4-Monochlorobiphenyl (PCB3) induces mutations in the livers of transgenic Fisher 344 rats

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4-Monochlorobiphenyl (PCB3) is found in small amounts in commercial PCB mixtures, indoor and outdoor air, and in food. In contrast to highly chlorinated congeners that are more resistant to metabolic attack, PCB3 is more readily converted by xenobiotic-metabolizing enzymes to monohydroxy-PCBs and further to dihydroxy-metabolites, which can be oxidized to quinones. Our recent studies demonstrated the initiating action of PCB3 in the livers of male rats. Therefore we hypothesized that PCB3 and/or its metabolite(s) are mutagenic in rat livers in vivo. To investigate the mutagenicity and the types of mutations generated by PCB3, male Fischer 344 BigBlue® rats, transgenic for the lacI gene, were injected intraperitoneally with PCB3 (600 μmol/kg), 4-hydroxy-PCB3 (4-HO-PCB3, 400 μmol/kg), 3-methylcholanthrene (3-MC, 300 μmol/kg, positive control) and corn oil (negative control) once per week, for 4 weeks. Animals were killed 17 days after the last injection and the mutant frequency of the liver lacI gene determined. 3-MC induced a 4-fold increase of the mutant frequency of the lacI gene in the liver. The mutant frequency in PCB3-treated animals was also significantly elevated. In contrast, 4-HO-PCB3 induced a non-significant doubling of the mutant frequency. The mutation spectrum of solvent control mutants was characterized by transitions, whereas in 3-MC-animals, transversion and frameshift mutations predominated. The PCB3-induced mutation spectrum was similar to that of the 3-MC-induced mutants. In contrast, the mutation spectrum of the 4-HO-PCB3 group hardly differed from that of the control animals. This study demonstrates for the first time the mutagenicity of a PCB in vivo.

Introduction

Polychlorinated biphenyls (PCBs) were produced worldwide from 1929 to the early 1980s. During this period, an estimated 2 million tons of commercial PCB mixtures were produced, of which about 0.2 million tons remain in mobile environmental reservoirs (1). Despite the production ban and heavily restricted use, PCBs can still be found even in the most remote areas like polar regions and mountain lakes due to atmospheric transport and precipitation (2–5). Vaporization of PCBs from landfills (6,7), contaminated surface water (8–10) and construction material for public buildings (11–15) are main sources for outdoor and indoor air contamination. Human exposure to PCBs occurs to 90% via food; inhalation contributes the remaining 10% (1). In some populations, however, inhalation exposure was found to be the major source for daily PCB uptake (16).

Commercial PCBs are mixtures of the 209 possible congeners, which differ in the number and position of chlorines bound to the biphenyl core. PCB congeners that accumulate in the food chain are mainly tetra- to hepta-chlorinated, are stored preferably in fatty tissues, and are only slowly metabolized. In contrast, airborne PCBs are lower chlorinated, and are susceptible to metabolic attack. They occur in commercial PCB mixtures (17,18), in the atmosphere (18), especially in cities, in buildings, and near waste sites (19). They can also be found in food, however in a lower proportion and more likely in/on food items like vegetables, possibly through air deposition, in vegetable oils and in dairy products (20,21). Congener-specific analysis of indoor air in houses built on soil contaminated with Aroclor 1260, one of the highest chlorinated commercial PCB mixtures, showed that the congeners measured in indoor air were mostly 4-monochlorobiphenyl (PCB3, Figure 1) and 2-monochlorobiphenyl (PCB1) (22).

