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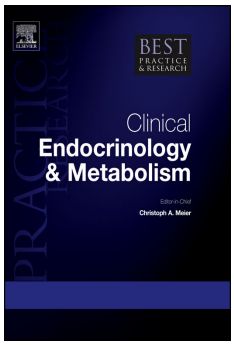

Endocrine disrupting chemicals and bone

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Endocrine disrupting chemicals and bone

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Abstract

Endocrine-disrupting chemicals (EDCs) are defined as chemicals that interfere with the function of the endocrine system. EDCs exert their hormonal effects through several mechanisms; modulating hormone receptors or changing metabolism of different hormones. EDCs also influence multiple signalling pathways while effecting the hormonal systems and possess complex dose–response curves. EDCs can exert deleterious effects on bone tissue through changing bone modelling and remodelling via altering bone paracrine hormone synthesis, the release of systemic hormones, cytokines, chemokines and growth factors, and effecting stem cell fate, as well as bone marrow mesenchymal stem cell differentiation. Evidence is accumulating of the bone disrupting effect of different groups of EDCs, such as; the perfluoroalkyl substances, the phthalate esters, the bisphenol A, the organotin compounds, the alkylphenols and the dioxin and dioxin-like compounds. This review highlights the recent discoveries of the effects of commonly found environmental chemicals on bone from basic molecular findings to clinical implications.

Keys words: Endocrine disrupting chemicals, bone, perfluoroalkyl substances, phthalate esters, dioxins, dioxin-like compounds, bisphenols, organotin compounds, alkylphenols

Practice Points

- EDCs are detected in many tissues in the body including bone. Some EDCs show a consistent tissue pattern of distribution and deposition.
- Exposure to EDCs is consistent and life-long. Some of the EDCS are persistent and accumulate in the body and environment and food chain.
- Considerable evidence, from in vivo studies to epidemiological data, suggests an adverse effect on bone of different EDCs.
- The prenatal and early childhood periods are of particular interest for potential hazardous effects of EDCs.
- EDCs exert effect on different receptors and different pathways, thus, when the body expose to one EDC, single pathway in different organ systems or different pathways in different organ systems are altered.

Research Agenda

- EDCs are found ubiquitously in the environment and simultaneous exposure to multiple EDCs are very likely in every day.
- Accumulation and interactions of different EDCs in the body and their effect on different systems and pathways deserve further study. Defining the interaction of different EDCs at the population level and both in vivo and in vitro systems will be needed.
- For every EDC, safe dose of exposure, low and high dose effect should be determined, however, in the era of exposures to the mixtures of EDCs, more detailed calculations will be required.

Introduction

Endocrine-disrupting chemicals (EDCs) are defined as chemicals that interfere with the function of the endocrine system. The Endocrine Society also described EDCs as: "An exogenous (non-natural) chemical, or mixture of chemicals, that interferes with any aspect of hormone action." (1, 2). It is a specific concern due to their exogenous effects which is independent from biofeedback loops, leading to potentially harmful consequences (2, 3).

Synthetic EDCs are commonly designed and manufactured for another purpose and their endocrine effects are subsequently discovered. EDCs are widely found in environment and, human and animals expose several EDCs in varying degrees through food and food packaging, water, personal care products, household goods, detergents, fabrics, electronics, medical equipment, pesticides and ambient air (1,2, 4-8). Additionally, many pharmaceutical agents are produced to target the endocrine system for therapeutic reasons, and their release leads to environmental contamination and also to potential endocrine disruption (9-12).

More than 1800 chemicals have been identified that disrupt at least one of three endocrine pathways, namely oestrogen, androgen, and thyroid function (13). Screening of 320 of 575 chemicals with the instruction of the European Commission revealed strong or potential evidence for endocrine disruption (14).

EDCs exert their hormonal effects by several mechanisms such as binding to hormone receptors or changing metabolism of different hormones (15). More recently, key characteristics of EDCs have been defined by an Expert Consensus Statement which characterises chemicals according to their ability to interact with key regulatory steps of hormone system (15).

EDCs act on the multiple signalling pathways while effecting the hormonal systems and possess complex dose–response curves (5, 16). EDCs also modulate hormone receptors by disrupting their biosynthesis, expression and signal transduction, thus exert agonistic or antagonistic effect by altering hormone delivery to target tissues (1, 15-17). EDCs can selectively bind to some nuclear hormone receptors (NRs) including sex steroid receptors, thyroid hormone receptors, retinoid X receptor (RXR) and the peroxisome proliferator activated receptors (PPARs) (17). EDCs–receptor interaction activates several signalling cascades in a direct or indirect way, and may even cause epigenetic changes such as DNA

methylation and histone acetylation (15, 16). In rodents, these epigenetic modifications can show transgenerational effects which can be transmitted for up to four generations (15-18).

In general, environmental EDCs can interfere with thyroid hormones, oestrogen, androgen actions and a plethora of other receptors (19). Other consequences of EDCs exposure are a variety of metabolic disorders such as diabetes type 2 and obesity, hormone-related cancers and the reproductive problems. EDCs can exert deleterious effects on bone tissue through the changing bone modelling and remodelling and bone paracrine hormone production and, also altering the release of systemic hormones, cytokines, chemokines and growth factors (20). Stem cell fate, as well as bone marrow mesenchymal stem cell (BMSC) differentiation and bone marrow niche organization can be disrupted with EDC exposure (20, 21).

