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Polychlorinated biphenyls and nonalcoholic fatty liver disease

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Abstract

Polychlorinated biphenyls (PCBs) have been associated with abnormal liver enzymes and suspected nonalcoholic fatty liver disease (NAFLD) in cohort studies. NAFLD affects greater than 25% of the global population and may result in liver-related mortality. Both dioxin-like and nondioxin-like PCBs have been associated with NAFLD, but their effects and mechanisms differ. Dioxin-like PCBs altered the gut–liver axis and microbiome and caused hepatic steatosis by disrupting hepatic lipid metabolism. In contrast, nondioxin-like PCBs reduced the liver's protective responses to promote diet-induced NAFLD. Mechanisms included the disruption of phosphoprotein signaling resulting in altered nuclear receptor function.

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Introduction

Polychlorinated biphenyls (PCBs) have been associated with liver injury since 1937, when three cases of fatal jaundice were reported in workers exposed to PCBs and chlorinated naphthalenes [1]. In 1981, dose-dependent liver enzyme elevation and hepatomegaly was reported in PCB-exposed electrical workers [2]. In the United States, PCBs were manufactured by Monsanto under trade names including Aroclor® for industrial applications including dielectric and hydraulic fluids. Monsanto's material safety data sheet (MSDS #M00018515) reported that "The consistent finding in animal studies is that PCBs produce liver injury following prolonged and repeated exposure by any route, if the exposure is of sufficient degree and duration. Liver injury is produced first, and by exposures that are less than those reported to cause cancer in rodents. Therefore, exposure by all routes should be kept sufficiently low to prevent liver injury." Despite their historical role in occupational hepatotoxicity, PCBs were only recently associated with an environmental liver disease consistent with nonalcoholic fatty liver disease (NAFLD) [3,4]. Indeed, PCBs were among the most potent chemicals associated with hepatic steatosis in archived rodent toxicologic pathology studies [5].

Structurally, PCBs are a thermodynamically stable chlorine-substituted biphenyl ring. Approximately 130 of 209 theoretical PCB congeners were commercially produced before PCBs were banned. PCBs are persistent organic pollutants which continue to contaminate the environment, the food supply, breast milk, and even the air in homes and schools. PCBs ranked #5 on the Agency for Toxic Substances and Disease Registry's 2017 Substance Priority List. All analyzed adult participants in the National Health and Nutrition Examination Survey (NHANES) 2003–2004 had detectable PCB levels in blood [6]. PCB congeners have been classified as either dioxin-like (DL) or nondioxin-like (NDL) based on their ability to activate the aryl hydrocarbon receptor (AhR) [7]. Both PCB types were associated with suspected NAFLD in a cohort study [3]. PCBs concentrate in adipose and liver. The more highly chlorinated congeners are more resistant to environmental degradation and hepatic metabolism resulting in their bioaccumulation. PCBs undergo enterohepatic circulation, so it is not surprising

Abbreviations

ACHS	Anniston Community Health Survey
AhR	aryl hyodrocarbon receptor
AKT	protein kinase B
ALT	alanine aminotransferase
AMPK	AMP-activated protein kinase
AOP	adverse outcome pathway
ApoB-100	apolipoprotein B-100
CAR	constitutive androstane receptor
CK2	casein kinase 2
CK18	cytokeratin 18
CREB-1	cyclic AMP responsive element binding protein 1
Cyp7a1	cytochrome P450 family 7 subfamily A member 1
DL	dioxin-like
EDCs	endocrine disrupting chemicals
EGF	epidermal grow factor
EGFR	epidermal growth factor receptor
EPA	Environmental Protection Agency
ERK	extracellular-regulated protein kinase
ESR1	estrogen receptor 1 or α
FXR	farnesoid x receptor
FGF-15	fibroblast growth factor-15
FGF-21	fibroblast growth factor-21
GLP-1	glucagon-like peptide-1
GCG	glucagon
GCGR	glucagon receptor
GSH	glutathione
HFD	high fat diet
HNF4α	hepatocyte nuclear factor 4-alpha
INSR	insulin receptor
IL6	interleukin 6
IL6R	interleukin 6 receptor
IR	insulin resistance
LXRα/β	liver X receptor α/β
MCD	methionine-choline deficient
MDCs	metabolism disrupting chemicals
mTOR	mammalian target of rapamycin
MSDS	material safety data sheet
LEPR	leptin receptor
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NDL	non-dioxin-like
NHANES	National Health and Examination Survey
NRF2	nuclear factor erythroid 2-related factor
PAI-1	plasminogen activator inhibitor-1
PCBs	polychlorinated biphenyls
PEPCK	phosphoenolpyruvate carboxykinase
PKA	protein kinase A
PNPLA3	patatin-like phospholipase domain-containing protein 3
PPARα/δ/γ	peroxisome proliferator-activated receptor α/δ/γ
PXR	pregnane X receptor
RXR	retinoid X receptor
SDCs	signaling disrupting chemicals
STAT3	signal transducer and activator of transcription 3
TAFLD	toxicant associated fatty liver disease
TASH	toxicant associated steatohepatitis
1,4-Bis-[2-(3,5-dichloropyridyloxy)]benzene	3,3′,5,5′-Tetrachloro-1,4-bis(pyridyloxy) benzene, TCPOBOP
TGF-β	transforming growth factor β
TRα	thyroid hormone receptor α

