999-2000 and 2000-2001 subsamples reflect a diet lower in isoflavones than lignans, consistent with consumption of a Western diet in which whole grains and cereals rather than soybean products contribute the bulk of phytoestrogens. Enterolactone levels were highest followed by daidzein, genistein, enterodiol, equol, and O-desmethylangolensin. Isoflavone levels at the higher percentiles may reflect dietary supplementation with soy products.

In NHANES 2001-2002, both urinary genistein and daidzein levels were higher in the group aged 6 - 11 years than in either of the groups aged 12-19 years or 20 years and older. Females and males had similar urinary levels of genistein.

What has the IARC determined about genistein and carcinogenesis?

The International Agency for Research on Cancer (IARC) has not determined phytoestrogens to be carcinogenic to humans. The IARC is part of the World Health Organization (WHO).

Has the federal government made recommendations to protect human health?

Yes.

FDA

In October 1999, the FDA approved a health claim that can be used on labels of soy-based foods to tout their heart-healthy benefits. The agency reviewed research from 27 studies that showed soy protein's value in lowering levels of total cholesterol and low-density lipoprotein (LDL, or "bad" cholesterol).

Since 1999, food marketers can now use the following claim, or a reasonable variation, on their products: "Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease. One serving of (name of food) provides __ grams of soy protein."

To qualify for the claim foods must contain per serving:

- 6.25 grams of soy protein
- low fat (less than 3 grams)
- low saturated fat (less than 1 gram)
- low cholesterol (less than 20 milligrams)
- sodium value of less than 480 milligrams for individual foods, less than 720 milligrams if considered a main dish, and less than 960 milligrams if considered a meal.

Foods made with the whole soybean, such as tofu, may qualify for the claim if they have no fat other than that naturally present in the whole bean.

NIEHS

An independent panel of 14 scientists was convened in March 2006 by the Center for the Evaluation of Risks to Human Reproduction (CERHR) (31) to evaluate whether genistein or soy formula is hazardous to human development or reproduction.

The final report is available at http://cerhr.niehs.nih.gov/chemicals/genistein-soy/genistein/Genistein_Report_final.pdf (25).

The report found that: "There are no human data available on developmental or reproductive toxicity of purified genistein. Available experimental data are sufficient to conclude that purified genistein can produce reproductive and/or developmental toxicity in rats and mice."

Breast Cancer and the Environment Research Centers Community Outreach and Translation Cores http://www.bcerc.org/cotc.htm

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