Review

Gut Microbiota, Endocrine-Disrupting Chemicals, and the Diabetes Epidemic

Ganesan Velmurugan,1,* Thamarajan Ramprasadath,1 Mithieux Gilles,2 Krishnan Swaminathan,3 and Subbiah Ramasamy1,*

Diabetes is rapidly emerging as one of the biggest health concerns worldwide, with profound implications for disability, mortality, and costs. This suddenly escalating rate of diabetes correlates with global industrialization and the production of plastics, pesticides, synthetic fertilizers, electronic waste, and food additives that release endocrine-disrupting chemicals (EDCs) into the environment and the food chain. Emerging evidence indicates an association between exposure of EDCs and diabetes. In humans, these chemicals are also metabolized by the gut microbiota and thereby their toxicodynamics are altered. In this review we highlight studies that focus on the role of gut microbiota in EDC-induced hyperglycemia and dysregulated glucose homeostasis. We also discuss the translational implications of understanding EDC–microbiota interactions for the diagnosis and treatment of diabetes.

Diabetes Epidemic: A Global Health Emergency

The diabetes epidemic (see Glossary) refers to the recent rapid increase in diabetes prevalence that is considered to represent one of the largest global health emergencies of the 21st century [1]. An estimated 422 million adults (8.5%) were living with diabetes in 2014, compared to 108 million (4.7%) in 1980 worldwide [2]. In addition, 318 million (6.7%) adults have glucose intolerance, which puts them at high risk of developing diabetes in the future. The World Health Organization (WHO) projects diabetes prevalence to expand from its current level to 592 million (12%) in 2035 [2]. This chronic hyperglycemic condition is associated with long-term damage to various organs, notably the eyes, kidneys, nerves, heart, and blood vessels. These complications are a major cause of mortality worldwide. Independently of other conventional risk factors, diabetes is associated with a twofold increased risk for a wide range of vascular diseases including myocardial infarction and stroke [3]. In addition to the impact on individuals, diabetes represents a huge challenge to sustainable economic development in all countries because of healthcare costs and productivity loss [2].

Common Risk Factors May Not Be the Only Contributors to Diabetes Burden

The major risk factors for diabetes proposed by physicians and regulatory agencies are age, sex, genetics, lifestyle (smoking, high-fat diet, physical inactivity) and physiological factors (obesity, hypertension, high cholesterol). Efforts to prevent and control diabetes have been largely directed at addressing these factors [1,2]. However, these traditional risk factors alone may not be sufficient to explain the massive rise in diabetes prevalence. The increased prevalence over the period 1980 to 2014 has not been similar in all nations, and the past

Trends

In recent decades the rate of diabetes rate has increased significantly, particularly in middle- and low-income countries where common risk factors such as obesity are of low prevalence.

During this period of diabetes escalation the world has witnessed a massive production and release of EDCs.

On exposure to EDCs, the gut microbiota undergoes a series of changes including microbial dysbiosis and the induction of xenobiotic pathways and associated genes, enzymes, and metabolites that cause biotransformation of EDCs.

The microbial products and byproducts of metabolism of EDCs are taken up by the host and affect glucose homeostasis, primarily by influencing hepatic gluconeogenesis.

Remediation of EDC-induced microbrial changes may be a potent therapeutic option for the control and prevention of diabetes.

[1] Department of Molecular Biology, Centre for Excellence in Genomic Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, Tamil Nadu, India
[2] Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 1213, Lyon, France
[3] KMCH Research Foundation, Koval Medical Centre and Hospital (KMCH), Coimbatore, Tamil Nadu, India
few decades have predominantly witnessed an increase in diabetes prevalence in middle- and low-income countries (Figure 1A) and the rural world [2]. Although rapid urbanization and changing lifestyle may still play a role in this explosion of diabetes, we believe that these factors.

---

**Figure 1.** Growth of Diabetes Incidence, Common Risk Factors, and the Production of Endocrine-Disrupting Chemicals (EDCs) in the Period 1980–2014. (A) Increase in diabetes prevalence in countries classified based on their income [2]. The increase in diabetes prevalence is given above the bars. (B) The prevalence of diabetes and its common risk factors in India in the period 1976–1980 [3–5]. (C) Correlation between global diabetes prevalence [2] and the production of plastics [76,77], chemical fertilizers [78], pesticides [79], e-waste [80], and food additives [81].
alone cannot explain the diabetes epidemic in middle- and low-income countries, especially in rural areas.

India is home to 17% of the world population, and to 15.3% of the diabetic population, and hence can be considered as an ideal reference for middle-income countries [2,3]. India witnessed an approximate doubling in its diabetic population over the period 1980–2014, but there was no significant increase in the level of common risk factors such as obesity, hypertension, hypercholesterolemia, and smoking (Figure 1B) [3–5]. An increased prevalence of diabetes and pre-diabetes particularly among the people involved in chemical-based farming was observed in rural communities [6–9]. On regression analysis, no linear association was observed between glycated hemoglobin and common risk factors [body mass index, systolic and diastolic blood pressure, low-density lipoprotein (LDL)-cholesterol, and physical inactivity] in rural India [7–9]. These findings indicate that, in addition to the common risk factors, several other factors play a determining role in the etiology of the diabetes epidemic.