that they may affect bile acids, the microbiome, and fecal metabolites [8–12].

Global NAFLD prevalence exceeds 25%, and NAFLD may result in liver-related morality or transplantation [13]. NAFLD represents a pathologic spectrum ranging from steatosis to steatohepatitis (NASH) with or without fibrosis, cirrhosis, and hepatocellular carcinoma [14]. A two 'hit' hypothesis has been proposed to explain why only some subjects with NAFLD develop progressive liver disease. Classically described second 'hits' include insulin resistance (IR), oxidative stress, proinflammatory cytokines, organelle dysfunction, as well as alterations in organokines and the intestinal microbiome. NAFLD is often considered the hepatic manifestation of metabolic syndrome. Thus, it is hardly surprising that PCBs, which are endocrine, metabolism, and signaling disrupting chemicals have been associated with NAFLD [15–17]. Recently, PCBs were shown to alter organokines previously implicated in NAFLD pathogenesis including fibroblast growth factor-21 (FGF-21), FGF-15, glucagon peptide 1, leptin, and adiponectin [4,8,10,17–22]. Metabolism disrupting chemicals are defined as chemicals that promote obesity, diabetes, fatty liver, and/or alterations in lipid and glucose metabolism but may require a second 'hit' such as increased dietary sugar or fat [15]. NDL PCBs including PCB 153 and the Aroclor 1260 PCB mixture worsened diet-induced NAFLD but did not cause NAFLD in mice fed with a normal diet [5,17,18,23–27]. In contrast, DL PCB 126 not only caused NAFLD in rodents fed with normal diet but also worsened diet-induced NAFLD [5,8,9,11,18–20,28–30]. Signaling disrupting chemicals alter intracellular signaling regulating normal hepatic metabolism, cell survival, inflammation, and fibrosis. PCBs inhibited epidermal growth factor receptor (EGFR) signal transduction to alter downstream protein kinase and transcription factor phosphorylation and function in NAFLD models [16,17,21–23].

The terms toxicant-associated fatty liver disease (TAFLD) and toxicant-associated steatohepatitis (TASH) were coined to describe the fatty liver disease associated with industrial chemical exposures [14]. Here, TAFLD will be used interchangeably with NAFLD. The U.S. Environmental Protection Agency (EPA)'s hepatic steatosis adverse outcome pathway (AOP) proposed disruption of crosstalking xenobiotic receptors to be the molecular initiating event for environmental chemicals in TAFLD [31]. Indeed, nuclear receptor dysregulation is a hallmark of NAFLD, and

several nuclear receptor agonists are currently in phase III therapeutic clinical trials for this liver disease [32]. Not surprisingly, PCBs altered normal hepatic nuclear receptor signaling [11,17–20,22–25,28,29,33,34] and AhR function [7,18–20,22,28,29,33] in NAFLD models. These and other recently described mechanisms are summarized in the Graphical Abstract and Table 1.

