The Adverse Cardiac Effects of Di(2-ethylhexyl) phthalate and Bisphenol A

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Abstract

The ubiquitous nature of plastics has raised concerns pertaining to continuous exposure to plastic polymers and human health risks. Of particular concern is the use of endocrine-disrupting chemicals (EDCs) in plastic production, including Di(2-ethylhexyl) phthalate (DEHP) and Bisphenol A (BPA). Widespread and continuous exposure to DEHP and BPA occurs through dietary intake, inhalation, dermal and intravenous exposure via consumer products and medical devices. This article reviews the literature examining the relationship between DEHP and BPA exposure and cardiac toxicity. In vitro and in vivo experimental reports are outlined, as well as epidemiological studies which examine the association between these chemicals and cardiovascular outcomes. Gaps in our current knowledge are also discussed, along with future investigative endeavors that may help resolve whether DEHP and/or BPA exposure has a negative impact on cardiovascular physiology.

Introduction

The incorporation of plasticizers and other additives has promoted versatility in plastic materials which, when combined with the low cost of production, has led to mass plastic production – exceeding 300 million tons in 2010[1]. Plastics are indispensable materials; yet, the ubiquitous use of plastic products has raised valid concerns pertaining to continuous exposure and human health risks. Health concerns primarily arise from the building blocks of plastics (i.e., BPA) and plastic additives (i.e., DEHP), both of which have endocrine-disrupting properties[1,2]. Endocrine disrupting chemicals are exogenous compounds that interfere with hormone homeostasis[3]. These chemicals initiate downstream effects through interaction with nuclear receptors, hormone receptors, orphan receptors, or by modifying enzymatic pathways involved in steroid biosynthesis or metabolism[3]. Despite the increasing popularity of BPA-free and phthalate-free plastics, these compounds are found in many consumer products, including food and beverage containers, electronics, and medical devices[4–7]. As a result, exposure to these EDCs has become virtually continuous and essentially unavoidable, a fact that is highlighted by numerous human biomonitoring studies[8–13].

Conflicts of Interest: None declared

Accumulating evidence suggests a link between EDC exposure and adverse human health outcomes, including cardiovascular conditions. Epidemiological studies have shown positive correlations between EDC exposure and coronary artery disease, hypertension, atherosclerosis, and myocardial infarction[14–21]. These associations are even more worrisome for patient populations who are more susceptible to cardiac disturbances, including neonates and infants, the elderly, and those with pre-existing heart conditions. Since increased EDC exposure is only associated with these disorders, and has not been recognized as causative, their potential toxicity is hotly debated. As a recent example, the appropriateness of using cross-sectional datasets to identify associations between environmental chemical exposure and complex diseases has been questioned by at least one group[22]. However, the financial support of this study by the chemical industry (Polycarbonate/BPA global work) adds further complexity to this debate. Consequently, there is a need on behalf of the public, scientific, medical and regulatory communities to resolve this debate by directly assessing the impact of EDCs on cardiac physiology and to identify the risks to both general and vulnerable patient populations.

Di-2(ethylhexyl)phthalate (DEHP)

I. Exposure

DEHP is a commonly used phthalate ester plasticizer that is used to impart flexibility and elasticity to polyvinyl chloride (PVC) products. Human exposure to DEHP occurs through contact with food packaging, toys, personal care products and medical devices. Exposure routes include ingestion, dermal uptake, inhalation, and direct release into the body from medical products (subcutaneous, intravenous). In addition to exposure via consumer products, DEHP is also the most widely used phthalate in FDA-approved medical devices and intravenous bags, including: bags containing blood, plasma, intravenous fluids, and total parenteral nutrition, tubing associated with their administration, nasogastric tubes, enteral feeding tubes, umbilical catheters, extracorporeal membrane oxygenation (ECMO) circuit tubing, hemodialysis tubing, respiratory masks, endotracheal tubes, and examination gloves. DEHP can contribute up to 40% by weight of intravenous bags and up to 80% weight in medical tubing[1,23].

DEHP's use in medical products is of particular concern, as exposure to DEHP increases dramatically in patients undergoing multiple medical interventions, such as bypass, hemodialysis circuits or long-term use of tubing in intensive care units[24]. This is because DEHP is not covalently bound to the PVC polymer and is hydrophobic, making it highly susceptible to leaching when in contact with blood, plasma, total parental nutrition solution, formulation aids used to solubilize medications, and other lipophilic fluids[24]. The rate at which DEHP migrates from the plastic product is dependent upon the storage conditions (temperature, volume of solution, contact time, and extent of shaking or flow rate of the fluid) and the lipophilicity of the fluid[25,26]. DEHP leaching has been reported to vary between 0.25–0.40 mg/100 mL/day for whole blood stored more than 21 days at 4°C, to 6 mg/unit of platelet concentrate stored at room temperature[25,27,28]. Furthermore, DEHP tubing has been shown to disintegrate with time, with one study measuring 6–12% less

weight after product usage[29], and another study reporting large particle debris from tubing degradation[30]. Of interest, both studies attributed medical complications to these findings.

DEHP exposure levels (Table 1) range depending on lifestyle factors[9,20,31–41] and medical device use[8,13,25,42–51], with high concentrations detected in blood samples from patients undergoing medical interventions (20–30 μg/mL range)[47–51]. Moreover, clinical studies frequently report DEHP concentrations in serum samples, which may actually underreport total exposure, as, a significant amount of DEHP binds to the membrane and cytosolic fraction of red blood cells[48]. Even higher exposure levels have been routinely detected in blood storage bags (150 – 300 μg/mL range)[25,47,48,52]. For simplicity, we perceive 300 μg/mL as a threshold concentration for experimental toxicology studies assessing DEHP's effects on cardiac physiology.