Exposure to Environmental Chemicals and Diabetes Incidence

Concurrently with the escalation of diabetes prevalence, the world has witnessed a massive production and release of toxic chemicals into the environment through industries, chemical-based agriculture and food production, and electronic wastes (Figure 1C) [10]. Many chemicals released by these practices interfere with the endocrine system by altering the production, release, transport, and action of hormones; these are termed endocrine-disrupting chemicals (EDCs) (Box 1). A growing body of evidence suggests that EDCs play an important role in the etiology of diabetes and metabolic disorders [11,12]. The effects of EDCs from industry are generally compartmentalized into small-exposure groups, but chemicals from agro- and food products pose a risk for a much larger group via the food chain. Any chemical that disturbs the pancreatic endocrine system and glucose metabolism is defined as a diabetogen. Several experimental and epidemiological studies have demonstrated an association between EDCs and hyperglycemia, glucose intolerance, and insulin resistance [10–13]. The proposed modes of action include interactions with the aryl hydrocarbon receptor (AhR) and nuclear hormone receptors including estrogen receptors, alteration of ERK/Akt signaling pathways, and the induction of oxidative and nitrosative stress, pancreatitis and dysregulated hepatic metabolism [14]. Although accumulating data indicate that these molecular mechanisms underpin the effects of EDCs on diabetes development, a holistic view of their mode of action is still lacking.

Metabolism of EDCs by Gut Microbiota

Oral exposure to EDCs particularly via the food chain is the major pathway for their entry into the human body. The gut microbiota comprises trillions of bacteria, fungi, and viruses and is recognized as a key player in the microbial metabolism of dietary compounds, drugs,

Box 1. History of EDCs

Although the terminology ‘endocrine disruptor’ was first coined in 1991 at the Wingspread conference, disruption of hormone action by environmental chemicals was documented from the beginning of 20th century. In Silent Spring [74], Rachel Carson quotes the reproductive defects among birds exposed to organochlorine and organophosphate pesticides, and thereby sowed the seeds for the concept of environmental endocrine disruptors. Our Stolen Future [75], a book published in 1996, prompted the enactment of new laws and regulations governing the production and release of EDCs around the world. At the 2001 Stockholm Convention the global community agreed to reduce or eliminate the production, use, and release of major persistent organic pollutants, primarily because of their endocrine disrupting potential. The Endocrine Society has released two position statements over the past 6 years on the role of EDCs in human health and disease [12,13]. Increasing public awareness and research data from experimental and epidemiological studies have led to progressive replacement of strong endocrine disruptors such as DDT. Despite increased awareness of environmental chemicals, the inflow of EDCs into environment has increased over the past few decades through sources such as electronic equipment, modern food preparation practices, and household and personal sanitary products.

Glossary

**Actinobacteria:** a phylum of Gram-positive bacteria that includes **Corynebacterium**, **Propionibacterium**, **Rothia**, **Actinomyces**, **Streptomyces**, and **Bifidobacterium**. These bacteria are present in all body surfaces from skin to mucosal layers.

**Bacteroidetes:** one of the three predominant phyla in the human gut; comprises three large classes of Gram-negative bacteria: **Cytophaga**, **Flavobacterium**, and **Bacteroides**. Bacteroides spp. in particular are highly abundant in gut flora.

**Diabetes epidemic:** the rapid increase in recent years of the prevalence of diabetes, a disorder characterized by elevated blood glucose levels.

**Diabetogens:** EDCs that specifically disrupt the action of hormones related to glucose metabolism and thereby cause diabetes.

**Endocrine-disrupting chemicals** (EDCs): exogenous agents that interfere with the synthesis, secretion, transport, metabolism, action, or elimination of natural blood-borne hormones and thus cause adverse health effects.

**Fecal transplantation:** a therapeutic procedure in which stool samples or their cultures from healthy donors are introduced into patients for remediation of the gut microbial ecosystem.

**Firmicutes:** one of the predominant bacterial phyla in the gut that encompasses different genera of Gram-positive bacteria. It includes beneficial probiotic bacteria such as **Bacilli** (e.g., **Lactobacillus**).

**Glucogeneosis:** the biochemical process that leads to production of glucose from non-carbohydrate sources such as proteins, fatty acids, and other metabolites. This process primarily occurs in the liver, but under specific conditions also occurs in intestine and kidney.

**Microbial dysbiosis:** defined as an imbalance of microbial ecology that leads to domination of the microbiota by specific groups of microbes, and elimination of other groups. This process leads to a change in the physiology and metabolism of the microbial community.