Bone remodelling is a highly regulated process with bone formation and bone resorption occurring in bone remodelling units, by the respective coordinated function of osteoblasts and osteoclasts. Secreted factors construct a reciprocal interaction between osteoblasts and osteoclasts (22). When an imbalance occurs between bone formation and resorption, such bone alterations can result in low or high bone mass disorders depending on the balance (23-25). Osteoblasts and osteoclast derive from different progenitor cell lines, which are pluripotent BMSC for osteoblasts and monocyte/macrophage lineage of hematopoietic stem cells (HSC) for osteoclasts (22-24)

The sequential expression of two transcription factors (RUNX2 and OSX1) are required for the differentiation of osteoblasts from BMSC (23). Other key factors of osteogenic differentiation and maintenance are WNTs and their membrane-associated Frizzled receptors with their co-receptors low-density lipoprotein receptor related protein 5 (LRP5) or LRP6 in RUNX2/OSX1 expressing cells through the activation of intracellular pathways, including β-catenin dependent signalling (23, 25).

For osteoclasts, the monocyte/macrophage progenitors first differentiate into tartrate-resistant acid phosphatase (TRAP)-positive preosteoclasts, then, preosteoclasts fuse with each other to form multinucleated osteoclasts (24). Binding of the macrophage colony-stimulating factor and the nuclear factor kappa B (NF-κB) ligand (RANKL) to their receptors and activation is required for the proliferation and survival of osteoclast precursors and, during these processes, several kinases are activated which in turn, induce the transcription for osteoclast

differentiation and function (26). Another signalling pathway, Wnt/β-catenin signalling, has a stimulatory role on osteoprotegerin (OPG) which is the endogenous decoy receptor of RANKL and thus an inhibitor for osteoclastogenesis (22, 24, 26, 27).

Sex steroids are the main role players in bone mineral metabolism for attainment of peak bone mass during puberty and maintaining bone mass and strength during adulthood by balancing bone remodelling between resorption and formation (28). The oestrogens and androgens exert their effect on bone via their receptors expressed in osteoblast and osteoclast progenitors and their descendants (28). Since EDCs have a potential to alter both oestrogen and androgen production and, their receptor function, they have a potential to disrupt bone mineralization and bone remodelling. Other than oestrogen and androgen receptors (ER and AR), the differentiation and function of osteoblasts and osteoclasts is also modulated by a family of interacting NRs which are also influenced by EDCs. The PPARγ, liver X receptors (LXRs) and RXRs are members of the NR superfamily and expressed in osteoblasts and osteoclasts (29). Activation of PPARγ in osteoblast precursors by synthetic analogues induces differentiation to the adipocyte lineage with suppression of osteoblast differentiation (30). Additionally, PPARγ, LXR and RXRα influence the differentiation of osteoclasts through RANKL signalling (31-33).

Moreover, EDC exposure during different developmental periods of life may cause more severe and persistent damage, which may even occur at lower doses than in full mature organisms (34). It is noteworthy that exposure to EDCs in utero or in early life stages can induce organ abnormalities, behavioural disorders and tumour formation (17, 35). Puberty is unique period of time for hormonal activation and growth and also most important period for peak bone mass attainment in which EDCs have a potential for widespread impact on bone (28, 35).

EDCs classification is based on their chemical properties and their peculiar effects on organs and tissues. Evidence for bone disrupting effect has been found in different groups of EDCs, such as; the perfluoroalkyl substances, the bisphenols, the alkylphenols, the phthalate esters, the organotin compounds, the dioxin and dioxin-like compounds (Table 1) (16, 20). This review highlights recent findings related to the effects of commonly found environmental chemicals on bone from basic molecular findings to its clinical implications.

A. Perfluoroalkyl Substances

Per- and polyfluorinated alkyl substances (PFASs) comprise a large group of synthetic organic chemicals which have been used for more than six decades in industry for their high thermal and chemical stability and low surface tension properties (16, 36). PFASs have been used as emulsifiers, surfactants, nonstick coatings, polymers, components of pharmaceuticals, fire retardants, lubricants, adhesives, cosmetics, paper coatings, and insecticides, especially in protective water- and stain-resistant coatings on clothing, carpets, furnishing and even in food containers (36-39). While main routes exposure to PFASs are foods and drinking water, the tap water consumption is correlated with serum levels of PFAS in the contaminated areas (40), fish and seafood are the major PFASs contributors to the diet in all the countries (41). House dust and outdoor/indoor air are other routes of exposure (42).

Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the most commonly studied compounds among the thousands of PFASs and, an estimated daily exposure per body weight is varying from 7 to 219 ng/kg/day for PFOS and 0.4 to 128 ng/kg/day for PFOA in North America (38, 39). PFOA and PFOS together with perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) were detected in 97–100% in the population aged 12 years and older in U. S. National Health and Nutrition Examination Survey (NHANES) conducted in 2011–2012 (43).

After exposure, PFASs enter the circulation and are deposited in the liver, kidneys, spleen, and accumulate in gall bladder, testicles and the skeleton with a biological half-life of 3–7 years (44-46). Many PFASs show cumulative toxic effect and leading to multiorgan damage including liver, reproductive organs, spleen, immune system and skeletal system and even causing developmental defects in animal models and humans (37, 38, 43-47).

Animal exposure studies and human autopsies have shown deposition of PFASs in bones (44, 45). The amount and type of PFAS accumulation show different trends in different tissues, but, display some common trends (45). Bone was found to be the tissue with the lowest burdens of PFASs and the PFAS profile shows considerably differences from those of the other tissues. While PFOA, PFNA, PFOS were the main PFAS deposited in bone, considerable differences of PFAS distribution in the bone samples from different countries

has been detected likely reflecting the amount of background exposure to the different PFASs in different environments (45, 46).