II. Adverse health effects

A number of studies have reported toxic effects of DEHP and its metabolites (reviewed by Halden[1] and Carlson[53]). Because of DEHP's endocrine-disrupting properties, most reports have concentrated on its risk of carcinogenicity, reproductive and developmental toxicities. Increased exposure to phthalates has been linked to adverse health outcomes in humans, including reduced anogenital distance in males, decreased sperm count, testicular dysfunction, early puberty in girls (a risk factor for breast cancer), increased waist circumference, insulin resistance, shorter pregnancy duration and low birth weight[33,34,54–60]. Indeed, the risk of testicular toxicity and the ensuing negative impact on fertility has warranted the use of DEHP-free tubing for premature boys[61,62].

Phthalate leaching is a source of concern for children's health[63], but even more so for patients in neonatal intensive care units (NICUs). There are two main reasons for this. First, critically ill neonates undergo multiple medical interventions over a prolonged period of time and these procedures frequently employ the use of stored fluids for transfusion and flexible tubing. Multiple medical interventions can result in high DEHP exposure, and it is estimated that NICU patients have a 26-fold higher phthalate exposure compared to the average child's environmental exposure[8,64]. The second reason is a curtailed glucuronidation pathway, which is not fully established in young children. Because glucuronidation facilitates urinary excretion of phthalates, and other xenobiotics, underdevelopment of this pathway increases the duration of exposure due to slow excretion[65]. Various regulatory agencies (Federal Drug Administration, Center for the Evaluation of Risks to Human Reproduction, Department of Human Health and Services, American Academy of Pediatrics) have concluded that critically ill neonates and other groups of patients exposed to DEHP over prolonged periods of time, can experience the adverse effects of phthalate esters[24,66–68]. As one example, use of DEHP-containing infusion systems for total parenteral nutrition was associated with a 5.6-fold increased risk of cholestasis among NICU infants and the incidence of hepatobiliary dysfunction declined from 50% to 13% after switching to DEHP-free tubing systems[69].

III. Cardiac toxicity

A. In vitro exposure studies—Forty years ago it was noted that DEHP [4 μg/mL] caused cessation of contractile function in chick embryonic cardiomyocytes after acute exposure, and cell death ensued after 24 hours exposure[28] (Table 2). Notably, this concentration is well within the range of reported clinical exposure levels (Table 1). Our laboratory has also reported adverse effects on cardiomyocyte function, specifically, DEHP exposure [1 – 50 μg/mL] resulted in a concentration-dependent decrease in conduction velocity beginning 24 hrs after exposure and asynchronous cell beating after 3-days treatment[70]. This effect was predominately attributed to a loss of gap-junctional connexin-43 protein expression. DEHP exposure [250 μM] has also been shown to modify cardiac electrical conduction in isolated perfused rat hearts, including a decrease in heart rate, and prolongation of PR and QT intervals[71]. Additionally, in situ studies have pointed to the potential cardiotoxic effects of Mono(2-ethylhexyl) phthalate (MEHP), the primary metabolite of DEHP. Specifically, Schulpen, et al. observed a significant decrease in human embryonic stem cell viability, along with a reduction in cardiac differentiation, following MEHP exposure[72]. And in situ studies have shown a concentration-dependent negative inotropic effect on human atrial trabeculae and a negative inotropic and chronotropic effect on isolated perfused rat hearts following MEHP exposure [15 – 200 μg/mL] [42,73,74].

B. In vivo exposure studies—Despite broad phthalate distribution throughout the body, including heart tissue[75], only a few reports have examined the effect of DEHP or its metabolites on the intact cardiovascular system[73,76–78]. Conflicting results have been reported related to MEHP and DEHP's effect on blood pressure in adult[73,78] and postnatal[76] animals. Specifically, Martinez-Argulles, et al. reported a *decrease* in systolic and diastolic blood pressures in male offspring following in utero exposure to DEHP [300 mg/kg/day][76]. While Wei, et al. observed an *increase* in blood pressure in rat offspring following maternal exposure to DEHP from gestational day 0 – postnatal day 21 [0.25 – 6.25 mg/kg/day][77].

To the best of our knowledge, experimental studies examining the effects of in vivo DEHP exposure on cardiac electrical conduction, ventricular pressure or contractility have not been performed. Additional in vivo studies that take into account the indirect effects between organ systems and the impact of the metabolic system are necessary to fully discern whether the observed in vitro cardiac effects are relevant to living subjects.

C. Potential mechanisms—Functional assays and genomic expression analysis have hinted to the mechanisms underlying these observed cardiac effects. We have shown that microarray analysis on DEHP-treated neonatal rat cardiomyocytes has revealed global changes in mRNA expression, including genes associated with cell electrical activity, calcium handling, adhesion, microtubular transport, and metabolism[79,80]. The latter was investigated further, and DEHP was shown to upregulate genes associated with fatty acid transport, esterification, mitochondrial import and beta-oxidation[80]. The functional outcome was an increase in myocyte fatty acid-substrate utilization, oxygen consumption, mitochondrial mass, PPARα expression, extracellular acidosis and a decrease in glucose oxidation. These results are consistent with Aronson, et al., which showed that lactate concentration (acidosis) increased 400% following DEHP-treatment of Langendorff-

perfused rat hearts[71]. Similarly, lactational exposure to DEHP [100 mg/kg/day] has been shown to impair insulin signaling and glucose oxidation in cardiac tissue[81]. Moreover, Feige et al. observed reduced fat reserves and increased hepatic fatty acid oxidation in DEHP-treated mice compared with controls[82]. Of interest, this phenomenon was reversed when mice were genetically engineered to carry human PPARα, suggesting a species-specific effect. Notably, in our studies, treatment with a PPARα agonist only partially mimicked the effects of DEHP exposure[80], suggesting that multiple mechanisms are likely at play. Even so, these studies suggest that DEHP exposure leads to a fuel switch that may be regulated at both the gene expression and posttranscriptional levels.

