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Endocrine Disruptive Activity of Bisphenol A and its Relationship to Metabolic Syndrome: Explaining Ubiquitous Exposures and Elusive Effects through Animal Studies

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An abstract of
A thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

Endocrine Disruptive Activity of Bisphenol A and its Relationship to Metabolic Syndrome: Explaining Ubiquitous Exposures and Elusive Effects through Animal Studies
By Kirsten Hesla

The pathways and processes of endocrine disrupting chemicals are extremely species specific (Ben-Jonathan et al, 2009). The ubiquitous chemical and endocrine disruptor bisphenol A (BPA) has been implicated in several exposure-disease relationships. There is substantial research suggesting that BPA has the potential to alter metabolic processes. *In vivo* and *in vitro* experimental studies isolate specific variables and explore exposure pathways in different taxonomic groups; they provide a basis for determining an exposure-disease causal relationship and insight into relevant doses and molecular mechanisms of operation. This meta-analysis integrates the findings from multiple *in vivo* and *in vitro* animal studies to provide a probability estimate of an association between exposure dose of BPA, and the symptoms characteristic of metabolic syndrome. The results indicate that low-dose exposures are significantly more likely to result in a metabolic effect that is deleterious to health (NTP, 2001; The Endocrine Society, 2009). The probability coefficient that determined the relationship between dose and metabolic effect was statistically significant ($p=0.033$) and demonstrated that that probability of a metabolic effect is higher in the low dose range. The probability that BPA will induce insulin resistance is more likely at low-doses but the probability of adipose tissue development is higher than insulin resistance at every dose level. BPA at low-doses was also shown to have a greater variety of metabolic effects as opposed to high-doses. The results of this meta-analysis suggest that there is a correlation between BPA and metabolic syndrome, especially at low-dose exposures.

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INTRODUCTION

BPA is the monomer used in plastics manufacturing because of its properties as a cross-linking chemical; it was widely chosen by the chemical industry to produce plastic polymers, mainly poly-carbonates (Alonso-Magdalena et al, 2006) This chemical is produced in extremely high volumes and has been found in the resin that lines food and beverage cans, dental sealants, and is a common additive in other plastic products (Kwon et al, 2000). Over 2 billion pounds of BPA are used in the production of epoxy resins and polycarbonate plastics every year; these plastic products are often used in food and drink packaging creating a direct ingestion exposure pathway. The resins are used as lacquers for metal cans, bottletops, and groundwater pipes (Takai et al, 2001). BPA is one of the highest volume chemicals produced worldwide (Alonso-Magdalena et al, 2006; Vogel et al, 2009).Global BPA production capacity in 2003 was 2.2 million metric tons (over 6.4 billion pounds). "The ester bond linking BPA molecules in polycarbonate and resins undergoes hydrolysis, resulting in the release of free BPA into food, beverages, and the environment, and numerous monitoring studies now show almost ubiquitous human exposure to biologically active levels of this chemical (Welshons et al, 2006)" Heat and extreme acidity are conditions that can accelerate hydrolysis of the ester bond linking BPA monomers, leading to release of BPA and the potential for human and environmental exposure (Burridge et al, 2003). BPA is also used in dentistry fillings and human exposure to the chemical may be significant because microgram amounts have been detected in the liquid of lined cans and in the saliva of people with dental sealants (Takai et al, 2001). The estimated daily human consumption of BPA, from epoxy-lined

cans alone is approximately 6.6 micrograms/kilograms (μg/kg) (The Endocrine Society, 2009).

BACKGROUND

Endocrine Disrupting Potential of Bisphenol A

BPA has been shown to mimic the actions of estrogenic compounds; it was the first synthetic estrogen without a steroid structure (Alonso-Magdalena et al, 2006; Dodds et al, 1936). While its endocrine disrupting potential was discovered fairly recently, BPA has been used consistently for decades; it is one of the most commonly used and widespread endocrine disrupting chemicals. Studies conducted in Japan and the United States have shown that BPA accounts for the majority of estrogenic activity that leaches from landfills into the surrounding area (Coors et al, 2003; Kawagoshi et al, 2003). It is well established that many environmental chemicals can interfere with complex endocrine signaling pathways and cause adverse consequences in the organism's development (Colborn et al, 1993). The Endocrine Society describes endocrine-disrupting chemicals (EDC) as "substances in our environment, food, and consumer products that interfere with biosynthesis, metabolism, or action resulting in a deviation from normal homeostatic control or reproduction." One distinguishing characteristic of endocrine disruptors are the infinitesimally small levels of exposure shown to elicit effects (NTP, 2001; The Endocrine Society, 2009). Endocrine disruptors exert uncharacteristic exposure-effect relationships, because low doses often exert more potent effects than higher doses (NTP, 2001). The National Institute of Environmental Health Sciences (NIEHS) defined "low-doses" of endocrine disrupting chemicals as doses below

the accepted No Observable Effect Level (NOAEL); the NOAEL for BPA is 50 mg/kg/day (Vandenberg et al, 2007). The Reference dose (RfD) currently recommended by the United States Environmental Protection Agency (EPA) is set at 50 μg/kg (EPA, 1993; Lang et al, 2008). The RfD is calculated as the level 1000-fold below the NOAEL and it is considered the acceptable level of daily human exposure (Vandenberg et al, 2007). While concern about endocrine disrupting chemicals initially focused on reproductive and carcinogenic effects, it is now known that multiple organ systems are affected by endocrine disrupting chemicals including the cardiovascular and neuro-endocrine systems.

Endocrine disrupting chemicals have been indicated as environmental pollutants that contribute in the pathogenesis of metabolic diseases. Most recently, an association between exposure to environmental chemicals and development of obesity has been proposed (Baillie-Hamilton et al, 2002; Newbold et al 2007). Adipose tissue metabolism is regulated by hormones including sex steroids under the sympathetic nervous system. Environmental estrogens may impact adipose tissue through direct modulation of lipogenesis, lipolysis, and adipogenesis, or indirectly through food consumption and leptin secretion (The Endocrine Society, 2009).

Characteristics of Metabolic Syndrome

Mayo Clinic defines metabolic syndrome (MetS) as “a cluster of conditions that occur together, increasing [the] risk of heart disease, stroke, obesity and diabetes.” The symptoms occur as a result of insulin resistance, hypertension, and hyperlipidemia (Newbold et al, 2009). Obesity and diabetes are quickly becoming significant health

problems worldwide. The incidence of diabetes mellitus has tripled during the last two to three decades; it is estimated that there are over 177 million cases worldwide (The Endocrine Society, 2009). Based on the links between endocrine disruptors and reproductive abnormalities, disturbed metabolism, and cancer it is reasonable to propose a connection between endocrine disrupting chemicals and diabetes or insulin resistance. In fact, BPA exposure causes deleterious effects on pancreatic β-cell function resulting in temporary hyperinsulinemia with acute exposure and chronic insulin resistance with longer exposures (Alonso-Magdalena et al, 2006).