Age also effect serum levels of PFASs and the amount of tissue accumulations, in general, older people showed higher concentrations of PFASs (45, 48). However, some young people may have higher tissue or serum PFASs levels which might be explained by environmental factors they encounter, like dietary intake, living habits, and/or contaminated environment (45, 47). Nevertheless, not all studies show a linear correlation between the bone PFAS concentration and age and nor was this observed with micro-computerized tomography/morphological parameters, but a negative trend was observed between bone PFOS concentration and relative bone volume (46). Tissue findings are in accordance with the population based studies, where, a negative association between serum PFOS, PFOA, PFHxS, PFNA and perfluoroundecanoic acid (PFUnDA) concentrations and bone mineral density (BMD) were detected (47-49).

A NHANES study evaluating serum PFOS and PFOA levels in 8 year and older population between 2005 and 2008 found that a higher serum concentration of PFOS is associated with a decrease in total lumbar spine bone mineral density (LS-BMD) predominantly in premenopausal women. No association was detected between serum PFOA, PFOS concentration, and femoral neck BMD (FN-BMD) or fracture risk (48). By contrast, another NHANES study evaluating PFOA, PFOS, PFHxS and PFNA concentrations in 12-80 years old population between 2009–2010 did not show any clear association between LS-BMD with any of the PFASs. However, only PFOS was found to be associated with lower FN-BMD in men, while PFOS, PFHxS, PFOA and PFNA were associated with lower FN-BMD or total femur (TF-BMD) or osteoporosis in women (49). In contrast to a previous NHANES cohort, BMD and PFASs associations were stronger among postmenopausal women (49). The potential reasons for the discrepant relationships between PFASs and BMD between two studies could be the differences in the NHANES survey cycles examined, the differences in sample size, age range, and covariates included (48, 49). Additionally, the decreasing mean serum PFOS concentration in the U.S. population through the years could have also affected the association with LS-BMD in the two studies. Nonetheless, osteoporosis risk increment in individuals having serum concentration of PFASs at lowest quartile to highest quartiles change from 2.3 to 96 folds (47, 49)

There are several potential mechanisms for the bone effect of PFASs. The first is the endocrine disruptive effect of PFASs, which is concluded from the sex specific differences of association between PFASs and BMD. Almost all studies showed females, especially if premenopausal, were more sensitive to PFAS toxicity than males, although males having higher serum levels of PFASs (47-49). Several supportive studies for this effect of EDC has been found in experimental studies show that PFAS have the ability to interfere with the biological effects of sex hormones (50, 51). Epidemiological studies have also shown that PFASs are associated with the delayed onset of puberty, earlier age of menopause, and lower serum oestradiol concentration (52, 53). All described hormonal receptor interferences and associated pubertal and hormonal changes could be explanatory for the negative association of serum/tissue PFASs on BMD and microarchitectural changes. However, not only the sex hormones but also thyroid hormones are affected by PFASs. Thyroid hormones also play a crucial role in bone health and remodelling and, epidemiological studies showed an association between PFAS exposure and serum thyroxine (T_4) and triiodothyronine (T_3) levels with a positive association between the serum PFHxS and subclinical hyperthyroidism in women (16, 20, 38, 43).

The other mechanisms of the bone effect of PFAS are possible direct effects on bone. Current in vitro and in vivo studies have shown that PFOA directly targets bone and bone marrow cells, such as abnormal stimulation the resorption activity of osteoclasts at lower PFOA concentrations, however bone resorption decreased and eventually stopped at higher concentrations (46, 54). Moreover, in preosteoblastic cultures, low concentrations of PFOA increased osteocalcin (OCN) expression while decreased at higher PFOA concentrations (54). It is important to underline that PFOA doses used in these studies were environmentally relevant concentrations, which were detected in human bone samples (45, 46, 54). Additionally, bone marrow accumulation is more evident than cortical or trabecular bone and, compromise the HSCs differentiation and commitment of BMSCs (44). The possible mechanisms that underpin osteoclast and osteoblast changes related to the PFOA exposure are unknown. However, it has been claimed in general that PFOA showed the toxic effects through the PPARα subtype and also down regulate Wnt/ β-catenin signalling which are expressed in both osteoclasts and osteoblasts (29, 54).

Additionally, more recently there is evidence of PFOAs direct binding to hydroxyapatite crystals and also interference with vitamin D action (56). In vitro studies using human

osteoblast cell model demonstrated competition of PFOA with calcitriol for the vitamin D receptor (VDR) at the same binding site, interference with receptor function with altering vitamin D-responsive gene, and also reducing mineralization. Finally, elevated PTH levels have been detected in a PFOA exposed population without any change in vitamin D and calcium levels, suggesting a compensatory PTH elevation overcomes functional hypovitaminosis D as a PFOA effect (56).

Furthermore, prenatal exposure studies in mice showed PFOA accumulates in bone till old age and consequently exerts a persistent deleterious effect on bone geometry and mineral density (46). PFAS exposure during foetal life and the newborn period is a specific concern due to the vulnerability of early life stages to toxic effects. Moreover, studies examining neonates showed reduced birth weight could be associated with prenatal exposures to PFASs, but this effect is not consistent in all examined studies. However, it has been shown that prenatal PFAS exposure is associated with bone mass and size in adolescent girls; but it is unclear whether these associations are mostly explained by the effects of PFAS on body size (57). Additionally, children aged 3–11 years in the NHANES 2013–2014 cohort had decreasing height with increasing PFAS levels predominantly in boys (58). Although height is not the subject of this review, the height is mentioned since longitudinal growth is determined by bones.