PCBs have repeatedly been shown to be complete carcinogens in rodents (23,24). In addition, commercial PCB mixtures as well as individual congeners have tumor promoting activity in two stage hepatocarcinogenesis assays (25–27). Generally, those PCB congeners that induce cytochrome P-450 dependent monooxygenases (CYPs) in the liver, e.g. PCB77, PCB126 and PCB153, are efficacious as hepatic tumor promoters (28–30). PCBs were reported to have no or only little genotoxic activity in most in vitro genotoxicity tests (24,31) and their potential as cancer initiators was therefore questioned. Recently, however, evidence for tumor initiating activity of PCB3 and other lower chlorinated PCBs that can be metabolically activated (PCB15, PCB52 and PCB77) was provided using a modified Solt-Farber protocol in male Fischer 344 rats (32,33). Thus PCB mixtures could be complete carcinogens due to the initiating activity of their lower chlorinated congeners and the promoting activity of the higher chlorinated congeners.

PCB3 is a substrate for hepatic CYPs and can be activated to electrophiles, namely arene oxides and quinones (34). These reactive metabolites can bind to cellular nucleophiles like glutathione and macromolecules like DNA, RNA, protein and hemoglobin (35–38). The generation of reactive...
oxygen species during oxidative metabolism as well as the resulting formation of 8-oxo-deoxyguanosine and DNA strand breaks have been demonstrated in vitro (39–41). However, the question whether PCB3 or one of its metabolites is mutagenic in vivo was still unanswered. Therefore, we investigated the mutagenicity of PCB3 in the livers of transgenic male Fischer 344 (BigBlue®) rats which contain 30–40 copies of the bacterial lacI gene in every cell as a mutable target sequence. Animals were treated with corn oil (negative control), the mutagen 3-methylcholanganthrene (3-MC, positive control), PCB3, or its main metabolite 4-hydroxy-PCB3 (4-HO-PCB3) (34). Mutated lacI sequences were detected after transfection into Escherichia coli based on a blue plaque phenotype using 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), and the type of mutational event was determined by sequence analysis of the lacI gene. PCB3 as well as the mutagen 3-MC induced a significant increase in mutant frequency in the liver of BigBlue® rats, thus demonstrating for the first time the mutagenicity of PCB3 in vivo.

Materials and methods

Chemical substances

PCB3 and its hydroxylated metabolite 4-OH-PCB3 (42) were synthesized, purified and characterized as described (32). 3-MC was purchased from Sigma (Cat # M-6501; St. Louis, MO) and X-gal from Research Products International (Prospect, IL). DNA isolation and transpackaging kits were obtained from Stratagene (La Jolla, CA). All other reagents, media and media supplements were from Fischer Scientific (Pittsburgh, PA), if not stated otherwise.

Animals and treatment protocol

Sixteen male Fisher 344 BigBlue® rats, homozygous for lacI transgene, were purchased from Stratagene (La Jolla, CA) and obtained from the breeder, Taconic Laboratories, Germantown, NY at postnatal day (PND) 30. The animals were kept on a 12 h light/dark cycle and were provided with a standard 7013-NIH-13 Modified Open Formula Rat diet and water ad libitum. After one week of acclimatization animals were weighed and distributed at random into four groups with four animals each. Each animal received once weekly i.p. injection on PND 37, 44, 51 and 58 of either 298 μmol (80 mg/kg) BW PCB3, 600 μmol (113 mg/kg) BW 3-MC (positive control), 600 μmol (82 mg/kg) BW 4-HO-PCB3 in corn oil or 5 ml/kg BW corn oil alone (negative control). The doses of the PCB and metabolites were selected based on previous studies (32,33). Throughout the treatment period animals were monitored daily for well being and weighed twice per week. Seventeen days after the last injection (PND 75), all animals were euthanized by CO2 asphyxiation followed by cervical dislocation. The livers were immediately removed and weighed. Small tissue samples were quickly excised from the central part of the large lobe of the liver. These samples and the remaining liver were snap frozen in liquid nitrogen and stored at −80°C until use for analysis of lacI mutation frequencies and mutant analysis as described below. Parts of the remaining liver were used to prepare slides for histological analysis. Four micron sections were prepared, placed on glass slides and stained with conventional hematoxylin and eosin stains. Slides were then examined using an Olympus BX40 light microscope. All experiments were conducted with the approval of the University of Iowa Institutional Animal Care and Use Committee.

