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The Involvement of Environmental Endocrine-Disrupting Chemicals in Type 2 Diabetes Mellitus Development

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Additional information is available at the end of the chapter

1. Introduction

The incidence of diabetes and associated metabolic disorders has tripled over recent decades and continues to rise at an alarming rate. Currently, 382 million individuals worldwide are estimated to have diabetes and this number is believed to increase to 592 million by 2035 [1]; the vast majority of the cases is type 2 diabetes mellitus (T2DM).

Taking into account the number of patients impacted by T2DM and its long-term consequences in terms of morbidity, mortality and economic costs, there is considerable interest in under-standing the contribution of non-traditional risk factors to the diabetes epidemic, especially concerning environmental chemicals and particularly endocrine-disrupting chemicals (EDCs).

Researches addressing the role of environmental chemicals in the development of metabolic disorders, like obesity and T2DM, have rapidly expanded. Epidemiological and experimental evidence suggest an association between exposure to EDCs and T2DM, especially since the exposure to chemicals increased massively in the last decade.

In this chapter we tried to elucidate the following issues: (1) the concept of EDCs; (2) human exposure to EDCs; (3) particular concepts related to EDCs; (4) mechanisms of EDCs action involved in the development of T2DM; (5) evidence of T2DM in animal models; (6) epidemio-logical data linking EDCs exposure to T2DM and (7) challenges in EDCs research.

2. The concept of EDCs

Originally articulated in the early 1990s by Colbron [2], the theory of *endocrine disruption* referes to exogenous chemicals present in the environment and/or diet that interfere and disrupt physiological hormonal systems, inducing adverse effects on human and wildlife health. The term "endocrine disruptor" was first used at the Wingspread Conference in Wisconsin, USA in 1991 for those EDCs, which may lead to an adverse health effect [3]. Initially the term focused on chemicals with estrogenic activity that would alter reproductive function, commonly referred to as environmental estrogens or xenoestrogens; it has expanded to compounds with androgenic activity, as well as thyroid-active chemicals [4]. Consequently, different variable terms appeared, e.g. endocrine disrupter or endocrine disruptor, hormone mimics, hormone inhibitors or endocrine modulators [5]. Today, these compounds are commonly referred to as EDCs.

Various attempts to set up a scientific definition of an EDC have been made. Today is generally wide acceptance of using the WHO definition [6], which is similar with the EU definition [7]: „*An endocorine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

EDCs interfere and disrupt physiological hormonal balance inducing adverse effects on human health through different mechanisms including direct interaction with hormone receptors, competition on binding and transport proteins or interference with hormone metabolism (blocking or inducing the synthesis of the hormones). Starting from this definition there are clearly two requirements for a substance to be defined as an EDCs, namely the demonstration of an adverse effect and an endocrine disruption mode-of-action. Additionally, the definition implies proof of causality between the observed adverse effect and the endocrine disruption mode-of-action.

It is important to underline that the definition of EDC includes the term "adverse" which was considered as a key criterion to differentiate a genuine EDC from a mere endocrine modulator (that elicits an adaptative reversible response in endocrine homeostasis). According to WHO the term "adversity" means: "*a change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences*." [8].

This definition is not covering the potential or indicates EDC. A potential (or suspected) EDC may alter function(s) of the endocrine system and consequently may cause adverse health effects, while a substance with indication of endocrine disrupting properties (called indicated EDC) might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations.

The need for an expansion of the general term to potential EDCs is reflected on the new Guidance document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD 2011) [9]: "*A possible endocrine disrupter is a chemical that is able to alter the*

functioning of the endocrine system but for which information about possible adverse consequences of that alteration in an intact organism is uncertain".

Recently, the Endocrine Society published a statement of principles on endocrine disruptors [10] in which another definition of an EDC has been proposed: *"An EDC is an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action."* This definition empha-sizes that the ability of a chemical to interfere with hormone action is of itself a reliable predictor for adverse outcomes.

A wide variety of chemicals act as EDCs. The Endocrine Disruption Exchange List (TEDX) to date lists almost 1000 endocrine disruptors [11]. The group of EDCs is highly heterogeneous and includes synthetic chemicals used as industrial solvents/lubricants and their by-products (persistent organic pollutants – POPs, dioxins like 2,3,7,8-tetrachlorodibenzo-p-dioxin – TCDD), plastics (bisphenol A – BPA), pesticides (as dichlorodiphenyltrichloroethane – DDT), plasticizers (phthalates like diethylhexyl phthalate – DEHP), heavy metals (arsenic, cadmium), tributyl tin (TBT), fungicides (vinclozolin) and pharmaceutical agents (diethylstilbestrol – DES). POPs comprise a broad class of organohalides, including polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs) and organochlorine pesticides.

However, one should keep in mind that there is also a large number of EDCs of natural origin occurring in plants consumed as food and also some secondary metabolites from fungi that may contaminate food. Examples of EDCs with oestrogenic activity are present in soy (e.g. genistein and daidzein), mycotoxins in cereals (e.g. zearalenone), goitrogens in cabbage (with the potential to inhibit iodine uptake) and glycirrhizine in liquorice (with the potential to disturb the mineralocorticoid system) [12].

EDCs are widely dispersed in the environment. Some are persistent, having long half-lives [13], while others are rapidly degraded in the environment or human body or may be present for only short periods of time but at critical periods of development, considered windows of susceptibility.

The effects of EDCs are observed especially on sensitive groups (foetus and child), based on their susceptibility to hormonal effects [14]. Therefore, for these groups the adverse effects may occur at concentrations that are far below levels that would be considered harmful in the adult [15]

Of a special concern are EDCs such as phthalates [16], PBBs [17] and BPA, detected in pregnant women, fetuses and newborns, taking into account that exposure occuring early in pregnancy can have short-term health effects, while exposure later or during early childhood may induce cognitive and developmental deficiencies [18].

Published studies illustrate that *in utero* developmental period is a critically sensitive window of vulnerability. Disruptions during this time-frame can lead to subtle functional changes that may not emerge until later in life [19] or even later to the next generation.

Actually, is considered that *in utero* and early postnatal exposures play an important role in the development of reproductive defects, obesity and metabolic syndrome as a result of inheritable chemical-induced epigenetic changes [20, 21, 22]. As an example of epigenetic

change involved in reproductive defects one can note the developmental exposure to vinclo-zolin causing an increase in spermatogenic cell apoptosis in the adult rats [23]. This spermatogenic defect was found to be transgenerational at least to F4 generation due to a permanent altered DNA methylation of the male germ-line [21].

