CHAPTER 12

BISPHENOL A

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12.1 INTRODUCTION

This chapter reviews the latest research on bisphenol A (BPA), including human exposure, discussions of biomonitoring studies, and toxickinetic models, human health effects and research needs, and experimental studies on low dose effects on the reproductive, neurodevelopmental, and metabolic systems. While the objective here is not to conduct a risk assessment nor directly inform public policy, the emergent body of knowledge about BPA serves as an important resource for regulators and policy makers faced with the challenge of integrating new tools, technologies, and understandings of disease etiology into risk assessment methodologies and assumptions. The research on BPA reflects an important shift in toxicity testing toward studies that use dosing levels relevant to human exposure and end points of human relevance. The relevance and reliability of the research reviewed here provides a solid foundation of knowledge from which toxicologists, epidemiologists, public health practitioners, and policy makers may draw from and build upon to inform their research and decision making.

12.2 CHEMICAL AND BIOLOGICAL PROPERTIES OF BPA

BPA ($C_{15}H_{16}O_2$; CAS # 80-05-7) is one of the most common industrial chemicals produced worldwide. BPA was first synthesized in by the Russian chemist A.P. Dianin in 1891. The compound consists of two conjoined phenol

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functional groups, and is synthesized by the condensation of acetone (hence the suffix A in the name) with two equivalents of phenol. It is used primarily in the production of polycarbonate plastics and epoxy resins, but also has application in flame retardants, carbonless thermal paper, and other plastics (e.g., polyester resins, polysulfone resins, and polyacrylate resins). The world production capacity was 1 million metric tons in the 1980s, and more than 2.2 million metric tons in 2009.

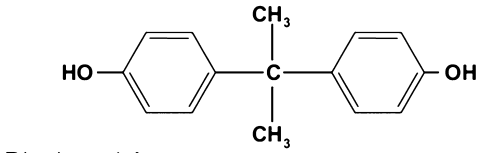
Epoxy resins are added to paints for metal coatings and applied to a wide array of metal items, from household appliances, cars, dairy equipment, office equipment and tools, to food cans and sanitation and sewage pipes, for the prevention of metal corrosion. They are also used in dental restorative work as adhesives in root canal work, adhering caps, bonding, and filling teeth. Polycarbonates are used in manufacturing computer components, compact discs, kitchen appliances, automobile parts, safety glazing, helmets and sports equipment, laboratory and hospital equipment, optical lens, and reusable food and beverage containers.[1] BPA is also used as a color developer in carbonless thermal receipt paper.

BPA has been detected in air, soil, water, landfill leachate, and the human body. Accordingly, BPA is found in the serum, milk, saliva, and urine of humans at nanomolar concentrations.[2,3] BPA has also been measured in amniotic fluid at concentrations fivefold higher than those measured in maternal plasma.[4] Fetal and perinatal exposures to BPA in rodents have been shown to affect the brain, mammary gland, and reproductive tract, including hormone-dependent cancer.[5–11]

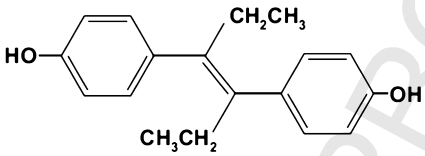
12.2.1 Endocrine Disrupting Properties of BPA

The estrogenic properties of BPA were first demonstrated in studies using ovariectomized rats in the 1930s during a search for synthetic estrogens. However, it was abandoned for pharmaceutical use when diethylstilbestrol (DES) was determined to be much more potent.[12] Biochemical assays have since shown that BPA does fit within the estrogen receptor (ER) binding pocket, and that it binds to both ERα and ERβ, with approximately 10-fold higher affinity for ERβ.[13,14] However, the binding affinity of BPA for both ER isoforms is nearly 10,000-fold weaker than that of estradiol (EC_{50} 2–7 × 10^{-7} M vs. 1–6 × 10^{-13} M for estradiol)[14,15] (Figure 12.1).

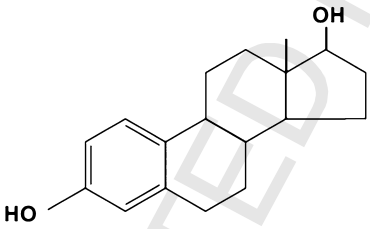
Several membrane-bound forms of the estrogen receptor (mER) and a transmembrane ER called G protein-coupled receptor 30 (GPR30), that is structurally dissimilar to nuclear ERs, have been described.[16–18] Studies have shown that BPA binds to both mER and GPR30, leading to nongenomic steroid activity.[17,18] For instance, GH3/B6 pituitary cells, which naturally express mER, respond to picomolar levels of BPA by initiating a calcium flux that results in prolactin release.[17] As well, pancreatic α-cells treated with BPA also demonstrate nongenomic signaling that is not specific to individual cell types.[19,20] And while other studies have shown that BPA displays nongenomic



Bisphenol A



Diethylstilbestrol (DES)



Estradiol

Figure 12.1. Chemical structures of BPA, DES, and estradiol.

signaling through activation of mER,[21] much of the estrogenic properties of this compound cannot be traced solely to membrane, nuclear, or cytosolic functions of ER. Based on this evidence, it is widely accepted that BPA acts as a xenoestrogen through multiple mechanisms.

Until the late 1990s, BPA was characterized as weakly estrogenic with low affinity to the nuclear ER compared with estradiol[22] and as estrogenic as o'p'-DDT.[23] Current *in vitro* research, however, details a far more complex understanding of mechanistic activity. BPA binds to cell membrane ERs at levels equipotent to estradiol; acts as an androgen receptor antagonist; interacts with the thyroid receptor; and alters hormone metabolism, concentrations, and synthesis.[24] BPA is, therefore, not simply a weak estrogen. As the National Toxicology Program (NTP) noted, "interpreting the toxicological effects of bisphenol A solely within the context of their consistency with a classic estrogenic mechanism of action is overly simplistic."[12] In addition to its estrogenic activity, there is mounting evidence that BPA interacts with other nuclear receptors, albeit at higher levels. For example, one study found that BPA binds to the thyroid hormone receptor (TR) with a lower affinity than the ER.[25] However, others believe BPA acts as an indirect antagonist of thyroid hormone (TH), and that its effects on TH action *in vivo* are likely dependent on the

TABLE 12.1. BPA Interactions with Various Nuclear Receptors

Receptor	Location	Mechanism	References
ERα	Nucleus	Receptor binding alters recruitment of coactivators	13, 32, and 33
ERβ	Nucleus	Receptor binding alters recruitment of coactivators	14, 32, and 33
ERR-γ	Nucleus	Receptor binding preserves basal activity	28 and 34
mER	Cell membranes	Nongenomic action	16
GPR30	Cell membranes	Nongenomic action	17 and 18
AhR	Nucleus	Receptor binding, unknown consequences	35
TR	Cellular	Potentially acts as thyroid hormone agonist	25 and 36–38
AR	Nucleus	Unknown	30

composition and relative abundance of cofactors available in the cell.[26] Studies have also shown that BPA binds to the ubiquitous aryl hydrocarbon receptor (AhR).[27] This is not surprising because AhR is thought to be activated by many chemicals and likely mediates toxicity through several signaling pathways.[27]