**Microbial metabolism:** the process of metabolism by microbial enzymes and metabolites that causes activation/deactivation or degradation of dietary compounds or...
antibiotics, and environmental toxins [15–20]. Further, it has an important influence on digestion, immunity, development, physiology, and disease initiation and progression [18]. Hence, Pfugheot and Versalovic [21] stated ‘Human biology can no longer concern itself only with human cells’. The gut bacterial community predominantly comprises four major phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria [22]. Microbial fermentation of undigested carbohydrates, fiber, and other dietary and xenobiotic compounds produces short-chain fatty acids (SCFAs), choline, bile metabolites, and different gaseous compounds including hydrogen sulfide. These metabolites act as hormones that can influence different host metabolic processes, and hence the gut microbiota has been proposed to be a novel endocrine organ [23–25] (Box 2). Disruption of the structure of the microbiota is commonly observed in disease conditions. With regard to diabetes, changes in the Bacteroidetes/Firmicutes ratio were found to correlate with plasma glucose concentration [26]. Human metagenome-wide association studies carried out in patients with type 2 diabetes demonstrated highly significant correlations between diabetic status and specific intestinal bacteria, bacterial genes, and associated metabolic pathways [26, 27]. These microbial genes, proteins, and metabolites influence host metabolism by altering gluconeogenesis, glycogenolysis, lipogenesis, inflammation, and hormone action.

The role of the gut microbiota in the metabolism of dietary products and drugs is well established, and many reviews have covered this aspect [15–18]. Antidiabetic drugs also induce significant changes in the gut microbiota, and this may in part account for their antihyperglycemic effects [28]. Microbial metabolism of chemicals including EDCs by gut microbiota can be accompanied by microbial dysbiosis – a change in the microbial community structure, the induction of specific bacterial genes, and altered microbial transformation of molecules (Figure 2, Key Figure) [19, 20]. In addition, EDCs can be absorbed and transported to the liver, where they are conjugated and excreted back into the gut through bile secretion for further microbial metabolism. Enzymes such as azoreductases, esterases, methylases, thiolases, lipases, nitroreductases, β-glucuronidases, sulfatases, and β-lyases are known to be involved in the microbial metabolism of environmental chemicals [15–20].

Heavy Metals

Heavy metals are ubiquitous in the environment where they have accumulated as a result of both natural and human activities. Environmental levels of heavy metals have increased particularly through the widespread use of synthetic phosphate fertilizers that contain arsenic, cadmium, lead, and other heavy metals. Heavy metals are well known to be EDCs, and both epidemiological and experimental studies have revealed their role in diabetes development [12, 29].

Box 2. SCFAs – Novel Hormones of Glucose Regulation

From biochemical and physiological perspective, the gut microbiota is a versatile endocrine system that produces a wide variety of chemicals and regulates different metabolic processes in host. Candidate hormones produced by the gut microbiota include SCFAs, neurotransmitters (serotonin, dopamine, noradrenaline, GABA), secondary bile acids, choline metabolites, cortisol, and gastrointestinal hormones such as ghrelin, leptin, glucagon-like peptide-1, and PYY. These microbial metabolites impact on the gut-brain axis that regulates the neuroendocrine and metabolic pathways [23]. SCFAs (acetate, propionate, and butyrate) are major microbial metabolites produced by the fermentation of carbohydrates, fiber, and xenobiotics that act as novel hormones of glucose regulation. Propionate and butyrate activate intestinal gluconeogenesis (IGN) that has beneficial effects on glucose and energy homeostasis by mediating suppression of hepatic gluconeogenesis (HGN) [24] via the gut–brain axis. Unlike butyrate and propionate, only a small fraction of acetate is utilized in the intestine, and the remainder is transported to the liver and converted to glucose by gluconeogenesis. However, when HNG is strongly altered, for example when bile salts are released into the portal blood, HNG dominates over the regulatory action of IGN [25]. In addition, increased production of acetate by an altered gut microbiota in rodents leads to activation of the parasympathetic nervous system, which in turn promotes increased glucose-stimulated insulin secretion, increased ghrelin secretion, hyperphagia, obesity, and related sequelae [48]. The metabolism of EDCs by the gut microbiota produces SCFAs that act as signatures for metabolic disease, but further detailed investigations will be necessary to elucidate their role in glucose metabolism.
Heavy metals are metabolized by the gut microorganisms \textit{in vitro}, notably by methylation, to produce different inorganic derivatives \cite{30,31}. Arsenic (As) is recognized as a causative factor for metabolic disease, and As is regarded by WHO as being responsible for the largest mass poisoning worldwide. Incubation of As-contaminated soil or inorganic As in the SHIME (simulator of the human intestinal microbial ecosystem) causes biotransformation of As into monomethylarsonic acid (MMA\textsuperscript{V}), monomethylarsonous acid (MMA\textsuperscript{III}), and monomethylmonothioarsonic acid (MMMTA\textsuperscript{V}) (Figure 2) \cite{31}, indicative of the action of microbial methylases and thiolases. Exposure to As in mice induced a significant change in the gut microbiome and associated microbial metabolites. Although no change in \textit{Bacteroides} levels were reported,
several families of Firmicutes were found to be significantly decreased [32]. A large number of metabolites including indoles, glucuronides, fatty acids, carnitines, isoflavones, and bile acid intermediates were significantly perturbed in As-exposed mice (Table 1). These metabolite changes reflected As-induced changes in the biotransformation capacity of the gut microbiota [32] (Figure 2). The altered metabolic profile correlates with changes in microbial community structure, and the associated metabolomic changes are known to affect gluconeogenesis, adipogenesis, lipogenesis, and inflammation in the host [32].