DNA extraction and packaging

DNA extraction, packaging and plating for lacI mutant plaque detection were carried out in a blocked manner (i.e. one animal from each group per cycle) to avoid bias from day-to-day variations following the BigBlue® assay procedure according to the manufacturer’s instructions (Stratagene). Genomic DNA was extracted from the livers using the BigBlue® RecoverEase DNA isolation kit according to the manufacturer’s instruction manual. Briefly, 50 mg of liver tissue were homogenized with a Dounce homogenizer at 4°C, and nuclei were pelleted by centrifugation for 10 min at 11000 × g. RNA and proteins in the pellet were digested with RNase and Proteinase K, and the genomic DNA was purified by dialysis in TE buffer for 48 h.

For recovery of the lambda transgenic shuttle vector containing the lacI target, i.e. excision of the lambda vector and packaging into a lambda head, the Lambda Phage Transpack® packaging kit (Stratagene) was used with 10 μl of genomic DNA per reaction as described in the manual. The mixture, now containing the lacI transgene packaged into infectious phages (packaged lacI), was diluted to 1 ml with SM buffer [100 mM NaCl, 8 mM MgSO4, 50 mM Tris–HCl and 0.004% (w/v) gelatin], and stabilized by addition of 50 μl chloroform. Packaging efficiency was checked by infecting E.coli bacteria, strain SCS-8 (2 ml SCS-8 suspension, OD = 0.5) with 1 μl packaged lacI extract and plating the suspension with 3 ml top agarose onto 100 mm NYZ-agar plates. Infected bacteria result in plaques in the bacterial lawn.

Determination of lacI mutant frequency

For determination of the lacI mutant frequency, a volume of phage extract equivalent to ~12 500 plaque forming units (pfu) was mixed with 2 ml SCS-8 suspension (OD = 0.5) and incubated at 37°C for 15 min. A total of 50 μl of each 2 ml SCS-8/phages suspension were added to 2 ml fresh SCS-8 suspension and mixed with 35 ml top agarose (titer trays). The rest of the 2 ml SCS-8/phage suspension was mixed with 35 ml top agarose containing 1.5 mg/ml of X-gal. Top agarose containing infected and non-infected SCS-8 was poured onto 500 mm2 assay trays (Corning, Acton, MA) containing NZY-bottom agar (assay trays). The trays were vented for 30 min before incubation at 37°C for 20 h and scoring. Packaging and plating were repeated until 95 000–339 000 pfu were scored for each DNA sample.

Titer trays were scored for pfu and assay trays were inspected for blue plaques. To confirm the mutant phenotype, and for use in DNA sequence analysis, all putative mutants from the 500 mm2 assay trays were picked, diluted 1:100 with SM buffer and replated on 100 mm diameter plates with 3.5 ml of top agarose containing 1.5 mg/ml of X-gal. The lacI mutant frequency was calculated by dividing the number of verified mutant plaques by the total number of plaques analyzed and presented as number of mutants ± standard error per 106 pfu.

Sequence analysis of lacI mutants

The lacI genes of verified mutants were PCR amplified using the following primers (named according to the manufacturer’s instruction manual) and amplification conditions: 5’-GTATTACCGCCATGCATACTAG-3’ (forward PCR primer), 5’-CGTAATACGACTCACTATAG-3’ (forward PCR primer), 30 amplification cycles at 94°C for 30 s, 53°C for 30 s and 72°C for 60 s. Aliquots of the PCR products were characterized by agarose gel electrophoresis (1% in TAE buffer, 5 V/cm). Then, PCR products were purified using QiaQuick columns (Qiagen, Valencia, CA) and quantified by their OD at 260 nm. A total of 60 ng of this purified DNA were used per reaction to amplify the lambda shuttle vector containing the lacI gene. The sequencing products were analyzed on an Applied Biosystems automated capillary DNA sequencer Model 3700 following the procedure outlined by Applied Biosystems. All alterations in
Figure 2. Weight gain of male BigBlue® rats treated with corn oil (negative control), 3-MC (positive control), PCB3 and 4-HO-PCB3. Asterisks indicate significant differences to the untreated control group. Levels of significance: *P < 0.05; **P < 0.01; (Student’s unpaired t-test). Arrows, injection, PND.