D. Epidemiological studies—Although experimental data suggests toxic effects of DEHP and MEHP on cardiac physiology, only three epidemiological studies have investigated this link in humans (Table 3). Specifically, higher urinary phthalate levels have been linked to increased blood pressure in adolescent populations[20], and increased coronary risk in elderly populations[17,83]. The later found a significant association between higher MEHP urinary levels and LDL cholesterol levels[83], but not blood pressure. This group also found a positive association between MEHP urinary levels and the echogenicity of vascular plaques[17], which is an indicator of lipid infiltration and a predictor of future cardiovascular death. Prospective studies that comprehensively assess blood pressure changes, as well as inclusion of cardiac physiology indices (i.e., echocardiography, electrocardiography) would be beneficial to understanding the scope of DEHP's effects on the heart.

Bisphenol-A (BPA)

I. Exposure

BPA is a monomeric building block that is used in the production of polycarbonate plastics, polystyrene resins and dental sealants; it can also be used as an additive in PVC products. BPA is found in a vast amount of consumer products, including food and drink containers (including canned goods), water pipes, thermal paper and paper products (receipts, paper towels), toys, safety equipment, electronics, and medical devices[4]. Since some BPA monomers remain unbound during the manufacturing process, BPA can leach from these products under normal conditions of use[4,84,85]. Similarly to DEHP, the rate at which BPA migrates from the product is dependent upon storage conditions and frequency of use (i.e., temperature, prolonged contact with acidic/basic solutions)[85]. Due to its ubiquitous use, human biomonitoring studies have routinely detected BPA in >90% of the population, including both children and adults[8,11,12,86].

Similar to DEHP, BPA exposure (Table 4) ranges dramatically depending on lifestyle factors[11,41,87–98], with industrial workers and NICU patients having overall higher urinary BPA levels [4 – 8 μM range][12,99,100]. Human serum BPA levels are actively debated, with estimates ranging from 0.1 – 300 nM for adults[91,101–110]. Individuals undergoing multiple medical interventions- such as NICU patients- most likely have even higher levels of BPA in the blood, although blood samples from these patients have not been analyzed. As previously mentioned, these patients have greater exposure levels due to multiple medical interventions and reduced glucuronidation activity, which increases total BPA exposure and time. For simplicity, we perceive 0.3 μM (blood) and 8 μM (urine) as a threshold

concentration for experimental toxicology studies assessing BPA's effects on cardiac physiology.

II. Adverse health effects

Several studies have illuminated BPA's adverse effects (reviewed by Vandenberg[111]), particularly in relation to reproductive and developmental toxicities. Increased exposure to BPA has been associated with human health conditions, including cardiovascular disease, diabetes, reduced sperm quality, breast cancer, implantation failure, endometrial hyperplasia and polycystic ovarian syndrome[14,15,18,112–115]. Elevated BPA exposure in children is particularly worrisome[116], since EDCs can have varying effects based on exposure time in relation to development. Indeed, lower BPA doses are necessary to induce alterations in estrogen-target organs when administered prenatally versus during adulthood[117,118].

III. Cardiac toxicity

A. In vitro exposure studies—Recent experimental studies suggest that BPA may be arrhythmogenic, particularly in female subjects[119–121]. Acute BPA exposure [1 nM] increased the duration of sustained ventricular arrhythmias following ischemia-reperfusion injury in excised female hearts, and increased the incidence of spontaneous after contractions in isolated female rat ventricular cardiomyocytes[119]. These effects were exacerbated in the presence of estradiol and could not be replicated with male cardiac cells. BPA's pro-arrhythmic effects were abolished when samples were pretreated with an estrogen receptor (ER) antagonist, and also in an ERβ knockout model, suggesting mediation via ER signaling.

Our laboratory has also examined the effects of BPA on cardiac function using excised Langendorff-perfused hearts[122]. Although we did not observe arrhythmias in our studies, BPA exposure adversely affected cardiac electrical conduction in a concentration-dependent manner. Ex vivo exposure resulted in prolonged PR segment time and decreased epicardial conduction velocity [0.1 – 100 μM], prolonged action potential duration [1 – 100 μM] and delayed atrioventricular conduction [10 – 100 μM]. Importantly, these effects were observed after acute exposure (15min), underscoring the potential detrimental effects of continuous BPA exposure. The highest BPA concentration used [100 μM] resulted in prolonged QRS intervals, dropped ventricular beats and eventually resulted in complete heart block. We observed slowing in sinus rate after exposure to high BPA concentrations; such a decrease in rate has also been observed by others, using both in vivo models[123–125] and in vitro atrial preparations[126]. In the later, BPA [10 – 100 μM] exposure caused a decrease in atrial rate and force, which was attributed to involvement of the nitric oxide-guanylyl cyclase pathway. It is important to note that high BPA concentrations [>10 μM] exceed clinically-relevant exposure concentrations; however, reporting the observed effects may hint to BPA's underlying mechanisms and also allows for direct comparison between toxicological studies.

B. In vivo exposure studies—A few studies have examined the cardiac effects of BPA using an in vivo mammalian model[125,127]. Lifelong BPA exposure [0.5 – 5 mg/kg/day] was shown to modify cardiac structure and function in mice[127]. Similar to previous reports, Patel et al. identified sex-specific differences following BPA exposure, including concentric

remodeling (male), increased systolic and diastolic blood pressure (female) and modified calcium handling protein expression (male & female). Interestingly, the authors reported a reduced ability to remove and store calcium in female rats – a result that contradicts data from previously published in vitro studies[119,120]. These conflicting results may be due to a compensatory effect resulting from long-term BPA exposure, and highlight the importance of assessing toxicity using multiple models and time points. In vivo toxicity studies have also reported respiratory arrest, bradycardia and hypotension following intravenous injection with a lethal dose of BPA [40 mg/kg][125].