Criteria	Available data – according to OECD Conceptual Framework [29,30]
Category 1: EDCs	
• *in vivo data* (mode of action clearly linked to adverse effects)	• *In vivo* assays providing data on effects clearly linked to endocrine mechanisms (OECD, conceptual Framework (CF) level 5) • On a case-by-case basis, *in vivo* assays providing data about single or multiple endocrine mechanisms and effects (OECD, CF levels 3 & 4), combined with other relevant information • In special cases, categorization or QSAR approaches may provide the necessary data in combination with *in vivo* ADME information and *in vitro* data • Reliable and good quality evidence from human cases or epidemiological studies
Category 2a: Suspected EDCs	
• *in vivo data* (mode of action suspected to be linked to adverse effects) or • *in vitro* mode of action combined with toxicokinetic *in vivo* data	• *In vivo* assays providing data on effects linked to endocrine or other mechanisms (OECD, CF level 5), but where ED mode of action is suspected • *In vivo* assays providing data about single or multiple endocrine mechanisms and effects (OECD, CF levels 3 & 4) • In some cases, read across, chemical categorization and/or QSAR approaches may provide the necessary data in combination with *in vivo* ADME information and *in vitro* data • Good quality epidemiological studies showing associations between exposure and adverse human health effects related to endocrine systems.
Category 2b: Indicated EDCs	
• *Some in vitro/in silico* evidence	• *In vitro* assays providing mechanistic data (OECD, CF level 2) • QSAR, read-across, chemical categorization, ADME information (OECD, CF level 2) • System biology methods indicating associations between the substance and adverse human health effects related to endocrine systems.

Table 1. Classification of EDCs based on Danish criteria

Many other chemicals have also been implicated in promoting toxicity for multiple generations, including BPA [24] or pesticides [25], but in these cases the multigenerational effects involved direct exposures, therefore are not considered transgenerational because they are not transmitted solely through the germ cells. Only effects appearing in the F3 generation are considered to be truly transgenerational [26].

There are multiple classifications of EDCs, based on their mechanisms of action (on enzymes, transport proteins or receptors), the pathways modulated (e.g., xenoestrogens/antiestrogens,

xenoandrogens/antiandrogens), or the biological outcomes (simulation or inhibition) [27]. However, the best classification is the Danish criteria, based on available data (*in vitro*, *in vivo* and epidemiological studies), which is scientifically legitimated by Hass et al. [28]. Using this approach, EDCs are divided into 3 categories: *category 1*, for substances known to produce endocrine disrupting adverse effects in humans or animal species, *category 2a* for suspected EDCs, and *category 2b* for indicated EDC. Details regarding classification criteria are included in Table 1.

3. Human exposure to EDCs

Environmental human exposure occurs through a variety of routes and varies widely around the world. Food ingestion represents the major route by which people are exposed to EDCs. For example, diet is thought to account for up to 90% of a person's POPs body burden [31]. Taking into account that these pollutants are accumulated particularly in highly rank predators, like fish, in Sweden, consumption of fatty fish from the Baltic Sea is the major source of POPs, but also dairy products and meat contain these pollutants [32]. Also contaminated ground water is a major exposure source to inorganic arsenic in the general adult population in several regions, notably Bangladesh and India [33,34].

Regarding BPA exposure, small amounts of BPA can migrate from polymers to food or water, especially when heated. The human consumption of BPA from epoxy-lined food cans alone is estimated to be about 6.6 µg per person per day [35]. BPA has been found in concentrations of 1–10 ng/ml in serum of pregnant women, in the amniotic fluid of their fetus, and in cord serum taken at birth [36]. Moreover, BPA concentrations up to 100 ng/g were reported in placenta [37], but also in breast adipose tissue, taking into account its lipophilicity.

With the increase in household products containing pollutants and the decrease in the quality of building ventilation, indoor air has become a significant source of EDCs exposure, via inhalation [38]. Published studies [39,40] suggest that contaminated house dust may be the major source of PBBs body burden (up to 82%).

Other routes of exposure include the dermal contact (e.g. parabens or triclosan), via lactation or infants fed formula (especially for phytoestrogens as genistein or BHA). A published study [41] reported that urinary concentrations of genistein and daidzein were about 500-fold higher in infants fed with soy formula compared with those fed cow's milk formula.

4. Particular concepts related with EDCs

For decades, two major interrelated concepts are particularly addressed regarding EDCs: the low dose effect and non-monotonous dose–response relationships (e.g. "inverted U-shapes" of the dose–response curve).

Like hormones, some of EDCs act at low or very low doses, other variable, therefore their blood levels are not reflecting the real activity [42].

The traditional toxicological endpoints are not sufficient to preclude the adverse outcome. Therefore for the endocrine-sensitive endpoints it was suggested to set the NOAEL (no observed adverse effect level) or the LOAEL (lowest observed adverse effect level) from traditional toxicological studies or even below the range of human exposures, as the highest dose in experiments designed to test EDCs. For example, low-dose effects of BPA should be investigated in rodents exposed to 400 µg/kg bw/day BPA or lower, because this concentration produces levels of unconjugated BPA in the range of human blood concentrations [43]; this level is incomparable lower with the classical developmental studies, where LOAEL corresponds to 50 mg/kg bw/day [44]. Actually, most effects were seen at doses below 50 µg/kg [43], so even lower concentration than those normally detected in humans may induce adverse effects.

The effect of EDCs also depends on the type of tissue and the expression of hormone receptors on those cells, therefore the effect is considered to be tissue specific. Taking into account that some EDCs can exhibit different potencies on different receptors isoforms (e.g ERα or ERβ), the effect is also receptor-selective.

A well-known example is related to methyl-and propylparaben. *In vitro*, both parabens are binding to estrogen receptors (ERα and β), but methylparaben exhibits a weak estrogenic activity, while for propylparaben the estrogenic potential is a stronger [45,46]; *in vivo* parabens estrogenic potencies are comparable [46]. The relative activity of parabens compared with estradiol (E$_2$) is 1000 times lower [47]. Interesting, the estrogenic effects of parabens are not modulated only by estrogen receptors, but are also related to the inhibition of sulfotransferases in skin, elevating the local level of free estrogens [48].