There is also evidence that BPA mimics estrogen by binding to the estrogen-related receptor γ (ERR-γ).[28] This orphan receptor behaves as a constitutive activator of transcription. BPA binds to ERR-γ ($EC_{50} = 5.5 \times 10^{-9}$ M), preserving its basal constitutive activity.[28] However, recent studies have revealed alternative pathways through which BPA can stimulate cellular responses at very low concentrations, well below the levels of the binding affinity of BPA to ER receptors.[29] Finally, some reports indicate BPA exhibits antiandrogenic properties,[30] but additional studies are needed to determine whether this is due to androgen receptor (AR) binding or via ER binding due to its estrogenicity[31] (Table 12.1).

12.2.2 Human Exposure to BPA

Since the 1990s, several dozen studies have been dedicated to determining which consumer products contain BPA, and how much is released from these products into food and beverages under normal conditions of use.[39] In particular, studies have focused on the levels of BPA released from baby bottles and other consumer plastics, food contact papers, and the epoxy resins used both for dental sealants and the linings of metal food cans.

Many studies were dedicated to measuring BPA in canned foods, including vegetables, fish, soups, infant formulas, and milk, among others.[39] Recently, two market surveys have been conducted of canned foods in Canada and the United States.[40,41] These studies confirm that the majority of canned

foods contain measurable levels of BPA. A wide range of concentrations were reported, leading scientists to estimate that current human exposures from canned and bottled goods could be in the nanogram per kilogram range for children and adults and the microgram per kilogram range for bottle-fed infants.[39]

Yet the concentrations found in canned goods are not likely to produce the blood concentrations that have been repeatedly measured. Some scientists have proposed that nonoral exposures could be significant. For this reason, recent studies have explored nonoral exposures to BPA, and have proposed that thermal papers coated with BPA and cigarette filters could be two significant sources of nonoral exposures.[42–44] Importantly, BPA applied to the skin is absorbed and can be passed through all layers of skin, suggesting this could be a significant route directly to the bloodstream; the skin has limited ability to metabolize BPA.[45]

BPA has also been detected in a large number of environmental samples, including water, air, and dust (reviewed in von Goetz et al.[39]). It is also found in significant quantities in landfill leachates, likely due to the large numbers of plastic products distributed in these locations. At this time, it is unknown how these environmental sources contribute to human exposures, although the exposures of wildlife are likely significant. Humans are exposed to BPA through the food supply and the environment as a result of widespread use of the chemical in the production of polycarbonate plastics and epoxy resins, as well as in PVC plastics (as an antioxidant and to inhibit polymerization), flame retardants, polyester resins, polysulfone resins, polyacrylate resins, and thermal paper.[12] Epoxy resins, first produced in the late 1940s, are added to paints for metal coatings and applied to a wide array of metal items from household appliances, cars, dairy equipment, office equipment and tools, to food cans and sanitation, and sewage pipes, for the prevention of metal corrosion. Epoxy resins are also used in dental restorative work as adhesives in root canal work, adhering caps, bonding, and filling teeth. Commercial production for polycarbonates, a hard, heat-resistant plastic, began in the late 1950s and expanded considerably in the 1980s. Polycarbonates are used in manufacturing computer components, compact discs, kitchen appliances, automobile parts, safety glazing, helmets and sports equipment, laboratory and hospital equipment, optical lens, and reusable food and beverage containers.[1]

12.3 USE OF BIOMONITORING STUDIES IN BPA RESEARCH

The term "biomonitoring" has different connotations in different fields of study. With regard to BPA and other chemicals found ubiquitously in the environment, biomonitoring refers to the measurement of chemicals in human tissues and fluids via application of analytical chemistry methods.[46] As such, biomonitoring studies allow for the determination of actual levels of exposure from all possible sources, rather than suspected exposures from specific sources,

by examining internal and excreted concentrations of any given chemical. Biomonitoring studies are often used for two purposes: (1) to estimate daily exposure levels and (2) to inform risk assessments. The types of measurements and matrices examined are usually offset by the difficulty obtaining samples, as well as the cost associated with the analysis.[47] Typically, biological tissues and fluids are collected from individuals representing a population of interest; this can include relatively noninvasive measurements including urine, sweat, breast milk, semen, and saliva, and more invasive measures, including serum/blood, amniotic fluid, ovarian follicular fluid, and adipose tissue.

The chemical properties of the substance being examined also have an impact on the matrices that can be examined reliably, as some substances are altered by enzymes in blood, and other substances can break down in urine.[46] Additionally, the sensitivity and reliability of the methods used for analysis, the collection methods and materials, and the contamination of laboratory chemicals and equipment with the substance of interest are all important factors to be considered in biomonitoring studies. This is an important issue with regard to the biomonitoring of BPA, especially considering the ubiquitous nature of this chemical in laboratory equipment and environments.

Many techniques have been used to measure BPA in human urine, blood, and tissue samples, including gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), HPLC with electrochemical detection, HPLC with tandem MS, derivatization with different chemical agents followed by GC, and ELISA. Additionally, the U.S. Centers for Disease Control and Prevention (CDC) has developed a "gold standard" method for measuring BPA in urine: solid phase extraction, coupled with isotope dilution-HPLC-tandem MS[46]; CDC laboratories have been utilized by many researchers using biomonitoring samples to compare BPA concentrations among different populations.[48,49]

The best methods are those that can detect low concentrations (highly sensitive) that are also highly specific[22,23]; methods that cannot distinguish BPA from its metabolites may overestimate internal exposure to the unconjugated (active) form. For this reason, some scientists and regulatory agencies have challenged results obtained with ELISA because of the possibility of cross-reactivity of the antibodies used in this method.[23,24] Importantly, ELISA has been used in at least seven studies of human fluids and tissues and has produced results that are very similar to those obtained with analytical chemistry methods (reviewed in Vandenberg et al.[12]). This method is convenient and relatively inexpensive, making it useful for the screening of a large number of samples.

