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An Updated Weight of the Evidence Evaluation of Reproductive and Developmental Effects of Low Doses of Bisphenol A

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Summary

Bisphenol A (BPA) is used primarily as a building block to manufacture polycarbonate plastic and epoxy resins. Human exposures to minute levels of BPA occur mostly through food contact products, as polycarbonate plastic is used in certain water bottles, baby bottles, and food containers, while epoxy resins are used to coat the interior surface of food and beverage cans. These exposures are well below the intake levels set by government bodies that are considered to be without harm.

For many years it has been known that BPA is weakly estrogenic. Although BPA exhibits generally low toxicity, considerable controversy has surrounded the so-called "low-dose hypothesis" that very low doses of BPA may act as a synthetic estrogen and cause adverse reproductive and developmental toxicity. Several years ago an expert scientific panel convened by the Harvard Center for Risk Analysis to evaluate the weight of evidence supporting this hypothesis "found no consistent affirmative evidence of low-dose BPA effects for any endpoint."

Since the April 2002 cut-off date for studies evaluated by the Harvard panel, numerous relevant studies have been published. We recently participated in an expert scientific panel that critically reviewed the new studies and reached an updated weight-of-the-evidence conclusion. The panel's report has been published in the peer-reviewed journal Critical Reviews in Toxicology. (Goodman *et al.*, 2006). As summarized below, the panel's findings are consistent with the earlier Harvard study and government bodies worldwide—"Taken together, the weight of evidence does not support the hypothesis that low oral doses of BPA adversely affect human reproductive and developmental health."

"Taken together, the weight of evidence does not support the hypothesis that low oral doses of BPA adversely affect human reproductive and developmental health." This is consistent with earlier findings of a Harvard Panel and government bodies worldwide.

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The Low-Dose Controversy

The controversy began with a study conducted in the laboratory of Frederick vom Saal that found increased prostate gland weight in male mice at six months of age after exposure in utero to either 2 or 20 µg/kg-day—doses over 1000-fold lower than those previously found to cause adverse effects. Studies by other laboratories did not replicate this surprising result. These and many other subsequent studies have become the central focus of scientific debate on whether BPA can disrupt normal reproductive and developmental functions at doses far below those previously thought capable of causing such effects.

To address this controversy, a panel of scientific experts convened by the Harvard Center for Risk Analysis (Harvard Panel) critically reviewed the relevant studies and evaluated the overall weight of evidence regarding low-dose (≤5 mg/kg-day) reproductive and developmental effects of BPA. The Harvard Panel concluded that reported low-dose BPA effects were questionable, citing inconsistent responses across rodent species, a lack of adverse effects in two large multi-generational reproductive and developmental studies, and issues related to extrapolation from studies with non-oral routes of administration. Overall, the Harvard Panel "found no consistent affirmative evidence of low-dose BPA effects for any endpoint" (Gray *et al.*, 2004).

Since the cut-off date (April 2002) for studies evaluated by the Harvard Panel, more than 50 studies that examined reproductive or developmental endpoints in laboratory animals after exposure to low doses of BPA have been published. Numerous other papers with additional relevant information from animal and human studies have also been recently published. This continuing flow of research has stoked the low-dose controversy and highlighted the need for an updated weight-of-the-evidence evaluation.

Weight-of-the-Evidence Methods

We recently organized and participated in an expert scientific panel that conducted an updated weight-of-the-evidence evaluation of reproductive and developmental effects of low doses of BPA. Along with scientists from Gradient Corporation, the panel members included three prominent independent scientists: Dr. Ernest E. McConnell, Professor I. Glenn Sipes and Professor Raphael J. Witorsch. Three participants (Sipes, McConnell, Rhomberg) were also on the Harvard Panel. The panel conducted what now stands as the most up-to-date critical review of low-dose reproductive and developmental effects of BPA, the results of which, titled "An Updated Weight of the Evidence Evaluation of Reproductive and Developmental Effects of Low Doses of Bisphenol A," have been published in Critical Reviews in Toxicology (Goodman *et al.*, 2006).

From comprehensive literature searches we identified studies published from April 2002 through February 2006 that were peer-reviewed, included in vivo mammalian doses ≤5 mg/kg-day, examined reproductive and developmental endpoints, and were not previously reviewed by the Harvard Panel. Specific endpoints of interest included reproductive organ weights, anogenital distance, pubertal characteristics, teratogenic effects, and reproductive function. We reviewed more than 50 papers that met these criteria as well as many other papers that provided relevant information from animal and human studies.