In the US in 2008, a startling 60% of adults were overweight or obese (CDC, 2008). However, the US is not the only country that has experienced an increase in morbidity related to MetS, the prevalence of obesity has risen dramatically not only in wealthy industrialized countries but in poorer underdeveloped countries as well (Grun et al, 2009). This trend demonstrates that the current obesity epidemic cannot be explained solely by alterations in food intake and/or decrease in exercise. Furthermore, the symptoms of MetS were traditionally thought of as characteristics of older adults, but they have become more common in younger individuals (Flegal et al, 2010). While there may be a genetic predisposition to obesity, it is unlikely that population changes in genetics are solely responsible for this trend. Thus, environmental changes may have a larger role in the current obesity epidemic.

The environmental obesogen hypothesis claims that environmental pollutants disrupt normal development and interfere with the body's homeostatic controls. Exposures can initiate or exacerbate obesity by altering critical pathways involved in adipogenesis, lipid metabolism or energy balance (The Endocrine Society, 2009). There

is also a relationship between the increased use of industrial chemicals beginning in the 1940s and the significant increase in MetS symptoms and conditions. The introduction of many persistent chemicals in the environment coincides with the spike in obesity/diabetes incidence, and the number of chemicals that have been shown to cause weight gain by interfering with elements of the human weight control system continues to grow (Heindel, 2003).

HYPOTHESES

In the last few decades, BPA has become a ubiquitous chemical in the environment due to increased plastic production; meanwhile rates of the metabolic conditions such as obesity, weight gain and insulin resistance have increased correspondingly. The time-specific increase in exposure and disease, indicates a possible positive association between BPA and metabolic syndrome. If an association exists between exposure to BPA and the development of metabolic syndrome, it's more likely that BPA will demonstrate stronger and more frequent metabolic effects at lower doses than high doses because of the unique low-dose characteristics of endocrine disruptors (Richter et al, 2007; NTP, 2001).

METHODS

Literature Review and Study Selection

The scientific databases PubMed, ScienceDirect and ISI Web of Science were used to search the literature for studies on the metabolic effects of BPA using the

following search strategy: “metabolic effects of bisphenol A (BPA)” or “BPA and obesity/ insulin resistance.” Articles were limited to the English language and to studies involving animal subjects. Studies that demonstrated either a positive association with exposure and the metabolic syndrome, or a negative relationship were reviewed. The bibliographies of all studies obtained for data abstraction were reviewed, and through this process 10 studies that were not captured in database searches were identified. This literature review study identified 56 English-language studies that were potentially related to the metabolic effects of BPA exposure. Of those, 13 were excluded because of their focus on reproductive outcomes, 6 determined the effects on humans or human tissue explants, and 8 utilized incomparable methods of administering the chemical, leaving 29 studies for further review and analysis.

To help ensure that the studies demonstrated symptoms of metabolic syndrome rather than reproductive effects which can alter growth/maturation, only studies that demonstrated an increase in adipogenesis, enzymes that trigger lipid growth/storage, fat depots, body weight, or altered blood glucose/insulin sensitivity were included. Studies were excluded if they only weighed organs to determine if some level of reproductive damage or abnormality had occurred as a result of BPA exposure. In vivo studies that administered the chemical in a method other than oral ingestion or subcutaneous injection, such as intracisternal administration or other injection methods were also excluded from analysis because those forms of exposure do not represent the dominant or relevant environmental route of exposure.

A positive metabolic effect was defined by recognized endpoints that could lead to symptoms of metabolic syndrome including insulin resistance and increased adipose

tissue development. For this meta-analysis, insulin resistance was characterized by fluctuations in blood glucose transport or tolerance, an increase in insulin levels or sensitivity, or alteration or deterioration of pancreatic β-cells.

Meanwhile a positive metabolic response for adipose tissue development was characterized by the endpoints: adiponectin suppression, adipogenesis, adipocyte differentiation, increased body weight, increased lipid accumulation, increased triacylglycerol accumulation, increased LPL activity, and reduced leptin levels.

In order to have sufficient studies and dose measurements to complete a meaningful analysis, *in vivo* and *in vitro* study data were included. The doses of BPA administered in both study designs were very comparable. *In vitro* studies provide specific information about new modes of action to consider and test, while *in vivo* studies utilize living organisms to either validate, refute and suggest viable levels of exposure at which those modes of action operate. Progress towards understanding the actions of BPA requires that the two study designs depend on each other; therefore, they are both included in the data to assess a possible association between exposure and metabolic health effects.

Article Content and Quality Review

The studies were reviewed independently and the following data were extracted from all eligible studies using the same data collection form: study subject, exposure method, dose, metabolic effect of exposure and characteristic endpoints. Following the collection of studies, a table comparing specific characteristics of each study was

constructed (Table 1). While there is extensive literature on BPA, the studies relating exposure to metabolic effects are limited.

Statistical Methods

Every dose tested in any of the studies was recorded; individual doses were converted into a standard unit of measurement (μg/kg). Stoichiometric calculations using the recognized molecular weight of BPA ($C_{15}H_{16}O_2$, 228.29 g/mol) and density (1.20 g/cm^3) were used to convert varying dose measurements into consistent units (Staples et al. 1998; Richter et al, 2007) (Table 2).

STATA 11.0 (StataCorp 2009) statistical programming was used to conduct data analyses. To determine the relationship between exposure and the development of symptoms of metabolic syndrome a probit regression was used. The individual doses were assigned as the independent variable. The dependant variable was a binary variable based on metabolic or no metabolic effect. The binary value of 1 was given to positive metabolic effect, while no metabolic effect was 0 (Figures 1 & 2).

A similar probit regression was conducted using only the doses from the positive metabolic effect studies to determine the probability of certain endpoints. The specific endpoints can lead to one of two conditions characterized as metabolic syndrome: overweight/obesity and insulin resistance. Again the individual doses were the independent variable and the binary dependent variables were the endpoints “insulin resistance” and “adipose tissue development.” The binary value of 1 was given to adipose tissue and insulin resistance was 0 (Figure 3).

RESULTS

Metabolic Effect

The Probit 1 dose/metabolic effect data revealed the probability that a metabolic effect will occur at a given dose. The dose coefficient from the regression represents the slope of the line that corresponds to the dose/metabolic effect interaction. The predicted probabilities from the probit regression reveal that for the extremely low doses (0.0833 µg/kg to 33µg/kg) there is an 82.9% chance that exposure to the chemical BPA will result in a metabolic effect. Even the most infinitesimally small doses of BPA are capable of producing deleterious health effects related to insulin resistance, weight gain and adipose tissue production.