C. Potential mechanisms—BPA has been shown to act as an estrogen agonist via ERβ, whereas it has dual actions as an agonist and antagonist via ERα depending on cell type[128]. BPA's effect on estrogen receptors has been shown to increase contractility and spontaneous after contractions in female ventricular cells, and increase arrhythmia duration in female excised hearts via ERβ signaling[121,129–131]. These effects are likely mediated via protein kinase A and Ca2+/CaM-dependent protein kinase II signaling pathways, which modify the phosphorylation state of phospholamban and ryanodine receptors resulting in increased SR calcium leak and load[131]. The authors note that BPA's cardiac effects are gender specific, as similar effects were not observed in male ventricular myocytes. These cardiac effects were abolished in ERβ knockouts, overiectomized females, and by pretreatment with ER blockers, but not by pretreatment with L-NAME a nitric oxide (NO) synthase inhibitor. Conversely, Pant et al. observed a decrease in atrial rate and contractility following BPA exposure [1 – 100 μM]; these effects were blocked by pretreatment with methylene blue or L-NAME[126]. Importantly, alterations in NO/cGMP signaling are biphasic and concentration dependent, which may explain experimental differences with L-NAME pretreatment[132].

BPA has also been shown to interact directly with multiple ion channels. Using HEK293 cells, O'Reilly et al. showed that BPA binds directly to and blocks the human Nav1.5 sodium channel[133], which is responsible for phase 0 depolarization in ventricular myocytes. Inhibition of the fast sodium current by BPA could explain the reduction in epicardial conduction velocity we observed in our ex vivo heart studies. BPA has also been shown to block multiple voltage-activated calcium channels, including L-type calcium channels which are responsible for the plateau phase of ventricular action potentials and phase 0 depolarization in sinoatrial cells[134]. This effect may explain the decreased heart rate observed following BPA exposure. Finally, BPA was recently shown to activate Maxi-K channels in coronary smooth muscle cells[135]. Although these channels are found in the mitochondria of cardiomyocytes, a similar interaction with sarcolemma potassium channels could hyperpolarize and decrease cardiac excitability.

D. Epidemiological studies—Higher BPA urine concentrations have been associated with an increased risk of coronary artery disease[14,15,136], hypertension[16], carotid atherosclerosis[17], angina and myocardial infarction[14,18], and decreased heart rate variability[19]. Higher BPA urinary levels have also been associated with LDL and HDL cholesterol levels [83], and the echogenicity of vascular plaques[17]. Notably, these positive associations were observed at urinary concentrations that fall below the exposure levels observed in industrial workers and NICU patients[12,99]. To the best of our knowledge,

epidemiological studies examining a link between BPA exposure and cardiac toxicity has not been investigated in these highly susceptible populations. It is important to note that the associations between BPA urinary levels and cardiovascular disease were not reproducible in an alternative study, which analyzed the National Health and Nutrition Examination Survey (NHANES) data using alternative inclusion parameters[22]. This highlights the importance of conducting toxicological experimental studies to fully understand the impact of endocrine disrupting chemicals on cardiovascular disease.

Questions remaining

I. How does the presence of blood alter EDC adverse effects?

Drug efficiency is influenced by plasma protein binding, which diminishes drug uptake to target organs and interaction with target receptors. The bound or inactive form of BPA represents 90–95% of total BPA in the blood[137–139], whereas 80% of total DEHP is bound to lipoproteins and albumin[140–142]. Since many in vitro and ex vivo studies are conducted in serum-free or low albumin-containing solutions, additional studies are warranted to determine whether protein binding diminishes the bioavailability of these chemicals to such an extent that cardiotoxicity is irrelevant. Indeed, this question of bioavailability has been argued by researchers in the field[111,138,143].

II. Does chronic EDC exposure result in adverse cardiac effects in vivo?

Some argue that EDC exposure is not a significant risk to humans because DEHP and BPA are rapidly metabolized[143,144]. However, it is actively debated whether these EDCs are immediately cleared from the body[145–147] and pharmacokinetic studies are frequently based on acute exposure[138,148–150], which differs from typical everyday human EDC exposure (the latter is likely to be a continuous, low dose exposure via ingestion). Many of these studies also fail to take into account differences in age-dependent differences in metabolic capacity[151]. Published pharmacokinetic studies estimate DEHP and BPA half-lives of 1–7 hours, and total elimination time to be 1–6 days[148–150,152,153]. Importantly, changes in cardiac function have been observed following acute EDC exposure, within a time frame that is less than the above reported half-lives, by both our lab and others (Table 2 & 5). Moreover, chronic EDC exposure may result in tissue accumulation or longer elimination times[154,155] due to accumulation in lipid-rich tissues[156–158]. Indeed, at least one study detected postexchange serum DEHP that were higher than the corresponding concentration in blood units, which indicates accumulation in newborn patients[48]. To fully address the risk of EDCs to the cardiovascular system and the impact of metabolic system, additional in vivo experiments are needed that more closely mimic chronic human exposure.

III. Are high-risk subjects more susceptible to adverse EDC cardiac effects?

Experimental studies have revealed electrical abnormalities in excised hearts, ventricular and atrial cells exposed to DEHP or BPA. Even if exposure to these EDCs does not elicit detrimental phenotypes in healthy individuals, it is plausible that chronic EDC exposure may exacerbate conduction and contractile abnormalities in high risk subjects, such as neonates, the elderly, or those with pre-existing heart conditions. Indeed, conduction and contractility disturbances are a common complication of myocardial infarction and heart failure[159–167].

Alternatively, elderly patients are prone to cardiac fibrosis, which increases ventricular stiffness and impairs diastolic function, ultimately diminishing the heart's ability to meet increased demand[168]. Moreover, cardiac fibrosis can affect the conduction system resulting in slowed conduction velocity, reentrant currents and arrhythmias[169,170]. Such pathological phenotypes can be exacerbated by the adverse cardiac effects of EDCs. Furthermore, EDC exposure during fetal and infant development can produce a range of adverse effects resulting from altered endocrine function[63,116]. As previously mentioned, these patients are vulnerable to high levels of exposure in the medical setting[12,24], and questions regarding EDC metabolism and accumulation remain.