By comparison with native hormones, EDCs exhibits lower affinity for hormone receptors, with some exceptions, such as TBT, which is the most potent agonist of retinoid-X-receptor and PPARγ (peroxisome proliferator activating receptor subtype gamma) in the low nanomolar range [49].

The shape of the dose-response curve for EDCs does not follow the usual dose-response curve. The curve can have a sigmoidal shape (relationship between dose and effect occure based on the saturability of the receptors), but in general EDCs do act via a non-monotonic dose-response relationship [43]. In this case, the slope of the curve changes sign somewhere within the range of the examined doses. In other words, some effects can be seen at very low doses, while slightly higher doses can show no effects and then, at high doses, some different types of effects may be found.

For example, hypoglycaemic or hyperglycaemic effects of TCDD (tetrachlorodibenzo-p-dioxin) observed in animal models are dose-dependent. Repeated low-dose of TCDD (500 ng/kg p.o.) reduced glucokinase gene expression in mice [50], while higher dosage (12.8 µg/kg TCDD p.o) induced a significantly reduction of serum glucose levels [51]. Moreover, a higher dose of TCDD (116 µg/kg i.p.) impaired insulin-stimulated glucose uptake in mice [52].

Another example is BPA. On isolated pancreatic islets of Langerhans BPA induced an increase of insulin content following an inverted U-shape dose response curve, with a significant effect observed at 1 nM and 10 nM BPA compared to vehicle. Higher concentrations of BPA (1 μM) produced no increase in insulin content [53].

A similar non-monotonic behaviour is exhibited by BPA in animal studies where treatment with high dosage (BPA 100 μg/kg bw/day) twice per day for 4 days increased pancreatic insulin content, produced hyperinsulinemia, and induced insulin resistance in adult male mice [54] while sustained exposure of pregnant mouse dams to lower levels of BPA (10 μg/kg bw/day) from gestation day 9–16 impaired glucose tolerance, increased plasma insulin, triglycerides and leptin concentrations, thus revealing the ability of BPA to alter pancreatic function and metabolic parameters [55].

Also PBBs, especially PBDE-153 (polybrominated diphenyl ether) showed an inverted U-shaped association with metabolic syndrome in epidemiological study in humans [56].

So, the most important effects of EDCs observed in animal models are those that occur at low doses, similar with the level of human environmental exposure, therefore only these toxicological data should be corroborated with epidemiological studies.

We also should note that for the assessment of EDCs effects, the assumption of an experimental threshold (like a NOAEL) is questionable. A first reason is related to the lack of adversity for some endpoints investigated (e.g., uterotrophic assay). A second reason is connected with the difficulties to establish it. According to Blair et al. [57], a threshold could be established in the absence of endogenous hormone at some life stage, if the endogenous hormone induces no adverse effect or if there is effective homeostatic control. Even if a threshold does exist, for a certain endpoint, taking into account the population variability and the connection with already ongoing biological process (EDCs exhibit additive effects), the threshold will not be observable.

5. Mechanisms of action involved in development of T2DM

In addition to the classical pathway modulated by EDCs (as interaction with aryl hydrocarbon receptor (AhR) or nuclear hormone receptors, in particular estrogens, androgens and thyroid receptors), it was observed that EDCs exhibit the capacity to modulate signalling pathways involved in energy regulation, in general, and glucose homeostasis in particular. EDCs can decrease insulin sensitivity, impair β-cell insulin production, impair cellular insulin action or alter the intermediary metabolism. All these mechanisms contribute to the pathogenesis of T2DM. Experimental data revealed that one EDC acts on different levels and receptors, therefore the ultimate effect on insulin action may be the result of all pathways involved (Table 2).

Compound	Mechanisms of action	Primary effects
BPA	(+)ERα via ERK1/2	↑ β-cell insulin content
		↓ GLUT4
	(+) ER-GPR30	↑ β-cell insulin content
	↑ CREB phosphorylation	↑ $[Ca^{2+}]i$
	↑ Akt phosphorylation	↓ IRS activity
	↑ PI3-kinase activity	
	Potent antagonist of PPARγ	↑ TNF-α, IL-6; ↓ adiponectin release
	(-) PDI	↓ IR phosphorylation
PCBs	(+) AhR	↑ TNF-α, IL-6; ↑ MCP-1
(especially PCB-77)		↓ PEPCK
	(+) MAPK1/2	↑ [Ca]i
	(+) CaMK2	(-) IRS-1 phosphorylation
	(-)Akt phosphorilation	Insulin resistance
	↑ oxidative stress	↑ β-cell death

Legend: (+) activate; (-) inhibit; ↑ increase; ↓ decrease; ER-estrogenic receptors; ERK1/2 extracellular regulated kinase1/2; GLUT4 – glucose transporter type4; ER-GPR30-membrane G protein related estrogen-receptor; CREB-cAMP response element-binding protein; $[Ca^{2+}]i$ - intracellular calcium ion levels; IRS – insulin receptor substrate 1; PI3 - phosphatidylinositol-4,5-bisphosphate 3-kinase; PPARγ - peroxisome proliferator-activated receptor gamma; TNF-α - tumor necrosis factor-α; IL-6 interleukin-6; PDI - protein disulfide isomerase; IR – insulin receptor; AhR -aryl hydrocarbon receptor; MCP-1- monocyte chemoattractantprotein-1;PEPCK- phosphoenolpyruvate carboxykinase; MAPK1/2 - mitogen-activated protein kinase 1/2; CaMK2 - Ca²⁺/calmodulin-dependent kinase II

Table 2. Example of EDC that acts on multiple pathways

Interestingly, some EDCs such as TCDD [58], PCBs [59], inorganic arsenic [60] or cadmium [61] that modulate β-cell function, may also play a role in type 1 diabetes mellitus, as a result of β-cell destruction or dysfunction as well as promotion of β-cell death.

The following mechanisms of action can explain the development of T2DM: (1) activation of aryl hydrocarbon receptor (AhR) or interaction with estrogenic receptors (ERs); (2) β-cell dysfunction and impairment of insulin secretion; (3) impairment of cellular insulin action and (4) alteration of the intermediary metabolism.