Cohort studies investigating PCBs in liver disease

Cohort studies support the rationale to investigate the mechanisms of PCBs in NAFLD. Chronic liver disease mortality was increased in men, and liver cancer mortality was increased in women following the Yucheng and Yusho PCB-poisoning events [35]. Likewise, liver cancer mortality was increased in some, but not all, studies of PCB-exposed electrical workers [36]. In 2010, multiple PCB congeners were dose dependently associated with increased odds ratios for 'unexplained alanine aminotransferase elevation', a surrogate NAFLD biomarker in adult NHANES 2003–2004 [3]. Subsequent NHANES and other cohort studies confirmed the positive association between PCBs and liver enzymes [37–41]. In 2018, a novel combination of serologic liver disease biomarkers investigated TASH in the Anniston Community Health Survey (ACHS) [4]. The ACHS is a residential cohort from Anniston, AL, the historical location of a PCB-manufacturing complex. In addition to elevated PCB exposures, ACHS participants had a high prevalence of obesity and diabetes placing them at increased risk for steatohepatitis. Diet-induced NASH is characterized by apoptotic hepatocyte death, whereas TASH has frequently been associated with hepatocellular necrosis [14]. Indeed, Aroclor 1260 exposures caused secondary liver necrosis in model systems due to negative caspase-3 regulation by PCB-induced alterations in casein kinase 2 (CK2) structure and function [17]. To evaluate hepatocyte apoptosis and necrosis, the cytokeratin 18 (CK18) M30 and M65 serologic

Table 1 PCB mechansims and effects in NAFLD and liver disease.

Category	PCB mechanisms and effects		Dioxin-like PCBs	Nondioxin-like PCBs	PCBs*
Steatosis	↑ Steatosis (alone or in combination with either HFD or MCD diet)		[5,9,11,18–20,28–30]	[5,24,26,27,43]	[22]
Liver injury and cell death	↑ Liver enzymes		[3,9,11,28,37,38,40,41]	[3,4,25,37–41]	[2]
	↑ Hepatocyte necrosis		[4]	[4,17,25]	–
Inflammation	↑ Liver and/or systemic inflammation (e.g., cytokines)		[8,9,11,18,28,43]	[4,18,24,25,27,34,43]	[22]
Fibrosis	↑ Fibrosis and/or cytoskeletal remodeling		[11,18,28]	[18,23]	–
AhR and nuclear receptors	↑ AhR activation		[18–20,28,29,33]	–	[19,22]
	Δ Nuclear receptors (e.g., PXR, CAR, PPARα/δ/γ, HNF4α, FXR, ESR1, TRα, LXRα/β, RXR, etc.)		[11,18–20,28,29,33]	[17–19,23–25,33,34]	[19,22]
Endocrine disruption	Pancreatic hormones	Δ Insulin and/or insulin signaling	↑ [8]	↓ [4,17,18,25,34]	–
	Adipokines	↓ Leptin and/or STAT3 expression	[20]	[4,17,21]	[22]
	Enterokines	↑ FGF-15	–	–	[10]
		↓ GLP-1	[8]	–	–
	Hepatokines	Δ FGF-21	↓ [18–20]	↓ [19]	↑ [19]
		Δ Insulin-like growth factor 1(IGF-1)	↑ [19]	↑ [19]	↔ [19]
		Δ Betatrophin	↔ [19]	↑ [19]	↓ [19]
Metabolism disruption	Δ Hepatic lipid metabolism (e.g., mitochondrial dysfunction) and/or blood lipids		[9,11,18–20,28,42]	[17–19,23–25,27,34]	[19,22]
	↓ Gluconeogenesis		[18–20,28,29,42]	[18,25,26]	–
Phosphoprotein signaling disruption	↓ Activation of the EGFR, other kinases, and their targets (e.g., AMPK, AKT, mTOR, ERK, PKA, CREB-1, etc.)		[16,20]	[16,17,21,23]	[19,22]
Gut–liver axis and microbiome	Diet interaction		[18,28,42,43]	[17,18,23–27,43]	–
	Δ Microbiome or bacterial metabolites		[8,9,11,12,43]	[43]	[10]
	Δ Bile acids		[11]	–	[10]
	↑ Trimethylamine N-oxide (TMAO)		[28]	–	–
Oxidative stress	↑ Oxidative stress or ↓ antioxidant protection (e.g., ↓ NRF2)		[42]	[23,26,27]	–
Gene–environment interactions	Δ PNPLA3 expression		↓ [19]	↑ [19]	↓ [19,22]
Sexually dimorphic effects	Sex differences		[4,35]	–	[22]
Clinical outcomes	↑ Liver-related deaths		–	–	[1,35]
	↑ Hepatocellular carcinomas		–	–	[35,36]