Figure 1. The Probability of Metabolic Effect at a Given Dose

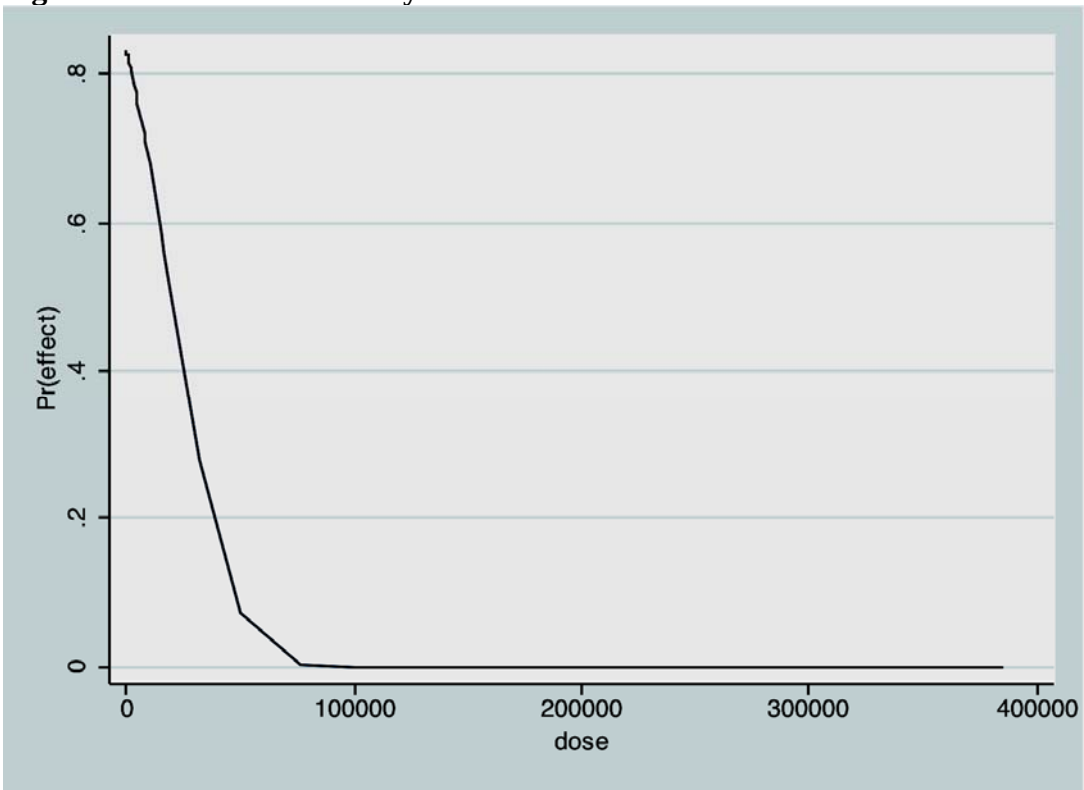

Figure 1. This is a graph of the probabilities of a metabolic effect occurring at specific doses that were tested. (The y-axis = the probability of metabolic effect. The x-axis = dose in (µg/mg))

The highest probability of observing a metabolic effect is at low doses. The coefficient for the dose was statistically significant (p=0.033) and the relationship between dose and metabolic effect was negative as expected, because endocrine disrupting chemicals are more effective at low doses. There was an R^2 value of 0.225 which means that 22.5% of the variation observed in this sample can be explained by the probit regression model (Table 3).

Figure 2. Distribution of Metabolic Effect Probabilities at a Given Dose

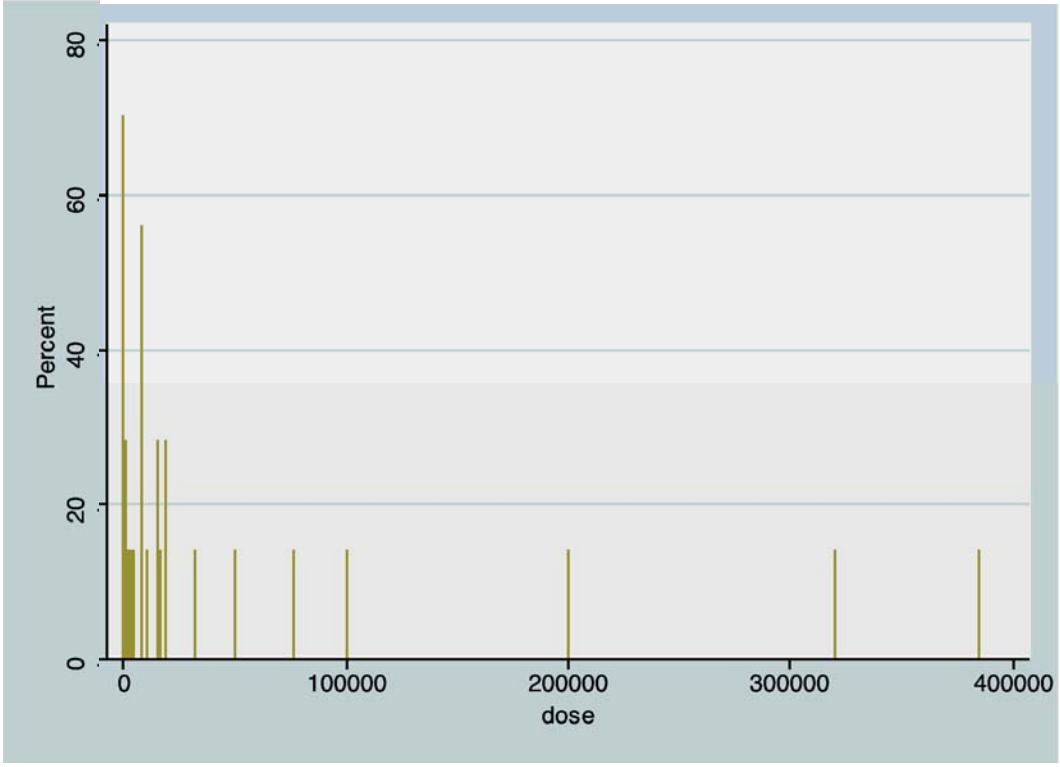

Figure 2. This is a histogram of the probabilities of a metabolic effect occurring at specific doses that were tested. (The y-axis = frequency of metabolic effect. The x-axis = dose in (μg/mg)) It's a dose-response curve for BPA; it shows the percent response against a range of doses.

The Histogram representation of the metabolic effect probabilities is representative of a dose-response curve for BPA. There were more responders at the low end of the dose range. The inverted U-shaped curve of low-dose chemicals is

characteristic of endocrine disruptors (The Endocrine Society, 2009). The peak of the inverted U represents the dose range that demonstrated a metabolic effect most frequently. The very smallest and largest doses did not elicit an effect; this pattern is similar to the normal distribution curve. BPA has a greater effect at low doses, so the majority of the effective doses were within the low-dose range which shifted the curve to the left.

Endpoints

The relationship between exposure to BPA and developing specific symptoms of metabolic syndrome was based on a dose-dependent Probit 2 regression; it shows the change in probability of developing increased adipose tissue depots or insulin resistance per μg/kg dosage. Only doses that were tested and showed a positive metabolic effect were included in this analysis, so that it would not be distorted by no effect studies. All of the endpoints recorded in the studies could be consolidated into symptoms that either promoted adipose tissue development, or induced insulin resistance. Doses were entered as the independent variable and the specific endpoints were explanatory dependent variables. Therefore, this probit regression describes the probability of increased adipose tissue development at given doses of BPA. Since the binomial was positive adipose tissue development versus positive induced insulin resistance, the inverse of the probability produced by the regression is the probability that insulin resistance developed.