5.1. Activation of AhR or interaction with ERs

EDCs can exhibit their metabolic effects through the classical pathways, such as the activation of AhR or the interaction with ERs. It is well known that AhRs are involved in the glucose homeostasis [50], therefore activation of AhR or its heterodimerization partner, called ARNT (AhR nuclear translocator) by EDCs could interfere with glucose uptake. Gunton et al. [62] revealed that abnormal ARNT expression causes impaired insulin release in human islets, but it is unclear if this effect is a cause or a consequence of T2DM.

PCBs [63] or PBDE [64] reduce primary hepatocyte glycogen levels and impair gluconeogenesis due to a specific down regulation of phosphoenolpyruvate carboxykinase (PEPCK) expression, a central regulator of gluconeogenesis. The alteration of PEPCK expression was

proportional to activation of the AhR, suggesting a direct correlation between AhR activation and perturbation in intermediary metabolism.

PCBs (especially PCB-77) also impaired glucose homeostasis through another AhR-dependent mechanism, associated with an adipose-specific increase in TNF-α (tumor necrosis factor-α) expression and interleukin-6 (IL-6) levels [65, 66]. In addition to its effects on TNF-α and IL-6, PCB-77 also increases expression of MCP-1 (monocyte chemoattractant protein-1), an adipocyte-secreted molecule with inflammatory function that contributes to global insulin sensitivity [67].

The implication of estrogenic receptors (especially ERα) in the pancreatic β-cell insulin content is confirmed by BPA studies. At physiologically relevant doses, BPA increases pancreatic β-cell insulin content, the effect being mediated by ERα activation via extracellular regulated kinase1/2 (ERK1/2) [53]. ERα is also implicated in β-cell survival [68], regulates the glucose transporter (GLUT4) in skeletal muscle [69] and insulin sensitivity in the liver [70]

Also the non-classical membrane G protein related estrogen-receptor (ER-GPR30), expressed in pancreatic islets is involved in the effects of estrogens on glucose metabolism, its deficiency inducing hyperglycemia, impaired glucose tolerance, and elevated blood pressure [71]. GPR30 activation by several EDCs, e.g. BPA could partly contribute to the increase of insulin after BPA exposure [72]

5.2. Beta-cell dysfunction and impairment of insulin secretion

Taking into account their reduced capacity to fight against chronic oxidative stress and the lack of detoxification mechanisms, β-cells are the perfect target for EDCs that disrupt their structure and function or promote death.

Oxidative stress is the mechanism implicated in T2DM induced by exposure to inorganic arsenic. At relatively low concentrations arsenic-induced oxidative stress produces impairment of glucose-stimulated insulin secretion [73], while exposure to high concentrations results in irreversible damage (including oxidative damage) to β-cells followed by apoptosis or necrosis [74]. Actually, the mechanism behind arsenic-induced oxidative stress is more complex. Chronic exposure to relative low concentration of arsenite (1–2 μM) produced an adaptive response, activating the transcription factor NF-E2–related factor 2 (Nrf2). Even if Nrf2 is generally considered a protective cellular component that induces antioxidant / detoxification enzymes [73], in this case Nrf2 activation that diminishes the reactive oxygen species (ROS) have a negative impact on insulin secretion. In normal cells, ROS signals produced during glucose metabolism increase the insulin secretion [75], thefore arsenic Nrf2-mediated response appears to play an important role in reduced glucose-stimulated insulin secretion. Inorganic arsenic also promotes β-cell apoptosis via induction of endoplasmic reticulum stress, but this mechanism is poorly studied and necessitates further investigations [76].

Regarding the interference and impairment of insulin secretion, different examples can be provided, especially taking into account that insulin secretion is a calcium-dependent process. On isolated pancreatic β-cells BPA at low concentration (10^{-9} M) increases the phosphorylation

of CREB (transcription factor cyclic adenosine monophosphate-response element-binding protein) via an alternative mechanism, involving a non-classical membrane estrogen receptor [77], which provokes the closure of K^+/ATP channels. As a result the plasma membrane depolarizes, opening the L-type voltage-dependent calcium channels and increasing intracellular calcium ion levels $[Ca^{2+}]i$ and triggering insulin secretion [78].

Also abnormal levels of $[Ca^{2+}]i$ and the impairment of insulin secretion were observed on isolated islet cells exposed to TBT and are associated with the disruption of protein-kinase A activity [79].

PCB treatment of RINm5F cells resulted in a rapid increase of $[Ca^{2+}]i$ as a result of $Ca^{2+}/$ calmodulin-dependent kinase II (CaMK2) and mitogen-activated protein kinase 1 and 2 (MAPK 1 and 2) activation [80]. In addition, RINm5F cells exposed to inorganic arsenic (III) exhibited a reduction of insulin secretion as a result of decreased calcium-dependent calpain-10 activity, a pathway that triggers insulin exocytosis [81]. Arsenic also reduces the β-cell line proliferation in a dose-dependent manner, as an indirect consequence of the decrease in insulin secretion.

5.3. Impairment of cellular insulin action

Taking into account that insulin signalling mechanisms are described in detail elsewhere [82], we present only a short analysis of the insulin signalling cascade in order to provide some insights into how EDCs might modulate insulin action.

Insulin acts on target cells and stimulates glucose uptake via membrane –bound tertrametric insulin receptor (IR) with tyrosine kinase activity. Binding to extracellular α-subunits of IR leads to activation of tyrosine kinase. Once the tyrosine kinase of IR is activated, it promotes autophosphorylation of the β subunit, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162, and Tyr-1163) is required for amplification of the kinase activity. Then tyrosine kinase phosphorylates the insulin receptor substrate proteins (IRS 1 and 2) and phosphotyrosine residues on IRS proteins become targets for the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3-kinase).

The activated PI3-kinase generates higher levels of phosphotidylinositides, such as phosphatidyl-inositol-3,4-bisphosphate (PIP2) and phosphatidyl-inositol-3,4,5-trisphosphate (PIP3), which bind to the phosphoinositidedependent kinase-1 (PDK1). PDK1 can directly phosphorylate all protein kinase C (PKCs).

Downstream from PI3-kinase, activation of Akt (protein kinase B) produces its effects, including those on gene transcription as well as glucose uptake through the translocation of facilitative glucose transporter 4 (GLUT4) to the cell membrane.