IV. Do EDCs affect human cardiomyocyte function?

With the exception of two reports, which showed that MEHP exposure had a negative inotropic effect on human trabeculae fibers and elicited arrhythmic patterns[42,74], DEHP and BPA exposure studies have been limited to chick and rodent models (Tables 2 & 5). Species differences in cardiac physiology are well documented[171,172], and highlight the importance of not automatically assuming human cardiac toxicity based solely on alternative species models. To further understand the clinical impact of EDCs on cardiac physiology, more systematic studies should be conducted that examine the electrical and mechanical effects of EDCs using either human cardiac tissue samples or human stem cell-derived cardiomyocytes (hESC-CM). hESC-CM are a viable option since these cells have been fully characterized in terms of electrophysiology, calcium handling, and receptor response[173], and have also been used as models to assess contractile impairment, arrhythmias, drug discovery, and as a toxicological screening tool[174–178]. Indeed, our recent studies suggest that human cardiomyocytes may be more sensitive to the effects of EDCs compared with rodent cells[179].

V. What about DEHP and BPA alternatives?

There is an increasing availability of alternatives to DEHP and BPA, due largely in part to consumer wariness of the potential health effects raised by scientific and advocacy groups. Specifically, 1,2-Cyclohexane dicarboxylic acid diisononyl ester (Hexmoll DINCH™), Tris(2-ethylhexyl) trimelliate (TOTM), Di(2-ethylhexyl) adipate (DEHA) and two citrates, Butyryl trihexyl citrate (BTHC) and Acetyl tributyl citrate (ATBC) are the main DEHP alternatives currently used in PVC-containing medical products. BPA polycarbonate plastic alternatives include Bisphenol S (BPS) and Tritan Copolyester™, and alternative liners, such as EcoCare™ and oleoresin. The appeal to these alternatives is two-fold; first and foremost, most of these chemicals appear to be less toxic compared with DEHP or BPA, and second, these alternatives can be marketed as "phthalate-free" which appeals to consumers. Unsurprisingly, all of these plastic additives have their own individual drawbacks[180–186], including: higher price, less efficiency, significant leaching, insufficient purity, and adverse health effects. The major drawback to these alternative products is that they are relatively untested and the toxicological data on these substitutes is extremely limited. To the best of our knowledge, there are no existing scientific studies that have examined these any of these alternatives with regard to cardiotoxicity.

Conclusion

Our current understanding of DEHP and BPA toxicity is primarily a result of human epidemiological observations and high dose effects observed in the laboratory. Epidemiological or cross-sectional studies are useful in identifying associations between EDC exposure and adverse health conditions, but they can only identify correlations and not causal links between exposure and disease. Whereas, research studies that employ high exposure levels hamper direct extrapolation to humans. Ideally, experimental studies should be designed to examine clinically-relevant concentrations in both an in vitro model, which allows one to elucidate the direct effects of EDC exposure, and in vivo model, since exposure can affect organ systems differently and because a fully functioning metabolic system is present. In addition, large scale epidemiological studies that are designed to comprehensively examine multiple cardiovascular parameters, including: blood pressure, incidence of plaques, echocardiography and electrocardiography, would greatly improve our understanding of toxicity to the general population.

Additional studies are necessary to clarify gaps in our current understanding of DEHP and BPA cardiac toxicity and determine the applicability of our findings to humans. These include, 1) determining the direct effect of EDCs on cardiac physiology at clinically-relevant concentrations and clarifying to what extent the presence of plasma-binding proteins negate these effects, 2) identifying whether long-term EDC exposure negatively impacts cardiovascular function in vivo, and 3) ascertaining patient populations that are most susceptible to EDC cardiac toxicity. These studies are particularly important, as sustained exposure to EDCs may cause and/or exacerbate conduction abnormalities in individuals with preexisting heart conditions (e.g., AV conduction dysfunction, bradycardia, atherosclerosis, myocardial infarction), or other high-risk populations (e.g., industrial workers, prenatal and neonatal patients with reduced metabolic capacity, elderly patients with substantial fibrosis), 4) determining the applicability of these previous findings with human cells and/or tissue. Thorough examination of DEHP and BPA cardiac toxicity using these experimental models should resolve the controversy as to whether EDC exposure negatively impacts the cardiovascular system, and provide the foundation for objective decision making by the public, scientific, medical and regulatory communities.

Acknowledgments

Abbreviations

BPA	Bisphenol A
DEHP	Di(2-ethylhexyl) phthalate
EDCs	Endocrine disrupting chemicals
MEHP	Mono(2-ethylhexyl) phthalate

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Table 1

Experimental studies which measured DEHP exposure in humans via blood or urine samples.

Notes			DEHP [µg/mL]	DEHP [mg/kg]	MEHP [µg/mL]	MEHP [mg/kg]	Reference
Blood samples							
		Pregnancy			0.17 – 6.74 (range)		Li, et al. 2013
		Pregnancy	1.15 ± 0.81		0.68 ± 0.85		Latini, et al. 2003a
		Pregnancy	0.3 – 1.3 (range)		1.2 – 3.5 (range)		Zhang, et al. 2009
		Coronary bypass		15.4 – 72.9 mg/day		2.2 – 8	Barry, et al. 1989
		Heart transplantation		2.3 – 167.9 mg/day		0.25 – 18.8	Barry, et al. 1989
Adult		Hemodialysis	0.3 – 7.6 (range)	10.2 – 154.3 mg/day	Patient blood: 0.9 – 2.83 (range)		Pollack, et al. 1985
		Hemodialysis	2–3 (range)	19 – 84.9 mg/day 0.02 – 0.36 mg/kg/day			Faouzi, et al. 1999
					0.885 – 2.83 (range)		Pollack, et al. 1985
			Plasma fraction: 36.8 – 84.9 (range)	1.7 – 4.2 (range)	3.03 – 15.64 (range)	0.2 – 0.7 (range)	Sjoberg, et al. 1985a
		Blood storage	Plasma fraction: 172.3 – 295.2 (range) 21 – 42 day storage Whole blood: 72.5 – 152.5 (range) 21 – 42 days		Whole blood: 1.1 – 18.7 (range) 21 – 42 days		Peck, et al. 1979
			Plasma fraction: 145 ± 2.9 (SD) 106 – 209 (range, 24 days)				Marcel, et al. 1973
			2.05 ± 1.47 0 – 4.71 (range)		0.68 ± 1.03 0 – 2.94 (range)		Latini, et al. 2003a
			1.19 ± 1.15 0 – 4.71 (range)		0.52 ± 0.61 0.39 – 0.66 (range)		Latini, et al. 2003b
		Cord blood			0.01 – 4.92 (range)		Li, et al. 2013 PLoS
Infant		Cord blood	187.16 µg/L (mean) 841.16 µg/L (95%)				Huang, et al. 2014 PLos
			0.1 – 1 (range)		0.9 – 3.4 (range)		Zhang, et al. 2009
		RBC exchange transfusion	Blood bag: 54.6 (mean) 36.82 – 84.92 (range) Patient plasma: 2.3 – 19.9 (range)	Calculated exposure: 1.7 – 4.2 (range)	Blood bag: 3.03 – 15.64 (range) Patient plasma: 1.1 – 15.6 (range)	Calculated exposure: 0.2 – 0.7 (range)	Sjoberg, et al. 1985b