DNA sequence were verified at least once. The same mutation at the same locus in DNA extracted from the same animal may be due to the same event and was therefore counted only once for the calculation of the mutation frequency, which is the number of independent mutations (different locus and/or different type as verified by sequence analysis) per total number of plaques analyzed and presented as number of mutations ± standard error per 10⁶ pfu.

Statistical analysis

Gains of body weight were analyzed by Student’s t-test. The lacI mutant frequencies and the frequencies of recovered mutations were analyzed by one-way ANOVA and Chi-square. Data are presented as means ± standard error (P < 0.05). Chi-square test and the statistical test described by (43) using the classes ‘transition’, ‘transversion’ and ‘frameshift’ were used to analyze the lacI mutation profiles of the four treatment groups.

Results

The objective of the present study was to clarify whether PCB3 and/or its monohydroxylated metabolite 4-HO-PCB3 are mutagenic at the lacI gene locus in the liver of transgenic male Fischer 344 BigBlue® rats. The established mutagen 3-MC (Figure 1) was used as a positive control throughout this study.

Development of animal and liver weight

The bodyweights of male BigBlue® rats administered corn oil (negative control group), 3-MC, PCB3 or 4-HO-PCB3 were monitored from 2 days before the first injection (PND 35) until euthanization at PND 75. At PND 35, bodyweights of all treatment groups were similar (average 71.5 ± 4.32 g). Bodyweights as well as gains of bodyweight did not differ significantly during the first 30 experimental days (Figure 2). However, at PND 65 and 75, the gain of bodyweight of animals treated with 3-MC were significantly reduced compared to the other treatment groups (Figure 2). In contrast, liver weights of all treatment groups (corn oil, 3-MC, PCB3 and 4-HO-PCB3) did not differ significantly at PND 75 (4.3 ± 0.37/100 g BW, 4.7 ± 0.64/100 g BW, 4.3 ± 0.26/100 g BW and 4.5 ± 0.33/100 g BW, respectively.

Liver histology

Liver samples were analyzed microscopically. Control animals had normal liver architecture, one with rare lymphoid aggregates in the parenchyma. PCB3-treated animals had only very minimal changes with or without small clusters of parenchymal lymphocytes and rare acidophil bodies. Animals that had received 4-HO-PCB3 showed dilated sinusoid and rare acidophil bodies. Three of four animals had signs of minimal portal tract inflammation. 3-MC-treated animals had mild to severe centrilobular necrosis with various degrees of lymphocytes and neutrophiles in the necrotic areas. The portal tracts were unaffected.

LacI mutant frequency in the livers of BigBlue® rats

Liver lacI mutant frequencies for BigBlue® rats exposed to corn oil (negative control), 3-MC (positive control), PCB3 and 4-HO-PCB3 were measured 17 days following the last injection (PND 75). For control animals, the mutant frequency was 17 ± 4 × 10⁻⁶ pfu (Figure 3 and Supplementary Table 1). Treatment with 3-MC induced a mutant frequency of 88 ± 15 × 10⁻⁶ pfu, approximately a 5-fold increase over the untreated control (P = 0.004). The mutant frequency in the liver of rats treated with PCB3 was 48 ± 4 × 10⁻⁶ pfu (P = 0.001). Although, elevated more than 2-fold, the mutant frequency for rats administered 4-HO-PCB3 (40 ± 12 × 10⁻⁶ pfu) was not significantly different from that of the control rats (P = 0.115) using ANOVA for analysis due to the very high variation within this group. When the Chi-square test was applied, the significance of 3-MC and PCB3 was confirmed and 4-HO-PCB3 was significant as well (P < 0.05).

