Chapter

Impact of PCBs, Furan and Dioxin on Hepatocarcinogenesis

Mohamed Helal, Sara Ghanem and Amany El-Sikaily

Abstract

Stockholm Convention defined polychlorinated biphenyls (PCBs) as a group of persistent organic pollutants (POPs) such as dioxin/furan, dichlorodiphenyltrichloroethane, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, aldrin, polychlorinated dibenzofurans and organometallic compounds (such as organotin and organomercury) which share the same characteristics of being persistent, bioaccumulative and toxic and can travel long distance through various media. They have diverse health impacts with different underlined molecular mechanisms. Recently, PCBs were referred as potent carcinogens with persistent existence in the environment. As the liver is the organ of detoxification, it is the major target organ for toxic effects induced by environmental contaminants, including PCBs. PCBs, furan and dioxin exert their hepatocarcinogenic effect through different mechanisms such as induction of oxidative stress, an increase of reactive oxygen species (ROS), mutagenic induction to oncogenes and epigenetic alteration to hepatic cells. In this chapter, we will provide an updated overview about PCBs, furan and dioxins, their impact on liver cancer initiation and progression on various *in vivo* and *in vitro* systems and its underlined molecular mechanisms. Also, a special emphasis will be directed to highlight zebrafish as *in vivo* model system to analyse the hepatocarcinogenic effect of these pollutants.

Keywords: polychlorinated biphenyls, Dioxin, Furan, hepatocarcinogenesis, zebrafish

1. Introduction

Polychlorinated biphenyls (PCBs) are related to a larger group of environmental pollutants named persistent organic pollutants (POPs) with intense ecological burden and toxicological problems [1, 2]. PCBs are halogenated aromatic hydrocarbons with special chemical formulas encompassing 209 congeners [3]. Based on the number of chlorine atoms and their location at the biphenyl, PCBs can be divided into lower and higher chlorinated congeners. Lower and higher chlorinated PCBs have different bioaccumulation rates [4]. PCBs are made of two cis-carbon rings linked by a single carbon bond and biphenyl molecule. Each PCB molecule consists of 12 carbon atoms alongside chlorine atoms substituted for hydrogen ones at any of 10 possible positions. Hence, theoretically, 209 possible PCB congeners can be found [5]. Due to their chemical characteristics, they have been used extensively in the industry as electric insulators, plasticizers, heat exchange and hydraulic fluids. The main problem of PCBs lies in their persistent nature, high degree of lipophilicity, slow transformation rate and low environmental degradation, which made them associated with a broad spectrum of human diseases such as reproductive,

immunological and carcinogenic [3, 6–8]. In 1987, International Agency for Research on Cancer and in 1996, the US environmental Protection Agency reported that PCBs are carcinogen in laboratory and wild animals and a possible carcinogen in humans [9–15].

Absorption of PCBs is feasible through ingestion, inhalation and dermal routes. The highest exposure pattern occurs through inhalation and skin absorption [16]. Once inside the body PCBs are transported to the liver. The main target of PCBs metabolism is the liver by the action of hepatic cytochrome p450 (CYP450). According to their chemical structure, PCBs can bind to different receptors [17] such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) and pregnane X receptor (PXR) [3]. Therefore, research was focused to study the impact of PCBs on liver function alteration and carcinogenicity [18]. Based on PCBs' chemical structure and receptor affinity, they can be categorized into dioxin-like and non-dioxin-like PCBs. Among different PCBs, Aroclors 1016, 1242, 1254 and 1260 are the most produced and used PCBs in the US during the 1958–1977 period [19].

One of the earliest incidences of human direct PCB ingestion was reported in Japan in 1968 and known as Yusho accident (oil syndrome in Japanese). This incident affected more than 1800 people who ingested rice oil contaminated with kanechlor-400 [20]. The average intake of PCBs in Yusho patients was 633 mg. The PCB concentration in adipose tissue was 46–76 ppm. After 34 years, the titre of non-ortho and mono-ortho PCBs in the blood of those patients reached 320 and 76 pg./g lipid, respectively [21]. Another famous PCB toxicity outbreak was reported a few years later after the Yusho incident in 1979 in Taiwan, known as the Yucheng incident (oil syndrome in Chinese). In this incident, around 2000 individuals consumed the same rice oil contaminated with Kanechlor-500 from one store [22]. Later, they developed skin problems and various health diseases. The concentration of PCBs in blood ranged from 3 to 1156 ppb [23]. A meta-analysis study of Yusho and Yucheng incidents showed that most patients exhibit a high degree of mortality due to lung, liver and skin cancers in both men and women [24, 25].

Before 1996, different assumptions have been made regarding PCBs' carcinogenicity. Sometimes all PCBs were considered carcinogens. Other assumptions indicated that mixtures with high chlorine content are only carcinogenic [26]. The carcinogenic potential of different PCBs is attributed to PCBs potency, which is affected by environmental processes (partitioning, chemical reaction, transformation and preferential bioaccumulation). Partitioning includes the fractionation of PCB mixture into different environmental compartments (air, water, sediment and soil). PCBs adsorption rate increases proportionally with their chlorine and organic content where low-chlorine content PCBs tend to be more volatile and hydrophilic and high-chlorine content is more persistent and lipophilic. Chemical transformation of PCBs in the environment occurs through the biodegradation process by the action of anaerobic bacteria in the sediments. These bacteria remove chlorines from *para* and *ortho* positions leading to a reduction of PCB toxicity [27]. PCBs bioaccumulation tends to concentrate in the adipose tissue with long-term stability and toxicity [28]. Due to the above-mentioned characteristics, EPA developed a new test approach to assess the cancer risk of different PCBs considering both environmental processes and PCBs toxicity [29]. Furthermore, Stecca *et al*. used different human cell lines (HuH6, HepG2 and DLD-1) to develop a suitable *in vitro* test battery to evaluate the cumulative effect of different PCBs mixtures. Their results showed that the best *in vitro* model to study toxicity is HepG2 and DLD-1 cell lines in terms of expression panel of several genes such as *AhR*, *AR*, *PXR*, *PPARγ*, *ERβ* and *THRα* (which showed the best representative expression upon PCBs mixture treatment) [30]. Most importantly, many of the genes identified by the Stecca group are also

implicated in lipogenesis and lipid homeostasis in the liver indicating a significant role of PCBs on lipid metabolism in the liver [31].

Based on several reports from different agencies, PCBs were referred as possible human carcinogens [32]. This assumption is based on experimental data from rodents where PCBs treatment increased neoplasm formation in different rodent tissues.

Commercial PCB mixtures such as Aroclors 1016, 1242, 1254 and 1260 (dietary PCBs concentration ranged from 25 to 200 ppm over a period of 24-month treatment) were found to induce not only alteration in liver function tests (AST, ALT and GGT) but also liver tumours with bile duct carcinoma (cholangiocarcinoma) in rats (female tumour incidence higher than male rats) after long-term feeding regime [33]. In addition, rats exposed to commercial mixtures with 60% chlorine through a dietary lifetime regime developed benign liver tumours that eventually progressed to malignant ones [34]. Furthermore, a mixture of PCB126 and PCB153 caused a mild increase in neoplastic liver lesions in mice. This was accompanied by an up-regulation of Cyp1a1 and Cyp2b10 (RNA and protein level) [35]. The same promotion of liver carcinogenesis was observed in partially hepatectomized rats challenged with a single DEN dose and subsequent intraperitoneal injection of PCB105, 126 or 153 [36]. Moreover, rats receiving a single dose of DEN followed by intraperitoneal injection of PCB77 or PCB153 (150 μmol/kg) alongside selenium-enriched diets feeding developed hepatic neoplasm. Selenium administration enhances the carcinogenic induction of PCB77 more than that of PCB153 as the number of positive placental glutathione s0transferase (PGST+) hepatic regions was higher in the former than the latter, respectively [37].

Single PCB compounds have a preferential binding affinity to different receptors. For example, PCB126 binds to AhR while PCB153 binds to CAR. Rats treated with a single dose of DEN followed by PCB126, PCB153 or in combination developed hepatic neoplasms most profoundly in single PCBs treatment only (PCBs combination treatments showed antagonistic results on liver neoplasm formation) as indicated with positive GST-P liver areas [38]. Cultured mouse hepatocytes treated with PCB126 exhibited reduced hepatocyte glycogen content in a dose-dependent manner and suppressed forskolin-stimulated gluconeogenesis from lactate. Interestingly, glycogen treatment of cells restored PCB127 effects, indicating that PCB127 could affect the terminal players in the gluconeogenesis cycle. Finally, PCB126 could activate AhR and its downstream effector phosphoenolpyruvate carboxylase. This suggests a possible role of PCBs as energy metabolism disruptor agents [39]. Other studies showed that PCB153 could induce hepatocarcinoma through induction of NF-kB in mice (this was inhibited by deleting the p50 subunit of NF-kB) [40] or induce mutation in β-catenin (*Catnb*) [41] or *ras* [42] oncogenes as a promoter of tumorigenesis.