12.3.1 BPA and Its Conjugates in Urine

When unconjugated (active) BPA enters the body, it is metabolized by the liver into at least two metabolites; the majority is conjugated to BPA-glucuronide, and a smaller but significant portion is conjugated to BPA sulfate.[1]

When BPA enters the body through the oral route, this metabolism has been reported to be very efficient because BPA is metabolized by the liver before it reaches the bloodstream. When BPA enters the body through nonoral routes, that is, absorption through the skin or respiratory tract, it can circulate through the body before reaching the liver.[29] This is an important distinction because only unconjugated BPA has estrogenic activity, although other toxicity information of conjugates is relatively lacking, and these metabolites can be deconjugated by enzymes present in tissues.[50–52] Because conjugation of BPA changes its water solubility, BPA metabolites are removed from the body in urine; in contrast, unconjugated BPA is not expected in urine.[53]

In 2001, the first biomonitoring study to measure BPA metabolites in urine was conducted at the CDC examining pools of urine collected from several individuals.[54] Following that initial observation, more than 35 additional studies have examined BPA and/or its metabolites in urine samples (reviewed in Vandenberg et al.[12]). In spite of the large differences in sample size and a wide variety of analytical methods employed in these studies, most of these studies report the presence of BPA metabolites in the majority of samples tested. The central tendencies of these studies collectively indicate concentrations of 1–3 ng BPA metabolites/mL urine.

Several large studies have been performed in reference samples, that is, groups that are representative of larger populations. These studies, conducted in the United States, China, Germany, and Canada, indicate that lifestyle factors can contribute to urinary BPA concentrations.[46,55,56] However, these factors are different by country, suggesting that there are regional differences that contribute to BPA exposure.

12.3.2 Blood and Other Tissues and Fluids Suggest Widespread Internal Exposure to Unconjugated BPA

Almost two dozen studies have examined blood and serum samples collected from pregnant women, nonpregnant adults, and fetal umbilical cords (reviewed in Vandenberg et al.[12]). These studies specifically measured unconjugated BPA in healthy individuals, and the majority used analytical chemistry methods. All but two of these studies were able to detect unconjugated BPA in at least some samples. Compared with the size of the studies examining urine, these studies were typically much smaller, although a few larger studies have been published recently.[56–58] Again, the central tendencies of blood concentrations ranged from 0.5 to 2 ng unconjugated BPA/mL blood. These concentrations are higher than those required to stimulate responses in cell cultures[59] and in the range of concentrations that affect development of rodents and other animals,[22] suggesting that these low levels could influence biological end points and development in humans.

Of the five studies that have measured BPA in umbilical cord blood, two detected concentrations in a similar range (approximately 1 ng/mL) to the levels measured in healthy adults.[58,60] The other three studies measured BPA

concentrations two to four times higher in fetuses than nonpregnant adults.[4,61,62] However, only two studies directly compared plasma/blood BPA concentrations in fetuses and nonpregnant adults,[4,60] so it is difficult to make any conclusive comparisons regarding internal concentrations, metabolism, or exposure in these two groups. BPA measurements were taken from different matrices in the studies, thus further complicating the ability to directly compare samples.

Additional human tissues and fluids, including amniotic fluid, placenta, breast milk, adipose tissue, and saliva have been examined for BPA content (reviewed in Vandenberg et al.[12]). Similar concentrations have been detected in these samples as have been reported for blood. Together, it can be concluded that the majority of individuals are exposed to BPA, and that internal exposures are typically in the 1 ng/mL range.

12.3.3 Toxicokinetics of BPA

In contrast to the dozens of studies measuring BPA and its metabolites in environmentally exposed individuals, only a few small studies have determined the kinetics of BPA metabolism following administration of BPA. In the first, healthy adult volunteers were administered 5 mg deuterium-labeled BPA (equivalent to 54–90 µg/kg body weight [BW]) and then monitored for blood and urinary BPA and BPA metabolites levels.[53] Unconjugated BPA was always below the limits of detection (LOD; for urine = 1.37 ng/mL, for plasma = 2.28 ng/mL), and BPA glucuronide concentrations fell below the LOD in both urine and blood 24–34 hours after BPA administration. The authors concluded that BPA was rapidly metabolized to BPA-glucuronide, and this conjugate is rapidly cleared from blood. In a second study, subjects were administered 25 µg BPA, and unconjugated and conjugated BPA were then measured in urine.[63] The authors concluded that there were no differences in kinetics based on age or sex.

These toxicokinetic studies have multiple limitations that have called into question their conclusions.[12] First, these studies, and the interpretation of these studies used for risk assessment purposes, did not consider the strong possibility that BPA toxicokinetics would be affected by constant versus acute exposures. This is important because human exposures are thought to be both ubiquitous and continuous. Second, BPA has been reported to have rapid nongenomic actions, which could occur in target tissues if metabolism is not 100% effective, as has been reported. Third, the toxicokinetic studies were designed to assess the metabolism of BPA following oral exposure because until very recently,[64] it was assumed that most, if not all, BPA exposure in humans was occurring via the oral route. There are likely multiple, unidentified sources of BPA exposure, so nonoral exposures cannot be discounted, and metabolism following dermal absorption of BPA is likely to be different.[65] Fourth, data from biomonitoring studies suggest that it is extremely likely that there are differences in BPA metabolism under different physiological condi-

tions, such as pregnancy. Sex and age are also factors that can influence metabolism. The toxicokinetic studies used small mixed-subject groups, limiting statistical power for subgroup analyses. Furthermore, the data does not support the conclusion that there were no differences by sex. Finally, there is the potential for deconjugation of BPA metabolites in utero by enzymes that are present in high concentrations in the placenta and various other tissues.[51,66]

Because of the potential limitations and unanswered questions left from the few toxicokinetic studies (reviewed further in Vandenberg et al.[12]), many studies have examined BPA metabolism in animal models, including nonhuman primates.[22,67,68] Several recent studies suggest that relatively large exposures are needed to produce the levels measured in human samples, and age can also influence metabolism.[22,67]

12.3.4 Open Questions

Work is needed to identify all products that contain BPA and all sources of human exposure. This would also allow for better understanding of routes of exposure, which is critical to determine how efficiently BPA is metabolized. We also still know very little about how different genetic variations influence BPA metabolism. Most important is exposure to fetuses and neonates, but others (i.e., those with genetic polymorphisms that could influence metabolism) are also in need of future study. And finally, it has become imperative to understand whether current exposure levels are associated with any diseases or dysfunctions. This topic will be covered later in this chapter.