PCB, polychlorinated biphenyl; NAFLD, nonalcoholic fatty liver disease; AMPK, AMP-activated protein kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; ERK, extracellular-regulated protein kinase; PKA, protein kinase A; CREB-1, cyclic AMP responsive element binding protein 1; PNPLA3, patatin-like phospholipase domain-containing protein 3; FGF, fibroblast growth factor; EGFR, epidermal growth factor receptor; NRF2, nuclear factor erythroid 2-related factor; DL, dioxin-like; NDL, nondioxin-like.

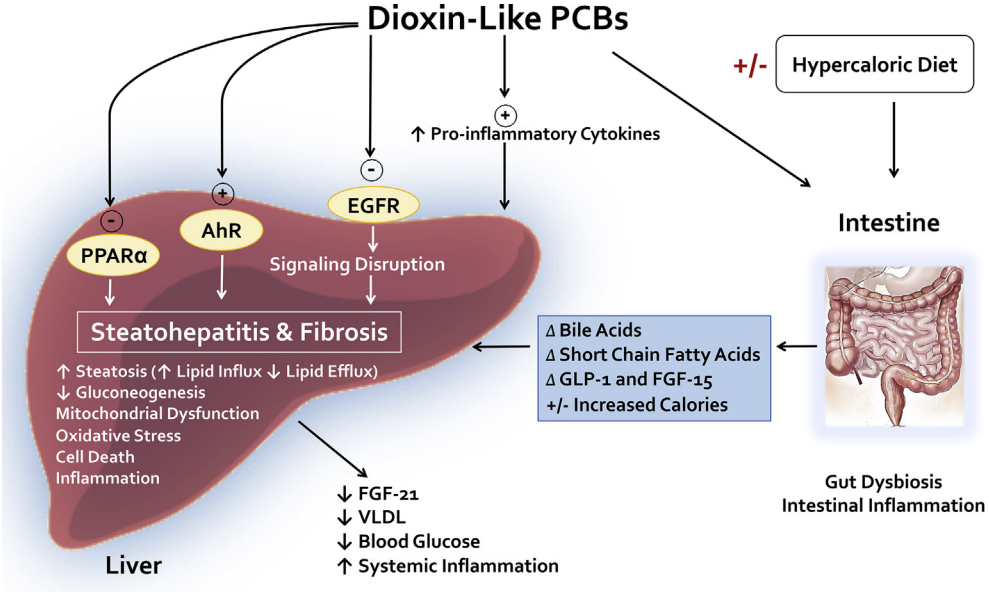
* PCBs in cohort studies where levels of specific PCB congeners were not reported or in animal studies investigating mixtures of DL and NDL PCBs.

biomarkers were measured in the ACHS [4]. CK18 is a cytoskeletal protein abundantly expressed in hepatocytes which spills into the blood as either a whole (M65) or caspase-3–cleaved fragment (M30) during cell death. Circulating M30 and M65 are both increased by apoptosis, but only M65 is increased by necrosis. Applying this biomarker combination, a high prevalence of necrotic liver disease (49%) was determined in the ACHS. Fifteen of the thirty-five measured PCB congeners were positively associated with necrotic liver disease and/or M65. Next, steatohepatitis second 'hit' mechanisms including IR and serum proinflammatory cytokines were assessed [4]. Subjects with necrotic liver disease had significantly increased homeostatic model assessment for insulin resistance (HOMA-IR), interleukin (IL)-1β, IL-6, and plasminogen activator inhibitor-1. Thus, ACHS participants had a high prevalence of liver necrosis positively associated with PCB exposures, IR, and proinflammatory cytokines consistent with TASH. ΣPCBs was inversely associated with serum insulin and leptin levels consistent with endocrine disruption. Interestingly, IL-6 and plasminogen activator inhibitor-1 were also increased, while insulin was decreased in an animal model of steatohepatitis due to Aroclor 1260 and high fat diet (HFD) coexposures [25]. Although leptin was not decreased in this model, PCBs greatly reduced signal transducer and activator of transcription 3 expression and leptin signaling [17,21]. Unfortunately, liver biopsy is not available to confirm the suspected steatohepatitis observed in the cohort studies.