PCBs administration could interfere with metals accumulation in the liver and affect their transport and excretion through kidneys. Mice fed different concentrations of PCBs with Cadmium (Cd)-enriched diets showed a reduction of Cd concentration in the liver. Also, liver histology of those mice revealed a characterized centrilobular enlargement of hepatocytes, hepatic focal necrosis and clear cytological signs of malignancy than the control group [43]. On the other hand, female rats fed a diet enriched with high-dose Aroclor-1254 and Aroclor-1260 for 78 weeks developed initial iron accumulation in the liver by week 52, induced hepatocyte proliferation and eventually liver carcinoma by the 78th week, indicating that iron accumulation in the liver is an early sign of hepatic neoplasm transformation induced by PCBs [44].

Human exposure cohort studies were also conducted to monitor the pathological aspects of PCBs. Workers in capacitor factories exposed to Aroclors mixtures

with 41–54% chlorine content had increased mortality rates from liver tumours (gall bladder and biliary tract) [45]. The same finding was reported in HCC Italian patients settled in areas highly polluted with PCBs [46, 47]. The burden of PCBs concentration in liver, lung and kidney tissues of Chinese cancer patients residing near e-waste disassembly sites was very high (257.9 to 455.1 ng g^{-1}), indicating a possible correlation between PCBs exposure and cancer incidence in those patients [48]. Another long-term cohort study was conducted in Germany in former PCB-exposed workers. The study linked the change in liver enzymes and morphology with PCB exposure level. There was a significant inverse connection between PCB concentration and γGT and a significant association between liver enlargement and PCBs level [49]. Another cohort study in the USA linked elevated levels of ortho-substituted PCBs and liver toxicant-associated steatohepatitis (TASH) in the former worker of PCB manufacturing complex. The authors reported that the increase in PCBs exposure was connected with an increase in liver disease burden, inflammation, steatohepatitis induction and hepatocyte apoptosis and fibrosis [50]. A large and extensive cohort study was conducted on 138,905 electricity workers exposed to insulating liquids of PCBs at five different electricity companies between the period of 1950 and 1986. Poisson regression was utilized to examine mortality of skin cancer (melanoma) and liver cancer in relation to PCBs exposure. Results showed that PCBs exposure was linked to melanoma development and in some workers hepatic cancers [51]. A controlled study was nested with two large prospective cohorts (one from Northern California Multiphasic Health Check-up (MHC) comprising 408 HCC cases and Norwegian Janus group comprising 84 HCC cases) from 1960 to 1980. Measuring 37 different congeners with GC-MS, the authors found that among measured congeners, PCBs (151, 170, 172,180,177 and 195) congeners were the highest with a concentration in HCC patients 1.3 to 1.4 ng/g lipid for the first group and 1.9 ng/g lipid for the second group, confirming a significant link between PCBs levels and HCC development [52].

PCBs can be indirectly accumulated in the human body through food chain by ingestion of aquatic animals contaminated with PCBs. For example, Delistraty study showed that PCBs titre in different aquatic animals in Columbia River, USA was significantly high. Sturgeon liver, whitefish fillet, carbs and smallmouth bass all showed significant high level of dioxin-like PCBs, non-dioxin like PCBs and total PCBs [53]. Another study showed that Bottlenose dolphin was stranded alive with high levels of different PCBs such as PCB 153,180, 187 and 138. Finally, large cell immunoblastic lymphoma was observed in the hepatic sinuses of these dolphins accompanied with liver enlargement. All previous studies indicate a direct correlation between carcinogenesis induction and levels of PCBs in those dolphins [9].

1.1 Possible hepatocarcinogenic mechanisms of PCBs

PCBs mode of action and the underlined molecular mechanisms of toxicity and carcinogenicity have not been deciphered so far [7]. Yet, studies on different animal models, *in vitro* cell lines and human cohort studies could give us a glimpse of the key molecular players responsible for different pathogenic outcomes.

2,3′4,4′,5-Pentachlorobiphenyl known as PCB118, one of the most persistent congener members, was found to promote hepatocellular carcinoma SMMC-7721 cell proliferation and glycolysis through AhR, which subsequently elevates the expression of pyruvate kinase M2 (PKM2) and stimulation of reactive oxygen species (ROS) production through nicotinamide adenine dinucleotide phosphate (NADPH). These effects were inhibited by treating cells with PKM2 shRNA and superoxide dismutase, respectively [6].

PCB126 (3,3′,4,4′,5-Pentachlorobiphenyl), a non-ortho-chlorinated congener, was found to increase the synthesis of ROS specifically. Treatment of HepG2 cells with this congener enhance their carcinogenicity by inducing an oxidative stress response that was underlined by activation of mitogen-activated protein kinases (extracellular signal-regulated kinase 1/2), p38, c-Jun phosphorylation, activating protein-1 (AP-1) and finally an expression of antioxidant-responsive element (ARE)-dependent genes [7]. In addition, Faust *et al*. demonstrated that rat progenitor liver cells (WB-F344) treated with PCB126 exhibit a differential transcriptional response over the treatment period. At 6-hour post-treatment (hpt), about 146 significant deregulated genes were identified under AhR direct targets. The number of deregulated genes was 658 and 968 after 24 and 72 hpt, respectively. The most identified genes through gene ontology analysis were affiliated to developmental, cell cycle, growth control and drug metabolism. The main targeted pathways were Wnt and TGF-β. Finally, they have also identified a novel target gene under the AhR signalling pathway such as *Fst*, *Btg2*, *Ctgf* and *Hbegf* [54]. AhR is an essential receptor controlling liver response to environmental toxicants. By using rat and human hepatocytes as *in vitro* cellular models to study PCBs toxicity, researchers found that rats fed Arochlor 1254 exhibit liver carcinoma through activation of AhR and downstream induction of *raf* effector in a MAPK-dependent pathway [55].

PCB47, 49, 52, 77 and 153 have a tumour promoting activity [32]. Also, some PCBs induce liver toxicity through induction of mixed function oxidases (phenobarbital, 3-methylcholanthrene) [56] and inhibition of anti-oxidant production such as PCB154, 155, 184 and 153 inhibit paraoxonase 1 (PON1) in treated rats [57].

Most PCBs mixtures with high chlorine content and their derived metabolites showed superior tumour-promoting characteristics. Yet, concern over low-chlorine content PCBs was raised after experiments showing that dihydroxy metabolites induce breast cancer by inducing oxidative DNA damage in breast cancer cells [58]. Another possible mechanism of PCBs carcinogenicity is their ability to suppress the immune system and cause endocrine disruption [59]. PCB104, 188 and their hydroxylated forms 4′-50, 4′-30, 4′-72, 4′-112 and 4′-121 disturb endocrine pathways in rainbow trout cultured hepatocytes and induced vitellogenin synthesis indicating altered liver physiology [60]. Human MCF-7 cells exposed to PCBs analogues showed a reduction in catechol-O-methyltransferase (COMT) activity on the transcriptional and translational level *via* the oestrogenic receptor. This could explain the PCB mode of liver tumour induction *via* modulation of oestrogen receptor response [61]. A comparative metabolomic study was conducted on rats fed a control diet and choline-deficient diet (as an inducer of liver non-alcoholic steatohepatitis) and subsequently exposed to PCB126. The addition of PCB127 promoted fatty liver development through dysregulation of glycerophospholipid metabolism, CoA biosynthesis pathway and glutathione metabolism. In addition to lipid metabolism disturbance, PCB127 down-regulated redox genes, and induced oxidative stress and mitochondrial dysfunction [62].

HepG2 cells co-treated with benzo-a-pyrene and different doses of Aroclor 1254 had a high degree of DNA damage (as indicated by DNA migration assay and formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG)), oxidative stress and elevated CYP1A activity [63]. In another experiment, HepG2 cells exposed to various PCBs concentration (0.01-10μM) exhibit aggressive carcinogenic behavious underlined by pERK Tyr204 and pMdm2 Ser166 which attenuated P53 activity in those cells [64].

Dioxin-like PCBs such as PCB 77 and 81 were shown to have direct genotoxic effects on Chinese hamster V79-derived cell line by inducing micronuclei formation, and induced expression of CYP1A1, CYP2E1 and γ-H2AX protein (a marker of DNA double-strand breaks) [65, 66].