12.4 HUMAN HEALTH STUDIES OF BPA EXPOSURE IN HUMANS

Normal human growth, development, and homeostasis are dependent on numerous potent hormonal messengers that act on evolutionarily conserved receptors.[69,70] Exposure to endocrine disrupting compounds, like BPA, may interact with hormonally mediated pathways that include the estrogen, thyroid, and androgen receptors, and in turn increase the risk of disease.[71] A large number of experimental studies in animals and a limited number of human studies suggest that BPA exposure may be associated with some adverse health outcomes. The association of prenatal and childhood exposure to BPA with neurodevelopment, obesity, and reproductive health has been understudied in humans.[72] Early-life exposure is of particular concern given the unique susceptibility of the fetus and child to environmental toxicant exposures; however, chronic low-level exposures may increase the risk of some adult health outcomes.[73] Since human BPA exposure is widespread, even small adverse effects of BPA could have large public health implications.[74]

In contrast to hundreds of experimental studies on animals assessing the effects of early-life BPA exposure, a limited number of human studies have

been conducted.[75] A total of 23 peer-reviewed epidemiological studies have examined relationships between BPA exposure and human health outcomes, and the majority ($n = 17$) were cross-sectional or case–control studies.[40–45,76–91] Only six epidemiological studies examined health outcomes in children. Few of the 23 studies examined the same health outcomes. However, outcomes can be grouped and evaluated as categories of cancer,[40] reproductive,[41,42,45,76–79,85,87–92] metabolic,[43,80] pubertal development,[81,84] infant and childhood growth,[44,83,86] and neurodevelopment outcomes.[82]

12.4.1 BPA and Cancer

One case–control study compared urinary BPA concentrations among Korean women with breast cancer and age-matched controls.[40] No information was provided on the type, stage, or grade of the tumors. Mean serum BPA concentrations were similar among cases and controls; however, women with breast cancer had higher median serum BPA concentrations than women without cancer. A major limitation of this study is the use of concurrent serum BPA concentrations (i.e., concentrations at the time of breast cancer diagnosis). Because BPA has a short half-life, current serum BPA concentrations may not reflect the etiologically relevant window of exposure for the development of breast cancer, which is years to decades before clinical recognition. In addition, women with breast cancer or breast cancer clinical evaluations may be exposed to more BPA containing plastics compared with women without breast cancer.

12.4.2 Reproductive Outcomes

Because BPA is a suspected endocrine-disrupting compound, several studies have examined relationships between BPA and reproductive end points in men exposed to BPA. Outcomes in these studies include sexual function and semen quality. Several epidemiological studies observed associations between urinary BPA concentrations and serum reproductive hormones, but the direction of the relationship between BPA and hormones has been inconsistent.[45,76,79,90] Three studies reported consistent associations between increasing urinary BPA concentrations and one or more measures of reduced semen quality.[45,91,92] Two additional studies found that occupational BPA exposure and urinary BPA concentrations were associated with decreased sexual function in Chinese men.[77,78] Mok-Lin and colleagues reported that urinary BPA concentrations were associated with decreased serum estradiol concentrations and number of oocytes among women undergoing IVF.[41] These findings suggest that BPA exposure may be associated with lower semen quality and increased sexual dysfunction in men. However, the clinical relevance of these findings remains uncertain, and results from some studies are based on men with higher than background levels of exposure.

12.4.3 Metabolic Outcomes

The correlation between urinary BPA concentrations and metabolic disorders was investigated in a nationally representative cross-sectional sample of U.S. adults from the National Health and Nutrition Examination Survey (NHANES).[43,80] Among 2948 adults participating in two cycles of the NHANES (2003/2004 and 2005/2006), urinary BPA concentrations were associated with increased prevalence odds of self-reported CVD and diabetes. However, associations between BPA and CVD and diabetes were stronger in the 2003/2004 cycle, when geometric mean BPA concentrations were higher (2.5 vs. 1.8 μg/L). Positive correlations between urinary BPA and serum liver enzyme concentrations were also observed. The interpretation of these results is limited by the cross-sectional design. CVD and metabolic disorders have long latency periods and contemporaneous urinary BPA concentrations may not reflect the relevant etiologic window for the development of cardiovascular and metabolic diseases, which is known to be years or decades earlier. In addition, time-dependent confounding (i.e., reverse causality) may be responsible for observed associations, since obese individuals are at an increased risk for CVD and metabolic disorders, and may consume more packaged and processed foods that contain BPA. Even in the face of these caveats, animal studies show that prenatal BPA exposure may influence the development of metabolic disorders.[93] Thus, fetal exposure to BPA may be more important to the development of metabolic disorders than later-life exposure.

12.4.4 Pubertal Development Outcomes

Early-life BPA exposure may increase the risk for altered pubertal development and in turn increase the risk factor for breast cancer.[94,95] Some rodent studies suggest that early-life BPA exposure may accelerate pubertal development and increase breast cancer risk.[96,97] Two studies have examined the relationship between BPA exposure and pubertal development in young girls.[81,84]

A cross-sectional study in New York, New York examined the association between concurrent urinary BPA concentrations with pubic hair and breast development in 192 9-year-old girls.[84] Higher urinary BPA concentrations were not associated with advanced breast or pubic hair development. However, BMI modified the association between BPA and breast development, where higher urinary BPA concentrations were more associated with delayed breast development among girls with lower BMI.

A multicenter prospective cohort of 1151 6- to 8-year-old female children from Cincinnati, Ohio, San Francisco, California, and New York, New York examined the relationship between urinary BPA concentrations and pubertal development 1 year later.[81] Compared with the lowest quintile of urinary BPA concentrations, the odds of advanced breast development were similar but slightly below the null for the top four quintiles, with no discernible

dose–response pattern. Compared with the first quintile of urinary BPA concentrations, the odds of advanced pubic hair development (stage 2+) were slightly increased in the second and third quintiles of urinary BPA concentrations and null in the fourth and fifth quintiles. The authors did not report whether BMI modified the association between urinary BPA concentrations and pubertal development.

These two studies suggest that childhood BPA exposure may have modest associations with altered pubertal timing in girls. However, given the relatively small effect sizes, there are several important limitations, including residual confounding by measured and unmeasured confounders, and the potential for differential pharmacokinetics among prepubertal and pubertal girls that may account for differences in urinary BPA concentrations.

12.4.5 Fetal and Childhood Growth Outcomes

Three studies examined associations between BPA exposure and infant/childhood growth.[44,83,86] One small cross-sectional study reported no association between serum BPA concentrations and birth weight or gestational age; however, numerical results were not presented. Closer examination of their data suggests lower birth weight among infants born to mothers with serum BPA concentrations >5 μg/L.[83] The associations between numerous nonpersistent environmental toxicants, including BPA, and birth outcomes were examined in a prospective birth cohort of 367 women in New York, New York.[44] Third trimester urinary BPA concentrations were associated with modest increases in birth weight, length, and head circumference, and no change in gestational age. While these findings were not statistically significant, the association between urinary BPA concentrations and birth weight was the largest, but one of the least precise estimates, of all the toxicant exposures examined. These two studies report different directions of effect between BPA exposure and infant size. Differences in exposure assessment methods (urine vs. serum) and timing (third trimester vs. birth) of BPA exposure may be responsible for these disparate findings.