Notes	DEHP [µg/mL]	DEHP [mg/kg]	MEHP [µg/mL]	MEHP [mg/kg]	Reference
	Patient plasma: 5.8 – 19.6 (range)	*Amount transfused*: 0.8 – 3.3 (range)	*Patient plasma*: 5 (maximum)	*Calculated exposure*: 0.05 – 0.2 (range)	Sjoberg, et al. 1985a
	Blood bag: 44.8 (mean) 4.3 – 123.1 (range) *Patient serum*: 6.1 – 21.6 (range)	*Calculated exposure*: 1.2 – 22.6 (range)			Plonait, et al. 1993
	ECMO circuit: 19.1 ± 7.5 10.13 – 30.83 (range) *Patient plasma*: 0 – 24.18 (range)	*Calculated exposure*: 10.5 – 34.9 (range) 3 – 10 days			Karle, et al. 1997
Extracorporeal membrane oxygenation	26.8 (14 days) 33.5 (24 days)	*Calculated exposure*: 42 – 140 (range) 3 – 10 days *DEHP in heart tissue*: 1 µg/g			Shneider, et al. 1989
Plateletpheresis	*Plateletpheresis concentrate*: 5.7 – 23.7 (range) *Patient serum*: 0.142 – 1.236 (range)	*Exposure*: 1.6 – 8.8 mg/ patient			Buchta, et al. 2005
Urine samples					
General population			16.5 ± 30.1 ng/mL (SD) 1 – 143.9 ng/mL (range)		Hoppin, et al. 2002
			13.8 ng/mL (> 20 years)		Silva, et al. 2004
	Sum of metabolites: 311 nmol/L (mean, 16 weeks) 245 nmol/L (mean, 26 weeks)		4.9 ng/mL (mean, 16 weeks) 4.2 ng/mL (mean, 26 weeks)		Yolton, et al. 2011
Adult			4.1 – 5.7 mg/mL (range)		Whyatt, et al. 2009
Pregnancy	*Sum of metabolite*: 35.6 ng/mL (male) 42.5 ng/mL (female)		5.2 ng/mL (male) 8.7 ng/mL (female)		Swan, et al. 2010
			4.6 ± 6.4 ng/mL (mean ±SD) 1.2 – 47.3 ng/mL (range) (12 – 18 months)		Brock, et al. 2002
General population			4.9 µg/L (6 – 11 years) 8.1 µg/L (12 – 19 years)		Silva, et al. 2004
Children			11.3 (mean) 0.6 – 272.7 (range) (6 – 10 years)		Teitenbaum, et al. 2011
	0.34 µM (Median) (8 – 19 years)				Trasande, et al. 2013

Notes	DEHP [µg/mL]	DEHP [mg/kg]	MEHP [µg/mL]	MEHP [mg/kg]	Reference
	Sum of metabolites: 0.16 – 20.766 (range)	*Calculated exposure:* 26.4 mg/kg/day	0.049 – 1.273 (range)		Su, et al. 2012
			86 mg/mL (median)		Green, et al. 2005
NICU patient			205 ng/mL (mean) 704 ng/mL (95%)		Calafat, et al. 2004
Infant		*Calculated exposure, high DEHP-product use:* 233 – 352 µg/kg bw/day	*Low DEHP-product use:* 4 (median), 18 ng/mL (75th%) *High DEHP-product use:* 86 (median), 171 ng/mL (75th%)		Weuve, et al. 2006

Table 2

Experimental studies examining the effect of DEHP or MEHP on cardiac physiology.