Another surprising finding of PCBs-induced hepatic carcinogenicity is their ability to inhibit intercellular community between liver cells. Mouse hepatoma cell line (Hepa1c1c7) treated with TCDD and different PCBs showed a rapid intercellular inhibition after 2 hrs. of treatment accompanied with AhR activation and induction of ethoxyresorufin O-deethylase (EROD) activity (an early marker of PCBs induced oxidative stress) [67]. Moreover, by using a quantitative polymerase chain reaction (qPCR) to quantify relative telomere length in lung and liver samples collected from rats treated with different PCBs (126, 153 and a mixture of them) showed larger relative telomere length, which is an early indication of euplastic or non-neoplastic pathogenic disease development [68].

2. Furan

Furan, a heterocyclic organic chemical, is considered as a human carcinogen and a liver toxicant in rodents [69]. It is found in a broad spectrum of common heat-treated and jarred foods in addition to tobacco smoke. It is also generated from numerous precursors such as amino acids, ascorbic acid and carbohydrates [70]. Infants received the highest furan exposure from ready-to-eat meals, while adults are exposed to furan by the dietary intake of coffee [69]. Furan is found mainly in the liver and is metabolized to the reactive metabolite, cis-but-2-ene-1,4-dialdehyde (BDA) through cytochrome P450 2E1 (CYP2E1). Reported studies have indicated that humans can convert furan to its reactive metabolite and cis-2-butene-1,4-dial (BDA), and consequently may be subjected to furan toxicity [71].

Being hepatotoxic, researchers [72] stated that furan is associated with cholangiofibrosis in rats and HCC & adenomas in mice. They also indicated that oxidative stress, alterations in gene expression, epigenetic modifications, inflammation and increased cell proliferation represent indirect mechanisms that are included in carcinogenesis. The carcinogenic effects of furan have been referred to as genotoxic and non-genotoxic modes of action. Epigenetic alterations are among the most important non-genotoxic alterations induced by furan since they are related to all other non-genotoxic events [69]. As a genotoxic furan could be linked to furan-into carcinogenicity, current human exposure levels to this hepatotoxicant may represent a risk to human health and required the necessity for its mitigation [73].

2.1 Possible hepatocarcinogenic mechanisms of furan

Metabolism of furan leads to the formation of protein adducts in the target organ. The first bioactivation step comprises the oxidation of cytochrome P450-catalysed of furan, which generates cis-2-butene-1,4-dial (BDA). BDA can react with lysine to form pyrrolin-2-one adducts [70]. This metabolite directly reacts with DNA nucleophiles and proteins [74]. It is also known as a bacterial mutagen in Ames assay strain TA104. According to metabolic studies, this reactive metabolite is formed *in vivo* [74]. BDA was found to react with glutathione (GSH) generating 2-(S-glutathionyl) butanedial (GSH-BDA), which reacts in turn with lysine forming GSH-BDA-lysine cross-links. Relative reactivity of these two intermediates was explored by the reaction of cytochrome *c* with BDA in the existence and absence of GSH [75]. Using MALDI-TOF mass spectrometry, BDA was found to react widely with cytochrome *c* forming adducts (which add 66 Da to the protein) according to pyrrolinone adducts formation. On the other hand, when GSH was added to the reaction, the overall extent of adduct formation was reduced. Briefly, the majority of adducts arose on lysine residues contributing to the carcinogenic hazard of furan [71]. By using liquid chromatography, tryptic peptides analysis clarified a cross-link

between GSH-BDA and lysine 107 of histone H2B isolated from male F344 rats' liver exposed to carcinogenic doses of furan. This cross-link was detected before the identification of epigenetic changes and occurred at a lysine residue that is known as a target for epigenetic modifications and crucial for nucleosome stability [76].

Being a hepatocarcinogen in mice and rats, furan induced an enhancement for cytotoxic pathways represented by signalling of stress-activated protein kinase (SAPK) and death receptor (DR5 and TNF-alpha), and proliferation through extracellular signal-regulated kinases (ERKs) and TNF-α. In addition, NF-kappa B and c-Jun (genes essential for liver regeneration) were involved in response to furan [76]. Previous studies applying furan high doses revealed that it induced tumours at nearly 100% incidence at all doses [77]. Fraction of H-ras codon 61 CAA to AAA mutation was increased in liver tumours of furan-treated mice [78]. Besides, furan has a deleterious impact on the activity of crucial target enzymes included in ATP synthesis, glycolysis, redox regulation as well as b-oxidation in rat liver. After treatment with a high dose of furan, it was found that glyceraldehyde-3-phosphate dehydrogenase was significantly inhibited and observed some metabolic changes reliable to blockage of the glycolytic breakdown of glucose in the liver of the rat. Despite an increase in enoyl-CoA hydratase activity, an enhancement of ketone bodies production and a reduction in the activity of succinate dehydrogenase were recorded as a result of furan treatment. These enzymatic changes were linked to impairments occurring in cellular processes affecting the metabolic pathways and antioxidant defence and indicate mitochondrial dysfunction as a serious incident in furan toxicity [79]. Moreover, targets of putative protein of furan reactive metabolites induced functional damage of numerous individual proteins and interference with pathways, especially that of mitochondrial energy production, redox regulation, and protein folding. This damage represented critical targets of furan toxicity and can combine together to disturb cell homeostasis and cause the cell death of hepatocytes [80].

The liver is the main target organ affected by furan as indicated by serum biomarkers changes, change in liver weights and histological lesions after exposure to furan. Accordingly, a dose of 0.03 mg/kg bw of furan was proposed to be the non-detectable serious effect for hepatic toxicity [80]. In addition, Selmanoğlu *et al.* [81] revealed a significant increase of LDL levels, a significant decrease in ALT and ALP levels and insignificant changes in liver MDA levels, catalase activities and superoxide dismutase in the liver of rat groups treated with furan comparing with control groups. They also indicated a significant change in liver weights of furan-treated groups and observed hyperaemic blood vessels in their hepatic tissue under the light microscope. Histopathologically, multifocal hepatocellular necrosis intermingled with pigment-laden Kupffer cells and reactive leukocytes, oval-cell hyperplasia enhancement, hepatocyte mitoses increase and hepatocyte injury were also observed in livers from furan-treated mice as a result of furan induction [82]. Furthermore, furan-enhanced Ki-67 and PCNA expression in hepatic tissues increased the content of ROS in addition to indices of oxidative damage and decreased the TAC in the serum level of exposed rats. Finally, exposure to furan was found to be linked to changes in the mRNA expression pattern of intermediate filament proteins in hepatic tissues and promoted fibrosis and proliferation of hepatocytes in the liver [82].

In addition, analysis of liver rats treated with furan by Comet assays showed breaks in both strands of DNA, an increase in oxidized purines and pyrimidines at cancer bioassay dosage represented by a near-linear dose-responsive manner [83]. Consequently, these findings postulated that furan induces cancer mainly in rats' liver through a secondary genotoxic mechanism including oxidative stress, a down-regulation in the expression of apoptotic, cell-cycle checkpoints as well as DNA-repair genes accompanied by inflammation and cell proliferation dosage [83].

Furthermore, glutathione S-transferase placental form-positive (GST-P) foci are considered as preneoplastic lesions markers in the hepatocarcinogenic rats. Using reporter gene transgenic rats, it was found that furan rapidly induces GST-Pþ foci formation without reporter gene mutation after short exposure [84]. On the other hand, GST-P foci development is probably due to cell proliferation other than the genotoxic mode of action in furan-treated rats. Based on the close association between neoplastic hepatocytes and GST-P, Hibi *et al*. [85] postulated that cell proliferation following signal transduction other than the pathway of mitogen-activated protein kinase (MAPK)/ERK may contribute in the early stage of furan-induced hepatocarcinogenecity.

Cholangiofibrosis is defined as a physical anomaly that occurs before cholangiocarcinoma development in some rodents. Some reports explained that severely affected areas of the liver representing injury due to furan administration were extended into the portal and capsular parts, resulting in a rapid ductular cells proliferation that extended into the parenchyma accompanied by a subtype of liver fibroblasts. These ductules were differentiated into hepatocytes lacking fibroblasts or developed to form tortuous ductular structures replacing much of the parenchyma, leading to cholangiofibrosis [86]. Moreover, furan-induced cholangiocarcinomas were proposed to develop from cholangiofibrosis areas as a consequence of indirect and chronic damage to DNA through oxygen radicals joined with persistent proliferative signals, including loss of connexin 32, which acts to translate this DNA damage to fixed mutations [87].