The weight-of-evidence approach used in this review followed the one used in the Harvard Panel's analysis. We critically reviewed each of the new studies and evaluated the overall weight of evidence. Our review included examination of the stated significance of responses, the adequacy of study design and statistical analysis, the presence of dose-response relationships, and an examination of evidence for and against modes of action that would be relevant to low-dose reproductive and developmental toxicity.

Studies with oral doses were given higher weight than studies using other exposure routes. Humans are exposed primarily via oral ingestion. Because first-pass metabolism of BPA in the intestinal wall and liver to a non-estrogenic metabolite is essentially complete, the dose of BPA reaching tissues is much smaller for oral exposures than for BPA given by other routes that bypass the intestine and

liver. In addition, evidence from several studies demonstrates that BPA causes different effects if given via different exposure routes.

Findings from Animal Studies

Our full report contains a detailed study-by-study review and the overall weight-of-evidence evaluation. Here we summarize our overall findings from animal studies and compare our conclusions with those of the Harvard Panel. A tabular summary of findings from all animal studies reviewed by either panel, with results organized by endpoint and order-of-magnitude dose, is presented in Tables 1 (oral exposure) and 2 (non-oral exposure).

In rats we found no consistent effect on body or reproductive organ weights or organ morphology associated with BPA exposure in utero, while nursing, or in adulthood. We also found no consistent finding on sperm count, and the majority of studies found no effect on sperm characteristics. None of the studies in our review indicated any BPA—related changes in anogenital distance or pubertal characteristics. Overall, there is no corroboration of positive findings for an association between low-dose BPA exposure and any effects on rats. The Harvard Panel came to a similar conclusion, which was that the weight of evidence does not support a role for low doses of BPA causing reproductive or developmental effects in rats.

In mice we found no consistent effect on body weight, reproductive organ weights, anogenital distance, puberty endpoints, or reproductive effects, all consistent with the Harvard Panel findings. It is particularly interesting that we found no reported effect on mouse epididymis or prostate weight, which are the endpoints that initiated the low-dose controversy. The Harvard Panel reviewed several studies reporting significant weight changes for these two organs, but concluded that "there was at best limited consistency" across the studies that evaluated prostate weight, and that the epididymis results did not form a convincing pattern. We found no additional evidence that would bolster the limited evidence for effects on mouse epididymis or prostate weight.

We found several oral and non-oral mouse studies reporting morphological changes in the testes (including seminiferous tubules), and several non-oral mouse studies reporting morphological changes in female reproductive organs. One of the mouse studies reviewed by the Harvard Panel also reported significant changes in mammary gland morphology, but none addressed morphology of male reproductive organs. We also found some oral and non-oral mouse BPA studies reporting effects on sperm characteristics, as did the Harvard Panel. These results were generally not repeated and results from non-oral studies are of limited relevance to effects of oral exposures owing to significant pharmacokinetic differences between routes. In addition, morphologic changes did not translate to negative reproductive outcomes in generational studies.

As is apparent in the data tables, no consistent effects are found for endpoints with data from multiple studies. Put another way, the most consistent findings are those showing no effects. For endpoints where effects have been reported, some effects are consistent with an estrogenic compound but others are not, or are seen in some settings but not others, or with marginal magnitude at a single low dose but no apparent effect at higher ones. Some effects are opposite to what one would expect from an estrogenic compound. Overall there is no consistent and repeatable pattern of effects that would be expected if BPA were acting as an estrogen at low doses.

Table 1. Outcome by Dose for Rat and Mouse Studies – Oral Administration

Endpoint	Dose (mg/kg–day) Order of Magnitude					
	$\leq 10^{-5}$	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1
Body and Organ Weights						
Body	0000	000000	+0000000 00000000 –++– –00	0000000000 0000000000 0000– –00	0000000+00 000000– – 000	0000000000– 0000000
Male						
Epididymis	00	00–0	0000– 0000–0	000000 0– 0000000+0	0000000000 –0	00000
Preputial gland			00+0	0000	000	0
Prostate and ventral prostate	00	00+0	000000+0 00+	000000 0+00–000+	000000000+	000000
Seminal vesicles	00	0000	00000–0– 000– –	0000000000 0000–	0000000000 00	00000
Testes	0000	000–00	0000000– 0000+00–	0000000– –00– 0000+00	0000000+00 0000	0000000– –
Female						
Cervix			0	000	00	
Ovaries		0	00000	00000000	000000+	00
Uterus		0	0000000	000000000+	0000000++	00000
Vagina			0	000	00	
Organ Morphology/Cytology						
Male		– –	–0	– –0000	00+00	0000–++
Female			00	0–00	00+0+– – –	0000000
Sperm Characteristics						
Sperm characteristics	000000	000– – – 000000–	0000– – – –0 00000– +–000000	000– –0– 00– – 00000000– 00–000–00+	000– 000000000– 0–0000	00–000–000
Perinatal						
AGD	00	00	000	000000	00000+	0
Puberty						
Male						
Preputial separation date	00	000	000	00000	0000	
Testis descent date		0	0	00	00	
Female						
Time until first estrus			–	00	00	
Vaginal opening date		0	00000	000000	00000	
Other Reproductive Endpoints						
Sex ratio		0	0000	0000000	000	000
Pup survival		0	00–0	000	000	00
Fertility	00	–00	00000000 00000+	0000000000 0000–0+++	000000000– +++	000000
Estrous cycle		00	00000	00000	0–000	–000

