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MUTAGENICITY OF 3-METHYLCHOLANTHRENE, PCB3, AND 4-OH-PCB3 IN THE LUNG OF TRANSGENIC BIGBLUE® RATS

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Abstract

Recent findings of high levels of predominantly lower chlorinated biphenyls in indoor and outdoor air open the question of possible health consequences. Lower chlorinated biphenyls are more readily metabolized to reactive and potentially harmful intermediates, acting as mutagens and cancer initiators. The goal of this study was to assess the mutagenicity of PCB3 in the lungs of rats. Male BigBlue® 334 Fisher transgenic rats, which carry the bacterial *lacI* gene as a target of mutagenicity, were given intraperitoneal injections of corn oil, 3-methylcholanthrene (3-MC, positive control), 4-monochlorobiphenyl (PCB3) or its metabolite 4-hydroxy-PCB3 (4-OH-PCB3) weekly for 4 weeks. Lungs tissue was harvested to determine mutant frequencies, mutation spectra, and pathological changes. 3-MC caused a 15-fold increase in mutant frequency and an increase in transversion type mutations; a very early occurrence of this type of mutation in lung tissue was previously identified in Ki-ras oncogenes of lung tumors from 3-MC exposed mice. The 2-fold increase in the mutant frequency after treatment with PCB3 and 4-OH-PCB3 was not statistically significant, but a shift in the mutation spectra, especially with PCB3, and an increase in mutations outside of the hotspot region for spontaneous mutations (bp 1-400), suggest that PCB3 and possibly 4-OH-PCB3 are mutagenic in the rat lung.

Keywords

4-monochlorobiphenyl; 3-methylcholanthrene; lung; mutagenicity; transgenic rat; BigBlue®

1. Introduction

Polychlorinated biphenyls are a class of organic compounds that were produced between 1929 and 1977 in the United States. An estimated two million tons of commercial PCB mixtures were produced world-wide during this period with roughly 0.2 million tons remaining in various environmental reservoirs (WHO, 2003). PCBs were used as coolants and lubricants, most commonly as dielectric fluids in transformers and capacitors, as additives in paints,

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plastics, adhesives, and in sealants. The major source of PCBs in the body is from ingestion of contaminated food, especially fish, where higher chlorinated congeners dominate. Another important route of exposure is by inhalation from contaminated indoor and outdoor air. In this case, lower chlorinated congeners are dominating. Common sources for outdoor contamination are vaporization from landfills (Vorhees et al., 1997) and contaminated surface water (Hornbuckle and Green, 2003). The main source of indoor contamination is vaporization of PCBs from construction materials. A study by Herrick and coworkers found that several buildings surveyed in the Greater Boston area contained caulking that exceed the Environmental Protection Agency limit of fifty parts per million by weight (Herrick et al., 2004). Another study reported that for children, inhalation exposure due to indoor exposure was greater than ingestion exposure (Wilson et al., 2001). Thus in specific situations the lung may play an important role in PCB toxicity. Bachour and coworkers examined the species and organ dependence of PCBs in fish, foxes, roe deer and humans and discovered, among others, high concentration of lower chlorinated biphenyls in the human lung (Bachour et al., 1998). They concluded that the lung may be a target organ for accumulation of metabolically activated lower chlorinated biphenyls. Indeed, a study examining retention of PCBs in rats after oral application found hydroxylated PCB metabolites in both, the lungs and livers (Bergman et al., 1994). Such activated biphenyls may result in the formation of DNA adducts and subsequently DNA damage (Dubois et al., 1995; Wong et al., 1979), a mechanism that could result in cancer initiation.

In rodents the most common tumor site after PCB-exposure is the liver (Mayes et al., 1998), but lung tumors were also described after exposure to PCB mixtures (Anderson et al., 1994; Nakanishi and Shigematsu, 1991) or individual PCB congeners (NTP, 2006a; NTP, 2006b). PCBs are known promoters of carcinogenesis (Silberhorn et al., 1990), but they are also complete carcinogens (Mayes et al., 1998). The question remains whether PCBs are indeed mutagenic, and therefore possible cancer initiators, in a potential target organ like the lung. The mutagenicity of 4-chlorobiphenyl (PCB3) in the liver of male 344 Fisher transgenic (Big Blue®) rats which contain the bacterial *lacI* gene as target sequence has been shown recently (Lehmann et al., 2007). The goal of this study was to assess the mutagenicity of PCB3 in the lung of exposed male Big Blue® rats. PCB3 and its metabolite 4-OH-PCB3 were the study compounds, corn oil and 3-methylcholanthrene (3-MC) served as negative and positive control, respectively. We report here that 3-MC is a very efficacious mutagen in the lung, inducing a 15-fold increase in mutant frequency compared to control. PCB3 and 4-OH-PCB3 caused a non-significant 2-fold increase in mutant frequency, which was, however, accompanied by significant changes in the mutation spectra which suggest that PCB3 and, with less likelihood, its metabolite 4-OH-PCB3 may be mutagenic in the lung.