Each step in this signaling cascade is a potential target for EDCs. EDCs interact and impair the cellular insulin effect acting at different levels: on IRS, PI3-kinase, Akt, PDK or PKC or through associated mechanisms. Some examples are included in figure 1.
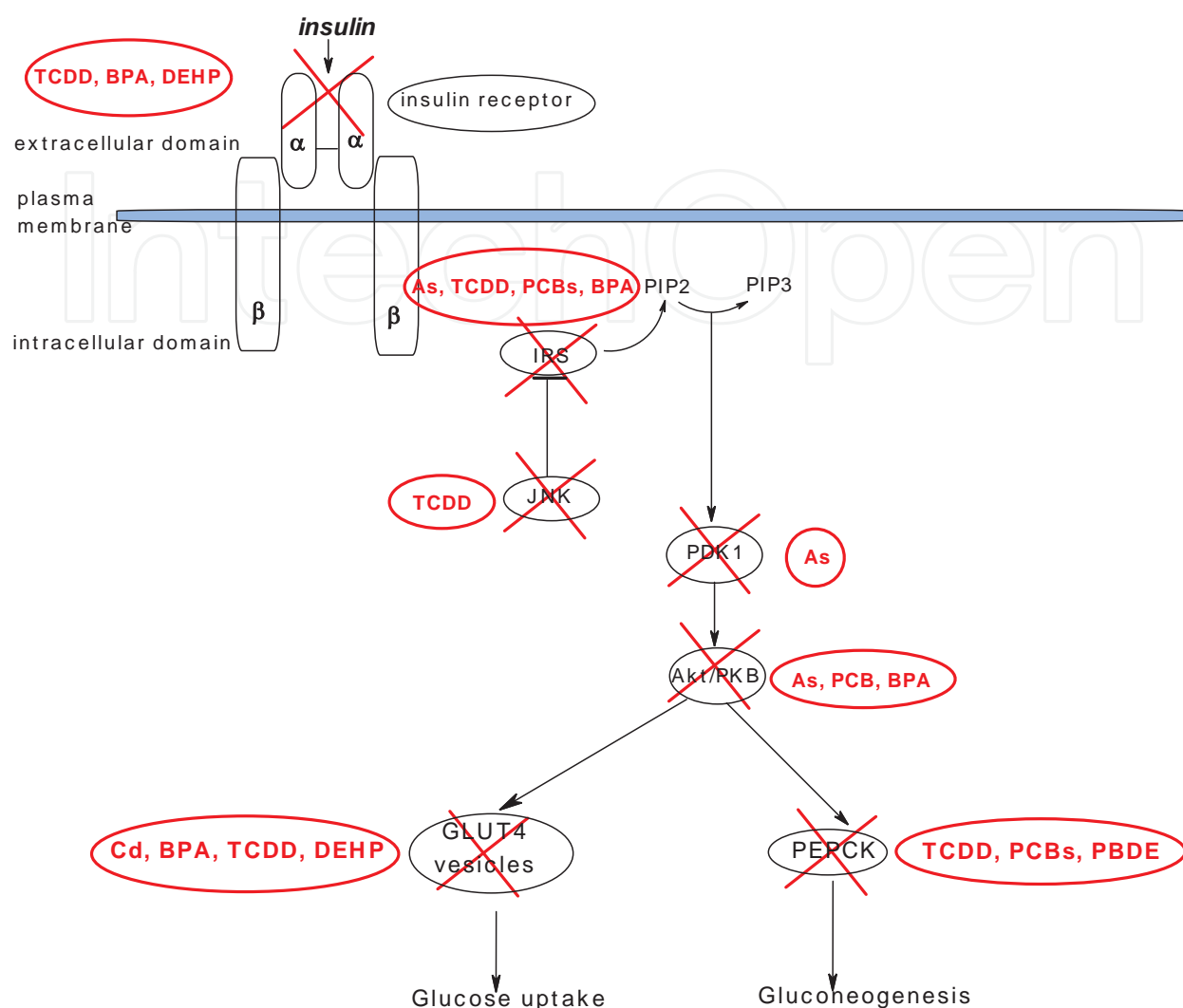
Figure 1. A schematic illustration of EDCs interference on insulin signaling pathways. Arrows represent an activation process; X represent an inhibition process

For example, TCDD, arsenic or PCB alter IRS activity (especially IRS-1 phosphorylation) through different mechanisms: TCDD increasing MAPK (mitogen-activated protein kinase) activity and JNK (c-Jun N-terminal kinase) activity [83], arsenic decreasing p70-S6-kinase activity [84] and PCBs increasing CaMK2 and MAPK 1 and 2 activity [80].

Other EDCs act on insulin-stimulated Akt phosphorylation. Akt phosphorylation is attenuated by PCB-77 [65] or BPA [55]. Arsenic (III) exposure was also associated with suppression of AkT phosphorylation and glucose uptake in 3T3-L1 adipocytes, causing an insulin resistant phenotype [85,86].

BPA acts not only on Akt phosphorylation, but also stimulates tyrosine phosphorylation via PI3-kinase, the global effect being the impairment of IRS activity [87].

Additional studies have demonstrated that TCDD [83], BPA [88] or DEHP [89] are modulating the insulin signalling cascade by down-regulation of the insulin receptors or acting on plasma membrane GLUT4 level and antagonizing insulin action [90].

Also, cadmium induces impaired glucose tolerance by down-regulating GLUT4 expression in adipocytes [91].

Inorganic arsenic (III) inhibits PDK-1 activity, thus suppressing PDK-1-catalyzed phosphorylation of PKB/Akt and p-PKB/Akt–mediated translocation of GLUT4 transporters to the plasma membrane [85,92].

5.4. Alteration of the intermediary metabolism

In addition to direct effects on the insulin signalling cascade, EDCs alter the intermediary metabolism, mainly the gluconeogenesis. TCDD [63], or PCBs [93] have been shown to down-regulate the expression of phosphoenolpyruvate carboxykinase (PEPCK), reducing its activity and inducing hypoglicemia. In the case of PCBs, the suppression of hepatic PEPCK expression was proportional to activation of the AhR, suggesting a direct correlation between AhR activation and perturbation of the intermediary metabolism.

Alternative mechanisms are implicated in the development of T2DM, such as inflammation or oxidative stress. For example, PCB-77 has been shown to promote expression of IL-6 and TNF-α, leading to impaired insulin signalling in endothelial cells [64]. In addition to its effects on TNF-α and IL-6, PCB-77 also increases expression of MCP-1, adipocyte-secreted molecule that contributes to global insulin sensitivity [66].