Cadmium (Cd), another toxic heavy metal, was reported to increase hepatic triacylglycerol, serum free fatty acids, and triglyceride levels, accompanied by an alteration of gut microflora structure (decreased Firmicutes and Proteobacteria) in mice. These microbial changes led to increased serum lipopolysaccharide and hepatic inflammation that may cause perturbations in energy homeostasis (Table 1) [33]. In another study, early-life exposure to Cd predominantly induced changes in male mice, including fat accumulation and an increase in the Bacteroidetes/Firmicutes ratio. Transplantation of fecal microbiota (fecal transplantation) from Cd-exposed mice to control mice resulted in increased body fat; antibiotic treatment prevented this phenotype, indicating that the gut microbiota plays a role in this process (Table 1) [34]. Exposure to lead (Pb) during gestation and lactation via maternal drinking water resulted in increased adult bodyweight in male offspring but not in female offspring. Gut microbiome analysis of offspring revealed an inverse shift in Bacteroidetes/Firmicutes ratio with Pb exposure without sex bias. This study indicates that exposure to environmental chemicals during pregnancy has a role in shaping the adult gut microbiota and its impact on body physiology (Table 1) [35].

**Persistent Organic Pollutants (POPs)**

POPs encompass a variety of lipophilic chemicals that are resistant to environmental degradation through chemical, biological, or photolytic processes. POPs include organochlorines, polychlorinated biphenyls, polychlorinated dibenzo-furans, and dioxins. Meta-analysis of 72 different epidemiological studies concluded that there is a positive association between diabetes and exposure to POPs [12,36].

Dichlorodiphenyltrichloroethane (DDT) is among the most widely used synthetic insecticides in human history. Coliform bacteria isolated from feces of rats cause dechlorination of DDT to dichlorodiphenylchloroethane (DDD); this conversion was observed in rats treated orally with DDT, but not in animals injected with DDT intraperitoneally, indicating that the gut microbiome is responsible for the conversion [37]. Both DDT and DDD are recognized as endocrine disruptors. Oral exposure to chlorothalonil, an organochlorine fungicide, induced microbial dysbiosis and the expression of oxidative metabolic genes, and decreased carbohydrate metabolism and peptidase activity in the microbiome of chlorothalonil-exposed bees [38]. Exposure to polychlorinated biphenyls (PCBs) in mice induced substantial changes in gut microbiome, with depletion of Proteobacteria. It is interesting to note that exercise training appeared to reverse PCB-induced changes in the gut microbiota [39].

AhR receptor is a key regulator of the metabolism of xenobiotics by the host [40]. POPs bind to the AhR and thereby affect host metabolism and immunity. Microbiota-derived tryptophan catabolites were shown to bind to and modulate AhR activity [41]. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affected the gut microbial community structure, with a decrease in the Firmicutes/Bacteroidetes ratio, but no dysbiosis was observed in Ahr<sup>−/−</sup> mice [42]. TCDF enhanced the levels of Flavobacteria and Butyribio spp., and depleted Clostridia and Oscillobacter spp.; these microbial changes were accompanied by an increase in bile acid metabolites (Table 1). A Flavobacteria species was reported to produce dehalogenases that can metabolize TCDF and other halogenated compounds [43]. TCDF was also found to inhibit farnesoid X receptor (FXR) signaling, thereby triggering inflammation and bacterial
Table 1. Effects of EDCs on Gut Microbial Ecology and Physiology, and Their Subsequent Impact on Host Glucose Metabolism