12.4.6 Neurodevelopmental Outcomes

A prospective birth cohort of 249 mothers and infants from Cincinnati, Ohio examined the association between three prenatal urinary BPA concentrations and childhood behavior at 2 years of age.[82] Mean gestational BPA concentrations and those from samples taken at 16 weeks gestation were positively associated with externalizing behaviors (aggression and hyperactivity) in children, but this association was stronger in girls than in boys. Previous animal studies observed sex-specific effects of BPA exposure, and some hypothesize that BPA may disrupt sex steroid-mediated brain development.[98,99] The magnitude of the observed associations was similar to those seen for other

environmental toxicants and neurodevelopment (e.g., environmental lead and IQ/behavior). While this study had multiple measurements of gestational BPA exposure, the persistence of the observed associations into later childhood and the role of childhood BPA exposures remain to be determined.

12.4.7 Limitations of Human Studies

Three major limitations emerge from this review of the human studies. First is the small number of studies with similar health end points. Future studies should utilize comparable health end points and measurement instruments to facilitate systematic reviews. The second is the potential for misclassification of BPA exposure due to the short biological half-life of BPA. Urinary BPA concentrations are believed to reflect recent exposure over the past 6–12 hours. Therefore, a single spot urine sample may not accurately classify long-term exposure (over weeks, months, or years), since data shows that BPA exposure is episodic and varies over time.[100] Finally, only six studies examined health outcomes in infants and children, whom may be more vulnerable to BPA toxicity.

12.4.8 Conclusions of the Human Studies

To date, most human studies of BPA have utilized cross-sectional designs that offer suggestive results, but cannot address the temporality of exposure and disease. For many diseases and disorders (e.g., asthma, neurobehavioral disorders, obesity, or early-onset puberty), gestational, childhood or pubertal BPA exposure may be more relevant to the development of disease than concurrent BPA exposures. Despite these limitations, results from cross-sectional results can be used to guide the design of stronger and more powerful studies. The limitation of BPA exposure misclassification can be addressed in prospective cohort studies by collecting multiple or 24-hour urine samples during the etiologic relevant window prior to disease onset.

A growing body of literature suggests that many adult diseases have in utero origins.[101,102] Given the unique susceptibility of the fetus and child to environmental exposures, studies examining the relationship between BPA exposure and diseases of childhood may offer insight into the potential effect of BPA exposure across the lifespan.

12.5 ANIMAL MODEL STUDIES OF BPA TOXICITY

12.5.1 Effects of Developmental Exposure to BPA on the Prostate in Male Rodents

In 1997, two studies were published that showed effects of maternal administration of very low doses of estradiol, diethylstilbestrol (DES), and BPA on the

development of the prostate in male mouse fetuses, as well as a permanent increase in prostate size and number of androgen receptors in adulthood.[103,104] These effects of BPA were caused by feeding pregnant mice very low doses of BPA (2 and 20 μg/kg/d), doses that were 2500 and 25,000-fold lower than any dose previously tested in experimental animals. The prediction that BPA would show biological activity at these low doses was based on studies focusing on the estrogenic activity of BPA, although it is now clear that BPA can act via multiple response systems in addition to ERs. The application of approaches used by endocrinologists to the study of an environmental chemical was a radical paradigm shift from the traditional approach in toxicology of testing BPA or other chemicals for acute toxic effects only at very high doses; the effects seen at high doses were then used to predict effects at lower doses.[103,105] The traditional toxicological approach is based on the assumption that all dose–response relationships for chemicals are monotonic, a prediction that is not correct based on data for virtually all hormonally active compounds.[29,106–108]

The low dose effects of BPA on the developing prostate were consistent with similar effects on the fetal prostate of a 0.2-μg/kg/d dose of DES fed to pregnant female mice and an experimental increase in free estradiol in of only 0.1 pg/mL (0.1 parts per trillion) in fetal mouse serum.[104] Subsequent research showed that the increase in prostate size was associated with basal epithelial cell hyperplasia in the primary prostate ducts in mouse fetuses caused by very low doses of BPA, ethinylestradiol, and DES.[109,110] In addition, at the serum concentrations of estradiol and BPA produced by administering BPA and estradiol in these prior studies,[67,104,111] estradiol and BPA increased both androgen receptor and ER alpha (Esr1) gene activity in fetal mouse prostate mesenchyme cells in primary culture.[112]

The initial findings that BPA altered prostate development raised concern because there is evidence that basal cells, whose differentiation during fetal life is altered by BPA, are the progenitor cell population that undergoes transformation during the development of prostate cancer,[113,114] and hyperplasia of progenitor/stem cells during critical periods in development in organs is associated with disease in adulthood, consistent with the developmental origin of adult disease hypothesis.[110,114] Indeed, treatment of neonatal rats with very low doses of BPA (10 μg/kg/d) either orally or by subcutaneous injection, results in early-stage prostate cancer when males are treated with estrogen in adulthood to mimic the increase that occurs during aging in men.[6,68]

An important aspect of the prostate findings was that the dose–response curves for both estradiol and DES, which were tested over a wide dose range, were nonmonotonic and formed an inverted U, with high doses inhibiting prostate differentiation and subsequent postnatal development[104,110,115]; a similar inverted-U dose-response relationship was reported for the stimulation of human prostate cancer cell proliferation by low doses of BPA[116] that were directly in the range of unconjugated BPA levels reported in human serum in multiple studies.[12] In fact, studies have shown nonmonotonic (U-shaped and inverted-U shaped) dose–response curves for BPA, which is an expected finding for any hormonally active chemical.[29,108]

Several studies showing significant stimulating effects of low doses of BPA and DES on the prostate in mice was published. One study included an *in vivo* analysis on the effects of fetal exposure to low doses of BPA and DES (via maternal feeding) on the prostate, as well as an analysis of the effects of these chemicals on the fetal prostate in primary culture.[109] This finding led to the conclusion that the initial findings by Nagel et al.[103] had been replicated.[117] Taken together, there are a series of findings that provide evidence for stimulating effects of maternal administration of 2, 10, 20, and 50 μg/kg/d BPA, as well as similar low dose effects of estradiol, DES, and ethinylestradiol, on prostate development in male mouse fetuses, with long-term consequences for the adult prostate.[6,68,103,104,109,110,118,119]

The ability to detect low dose effects of BPA is complicated by issues such as contamination from caging and water bottles.[120,121] The issue of contamination of control animals is a concern when a study attempts to find effects of exposure during development to a very low dose of BPA (e.g., 0.25 μg/kg/d) if the investigators house mice in polycarbonate cages and provide water in polycarbonate bottles.[122,123] In addition, there have been unexpected effects caused by components of feed that can mask low dose effects of chemicals such as BPA.[124,125] A recent study examined the effect of a feed (Purina soy-based 5002 chow) that was used in a number of studies of BPA that reported finding no significant low dose effects of BPA.[126–129] The 5002 feed interfered with the ability to detect effects of DES in mice, while effects of DES were observed using other commonly used soy-based Purina feeds.[130]

