RESEARCH ARTICLE

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Carcinogenic Potential of Bis phenol

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INTRODUCTION:

Bis phenol A (BPA) is used to make epoxy resins and clear polycarbonate plastic strong, heat and shatter resistant. It has been in the eye of a storm over its estrogen mimicking effects. Exposure to BPA has fueled health concerns including increasing incidence of some types of cancer due to exposure to BPA. Unless scientific evidence can prove conclusively that BPA exposure at current levels endangers human health, the use of BPA in plastics is likely to continue as few manufacturers may be willing to cut down the use of BPA over 'fears' alone.

BPA has courted controversy due to a number of factors-

1. Uncertainty about its mechanism of toxicity and dose response curve
2. Toxicokinetics of BPA including its binding to estrogen receptors, clearing from body tissues, conjugation to produce the glucuronide metabolite, potential for deconjugation in tissues, sex, age and individual specific differences in metabolism and susceptibility to BPA exposure,
3. Differences in research deign – strains of animals, route of administration, results of experiments with positive controls

BPA is a classic example of the prevailing scientific logjam over the potential risks of a xenobiotic.

EXPOSURE TO BPA:

Occupational exposure to BPA is possible but for the majority of the populace the most likely route of exposure to BPA would be through food contact containers. Von Goetz, *et al.,* [1] have estimated that polycarbonate baby bottles for infants and canned food for adults are the most important source of BPA exposure. They have also reported a pattern of decreasing exposure to BPA with increasing age. BPA leaching out of containers can either be the unbound/ unreacted monomer or may come due to the hydrolysis of the polymer itself [2, 3]. While the former can be dealt with by better manufacturing control the latter is dependent on the duration and characteristics of use and may be very difficult to predict or control under normal use conditions.

In infants, BPA exposure may occur due to the presence of BPA in transparent, polycarbonate baby feeding bottles or from epoxy lined formula feed containers. The exposure of pregnant females and lactating mothers to BPA containing containers may be an additional source of exposure for prenatal and breastfed neonatal children. Krishnan, *et al.,* [4] demonstrated that BPA could leach from polycarbonate flasks during autoclaving. In their study, BPA leached from polycarbonate flasks increased the rate of cell proliferation in human mammary cancer cells (MCF-7) *in vitro*.

Studies with food products are confounded by the low limit of detection in a food matrix and challenges in identifying the source of the BPA in food. Food stimulants like ethanol, acetic acid, oil etc., are widely used to study the release of BPA from food contact containers.

Table 1: BPA release in food stimulants

Food stimulant	Reference	Other variables	BPA release	Possible Implications
Oil	Biles et al., 1997 [5]	-	1.7 mg/l	-
Ethanol	Biles et al., 1997 [5]	8-50% ethanol, 64 degree centigrade	Levels rise from 0.87 mg/ l to 5.9 mg/l over ten days	BPA release may be dependent on temperature, duration and nature of food.
3% Acetic acid	ECB, 2003 [6]; Maragou et al., 2008 [7].	-	Below limit of detection	Minimum BPA release in food products pickled in acidic solutions like vinegar
Water	Brede et al., 2003 [8]	BPA release after washing boiling and brushing was estimated	0.23 microgram / L (new bottles), 8.4 micrograms / L (bottles washed 51 times) and 6.7 micrograms / L (bottles washed 169 times).	BPA release was lower than the EU tolerable daily intake level of 0.01 mg/L. Release of BPA may increase under typical use conditions.
Water	Maragou et al., 2008 [7]	BPA release after brushing of Polycarbonate bottles	2.4-14.3 µg / kg	BPA release did not increase after washing. Estimated exposure to BPA 2.2 µg / kg bw / day, (Tolerable Daily Intake - 50 µg/ kg / bw)
Tap water heated in a microwave	Biedermann-Brem and Grob, 2009 [9]		Increased from <0.0001 mg/l (50 degree C) to 0.0006 mg/l (at boiling temperature)	
Boiled Tap water heated in a microwave	Biedermann-Brem and Grob, 2009 [9]	pH of boiled water was 9.5 presumably due to offgassing of CO_2	Increased from <0.002 mg/l (50 degree C) to 0.033 mg/l (at boiling temperature)	Release of BPA increases at higher pH. Similar conclusions were drawn from another study by the same authors. (Biedermann-Brem and Grob, 2008) [10]

BPA concentration in food stimulants ranged from 13 to 368 % of the available BPA [5]. This suggests that besides un-reacted BPA, reacted BPA may also leach out of polycarbonate food containers. This is supplemented by studies showing increased release of BPA from used polycarbonate animal cages (310 µg/L from used cages versus 0.3 µg/ L) [11]. Maia, *et a.,l* [12] have demonstrated that BPA release from polycarbonate containers could increase by up-to 500 times after rinsing in detergents.