BPA augments secretion of IL-6 and TNF-α, but simultaneously inhibits the release of adiponectin in human adipose tissue explants [94]. The suppression of adiponectin release could promote insulin resistance and increase the risk of developing the metabolic syndrome. The same outcome is expected based on elevated IL-6 levels.

We should highlight the strong correlation between increased TNFα production and insulin resistance [95]. TNFα affects insulin resistance by downregulating the glucose transporter, interfering with IR phosphorylation and signaling, and by inhibiting transcription factors that affect insulin sensitivity.

Some EDCs are acting on other nuclear receptors involved in fat metabolism and regulation of glucose uptake, like PPARs (peroxisome proliferator-activated receptors), especially on PPARγ which are involved in the regulation of adipocyte differentiation, production of adipokines or insulin responsiveness [96]. By antagonizing PPARγ, EDCs significantly inhibit the release of adiponectin that has insulin-sensitizing effects, as it enhances inhibition of hepatic glucose output as well as glucose uptake and utilization in fat and muscle tissues. So, adiponectin levels are correlated with insulin sensitivity, therefore supressing its biological effects affects glucose homeostasis.

For example, BPA at 0.1 and 1 nM doses is a potent antagonist of PPARγ, which suppresses adiponectin release in human adipose tissue explants [97]. In the same time, BPA influences

adiponectin level via another mechanism that implies binding to protein disulfide isomerase (PDI), a critical player in the retention of adiponectin in cells [98]

Interestingly, *in vitro* it was observed that ERβ can act as a negative regulator of PPARγ, decreasing ligand-induced PPARγ and PPARγ induced adipogenesis [99], therefore it is obvious that PPARγ function is affected by EDCs directly interacting with the receptor, but also by EDCs that modulate ERβ activity. Also, TCDD inhibits adipogenesis through a suppression of PPARγ [100].

Other EDCs such as phthalates (DEHP) act as potent agonists of PPARα or PPARγ. In rodent models, PPARα appears to mediate high-dose DEHP-induced body weight loss [101], but these effects can not be extrapolated to humans, taking into account that the levels required to activate human PPARα are almost three times higher than the concentrations required to activate mouse PPARα, and the maximum-fold induction is less for human PPARα than for mouse PPARα [102].

In conclusion, the investigation of insulin signaling pathways may explain how EDCs modulate insulin action, especially in the case of exposure to singular compound; however, in the context of accidental or occupational exposures, humans are exposed to mixtures of compounds and this complicates understanding the global biological effects. For example, if different compounds are acting through the same pathway, but at different points, co-exposure is likely to have additive or synergistic effects that promote the development of insulin resistance and T2DM. Moreover, points of pathway convergence (e.g., IRS) might be the perfect target of drug intervention to treat environmentally-mediated diabetes.

6. Evidence of T2DM in animal models after EDC exposure

There are enough published data in animal models that investigated the correlation between EDCs exposure and T2DM. This correlation between exposure in the animal models and alterations in glucose homeostasis, including hyperglycemia and glucose intolerance is crucial, taking into account that epidemiological studies fail to establish a causality.

A number of examples are given below.

Repeated low dose of TCDD (500 ng/kg bw), administered orally, reduced glucokinase gene expression, predicting a rise in blood glucose levels on C57BL/6 mice [103], while in diabetic rats, a higher dose of 12.8 μg/kg bw TCDD had significantly reduced serum glucose levels by day 8 of treatment [51]. Moreover, a single but high dose of TCDD (116 μg/kg bw i.p.) impaired insulin-stimulated glucose uptake in C57BL/6 and DBA/2J mice [52].

Administration of 75 μg/kg bw DEHP for 14 days reduced insulin levels and raised serum glucose levels in exposed female Wistar Kyoto rats [104]. Almost similar results were obtained on male rats treated for a longer period (21 days) with diet supplemented with 2% (w/w) of DEHP [105].

Male mice treated orally with 0.5-50 μg/kg bw TBT for 45 days demonstrated hepatic steatosis, hyperinsulinemia, hyperleptinemia and a reduction in hepatic adiponectin levels, in a dose-dependent fashion, confirming PPARγ stimulation observed *in vitro* [106].

Similar results were obtained in adult Sprague-Dawley rats exposed for 28 days to crude salmon oil containing POPs [107]. The animals developed insulin resistance syndrome, abdominal obesity and hepatosteatosis, the contribution of POPs to insulin resistance being confirmed also by the same authors in cultured adipocytes. These findings are important since POPs are accumulating in the lipid fraction of fish, and fish consumption represents the main source of POP exposure to humans.

Also coplanar PCBs (e.g. PCB-77 and PCB-126), at dosage of 50 mg/kg orally, impaired glucose homeostasis in lean C57BL/6 mice and mitigate beneficial effects of weight loss on glucose homeostasis in obese mice [66], while inorganic arsenic (III) administered in the drinking water for 20 weeks, at doses of 25 or 50 ppm As/kg bw/day, impaired glucose tolerance in C57BL/6 mice in a dose-dependent manner [108].

In the animal studies mentioned before, not just blood glucose levels were investigated, but other markers of insulin regulation, such as HOMA-IR, pancreatic production of NO, SOD and CAT activity, in order to reflect the magnitude of the global disturbance. While most studies have demonstrated perturbations in insulin action, some of them have shown improved glucose tolerance or even hypoglycaemia. Acute exposure of adult male mice to high dosage of BPA (100 μg/kg bw/day) produced a rapid hyperinsulinemia based on significant increase in β-cell insulin content, as a direct result of BPA estrogenic properties [109], while sustained exposure to lower dosage (10 μg /kg bw/day) impaired glucose tolerance and reduced the hypoglycaemic effect of insulin, through a compensatory peripheral insulin resistance [54]. These results are easily correlated with non-monotonic dose response curve exhibited *in vitro* by BPA.

Taking into account that EDCs alter glucose homeostasis and endocrine pancreatic function not just in adult animals but also during pregnancy or in offspring, these effects were also investigated in pregnant animals. For example, prenatal exposure to high dosage of diisobutyl phthalate (600 mg/kg bw/day) from gestation Day 7 to Day 21 reduced plasma leptin and insulin levels in male and female offspring, complementary to sexual distrurbance [110]. Maternal glucose intolerance was observed in pregnant mice exposed to inorganic arsenic (V) at dosage of 9.6 mg/kg bw, this explaining the neural tube defects induced by arsenate [111].