Sequence analysis of lacI mutants

Sequence analysis of mutants provides information about the kind of mutation induced spontaneously or by treatment with test compounds. Sixteen of the 17 mutants (95%) from the vehicle-treated rats (Supplementary Table II), 47 of 56 (85%) from the 3-MC-treated rats (Supplementary Table III), 24 of 29 (84%) of the mutants from rats treated with PCB3 (Supplementary Tables IV) and 29 of 34 (83%) of those from rats treated with 4-HO-PCB3 (Supplementary Table V) provided lacI sequences that were complete, readable, and contained a mutation. In some instances the same mutation

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Forty-four different mutation sites were discovered in mutants from 3-MC-treated animals. One mutation was recovered three times and another one twice from the same animal, leaving 44 independent mutations. The predominant mutation types were G:C→T:A transversions and G:C→A:T transition mutations (27% each). Seven of the 12 transition mutations (58%) occurred at a CpG site. In addition, there were seven single-base frameshifts, three 2–5 bp insertions, and a 7 and a 67 bp deletion, leading to a frameshift mutation rate of 27% of the total mutations in this group (Table I and Supplementary Table III). The total number of transitions was significantly reduced in the 3-MC-treated animals compared to solvent controls (Chi-square test, Table I). However, using the test described by Cariello et al. (43), the 3-MC-induced mutation spectrum in total barely missed being significantly different from the lacI spectrum of untreated rats \(P = 0.058\).

Of the 17 PCB3-induced single-base pair substitutions, three mutations (18%) occurred at A:T base pair while 14 (82%) were at G:C base pair (Table I and Supplementary Table IV). Like the 3-MC spectrum, the predominant mutations in this group were G:C→A:T transition (30%; 7/23), with 43% of them occurring at CpG sites, and G:C→T:A transversion (30%; 7/23), followed by A:T→C:G transversion (9%; 2/23). Also, there was one (4%) A:T→T:A transversion mutation recovered from this group. No A:T→C:G or A:T→T:A transversions were detected in this group. In addition to base pair substitutions, 1 and 2 of the 16 total mutations from the control group were deletions or insertions, respectively, leading to frameshift mutations (19% of the total mutations; Table I and Supplementary Table II).

Characteristics of lacI mutations in the different treatment groups

The most frequent type of mutation in the negative control group was G:C→A:T transition (9/16; 56%), with 22% of these occurring at CpG sites. Also 2 G:C→C:G transversion (13%), 1 A:T→G:C transition (6%) and 1 G:C→A:T transversion (6%) mutations were recovered from this group. No A:T→C:G or A:T→T:A transversions were detected in this group. In addition to base pair substitutions, 1 and 2 of the 16 total mutations from the control group were deletions or insertions, respectively, leading to frameshift mutations (19% of the total mutations; Table I and Supplementary Table II).

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PCB3-treated rats (Table I), the PCB3-induced mutation spectrum was not significantly different from the spectrum of the control animals using the previously described statistical test method (43) \( (P = 0.117) \).

Of the 21 4-HO-PCB3-induced single bp substitutions, only three mutations (14%) occurred at an A:T base pair while 18 (86%) were at G:C base pairs (Table I). Like the control spectrum, the predominant mutations in this group were G:C→A:T transition (48%; 13/27), with 23% of them occurring at CpG sites, followed by G:C→T:A transversions (15%; 4/27), and A:T→G:C transition (7%; 2/27). There was only one (4%) A:T→C:G transversion recovered from this group and no A:T→T:A transversion. Additionally, there were two single-base frameshifts, three insertions (4–8 bp long), and a 21 bp deletion, leading to frameshift mutations (22% of the total mutations, Table I). Therefore, the 4-HO-PCB3-induced mutation spectrum was not significantly different from the spectrum of the control group.