Altogether, these findings illustrate several mechanisms leading to skeletal hazard and understanding mechanisms might provide a therapeutic target to overcome endocrine disruption by these chemicals in the future.

B. Bisphenols/ Bisphenols A

Bisphenols A (BPA) (4,4′-isopropylidenediphenol), is a class of synthetic monomers, which is the major component of epoxy and polystyrene resins and polycarbonate plastics. BPA is widely used in protective coatings, in food-packaging and in various household appliances, such as electronic devices/media, children toys, kitchen utensils, water pipes, reusable bottles and food storage containers, and in dentistry for dental sealants (1-16). Halogenated derivatives of BPA (i.e. tetrabromobisphenol A), are used as flame retardants for building

material. BPA had been also implemented in thermal paper used for the cash and billing receipts in very high concentration (20 mg BPA/g/paper). Exposure to BPA occurs directly through oral and topical routes via environmental pollution and food chain contamination (1-16). BPA is a water-soluble compound and absorbed from the gastrointestinal tract. Although it is metabolization in the liver, excreted from the body via urine, faeces and sweat, whose concentration is 70% higher than in serum (59, 60). BPA is also found in foetomaternal unit and in the lactating milk (59, 61). BPA has been detected in varying concentration in almost everyone's urine range due to its widespread usage (59, 60).

BPA exerts its effects by bindings to various receptors in the body including the bone. Initially, BPA was defined as a xenoestrogen because of its structural similarity with the endogenous 17β-oestradiol (E2), functioning through binding to both ERα and β (62). However, BPA shows estrogenic effects with ER binding affinity of approximately 2000 to 10000-fold weaker compared to E2 (62), so far, acts as a selective ER modulator and/or disruptor. Additionally, an anti-androgenic effect has been suggested related to the BPA exposure due to reduced luteinizing hormone levels and decreased steroidogenic enzyme gene expression in rat Leydig cells (63). Furthermore, BPA exerted indirect antiandrogenic effect by upregulating of aromatase enzyme and altering mRNA expression of 5α-Reductase isoenzymes. These changes result reduced testosterone levels, oestrogen testosterone imbalance and decreased tissue activity of testosterone (63). The complex association of BPA with sex hormones could have a significant biological and clinical implications for bone as a target organ of sex hormones. Moreover, BPA induces inflammation by stimulating production of pro-inflammatory cytokines and inhibiting the production of anti-inflammatory cytokines and, produces reactive oxygen species which subsequently causes oxidative DNA damage and cell death (64). A link between BPA exposure and inflammation and oxidative stress was found in cross-sectional studies (65). Thus, in addition to endocrine effects, both inflammation and oxidative stress related to the BPA exposure could be destructive to the bone.

Furthermore, BPA binds the oestrogen-related receptor gamma (ERγ), which is an orphan receptor with unclear physiological ligand. The binding of BPA to the ERγ receptor regulates the expression of mitochondrial genes. ERγ receptor is reported to act on RUNX2 and bone morphogenetic protein 2 which are important for osteoblast differentiation and mineralization (66). BPA also disrupts receptor activation of RANKL and the Wnt/β-catenin pathway (67).

Although there are several implications for BPA influences on bone, no strong evidence for bone effect of BPA has been found. While small scale population study showed no association between BPA levels and BMD, one recent large NHANES study including a population study from 2005 to 2010, displayed an inverse association between BPA levels and the prevalence of osteopenia and osteoporosis at lumbar spine in postmenopausal women yet, no association in premenopausal women (68). Furthermore, a population-based cohort study evaluating 1.362 mother-child pairs demonstrated that increase in maternal first trimester bisphenol S (BPS) concentrations was associated with lower BMD and aBMC at 10 years, but not 6 years of age (69). The authors claimed this age dependent associations were related to the variability of BMD measurements at different ages, or manifesting bone effect of bisphenols by age. Although results must be more carefully evaluated and more data is required to make strong conclusions, yet, it must be kept in mind that BPS exposure in the early life periods might have persistent effects.

Previous animal studies showing effect of BPA on bone development and sex-specific changes are not consistent. Maternal exposure studies with tolerable high doses of BPA revealed the direct effects of BPA on embryonic bone formation are dose dependent, and lead to retardation of bone ossification at lower doses (85, 100, 125, and 300 mg/kg) while disruption of skeletal development at higher doses (1,000 mg/kg) (16, 20, 67). However, lower maternal exposure doses (10-25 mg/kg/day) showed sexual dimorphic changes on bone geometrics and increment femoral length (16, 20, 67). Conversely, prenatal exposure of much lower BPA at doses similar to the range of daily human exposure (0.1–1.5 μg/kg/day) for longer periods, through the pregnancy and lactation, led to shorter femurs, with reduced trabecular area and total cross-sectional area in male offspring, indicating the sexual dimorphic effects of BPA (16, 20, 67). Moreover, the harmful effect of BPA on bone is a highly controversial issue. Even beneficial effects of BPA have been found as it prevents of bone loss in oestrogen deficient mice model (*Aromatase knock-out)*. It is possible in an oestrogen-lacking environment, the outcomes of BPA exposure may not be detrimental and, serves an oestrogen-like function in skeletal metabolism and bone mass maintenance (16, 20). Additionally, BPA do not protect against trabecular bone loss which could be explained by BPAs different receptor affinities to ER-β and ER-α (66).

As a result, the mechanism on bone health is multifactorial for BPA which depends on the dose and critical time period of exposure, hormonal status of host, duration of exposure. So far, we can say that BPA is potentially harmful for bone although it is difficult to make a strong conclusion.

