4-Chlorobiphenyl (PCB 3) as initiating agent of liver cancer

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Abstract: Polychlorinated biphenyls (PCBs) have long been studied as cancer related chemicals, but it was not until recently that the PCBs are considered as initiating agents of cancer. This paper attempts to provide an overview of the initiating ability of one of the most studied congers of PCBs, 4-chlorobiphenyl (PCB3). During the metabolism pathway of PCB3, several noticeable metabolites and byproducts capable of adduct formation were detected. These chemicals were evaluated separately, and quinones were identified as the ultimate cause of adduct formation, with semiquinones and Reactive oxygen species (ROS) involved at a less significant level. The carcinogenic effect caused by DNA damage as a result of the adduct formation was considered. Several studies with rodent and human liver cells were also evaluated to provide experimental support for the initiating capacity of PCB3. These results suggest both theoretically and empirically that PCB3 is capable of initiating cancer. This review is able to conclusively identify PCB3 as an ultimate carcinogen for liver cancer, and reveal some areas that need further studies for clarification.

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of synthesized organic compound. Commercial production of PCBs began in the early 1920s, and they are mass-produced until their commercial production was banned in 1970s. For their excellent physical properties and chemical stabilities, PCBs are widely used as coolants and lubricating agents. However, PCBs are also a Persistent Organic Pollutants (POPs) because they are not readily degraded by the physical and biologic processes in the environment [1]. The major source of PCBs for the general population is the food supply [2-3]. A second and often overlooked source of exposure to PCBs is city air and the air of buildings that were constructed using PCBs in sealants, caulking, and other building materials [4]. These hydrophobic compounds will bioaccumulate in our fat layer before being metabolized.

PCBs are mixtures of congeners which differ in the number and position of chlorines on the biphenyl rings. There are 209 congeners of PCBs, numbered PCB1 to PCB209 based on the number and position of chlorine atoms around the biphenyl rings [5]. Because of the different structures of PCB congeners, the metabolism of them varies greatly. At most time, the PCBs will be transformed to be more hydrophilic for better excretion. However, metabolic processes may also produce electrophilic metabolic intermediates such as arene oxides or (semi) quinions which are form by the oxidation of OH-PCBs. These metabolites may react with biomacromolecules like protein and DNA, causing unreversible damage to our health. Additionally, metabolites like OH-PCBs, PCB sulfates, and PCB methyl sulfones (MeSO2-PCBs) might be equally persistent as parent congeners and elicit their own toxicities.

2. The Metabolism and Toxicity of PCBs and Their Metabolites

2.1 General introduction of the PCBs and their metabolites

Since there are 209 structural different PCBs, the metabolism of PCBs varies from congener to congener. The structure of the PCBs will affect the metabolic pathway in human bodies. For example, the rate and extent of PCB metabolism depends on the number and positions of chlorines in the molecule [6-7] OH-PCBs are Phase I biotransformation products of PCBs. They are characterized by the addition of one or more hydroxyl groups to the chlorinated biphenyl skeleton. PCB sulfate and PCB glucuronide are two representative PCB metabolites which undergo Phase II reaction. There are also some short live, but quite active and harmful metabolites, including PCB arene oxides and (semi) quinions.

2.2 The oxidation of PCBs and futher reactions in metabolic processes

1) Phase I: In humans, cytochrome P450 families 1 and 2 contain major inducible isoforms participated in the Phase I metabolism of PCBs. To understand the mechanism of CYP450 enzymatic reactions with PCBs, early studies focused on the mono-chlorinated PCBs as substrates [8-9]. From the results of the study, scientists discovered that CYP450 involve in two different oxidative processes. One of the processes, the direct way, is simply inserting a hydroxyl group into PCBs. The other process, a more indirect way, is generating a transient arene oxide intermediate which rearranges to form the OH-PCBs [10]. The intermediacy of an arene oxide may lead to an NIH shift, a process characterized by the movement of neighboring substituents [11].

2) Phase II: Biotransformation process is a way that the metabolic system increases the polarity of compounds for better excretion. During the Phase II reaction, the OH-PCBs that produced in the Phase I reaction are inserted with several kinds of functional groups in order to polarize OH-PCBs. Glucuronidation and sulfation are the two most common conjugations, both processes are achieved by transferring a glucuronosyl group or a Sulfuryl group onto the phenyl. The addition will further facilitate the metabolism. However, some congeners with the phenolic hydroxyl group in an ortho-position with respect to the biphenyl ring-junction have been found to hinder conjugation reactions by inhibiting sulfotransferases and UDP- glucuronosyltransferases [12-13]. This phenomenon may cause various health problems including metabolic, endocrine, and developmental defects [14].