Figure 3. Probability of Adipose Tissue Development versus Insulin Resistance Given a Positive Metabolic Effect Dose

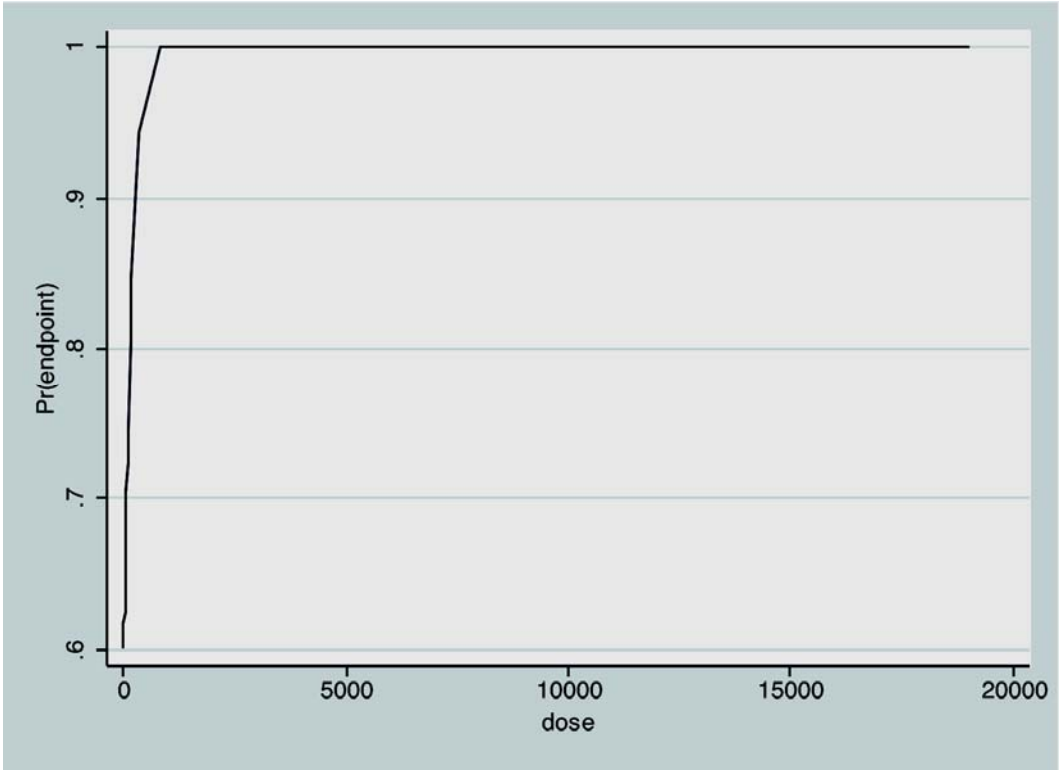

Figure 3. This graph shows the probability of developing either increased adipose tissue or insulin resistance given that the dose elicited a positive metabolic effect. (The y-axis = probability of adipose tissue development. The binary value of 1 was given to adipose tissue development. The x-axis = dose in (μg/mg))

The upward trend of the adipose tissue probabilities reveals that if a metabolic effect was observed at a low dose, the result very well could have been insulin resistance. However if a metabolic effect was observed at a high dose, which was infrequent, it was most likely adipose tissue development.

The coefficients for dose in Probit 2 had a p-value of ($p=0.243$) and there was an R^2 value of 0.1959 which means that 19.59% of the variation observed in this sample can be explained by the probit regression model (Table 4).

DISCUSSION:

Determining a New "Safe Dose"

Exposure of mice to BPA, at doses about 1,000-fold less than the LOAEL established by the EPA, alters blood glucose homeostasis in vivo (Alonso-Magdalena et al, 2006; Ropero et al, 2008; Adachi et al, 2005). This analysis demonstrated that the probability of developing insulin resistance is highest at the low-dose range. In Humans, Type II diabetes mellitus is characterized by insulin resistance, which results in lower levels of blood glucose uptake into target tissues in spite of increased insulin production. As a result, blood glucose levels increase and more insulin is released, producing hyperinsulinemia; that state of increased insulin manifests early in type II diabetes (Alonso-Magdalena et al, 2006). Hypersecretion of insulin is the primary defect of type II diabetes and often insulin resistance develops secondarily to chronic hyperinsulinemia. There is credible evidence that low doses of BPA exposure can elicit effects on certain endpoints, which is supported by the probability of insulin resistance at low doses in this study (NTP, 2001).

There is debate about determining the safe level of BPA exposure and whether there is a need for a new risk assessment. The FDA recently changed its stance on the safety of BPA to a chemical that is of concern. Standardized toxicity tests once supported the safety of low level human exposure to BPA. However, novel toxicity tests for subtle effects, from the Nationals Toxicology Program and the National Institutes of Health and the FDA have caused concern about exposure (FDA, 2010). Results of recent studies using novel approaches and different endpoints describe BPA effects in laboratory animals at very low doses corresponding to some estimated human exposures. It was

demonstrated that BPA can have harmful effects on the brain, behavior, and prostate for fetuses and children. Many of these new studies evaluated developmental or behavioral effects that are not typically assessed in standardized tests, but should be of concern to health agencies and the public (FDA, 2010). The National Institute of Environmental Health Sciences is investing $30 million for new research on the effects of BPA; researchers in the government and academia will be conducting both human and animal studies to assess a variety of health effects especially at critical periods of development (Smolonsky, 2010).

There have been many published in vivo and in vitro mechanistic studies that demonstrate the effects of BPA that are observed at low doses, but are not observed at higher doses (Takai et al, 2000; Suzuki et al, 2002). This type of dose-response relationship is known as an inverted-U-shaped curve. For a long time, toxicologists assumed that the only valid dose-related effect is a monotonic dose-response relationship. However, as toxicology has advanced and investigated more sub-lethal endpoints and more extensive dose ranges, non-monotonic dose-response relationships have been observed with increasing frequency (Richter et al, 2007). It has been difficult to make a persuasive argument that BPA is hazardous to health because the mode of action BPA exerts is not well understood, and the lack of a linear dose-dependent effect is unusual. However, BPA has been found to be active at environmentally relevant concentrations (approximately 0.48 to 1.6 μg/kg), which is within the range of doses that had the highest probability for metabolic effect and most frequently demonstrated a positive relationship between exposure and metabolic health effects in this study (Ben-Jonathan et al, 2009; Vandenberg et al, 2007).

In developmental exposure, BPA produces effects that persist long after the causal agent is removed (Rubin et al, 2001). Therefore, the adipose tissue development data in this study suggest that low-dose exposure to this compound may in fact be more detrimental than high doses and that persistent exposure to low doses of the compound over long periods may be as effective as shorter exposures to somewhat higher levels. These findings indicate the compelling need for reevaluation of the end points used for the toxicological assessment of BPA, of the acceptable levels of exposure to this compound, and of other xenoestrogens present in the environment.