2. Materials and Methods

2.1. Chemical Substances

PCB3 and the hydroxylated metabolite, 4-OH-PCB3 were synthesized, purified, and characterized as described (Espandiari et al., 2003). Agar and 3-MC were purchased from Sigma (St. Louis MO). 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-gal) was purchased from Research Products International (Prospect, IL). The DNA isolation RecoverEase kit and Transpack® kit were supplied by Stratagene (La Jolla, CA). All other chemical substances used were purchased from Fisher Scientific.

2.2. Exposure Protocol

The exposure protocol used was as described by Lehmann et al (2007). Briefly, a total of sixteen male Fisher 334 Big Blue® rats which contain 30-40 copies of the bacterial *lacI* gene in each cell, were purchased from Stratagene (La Jolla CA) and received from the breeder Taconic

Laboratories (Germantown NY) at postnatal day 30. Animals were provided with 7013-NIH-13 Modified Open Formula Rat diet and water ad libitum and kept on a 12-hour light/dark cycle. On postnatal day 37 animals were weighed, distribution into four exposure groups with four animals each, and received the first of 4 weekly intraperitoneal injections of either corn oil (5 ml/kg body weight (bw)), 3-MC (80 mg/kg bw), PCB3 (113 mg/kg bw) or 4-OH-PCB3 (82 mg/kg bw). The doses of PCB3 and 4-HO-PCB3 were based on previous studies (Espandari, et al. 2003; Espandari et al. 2004). During the entire exposure period, animals were monitored daily to assess well-being and weighed twice a week. The last injection was given on postnatal day 58. Seventeen days later all animals were euthanized and the lungs were excised and frozen in liquid nitrogen and then stored at -80°C until use. The right lobe was used to prepare histological slides at the University of Iowa Pathology Department. For each animal four micron sections were cut, fixed on glass slides, stained with hematoxylin-eosin stain, and analyzed by light microscopy. The left lobe was used for DNA extraction. All experiments were conducted with the approval of the University of Iowa Institutional Animal Care and Use Committee.

2.3. DNA Extraction and Packaging

DNA was extracted from the lung tissue using the Big Blue ® RecoverEase DNA isolation kit (Stratagene) as described in the manual. Each extraction run included one animal from each exposure group to prevent bias from day-to-day variations in the assay. Lung tissue, 175 mg of the left lobe of each animal, was minced into smaller pieces, which was necessary due to the fibrous nature of the lung, and added to 5 ml ice-cold lysis buffer, provided in the kit, in a Dounce tissue homogenizer. Using the “loose” pestle, the tissue was ground using 10-15 strokes, followed by 8 strokes using the “tight” pestle. Twisting of the pestle was avoided to prevent shearing of the genomic DNA. The entire process was performed at 4° C. The homogenate was centrifuging at 1100g for 12 min. RNA and protein in the pellet were digested with RNAase and Proteinase K and the genomic DNA was purified by dialysis in Tris-EDTA (TE) buffer for 48 hours.

The excision of the lambda transgenic shuttle vector containing the *lacI* target gene and packaging in phage heads was performed using the Lambda Phage Transpack® packaging kit (Stratagene) as instructed by the manufacturer. Each DNA sample was transpackaged and subsequently plated a minimum of three times. After the completion of the Transpack® reaction, the mixture contained the *lacI* transgene packaged into phages was diluted to 1 ml with SM buffer (100 mM NaCl, 8 mM MgSO₄, 50mM Tris-HCl, 0.004% gelatin) which 50 µl of chloroform for stabilization before storage at 4° C until used. The packaging efficiency was determined by infecting 200 µl of E. coli strain SCS-8 suspension (OD = 0.5) with 1 µl of the Transpack® reaction and plating of this mixture in 3 ml of top agarose onto 100 mm NZY-agar (bottom agar) plates. Transpackaging efficiency was determined as the number of plaques produced by infected bacteria in the bacteria lawn.