Reference	Concentration	Model & Approximate Exposure Length	Result
Aronson, et al. 1978	250 μM DEHP	Excised rat heart; 60 min	DEHP ↓ heart rate, ↓ coronary flow, ↓ systolic tension and ↑ diastolic tension, prolonged PR and QT intervals. DEHP ↑ lactate levels in tissue and perfusate media.
Barry, et al. 1989, 1990	15 – 300 μg/mL MEHP	Human atrial trabeculae; 10 – 120 min	MEHP reversibly ↓ contractility (IC_{50} = 85 μg/mL) and ↑ arrhythmia incidence. MEHP may act via cholinergic receptors (atropine shifts IC_{50} = 120 μg/mL).
Calley, et al. 1966	350 mg/kg DEHP	Rabbit; 3 min	DEHP ↓ blood pressure
Gillum, et al. 2009	1 – 50 μg/mL DEHP	Neonatal rat cardiomyocytes; 24 – 72 hours	DEHP ↓ conduction velocity, ↓ cell synchronicity, ↓ gap-junctional connexin-43 expression, and modified mechanical movement of cell layers
Mangala, et al. 2013	0 – 100 mg/kg/day	Female rat; 21 days	Postnatal DEHP exposure (via lactation) impaired insulin signal transduction and glucose oxidation in cardiac muscle in female progeny.
Martinez-Arguelles, et al. 2012	300 mg/kg/day DEHP	Male rat; 7 days	Prenatal DEHP exposure ↓ heart rate, ↓ systolic & diastolic blood pressure in adult males.
Posnack, et al. 2012	50 – 100 μg/mL DEHP	Neonatal rat cardiomyocytes; 72 hours	DEHP ↑ fatty acid substrate utilization, ↑ oxygen consumption, ↑ mitochondrial mass, ↑ PPARa expression and ↑ extracellular acidosis. Effects partially mimicked by PPARa agonist.
Posnack, et al. 2011	1 – 50 μg/mL DEHP	Neonatal rat cardiomyocytes; 24 – 72 hours	DEHP modified gene expression related to cell electrical activity, calcium handling, adhesion and microtubular transport. DEHP also modified adhesion and transport proteins.
Rock, et al. 1987	0 – 100 mg MEHP	Rat; < 15min	MEHP ↓ heart rate (LOAEL = 10 mg) and ↓ blood pressure (LOAEL = 55 mg).
Rubin, et al. 1973	4 μg/mL DEHP	Chick embryonic heart cells; 30 min – 24 hour	DEHP stopped cell contractions (30 min), and resulted in 97–98% cell death within 24 hours.
Schulpen, et al. 2013	0.004 – 4.4 mM MEHP	Embryonic stem cells; 5 – 7 days	MEHP ↓ cell viability and cardiac differentiation, and genes associated with these endpoints.
Wei, et al. 2012	0.25 – 6.25 mg/kg/day DEHP	Rat; 40 days	Prenatal and postnatal DEHP exposure ↓ renal protein expression, ↑ blood pressure

Table 3

Epidemiological studies examining the association between higher urinary concentrations of DEHP metabolites and cardiovascular disease.

Reference	Result
Lind, et al. 2011	Cross-sectional analysis of PIVUS data. Higher serum MEHP concentration was associated with echolucent intima-media complex and overt plaques. 4.53 ng/mL serum MEHP (mean)
Olsen, et al. 2012	Cross-sectional analysis of PIVUS data. Higher serum MEHP concentration was associated with LDL cholesterol levels, but not associated with deviations in blood pressure. The effect on cholesterol levels was not significant after multiple-testing correction.
Transande, et al. 2013	Cross-sectional analysis of NHANES survey data. Higher urinary concentration of DEHP metabolites associated with increased blood pressure in children aged 6–19 years old. Median urinary DEHP metabolite molar concentration: 0.337

Table 4

Experimental studies which measured BPA exposure in humans via blood or urine samples.

Notes		BPA [ng/mL, mean ± SEM]	Reference
Blood samples			
Adult	General population	3.83 ± 1.98 (SD) 1.30 – 8.17 (range)	Aris, A. 2013
		0.2 ± 0.18 (SD) 0.1 – 2.27 (range)	Zhang, et al. 2013
	Pregnancy	5.9 ± 0.94 ND – 22.3 (range)	Padmanabhan, et al. 2008
		4.4 ± 3.9 0.3 – 18.9 (range)	Schonfelder, et al. 2002
		9.04 ± 0.81 ND – 66.48 (range)	Lee, et al. 2008
		1.36 ± 1.18 (SD) ND – 4.46 (range)	Aris, A. 2013
		3.58 ± 4.27 (SD) 0.10 – 29 (range)	Zhang, et al. 2013
	Hemodialysis	10 ± 6.6 (maintanence dialysis)	Krieter, et al. 2012
		0 – 15.5	Sajiki, et al. 2008
		5.3 ± 0.3	Kanno, et al. 2007
	Endometriotic patients	2.91 ± 1.74 0 – 7.12 (range)	Cobellis, et al. 2009
Children	General population	3.18 ± 1.66 (SD) 1.20 – 8.76 (range) (1 – 5 years)	Zhang, et al. 2013
Infant	Cord blood	2.9 ± 2.5 0.2 – 9.2 (range)	Schonfelder, et al. 2002
		1.13 ± 0.08 ND – 8.86 (range)	Lee, et al. 2008
		1.23 ± 1.04 (SD) ND – 4.60 (range)	Aris, A. 2013
		0.13 ± 0.12 (SD) 0.1 – 0.79 (range)	Zhang, et al. 2013
Urine samples			
Adult	General population	1.15 mean	Bushnik, et al. 2010
		2.4 mean	Calafat, et al. 2008
		1.5 mean	Mendiola, et al. 2010
		ND - 42	Genuis, et al. 2012
		2.61	Heffernan, et al. 2013
		2.61 mean	Heffernan, et al. 2013
		0.5 – 15.5	Calafat, et al. 2008
		1.90 ± 1.23 (SD) 0.10 – 8.70 (range)	Zhang, et al. 2013
	Industrial worker	55.73 ± 5.48 (SD) 5.56 – 1934.85 (range)	Wang, et al. 2012
	Pregnancy	1,250 (single patient)	Sathyanarayana, et al. 2011

	Notes	BPA [ng/mL, mean ± SEM]	Reference
		70 (mean), pre-term birth 30 (mean), normal gestation time	Patel, et al. 2013
Children	General population	2.98 0.65 – 265 (range) (0 – 5 years)	Heffernan, et al. 2013
		3.9 (1 year), 2.9 (2 year), 2.9 (3 year) 0.4 – 616 (range)	Braun, et al. 2011
		8.9 ± 23.6 (SD) 0.4 – 211 (range) (2 – 5 years)	Morgan, et al. 2011
		2.7 (mean) (3 – 14 years)	Becker, et al. 2009
		3.6 (mean) 0.3 – 40 (range) (6 – 10 years)	Teitelbaum, et al. 2008
		3.6 (mean) (6 – 11 years) 3.7 (mean) (12 – 19 years) 0.4 – 149 (range, all ages)	Calafat, et al. 2008
Infant	General population	0 – 17.85	Volkel, et al. 2011
	NICU patient	*Total BPA* 30.3 ± 4.8 (SD); 1.6 – 946 (range) *Free BPA:* 1.8 ± 3.2; ND – 17.3 (range) *Calculated max exposure:* 35.95 ug/kg/day	Calafat, et al. 2009
		2 – 196 (range) *High medical device use:* 18.5 (median), 47.3 (75th%) *Low medical device use:* 13.2 (median), 38.2 (75th%) *Calculated max exposure:* 7.45 µg/kg/day	Duty, et al. 2013

Table 5

Experimental studies examining the effect of BPA on cardiac physiology.