<table>
<thead>
<tr>
<th>EDC</th>
<th>Changes in gut microbial diversity</th>
<th>Changes in gut microbial physiology</th>
<th>Effect on host glucose metabolism</th>
<th>Study model</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic (heavy metal)</td>
<td>No changes in Bacteroidetes but Firmicutes were decreased (Eubacterium, Faecalibacterium, and Roseburia were decreased)</td>
<td>Methylenes transforms arsenic into methylated derivatives. Indole-containing metabolites were significantly altered. Glucuronide metabolites and fatty acid carnitines were reduced in urine</td>
<td>These microbial changes can affect energy harvesting, gluconeogenesis, lipogenesis, and adipogenesis</td>
<td>In vitro incubation (SHIME) and C57BL/6 mice</td>
<td>[31,32]</td>
</tr>
<tr>
<td>Cadmium (heavy metal)</td>
<td>Increase in Bacteroides levels and decreased Firmicutes and Proteobacteria. The changes were predominant in male mice</td>
<td>Increase in serum lipopolysaccharides</td>
<td>Increased body fat, triacylglycerol, serum levels of free fatty acids and triglycerides, and hepatic inflammation</td>
<td>C57BL/6 mice</td>
<td>[33,34]</td>
</tr>
<tr>
<td>Lead (heavy metal)</td>
<td>Reduction in the Firmicutes/Bacteroidetes ratio. Increased Desulfovibrionaceae, Barnesiella, and Clostridium XIVb, and decreased Lactococcus, Enterorhabdus, and Clostridiales</td>
<td>Induces fermentation of sugars and the production of SCFAs including butyrate and propionate. Production of bacterial dehalogenase that metabolizes TCDF and other halogenated compounds</td>
<td>Triggers inflammation and alters hepatic lipogenesis, gluconeogenesis, and glycogenolysis in an AhR-dependent manner</td>
<td>C57BL/6 wild-type and Ahr−/− mice</td>
<td>[42]</td>
</tr>
<tr>
<td>2,3,7,8-Tetrachlorodibenzofuran (persistent organic pollutant)</td>
<td>Reduction in the Firmicutes/Bacteroidetes ratio with enrichment of Flavobacteria and Butyrivibrio spp., and depletion of Clostridia and Oscillibacter. No dysbiosis noted in Ahr−/− mice</td>
<td>Reduces organophosphate degradation genes (esterases, hydrolases, and lipases) and produces SCFAs, specifically acetate</td>
<td>Gluconeogenesis is induced and accounts for glucose intolerance and diabetes</td>
<td>BALB/c mice and human samples</td>
<td>[7]</td>
</tr>
<tr>
<td>Monocrotophos (organophosphate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazinon (organophosphate)</td>
<td>Sex-specific microbial changes. Bacteroides, Burkholderiales, Clostridiaceae, Erysipelotrichaceae, and Coprobacillus were observed, and Lachnospiraceae and Staphylococcaceae were completely inhibited in males</td>
<td>In male mice, microbial secondary metabolism, potassium metabolism, cell signaling, motility and chemotaxis, cell wall and capsule, and respiration were all significantly perturbed</td>
<td>Increased inflammation and no change in serum glucose. Increase in the level of total cholesterol and triglycerides</td>
<td>C57BL/6 mice</td>
<td>[49]</td>
</tr>
<tr>
<td>Carbendazim (carbamate)</td>
<td>Abundance of Firmicutes and Proteobacteria, increased levels of Actinobacteria, and decreased Bacteroidetes</td>
<td></td>
<td></td>
<td>ICR mice</td>
<td>[53]</td>
</tr>
<tr>
<td>EDC</td>
<td>Changes in gut microbial diversity</td>
<td>Changes in gut microbial physiology</td>
<td>Effect on host glucose metabolism</td>
<td>Study model</td>
<td>Refs</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>----------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>Causes dysbiosis with induction of Helicobacteraceae and reduction of Firmicutes and Clostridia</td>
<td></td>
<td>Changes in microbial dysbiosis reflect the changes seen in high-sucrose and high-fat fed mice</td>
<td>CD-1 mice</td>
<td>[55]</td>
</tr>
<tr>
<td>Saccharin (artificial sweetener)</td>
<td>Causes dysbiosis with an abundance of the genus Bacteroides and the order Clostridiales</td>
<td>Induction of the glycan degradation pathway and SCFAs such as acetate and propionate</td>
<td>Induces glucose intolerance and diabetes</td>
<td>C57BL/6 mice and human samples</td>
<td>[61]</td>
</tr>
<tr>
<td>Carboxymethyl cellulose, polysorbate-80 (dietary emulsifiers)</td>
<td>Reduced levels of Bacteroidales and increased Ruminococcus gravis</td>
<td></td>
<td>Increased body mass, fat mass, and fasting glucose levels and glucose intolerance. Colitis induced in Il10−/−, Tlr5−/− mice</td>
<td>C57BL/6 wild type, Il10−/−, Tlr5−/− mice</td>
<td>[62]</td>
</tr>
<tr>
<td>Trichloroacetamide (disinfectant)</td>
<td>Decrease in the Firmicutes/ Bacteroides ratio</td>
<td>Induction of genes associated with amino acid metabolism, energy production, and secondary metabolites, but repression of genes related to lipid metabolism. Alteration in urine metabolite profile including SCFAs</td>
<td>The changes in microbial and metabolite profile can influence host glucose and lipid metabolism</td>
<td>C57BL/6 mice</td>
<td>[64]</td>
</tr>
</tbody>
</table>
fermentation. Increased levels of SCFAs including butyrate and propionate were observed in fecal and cecal contents of TCDF-exposed mice. These changes in the SCFA profile were AhR-dependent and may be responsible for altered hepatic lipogenesis, gluconeogenesis, and glycogenolysis (Figure 2) [42].