2.4. Determination of the mutant frequency

DNA package sample equivalent to ~12,500 pfu (plaque forming units) was added to 2 ml SCS-8 suspension (OD = 0.5) and incubated for 15 min. Fifty µl of this SCS-8/DNA mixture with 2 ml fresh SCS-8 suspension and 35 ml top agarose were used to prepare titer plates. The rest of the SCS-8/DNA mixture was added to 35 ml of top agarose containing 1.5mg/mL of X-gal and poured onto 500 mm² assay trays (Corning, Acton, MA). Assay trays were vented for 1 h to prevent excess moisture. All plates were incubated in 37° C for 16-20 h. All colorless plaques on the titer plates were counted and used to determine the number of plaque forming units (pfu) on each plate. Assay plates were examined for mutant plaques with a blue phenotype. Mutants from blue plaques were picked and placed in SM buffer with chloroform, and later retested twice in the same manner as described previously except that 100 mm² trays and five

milliliters of X-gal containing top agar was used. Any mutants that were confirmed after the second re-plating were used to calculate the mutant frequency by dividing the number of confirmed mutants by the number of plaques as determined from the titer plates. Mutants from the second replating were isolated and stored in DMSO at -80° C until use for sequencing analysis.

2.5. Amplification and Sequencing

To establish the mutation spectra, all verified mutants were amplified by PCR and sequenced. Two primers were used during the amplification as outlined in the instruction manual, one forward primer (5'-GTATTACCGCCATGCFATACTAG-3') and one reverse primer (5'-CGTAATCATGGTCATAGCTG-3'). The amplification program was set as 30 amplification cycles at 94° C for 30 seconds, 53° C for 50 seconds, and 72° C for 60 seconds, using Taq Hot Start Polymerase (Quiagen). Each PCR product was purified using a PCR Purification Kit (Quiagen). Successful amplification was confirmed by 1% agarose gel electrophoresis if the product was roughly 1000 bp in length. Each mutant was sequenced in both directions using four primers: forward PCR primer, #5 primer (5'-TCTGGTCGCATTGGGTC-3'), reverse PCR primer, #12 primer (5'-AGAACTTAATGGGCCCCG-3'). Each complete, readable forward and reverse sequence was compared with an established *E. coli lacI* sequence using the BLAST2 Sequence function accessed through the National Center for Biotechnology Information (NCBI) website. Identical mutations from the same animal were considered possible siblings and eliminated from the final calculation of the mutation frequency for each treatment group which was calculated as the corrected number of mutant plaques per 100,000 plaques analyzed.

2.6. Statistical Methods

A Student t-test was used to determine if there was a significant difference between the mean lung weight for the control group and the treatment groups. A Poisson regression analysis was performed to assess if there was a significant increase in the mutation frequency in the treatment groups. The Pearson Chi-square test was used to determine significant differences between the mutation spectra.

3. Results

3.1 Body and Lung Weights

The final body weight varied between 131 (animal II-1) and 257g (I-1) (Table 1). The average weight gain in the four groups from post-natal day (PND) 35 to PND75 was 159.7 ± 7.6 (I), 90.4 ± 25.6 (II), 146.1 ± 8.0 (III), and 151.6 ± 17.5 (IV). The reduced weight gain and lower final b.w. in group II was due to lower weight gain during the last 10-14 days of the experiment (data shown in Lehmann et al., 2007). Lungs from all animals were removed, weighed, and macroscopically inspected by eye for visible changes. No gross morphological differences were observed. Lung weights and lung weight to body weight ratios are given in Table 1. No statistically significant difference in lung weights ($p > 0.19$, $p > 0.16$, $p > 0.42$ for 3-MC, PCB3 and 4-OH-PCB3 vs control) or ratio of lung to b.w. were seen.

3.2 Pathology Results

Changes seen in the lungs are shown in Table 2. The most common pathological observation, seen in most animals of all groups, was perivascular or peribronchial lymphocytes, which is an accumulation of lymphocytes around veins or bronchi. Intra-alveolar macrophages were seen in 2 animals from different groups. None of these findings are significant pathological changes or indications of specific health problems in the animals. No specific treatment changes were seen in any of the groups, with the possible exception of peribronchial lymphocytes in

group III, nor did any change correlate with an unusual high or low body or lung weight (Table 1).

3.3 Mutant and mutation frequency

Several hundred thousand plaques per treatment group were analyzed for the occurrence of mutant plaques. Identified mutant plaques were verified by repeated re-plating. A total of 75 mutants were observed in close to 2 million plaques analyzed. The number of mutants per 100,000 plaques varied between ~1.1 in the control and 2.1 in the PCB3 and 4-OH-PCB3 groups to over 12 mutants/100,000 plaques in the 3-MC group (Table 3). Prior to sequencing, the mutants were considered to be “uncorrected mutants” and used to calculate the “uncorrected” mutant frequency.