Mechanisms of DL PCBs in fatty liver disease

PCB 77 and PCB 126 caused steatosis or worsened diet-induced steatosis by increasing hepatic triglycerides, free fatty acids, and cholesterol in rodent models [9,11,18–20,28–30]. Applying EPA's NAFLD AOP, the apical key events responsible for DL PCB-induced steatosis were (i) increased lipid influx (*via* upregulation CD36 and fatty acid binding protein-1); (ii) decreased lipid efflux (*via* downregulation of apolipoprotein B-100); (iii) decreased fatty acid oxidation (*via* downregulation of peroxisome proliferator-activated receptor α [PPARα]); despite (iv) inconsistently decreased lipogenesis (*via* downregulation of fatty acid synthase). The downregulation of apolipoprotein B-100 was profound in mice fed with methionine-choline deficient diet [28] and may have been responsible for the reduced serum lipid levels observed in some studies [11,19,28]. PCB 126 reduced hepatic gluconeogenesis (*via* phosphoenolpyruvate carboxykinase downregulation), and this was associated with fasting hypoglycemia in some studies [18–20,28,29,42]. Liver cell death [3,4,9,11,28,37,38,40,41], inflammation [8,9,11,18,28,43]; and fibrosis [11,18,28] were increased by DL PCBs.

Figure 1



Schematic diagram depicting mechanisms of dioxin-like PCBs in NAFLD. Dioxin-like (DL) PCBs induce proinflammatory cytokines and directly activate the AhR while inhibiting EGFR, leading to signaling disruption and transcriptional reprograming that facilitate hepatic lipid accumulation, inflammation, and cell death. DL PCBs, with or without a high caloric diet, alter intestinal microbiota leading to gut dysbiosis thereby promoting host inflammation, disruption of bile acid, and short-chain fatty acid metabolism, and enterokine production. This, in turn, affects liver function and behavior, further exacerbating NAFLD symptoms. PCB, polychlorinated biphenyl; NAFLD, nonalcoholic fatty liver disease; AhR, aryl hyodrocarbon receptor; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; PPAR, peroxisome proliferator-activated receptor; VLDL, very low density lipoprotein.

Recently described mechanisms for DL PCBs in NAFLD include mitochondrial dysfunction and oxidative stress with antioxidant depletion [42]; decreased expression of the protective hepatokine, FGF-21 [18–20]; and phosphoprotein signaling disruption [16,20]. Of all congeners tested, PCB 126 was the most potent inhibitor of EGFR activity [16], and it also reduced downstream protective AMPK and cyclic AMP–responsive element–binding protein 1 (CREB-1) signaling [20].