2.1.1 Epigenetic alterations and the non-genotoxic mechanism of furan in liver

The carcinogenic effect of furan has been referred to as a genotoxic and non-genotoxic mechanism comprising epigenetic alterations in liver tissue [88]. Some reports postulated that furan carcinogenicity is caused by a non-genotoxic mechanism since it was not genotoxic in *in vivo* or *in vitro* micronucleus assay [89, 90]. Other studies indicated that BDA is not directly responsible for the effects of furan on mutational spectra *in vivo.* Therefore, an indirect mechanism of genotoxicity was hypothesized in which chronic toxicity was followed by inflammation and secondary cell proliferation that triggers the development of cancer in furan-exposed models [82].

In addition, epigenetic alterations involving DNA and microRNA (miRNA) methylation play a fundamental role in inducing furan carcinogenicity. It was indicated that DNA methylation changes and miRNA modulation followed by a DNA-damage response are the most pronounced alterations resulted from the use of 3-month furan treatment at a carcinogenic dose suggesting that non-genotoxic mechanisms are crucial for furan carcinogenicity [91]. It was found that gene-specific DNA methylation alterations have an essential role in the contribution of furan hepatotoxicity and hepatocarcinogenicity [88]. Other studies indicated that aberrations in microRNAs (miRNAs) expression are one of the non-genotoxic alterations induced by furan exposure, which highlighted the role of epigenetic impairments in the furan hepatotoxicity mechanism [69].

Moreover, Conti *et al*. [92] mentioned that epigenetic modifications which occurred in hepatotoxicity and carcinogenicity of furan are dose and time-dependent. They noted some epigenetic aberrations represented by DNA methylation, histone lysine acetylation and methylation, gene-specific methylation and alteration of chromatin-modifying genes expression in male Fisher rats treated with furan. Their findings indicated that sustained alterations in histone lysine acetylation (which is responsible for the ability of cells to maintain and control correctly the expression of genetic information) represent the adverse effects of furan

induction. Some reports indicated that gene expression alterations resulted from furan exposure were irreversible [91]. Using whole-genome transcriptomic analysis, Tryndyak *et al*. [88] demonstrated differential gene expression alterations in liver lesions induced in male rats treated with furan. These alterations are essential in key pathways linked with the diverse aspects of liver pathology. Furthermore, it was noted that the continuous exposure to furan induced noticeable changes in the expression of miRNA represented by over-expression of hepatic miRNAs (miR-34a, miR-93, miR-200a, miR-200b and miR-224), and down-regulation of miR-375. In addition, hypermethylation of cytosine DNA and the lysine methylation of histone H3K9 and H3K27 at the MiR-375 genes were increased due to the reduction in miR-375 expression. It was revealed that the significant miR-375 inhibition was accompanied by toxicity and carcinogenicity of furan-induced liver leading to an up-regulation in Yes-associated protein 1 (YAP1), which is one of the principal events in liver carcinogenesis [69].

Since the mammalian genome is transcribed into mRNAs that code for protein and other non-coding RNA products [93]. Long non-coding-RNAs (lncRNA) are known as ncRNA species >200 nucleotides long, which represent significant epigenetic regulators of gene expression and are included in a wide spectrum of biological processes related to toxicology. Recio *et al*. [93] indicated that lncRNAs are transcriptional targets in the cytotoxic levels of furan exposure inducing cell proliferation. They also hypothesized that lncRNAs are considered as epigenetic biomarkers of carcinogenic exposure.

3. Dioxins

Dioxins are considered as representative toxic agents among persistent organic pollutants and a large family of halogenated aromatic hydrocarbons, which composed of tricyclic aromatic compounds [94]. These compounds are produced by industrial wastes and can accumulate in soil, sediments as well as food chains with long half-life of numerous years, affecting human health [95]. 2,3,7,8-Tetrachlorodibenzo -p-dioxin (TCDD) is a typical representative and the most toxic substance of dioxins, which exhibits systemic hepatotoxicity, carcinogenicity, immunotoxicity, teratogenicity, endocrine disruption and also affects pathology and physiology of human skin [96]. Being with four chlorine atoms in lateral positions, 2,3,7,8 Tetrachlorodibenzop-dioxin (TCDD) is the most biologically active isomer of dioxins [97]. It is a widespread and persistent pollutant in the environment originated from waste incineration or metal industries, plastics manufacturing and paper processing [98]. Moreover, it plays a significant role by binding to AhR for endocrine changes in experimental animals [99]. Besides, Türkez, Türkez *et al.* [100] suggested that oxidative stress has a crucial role for toxic effects of TCDD with AhR.

3.1 TCDD exposure and dioxin receptor

The aryl hydrocarbon receptor (AhR) is considered as a ligand-activated receptor which enables environmental pollutant toxicity like 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [101]. It is also known as xenobiotic receptor or dioxin receptor and is a member of the basic helix-loop-helix/period AhR nuclear translocator single-minded family [102]. AhR translocates to the nucleus after binding to TCDD, dimerizes with AhR nuclear-translocator protein, binds to dioxin-responsive elements and up-regulates a series of genes expression that encode xenobiotic-metabolizing enzymes, such as cytochrome P450s (e.g. CYP1A1, CYP1A2), NAD(P)H

quinone oxidoreductase as well as a form of UDPglucoronosyl-transferase-6 [103]. Even though AhR may serve as part of an adaptive chemical response, numerous studies reported that this dioxin receptor has important functions in liver, cell proliferation, cardiac development [104, 105] and the ubiquitin-proteasome system [106]. AhR plays a fundamental role in three biological aspects including xenobiotics metabolism, the toxic responses related to TCDD (dioxin) exposure and the vascular remodelling of the developing embryo. Using *Cre*-lox technology, Walisser *et al*. [107] examined the role of AhR signalling in hepatocytes and endothelial cells. They revealed that AhR signalling in hepatocytes is crucial to produce adaptive and toxic hepatic responses due to TCDD exposure.

Being a generally expressed ligand-dependent transcription factor, AhR mediates cellular responses to dioxins. Boutros *et al*. [108] demonstrated that AhR mediated all effects of dioxin on hepatic mRNA levels and revealed the alteration of 297 genes including many well-established AhR target genes due to dioxin exposure in mice liver. They also indicated that AhR genotype remodelled hepatic transcriptomes suggesting the existence of a basal AhR gene battery. Results of Boutros *et al*. [108] highlighted the fundamental role of this dioxin receptor in the liver tissue.

In response to dioxin, Kennedy *et al*. [109] also explained that the signal transduction pathways that mediate tumour promotion of liver by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are accomplished by the linked action of two receptor systems, the AhR and the receptors for the "IL-1-like" cytokines. However, Yamaguchi and Hankinson [110] indicated that TCDD might suppress the cell growth of liver cancer through numerous signalling pathways, mediated by AhR and its related co-factors. In addition, they found that the impact of TCDD was accomplished by gemcitabine (responsible for nuclear DNA damage in cancer cells), suggesting that their use as a combination may be considered as a suppressor of tumour cell growth *in vitro*.

TCDD can induce hepatic fibrosis through a sequential events of steatosis followed by steatohepatitis. Lee, Wada [102] investigated the role of AhR in liver steatosis in wild type and transgenic mice. They concluded that AhR activated in liver cells induced CD36 expression, enhanced the uptake of fatty acids and steatosis induciton [102].

Cytochrome P4502E1 (CYP2E1) mainly expressed in liver, is involved in the metabolic activation of carcinogens and hepatotoxins such as TCCD and CCl4. At post-transcriptional levels, CYP2E1 is induced and exerted mostly through mRNA and protein stabilization, while xenobiotic induction is found to be very limited at the transcriptional level [101]. Since the effect of xenobiotics on CYP2E1 liver, expression is of significant attention. Therefore, Mejia-Garcia *et al*. [101] studied the effect of TCDD on CYP2E1 liver of mouse and the impact on CCl4 that induced hepatotoxicity. They found that TCDD augmented levels of mRNA and protein in hepatic tissue of mouse CYP2E1 in an AhR-dependent manner and CYP2E1 was induced causing CCl4-induced hepatotoxicity.