C. Alkylphenols

Alkylphenol ethoxylates (APEs) are the nonionic surfactants and classified as xenoestrogens. APEs are used in the production of chemical in industry like paints, detergents, plastics and pesticides (1-16). The major degradation products of APEs are 4-tert-octyphenol (OP) and 4-nonylphenol (4-NP) which are widely dispersed into the environment and commonly found in water sources includes the wastewater, in river sediments and in drinking water (1-16, 70). The toxicity and endocrine disruptive effects by mimicking oestrogen have been identified in various tissues, such as the liver, kidney, spleen, and blood (1-16, 71). Although bone effect of APEs has been extensively studied in cell culture and animal studies, the human implications have not been described. However, in vivo and in vitro studies showed APEs influenced bone architecture through the downregulation of critical factors involved in osteoblasts and osteoclasts differentiation (72-75).

First of all, it was shown that in vitro administration of NP and OP in different cell culture systems resulted in suppressed osteoclast formation without remarkable change on osteoblast population, yet, high doses of OP exposure resulted in the impaired differentiation of the multipotent cells to osteoblasts (72, 74). Additionally, NP exposure in osteoblast cell cultures caused massive cell death and activation of the extrinsic and the intrinsic apoptotic pathway in a dose dependent manner (71). Moreover, in vivo administration of NP and OP (0.1 mg/kg of body weight) to pregnant mice has revealed an accelerated ossification in exposed foetuses (72). However, further in vivo studies, based on perinatal and postnatal OP exposure (1 or 10 ug/ml in drinking water) revealed a reduction in bone growth in width. Moreover, dose specific bone effect has been observed as decreased in the cortical bone circumference at diaphysis with low OP doses, while decreased in trabecular bone area with high doses (73). Additionally, it is noteworthy that the adverse effects of OP show sexual dimorphic pattern with a female predominant effect (73).

APEs exert weak oestrogenic effect by binding to the ERs. It was shown that NP also interferes the E2 effect through the decreasing expression of ERs which normally increased with oestrogen exposure but not with co-exposure of E2 and NP (71). Thus, APEs have several effects on bone metabolism which are potentially hazardous.

In humans, daily intake of NPs has been calculated as around 7.5 ug with intakes for breast-fed and bottle-fed infants of 0.2 and 1.4 ug/day, respectively. This intake doses could be lower than the doses used in mice studies. However, OP and NP concentration of human milk samples is dependent on the maternal diet habits and showed strong association with amount of cooking oil, fish oil, fish and processed meat products intakes (70). As a conclusion, although any bone effect related to the expose of APEs in human has been studied and/or shown yet, potential harmful effect should be considered from in vitro and in vivo studies.

D. Phthalate esters

Phthalate esters (PEs) are ubiquitously found in environment and moderately resistance to degradation. Worldwide the enormous amount of phthalate production which occurs each year is primarily used in the food industry, toys, car seats and blood bags (1-16, 75). High molecular weight (HMW) phthalates, namely di-(2-ethylhexyl)-phthalate (DEHP) and benzyl butyl phthalate (BBP), are used mainly in polyvinylchloride (PVC) to provide flexibility of plastic products, like in vinyl floor tiles, food packaging, medical devices, latex adhesives, and solvents (1-16, 75). Low molecular weight (LMW) phthalates, such as diethyl phthalate (DEP) and di-n-butyl phthalate (DBP) are widely used in personal care products and cosmetics, especially perfumes, nail polish, and insect repellent, and also used in drugs industry as drug coating (1-16, 75). It is believed that exposure to phthalates is relatively constant in human through the ingestion, inhalation of indoor air, and dermal exposures, since many consumer products contain phthalates.

Phthalate diesters are hydrolysed by salivary esterases in human saliva as well as in the intestines to their monoesters following the intake, and then absorbed to the body (76). Nevertheless, some of metabolic products are biologically active. Phthalate metabolites are excreted in the urine after glucuronidation in the liver (76). Single phthalate may produce multiple metabolites and, some metabolites are products of more than one parent compound. Additionally, certain phthalate metabolites may show extensive interpersonal or intra-person variability between days and within the day (77).

Bone effect of phthalate metabolites were studied in population-based studies and, a negative effect of urinary phthalate metabolites on the total spine BMD in post-menopausal women has been detected in NHANES populations from 2005 to 2010. It was shown that mono-ethyl phthalate (MEP), molar sum of LMW metabolites, molar sum of estrogenic metabolites and estrogenic equivalency factor were negatively associated with spinal BMD (78). Another study also based on the NHANES data from 2005–2008 demonstrated the higher the urinary mono-n-butyl phthalate (MNBP), mono-(3-carboxypropyl) phthalate (MCPP) and monobenzyl phthalate (MBZP) levels had negative effect on TF- and FN-BMD (79).

These studies are based on the fact that oestrogen deficiency is the main etiologic factor for the post-menopausal osteoporosis and PEs are the oestrogen modulating compound and their effect could be more pronounced in oestrogen deficient era. However, no information was found the younger women and men, but children. Population cohort studies examining the effect of maternal exposure on offspring bone health showed, although associations lost its significances after multiple regression, maternal third trimester LMW phthalate and trimester di-n-octylphthalate (DNOP) concentrations were associated with higher aBMC at 6 years and, with lower aBMC at 10 years, respectively (69). Additionally, another study evaluating maternal exposure of PEs and prenatal and postnatal growth in the first five years of life in boys, showed the maternal monocarboxyisononyl phthalate (MCNP) concentrations at the second and third trimesters of pregnancy were associated with increased foetal femoral length and also with increased birth length. After birth, there was a positive association between MBZP concentration and height in the first two years of life and, between MBZP, monoisobutyl phthalate (MIBP) concentrations and growth velocity at three months, consistent with their association with length at one year (80).