Modes of Endocrine Action Inducing Metabolic Syndrome

Environmental endocrine disruptors can influence insulin resistance, as well as adipogenesis and obesity. The specific modes of action that promote insulin resistance typically involve the pancreas because it is partly composed of endocrine pancreatic islet cells, which secrete hormones into the bloodstream (Norman, 2010). The islet of Langerhans is the region of the pancreas that contains endocrine cells. The islets contain four different types of cells (β-cells, α-cells, δ-cells, and PP-cells), which are responsible for secreting insulin, glucagon, somatostatin and pancreatic polypeptide, respectively (Ropero et al, 2007). BPA dramatically affects how effectively the islet of Langerhans functions, which disrupts numerous glucose metabolism processes, because the isle of Langerhans is the main physiological unit of the endocrine pancreas (Ropero et al, 2007). Pancreatic β-cells are receptive to acute insulin changes through ion channels and play a major role in the release of insulin. When continued insulin resistance occurs, β-cells increase insulin output but plasma glucose levels continue to increase into the diabetic

range (Fonseca, 2009). The hormone insulin is critical to maintaining glucose homeostasis, and the synthesis and storage of fat (Ropero et al, 2007). Thus, altering the functionality of β-cells to secrete insulin, or increasing the levels of the insulin in the bloodstream can cause significant metabolic effects.

BPA is a chemical obesogen that inappropriately regulates lipid metabolism and adipogenesis to promote obesity; the specific mechanisms of adipogenesis are more variable than the modes of insulin resistance (Newbold et al, 2007). Adiponectin is an adipocyte-specific hormone that is critical in preventing metabolic syndrome because it reduces tissue inflammation and promotes insulin responses (Hugo et al, 2008). In humans, it is common for serum adiponectin levels to be lower in obese animals and individuals just before the development of type II diabetes (Trujillo et al, 2005). Therefore, a chemical such as BPA which suppresses its release increases susceptibility of developing metabolic syndrome and especially abdominal obesity (Hugo et al, 2008). Adipogenesis is the development of fat cells from preadipocytes; endocrine disruptors promote adipogenesis by targeting key cellular regulators of transcription in the adipogenic pathways (Grun et al, 2009). A group of transcription factors called nuclear receptors are involved in nearly all essential cell functions including homeostasis, metabolism and response to xenobiotic chemicals (Swedenborg et al, 2009). Specific nuclear receptors are implicated in adipogenesis based on studies that showed an increase in the number of 3T3-L1 type preadipocytes into differentiating into adipocytes (Grun et al, 2006). The estrogen receptors are implicated in many of the endocrine actions of BPA; while they are not considered classical targets of estrogen, adipocytes and pancreatic islets both have functional estrogen receptors (Ben-Jonathan et al, 2009). Interfering with

the control of adipogenesis promotes weight gain and obesity. Lipid accumulation can be measured to asses the degree of adipocyte differentiation, because adipocyte differentiation is the main mode of adipogenesis and weight gain (Grun et al, 2006). In several studies triacylglycerol accumulation was measured because it is a marker of lipid accumulation in mature adipocytes, that have differentiated from 3T3-L1 preadipocytes (Wada et al, 2007). Lipid metabolism in adipose tissue can be altered by estrogens. Mechanistic studies have further described the disruptive effects of environmental chemicals on normal adipocyte development, and homeostatic control over adipogenesis and early energy balance (Grun et al, 2009). Adipocytes were once thought to function simply as storage depots, however their role in leptin synthesis demonstrates that they actually function as an endocrine organ (Newbold et al, 2009). Leptin is an adipocyte-derived hormone that communicates information about energy reserves in adipocytes to other organs and the central nervous system. Phrakonkham et al. 2008, explained that leptin concentration is carefully regulated by nutritional and hormonal factors including glucocorticoids, insulin and sex hormones and concluded that exposure to BPA reduces leptin levels. So in addition to being a target for circulating and exogenous estrogens, adipose tissue is in itself a source of estrogen production (Mattsson et al, 2007).

Lipiprotein Lipase (LPL) is the enzyme that catalyzes the breakdown of fat-carrying molecules called lipoproteins and plays a critical role in fat transportation (Mead et al, 2002). It hydrolyzes the triacylglycerol component of lipoproteins, which releases fat molecules for energy use or storage in adipose tissue (Mead et al, 2002). LPL is primarily synthesized and stored in adipose tissue and BPA has the ability to significantly increase LPL activity which demonstrates the chemical's affinity for adipose tissue

(Masuno et al, 2002). Since BPA artificially stimulates the rate fat is created, the body's energy requirements are exceeded and the excess fat molecules end up being stored in adipose tissue deposits. If LPL activity is increased for an extended period of time, the build up of excess fat molecules can initiate atherosclerosis and increase the risk of heart disease, obesity and insulin resistance (Mead et al, 2002).

Considerations

Since the dose/metabolic effect binary in Probit 1 only has a definitive cut-off between positive and negative metabolic effect, relative changes in the underlying mechanisms may be obscured.

While an effect was sometimes observed at high doses in Probit 2, and it was always more likely to be adipose tissue development than insulin resistance, the number of positive effect doses in the high-dose range were limited. There were also more studies that tested for adipose tissue development compared to those which used insulin resistance as the main endpoint for metabolic effect.

Study Comparisons

While the results of this analysis show that the majority of the studies demonstrated a positive relationship between BPA exposure and metabolic effect, there are differences in study design and biological factors, which might account for the observed difference in study outcomes. Some of the no effect studies were multigenerational, so the mice/rodents were chronically exposed to BPA. Animals in these studies were given the opportunity to develop resistance and may have adapted, so

that they did not show a response to BPA at some critical time. However, there were other studies that failed to observe a low dose effect of BPA, which utilized an exposure paradigm designed to reproduce the short-term exposure studies. Additionally, species and strains differ greatly in their sensitivity to different hormonally active compounds. (Richter et al, 2007).

Differences in the strains of mice used could in theory have contributed to different responses to low doses of BPA. For instance, it has been suggested that the Sprague-Dawley rats from different breeders cannot be assumed to have the same sensitivity to exogenous estrogens, and that CD-1 (ICR) mice are very sensitive to low-doses of BPA during development (Richter et al, 2007).

Often exposure is considered to be constant over time, with the total dose estimated by the cumulative exposure; it is calculated as the product of concentration and time. However, if exposure is not constant over time, the same total cumulative exposure, delivered in different patterns, may produce different biologic effects. For several of the studies [(Ashby et al, 1999), (Kwon et al, 2000), (Miyawaki et al, 2007), (Somm et al, 2009)] mothers were exposed to one concentration of BPA throughout their entire pregnancy, and then the pups were exposed to a different concentration of BPA. Since all of the studies considered the metabolic effects of BPA on the pups, and only a few measured characteristic symptoms in the mothers only the effects that occurred on the pups were considered for analysis. None of the studies that exposed mothers and pups to different concentrations explained the rate BPA is metabolized in adult rats as compared to pups. It seems feasible that the reduced concentrations the pups were exposed to could be similar to the concentration that they were exposed to after the dose administered to

the mother was partially metabolized, but this was never explicitly stated. For these studies the daily predicted average exposure dose(s), were considered the most accurate and used in analysis. The administration of the dose is also an important factor to consider.