3) Other reactions: OH-PCBs have also been shown to undergo multiple oxidation reactions leading to more than a single hydroxyl substituent on the biphenyl structure [14-15]. One of the reaction is the dihydroxylation, which can result in the formation of catechols and other hydroquinones [11]. One-electron oxidation of a PCB hydroquinone or catechol, or single-electron reduction of a PCB quinone, results in a semi-quinone radical with subsequent formation of reactive oxygen species (ROS) and the PCB quinone [16]. Quinones are toxical because they can can bind with cellular nucleophiles such as protein and nonprotein sulfhydryls and redox cycle with the creation of oxidative stress.

3. The Initiation of Liver Cancer by PCB3 Metabolites

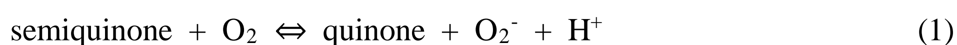
3.1 The formation of DNA adducts from PCB3 metabolites

During the metabolism pathway of PCB3, there are several metabolites and byproducts that are capable of forming DNA adducts and thus initiate cancer. The first noticeable products are the arene oxides, which are epoxides of arenes. Since they are the only products formed in the Phase one metabolism that are able to cause adduction, its participation in this issue can be examined simply by looking at the timing of adduct formation. In one experiment with PCB4, another PCB with the similar structure as PCB3, researchers identified that the adduct formation linearly increased to 80 minutes, whereas the arene oxides formed and degraded during the first 40 minutes, proving that they do not play a major role in the formation [17]. Similarly, the involvement of arene oxide in the carcinogenicity of PCB3 should also be limited.

Quinone metabolites are the final product of the metabolism pathway of PCB3, and they are formed from hydroquinone metabolites via semiquinone intermediates. In one early experiment with 32P-

postlabeling, a technique that detects the level of DNA adduction, the involvement of both products were confirmed [11]. The researchers were able to show that the Phase two metabolism plays a crucial role in the formation of DNA adducts by using peroxidase, the enzyme that activates the Phase two reactions. Since quinones and semiquinones are the only products of the Phase two metabolism, they must be involved in the formation as well.

Later, another experiment was performed in the attempt to identify the specific roles that the two metabolites play in the adduct formation [18]. The experiment focused on a redox reaction that produces quinones by using semiquinone intermediates [19]:



$$\text{semiquinone} + O_2 \Leftrightarrow \text{quinone} + O_2^- + H^+ \quad (1)$$

When applying peroxide dismutase, an enzyme that uses superoxide to produce oxygen and can thus shift the equilibrium equation to the right side, researchers identified a major increase in the level of major DNA adducts along with a decrease in that minor DNA adducts [18]. This change indicates that quinone metabolites are possibly responsible for the formation of major DNA adducts, while semiquinone intermediate in the formation of minor DNA adducts. One additional experiment identified 1200 adducts/108 nucleotides with quantitation by HPLC/MS/MS for 4′ClPh-BQ, a PCB3 quinone metabolite [20]. This high level of adduction further proves the major role quinone metabolites of PCB3 plays in the formation of DNA adducts.

Reactive oxygen species (ROS) are a type of highly reactive molecule that contains oxygen [21]. During the redox reactions between catechols/hydroquinones and quinones, several ROS, such as the aforementioned superoxide, are created as byproducts [22]. These reactions can be activated via two routes, by using enzymes in the organisms, or using transition metals. In one experiment, copper ions Cu+ and Cu2+ were used to initiate the reactions and thus produce more ROS [23]. Researchers were able to identify a total of 21 new DNA adducts with different metabolites of different PCBs. The hydroquinone metabolite of PCB3 exhibited the highest adduct level, at 147 adducts/106 nucleotides, a stunning high result. However, the chemicals used in this experiment are mediated by copper ions to produce more ROS than the normal amount, and thus cannot be compared with other products directly. That being said, it still reveals that these ROS may play a causal role in adduct formation.