Recent studies revealed that TCDD exposure had caused increased productions of lipid peroxidation, reactive oxygen species and histopathological injury in the liver of both rats and mice [111]. This exposure also enhances oxidative stress and diminishes the fluidity of hepatic membrane and glutathione (GSH) content, as well as imbalances the antioxidant enzymes in the liver [112, 113]. Moreover, an increase in the relative weight of the liver, a significant increase in all of the hepatic biomarker levels (glucose, cholesterol, triglycerides, AST, ALT and LDH) in the serum and a decrease of the antioxidant enzyme activities (catalase, glutathione peroxidase and superoxide dismutase) were observed under dioxin effect in hepatic tissue of rat [114]. Additionally, Bentli *et al.* [99] revealed that immunotoxicity associated with altered cytokine levels is among the other TCDD-induced toxicity prominent symptoms. Finally, Ciftci and Ozdemir [115] indicated that one of the

main regulated pathways of TCDD toxicity is the elevated levels of the inflammatory cytokine. Using the real-time polymerase chain reaction (PCR), several studies indicated that heat shock proteins (mortalin, α-B-crystallin, Hsp105, Hsp27 and Hsp90s) and antioxidant enzymes (GST, SOD-3 and catalase) in livers of rats were induced suggesting protective mechanisms against 2,3,7,8-TCDD which induced hepatotoxicity [116]. Moreover, Czepiel *et al*. [117] mentioned that TCDD impaired the liver of rats and the activity of CYP1A1 in a dose-dependent manner. Parenchymal degeneration of hepatic lobules, hepatocytes vacuolation in prominent and peripheralized nuclei, hepatocellular hypertrophy and turgor of the vein in the centriacinar regions were also observed in rats' liver that received a high dose of dioxin [114]. TCDD also induces CYP1A1 activity by elevating the immunohistochemical reactivity of central areas of hepatic lobules located around the central vein in the rat liver [117].

4. Zebrafish models of PCBs-, furan- and dioxin-induced hepatocarcinogenesis

Viluksela and Pohjanvirta [118] reported that paternal exposure to TCDD was considered as the most effective congenator of dioxins in laboratory rodents and zebrafish as it can lower the reproductive performance and reduce the male/female ratio of offspring. Therefore, it will affect subsequent generations *via* both paternal and maternal germlines. These adverse effects have been accompanied by epigenetic alterations in sperm cells and/or placenta, including variations in methylation patterns of imprinted genes.

Besides, previous studies have demonstrated that dioxins broadly alter hepatic mRNA levels [119]. Unexpectedly, Boverhof, Burgoon [120] found that responses of mouse and rats to TCDD exposure revealed that rat and mouse responses diverge significantly through analysis of a limited portion of their transcriptome. Accordingly, it was suggested that both mice and rat models should be applied to detect the acute hepatotoxicity of xenobiotics [120].

Under fabp10a promoter, Zhang [121] also established a line of transgenic zebrafish line (LiPan) characterized with the expression of liver-specific red fluorescent protein (DsRed), which enables the observation of liver in live LiPan fry. They revealed that TCDD could significantly increase both liver red fluorescence and size in LiPan fry. Thus, LiPan transgenic fry offers a suitable and rapid hepatotoxicity assay *in vivo* that should be used to monitor the effect of environmental contaminants from the chemical mixture [121].

Furthermore, by using the inducible *kras* transgenic zebrafish model of hepatocarcinogenesis, Qiqi *et al.* have shown that PCB12 and TCDD alongside other environmental pollutants could accelerate HCC induction and inflammation of the liver [122]. Zebrafish is a very promising model of environmental and molecular toxicology. Further studies and more deep studies should be carried out using this model to provide more insightful information about carcinogenic potential and mechanisms of PCBs, furan and dioxins.

5. Conclusion

Environmental pollutants are a severe persistent burden, which cause a broad spectrum of health problems not only to aquatic animals but also to humans. Among them, PCBs, furan and dioxin are organic pollutants that were widely used in different applications before they were banned due to their carcinogenic

potential. Different studies using different model animals and screening systems (*in vivo* and *in vitro*) indicated their correlation with liver tumour induction and promotion. In this chapter, we highlighted the collective and recent updates linking these groups of pollutants with the pathology of hepatocellular carcinoma. Although extensive research has been done, yet the exact potential and molecular mechanisms of these pollutants are to be discovered and deciphered.

Author details

Mohamed Helal*, Sara Ghanem and Amany El-Sikaily
National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt

*Address all correspondence to: m.helalf@gmail.com

IntechOpen

References

[1] Dirinck E, Jorens PG, Covaci A, Geens T, Roosens L, Neels H, et al. Obesity and persistent organic pollutants: Possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. Obesity (Silver Spring, Md). 2011;**19**(4):709-714

[2] Markowitz G, Rosner D. Monsanto, PCBs, and the creation of a "world-wide ecological problem". Journal of Public Health Policy. 2018;**39**(4):463-540

[3] Faroon O, Ruiz P. Polychlorinated biphenyls: New evidence from the last decade. Toxicology and Industrial Health. 2016;**32**(11):1825-1847

[4] Safe S. Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: Progress and problems. Environmental health perspectives. 1993;**100**:259-268

[5] Carpenter DO. Polychlorinated biphenyls (PCBs): Routes of exposure and effects on human health. Reviews on Environmental Health. 2006;**21**(1):1-23

[6] Liang W, Zhang Y, Song L, Li Z. 2,3′4,4′,5-Pentachlorobiphenyl induces hepatocellular carcinoma cell proliferation through pyruvate kinase M2-dependent glycolysis. Toxicology Letters. 2019;**313**:108-119

[7] Song MO, Freedman JH. Activation of mitogen activated protein kinases by PCB126 (3,3′,4,4′,5-pentachlorobiphenyl) in HepG2 cells. Toxicological Sciences. 2005;**84**(2):308-318

[8] Knerr S, Schrenk D. Carcinogenicity of "non-dioxinlike" polychlorinated biphenyls. Critical Reviews in Toxicology. 2006;**36**(9):663-694

[9] Jaber JR, Pérez J, Carballo M, Arbelo M, Espinosa de los Monteros A, Herráez P, et al. Hepatosplenic large cell immunoblastic lymphoma in a bottlenose dolphin (Tursiops truncatus) with high levels of polychlorinated biphenyl congeners. Journal of Comparative Pathology. 2005;**132**(2-3):242-247

[10] Program NT. Toxicology and carcinogenesis studies of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (Cas No. 57117-31-4) in female Harlan Sprague-Dawley rats (gavage studies). National Toxicology Program Technical Report Series. 2006;**525**:1-198

[11] Program NT. Toxicology and carcinogenesis studies of a binary mixture of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Cas No. 57465-28-8) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) (CAS No. 35065-27-1) in female Harlan Sprague-Dawley rats (gavage studies). National Toxicology Program Technical Report Series. 2006;(530):1-258

[12] Program NT. Toxicology and carcinogenesis studies of a mixture of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Cas No. 1746-01-6), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (Cas No. 57117-31-4), and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Cas No. 57465-28-8) in female Harlan Sprague-Dawley rats (gavage studies). National Toxicology Program Technical Report Series. 2006;(526):1-180

[13] Program NT. Toxicology and carcinogenesis studies of a binary mixture of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Cas No. 57465-28-8) and 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) (Cas No. 31508-00-6) in female Harlan Sprague-Dawley rats (gavage studies). National Toxicology Program Technical Report Series. 2006;**531**:1-218

[14] Program NT. Toxicology and carcinogenesis studies of

2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) (CAS No. 31508-00-6) in female harlan Sprague-Dawley rats (gavage studies). National Toxicology Program Technical Report Series. 2010;**559**:1-174

[15] N Program NT. TP toxicology and carcinogenesis studies of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in female Harlan Sprague-Dawley rats (Gavage Studies). National Toxicology Program Technical Report Series. 2006;**520**:4-246

[16] Bosetti C, Negri E, Fattore E, La Vecchia C. Occupational exposure to polychlorinated biphenyls and cancer risk. European Journal of Cancer Prevention: The Official Journal of the European Cancer Prevention Organisation (ECP). 2003;**12**(4):251-255

[17] Oliver JD, Roberts RA. Receptor-mediated hepatocarcinogenesis: Role of hepatocyte proliferation and apoptosis. Pharmacology & Toxicology. 2002;**91**(1):1-7

[18] Ludewig G, Robertson LW. Polychlorinated biphenyls (PCBs) as initiating agents in hepatocellular carcinoma. Cancer Letters. 2013;**334**(1):46-55

[19] Mayes BA, McConnell EE, Neal BH, Brunner MJ, Hamilton SB, Sullivan TM, et al. Comparative carcinogenicity in Sprague-Dawley rats of the polychlorinated biphenyl mixtures Aroclors 1016, 1242, 1254, and 1260. Toxicological Sciences. 1998;**41**(1):62-76

[20] Kashimoto T, Miyata H, Fukushima S, Kunita N, Ohi G, Tung TC. PCBs, PCQs and PCDFs in blood of yusho and yu-cheng patients. Environmental Health Perspectives. 1985;**59**:73-78