All mutants were sequenced and any identical mutants from the same animals were removed from the total numbers of mutants, which reduced the number of mutations in group I, III, and IV. In addition, 2 mutants in group II had more than 1 mutation in the sequence (confirmed by sequencing in both directions), which increased the number of mutations in this group (Table 3). The total number of mutations after sequencing was used to calculate the “corrected” mutation frequency.

The statistical analysis of the mutation frequency of groups II to IV vs control by Poisson regression showed p values of 0.000000217 for group II, and 0.244 and 0.208 for groups III and IV, respectively.

3.4 Mutation Spectra

Individual base pair mutations were the most frequent type of mutation, ranging from 75% in group I and IV to 80 and 87% in groups III and II, respectively (Table 4). The type of bp substitution differed strongly between the treatment groups. In control animals (I), 50% of mutations were transitions, whereas this number fell to 42% with 4-OH-PCB3, 20% with PCB3 and only 18% with 3-MC. Both kinds of transitions were about equally represented. On the other hand, transversions increased from 25% in the control to 33% (4-OH-PCB3), 60% (PCB3) and 69% (3-MC). The majority of transversions were from G:C → T:A (or C:G → A:T), which increased to 44% of all mutations in the 3-MC group. G:C → C:G was well represented (30%) in the PCB3 group, whereas A:T → T:A mutations were best represented in the 4-OH-PCB3 group. All frameshift mutations were deletions, no additions were found. All bp and frameshift mutations in the control group were in the first 400 bp section of the *lacI* gene, whereas this number went down to 75%, 70%, and 62% in groups IV, III, and II, respectively. The small number of mutations that were sequenced did not allow for the identification of “hot spots” of mutations between bp 400 and 1200 for these groups. The Pearson Chi-square test of the mutation spectra (transitions/transversions/deletions) did not show any significant differences between the 4 treatment groups.

4. Discussion

Several low chlorinated biphenyl congeners, including PCB3, were positive in the modified Solt-Farber protocol (Espandari et al., 2003). A follow up study revealed that in addition to PCB3, its 4-OH and the 3,4-quinone metabolites were positive in this assay (Espandari et al., 2004). The modified Solt-Farber assay is designed to indirectly measure the initiating activity of a compound by examining the appearance of GGT-positive foci. Initiation requires a mutagenic event, and the mutagenicity of PCB3 in the liver of male 344 Fisher transgenic rats has been established (Lehmann et al., 2007). The primary purpose of this study was to determine the mutagenicity of PCB3 or 4-OH-PCB3 in the lung of transgenic Big Blue® Fisher 344 male rats. In addition, the same type of mutation that was found in oncogenes of lung

tumors from 3-MC-exposed mice was identified in lung tissue of rats only weeks after exposure to this carcinogen.

4.1 Pathology

Neither PCB3 nor 4-OH-PCB3 had any detectable toxic effect on body weight gain with the exposure regiment used in this study. The positive control 3-MC caused a reduction of weight gain in the final phase of the exposure time, which may indicate some general health issues with this treatment. None of the 3 test compounds had a significant effect on lung weight or morphology. Minimal perivascular lymphocytes and, in 2 animals, intra-alveolar macrophages were found. These increased levels of macrophages and lymphocytes indicate low grade inflammation in the lung. Topinka et al (2004) theorized that persistent inflammation and cell proliferation could play a role in the mutagenicity of amosite asbestos. However, in most animals these changes were only very mild and they were also observed in the control group, which excludes a causal relationship to the mutagenicity seen in some treatment groups. The only group-specific change could be the increase in peribronchial lymphocytes in 3 of 4 animals treated with PCB3 (group III). The physiological mechanism or significance of this change is not clear at this moment. It should be kept in mind, however, that the amount of inflammation may increase if inhalation exposure is used instead of IP injection, since more PCB molecules could possibly reach the lung. Therefore pathological examination of lung tissue should always be included after inhalation exposure studies with PCBs. The important point for this study is, however, that none of these pathological changes are expected to have an influence on the mutant frequency or mutation spectra observed here.