In conclusion, all these examples regarding *in vivo* effects in animal models are highly suggestive, taking into account that the experimental exposure is very close or even similar with environmental human exposure. However, data on co-exposure are lacking, therefore new studies should focus on this issue, in order to reveal possible additive, synergistic or antagonistic effects exhibited by the mixtures.

7. Epidemiological data linking EDCs exposure to T2DM

There is growing concern in the scientific community that EDCs may be contributing to the high incidence of diabetes, particularly in young people.

Epidemiological studies (as occupational or population-based studies) but also disasters tried to link, at least partially, the environmental exposure to EDCs with the development of T2DM.

We collected and compiled from a comprehensive scientific literature the most relevant epidemiological studies concerning the T2DM and exposure to EDCs like TCDD, arsenic, phthalates or BPA.

Disasters such as Seveso accident or exposure of military personnel during the Vietnam War and follow-up studies have suggested a link between TCDD exposure and a higher incidence of diabetes [112, 113, 114]. Other cross-sectional studies [115, 116] did not revealed such correlation, while longitudinal studies that have been conducted are inconsistent [117].

Some poisoning cases reported during late 1970s have involved contaminated rice oil with PCBs. PCB exposure was associated with an increased prevalence of diabetes in women [118]. Other prospective studies on PCB153 showed a positive association with T2DM, but taking into account the variation across studies, it did not allow a metaanalysis. For example, five studies used different diagnostic strategies and several approaches to address serum lipid levels [119]. In addition, the age varied between cohorts from 18 to 30 years [119] to 70 years [120] while gender was also inconsistent, exclusively female in one study [121], exclusively male in another [119] and mixed in the remaining studies [120; 122, 123]. The temporal and geographic variation among the studies induced significant differences in the exposure assessment especially on duration of exposures or on the composition of the mixtures. However, other variables must be considered in the interpretation of PCBs studies, such as the use of PCB153 as a surrogate for total PCBs or the lack of data regarding kinetics of different PCBs (especially on accumulation) that influence their current serum levels.

A closer evaluation of the cohorts described before revealed the non-monotonic exposure-response relationships exhibited by PCBs: the risk of diabetes was significantly increased with small increases within the lower ranges of PCBs concentrations, but only slightly increased with significant increases in concentrations of PCBs. This non-monotonic relationship exhibited by PCBs in cohorts was also observed in brominated flame retardants studies, like those conducted on PBDE-153 [124], but not in BPA cohorts, where BPA urinary levels were associated with diabetes incidence in a dose-dependent manner [125].

Evaluation of studies conducted on EDCs (PCBs or TCDD) reveal that many of them focused specific populations (e.g. occupational studies or exposure through industrial accidents or disasters), so they might not reflect the actual risk of the general population. However, recent investigations were done on representative sampling of the US population, using data from the National Health and Nutrition Examination Survey (NHANES). For example, Lee et al. [126] reported strong and highly significant associations, among participants in the NAHNES study, between serum concentrations of POPs and the HOMA-IR insulin resistance values, after correction for age, sex, BMI, and waist circumference.

Study design	Diagnosis	Findings (95% CI)	As in drinking water (µg/L)	Exposure	Ref.
Low-to-moderate exposures (< 150 µg/L drinking water)					
Cross-sectional [male 225 nonsmokers 209 smokers]	Self-report	Increased urinary As in nonsmoking diabetics	n.r.	Nonsmokers: 5.59 (diabetics) vs. 4.7 (nondiabetics) µg As/L Smokers: 7.27 (diabetics) vs. 5.41 (nondiabetics) µg As/L (urine)	127
Cross-sectional [n=11,319]	Self-report prior to baseline	adjOR=1.24 (0.82- 1.87)	0.1–864	41–92 (Q3) vs. 0.1–8 (Q1) µg As/L drinking water, CEI	128
Case–control [n=144 female]	Fasting blood glucose, OGTT	Increased As in urine from diabetics	n.r.	4.13 (diabetics) vs. 1.48 (nondiabetics) µg As/L in urine	129
Retrospective [41,282 male 38,722 female]	Death certificate	Male SMR =1.28 (1.18-1.37) Female SMR =1.27 (1.19- 1.35)	1.27–11.98	6 counties vs. state µg As/L (drinking water)	130
Case–control [n=87]	Not reported	RR=0.87 (0.5- 1.53)	n.r.	75th vs. 25th percentile µg As/L (urine)	131
Retrospective [n=1,074 deaths]	Death certificate	RR=1.6 (0.36- 7.16)	n.r.	Residence time within 1.6 km (1 mi): ≥ 10 years vs. < 1 year	132
Cross-sectional [n=235]	Hospital records	RR=1.098 (0.98- 1.231)	16–272	21–272 (range) vs. 16–38 (range) µg As/L (drinking water)	133
Case–control [n=117]	Not reported	RR=1.09 (0.79- 1.49)	n.r.	75th vs. 25th percentile µg As/mL (plasma)	134
Cross-sectional [n=1,185]	Self-report	adjOR=1.02 (0.49 - 2.15)	0–2,389	> 10 vs. < 2 µg As/L (well-water)	135
Cross-sectional [n=788]	Fasting blood glucose, self-report, medication	adjOR=3.58 (1.18- 10.83)	-	18 (≥ 80th) vs. 3.5 (≤ 20th percentile) µg As/L (urine)	136
Cross-sectional [n=1,279]	Fasting blood glucose, self-report, medication	adjOR=2.60 (1.12 - 6.03)	-	7.4 (80th) vs. 1.6 (20th percentile) µg As/L (urine)	137
Cross-sectional [n=795]	Fasting blood glucose, self-report, medication	adjOR=1.15 (0.53 - 2.50)	-	12 (≥ 80th) vs. 2.7 (≤ 20th percentile) µg As/L (urine, not adjusted for creatinine)	138
Study design	**Diagnosis**	**Findings (95% CI)**	**As in drinking water (µg/L)**	**Exposure**	**Ref.**
High exposure (≥ 150 µg/L drinking water)					
Cross-sectional [n=11,319]	Self-report prior to baseline	adjOR=1.11 (0.73- 1.69)	0.1–864	176.2–864 (Q5) vs. 0.1–8 (Q1) µg As/L drinking water, CEI	134