[21] Todaka T, Hirakawa H, Hori T, Tobiishi K, Iida T, Furue M. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and non-ortho and mono-ortho polychlorinated biphenyls in blood of Yusho patients. Chemosphere. 2007;**66**(10):1983-1989

[22] Hsu ST, Ma CI, Hsu SK, Wu SS, Hsu NH, Yeh CC, et al. Discovery and epidemiology of PCB poisoning in Taiwan: A four-year followup. Environmental Health Perspectives. 1985;**59**:5-10

[23] Guo YL, Ryan JJ, Lau BP, Yu ML, Hsu CC. Blood serum levels of PCBs and PCDFs in Yucheng women 14 years after exposure to a toxic rice oil. Archives of Environmental Contamination and Toxicology. 1997;**33**(1):104-108

[24] Onozuka D, Nakamura Y, Tsuji G, Furue M. Mortality in Yusho patients exposed to polychlorinated biphenyls and polychlorinated dibenzofurans: a 50-year retrospective cohort study. Environmental Health. 2020;**19**(1):119

[25] Li MC, Chen PC, Tsai PC, Furue M, Onozuka D, Hagihara A, et al. Mortality after exposure to polychlorinated biphenyls and polychlorinated dibenzofurans: A meta-analysis of two highly exposed cohorts. International Journal of Cancer. 2015;**137**(6):1427-1432

[26] Cogliano VJ. Assessing the cancer risk from environmental PCBs. Environmental Health Perspectives. 1998;**106**(6):317-323

[27] Abramowicz DA. Aerobic and anaerobic PCB biodegradation in the environment. Environmental health perspectives. 1995;**103**(Suppl 5):97-99

[28] Gilbert JM, Baduel C, Li Y, Reichelt-Brushett AJ, Butcher PA, McGrath SP, et al. Bioaccumulation of PCBs in liver tissue of dusky Carcharhinus obscurus, sandbar C. plumbeus and white Carcharodon carcharias sharks from south-eastern Australian waters. Marine Pollution Bulletin. 2015;**101**(2):908-913

[29] Cogliano VJ. PCBs: Cancer Dose-Response Assessment and Application to Environmental Mixtures. Washington, DC, EPA/600/P-96/001F., editor: U.S. Environmental Protection Agency OoRaD, National Center for Environmental Assessment, Washington Office; 1996

[30] Stecca L, Tait S, Corrado F, Esposito M, Mantovani A, La Rocca C. Development of an in vitro test battery model based on liver and colon cancer cell lines to discriminate PCB mixtures by transcription factors gene expression analysis. Toxicology In Vitro. 2016;**34**:204-211

[31] Shan Q, Li H, Chen N, Qu F, Guo J. Understanding the Multiple Effects of PCBs on Lipid Metabolism. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2020;**13**:3691-3702

[32] Silberhorn EM, Glauert HP, Robertson LW. Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. Critical Reviews in Toxicology. 1990;**20**(6):440-496

[33] Brunner MJ, Sullivan TM, Singer AW, Ryan MJ, Toft Ii JD, Menton RS, et al. An Assessment of the Chronic Toxicity and Oncogenicity of Aroclor-1016, Aroclor-1242, Aroclor-1254, and Aroclor-1260 Administered in Diet to Rats: Chronic Toxicity and Oncogenicity Report. Vol. 1. Columbus, OH: Battelle; 1997 Report No.: Battelle Study No.SC920192

[34] Norback DH, Weltman RH. Polychlorinated biphenyl induction of hepatocellular carcinoma in the Sprague-Dawley rat. Environmental Health Perspectives. 1985;**60**:97-105

[35] Rignall B, Grote K, Gavrilov A, Weimer M, Kopp-Schneider A, Krause E, et al. Biological and tumor-promoting effects of dioxin-like and non-dioxin-like polychlorinated biphenyls in mouse liver after single or combined treatment. Toxicological Sciences. 2013;**133**(1):29-41

[36] Haag-Grönlund M, Johansson N, Fransson-Steen R, Hâkansson H, Scheu G, Wärngård L. Interactive effects of three structurally different polychlorinated biphenyls in a rat liver tumor promotion bioassay. Toxicology and Applied Pharmacology. 1998;**152**(1):153-165

[37] Stemm DN, Tharappel JC, Lehmler HJ, Srinivasan C, Morris JS, Spate VL, et al. Effect of dietary selenium on the promotion of hepatocarcinogenesis by 3,3′, 4,4′-tetrachlorobiphenyl and 2,2′, 4,4′, 5,5′-hexachlorobiphenyl. Experimental Biology and Medicine (Maywood, NJ). 2008;**233**(3):366-376

[38] Dean CE Jr, Benjamin SA, Chubb LS, Tessari JD, Keefe TJ. Nonadditive hepatic tumor promoting effects by a mixture of two structurally different polychlorinated biphenyls in female rat livers. Toxicological Sciences. 2002;**66**(1):54-61

[39] Zhang W, Sargis RM, Volden PA, Carmean CM, Sun XJ, Brady MJ. PCB 126 and other Dioxin-like PCBs specifically suppress hepatic PEPCK expression via the Aryl hydrocarbon receptor. PLoS One. 2012;**7**(5):e37103

[40] Glauert HP, Tharappel JC, Banerjee S, Chan NL, Kania-Korwel I, Lehmler HJ, et al. Inhibition of the promotion of hepatocarcinogenesis by 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) by the deletion of the p50 subunit of NF-kappa B in mice. Toxicology and Applied Pharmacology. 2008;**232**(2):302-308

[41] Strathmann J, Schwarz M, Tharappel JC, Glauert HP, Spear BT, Robertson LW, et al. PCB 153, a non-dioxin-like tumor promoter, selects for beta-catenin (Catnb)-mutated mouse liver tumors. Toxicological Sciences. 2006;**93**(1):34-40

[42] Vincent F, de Boer J, Pfohl-Leszkowicz A, Cherrel Y, Galgani F. Two cases of ras mutation associated with liver hyperplasia in dragonets (Callionymus lyra) exposed to polychlorinated biphenyls and polycyclic aromatic hydrocarbons. Molecular Carcinogenesis. 1998;**21**(2):121-127

[43] Andersen O, Lindegaard P, Unger M, Nordberg GF. Effects of liver damage induced by polychlorinated biphenyls (PCB) on cadmium metabolism in mice. Environmental Research. 1985;**38**(2):213-224

[44] Whysner J, Wang CX. Hepatocellular iron accumulation and increased cell proliferation in polychlorinated biphenyl-exposed Sprague-Dawley rats and the development of hepatocarcinogenesis. Toxicological Sciences. 2001;**62**(1): 36-45

[45] Brown DP. Mortality of workers exposed to polychlorinated biphenyls--An update. Archives of Environmental Health. 1987;**42**(6):333-339

[46] Donato F, Moneda M, Portolani N, Rossini A, Molfino S, Ministrini S, et al. Polychlorinated biphenyls and risk of hepatocellular carcinoma in the population living in a highly polluted area in Italy. Scientific Reports. 2021;**11**(1):3064

[47] Zani C, Gelatti U, Donato F, Capelli M, Portolani N, Bergonzi R, et al. Polychlorinated biphenyls in serum, liver and adipose tissue of subjects with hepatocellular carcinoma living in a highly polluted area. Chemosphere. 2013;**91**(2):194-199

[48] Zhao G, Wang Z, Zhou H, Zhao Q. Burdens of PBBs, PBDEs, and PCBs in tissues of the cancer patients in the e-waste disassembly sites in Zhejiang, China. Science of the Total Environment. 2009;**407**(17):4831-4837

[49] Kaifie A, Schettgen T, Gube M, Ziegler P, Kraus T, Esser A. Functional and structural liver abnormalities in former PCB exposed workers – analyses from the HELPcB cohort. Journal of Toxicology and Environmental Health, Part A. 2019;**82**(1):52-61

[50] Clair HB, Pinkston CM, Rai SN, Pavuk M, Dutton ND, Brock GN, et al. Liver disease in a residential Cohort with elevated polychlorinated biphenyl exposures. Toxicological Sciences. 2018;**164**(1):39-49

[51] Loomis D, Browning SR, Schenck AP, Gregory E, Savitz DA. Cancer mortality among electric utility workers exposed to polychlorinated biphenyls. Occupational and Environmental Medicine. 1997;**54**(10):720-728

[52] Niehoff NM, Zabor EC, Satagopan J, Widell A, O'Brien TR, Zhang M, et al. Prediagnostic serum polychlorinated biphenyl concentrations and primary liver cancer: A case-control study nested within two prospective cohorts. Environmental Research. 2020;**187**:109690