Differences in the method of administration of BPA could alter results; however this was controlled for by only including studies that utilized oral methods of ingestion and subcutaneous injection for analysis. These routes of exposure are most likely to yield metabolic effects, rather than reproductive or neuroendocrine effects from other injection sites.

Exposures to endocrine disruptors have different effects depending on the life stage of the exposed animals. In general, the period of highest susceptibility to adverse effects from environmental exposures is the *in utero* period because rapid structural and functional processes are occurring (The Endocrine Society, 2009). *In utero* exposures result from mobilization of chemical agents in the mother's body that can cross the placenta and enter into the fetal blood flow. Effects resulting from exposure during organ development (beginning during prenatal development and continuing in postnatal life through puberty) may result in persistent alterations of the affected systems, even in the absence of subsequent exposure (Richter et al, 2007).

Human Evidence

The debate about the human health effects of BPA exposure is limited by a lack of epidemiological data. Currently, there is not sufficient statistical power to detect low-dose effects or determine all the health consequences of exposure to BPA in humans

(Lang et al, 2008). Although the value of murine and rodent animal models is beneficial, there are enough differences in adipocyte biology between rodents and humans to warrant discretion (Ben-Jonathan et al, 2008). Endocrine activity is extremely species specific and acutely sensitive. To firmly establish the metabolic effects of BPA it is necessary to document, recognize and understand all modes of actions on human fat (Ben-Jonathan et al, 2008). However, provided the necessary precautions about extrapolating the results from animal studies to humans are taken, the consistent associations between exposure and characteristics of metabolic syndrome are difficult to ignore.

BPA is a ubiquitous synthetic chemical in the environment and is present in nearly all human serum samples from developed countries (Welshons et al, 2006). BPA has been measured in human serum, urine, amniotic fluid, placental tissue and umbilical cord blood. In most cases, the levels measured in human blood and other fluids are higher than the concentrations used to stimulate a number of molecular endpoints in cell culture *in vitro*, and *in vivo* animal studies (Vandenberg et al, 2007). BPA at low nanomolar concentrations suppressed adoponectin release from human adipose tissue explants, as well as from isolated mature adipocytes (Hugo et al, 2008). Adiponectin increases insulin sensitivity and reduces tissue inflammation; therefore any xenobiotic chemical that has the ability to suppress adiponectin release could lead to insulin resistance and increased susceptibility to obesity-related diseases (Hugo et al, 2008). In a cross-sectional study of the associations between urinary BPA concentrations and adult health status in the general US population, higher BPA concentrations were associated with an increased risk of diabetes, OR = 1.39 (Lang et al, 2008). Given its ability to alter adipogenesis through suppressing adiponectin, and to promote insulin resistance through altered glucose

tolerance, BPA may in fact be an endocrine disruptor that adversely affects metabolic homeostasis (Alonso-Magdalena et al, 2006; Ben-Jonathan et al, 2009).

Future Research

There is a definite relationship between exposure to BPA and other endocrine disrupting chemicals, but further research is needed to determine all of the biological systems they affect and their specific modes of action. Accordingly, this has been proposed by the FDA and the topics they have proposed to investigate further such as the effects at low-dose exposures and a special focus on metabolic conditions are consistent with the findings of this analysis.

Table 1. Summary Data of Studies Evaluated for BPA Exposure and Metabolic Effects

Authors	Year	Animal	Exposure, Vehicle	Doses Tested	Detection Method	Metabolic Effect	Endpoints
Nagel et al.	1997	CFI mice	dissolved in corn oil	2 µg/kg/day 20 µg/kg/day	Body weight (RBA-SMA) assay	No effect: low dose caused significantly lower body weight in BPA exposed and the high dose had no effect on weight between exposed and controls	body weight
Ashby et al.	1999	CFI mice (pregnant mothers and pups)	drip fed from a pipette	2 µg/kg/day 20 µg/kg/day	Body weight of pups	Significant increase in body weight (both low and high doses)	epididymis weight and body weight
Howdeshell et al.	1999	Pregnant CF-1 mice	Fed BPA dissolved in oil	2.4 µg/kg	Body weight at weaning age	Weight of pups exposed to highest BPA level increased by 22% compared to intermediate group	Body weight after prenatal exposure
Fialkowski et al	2000	Sprague-Dawley	oil vehicle	20 µg/kg 100 µg/kg 50,000 µg/kg	Body weight	Increased body weight at lower doses	Body weight, sexual maturation characteristics
Kwon et al.	2000	Sprague-Dawley rats (pregnant mothers and pups)	drinking water	3.2 mg/kg/day 32 mg/kg/day 320 mg/kg/day	Body weight of mothers through pregnancy and pups	No effect: maternal weights during pregnancy, lactation, or at termination were no different from controls, there was also no	Estrogen-mediated endpoints

						significant effect on the weight of male or female pups	
Takai et al.	2000	B6C3F1 mice	embryo cultures	100 pM 100 μM	Ultraviolet light observations using fluoroscein microscopy	Rate of in vitro development of 2-cell embryos for BPA exposed group was significantly higher than control group	rate of cell growth in presence of BPA, cell morphologically
Nunez et al.	2001	Sprague-Dawley rats	osmotic, pump	5000, 18000 23,000	body weight, high performance liquid chromatography	No effect: reduced body weight	Body weight, blood and tissue levels of BPA
Takai et al.	2001	B6C3F1 mouse	during IFV	1 nM	body weight	Exposed had significantly increased body weight	Postnatal weight gain increase in culture medium
Rubin et al.	2001	Sprague-Dawley rats	drinking water	0.1 mg/kg 1.2 mg/kg	Body weight during neonatal period and at weaning age	Pups exposed to both low and high dose BPA weighed more (those exposed to the low dose were heavier than those exposed to the high dose)	Body weight
Al-Hiyasat et al.	2002	adult Swiss mice	orally administered 100 ng kg^{-1}	5 ng kg^{-1} 25 ng kg^{-1}	Organs were excised and weighed	No effect: body weight decreased with increased exposure	Body weight
Honma et al.	2002	ICR mice	oil vehicle	2 μg/kg 20 μg/kg	Body weight	In utero exposure increased body weight gain, early puberty	Steroid hormone concentrations, post-natal body weight