12.5.2 Effects of Developmental Exposure to BPA on the Ovary and Reproductive Tract in Female Rodents

Hunt and colleagues published data showing that the BPA-disrupted alignment of chromosomes in oocytes undergoing meiotic division during progression from meiosis I to meiosis II after puberty,[121] as well as during the initial entry into meiosis during fetal development.[131] A number of other studies have been published that reported effects of BPA on meiosis in oocytes, although there are some differences in some of the findings and in some of the methods used. In a review of the data on BPA and oocyte meiosis, Hunt and colleagues identified that "all studies reported that BPA induces detectable meiotic disturbances in the periovulatory egg." In addition to adverse effects on oocytes, neonatal exposure to BPA (ovarian differentiation spans prenatal—neonatal life in rodents) has been shown to lead to ovarian cysts in rats and mice.[10,132] Only one study has examined the effects of prenatal exposure to BPA in middle-aged rodents. Specifically, mice were subcutaneously injected (sc) on days 9–16 of gestation with BPA (0.1, 1, 10, 100, or 1000 μg/kg/d), and the offspring were examined when 18 months old (middle age) for adverse effects on reproductive tissues. Ovarian cysts were significantly increased in the 1-μg/kg BPA group; ovarian cyst adenomas were also seen in the other BPA-treated groups, but not in controls. Proliferative lesions of the oviduct were also observed.[11]

12.5.3 Effects of Exposure to BPA on the Mammary Gland in Female Rodents

Beginning in 1993 with a study showing that BPA leaching from polycarbonate plastic could stimulate human breast cancer (MCF-7) cell proliferation,[133] there has been interest in BPA exposure during perinatal period of mammary gland differentiation in rats and mice. BPA exposure during this time was associated with mammary gland cancer later in life.[11,134] There is now an extensive literature showing that exposure to BPA (via Alzet pumps that continuously release BPA, subcutaneous injection, or oral administration) alters mammary gland differentiation and subsequent adult function, and increases the risk of developing neoplastic lesions. Studies have shown effects of BPA in female fetuses, similar to effects described for the prostate in male fetuses exposed to BPA.[135] In numerous studies that examined mammary gland gene expression, low-dose prenatal BPA treatment resulted in ductal hyperplasia and carcinoma *in situ,* increase in mammary ducts, terminal end buds, and alveolar buds in adult rodents.[9,136,137] Perinatal oral administration of BPA to pregnant and lactating rats also altered key proteins involved in signaling pathways involved in mammary gland proliferation in female offspring.[138] In addition, a number of studies have shown that developmental exposure to BPA increases the rate at which mammary tumors are induced by the treatment of female rats in adulthood with the carcinogens N-nitroso-N-methylurea (NMU)[5] and dimethylbenzanthracene (DMBA).[139]

The developing fetus is particularly sensitive to chemicals with estrogenic activity, as illustrated by the benign and carcinogenic effects of prenatal exposure to DES. Recently, Newbold et al. demonstrated that neonatal exposure to BPA (subcutaneous doses of pups ranging from 10 to 1000 μg/kg for 5 consecutive days) resulted in long-term effects in reproductive tissues of adult animals.[10] Specifically, BPA exposure leads to development of cystic ovaries, adenomyosis, leiomyomas, atypical hyperplasia, and stomal polyps. In a separate study, the same group showed that neonatal exposure to environmentally relevant doses of BPA (0.1–1 10–1000 μg/kg) resulted in similar reproductive abnormalities in adult female mice.[11] These studies highlight the notion that low-dose exposure to endocrine-disrupting compounds during critical stages of development can cause irreparable harm. Additional studies are needed to determine the potential adverse effects of BPA exposure to human fetuses and newborns.

12.5.4 Route of Administration of BPA: Differences between Adults and Neonates in Rodents

An issue raised as a criticism of some experimental research involving developmental exposure to BPA is that some studies did not administer BPA by an oral route. The past assumption that has been accepted by all regulatory agencies is that using subcutaneous injection or constant administration via a

mini-pump to expose either fetuses (via maternal treatment) or neonates to BPA would lead to unrealistically high exposures to BPA. However, one recent study showed little or no difference[111] in serum levels of biologically active (unconjugated) BPA based on route of administration in newborn mouse or rat pups.[68] In addition, there was no impact of route of administration (subcutaneous or oral) on findings of prostate cancer in adult male rats when very low doses of BPA were administered during the early neonatal period.[68] Support for a lack of difference by route of exposure was that both oral and sc administration of genistein to neonates lead to the same adverse outcomes,[11,140,141] similar to data concerning these different routes of administration of BPA. Thus, route of administration of BPA to the future development of neonates is less important than in the human.

One reason that the route of administration has a greater impact on adult internal levels of hormonally active chemicals relative to neonates is that the phase II metabolizing enzymes are not expressed at adult levels in neonates, so differences in pharmacokinetics observed in adults based on route of administration are not observed in neonates.[68,111] It has been recognized for a long time that infants and children are not little adults with regard to the capacity to metabolize drugs or chemicals.[142] Another issue has been the degree to which rodents are relevant for examining the effects of BPA at similar serum levels reported in human biomonitoring studies (Vandenberg et al.[12]) in relation to the dose required to produce those levels. Pharmacokinetics of BPA in rodents, monkeys, and humans is very similar,[67] and rodents are appropriate models for assessing the effects posed by BPA, based on consensus statements about BPA from a German EPA meeting[143] and a World Health Organization (WHO) meeting.[144] The WHO panel concluded that: "Despite some differences between BPA metabolism and disposition in rodents and primates, internal exposures to aglycone (unconjugated) BPA are remarkably similar for adult rodents, nonhuman primates, and humans. This apparent lack of requirement for allometric scaling is atypical in the therapeutic drug and general chemical literature, and suggests that a specific adjustment for interspecies differences in toxicokinetics is not required."