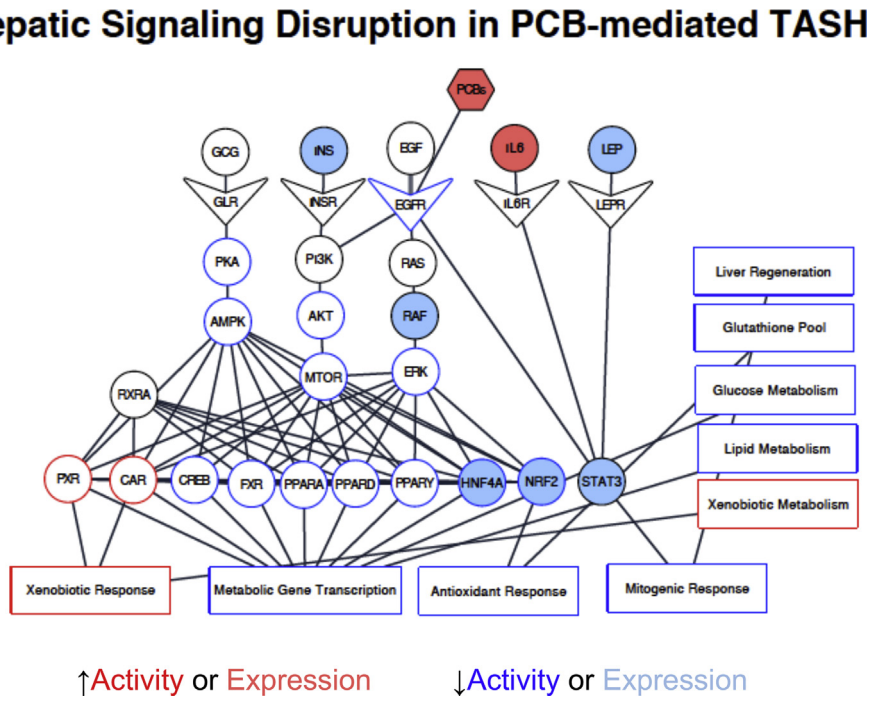
Gut bacteria–derived indoles can activate AhR, thereby implicating the AhR in maintenance of intestinal bacteria–host homeostasis [44]. Therefore, it is not surprising that DL PCBs impacted the gut–liver axis and microbiome. Indeed in animal models, DL PCBs altered gut bacteria and their metabolites [8–12,43], bile acids [10,11], and enterokines [8,10]. PCB 126 increased the Firmicutes:Bacteroidetes ratio, decreased alpha diversity and glucagon peptide 1, and altered microbial production of short-chain fatty acids [8,9,12,43]. PCB 126–induced gut dysbiosis correlated with fifteen different NAFLD biomarkers involving hepatic steatosis, metabolism, and inflammation [9]. PCB 77 increased the Firmicutes:Bacteroidetes ratio; serum bile acids; and hepatic steatosis, inflammation, and fibrosis [11,43]. The dose-dependent intestinal dysbiosis induced by a PCB mixture quantitatively and qualitatively altered bile acids as well as ileal FGF-15 and hepatic Cyp7a1 expression [10].

In contrast to the experimental data, DL PCBs were inversely correlated with the degree of hepatic steatosis and inflammation at baseline in bariatric surgery cohorts [40,41]. However, PCB levels positively correlated with liver enzymes at 12 months postoperatively suggesting impaired NAFLD resolution with weight loss [41]. While confirmatory data are required, the reported species differences could be due to differential exposures and potencies for AhR activation [45]. Figure 1 summarizes the mechanisms of DL PCBs in NAFLD which included both AhR-dependent and AhR-independent pathways.

Mechanisms of non-DL PCBs in NAFLD

Rather than causing NAFLD, NDL PCBs attenuate the liver's protective responses against the deleterious effects

Figure 2



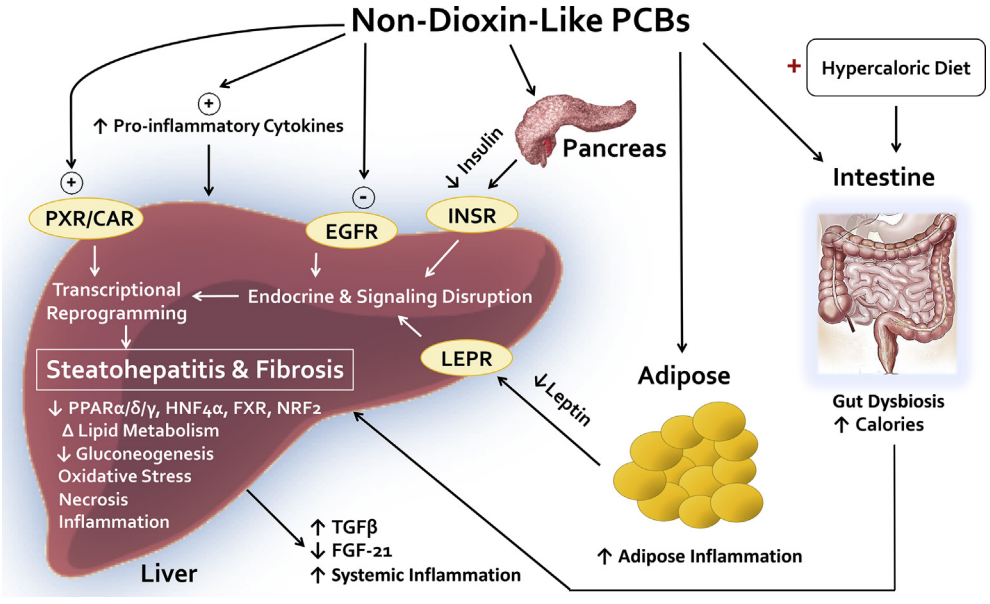
Schematic diagram highlighting signaling disruption by PCBs in NAFLD. Nondioxin-like (NDL) PCBs inhibit the EGFR pathway which negatively affects HNF4a and NRF2 activity leading to deficits in metabolism and antioxidant response, respectively. NDL PCBs also diminished hepatic PKA-AMPK-CREB pathway and hepatic FXR and PPARa/d/g activity, further causing metabolic disruption. Both circulating insulin and leptin are diminished with NDL PCBs contributing to diminished activity of the insulin signaling pathway and effector STAT3. In contrast, IL-6 is elevated with NDL PCB exposures contributing to inflammation. NDL PCBs activate xenobiotic receptors, CAR, and PXR; these nuclear receptors have agonists in clinical trials currently and could serve as potential targets for TASH to restore metabolic reprogramming of the liver. PCB, polychlorinated biphenyl; NAFLD, nonalcoholic fatty liver disease; NRF2, nuclear factor erythroid 2-related factor; HNF4a, hepatocyte nuclear factor 4-alpha; PKA, protein kinase A; CREB-1, cyclic AMP responsive element binding protein 1; AMPK, AMP-activated protein kinase; PPAR, peroxisome proliferator-activated receptor; EGFR, epidermal growth factor receptor; STAT3, signal transducer and activator of transcription 3; IL. interleukin; CAR, constitutive androstane receptor; PXR, pregnane X receptor; TASH, toxicant-associated steatohepatitis.