Study design	Diagnosis	Findings (95% CI)	As in drinking water (µg/L)	Exposure	Ref.
Cross-sectional [n=891]	Self-report, OGTT, treatment history	adjOR=10.05 (1.3-77.9)	700–930	≥ 15 vs. 0 ppm-year drinking water, CEI	141
Case–control [n=235]	Glucose, blood	OR=2.95 (0.954, - 9.279)	3–875	218.1 µg As/L vs. 11.3 µg As/L(mean)	140
Cross-sectional [n=1,107]	Self-report, OGTT, glucosuria	adjPR=5.2 (2.5- 10.5)	10–2,100	Keratosis vs. non-keratosis	141
Retrospective [n=19,536]	Death certificate	SMR=1.46 (1.28- 1.67)	250–1,140	Blackfoot endemic region vs. national reference	142
Prospective [n=446]	Fasting blood glucose, OGTT	RR=2.1 (1.1- 4.2)	700–930	≥ 17 vs. < 17 mg/L-year As (drinking water, CEI)	143
Cross-sectional [n=706,314]	Insurance claims	adjOR=2.69 (2.65- 2.73)	350–1,140	Endemic vs. non-endemic region	144

Abbreviations: 95% CI – confidence interval 95%; adjOR-adjusted odds ratio; adjPR-adjusted prevalence ratio; As-arsenic; CEI, cumulative exposure index; OGTT-oral glucose tolerance test; Q-quintile; RR-relative risk; SMR-standardized mortality ratios; n.r. – not reported

Table 3. Association between arsenic and diabetes

The correlation between the level of arsenic in drinking water and the incidence of T2DM was extensively investigated. The published cohorts were categorized based on the level of exposure (table 3) in order to identify the correlation between exposure and critical endpoints. In addition to diabetes, epidemiological studies have associated exposure to arsenic with other measures of disturbed glucose homeostasis, such as glucose tolerance or metabolic syndrome.

Preliminary analysis on the existing human data provide limited support for an association between arsenic and diabetes in populations exposed to relatively high levels (≥ 150 µg As/L in drinking water), but the evidence is insufficient to conclude that exposure to low to moderate level is associated with diabetes. However, a major gap is obvious. The measurement of arsenic in drinking water supplies, which was often used to assess arsenic exposure, is not appropriate to calculate the internal dose, taking into account individual variation in arsenic uptake and metabolism. Also, individual information on the duration and timing of exposure, which is critical, especially for estimating cumulative exposure, are missing.

Regarding phthalates, cohort studies were mainly focused on correlation between exposure and obesity and less on T2DM. However, those found were done on representative sampling of the US population, using data from NHANES. For example, Stahlhut et al. [145] investigated 1,292 adult US male participants in the NHANES 1999–2002 and revealed that urinary concentrations of three phthalate metabolites (mono-n-butyl phthalate, monobenzyl phthalate and monoethylphthalate) were associated with increased insulin resistance, assessed by HOMA-IR. In addition, phthalates levels were associated with increased waist circumference. A similar association between urinary phthalate metabolite concentrations, body mass index

and waist circumference was found in another cross-sectional study of NHANES data [146]. However, considering the methodological limitations of the existing data, there is no sufficient evidence to conclude there is a correlation between phthalates and diabetes or obesity.

The epidemiological data on BPA and T2DM is less consistent compared with POPs, but is growing. There are two cross-sectional analyses of NHANES data 2003-2008 that reported a positive associations of BPA exposure (median 2.5 and 1.8 μg/l) with self-reported diagnosis of diabetes [125, 147]. However, these analyses have an important weakness that limits their value: the use of a single spot urine sample collected concurrent with the information on diagnosis of diabetes. The single spot sample reflects only recent BPA exposure, so cannot be extrapolated to longer period (like years or decades) which is relevant for the development of diabetes. Other large cross-sectional studies on BPA in China provide conflicting data [148,149].

A closer evaluation of all epidemiological studies on EDCs reveals some weaknesses, such as the assessment of one compound as a surrogate for total mixture (in case of PCBs), the lack of data regarding kinetics, especially on accumulation in lipid-rich tissues (in case of POPs), limited type of biological material used for direct measurement EDCs (serum or urine) or environmental measurement which is not appropriate to calculate the internal dose (in case of As). Other caveats must be considered in the interpretation of studies, such as heterogeneity in the definition of diabetes or insulin resistance.

8. Challenges in EDCs research

There are a number of challenges limiting our understanding of the impact of EDCs on T2DM related to the physical properties of EDCs: the selection of experimental models to assess effects on glucose homeostasis or coexisting risk factors on the exposed individuals included in the epidemiological studies.

The thousands of chemicals released into the environment create the real scenario of human co-exposure and an enormous analytical challenge in the assessment. Sometimes the physical properties of EDCs such as lipophilicity contribute to their accumulation and persistence in human tissues, even after the exposure has terminated. In this case biomonitoring is the key for the assessment of EDCs. Regarding the types of sample used in analysis, these must be expanded beyond urine and serum to lipid-rich organs (e.g., POPs are accumulated in brain and adipose tissue) as well as tissues relevant to *in utero* and early postnatal stages of exposure (e.g., human breast milk). Also the development of clinical biomarkers it will be useful to identify chemically exposed population.

Although environmental and tissue levels of certain EDCs (e.g., PCBs) have declined in some countries in response to EU regulations, they remain of concern in other countries, and uncertainty still exists regarding future trends.

Another important challenge is related with the lack of clear structure-function relationships that excludes a possible *in silico* prediction of endocrine disrupting effects and demands the use of bioassays to characterize the physiological effects of the exposure.

The experimental design is further complicated by non-monotonic dose response correlation, multiple mechanisms of action for a single compound, potential additive, synergistic or antagonistic effects observed during co-exposure or the lack of adversity for some endpoints defined in OECD guideline (e.g. uterotrophic assay).

The main gaps of epidemiological studies were already addressed. Still other factors like geographic and temporal variation among the studies can induce differences in the exposure assessment, especially on the composition of chemical mixtures (especially for POPs) and duration of exposure.

Also inter-individual variation, transgenerational effects or predisposing factors (such as obesity or a family history of diabetes) may influence the metabolic effects observed in the epidemiological studies.

9. Conclusion

More studies are necessary to establish the exact mechanisms through which EDCs determine impairments of glucose homeostasis; these studies are imperatively important in order to impose international guidelines that will lead to a reduction of the incidence of T2DM cases induced by chemical exposure.

Acknowledgements

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