12.5.5 BPA and Neurobehavior

Early-life sex steroid exposure influences postnatal behaviors by acting on evolutionarily conserved receptors.[69] Male and female animals display sexual dimorphisms in play behavior, aggression, anxiety, exploration, visual–spatial ability, and some aspects of cognition. Sexually dimorphic human disorders like attention-deficit/hyperactivity disorder (ADHD) and depression may be clinical correlates to some of these animal behaviors.[145] These disorders may be related to early-life disruption of the endocrine system, but there is relatively little known so far.[146] Some evidence suggests that gestational exposure to androgens masculinizes play behavior in girls, but not boys.[147] These observations and BPA's potential estrogenic activity suggest that sexually dimorphic

behavioral traits may serve as sensitive neurobehavioral end points in epidemiological studies. Experimental studies using animals report that early-life BPA exposure disrupts normal neurodevelopment, impacting sexually dimorphic behaviors, like aggression, anxiety, exploration, and spatial memory.[72,99,148]

12.5.6 Potential Mechanisms Mediating Effects of Developmental Exposure to Low Doses of BPA

One of the major topics of interest has been the issue of the "developmental origin of adult disease" and the potential for environmental chemicals to alter epigenetic programming during critical periods in development when cells differentiate from embryonic cells to all of the different types of cells found in an adult. The findings described above involved exposure to very low doses of BPA during critical periods in organ development, with the effects being observed much later in life. The determination that endogenous hormones, as well as environmental endocrine-disrupting chemicals, can cause epigenetic changes (i.e., changes in the expression of genes rather than changes to the sequence of bases), has provided the mechanistic explanation for how exposure during very brief periods in development to environmental endocrine-disrupting chemicals, such as BPA, can lead to organ and system dysfunction later in life, resulting in diseases expressed in adulthood.[6,149,150]

There is evidence that fetuses and infants are more sensitive than adults to disruption by endocrine-disrupting chemicals.[151] One obvious basis for this heightened sensitivity during early development is the limited ability of the fetal/neonatal liver to metabolize environmental chemicals and drugs relative to adults.[111,142,151] The permanent impacts of chemicals on differentiating tissues is referred to as "organizational" effects, while impacts on adults, when exposures tend to alter function while the chemical is present, but do not result in permanent effects once exposure to the chemical ceases, are referred to as "activational" effects. An example of an activational effect would be the short-term use of oral contraceptives by women, which when present alter the functioning of the reproductive system, but once withdrawn have no lasting effect on fertility and the reproductive system returns to normal function. In sharp contrast, transient fetal exposure to the same doses of oral contraceptive hormones used by adult women causes permanent damage to the developing fetal reproductive system.[119] However, the dichotomy of activational and organizational may be overly simplistic, since systems involved in activation and organization overlap and interact.

Beginning in 1997, investigators from neurobiology and aquatic toxicology determined that low doses of BPA were able to cause adverse effects in laboratory animals, including mammals, fish, and invertebrates,[152–155] and by 2010, over 1000 studies concerning BPA have been published in peer-reviewed journals.

While the focus of the majority of BPA research has been on organizational effects, there are also many studies that have shown that adult exposure to

low doses of BPA can disrupt organ function, although the assumption (typically not tested by examining animals for long periods after exposure) is that the effects observed are not permanent. However, to challenge the generality of the "activational" hypothesis as stated above, a long-term adverse consequence of exposure of adult pregnant females to low doses of BPA was provided by a study reporting disruption of insulin and glucose regulation in the adult female mice that required a long-latency prior to being observed[93] months after treatment. However, the fetuses carried by these exposed mothers responded to a dose of BPA 10-fold lower than the dose required to see a long-term effect on the adult pregnant female, again revealing the heightened sensitivity of fetuses relative to adults to environmental chemicals such as BPA.

12.6 REGULATORY CONCERNS

As detailed in this chapter, biomonitoring studies indicate ubiquitous exposure to BPA in adults, adolescents and children Toxicology studies have determined that the maximum tolerated dose of BPA is 1000 mg/kg/d,[156] while others have shown that hormonal effects were measured at concentrations as low as 2–5 ppb (2–5 μg/L)[133] . BPA exposure has been linked to a variety of physiological problems in animal studies, such as infertility, weight gain, behavioral changes, early onset puberty, prostate and mammary gland cancer, and diabetes.[157]

The regulatory debate began in earnest several years ago after a group of BPA scientists released a consensus statement on the state of the evidence. The Chapel Hill Consensus statement stated that "the commonly reported circulating levels in humans exceed the circulating levels extrapolated from acute exposure studies in laboratory animals."[72] In reaching these conclusions, the group examined the entire body of scientific data, including more than 40 human biomonitoring studies available at the time and the two human toxicokinetic studies. The panel concluded that humans, including children, adult men and women, and pregnant women, have measurable levels of unconjugated BPA in their bodies, stating succinctly that "[h]uman exposure to BPA is widespread."[72] Additionally, a subpanel of experts concluded that: "Unconjugated BPA has been measured repeatedly in human blood (serum and plasma) with a central measure of the distribution in the 0.3–4.4 ng/mL range (1–19.4 nM), and in breast milk, amniotic fluid, and placental tissue in the low [nanograms per milliliter] or [nanograms per gram] range."[135]

During this same time period, the NTP Center for Research on Human Reproduction (CERHR), established a panel of scientific experts (but not researchers publishing on BPA effects) to assess the data on BPA toxicity. The 2007 CERHR report, and several subsequent drafts,[158] were challenged and criticized by a group scientists because they felt that arbitrary criteria was used to evaluate animal studies, applied unevenly to different studies, and contained

scientific errors and misinterpretations of published data (reviewed by Vandenberg et al.[71]). In the spring of 2008, the NTP undertook its own review of the BPA literature, including recommendations from the 2007 CERHR report and comments from the public; however, the review was limited to studies related to risks for human reproduction. The NTP concluded that "there are data reporting bisphenol A concentrations in urine, breast milk, and amniotic fluid."[159] Yet the NTP also stated that the many biomonitoring studies may be unreliable because BPA conjugates can be unstable under some storage conditions and because laboratory equipment may leach BPA: "it is possible that free bisphenol A concentrations measured in biological samples may be overestimated."[72]

In 2008, the FDA, focusing primarily on guideline studies, reported, "Based on our ongoing review, we believe there is a large body of evidence that indicates that FDA-regulated products containing BPA currently on the market are safe and the exposure levels to BPA from food contact materials, including for infants and children, are below those that may cause health effects."[160] The FDA[160] largely avoided the issue of current human exposure levels, giving very little attention to either the available biomonitoring studies or toxicokinetic studies.[161] The FDA[160] stated: "There are several publications detailing measurements in biological fluid for BPA. Although [the] FDA is aware of these data and considers them extremely useful, [the] FDA also understands the experimental limitations that have been identified with regard to these data. . . . [The] FDA's updated safety assessment is focused on a subpopulation, infants. Accordingly, the currently available data, which consider exposure to adults or young children (6 years of age or older), were not used or relied upon in FDA's safety assessment."[160] Thus, to base their decision, the FDA did not address biomonitoring studies that indicated internal exposures to unconjugated BPA.[12]

In 2010, the European Food and Safety Association (EFSA) received a request from the European Commission to review scientific arguments supplied by Denmark in support of the government's decision to ban the use of BPA in food contact materials for infants aged from 0 to 3 years. The Danish risk assessment was based mainly on the study by Stump looking at possible neurodevelopmental effects of BPA at a range of different dose levels.[127] Following a comprehensive review of recent scientific literature and studies on the toxicity of BPA at low doses, EFSA's CEF Panel concluded they could not identify any new evidence that would lead them to revise the current European guidelines of total daily dietary intake (TDI) for BPA of 0.05 mg/kg BW.[162] The panel also stated that the data currently available do not provide convincing evidence of neurobehavioural toxicity of BPA.[162]