Overall, for these four types of products derived from PCB3, arene oxides were determined to be insignificant in the formation of DNA adducts; quinone metabolites were identified as the major cause of DNA adduction; semiquinone intermediates were identified to be responsible for the formation of minor adducts; ROS were proved to be involved in the process, while the scale is yet to be determined.

3.2 The carcinogenic effect of DNA adducts of PCB3 on liver

The significance of DNA adduct formation is the carcinogenic effect that it can cause via damage to DNA, either directly or indirectly. One standard technique designed to detect this damage is the COMET assay, which does so by measuring the strand breaks in cells [24]. One experiment employed this method on hydroquinone and quinone metabolites of PCB3 to determine the carcinogenicity of which [25]. The study showed a reliance on peroxidase for the hydroquinone metabolites to cause DNA damage, whereas quinone metabolites can cause the damage via directly binding with DNA or indirectly depleting glutathione and allowing ROS to bind with DNA or protein. These observations correlate with the aforementioned assumption that quinone metabolites are the ultimate metabolites responsible for DNA adduction, and also suggest the importance of ROS in quinone-mediated DNA damage since about half of the damage for both metabolites are caused by ROS.

An additional experiment, the PCB3 was also converted into hydrocarbon and quinone metabolites, and its result also coincided with the former results: PCB3 itself had limited effect, and quinone metabolites yielded better results than hydroquinones [26]. Moreover, the researchers in this experiment were able to identify an induction of gene mutation at the HPRT locus in rodent livers, and the quinone metabolites were identified as the ultimate mutagens, though which quinone metabolite particularly was not determined. This result strongly suggests the possibility that the quinone metabolites of PCB3 are able to initiate cancer via gene mutation.

In addition to mutation caused by simple strand break, some experiments also suggest PCB3 can cause cancer via other routes with DNA adduction. One study emphasized on the telomeres section of DNA, and its result poses the possibility that the quinone metabolites of PCB3 can cause telomeres shortening by using ROS [27]. Some other studies focus on the DNA damage caused by PCB3 during the replication stage of DNA, suggesting that PCB3 can influence the process by making cells vulnerable [28]. That being said, these areas are studied less than the strand break, as it is the most promising way for PCB3 to initiate cancer, and all of them require further research for more detailed and decisive conclusions.

4. Mechanisms of PCB3 induced liver cancer

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4.1 Liver cancer induced through sequential hydroxylation reactions

Scientists have completed preparations and experiments using all PCB 3 metabolites to study and explain the activation mechanism of PCB3 [29]. They used various diminishing dosages of metabolites to see how this process works. They assessed every metabolite using monohydroxy metabolites, dihydroxy metabolites, and the para- and ortho-quinones.

For example, the 2-OH PCBs, the orthoquinones 2,3- and 3,4-BQ PCB 3, and the 2,3-diOH PCB 3 were examined in the third experiment. Only 3,4-BQ induced an increase in GGT-positive foci volume and number (Table 1).

Table.1. Effects of PCB3 Metabolites, 2-OH PCB 3, 2,3-diOH PCB 3, 2,3-BQ PCB3, and 3,4-BQ PCB 3, on the Production of Glutamyltranspeptidase-Positive Foci and on the Induction of Hepatocellular Adenomas [29]

Treatment	Foci/cm3	Foci/liver	Focal Volume (% of liver)	Rats with adenomas (microscope + visible) / total rats (%)
Corn oil	26±4	293±48	0.08±0.01	0/10(0%)
(5ml/kg, n=10)				
2-OH PCB 3	1.6±0.4	19±5	0.004±0.001	0/13(0%)
)400 μmol/kg, n=13)				
2,3-diOH PCB3	13±2	147±18	0.034±0.005	0/16(0%)
(200 μmol/kg, n=16)				
2,3-BQ PCB3	9.9±1.7	111±19	0.12±0.03	0/16(0%)
(100 μmol/kg, n=16)				
3,4-BQ PCB 3	62±12*	1214±157*	0.36±0.04*	0/13(0%)
(100 μmol/kg, n=13)				
DEN				
(20 mg/kg, n=6)	40±12*	489±155*	0.91±0.33*	1/6(16%)
(40 mg/kg, n=3)	227±29*	3046±495*	18±5.7*	3/3*(100%)

In addition, DEN ‘truly' made a big difference when it came to increasing the number of GGT-positive foci in the liver and the amount of space taken up by these foci. Most rat livers showed at least one of two cellular changes, which were, basophilic, eosinophilic, or mixed. Both were consistently present in rats with substantially enlarged GGT-positive foci or adenomas.