Altogether, these results suggest that PEs exposure in humans, especially during pregnancy, may have persistent effects on bone health. Several mechanisms for the bone effects of PEs have been proposed based on the in vivo and in vitro data. Animal studies have demonstrated that exposure to phthalate metabolites can cause a significant increase in skeletal malformations in foetuses which appears to be dose- and time dependent (16, 20).

In vitro studies showed PEs can enter osteoblasts, accumulate, and exhibit mitogenic effects which eventually lead to decrease the osteoblast cell viability through the micro-filament disruption, DNA damage, and an increase in p53 and apoptotic proteins. Moreover,

phthalates also decreased collagen and alkaline phosphatase expression in primary osteoblast cultures demonstrating their effect on the osteoblast differentiation (81).

EDC effect of phthalates might be via direct their oestrogenic modulation or anti-androgenic activities (82). Anti-androgenic effect also has indirect impact on oestrogen action via a reduction of the substrate for aromatization reaction to the E2. Phthalates also had agonistic effect on the PPARs and, increased PPAR-γ is correlated with decreased osteogenesis and bone density in human (83).

In summary, phthalates could affect bone cells through a variety of pathways, so far, exposure to different phthalate metabolites might cause marked changes in bone homeostasis and development depending on dose and time of exposure.

E. Organotin compounds

Organotins are antifouling agents showed powerful biocidal activity against fungi and bacteria and used in a variety of domestic, industrial, and agricultural products as agricultural pesticides, wood preservatives, and plastics manufacturing. Tributyltin (TBT) is the structurally tin (Sn) containing organotin and are used for wood preservation, antifouling paints for boats and ships, disinfection of circulating industrial cooling water, and slime control in paper mills since the 1960s (1-16). Although TBT is a very efficient protective agent for biological attachment on the hulls of ships and boats, it is reported as a cause of masculinization of the female genitalia (imposex) in several species of meso- and neogastropods (84). Therefore, the use of TBT in antifouling paints has been prohibited by the International Maritime Organization since 2008 (85). Although TBT has been alleviated from marines, organotins remain to be used in food crop fungicides, wood preservatives and plastics manufacturing (1-16). Organotins are ubiquitous environmental contaminant and found to be measurable quantities in house and human exposure is proven by the presence of organotins in liver, milk and blood at concentrations varying from 0.05 to 450 nM (86). Human exposure occurs primarily through dietary sources like seafood intake, food wrap products, and, timber products (86).

Although, there is no proven bone effect investigated in human, several in vitro and in vivo studies showed clear effects of the organotins on bone. Experimental studies displayed a profound effect of TBT on several endocrine gland and hormonal action including

hypothalamic-pituitary-adrenal axis, steroidogenesis, reproductive organ and hypothalamic-pituitary-gonadal axis as well (87, 88).

DXA measurements of TBT treated female adult rats showed a 20% increase in BMD relative to their untreated controls (89). Furthermore, microCT images of vertebral bones from TBT-treated animals demonstrated more soft tissue while the remaining bone was denser than bones obtained from untreated rats (89). Moreover, parallel to these findings, TBT treatment in mice caused a vigorous increase in the amount of trabecular bone with increased tissue mineral density (TMD), connectivity density and the number of trabeculae while no change in trabecular thickness (90). Conversely, cortical bone was adversely affected by TBT exposure as decreased in cortical cross-sectional area on visual inspection of the 3D reconstructions and significant decrease in cortical thickness, cortical area, medullary area, while no change in cortical TMD (90). Furthermore, ultrastructural analysis of the vertebral bones with scanning electron microscopy displayed an impaired cartilaginous tissue outside the bone and, bone matrix defect with disorganized and thinner collagen fibers, in comparison to untreated control rats (89).

Additionally, TBT treated rats was shown to have increased urinary excretion of calcium, magnesium and phosphate, however, serum calcium and magnesium levels were compatible with control but phosphate which was increased in treatment group (89). However, TBT treatment lead to renal impairment in these animals with reduced creatinine clearance and, which can be explanatory for hyperphosphatemia.

Moreover, serum and bone Sn concentration and urinary Sn excretion were significantly elevated in TBT treated group. It was speculated that Sn presumably Sn^{2+} could interfere with Ca^{2+} and/or Mg^{2+} in hydroxyapatite mineral matrix and disturb bone turnover and mineralization (89).

It was demonstrated that organotins, including TBT, triphenyltin (TPhT) and dibutyltin have agonistic effect on both PPARγ and its heterodimerization partner retinoid RXRα/β and, activate RXR homodimers, as well as PPARγ:RXR and LXR:RXR heterodimers (91). Activation of these systems have toxic effect on bone. In vitro studies showed that TBT impaired the osteoblast formation from BMSCs but promote the differentiation to the adipocytes through the PPARγ, similar to rosiglitazone which is a strong PPARγ agonist (91). The agonizing effect of TBT on PPARγ was proven by a PPARγ antagonist (PPARγ specific

small hairpin RNA) which suppressed TBT-induced differentiation (91). Additionally, a sexual dimorphic effect of TBT has been found in invitro studies, as there was a greater effect on both lipid accumulation and osteogenesis in female-derived cultures versus in male-derived cultures. Moreover, TBT at lower concentration (10 nM) was sufficient to suppress osteoblastic markers and mineralization in female-derived cultures, while higher concentration (50 nM) of TBT was required for suppression in male-derived cultures. Although, TBT had a minimal effect on gene expression in osteocytes, TBT inhibited osteoclast differentiation from primary bone marrow macrophage cultures as well (90). Although TBT exposure impairs osteoblast differentiation, it was found that trabecular bone mass increased in TBT treated animal models which might be explained by changing balance between bone formation and resorption in the favour of bone formation with impairment of osteoclast.