Dose groups are identified in order-of-magnitude categories across the columns. For each row, results from all relevant studies are summarized as follows: a "0" indicates a dose group showing no effect, a "-" indicates a dose-group with an outcome significantly lower than controls, and a "+" indicates a dose-group with an outcome significantly higher than controls. These tables include studies reviewed by the Gradient Panel and the Harvard Panel combined.

Table 2. Outcome by Dose for Rat and Mouse Studies – Non–oral Administration

Endpoint	Dose (mg/kg–day) Order of Magnitude					
	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1
Body and Organ Weights						
Body	0	–00	00	0000000000 0000	00000000+ 000000000	00000000– – 0000
Male						
Epididymis			0	0000000	00000	00000
Penis			0	00	0	
Preputial gland				000	00	0
Prostate and ventral prostate			0	000000	0000	+0
Seminal vesicles			0	0000000	0000000	00000
Testes			0	000000000	00000000	000000
Female						
Ovaries	0	0		000	+00	00+00
Uterus	000	00–0	0	0000+	0+0000	0000000000 0
Vagina	00	–0				
Organ Morphology/Cytology						
Male		0	0	+–+0+0	+– +0+0+++0 0	0000+0
Female	00000000++ +–00	–00000+– ++0–+0	0	000++++00	0++0+++0 000–+00	0000000
Sperm Characteristics						
Sperm characteristics				0–+000– 0000	– –+00– – – –0000++	–0–000+00– –00+
Perinatal						
AGD			0	000	0	
Puberty						
Male						
Preputial separation			0	00	0	
Female						
Mammary gland maturation date	+					
Age at first estrus			0	–		
Vaginal opening date	0	0	0	–	0+	00–
Other Reproductive Endpoints						
% of time in diestrus					+	
Sex ratio	0	0	0	00000	00000	000
Pup survival		–		0	00	0
Fertility	0	00	0–000	0000000000 000	0000000000 000	0000000
Estrous cycle (offspring)	+	+	0+	+	+	

Dose groups are identified in order-of-magnitude categories across the columns. For each row, results from all relevant studies are summarized as follows: a "0" indicates a dose group showing no effect, a "-" indicates a dose-group with an outcome significantly lower than controls, and a "+" indicates a dose-group with an outcome significantly higher than controls. These tables include studies reviewed by the Gradient Panel and the Harvard Panel combined.

Findings from Human Studies

Several studies have estimated BPA intake based on urine concentrations, finding that, for a 60 kg person, approximate intakes are 0.00002 to .00006 mg/kg-day (20-60 nanograms/kg-day). These values are well below the intake levels set by government bodies that are considered to be without harm during a lifetime and even well below the doses examined in almost all "low-dose" animal studies. Moreover, BPA is entirely excreted by humans in urine, avoiding enterohepatic circulation that occurs extensively in rodents and resulting in lower human bioavailability of BPA for a given oral intake. In addition, BPA is efficiently converted to a non-estrogenic metabolite after oral exposure, resulting in little or no systemic exposure to BPA itself.

Only a few studies have examined human health outcomes and BPA exposure. These studies were all conducted with a small number of subjects and used an analytical method that has been reported to be unsuitable for measurement of BPA levels in biological samples. Studies reporting statistically significant effects have major methodological shortcomings and it is not evident whether the findings are biologically meaningful. No credible human findings of reproductive toxicity at any BPA exposure level have been reported.

Finally, circulating endogenous hormone levels are much higher in humans than in rodents during pregnancy. Thus, if an animal and a human received the same dose of BPA, the relative BPA concentration in comparison to endogenous estrogens would be substantially lower in humans compared to animals. This would likely result in less potential for an effect (if any) in humans.

Given the limited and inconsistent results from human and animal studies and the low exposure levels in humans, it is unlikely that exposure to BPA causes adverse effects on human health.