Organophosphates (OPs)
OPs are a group of non-persistent chemicals that are used as insecticides, herbicides, chemical weapons, plasticizers, oil additives, and lubricants. Owing to their biodegradable nature, these OPs have replaced persistent organochlorine pesticides. Several human studies have reported an association between OP exposure and diabetes prevalence [7–9, 44, 45]. Intestinal bacteria such as Lactobacillus lactis, Lactobacillus fermentum, Lactobacillus plantarum, Escherichia coli, and Enterococcus faecalis were found to degrade chlorpyrifos, a commonly used OP insecticide [46].

The broadly accepted mode of action of these OP insecticides is inhibition of acetylcholine esterase (AChE). Prolonged intake of the OP insecticide monocrotophos induced hyperglycemia, dyslipidemia, cardiac oxidative stress, and myocardial infarction in rats [47]. A positive linear correlation was observed between plasma OP residues and glycated hemoglobin in a rural farming population from South India. However, no significant changes in AChE were recorded in the same population [7]. Consistently, chronic exposure of mice to monocrotophos induced hyperglycemia, glucose intolerance, and oxidative stress with no change in AChE. Fecal transplantation from mice exposed to OP induced glucose intolerance in recipient mice, indicating that the gut microbiota underlies this effect [7]. Metatranscriptomics and metabolomics analyses have revealed the OP exposure leads to the induction of xenobiotic-metabolizing enzymes, particularly esterases. These enzymes degrade OPs into SCFAs, particularly acetate, which are converted into glucose via gluconeogenesis. Increased hepatic glucose 6-phosphatase activity in mice treated with OPs indicates induction of gluconeogenesis. Levels of plasma OP residues correlated positively with fecal esterase activity and acetate levels in human diabetes. Collectively, these results indicate that altered hepatic gluconeogenesis mediated by OP-degrading gut microbiota is the key mechanism underlying OP-induced hyperglycemia [7] (Figure 2). Increased production of acetate by an altered gut microbiota leads to the activation of parasympathetic nervous system, which in turn promotes hyperglycemia, insulin secretion, increased ghrelin secretion, hyperphagia, obesity, and related sequelae [48]. In another study, diazinon insecticide exposure in mice perturbed the gut microbiome community structure, functional metagenome, and associated metabolic profiles in a gender-specific manner [49] (Table 1). As observed for Cd and Pb, significant microbial dysbiosis and associated changes in microbial metabolism were only seen in male mice [49].

Glyphosate is a widely used OP herbicide worldwide. Glyphosate binds to and blocks the activity of enolpyruvylshikimate-3-phosphate synthase (EPSPS) in plants, leading to inhibition of the shikimic acid pathway [50]. Although this enzyme is not present in animals, gut microbes possess an EPSPS enzyme which converts carbohydrate derivatives from glycolysis and pentose phosphate pathway to aromatic amino acids. In fecal culture studies, glyphosate was found to inhibit Enterococcus faecalis, whereas pathogenic bacteria such as Salmonella and Clostridium spp. were highly resistant to this herbicide [51]. Glyphosate suppresses gut microbiota cytochrome P450 enzyme activity, drug detoxification, and amino acid biosynthesis [52], pathways that are known to influence adipogenesis and gluconeogenesis [50, 52].

Carbamates and Pyrethroids
Carbamates and pyrethroids represent third-generation pesticides that are relatively less toxic. In addition to their use in agriculture, they are also used for household insect control. Carbendazim (CBZ) is a widely used broad-spectrum carbamate fungicide for control of fungal
diseases in agriculture. CBZ is classified as an endocrine disruptor and is known to cause diabetes as well as hepatic and reproductive toxicity in experimental animals [12]. On oral exposure in mice, CBZ causes a decrease in the level of Bacteroidetes accompanied by proliferation of Firmicutes, Proteobacteria, and Actinobacteria [53] (Table 1). CBZ also leads to significantly increased inflammation, hepatic lipid accumulation, and triglyceride levels in mice, but whether the microbiota plays a role in the altered hepatic metabolism was not established [53]. Bee experiments with tau-fluvalinate (a synthetic pyrethroid insecticide) altered bacterial diversity but not the fungal composition. A reduction in the level of Bifidobacteriales and an increase in the level of Rhizobiales in the bee microbiome was observed [38] but their influence on host metabolism was not studied.