of diet-induced obesity to exacerbate diet-induced NAFLD [17,18,23–27,43]. PCB 153, the single most abundant PCB congener in humans, was a diet-dependent obesogen which increased hepatic steatosis (*via* increased lipid synthesis, influx), oxidative stress, inflammation (*via* NFKB activation), and the intestinal Firmicutes:Bacteroidetes ratio [24,26,27,43]. Aroclor 1260 exposures mediated the transition from diet-induced steatosis to steatohepatitis by increasing hepatic necrosis [17,25], inflammation [18,25,34], and fibrosis [18,23]. Aroclor 1260 altered hepatic lipid metabolism and decreased gluconeogenesis consistent with metabolic disruption [17,18,23,25,26,34]. Insulin, leptin, and FGF-21 levels and/or signaling were reduced consistent with endocrine disruption [4,17–19,21,25,34]. Increased hepatocyte-derived transforming growth factor β activated profibrotic pathways [23].

Pollutant-induced hepatic nuclear receptor activation and crosstalk were proposed to be molecular initiating events in EPA's NAFLD AOP [31]. The pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) are nuclear receptors implicated in hepatic xenobiotic/intermediary metabolism, inflammation, and NAFLD [32]. NDL PCBs ligand-activated human PXR and CAR variants as well as mouse PXR [25,33]. PCBs indirectly activated mouse and human CAR *via* high-affinity hydrophobic binding at EGFR's ligand binding domain to prevent ligand-induced receptor endocytosis and tyrosine kinase activation leading to downstream CAR dephosphorylation and activation [16,17,21,25,33]. Perhaps, owing to similarities with the insulin receptor, the EGFR also regulates numerous pathways involved in liver metabolism, regeneration, and gene expression [46]. By impacting these pathways, PCB-mediated EGFR signaling disruption may explain why Aroclor 1260 worsened HFD-induced NAFLD even in CAR null mice, while the direct CAR agonist, TCPOBOP, was protective [34,47].

Recently, a proteomics approach determined that Aroclor 1260 negatively regulated the activities of several protective nuclear receptors [23]. These included hepatocyte nuclear factor 4-alpha (HNF4α), farnesoid x receptor (FXR), PPARα/δ/γ, thyroid hormone receptor α (TRα), and others. Agonists for several of these receptors (e.g., FXR, PPARα/δ) are currently in clinical trials for the treatment of NAFLD, suggesting that their negative regulation by PCBs could worsen fatty liver disease. Aroclor 1260 antagonized human PPARα, but not FXR or PPARγ, *in vitro* suggesting that both direct and indirect mechanisms may have contributed to the observed reductions in nuclear receptor activities [33]. PCBs interacting with diet decreased HNF4α protein and/or mRNA levels [22,23]. HNF4α is a critical identity gene regulating the expression of the liver's specific metabolic