**Discussion**

The present study demonstrates for the first time that PCB3 is mutagenic in vivo.

**Mutant frequencies and mutation spectra of the negative control**

In our experiments, the mutant frequency as well as the mutation frequency of the lacI gene in the liver of untreated BigBlue® rats was \( 17 \pm 4 \times 10^{-6} \) pfu which is in concordance with the finding derived from the analysis of 35 independent experiments by Lambert et al. (44), which yielded an average spontaneous mutation frequency of the lacI gene in the liver of BigBlue® rats of \( 35 \pm 3 \times 10^{-6} \) pfu with a range of \( 10–85 \times 10^{-6} \) pfu.

The dominating kind of mutation in all four groups was base pair substitutions (73–81%, compared to 19–27% frameshift mutations). Most of these base pair substitution mutations in untreated (69%), 3-MC-treated (59%), PCB3-treated (53%) and 4-HO-PCB3-treated (62%) rats were found within the first 360 bp of the lacI gene, which is in accordance with observations of other laboratories (45). This contains the DNA binding domain (or negative complementing region) of the lacI gene (46).

The most frequent type of mutation in the control group was G:C→A:T transition (56%) which is in agreement with the findings of deBoer and Glickman (45). Sixty-six percent (4/6) of the G:C→A:T transitions recovered from our control animals occurred in the first 400 bp and 50% of these (2/4) occurred at CpG sites (Supplementary Table II). G:C→A:T transitions are known to be particularly concentrated in the first 400 bp, where 65–85% of them are located at 5′-CpG-3′ sites due to spontaneous deamination of methylated cytosines (47) and the unusual malleability of CpG steps (48,49).

In contrast, deletion events are 3-fold more common after the first 400 bp (45). The three frameshift mutations recovered from the 16 mutants of the control animals occurred within this region (Supplementary Table II). Deletion of DNA is frequently mediated by repeated sequences and both insertions and deletions can be explained by a slipped-mispairing mechanism (50). For additional information, we added the location of the frameshift mutation in Supplementary Tables II–V.

**Mutations in 3-MC-treated animals**

The mutant frequency in the liver of 3-MC-treated rats in our experiments was \( 88 \pm 15 \times 10^{-6} \) pfu. Sequence analysis revealed that most of them, 93%, were true independent mutations. If we subtract 7% from those 88 mutants to account for possible siblings we obtain an estimated mutation frequency for the 3-MC group of about \( 82 \times 10^{-6} \) pfu, giving a 5-fold induction of mutations above background levels. Rihn et al. (51) analyzed the mutagenicity of 3-MC in the liver of male BigBlue® mice. Fourteen days after a single injection of 80 mg 3-MC/kg BW, a mutation frequency of \( 203 \pm 29 \times 10^{-6} \) pfu was observed. This seems to be higher than in our experiment with BigBlue® rats using four once a week injections of 80 mg 3-MC/kg BW, however, the mutation frequency in the liver of the control animals (76 ± 27 \( \times 10^{-6} \) pfu) was also much higher than in our study, resulting in a less than 3-fold induction of mutations by 3-MC. These differences between this mouse and our rat study may be due to the differing species and treatment protocols.

The predominant mutations among 3-MC-induced total mutations were G:C→T:A transversion and G:C→A:T transition mutations. Compared to the negative control, the 3-MC-induced mutation spectrum showed a dramatic increase in G:C→T:A transversions (Table I) and –1 frameshift mutations (Table I). G:C→T:A transversions were also the predominant mutations recovered from the 3-MC-induced lambda lacI mutant plaques sequenced by Rihn et al. (51). Metabolically activated polycyclic aromatic hydrocarbons are known to react with the exocyclic amino group of G, A, and - to a lesser extend - C (52), resulting in G:C→T:A and A:T→T:A transversions and G:C→A:T transition mutations.