Comparison with Other Recent Reviews

In recent years, government bodies worldwide have examined the scientific evidence supporting the safety of BPA. These assessments all support the conclusion that BPA is not a risk to human health at the low levels to which people might be exposed. No government body worldwide has banned or restricted the use of BPA, polycarbonate plastic, or epoxy resins. The findings of our weight-of-the-evidence analysis are consistent with and further support the conclusions of these government assessments.

- A comprehensive risk assessment conducted by the Japanese National Institute of Advanced Industrial Science and Technology, which is a public research organization affiliated with the Japanese Ministry of Economy, Trade and Industry, established a NOAEL of 50 mg/kg-day for reproductive and developmental toxicity based on the results of a multi-generation study in rats (AIST, 2005). The report further concluded "An additional uncertainty due to the low-dose effects was not incorporated because the findings in the low-dose studies were not robust, while those in negative studies were consistent."
- The Japanese Ministry of Environment (MOE, 2005) conducted their own low-dose tests on BPA, including a comprehensive reproductive test in laboratory animals, and concluded there were no clear endocrine disrupting effects at low doses and that no regulatory action is required to manage risks.
- A comprehensive risk assessment report published by the European Union established an overall NOAEL of 50 mg/kg-day based on a multi-generation study of BPA in rats (EU, 2003).
- The EU Scientific Committee on Toxicity, Ecotoxicity and the Environment, an independent scientific committee, affirmed the key conclusions of the EU risk assessment in their detailed opinion (CSTEE, 2002). Regarding low-dose effects, the CSTEE "agrees with the conclusion of the RAR [Risk Assessment Report] that there is no convincing evidence that low doses of bisphenol A have effects on developmental parameters in offspring."
- In a detailed assessment of BPA focused on food contact applications, the EU Scientific

Committee on Food, an independent scientific committee that advises the European Union on food-safety matters, established a Tolerable Daily Intake (TDI) of 0.01 mg/kg-day based on a multi-generation study of BPA in rats and further concluded that worst-case human exposures to BPA are well below the TDI (SCF, 2002).

- In an early attempt to address the low-dose controversy, a scientific panel organized by the US National Toxicology Program (NTP, 2001) conducted a peer review of the scientific evidence on low-dose reproductive and developmental effects for several chemicals. Overall, a subpanel focused specifically on BPA concluded "There is credible evidence that low doses of BPA can cause effects on specific endpoints. However, due to the inability of other credible studies in several different laboratories to observe low dose effects of BPA, and the consistency of these negative studies, the subpanel [was] not persuaded that a low dose effect of BPA has been conclusively established as a general or reproducible finding."

In stark contrast to the views of government bodies worldwide, vom Saal and colleagues have published two separate summaries of the low-dose BPA literature in which they refer to over 100 studies reporting adverse effects of low doses of BPA (vom Saal and Hughes, 2005; vom Saal and Welshons, 2005). However, these summaries do not offer critical evaluations or analyses of this literature and most of the cited studies focus on estrogenic activity of BPA at the biochemical level rather than in vivo reproductive and developmental effects. Neither the rigor and reliability of the cited studies nor their bearing on the safety of low-dose human exposures to BPA are examined.

Although it is implied that because there are many citations there must be some actual impact on human health, individual study outcomes do not give a clear scientific picture. Rather than just citing individual studies supporting or refuting each claimed effect of BPA, it is necessary to evaluate the whole body of studies, positive and negative, considering strengths and weaknesses of each, and weighing their points of agreement and contradiction to arrive at an overall scientific assessment of the evidence for the existence of disruption of normal reproductive and developmental function by ultra-low doses. This is especially so for a bold hypothesis that aims to revise established scientific understanding about the ability of chemicals to act at tiny fractions of the doses previously understood to be without adverse effect.

Conclusions

Overall, our findings in this updated weight-of-the-evidence assessment are consistent with the findings of the Harvard Panel. Some statistically significant findings on specific endpoints in rats and mice exist, but they are generally countered by more numerous studies showing no effect for the same or similar endpoints. No effect is marked or consistent across species, doses, and time-points. Some mouse studies report morphological changes in testes and sperm and some non-oral mouse studies report morphological changes in female reproductive organs. Owing to the lack of first-pass metabolism, results from non-oral studies are of limited relevance to oral human exposure. In addition, morphological changes did not translate to negative reproductive outcomes in generational studies.

Human biomonitoring studies indicate exposures well below the "low" doses in the reviewed animal studies. Studies in humans have also shown significant pharmacokinetic differences from rodents, leading to lower internal doses in humans at similar oral intake levels. Human studies that have examined health effects have shown inconsistent and weak results.

Taken together, and consistent with the findings of the Harvard Panel and government bodies worldwide, we conclude that the weight of evidence does not support the hypothesis that low oral doses of BPA adversely affect human reproductive and developmental health.

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About the Panel

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