The theory behind the metabolic activation was accomplished via a series of hydroxylation reactions, followed by oxidation to the final mutagen/carcinogen, a quinone structure. Most PCBs are hydroxylated due to the cytochrome P450 enzymes. Alternatively, monohydroxy metabolites could be produced through hydroxyl group insertion or an arene oxide intermediary are also possible [30]. There

are two types of hydroxylation performed by cytochromes P450: one hydroxylation makes catechols and hydroquinones [31]. In the case of catechols and hydroquinones, the placement of two adjacent hydroxyl groups affects the outcome of subsequent oxidation, since the positions influence the formation of new electrophiles, ortho- and paraquinones. It was found that PCB 3 metabolites had various hydroxylations [31] Also, Induced microsomal enzymes complete their metabolism of 4-chlorobiphenyl through a shared metabolic intermediate that is a product of both cytochrome P-448 and cytochrome P-450.

4.2 PCB3 induces mutation in the liver of transgenic Fisher 344 rats

In an in vivo study with Fischer 344 rats, researchers discovered PCBs cause cancer in rodents [32]. PCB mixes and homologues may increase tumor development in two-stage hepatocarcinogenesis assays, and they accomplish so via activating cytochrome P450 dependent monooxygenases. By exposing male Fischer 344 rats to PCB3 and other lower chlorinated PCBs (PCB15, PCB52, and PCB77) using the Solt-Farber technique, the initiating ability of tumors of PCBs is identified. Therefore, a PCB mixture may be a complete carcinogen owing to its lower chlorinated congeners' initiating and promoting actions. PCB3 may also be used to make arene oxides and quinones, which may bind with Molecules such as DNA, RNA, and protein.

Table.2. Summary of Sequenced, Independent Lacl Mutations in the Livers of BigBlue® Rats treated With Corn Oil, 3-MC, PCB3 and 4-HO-PCB3 [32]

Type of mutations	Number of mutations (% total mutations)			
	Corm oil	3-MC	PCB3	4-HO-PCB3
Transitions (total)	10(63)	13(30)*	7(30)*	15(56)
G:C→A:T.	9(56).	12(27)	7(30)	13(48)
A:T→G:C	1(6).	1(2)		2(7)
Transversions (total)	3(19)	19(43)	10(43)	6(22)
G:C→T:A	1(6)	12(27)	7(30)	4(15)
G:C→C:G.	2(13)	4(9)		1(4)
A:T-C:G		2(5)	2(9)	1(4)
A:T→T:A		1(2)	1(4)	
Frameshifts (total)	3(19)	12(27)	6(26)	6(22)
- 1 frameshift		6(14)	3(13)	1(4)
+ 1 frameshift	1(6)	1(2)	1(4)	1(4)
Insertions	1(6)	3(7)	1(4)	3(11)
Deletions	1(6)	2(5)	1(4)	1(4)
Total	16(100)	44(100)	23(100)	27(100)
Significance versus control		P=0.058(0.047-0.069)	P=0.117(0.102-0.132)	P=1.000

The researchers used 16 lacI-transgenic male Fisher 344 Big Blue rats, each with the same diet and limitless water. After a week of acclimation, four groups of four animals were chosen at random. On postnatal day (PND) 37, 44, 51, and 58, each animal received an injection of 3-methylcholanthrene (3- MC) (positive control), PCB3, 400 mmol, 4-hydroxy-PCB3 (4-HO-PCB3), or corn oil (negative control) [4]. The animals were weighed twice a week throughout treatment. Upon discovery, the livers were removed, weighed and assess LacI mutation frequencies and mutant analyses.

The body weights of male rats fed corn oil, 3-MC, PCB3, or 4-HO-PCB3 were assessed from PND 35 to PND 75. At PND 35, all treatment groups weighed the same. At PND 65 and 75, mice treated with 3-MC gained less weight than animals given with the other treatments. The weights of all treatment groups (3-MC, PCB3, and 4-HO-PCB3) were not significantly different at PND 75 liver histology. As for mutant frequency, PCB3 treated rats exhibited high frequencies at $48 +4\times10^{-6}$ pfu. Despite being lower than the frequency of 3-MC, it was significantly higher than the frequency of the control group. In contrast, the frequency of 4-HO-PCB3 was identified as insignificant. The research

also analyzed the lacI mutation in different treatment groups. However, the PCB3-induced mutation spectrum did not vary significantly from the control animals (P14 0.117). Three (14%) of the 21 4-HO-PCB3 single base pair changes occurred at A: T base pairs, whereas 18 (86%) occurred at G:C base pairings (Table 2). Thus, the 4-HO-PCB3-induced mutation spectrum was comparable to the control group's spectrum, while the PCB3-induced mutation spectrum was similar to the 3-MC group's spectrum, indicating that it is a liver cancer starting agent.