[53] Delistraty D. Ecotoxicity and risk to human fish consumers of polychlorinated biphenyls in fish near the Hanford Site (USA). Science of the Total Environment. 2013;**445-446**:14-21

[54] Faust D, Vondráček J, Krčmář P, Smerdová L, Procházková J, Hrubá E, et al. AhR-mediated changes in global gene expression in rat liver progenitor cells. Archives of Toxicology. 2013;**87**(4): 681-698

[55] Borlak J, Jenke HS. Cross-talk between aryl hydrocarbon receptor and mitogen-activated protein kinase signaling pathway in liver cancer through c-raf transcriptional regulation. Molecular Cancer Research. 2008;**6**(8):1326-1336

[56] Buchmann A, Kunz W, Wolf CR, Oesch F, Robertson LW. Polychlorinated

biphenyls, classified as either phenobarbital- or 3-methylcholanthrene-type inducers of cytochrome P-450, are both hepatic tumor promoters in diethylnitrosamine-initiated rats. Cancer Letters. 1986;**32**(3):243-253

[57] Shen H, Robertson LW, Ludewig G. Regulatory effects of dioxin-like and non-dioxin-like PCBs and other AhR ligands on the antioxidant enzymes paraoxonase 1/2/3. Environmental Science and Pollution Research International. 2016;**23**(3):2108-2118

[58] Oakley GG, Devanaboyina U, Robertson LW, Gupta RC. Oxidative DNA damage induced by activation of polychlorinated biphenyls (PCBs): Implications for PCB-induced oxidative stress in breast cancer. Chemical Research in Toxicology. 1996;**9**(8):1285-1292

[59] Birnbaum LS. Endocrine effects of prenatal exposure to PCBs, dioxins, and other xenobiotics: Implications for policy and future research. Environmental Health Perspectives. 1994;**102**(8):676-679

[60] Andersson PL, Blom A, Johannisson A, Pesonen M, Tysklind M, Berg AH, et al. Assessment of PCBs and hydroxylated PCBs as potential xenoestrogens: In vitro studies based on MCF-7 cell proliferation and induction of vitellogenin in primary culture of rainbow trout hepatocytes. Archives of Environmental Contamination and Toxicology. 1999;**37**(2):145-150

[61] Ho PW, Garner CE, Ho JW, Leung KC, Chu AC, Kwok KH, et al. Estrogenic phenol and catechol metabolites of PCBs modulate catechol-O-methyltransferase expression via the estrogen receptor: Potential contribution to cancer risk. Current Drug Metabolism. 2008;**9**(4):304-309

[62] Deng P, Barney J, Petriello MC, Morris AJ, Wahlang B, Hennig B. Hepatic metabolomics reveals that liver injury increases PCB 126-induced oxidative stress and metabolic dysfunction. Chemosphere. 2019;**217**:140-149

[63] Yuan J, Lu WQ, Zou YL, Wei W, Zhang C, Xie H, et al. Influence of aroclor 1254 on benzo(a)pyrene-induced DNA breakage, oxidative DNA damage, and cytochrome P4501A activity in human hepatoma cell line. Environmental Toxicology. 2009;**24**(4): 327-333

[64] Al-Anati L, Högberg J, Stenius U. Non-dioxin-like-PCBs phosphorylate Mdm2 at Ser166 and attenuate the p53 response in HepG2 cells. Chemico-Biological Interactions. 2009;**182**(2-3): 191-198

[65] Chen Y, Wu Y, Xiao W, Jia H, Glatt H, Shi M, et al. Human CYP1B1-dependent genotoxicity of dioxin-like polychlorinated biphenyls in mammalian cells. Toxicology. 2020;**429**:152329

[66] Hu K, Yu H, Li Z, Jin G, Jia H, Song M, et al. Human CYP2E1-activated mutagenicity of dioxin-like PCBs 105 and 118-Experimental data consistent with molecular docking results. Toxicology. 2020;**437**:152438

[67] De Haan LH, Simons JW, Bos AT, Aarts JM, Denison MS, Brouwer A. Inhibition of intercellular communication by 2,3,7,8-tetrachlorodibenzo-p-dioxin and dioxin-like PCBs in mouse hepatoma cells (Hepa1c1c7): Involvement of the Ah receptor. Toxicology and Applied Pharmacology. 1994;**129**(2):283-293

[68] VanEtten SL, Bonner MR, Ren X, Birnbaum LS, Kostyniak PJ, Wang J, et al. Telomeres as targets for the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polychlorinated biphenyls (PCBs) in rats. Toxicology and Applied Pharmacology. 2020;**408**:115264

[69] de Conti A, Beland FA, Pogribny IP. The role of epigenomic alterations in furan-induced hepatobiliary pathologies. Food and Chemical Toxicology. 2017;**109**:677-682

[70] Lu D, Sullivan MM, Phillips MB, Peterson LA. Degraded protein adducts of cis-2-butene-1, 4-dial are urinary and hepatocyte metabolites of furan. Chemical Research in Toxicology. 2009;**22**(6):997-1007

[71] Grill AE, Schmitt T, Gates LA, Lu D, Bandyopadhyay D, Yuan J-M, et al. Abundant rodent furan-derived urinary metabolites are associated with tobacco smoke exposure in humans. 2015;**28**(7):1508-1516

[72] Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, et al. Risks for public health related to the presence of furan and methylfurans in food. EFSA journal European Food Safety Authority 2017;**15**(10):e05005

[73] Mariotti MS, Granby K, Rozowski J, Pedreschi FJF. Furan: a critical heat induced dietary contaminant. Food & Function. 2013;**4**(7):1001-1015

[74] LAJ P. Electrophilic intermediates produced by bioactivation of furan. Drug Metabolism Reviews. 2006;**38**(4):615-626

[75] Phillips MB, Sullivan MM, Villalta PW, Peterson LA. Covalent modification of cytochrome c by reactive metabolites of furan. Chemical Research in Toxicology. 2014;**27**(1):129-135

[76] Nunes J, Martins IL, Charneira C, Pogribny IP, de Conti A, Beland FA, et al. New insights into the molecular mechanisms of chemical carcinogenesis: In vivo adduction of histone H2B by a reactive metabolite of the chemical carcinogen furan. 2016;**264**:106-113

[77] Jackson AF, Williams A, Recio L, Waters MD, Lambert IB, Yauk CLJT, et al. Case study on the utility of hepatic global gene expression profiling in the risk assessment of the carcinogen furan. 2014;**274**(1):63-77

[78] Gill S, Kavanagh M, Barker M, Weld M, Vavasour E, Hou Y, et al. Subchronic oral toxicity study of furan in B6C3F1 Mice. Toxicol Pathol. 2011;**39**(5):787-794

[79] Ramm S, Limbeck E, Mally A. Functional and cellular consequences of covalent target protein modification by furan in rat liver. Toxicology. 2016;**361**:49-61

[80] Moro S, Chipman JK, Antczak P, Turan N, Dekant W, Falciani F, et al. Identification and pathway mapping of furan target proteins reveal mitochondrial energy production and redox regulation as critical targets of furan toxicity. 2012;**126**(2):336-352

[81] Selmanoğlu G, Karacaoğlu E, Kılıç A, Koçkaya EA, Akay MT. Toxicity of food contaminant furan on liver and kidney of growing male rats. Environmental Toxicology. 2012;**27**(10):613-622

[82] Terrell AN, Huynh M, Grill AE, Kovi RC, O'Sullivan MG, Guttenplan JB, et al. Mutagenicity of furan in female Big Blue B6C3F1 mice. 2014;**770**:46-54

[83] Ding W, Petibone DM, Latendresse JR, Pearce MG, Muskhelishvili L, White GA, et al. In vivo genotoxicity of furan in F344 rats at cancer bioassay doses. 2012;**261**(2):164-171

[84] Takasu S, Ishii Y, Kijima A, Ogawa K, Nakane S, Umemura T. Furan induced characteristic glutathione S-transferase placental form-positive Foci in terms of cell kinetics and gene expression. Toxicologic Pathology. 2020;**48**(6):756-765

[85] Hibi D, Yokoo Y, Suzuki Y, Ishii Y, Jin M, Kijima A, et al. Lack of genotoxic

mechanisms in early-stage furan-induced hepatocellular tumorigenesis in gpt delta rats. 2017;**37**(2):142-149

[86] Hickling K, Hitchcock J, Chipman J, Hammond T, Evans JJ. Induction and progression of cholangiofibrosis in rat liver injured by oral administration of furan. Toxicologic Pathology. 2010;**38**(2):213-229