Masuno et al.	2002	3T3-L1 cells (Swiss albino mice)	Culture medium	2 μg/ml 10 μg/ml 20 μg/ml	Oil Red O Staining LPL activity assay	BPA at 10 μg/ml increased LPL activity by 170% and 20 μg/ml increased LPL activity by 560%	Rate of conversion 3T3-L1 fibroblasts to adiposytes
Suzuki et al.	2002	ICR/Jcl mice	dissolved in oil	10 mg/kg 100 mg/kg 15 μg/kg 150 μg/kg	Body weight	Increase in weight of excised fat samples and organs	Body weight
Kabuto	2003	ICR mice (pregnant mothers and pups)	drinking water	5 μg/mL 10 μg/mL	Body weight, brain liver, kidney and testis were also weighed	No effect: BPA administration did not alter overall body weight	Weight of BPA brains was significantly lower than controls
Takagi et al.	2003	Sprague-Dawley	mixed in CRF-1 soy free diet	60 ppm 600 ppm 3000 ppm	Body weight	Increased body weight at lower doses	Post-natal body weight (males and females)
Sakurai et al.	2004	mouse 3T3-F44 2A adipocytes		10^{-9} M 10^{-8} M 10^{-7} M 10^{-6} M 10^{-4} M	Deoxyglucose uptake assay	Altered glucose transport in adipocytes	Glucose levels in adipocytes (basal glucose uptake)
				10^{-9} M 10^{-8} M 10^{-7} M 10^{-6} M 10^{-4} M		Enhanced basal glucose levels and insulin stimulated glucose uptake increased	Insulin stimulated glucose uptake

Adachi et al.	2005	Wistar rats	Pancreatic islets batch-incubated	0.1 µg/l 1 µg/l 10 µg/l 100 µg/l	Collagenase digestion method Insulin levels assayed by radioimmunoassay	Long-term (24 hr) exposure significantly increased insulin secretion	Insulin secretion from islets via cytosolic/ nuclear estrogen receptors
Masuno et al.	2005	3T3-L1 cells (Swiss albino mice)	Culture medium	80 µM BPA	Oil Red O staining	Presence of BPA increased percentage of lipid-positive cells to 85% of all cells	lipid accumulation adipogenesis
Seidlova-Wuttke et al.	2005	Sprague-Dawley rats	pelleted food (pups fed BPA food for 3 mo)	33 µg/kg/day 333 µg/kg/day	Body weight, % fat depots, serum leptin levels, lipid levels, triglycerides	Increase in adipose tissue weight from adipose depots	Reduced leptin levels, body weight
Alonso-Magdelena et al.	2006	OFI mice	injection, oil	1, 10, 100	Accu-check compact glucometer	BPA induces rapid dose-dependant decreases in glycemia, rise in plasma insulin	Insulin release, insulin sensitivity
Ishido et al	2007	Wistar rats	Orally Administered	600 µg	Supermex sensor	Body weight and growth characteristics were indistinguishable	body weight, hyperactivity dopamine release
Miyawaki et al.	2007	ICR mice (pregnant mothers and pups)	drinking water	1 µg/mL 10 µg/mL	Body weight, parametrial, epididymal adipose tissue weight, serum lipid levels of pups measured	Mean adipose tissue weight increased by 132%, increased acids, body weight, cholesterol, adipose tissue and triacylglycerol level	Serum total cholesterol triacylglycerol, fatty glucose serum leptin
Wada et al.	2007	3T3-L1 cells (Swiss albino Mice)	Culture medium	10 µM	Oil Red O staining	BPA significantly stimulated the accumulation of triacylglycerol	triacylglycerol levels, lipid accumulation, adipocyte differentiation

Newbold et al.	2008	CD-1 mice	subcutaneous injection	.001 mg/kg 1 mg/kg	Body weight	Significant increases in body weight for males and females	Body weight at birth, body weigh at puberty
Phrakonkham et al.	2008	3T3-L1 mouse embryo fibroblasts	cells treated with hormone cocktail	80 μM	Triglyceride assay Oil Red O Staining Leptin assay	induced expression of adipocyte-specific genes and leptin levels	DNA and triglyceride levels, leptin secretion and expression
Ropero et al.	2008	mice	single injection (for 4 days)	10 μg/kg	fasting glucose tolerance test	Altered glucose levels and lipid metabolism	Plasma glucose level
				100 μg/kg	insulin tolerance test		Plasma insulin level, insulin secretion in intact islets
Sargis et al.	2009	3T3-L1 cells (Swiss albino mice)	Culture medium	1 μmol/L	Oil Red O staining	Lipid accumulation was increased by 61-70%	lipid accumulation, adipogenesis, preadipocyted to adipocyte differentiation
Somm et al.	2009	Sprague-Dawley rats (pregnant mothers and pups)	drinking water	70 μg BPA/kg/ day	Epididymal, parametrial, brown adipose tissue fat depots from anesthetized pups weighed Weekly body weight, food intake measured from pup study population	Increased body weight and adipogenesis	Adipocytes production, change in expression of genes involved in metabolism, body weight
Zhu et al.	2009	nude bearing Mice	oral gavage	200 mg/kg	Body weight, tumor growth	No increased body weight but significant increase in tumor weight	Adipocyte and SK-N-SH cell growth

Table 2. Dose-Conversion and Specific Endpoint Data Based on Study

Study Summary Data								
study	dose	conversion ug/kg	low-high	study-based on dose		metabolic effect (Y/N)	endpoints	binomial endpoints
alonso-magdelena	1	1	0.00000019	sakurai	0	no		
alonso-magdelena	10	10	0.0000019	sakurai	0	no		
alonso-magdelena	100	100	0.000019	sakurai	1	yes	increased glucose transport	insulin resistance
phrankonham	80 uM	15219.33	0.00019	sakurai	1	yes	significant increase in glucose transport	insulin resistance
ropero	100	100	0.005	al-hiyasat	0	no		
ropero	10	10	0.019	sakurai	1	yes	significant increase in glucose transport	insulin resistance
takai	100 uM	19024.17	0.025	al-hiyasat	0	no		
takai	1 nM	0.19	0.0833	adachi	1	yes	increased insulin secretion	insulin resistance
rubin	.1 mg/kg	100	0.1	al-hiyasat	1	yes	significant increase in adipose tisssue weight (testis)	adipose tissue
rubin	1.2 mg/kg	1200	0.19	takai	1	yes	significantly increased body weight	adipose tissue
sakurai	10^-9 M	1.9E-07	0.19	takai 2000	1	yes	significantly increased body weight (pregnant mothers)	adipose tissue

sakurai	10^-8 M	1.9E-06	0.57	takai 2000	1	yes	significantly increased body weight (pregnant mothers)	adipose tissue
sakurai	10^-7 M	0.000019	0.833	adachi	1	yes	increased insulin secretion	insulin resistance
sakurai	10^-6 M	0.00019	1	alonso-magdelena	1	yes	decrease in blood glucose	insulin resistance
sakurai	10^-4 M	0.019	1	newbold	1	yes	significantly increased body weight (females)	adipose tissue
howdeshell	2.4	2.4	1	newbold	1	yes	significantly increased body weight (males)	adipose tissue
masuno 2002	2 ug/mL	1666	2	nagel	0	no		
masuno 2002	10 ug/mL	8330	2	ashby	1	yes	significant increase in body weight (pups of exposed mothers)	adipose tissue
masuno 2002	20 ug/mL	16660	2	honma	1	yes	increased body weight (males)	adipose tissue
wada	10 uM	1902.42	2	honma	1	yes	increased body weight (females)	adipose tissue
somm	70	70	2.4	howdeshell	1	yes	significantly increased body weight	adipose tissue
somm	70	70	8.33	adachi	1	yes	significantly increased insulin secretion	insulin resistance
Ishido 2007	600	600	10	alonso-magdelena	1	yes	significantly decreased blood glucose significantly increased plasma insulin level, (altered glucose tolerance)	insulin resistance
miyawaki	1 ug/mL	833	10	ropero	1	yes	increased plasma insulin level	insulin resistance
miyawaki	1 ug/mL	833	15	suzuki	1	yes	increased body weight	adipose tissue
miyawaki	10 ug/mL	8330	20	nagel	0	no		