In summary, organotin compounds are powerful EDCs and effect several mechanisms in bone-mineral metabolism along with many organ systems including liver and kidney which also effect bone metabolism indirectly. Albeit no data exists for bone effect of organotin compounds according to best of my knowledge, in vivo studies demonstrated the bone changes with the exposure doses within the detected ranges of concentrations in human samples.

F. Dioxin and dioxin-like compounds

Dioxins include a group of 75 polychlorinated dibenzo-p-dioxin (PCDD) -among these, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) plays a key role- and 135 polychlorinated dibenzofuran (PCDF) congeners. Additionally, non-ortho- and mono-ortho- substituted polychlorinated biphenyls (PCBs) display similar toxic properties to dioxins, therefore, they are classified as ‘dioxin-like compounds (DLCs) (93). Dioxins and DLCs (D/DLCs) are lipophilic compounds and extremely resistant to degradation, so far, which persist in environment in human body for decades (94). These contaminants are ubiquitous in the environment and found in cigarette smoke, herbicides, and also in the food chain, mainly in meat, fatty milk, and fish products and even released from forest fires and volcanoes (93). Almost all exposure of D/DLCs occur through the diet, and, due to their lipophilic nature, they accumulated in the body and adverse effects are likely observed several years after. D/DLCs are detected in many human tissues and highly toxic to several organ systems,

including immune, neurologic, gastro intestinal and reproductive systems (16, 20, 93). Several population based studies related to environmental exposures of different D/DLCs have yielded inconsistent results whether they caused low or high BMD or sexual dimorphic effect and time period of exposure (16, 20, 95).

Bone effects of D/DLCs have been detected with persistent organochlorine exposure after environmental incidents. High-level, accidental dietary exposure to hexachlorobenzene resulted acquired porphyria with skin changes. Additionally, bone lesions had been described as small hands with small/resorbed phalangeal, carpal, and metacarpal bones and almost completely reabsorbed terminal phalanges which were presumable related to the osteoporosis (94). In case of toxic exposure, most severe symptoms occurred in children and even deadly in infants and young children. Elevated hexachlorobenzene levels in breast milk were detected even after 20-30 years of exposure. Furthermore, infants exposed *in utero* to high concentrations of PCBs and PCDFs due to the contamination food chain, developed congenital Yusho which was characterized by skin pigmentation, low birth weight, facial oedema with secretion from eyes, natal teeth with gingival hypertrophy, large fontanelles and widely sagittal sutures with irregular calcification of cranial bones (96).

Furthermore, TCDD exposure during the gestational and postnatal period in rats is related to the reduced cross-sectional area of the tibia and the femur with decreased endosteal and periosteal circumference. However, in utero short term administration of TCDD cause bone stiffness (16, 20). Additionally, it was found that craniofacial development and growth is affected by TCDD exposure in rodents, depending on the timing and the duration of exposure. The effect size has been changing from craniofacial size reduction to altered craniofacial geometric parameters such as suture morphology and undulant asymmetry (97). Intrauterine, early postnatal and growth periods are the critical periods of time for the effect of toxicants and, during these periods TCDD exposure induced bone changes occur at lower doses than during adulthood (97). In human, it is possible that maternal burden of toxic compounds can affect newborn baby either through placental transfer or the mother's milk. It has been also postulated that craniofacial defect is caused by maternal exposure to environmental dioxins (98). Furthermore, the concentrations of dioxins in milk of breastfed women from different region of World and polar bear are comparable to contamination of environment or their food chains and, several times higher than the permissible daily intake for an adult (93, 99). All these findings show that the bioaccumulation and the dioxins in the food chain contain very

serious health threats for the population and for the next generations who are highly vulnerable to persistent health hazards and malformations.

The structure of dioxins is similar to steroid hormones so far main action through the steroid hormone pathways either effecting hormone production or modulating receptor functions including sex steroids. Dioxins also act on the aryl hydrocarbon receptors (AhR), main receptor for xenobiotics and expressed in both osteoblast and osteoclast (16, 20, 100). TCDD action on AhR has been claimed to inhibit osteoblastogenesis through the ERK activation which suppresses of Wnt-β-catenin signalling (16, 20, 100). However, the effects of TCDD and AhR ligands on osteoclastogenesis are inconsistent and in vitro studies showed both impairment of osteoclast maturation and increased osteoclast differentiation and activation (16, 20, 100). It was shown that 3,3′,4,4′,5-pen- tachlorobiphenyl (PCB 126), a dioxin-like coplanar PCB congener, exposure reduced long bone length and diameter, but, increased trabecular thickness and volume, together with decreased serum osteocalcin but no change in bone resorption marker (100). Furthermore, the suppression of osteoblastogenesis could be blunted by the AhR antagonist in the presence of PBC 126, which proved the crucial role of AhR. Additionally, PCB 126 exposure significantly reduced the serum calcium levels with significant parathyroid elevation which is an appropriate response to the calcium reduction. The reason for hypocalcaemia was found to be due to decreased intestinal calcium absorption, demonstrated by inhibition of mRNAs for the calcium transporters TRPV6 and PMCA1b in human intestinal cells with the exposure of PCB 126 in the presence of vitamin D3 (100). PCB exposure also disrupted the growth hormone response (100). These findings suggested new mechanism for skeletal toxicity with DLC exposure as the disruption of calcium homeostasis and the growth hormone axis, and also provides evidence for the direct AhR-mediated effects on bone formation.