Tab. 2: Various estimates of exposure to BPA in children

Study	Population	Estimated BPA Exposure
SCF (2002) [13]	0-4 months	0.0016 mg/kg bw/day
	6-12 months	0.0008 mg/kg bw/day
EFSA (2006) [14]	Breast fed infants 0-6 months old	0.0002 mg/kg bw/day
	Babies fed with non polycarbonate bottles	0.0023 mg/kg bw/day
	Babies fed with polycarbonate bottles	0.011 mg/kg bw/day
	6 – 12 months	0.013 mg/kg bw/day
EU Risk Assessment Report (ECB, 2008) [15]	1-2 months	0.008 mg/kg bw/day
	4- 6 months	0.007 mg/kg bw/day
	6-12 months	0.004 mg/kg bw/day
US Food and Drug Administration (FDA) draft assessment of BPA (US FDA, 2008) [16]	0-2 months	0.002 mg/kg bw/day
	< 2 months	0.0002-0.0006 mg/kg bw/day
Von Goetz et al., (2010) [1]	infants fed on milk from PC bottles	0.0008 mg/kg bw/day

Ikezuki *et al.* [17] reported that BPA levels in serum and follicular fluids of human females were 1-2 ng/ ml (0.001-0.002 mg/ L) but at 15 to 18 weeks of gestation the concentration in amniotic fluids was 8.3 $\pm$ 8.7 ng/ ml (0.0083 $\pm$ 0.0087 mg/L), a rise of almost five times. This may be due to repeated maternal exposure to BPA.

Atkinson and Roy (1995) demonstrated that *in vitro* BPA metabolites can bind to DNA [18]. Dekant & Völkel, 2008 [19] have reported that in human and rodent intestine BPA is rapidly absorbed and converted to BPA-glucuronide and BPA-sulphate. Some scientists [20, 21] hypothesize that metabolic transformation of BPA would lead to reduction in the estrogenic potential of BPA rendering it relatively safe in human beings. Ginsberg et al., (2009) [22] have pointed out that β-glucuronidase and arylsulfatase C could potentially reconvert BPA – glucoronide and BPA – sulphate respectively to free BPA thus negating its metabolic conversion in the intestine.

THE EVIDENCE SUMMARIZED ABOVE INDICATES THAT:

1. BPA released from food containers may be either unreacted BPA or may come from hydrolysis of polycarbonate.
2. Alkaline pH, high temperature, duration of exposure and aging of polycarbonate products are factors likely to lead to increased leaching of BPA from food containers.
3. Residual detergents may cause increased release from PC containers and manual washing alone may be a safer alternative as far as BPA release from polycarbonate containers is concerned.
4. Exposure to BPA is highest in children and falls progressively with age. Maximum reported estimated exposure in children is around 0.013 mg/kg bw/day. While these are lower than Tolerable Daily Intake (TDI) values, the effect of low levels of BPA exposure, particularly in children, needs the attention of the scientific community.
5. Estimated exposure to BPA is higher in European Union than in USA. It is likely to be due to differences in the quantum of BPA released from food containers in European Union and USA probably because of the lower incidence of use of epoxy resin lined cans to store baby formula food in United States. Powdered formula may not need to be heat sterilized like liquid formula feed and the need to pack it in epoxy lined cans may therefore be obviated thus reducing a relatively large source of BPA in infant diet.
6. The estimated exposure to BPA is lower in infants fed with non-polycarbonate bottles.
7. Highest exposure to BPA occurs in children and neonates therefore, there is a need to reassess TDI limits based on children body weight.
8. The impact of high pH, overnight food storage in polycarbonate bottles and microwaving to reheat milk need to be better assessed for data that simulates worst case real time release of BPA.
9. There is a need to assess prenatal exposure to BPA to better assess risk to human infants from maternal exposure to BPA.
10. There is considerable disagreement on the metabolism of BPA and how the differences in human and rodent metabolism may affect circulating levels of free BPA or the projection of results from rodent studies to human beings.

STUDIES OF THE BPA CANCER LINK

The evidence for the link between BPA and carcinogenicity comes from multiple sources. Huff, (2001) [23] reported that when diets containing 1000 and 2000 ppm BPA were fed to Fischer rats, and 1000, 2000, 5000 and 10000 ppm BPA were fed to B6C3F1 mice there was increased incidence of leukemia in male and female rats, interstitial cell tumors in male rats, mammary gland fibroadenomas in male rats and lymphoma and leukemia in male mice. This report was based on tests conducted by the National Toxicological Program (1982) [24]. The EPA designated daily intake limit of 50 µg/kg/day is based on a 1000 fold reduction of the estimated daily dose calculated from these studies.