**Mutant frequencies and mutation spectra of PCB-induced mutants**

The purpose of this study was primarily to analyze whether PCB3 and one of its metabolites, 4-HO-PCB3, induce gene mutations in the livers of male Fischer 344 rats. The basis for this question was the observation that both compounds and a downstream metabolite, the 3,4-quinone (see Figure 4) induced \( \gamma \)-glutamyltranspeptidase-positive foci, which are considered to be pre-neoplastic stages and adenoma (only PCB3) in the livers of male Fischer 344 when applied as an initiating agent in a modified Solt-Farber protocol (32,33). Similar to the Solt-Farber experiment, a slightly lower dose of the 4-HO-PCB was used (four injections of 400 \( \mu \)mol/kg BW) than of the PCB3 (four injections of 600 \( \mu \)mol/kg BW) to account for the fact that 4-HO-PCB3 is expected to be one metabolic step closer to the proposed ultimate mutagen, the 3,4-quinone of PCB3, and the expected higher toxicity of the hydroxylated metabolite compared to the parent compound.

In this present study, PCB3 indeed significantly induced mutations in the liver of transgenic male BigBlue® rats. We did not observe any signs of liver toxicity due to PCB3-treatment, neither morphologically nor histologically, which argues against indirect effects as cause for this increase in mutations. Moreover, the mutation spectrum was significantly different compared to that of control animals, with transitions, which are typical for spontaneous mutations, contributing significantly less to the PCB3-induced mutation spectrum than in controls. This characteristic was shared with 3-MC, our positive control and a known mutagen and
carcinogen in rodents. In the 4-HO-PCB3 group on the other hand, the mutant frequency was elevated 2-fold, which was not statistically significant. This lack of significance may in part be due to the large variability in mutation frequency between animals within this group and the use of only four animals per treatment group. However, the mutation spectrum of mutants isolated from animals treated with 4-HO-PCB3, was also not significantly different from that of the control animals. A slight shift towards more transversion and frameshift mutations may be present, but it is nowhere near the one seen with 3-MC or PCB3. Even though the mutant frequency may be underestimated by inadvertently omitting light colored mutant plaques, the mutational spectra are unlikely to be biased (45). Of course, certain deletion or insertion mutations may not be detected in phage based transgenic rodent systems because of packaging constraints. Thus, agents whose genotoxicity arises primarily through clastogenic events are less likely to be detected in transgenic rodent systems (44). For these compounds existing clastogenic events are less likely to be detected in transgenic rodent systems because of packaging constraints. Several reports show metabolic activation of radiolabeled PCBs to result in protein, RNA and DNA binding of radioactivity in cell-free systems (54), in cultured cells (55) and in vivo (37). The formation of such adducts by monochlorinated biphenyls has been explained by activation through different metabolic pathways (34,39,56,57).

$^{32}$P-postlabeling studies with PCB3 and a metabolizing system showed that similar but also unique adducts were formed depending on whether the incubation environment was oxidizing or reducing (36). It was suggested that some DNA adducts of lower chlorinated biphenyls may be derived from arene oxides (reducing environment), but others may be formed from the oxidized products of catechol and $p$-hydroquinone, i.e. semiquinones and quinones (oxidizing environment). PCB-quinones form adducts with nitrogen and sulfur nucleophiles (40,56) as well as with guanosine or isolated DNA in vitro (36,40,53). Recently, the structure of the reaction product of PCB3-2,5-quinone with N$^2$ of guanine was identified (53). In addition to the cell-free experiments mentioned above, the induction of DNA adducts in cultured human hepatocytes by PCB3 has been demonstrated by the $^{32}$P-postlabeling assay (35) and quinonoid protein adducts of 2,5,2',5'-tetrachlorobiphenyl (PCB52) were discovered in the liver and brain of treated rats (58).