[87] Hickling KC, Hitchcock JM, Oreffo V, Mally A, Hammond TG, Evans JG, et al. Evidence of oxidative stress and associated DNA damage, increased proliferative drive, and altered gene expression in rat liver produced by the cholangiocarcinogenic agent furan. Toxicol Pathol. 2010;**38**(2):230-243

[88] Tryndyak V, de Conti A, Doerge DR, Olson GR, Beland FA, Pogribny IP. Furan-induced transcriptomic and gene-specific DNA methylation changes in the livers of Fischer 344 rats in a 2-year carcinogenicity study. Archives of Toxicology. 2017;**91**(3):1233-1243

[89] Durling JKL, Svensson K, Abramsson-Zetterberg L. Furan is not genotoxic in the micronucleus assay in vivo or in vitro. Toxicology Letters. 2007;**169**(1):43-50

[90] McDaniel LP, Ding W, Dobrovolsky VN, Shaddock JG Jr, Mittelstaedt RA, Doerge DR, et al. Genotoxicity of furan in Big Blue rats. 2012;**742**(1-2):72-78

[91] Chen T, Williams TD, Mally A, Hamberger C, Mirbahai L, Hickling K, et al. Gene expression and epigenetic changes by furan in rat liver. 2012;**292**(2-3):63-70

[92] Conti A, Kobets T, Escudero-Lourdes C, Montgomery B, Tryndyak V, Beland FA, et al. Dose-and time-dependent epigenetic changes in the livers of Fisher 344 rats exposed to furan. 2014;**139**(2):371-380

[93] Recio L, Phillips SL, Maynor T, Waters M, Jackson AF, Yauk CL. Differential expression of long noncoding RNAs in the livers of female B6C3F1 mice exposed to the carcinogen furan. Toxicological Sciences. 2013;**135**(2):369-379

[94] Wong MH, Armour M-A, Naidu R, Man M. Persistent toxic substances: Sources, fates and effects. Reviews on Environmental Health. 2012;**27**(4):207-213

[95] English JSC, Dawe RS, Ferguson JJ. Environmental effects and skin disease. British Medical Bulletin. 2003;**68**(1): 129-142

[96] Ju Q, Zouboulis CJB. Effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin on the skin. 2013;**2, 113** 2167-0331.1000113

[97] Pelclová D, Urban P, Preiss J, Lukáš E, Fenclová Z, Navrátil T, et al. Adverse health effects in humans exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). 2006;**21**(2):119-138

[98] Çiftçi O. The Investigation of effect mechanism, chemical structure and toxicokinetics properties of dioxins compounds. Annals of Medical reaserch. 2010;**17**(4):413-422

[99] Bentli R, Ciftci O, Cetin A, Otlu AJT. Montelukast, an antiinflamattory agent, can prevent toxic effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in terms of oxidative stress and histological alterations in liver and serum cytokine levels. Toxicology and Industrial Health. 2013

[100] Türkez H, Geyikoğlu F, Yousef MI, Toğar B, Vançelik SJT. Propolis alleviates 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin-induced histological changes, oxidative stress and DNA damage in rat liver. Toxicology and Industrial Health. 2013;**29**(8):677-685

[101] Mejia-Garcia A, Sanchez-Ocampo EM, Galindo-Gomez S,

Shibayama M, Reyes-Hernandez O, Guzman-Leon S, et al. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin enhances CCl4-induced hepatotoxicity in an aryl hydrocarbon receptor-dependent manner. 2013;**43**(2):161-168

[102] Lee JH, Wada T, Febbraio M, He J, Matsubara T, Lee MJ, et al. A novel role for the dioxin receptor in fatty acid metabolism and hepatic steatosis. 2010;**139**(2):653-663

[103] Gonzalez JF, Fernandez-Salguero P. The aryl hydrocarbon receptor: Studies using the AHR-null mice. Drug Metabolism and Disposition. 1998;**26**(12):1194-1198

[104] Elizondo G, Fernandez-Salguero P, Sheikh MS, Kim G-Y, Fornace AJ, Lee KS, et al. Altered cell cycle control at the G2/M phases in aryl hydrocarbon receptor-null embryo fibroblast. 2000;**57**(5):1056-1063

[105] Sato S, Shirakawa H, Tomita S, Ohsaki Y, Haketa K, Tooi O, et al. Low-dose dioxins alter gene expression related to cholesterol biosynthesis, lipogenesis, and glucose metabolism through the aryl hydrocarbon receptor-mediated pathway in mouse liver. 2008;**229**(1):10-19

[106] Reyes-Hernandez O, Mejia-Garcia A, Sánchez-Ocampo E, Cabanas-Cortes M, Ramirez P, Chávez-González L, et al. Ube2l3 gene expression is modulated by activation of the aryl hydrocarbon receptor: Implications for p53 ubiquitination. 2010;**80**(6):932-940

[107] Walisser JA, Glover E, Pande K, Liss AL, Bradfield CA. Aryl hydrocarbon receptor-dependent liver development and hepatotoxicity are mediated by different cell types. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(49):17858-17863

[108] Boutros PC, Bielefeld KA, Pohjanvirta R, Harper PA. Dioxin-dependent and dioxin-independent gene batteries: Comparison of liver and kidney in AHR-null mice. Toxicological Sciences. 2009;**112**(1):245-256

[109] Kennedy GD, Nukaya M, Moran SM, Glover E, Weinberg S, Balbo S, et al. Liver tumor promotion by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin is dependent on the aryl hydrocarbon receptor and TNF/IL-1 receptors. 2014;**140**(1):135-143

[110] Yamaguchi M, Hankinson O. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin suppresses the growth of human liver cancer HepG2 cells in vitro: Involvement of cell signaling factors. International Journal of Oncology. 2018;**53**(4): 1657-1666

[111] Türkez H, Geyikoglu F, Yousef MI. Ameliorative effect of docosahexaenoic acid on 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-induced histological changes, oxidative stress, and DNA damage in rat liver. Toxicology and Industrial Health. 2012;**28**(8):687-696

[112] Alsharif NZ, Hassoun EA. Protective effects of vitamin A and vitamin E succinate against 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD)-induced body wasting, hepatomegaly, thymic atrophy, production of reactive oxygen species and DNA damage in C57BL/6J mice. Basic & Clinical Pharmacology & Toxicology. 2004;**95**(3):131-138

[113] Hassoun EA, Vodhanel J, Abushaban A. The modulatory effects of ellagic acid and vitamin E succinate on TCDD-induced oxidative stress in different brain regions of rats after subchronic exposure. Journal of Biochemical and Molecular Toxicology. 2004;**18**(4):196-203

[114] Ahd K, Dhibi S, Akermi S, Bouzenna H, Samout N, Elfeki A, et al. Protective effect of ginger (Zingiber

officinale) against PCB-induced acute hepatotoxicity in male rats. 2019;**9**(50):29120-29130

[115] Ciftci O, Ozdemir I. Protective effects of quercetin and chrysin against 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) induced oxidative stress, body wasting and altered cytokine productions in rats. 2011;**33**(3):504-508

[116] Kim H-S, Park S-Y, Yoo K-Y, Lee SK, Jung W-W. Induction of heat shock proteins and antioxidant enzymes in 2, 3, 7, 8-TCDD-induced hepatotoxicity in rats. The Korean Journal of Physiology & Pharmacology. 2012;**16**(6):469-476

[117] Czepiel J, Biesiada G, Gajda M, Szczepański W, Szypuła K, Dąbrowski Z, et al. The effect of TCDD dioxin on the rat liver in biochemical and histological assessment. 2009;**58**(1-2):85-90

[118] Viluksela M, Pohjanvirta R. Multigenerational and transgenerational effects of dioxins. International Journal of Molecular Sciences. 2019;**20**(12):2947

[119] Boutros PC, Yan R, Moffat ID, Pohjanvirta R, Okey AB. Transcriptomic responses to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in liver: comparison of rat and mouse. BMC Genomics. 2008;**9**(1):1-17

[120] Boverhof DR, Burgoon LD, Tashiro C, Sharratt B, Chittim B, Harkema JR, et al. Comparative toxicogenomic analysis of the hepatotoxic effects of TCDD in Sprague Dawley rats and C57BL/6 mice. 2006;**94**(2):398-416

[121] Zhang X, Li C, Gong Z. Development of a convenient in vivo hepatotoxin assay using a transgenic zebrafish line with liver-specific DsRed expression. 2014;**9**(3):e91874

[122] Yang Q, Salim L, Yan C, Gong Z. Rapid Analysis of Effects of Environmental Toxicants on Tumorigenesis and Inflammation Using a Transgenic Zebrafish Model for Liver Cancer. Marine Biotechnology (New York, NY). 2019;**21**(3):396-405