Scientists have argued that BPA and other synthetic estrogen mimicking compounds may not necessarily follow a monotonic dose response curve and that BPA can cause cancer at low doses that are within the range of reported values of BPA exposure [25, 26]. It is possible to argue that given the differences in routes of exposure, uptake from alimentary canal and its subsequent metabolism, the concentration of unconjugated BPA in the

serum is a better measure for assessing the risk from exposure to BPA. Unconjugated BPA concentration in normal human serum has been reported to range between 0.2-24 ng/ml [27, 28, 29] and far higher values of 8.3 ± 8.9 ng/ ml have been reported in amniotic fluid [17].

Tab. 3: Dose/ serum concentration of BPA and Implications

Study	Animal Model	Dose/ serum concentration of BPA	Effect	Implications
Wetherill et al [30]	Mouse Xenograft model of prostate cancer	Highest serum concentration - 27 ng/ ml	Larger tumors in BPA treated groups	BPA at low serum concentrations can promote prostrate tumors *in vivo*.
Tsutsui et al [31]	Syrian Hamster Embryo (SHE) cells	50-200 µM	DNA adduct formation (at 50 µM dose)	Concentration of BPA used in this study was upto 10^4 times higher than reported human serum concentrations
Ho, S.-M. (2006) [32]	Sprague-Dawley	10 µg/ kg subcutaneous injections of BPA on post natal days 1, 3 and 5; chronic exposure to estradiol and testosterone	Intraepithelial neoplasias (PIN) increased from 35% to 100% .	Early postnatal exposure to BPA can increase sensitivity to the carcinogenic activity of steroids.
Murray, T.J. (2007)[33]	Female Wistar – Furth Rats	2.5-1,000 µg/kg/day delivered through osmotic pumps	BPA exposure from embryonic day 9 to post natal day 1 increased ductal hyperplasia and carcinoma.	Pre natal BPA exposure can increase the risk of developing cancer later in life.
Durando, M. (2006)[34]	Female Wistar Rats	25 µg/kg/day by subcutaneous osmotic pump implants; A single subcarcinogenic dose of N-nitroso-N-methylurea (NMU) (post natal day 50)	BPA exposure from gestation day 8 to gestation day 23 resulted in early puberty, increase in hyperplastic ducts, hyperplastic lesions	Prenatal BPA exposure can increase the susceptibility to subcarcinogenic doses of known chemical carcinogens.

Evidence suggests that at low doses BPA may contribute to carcinogenesis through four mechanisms

1. By disruption of endogenous endocrine regulation (Welshons *et al*) [26]
2. By promoting tumor progression (Wetherill *et al*) [30]
3. Genotoxicity (Tsutsui *et al*) [31]
4. By increasing susceptibility to other carcinogenic events. [33,34]

Tyl *et al* (2002) [35] conducted a three generation toxicity study of BPA. They used BPA free cages and monitored the amount of phytoestrogen in the diet. They did not find any increase in the incidence of cancer and no effects at low doses of BPA. The conclusions reached by Tyl et al [35] have been criticized on the grounds that the tests were conducted on Charles-River Sprague-Dawley (CD-SD) rats that are probably less susceptible to the estrogenic effects of BPA [25]. Vom Saal and Hughes [25] have further pointed out that while 90.4 % (96 % if studies with CD-SD rat strains are discounted) of government funded studies (94 out of 104) conducted till 2004 had demonstrated harmful effects of low dose exposure to BPA, none of the studies (11 studies) funded by private chemical corporation demonstrated harmful effects of low dose BPA exposure.

The preceding discussion indicates that research design, selection of animal strains, type of feed administered to test animals, prenatal exposure to BPA etc. can all potentially confound the results of research on the carcinogenic potential of low dose BPA. Selecting independent samples from a litter of rat pups is difficult because the relative position of the pup in the uterus has been linked to a 20% increase in prostrate weight and a threefold increase in androgen receptor density when there were two male neighbours compared to the condition when there was no male neighbour [36].

National Toxicological Program (2001) [37] and Harvard Center for Risk Analysis (HCRA) [38] have assessed the risk from BPA exposure. Their analysis suggested that the risk to human beings from BPA may be very limited. Yet the uncertainty prevailing about the potential toxicological responses to BPA is undeniable. The HCRA panel recommended "replication of existing studies under carefully controlled conditions and further study of BPA's pharmacokinetics and pharmacodynamics" [38].

There is a pressing need for carefully designed studies with animal models that most closely resemble human metabolism of BPA with well-planned positive controls and suitable rodent diet when conducting low dose studies with BPA [39]. The potential impact of prenatal exposure to BPA also needs to be assessed carefully. While the jury is still out on the safety of BPA Canada declared BPA a dangerous substance [40]. Perhaps the need to ensure the safety of consumers will be best addressed by evaluating the carcinogenic potential of not just BPA containing plastics but its alternates too.

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