Besides the reactivity of arene oxide and quinoid PCB metabolites towards DNA, the induction of oxidative stress might be another possible mechanism for the initiation potential of PCB3 and some of its metabolites: incubation of isolated DNA with dihalogenated PCB catechols in the presence of lactoperoxidase and metal ions induced 8-oxodeoxyguanosine formation (39). Several metabolites of PCB3 induce DNA strand breaks in vitro and reactive oxygen species in vitro as well as in cultured cells (41).

The G:C→T:A transversions recovered from PCB3-induced mutants in the present study are in concordance with both possible mechanisms: aryalkylating agents, such as activated PCB3 metabolites (e.g. quinones and epoxides) react preferably with the exocyclic amino group of G, A or C (52), which would generate a non-coding DNA lesion. When trying to interpret such a lesion during transcription, DNA polymerases usually use ATP instead of CTP resulting in G:C→T:A and A:T→T:A transversion, as well as G:C→A:T transition. However, G:C→T:A transversion is also the predominant mutation induced by 8-oxodeoxyguanosine (59). Thus both, oxidative stress and/or adduct formation, could have caused the observed increase in mutations. The fact that −1 frameshift mutations and A:T→C:G and A:T→T:A transversions also seem to be increased in PCB3-treated animals, similar to 3-MC-treated animals, may be additional support for aryalkylation as mechanism. Also, the strong mutagenicity of PCB3 compared to 4-HO-PCB3 seems to argue in favor of an arenoxide as mutagenic intermediate. However, it could also be argued that the 4-HO-PCB3 metabolite was already too reactive to reach the target organ, the liver, whereas the non-reactive PCB3 was not statistically significant. This lack of significance may in part be due to the large variability in mutation frequency between animals within this group and the use of only four animals per treatment group. However, the mutation spectrum of mutants isolated from animals treated with 4-HO-PCB3, was also not significantly different from that of the control animals. A slight shift towards more transversion and frameshift mutations may be present, but it is nowhere near the one seen with 3-MC or PCB3. Even though the mutant frequency may be underestimated by inadvertently omitting light colored mutant plaques, the mutational spectra are unlikely to be biased (45). Of course, certain deletion or insertion mutations may not be detected in phage based transgenic rodent systems because of packaging constraints. Thus, agents whose genotoxicity arises primarily through clastogenic events are less likely to be detected in transgenic rodent systems (44). For these compounds existing clastogenic events are less likely to be detected in transgenic rodent systems (44). For these compounds existing clastogenic events are less likely to be detected in transgenic rodent systems (44). For these compounds existing clastogenic events are less likely to be detected in transgenic rodent systems (44).

What is the mechanism of PCB3 activation to a mutagen? The second goal of this study was to analyze whether the mutation spectra of PCB3 and its metabolite 4-HO-PCB3
PCB3 induces mutations in the livers of transgenic Fisher 344 rats

easily transported to the liver and there bioactivated to a mutagenic species with or without the production of oxidative stress. Thus these in vivo experiments proved for the first time that PCB3 is indeed mutagenic in the organ where it produced pre-neoplastic lesions, but the ultimate mutagenic species and activation mechanism is still elusive.

PCB3 is found in the air of contaminated sites and in urban areas (18). It cannot be detected in human blood samples (60), indicating its susceptibility to metabolism that might involve the generation of mutagenic metabolites. In addition, many other lower chlorinated PCBs, which are ubiquitous in our environment, may have a similar potential to be activated to mutagenic and carcinogenic species as our model compound PCB3, as indicated by the observation that several of them cause pre-neoplastic foci in rat liver (32,33). Therefore, the here described mutagenicity of PCB3 in vivo might have strong implication for the potential carcinogenicity of this whole group of ubiquitous environmental pollutants.

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References