4.3 The formation of DNA adducts in the culture of primary human hepatocytes which can induce liver cancer

The production of DNA adducts in hepatocytes was assessed using five distinct donor cultures of human hepatocytes [33]. 4-CB, a key component of Aroclor 1016, was administered to cells of two out of five donors, while Aroclor 1254 and 1016 were applied to the cells of the other three donors (Table 3). Either NP1 or butanol enrichment methods recorded each donor's DNA adduct background level, and studies have shown that the methods identified quantitative and qualitative variations.

Table.3. Treatment Scheme for Human Hepatocytes [33]

Donor	PCB mixture	Concentration (μmM)	Time of exposure (h)
1	Aroclor 1254, Aroclor 1016	60, 90	24, 48
2	Aroclor 1254, Aroclor 1016	60/150, 90/230	24, 48
	4-CB		
3	4-CB	230	24, 48
4	Aroclor 1254, Aroclor 1016	3/15/60, 5/23/90	24, 48, 96
5	4-CB	23, 90	24, 48, 96

An increase of 2.4 adducts is seen in cultures treated with Aroclor 1254 after a one-sample, one-sided t test. This discovery was notably unique, with a p smaller than 0.036. Therefore, they observed a significant rise in overall DNA adducts after the exposure to the 60-μM Aroclor 1254 dosages.

The 32P-postlabeling method is able to identify one adduct in 10 to the power of 10 nucleotides without using a radiolabeled parent molecule. Additionally, it is very sensitive, able to detect just one adduct in 10 to the power 10 nucleotides [34]. The process that makes mono- and dichlorinated biphenyls create adducts has been determined, and there are many routes it may use [19]. In the majority of these investigations, Aroclor 1254 was used. This combination is comprised of mostly tetra-, penta-, and hexachloro- biphenyls, which have an average chlorine concentration of 54 percent. Little research has been done on using pure PCB isomers. Other investigations have shown that PCB quinones works very sluggishly with nitrogen nucleophiles [19]. When reacting with the sulfuryl nucleophiles N-acetyl-L-cysteine and glutathione, hydroquinone adducts are instantly produced. These adducts may add a second moiety by undergoing oxidation. Through their ability to bind to macromolecules and deplete glutathione and induce oxidative stress and quinone-mediated toxicities, PCB metabolites have been shown to alter how proteins interact with cells. It is thought that chlorinated biphenyls create DNA adducts by transferring their chlorine atoms to aromatic hydrocarbons, while other research points to phenol and p-hydroquinone oxidation products [35]. The results are well suited to the experimental data and the scientist proposes a comparable process for the synthesis of DNA in the development of rodents and human liver.

5. Conclusion

We have provided theoretical and empirical support for the initiating ability of PCB 3 of liver cancer. We identified the metabolism pathway of PCB3, and within the process found several chemicals possibly responsible for the DNA adduction. We used to study concerning these chemicals to evaluate their level of involvement, and we identified quinones to be the major cause of adduction, while both semiquinones and ROS were the minor causes. The carcinogenic effects were studied following the DNA adduction, which suggest the initiating potential of PCB3, though not conclusive. We also analyzed experiments with liver cells that proves that PCB3 can initiate liver cancer under

experimental conditions. Our findings strongly suggest that PCB3 is capable of initiating liver cancer via genetic mutations caused by DNA adduction. By identifying the initiating ability of PCB3, the danger and carcinogenic capacity of PCB3 can be reevaluated, and treatments can be studied for liver cancers caused by this chemical. In addition, we were able to identify several sections in the initiation process that lack statistical support or decisive conclusion. These areas, such as the mutagenic capacity of PCB3, still require further studies for clarification. In the future, the characteristics of PCB3-caused cancer will need to be studied in order to identify cancer patients with this cause and provide them with treatments.

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