miyawaki	10 ug/mL	8330	20	ashby	1	yes	increased body weight (pups of exposed mothers)	adipose tissue
adachi	.1 ug/L	0.0833	20	fialkowski	1	yes	increased body weight	adipose tissue
adachi	1 ug/L	0.833	20	honma	1	yes	increased body weight (males)	adipose tissue
adachi	10 ug/L	8.33	20	honma	1	yes	increased body weight (females)	adipose tissue
adachi	100 ug/L	83.3	33	seidlova-wuttke	1	yes	significant increase in adipose tisssue weight (paratibial depot)	adipose tissue
al-hiyasat	5 ng/kg	0.005	70	somm	1	yes	significantly increased body weight (males of exp mothers), increase in adipose tissue	adipose tissue
al-hiyasat	25 ng/kg	0.025	70	somm	1	yes	increased body weight (females of exposed mothers), significant increase in adipose tissue increased adipogenesis (adipocyte metabolism genes)	adipose tissue
al-hiyasat	100 ng/kg	0.1	83.3	adachi	1	yes	significantly increased insulin secretion	insulin resistance
takai 2000	1 nM	0.19	100	alonso-magdelena	1	yes	significantly decreased blood glucose	insulin resistance
takai 2000	3 nM	0.57	100	fialkowski	1	yes	increased body weight	adipose tissue
takai 2000	100 uM	19024.17	100	ropero	1	yes	significantly increased plasma insulin level, (altered glucose tolerance)	insulin resistance
nagel	2	2	100	rubin	1	yes	increased body weight	adipose tissue
nagel	20	20	150	suzuki	1	yes	increased body weight	adipose tissue
ashby	2	2	190.24	sargis	1	yes	increased adipocyte differentiation	adipose tissue

							significantly stimulated adipocyte lipid accumulation	
ashby	20	20	333	seidlova-wuttke	1	yes	significant increase in adipose tisssue weight (paratibial depot)	adipose tissue
nunez	4 mg	4000	600	ishido 2007	0			
nunez	5 mg	5000	833	miyawaki	1	yes	significantly increased body weight (females of exp mothers) significantly increased adipose tissue weight	adipose tissue
kwon	3.2 mg/kg	3200	833	miyawaki	1	yes	increased body weight (males of exp mothers) increased adipose tissue weight	adipose tissue
kwon	32 mg/kg	32000	1000	newbold	1	yes	significantly increased body weight (females)	adipose tissue
kwon	320 mg/kg	320000	1000	newbold	1	yes	significantly increased body weight (males)	adipose tissue
seidlova-wuttke	33	33	1200	rubin	1	yes	increased body weight	adipose tissue
seidlova-wuttke	333	333	1666	masuno 2002	1	yes	significantly increased LPL activity	adipose tissue
masuno	80 uM	15219.33	1902.42	wada	1	yes	significantly increased triacylglycerol accumulation	adipose tissue
sargis	1umol/L	190.24	3200	kwon	0	no		
kabuto	5ug/mL	4165	4000	nunez	0	no		
kabuto	10 ug/mL	8330	4165	kabuto	0	no		
suzuki	10 mg/kg	10000	5000	nunez	0	no		
suzuki	100 mg/kg	100000	7688	takagi	1	yes	increased body weight	adipose tissue

suzuki	15	15	8330	masuno 2002	1	yes	significantly increased LPL activity	adipose tissue
suzuki	150	150	8330	miyawaki	1	yes	significantly increased body weight (females of exp mothers) increased adipose tissue weight	adipose tissue
newbold	.001 mg/kg	1	8330	miyawaki	1	yes	significantly increased body weight (males of exp mothers) significantly increased adipose tissue weight	adipose tissue
newbold	1 mg/kg	1000	8330	kabuto	0	no		
newbold	.001 mg/kg	1	10000	suzuki	1	yes	increased body weight	adipose tissue
newbold	1 mg/kg	1000	15219.33	phrankonham	1	yes	increased adipocyte differentiation	adipose tissue
zhu	200 mg/kg	200000	15219.33	masuno	1	yes	increased adipocyte differentiation	adipose tissue
honma	2	2	16660	masuno 2002	1	yes	significantly increased LPL activity increased adipocyte conversion increased triacylglycerol accumulation	adipose tissue
honma	2	2	19024.17	takai 2000	0	no		
honma	20	20	19024.17	takai	1	yes	significantly increased body weight	adipose tissue
honma	20	20	32000	kwon	0	no		
fialkowski	20	20	50000	fialkowski	0	no		
fialkowski	100	100	76880	takagi	0	no		
fialkowski	50 mg	50000	100000	suzuki	0	no		
takagi	60 ppm	7688	200000	zhu	0	no		
takagi	600 ppm	76880	320000	kwon	0	no		
takagi	3000 ppm	384400	384400	takagi	0	no		

Table 3. Dose Specific Probit Regression of Metabolic Effect

Probit 1

probit effect dose

Iteration 0: log likelihood = -42.212519
Iteration 1: log likelihood = -34.894112
Iteration 2: log likelihood = -32.980303
Iteration 3: log likelihood = -32.728097
Iteration 4: log likelihood = -32.724898
Iteration 5: log likelihood = -32.724896

Probit regression — Number of obs = 71
LR chi2(1) = 18.98
Prob > chi2 = 0.0000
Log likelihood = -32.724896 — Pseudo R2 = 0.2248

effect	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
dose	-.0000482	.0000227	-2.13	0.033	-.0000926	-3.77e-06
_cons	.9502223	.1981118	4.80	0.000	.5619302	1.338514

Table 4. Probit Regression of Positive Metabolic Effect Doses and Specific Endpoints

Probit 2

probit endpoint dose

Iteration 0: log likelihood = -27.276352
Iteration 1: log likelihood = -24.38156
Iteration 2: log likelihood = -23.675809
Iteration 3: log likelihood = -22.685613
Iteration 4: log likelihood = -22.124126
Iteration 5: log likelihood = -21.95123
Iteration 6: log likelihood = -21.9331
Iteration 7: log likelihood = -21.933006
Iteration 8: log likelihood = -21.933006

Probit regression — Number of obs = 49
LR chi2(1) = 10.69
Prob > chi2 = 0.0011
Log likelihood = -21.933006 — Pseudo R2 = 0.1959

endpoint	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
dose	.004014	.0040169	1.00	0.243	-.003859	.011887
_cons	.2575173	.2578164	1.00	0.341	-.2477935	.7628281

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