**Bisphenol A (BPA)**

BPA is a widely used plasticizer that is present in plastic products including water bottles. BPA could contribute to the diabetes epidemic because it perturbs lipid metabolism and pancreatic β-cell function [12,54]. It is notable that dietary intake of BPA and a high-fat/high-sucrose diet both lead to similar changes in gut microbial community structure in mice. BPA favored the growth of Proteobacteria and Helicobacteraceae, with a decline in the populations of Firmicutes and Clostridia [55] (Figure 2 and Table 1). These microbiota alterations parallel the microbial structure in diabetes patients [26,27].

**Phthalates**

Phthalates, esters of phthalic acid, are used as plasticizers, stabilizers, dispersants, lubricants, and emulsifying agents. These chemicals are significant components of personal care products such as cosmetics, perfumes, and nail polishes. It has been reported that phthalates cause dysregulation of glucose metabolism, insulin resistance, and adipogenesis [12,56]. Urinary levels of phthalates in the National Health and Nutrition Examination Survey (2001–2010) participants were associated with a higher diabetes prevalence, particularly among women – the predominant users of personal care products [57]. Exposure of rats to diethyl phthalate led to reduction in Firmicutes (Bacilli) and an overabundance of Bacteroidetes (Prevotella). These diethyl phthalate-induced changes in the bacterial community were accompanied by consistent weight loss in rats, indicative of a role in lipid and glucose homeostasis [58]. Further studies will be necessary to explore the potential effect of phthalates on the skin microbiome and downstream effects on host metabolism.

**Non-Caloric Artificial Sweeteners (NAS)**

Food additives, synthetic chemicals that are used to preserve or enhance the lifetime, flavor, taste, or appearance of eatables, are less well recognized as EDCs but pose a potential risk to a large number of people via oral ingestion. NAS were introduced as an alternative safe option for diabetes patients to control glucose metabolism. By contrast, there is growing evidence indicate that NAS such as aspartame, sucralose, and saccharin induce significant dysbiosis in animals and humans, producing deleterious metabolic effects in the host [59]. Aspartame was found to directly alter the gut microbiota and its metabolites in rats, leading the authors to hypothesize that this might lead to an increase in hepatic gluconeogenesis [60].

NAS including saccharin induce glucose intolerance mediated by the gut microbiota [61]. These NAS-mediated deleterious metabolic effects are abrogated by antibiotic treatment. Fecal transplantation of microbiota from NAS-consuming mice, or of microbiota anaerobically incubated in the presence of NAS, into germfree mice resulted in the development of glucose intolerance. Saccharin induced strong dysbiosis, and more than 40 operational taxonomic units were significantly altered, with a specific overabundance of Bacteroides species and a decrease of Clostridiales (Table 1). Metagenomic sequencing revealed the induction of glycan-degradation pathways by NAS, and glycans are known to produce different metabolites
including SCFAs upon fermentation by bacteria (Figure 2). Consistent with this finding, increased levels of fecal acetate and propionate were observed in NAS-treated mice. In a human study it was noted that only half of individuals treated with saccharin developed glucose intolerance, and these responders were found to possess a specific microbial structure that was related to the changes microbial diversity observed in mice studies [19,61].

Emulsifiers
Emulsifiers are used for the preservation of food and other eatables. Mice administered the emulsifiers carboxymethylcellulose (CMC) or polysorbate-80 (P80) via drinking water had an altered microbiota composition, localization, and proinflammatory potential. Alterations included reduced levels of Bacteriodales and increased levels of several mucolytic operational taxonomic units including Ruminococcus gnavus (Table 1) [19,62]. Emulsifier treatments also increased fecal levels of bioactive lipopolysaccharides and flagellin in wild and Il10−/− mice. Emulsifiers induced gut chronic inflammation and colitis in Il10−/− and Tlr5−/− mice but not in wild-type mice. Both CMC and P80 resulted in significant overall weight gain and a marked increase in adiposity as measured by fat mass. Although the emulsifiers did not induce changes in bile acid levels, a reduction in mucus thickness was observed, which has an effect on host metabolism. Emulsifier treatment also impaired glycemic control as assessed by fasting blood glucose concentrations and glucose/insulin tolerance testing (Figure 2). Further studies indicated that an altered microbiota is necessary and sufficient for emulsifier-induced metabolic syndrome [62].

Disinfection Products
The use of disinfection products, particularly hand sanitizers, has increased in past few years, although scientific evidence to prove the health benefits of these compounds is so far lacking. Low-level exposure mimicking environmental exposure of triclosan in mice alters the intestinal microbiota and susceptibility to colitis [63]. Trichloroacetamide (TCAcAm), an emerging nitrogenous disinfection product, induces changes in host–gut microbiota co-metabolism in mice. The relative abundance of Bacteriodales was elevated with increased concentrations of TCAcAm (Table 1), and a dramatic perturbation of gut microbial metabolites in urine was noted in TCAcAm-treated mice [64], and the majority of these metabolites have been implicated in modulating host energy and glucose regulation.