Figure 3



Schematic diagram depicting mechanisms of nondioxin-like PCBs in NAFLD. Nondioxin-like (NDL) PCBs induce proinflammatory cytokines, activate CAR, and PXR and inhibit EGFR, leading to signaling disruption and transcriptional reprograming that facilitate diet-induced steatohepatitis and fibrosis. NDL PCBs also target extrahepatic organs including the intestine (in conjunction with a high fat diet), pancreas, and adipose tissue, thereby altering the gut microbiome, insulin levels, and leptin levels, respectively, further exacerbating NAFLD symptoms. PCB, polychlorinated biphenyl; NAFLD, nonalcoholic fatty liver disease; CAR, constitutive androstane receptor; PXR, pregnane X receptor; EGFR, epidermal growth factor receptor; PPAR, peroxisome proliferator-activated receptor; NRF2, nuclear factor erythroid 2-related factor; HNF4a, hepatocyte nuclear factor 4-alpha; FGF, fibroblast growth factor; TGF β, transforming growth factor β; INSR. insulin receptor; LEPR, leptin receptor.

genes as well as pancreatic insulin production [48]. Therefore, its downregulation may also explain the reduced insulin levels associated with NDL PCB exposures [4,17,18,25,34]. The activities of other hepatic transcription factors including nuclear factor erythroid 2 like 2 (NRF2) were also altered [23]. NDL PCB-induced NRF2 downregulation decreased hepatic glutathione levels, rendering the liver more susceptible to the oxidative stress imposed by the second 'hit' of diet induced obesity [23].

The reduction in intracellular phosphoprotein levels associated with PCB exposures has been termed signaling disruption [16,17]. Because the activity of many transcription factors is regulated by their phosphorylation status, signaling disruption contributed to PCB-induced hepatic transcriptional re-programming [23]. In an animal model of TASH, hepatic phosphoprotein levels were reduced up to 25% by Aroclor 1260, and these changes negatively impacted multiple steatohepatitis pathways [17]. HFD did not dramatically alter the hepatic signaling capacity of the liver but exacerbated the negative PCB effect. Decreased kinase function, rather than increased phosphatase activity, was responsible for the observed phosphoprotein downregulation [17]. Many, but not all, affected kinases were downstream of the EGFR. PCB binding sites were identified on the EGFR, and PCBs potently inhibited hepatic EGF-dependent EGFR signaling *in vitro*/*in vivo* and decreased placental EGFR phosphorylation human subjects [16,17,21,49]. NDL PCBs are likely responsible for the majority of PCB-induced EGFR inhibition because they have bioaccumulated to a greater degree in humans. In summary, PCBs antagonized the EGFR to cause signaling disruption (Figure 2) which contributed to the hepatic transcriptional reprogramming which promoted the development of diet-induced steatohepatitis (Figure 3).

Future directions

Emerging data implicate PCBs in liver fibrosis [11,18,23,28] and in the gene–environment interactions influencing NAFLD pathogenesis (e.g., patatin-like phospholipase domain-containing protein [PNPLA3] [19,22]). The hepatic effects of relevant PCB mixtures (e.g., DL plus NDL congeners) differed from the individual components [19]; and sexually dimorphic responses were reported [4,22,35]. The potential role of PCBs in the developmental origins of NAFLD remains unknown. These areas warrant future investigation.

Conclusions

While PCBs have long been associated with hepatotoxicity, they have only recently been associated with NAFLD. The responsible mechanisms included alterations in the following: (i) hepatic lipid metabolism, inflammation, and fibrosis; (ii) the gut–liver axis and microbiome; (iii) phosphoprotein signaling disruption resulting in transcriptional reprogramming with attenuated hepatoprotective responses; (iv) endocrine disruption including organokines; and (v) mitochondrial dysfunction with oxidative stress and antioxidant depletion. Interactions between diet and PCBs determined NAFLD severity. Thus, while PCB levels have decreased in the environment, PCBs may only now be impacting NAFLD due to the recent obesity epidemic. More PCB data are needed, particularly from human subjects with biopsy-proven NAFLD.

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Conflict of interest statement

The authors declare no conflict of interest.

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