Summary

Although population studies generally showed inconsistent findings for the bone effects of EDCs, there is a considerable amount of evidence from in vivo studies and human data from maternal and early childhood exposure to EDCs which demonstrate modifications of hormonal pathways or direct impact on bone homeostasis and formation by EDCs. In general, hazard ratio of EDCs seems highly dependent on the period of life of the exposure, duration and amount of exposure. Living creatures are vulnerable to the EDCs during growth periods, particularly prenatal period, in which organogenesis could be effected.

Another point to consider is the persistence of EDCs in environment and in the body. Lipophilic EDCs are deposited in fat tissue and persistent either in human body and transferred to offspring from mothers' system or accumulate in fish and meat products and contaminate the food chain. Several EDCs also deposited in different organ systems showed cumulative effect.

Additionally, one EDC can exert effect on different receptors and different pathways, thus, when the body expose to one molecule, single pathway in different organ systems or different pathways in different organ systems can be modified. Furthermore, EDCs are found ubiquitously in the environment and exposure to the multiple EDCs are very likely which means that our bodies are exposed to several compounds every day and many system or pathways are altered by different compounds simultaneously. So far, until now, the effects of several compounds have been studied extensively via in vitro and/or in vivo systems, nonetheless, several questions remain when the multiple compounds act on the system at the same time. Although we already have so much information about EDCs,yet it seems it is just the tip of the iceberg.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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Figure Legend

Figure 1. The main mechanisms for bone disrupting effects of EDCs; **A.** Altering sex steroid production/action: Perfluoroalkyl Substances (PFASs), Bisphenols A (BPA), Alkylphenols (APEs), phthalate esters (PEs), organotins, Dioxin and dioxin□like compounds **(**D/DLC). **B.** Elevated thyroid hormones: PFASs. **C.** Structural changes due to foetal exposure: PFASs, BPA, APEs,, PEs, D/DLC. **D.** Altering foetal/early life bone growth: PFASs, BPA, APEs, PEs, D/DLC. **E.** Effecting intestinal calcium absorption: D/DLC. **F.** Urinary mineral excretion: Organotins. **G.** Changing PTH secretion/action: D/DLC. **H.** Interfering Vitamin D action: PFASs. **I.** Changing osteoblast differentiation/function: PFASs, BPA, APEs, PEs, organotins, D/DLC. **J.** Changing osteoclast differentiation/action: PFASs, APEs, organotins, D/DLC. **K.** Changing osteoblast-osteoclast interaction: BPA. **L.** Bone matrix alterations: Organotins.

Table 1. Common Exposure sources, bone effect and main mechanisms for bone disrupting effects of EDCs

Compounds	**Sources of exposures**	**Interfering Mechanism**	**Bone effect**
Perfluoroalkyl Substances	Emulsifiers, surfactants, nonstick coatings, polymers, components of pharmaceuticals, fire retardants, lubricants, adhesives, cosmetics, paper coatings, insecticides, protective water□ and stain□resistant coatings on clothing, carpets, furnishing, food containers	• EDC effect (Sex steroids, thyroid hormones) • PPARα/Wntβ-catenin signalling • Vitamin D action	Human: Increased osteoporosis risk (2.3-96x) Prenatal exposure: Reduced birth weight? Decreased bone mass and size
Bisphenols/ Bisphenols A	Protective coatings, food□ packaging, electronic devices/media, children toys, kitchen utensils, water pipes, reusable bottles, food storage containers, dental sealants, cash and billing receipts	• EDC effect (Sex steroids) • Inflammation/Oxidative DNA damage • Oestrogen-related receptor γ /RANKL/the Wnt/β-catenin pathway	Human: Increase osteoporosis risk Animal: Dose dependent, sexual dimorphic effect Beneficial in oestrogen deficient condition

Alkylphenols	Water sources, paints, detergents, plastics and pesticides	• EDC effect (Sex steroids) • Induce apoptosis	Animal: Dose dependent, sexual dimorphic effect Decreased bone size and mass
Phthalate esters	Polyvinylchloride, vinyl floor tiles, food packaging, medical devices, latex adhesives, solvents, personal care products (cosmetics, perfumes, nail polish), insect repellent, drug coating	• EDC effect (Sex steroids) • Micro-filament disruption, DNA damage, • Increase in p53 and apoptotic protein • PPARs	Human: Negative effect on BMD Maternal exposure: increase bone length
Organotin compounds	Antifungal/antibacterial, agricultural pesticides, wood preservatives, plastics manufacturing wood preservation, antifouling paints for boats and ships, disinfection of circulating industrial cooling water, and slime control in paper mills	• EDC effect (Sex steroids) • Sn^{2+} • PPARγ/RXR/LXR	Animal: Sexual dimorphic effect Increased trabecular bone mass, disorganized bone structures
Dioxin and dioxin□like compounds	Cigarette smoke, herbicides, food chain (meat, fatty milk, fish products), released from forest fires and volcanoes	• EDC effect (Sex steroids) • Aryl hydrocarbon receptors (AhR), • Wnt-β-catenin signalling	Animal-human Prenatal exposures: Craniofacial defects, osteolysis/osteoporosis