Microbiome-Based Diagnostic and Therapeutic Perspectives
It is evident that increased exposure to EDCs may be a key etiological factor underlying the escalation of diabetes prevalence over the past few decades. Recent studies indicate that EDC-induced metabolic dysfunction can directly lead to diabetogenic changes in the host. Identifying changes in microbial content, enzymes, and metabolites may provide a novel approach to diagnosis of at-risk individuals, opening the way to personalized medicine [19,65]. Because EDCs perturb the structure of the gut microbiota, restoration of the microbial structure offers a promising therapeutic option. For example, the administration of plant flavonoids and polyphenols promotes a healthy gut microbiota [66], and the antioxidant tempol is reported to reduce host adiposity and insulin resistance in part by altering the interaction between the gut microbiota and the nuclear FXR receptor [67]. One common consequence of EDC exposure is a reduction in the level of beneficial bacteria such as Lactobacillus spp., and the administration of probiotics may therefore have therapeutic benefits [68]. Microcins, small peptide narrow-spectrum antibiotics that are effective against specific groups of bacteria, could conceivably be used as a targeted strategy to restore a healthy microbiota [69].

Furthermore, transfer of microbiota from EDC-exposed mice to normal mice reproduced the glucose dyshomeostasis of the donor [7,61], and fecal transplantation from healthy volunteers has been suggested as a possible antidiabetic strategy [70]. Several fecal transplantation
clinical trials for diabetes therapy are currently in progress [70,71]. In addition to microbes, the gut is also home to a large number of viruses that regulate the microbial diversity. The identification and characterization of bacteriophages targeting specific bacterial species may help in restoring the structure of the microbiota by phage therapy [72]. Another approach is to develop small-molecule inhibitors that target the microbial genes, enzymes, and metabolites that are responsible for EDC metabolism leading to glucose dyshomeostasis. β-Glucuronidase inhibitors are reported to block specific groups of bacterial enzymes and rescue the animals from drug toxicity [73]. Although these microbiome-based strategies provide novel options for future control of diabetes, further research into the efficacy and durability of these treatments will be essential.

Concluding Remarks
There is growing evidence that EDCs are likely to play an important role in the escalation of diabetes over recent decades [12,13]. Therefore, it is recommended that regulatory authorities including WHO should focus attention on regulating EDC production and release as a strategy for diabetes control. Because chemical toxicity is clearly modulated by the gut microbiota [15–20], the evaluation of new chemicals for their effects on the gut microbiota should be added to traditional toxicity assessment tests. Our current knowledge in the field is based on a small number of studies, and the role of the gut microbiota in the metabolism and detrimental effects of the majority of EDCs remains largely unexplored (see Outstanding Questions). Therefore, further research will be necessary to elucidate the interplay between EDCs, gut microbiota, and host glucose homeostasis. Moreover, an in-depth understanding would have important translational implications, and large-scale human epidemiological interventions coupled with exploration of EDC–microbiota interactions will undoubtedly contribute to long-term alleviation of the deleterious effects of environmental chemicals.

Acknowledgments
The authors acknowledge Interdisciplinary Program in Life Science (IPLS) and Rapid Grant for Young Investigators (RGYI) funding from the Department of Biotechnology (DBT), Government of India; University with Potential for Excellence (UPE), Centre for Advanced Studies (CAS), Centre for Excellence in Genomic Sciences (CEGS), and Networking Resource Centre in Biological Sciences (NRCBS) from the University Grants Commission (UGC); the Science and Engineering Research Board (SERB); and Promotion of University Research and Scientific Excellence (PURSE) award from the Department of Science and Technology, Government of India. G.V. is supported by the Council of Scientific and Industrial Research (CSIR) and the UGC, Government of India) through a Senior Research Fellowship and a Fellowship for Meritorious Students, respectively.

References

Outstanding Questions
Are common risk factors such as obesity, hypertension, hypercholesterolemia, and physical inactivity holistic causative agents for the diabetes epidemic?

Although WHO and other major agencies have released several reports on EDCs, there is no mention of EDCs in the WHO Global Report on Diabetes [2] or in the International Diabetes Federation Diabetes Atlas [1]. Why is the control and regulation of EDC release not included as a global measure for the control and prevention of diabetes?

How does the gut microbiota respond to simultaneous exposure to multiple EDCs, as commonly encountered in daily life, what is its impact on host endocrine system?

What are the precise microbial and host molecular mechanisms through which EDCs cause changes in the gut microbiota and subsequently affect host glucose metabolism? Understanding these mechanisms will pave the way for novel diagnostic and therapeutic strategies.

What is the efficacy of microbiome-based therapeutic approaches for diabetes, and how long do benefits persist following treatment? What are the ethical, societal, economic, and health implications of therapeutic approaches such as fecal transplantation?