However, a minority opinion expressed by one panel member noted that recent studies point to uncertainties regarding adverse health effects below the level used to determine the current total dailey intake (TDI; the amount of a substance ingested per BW over a lifetime that presents an acceptable

risk).[162] Although this panel member agreed with the rest of the Panel's general view that these studies could not be used to establish a lower TDI, the expert panel recommended that the current TDI should become a temporary TDI. The CEF Panel members acknowledged that some recent studies report adverse effects on animals exposed to BPA during development at doses well below those used to determine the current TDI. The panel member also noted that "the studies show biochemical changes in the central nervous system, effects on the immune system and enhanced susceptibility to breast cancer. However, these studies have many shortcomings."[162]

In response to evidence from animal research, growing public concern, and numerous pending liability cases, several manufacturers of baby bottles recently shifted from polycarbonate plastic to available "BPA-free" alternatives.[163] Five state governments in the United States, as well as France and Denmark, passed bills restricting or banning BPA in children's products. While markets and state legislators tend to respond more quickly and with greater precaution to laboratory research and public concerns, regulatory agencies in the United States, Europe, and Japan continue to uphold the safety of BPA at current human exposure levels based on traditional risk assessments.[164] The considerable scientific uncertainty about low dose effects, combined with the significant political and economic stakes of this debate, presents an opportunity to lay a foundation of knowledge about what is known about this chemical, where important research questions remain, and where uncertainty persists. It also presents a challenge to investigators studying the effects of BPA: what can they do to improve the impact of their research on the risk assessment process? It also presents a challenge to risk assessors and the regulatory agencies who are tasked with protecting the health of people around the world and who base their decisions on guideline studies and dismiss the investigator-initiated studies.

The above findings have to be considered in relation to the position regarding the predicted safe daily intake dose of BPA by U.S. and European regulatory agencies at that time, which despite data to the contrary remain an official position of regulatory agencies today; namely, that a daily oral dose of 5 mg/kg/d BPA is predicted to cause no significant effects in animals or humans.[165,166]

Evaluating low dose safety involves integrating a vast body of experimental and epidemiological research into health policy and regulatory decision making. As demonstrated in the case of BPA, this has proven to present a serious challenge to risk assessors. This is due in part to a number of important changes in environmental health research over the past decade that characterizes much of the low dose research on BPA. Some of these changes include the proliferation of data from human biomonitoring studies, and the increased use of genomic tools and technologies that allow researchers to rapidly screen BPA for its ability to activate or inhibit gene expression and protein production. Further, ongoing hypothesis testing of the fetal origins of disease shifts the focus of chemical testing further upstream in disease development.

Researchers working with BPA explore how even very low doses alter development early in life, and whether such changes lead to increased risk of disease or abnormal function later in life.[167]

Regulatory risk assessments assume that the principle route of exposure to BPA is through the food supply due to the chemicals' migration from epoxy resin liners and polycarbonate plastic, and that BPA is rapidly cleared from the body via first-pass glucuronidation. These two assumptions of oral exposure and rapid metabolism contribute to the exclusion of many low dose studies that employed nonoral routes of exposure from risk assessments.[50] Several researchers, however, question that oral exposures do not sufficiently account for exposure levels consistently reported in biomonitoring studies, and contend that other possible routes of exposure, including dermal and inhalation, are possible.[51] BPA is detectible in air, water, soil, and landfill leachate as a result of manufacturing, protection, and disposal. Data collected by the Toxics Release Inventory in the United States in 2007 report over 1 million pounds of total release with 122,965 lb to air, 6246 lb directly into water, 14,972 1b released on-site to land and 684,638 lb transferred offsite to land. Recent reports of BPA in thermal papers, such as those used in carbonless receipts, introduces the possibility of dermal and inhalation exposure in the general public. Dermal and inhalation exposure is also expected to occur in workers handling BPA and BPA-based products.[52]

12.7 ADDRESSING THE DATA GAPS

The discrepancy between the extensive harm shown in hundreds of studies at doses below 50 μg/kg/d and the acceptance of this dose as the NOAEL by regulatory agencies has led scientists,[29,107,108,168–170] scientific societies, such as the Endocrine Society,[171] as well as legislators around the world, to identify the urgent need for updating regulatory science used to assess the safety of endocrine disrupting chemicals. To specifically address this problem, the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, the major U.S. funding agency for research on the toxicity of BPA in animal and human studies, developed a research consortium to coordinate BPA research. Using funds from the American Recovery and Reinvestment Act (ARRA), NIEHS funded 12 researchers with experience studying BPA effects in human and animal to work together to develop studies of greater utility to the regulatory agencies. The researchers agreed to use multiple doses instead of single doses to measure internal dose levels of BPA, to address the issue of oral versus subcutaneous or continuous exposures via implanted pumps, and to add overlapping end points to their studies in order to provide more data using a variety of approaches. Today, this virtual consortium includes over 30 researchers, all working in smaller workgroups to assure coordination, collaboration, and data integration. Forthcoming published data

from these studies is expected to directly inform risk assessors and the regulatory community.

The NIEHS is also addressing the question of why good laboratory practice (GLP) regulatory guideline studies have not found low dose effects of BPA reported in investigator-initiated studies. Recently, the NIEHS invested approximately $14 million in Recovery Act funds on BPA-related research. This research will build upon existing NIEHS funded projects and in-house research, as well as NTP projects. Collectively, the results of these new ARRA-funded studies and ongoing studies should begin to chip away at uncertainties and provide a better understanding of the potential risks that exposure to BPA poses to public health. Additionally, working with the FDA and the NTP, the NIEHS is developing a guideline study with additional low doses of BPA (instead of three high doses) and the inclusion of a number of end points of concern identified in low dose studies. This experiment will provide insight into whether critical changes in the dosing regimen and end point selection are necessary to adequately modernize guidelines studies.

There are many lessons to be learned from the research and regulatory debate on BPA, and clear opportunities exist for change. It is important that we acknowledge and address the limitations of regulatory guidelines studies with respect to assessment of low-dose toxicity of endocrine-disrupting chemicals, such as BPA. It is also important to acknowledge the limitations of many investigator-initiated studies for use in risk assessment. We are hopeful that collaborative projects that couple regulatory guideline principles, together with rigorous academic research, will provide opportunities to address these data gaps. Finally, it is imperative that scientists, funding agencies, and the regulatory community work together to ensure that research addresses the critical questions that need to be asked to protect the public's health.

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