Reference	Concentration	Model & Exposure Length	Results
Asano, et al. 2010	10 – 100 μM	Human & canine coronary smooth muscle cells; 1 min	BPA activates maxi-K channels
Belcher, et al. 2011	1 pM – 1 nM	Female ventricular myocyte; 7 min	BPA ↑ contractility. Effect abolished in myocytes from ovariectomized females, and ERβ knockout mice. Effect not inhibited by L-Name pretreatment.
Deutschmann, et al. 2013	1 μM – 1 mM	Mouse cardiomyocytes & dorsal root ganglion neurons, rat endocrine GH3 cells, human HEK cells; 1 min	BPA reversibly blocks multiple calcium channels. Effect not due to intracellular signaling (PKA, PKC pathways). EC_{50} = 26 – 35 μM
Gao, et al. 2013	1 nM	Female ventricular myocyte; 15 min	BPA transiently alters ryanodine receptor phosphorylation at PKA site and phospholamban at CAMKII site. Effects abolished with ERβ or PKA blocker.
Lee, et al. 2012	1 – 100 ng/mL	Rice fish embryo; 2 – 4 days	BPA ↓ heart rate
O'Reilly, et al. 2012	0.1 – 1 mM	HEK cells; 2 min	BPA blocks hNav1.5 sodium channel in closed/resting-state. NOAEL = 100 nM, LOAEL = 1 μM, K_d = 25 μM
Pant, et al. 2011	0.1 – 100 μM	Rat right atria; 10min	BPA ↓ heart rate, ↓ force of contraction. Effect abolished with L-Name pretreatment or methylene blue. Effect not inhibited by atropine pretreatment.
Pant, et al. 2012b	LD_{50} = 841 mg/kg bw (i.p.), 35 mg/kg bw (i.v.).	Female rat; 7 min	Lethal BPA dose (40 mg/kg bw) produced respiratory arrest, hypotension and bradycardia.
Patel, et al. 2013	0.5 – 200 μg/kg/day	Male mice; 30 days – 4 months	Prenatal and postnatal BPA exposure resulted in concentric remodeling, ↑ velocity circumferential shortening, ↑ascending aorta velocity, and ↑ calcium mobility
	0.5 – 200 μg/kg/day	Female mice; 30 days – 4 months	Prenatal and postnatal BPA ↓ LV mass and wall thickness, ↑ blood pressure, ↓ calcium mobility
Posnack, et al. 2014	0.1 – 100 μM	Female excised whole heart; 15 min	BPA ↓ epicardial conduction velocity, ↑ atrioventricular delay, ↑ PR segment time, ↑ action potential duration, heart block at high doses
Schirling, et al. 2006	50 – 100 μg/L	Snail embryo; 9 days	BPA ↓ heart rate
Yan, et al. 2013	1 nM	Female excised whole heart; 60 min	BPA ↑ arrhythmia duration (ischemia reperfusion model). Effects abolished with ERα + ERβ blocker
	1 nM	Female ventricular myocyte; 2 hour	BPA ↑ spontaneous after contractions
Yan, et al. 2011	1 nM	Female ventricular myocyte; 7 min	BPA ↑ spontaneous after contractions, ↑ calcium leak and load in sarcoplasmic reticulum. Effects abolished in ERβ knockout mice

Table 6

Epidemiological studies examining the association between higher urinary concentrations of BPA and cardiovascular disease.

Reference	Result
Bae, et al. 2012	Higher urinary BPA concentration was associated with increased blood pressure and decreased heart rate variability. Mean urinary BPA: 1.2 μg/g creatinine
Khalil, et al. 2014	Higher urinary BPA concentration was associated with increased blood pressure in male children.
LaKind, et al. 2012	Cross-sectional analysis of NHANES survey data. Higher urinary BPA concentrations not associated with cardiovascular disease.
Lang, et al. 2008	Cross-sectional analysis of NHANES survey data. Higher urinary BPA concentrations associated with cardiovascular disease. 4.53 ng/mL (mean, males), 4.66 ng/mL (mean, females), 8 ng/mL (mean, reported cardiovascular disease).
Lind, et al. 2011	Cross-sectional analysis PIVUS study. Higher serum BPA concentration was associated with increased echogenicity of the intima-media complex and overt plaques. 3.76 ng/mL serum BPA (mean)
Melzer, et al. 2012a	Higher urinary BPA concentration was associated with severe coronary artery disease. 1.28 ng/mL (median) – normal arteries; 1.53 ng/mL (median) – severe coronary artery disease
Melzer, et al. 2012b	Longitudinal study examined association between higher urinary BPA concentration and incidence of coronary artery disease. 1.24 ng/mL (median)-normal; 1.35 ng/mL (median)-cases
Melzer, et al. 2010	Cross-sectional analysis of NHANES survey data. Higher urinary BPA concentrations associated with cardiovascular disease. 1.79 ng/mL urinary BPA (mean, all participants)
Olsen, et al. 2012	Cross-sectional analysis of PIVUS data. Higher serum BPA concentration was associated with LDL and HDL cholesterol levels, but not associated with deviations in blood pressure. The effect on cholesterol levels was not significant after multiple-testing correction.
Shankar & Teppala. 2012	Cross-sectional analysis of NHANES survey data. Higher urinary BPA concentrations associated with increased blood pressure.