4.2 Spontaneous mutations in the control group

The mutant frequency in the control group (I) was very low, 11×10^{-6} pfu, which is in agreement with other reports of a spontaneous mutant frequency in the Big Blue® rat lung of 8.8 and 30×10^{-6} pfu (Sato et al., 2000; Topinka et al., 2004). Half of the mutations were transitions, another quarter were transversions and deletions, which also is in very good agreement with the findings of others, who reported that 30 to 51% of mutations in their control group were transitions, 20 to 27% being transversions, and 21 to 30% deletions and/or insertions (Bottin et al., 2006; Sato et al., 2000). All mutations in the control group were in the first 400 bp of the *lacI* gene, which contains the DNA binding domain of the *lac I* gene and is a known hot spot region for spontaneous mutations (de Boer et al., 1998).

4.3 Mutagenicity of 3-MC

The highest number of mutants, 43, was seen in the 3-MC group, and in 2 of these mutants more than 1 mutation was identified. Overall there was a 15-fold increase in the mutant frequency after treatment with 3-MC. The mutagenicity of 3-MC in the Big Blue® mouse and rat liver has been described before (Lehmann et al., 2007; Rihn et al., 2000), but to our knowledge this is the first report about its mutagenic activity in the lung. Interestingly the same treatment dose and schedule that had induced 88×10^{-6} pfu in the liver of rats induced 125.5×10^{-6} pfu in the lung of these animals, up from 17 and 9×10^{-6} in the respective controls. This 15-fold increase of mutant frequency in the lung vs 5-fold increase in the liver suggests that 3-MC is a stronger lung than liver carcinogen in the rat after IP application, a finding that is confirmed by numerous carcinogenicity studies in mice that show a strong induction of lung tumors in 3-MC-treated animals (Miller et al., 1998; Wang and Witschi, 1995).

3-MC is a known carcinogen in the liver and lung of rodents. It was described that susceptibility for lung tumorigenesis after transplacental exposure seem to depend on sustained CYP 1A1 induction in the dams (Miller et al., 1990). 3-MC is a large polycyclic aromatic hydrocarbon that is activated by CYPs to metabolites that bind to the exocyclic amino group of guanine, adenine, and to some extent cytosines, and these bulky adducts cause misreading by

polymerases resulting in G:C → T:A and A:T → T:A transversions and G:C → A:T transitions (Dipple, 1995). Indeed, an increase in transversions from 17% in the controls to 65% in 3-MC treated animals was reported for the mouse liver (Rihn et al., 2000) and a shift from mostly transitions to mostly transversions (43%), predominantly G:C → T:A, in the liver of Big Blue® rats (Lehmann et al., 2007). In this study, an even more dramatic shift was seen in the lung of the rats, where 69% of all mutations were transversions, 66% of them from G:C → T:A. All other types of transversions were more frequent compared to solvent control or 3-MC-livers as well. Analysis of the lung tumors from mice which had been exposed to 3-MC in utero revealed that 79% of tumors contained mutations in codon 12 or 13 of Ki-Ras-2, the majority (84%) of which were G → T transversions (Miller et al., 1998). An earlier mouse tumor study had observed mostly G:C to C:G transversions at the first base of codon 12 and 13 of Ki-ras, followed by G to T transversion at the 2nd base of codon 12 (Wang and Witschi, 1995). In A/J mice, 44% of 3-MC-induced tumors and 94% of those from animals which had received 3-MC as initiator and butylated hydroxytoluene (BHT) as promoter had these types of mutations. This coincidence indicates that an elucidation of the type of mutations initiated by a compound may very well provide indications about the carcinogenic risk of this compound.

4.4 Mutant frequency and mutations caused by PCB3 and 4-OH-PCB3

Treatment with PCB3 created a 2.2-fold increase in the mutant frequency, similar to the 2.4-fold increase seen in the livers of PCB3-treated rats (Lehmann et al., 2007). This increase, which reached significance in the liver, was not statistically significant in the lung, probably because of the smaller sample size and the large variation within each group. This, however, does not necessarily mean that PCB3 is not mutagenic in the lung. A Big Blue® mutagenicity study with benzo(a)pyrene (B(a)P) did not find a significant increase in mutant frequency at early time points, but found differences in the mutation spectrum in the exposed animals (Shane et al., 2000a). The authors concluded that B(a)P was a mutagen. Similarly long term exposure to phenobarbital did not significantly increase the mutant frequency in the liver, but increased the percentage of G:C → T:A and G:C → C:G transversions, which was interpreted as an indicator of oxidative damage (Shane et al., 2000b). A similar strong shift in the mutation spectrum was observed in PCB3-lung mutations. The most common type of mutation in treatment group III (PCB3) was transversions (60%), followed by transitions and deletions with 20% each. This increase in transversions due to PCB3 treatment is even larger than the 43% transversions observed previously in the liver (Lehmann et al., 2007). In PCB3 lungs, the most frequent type of transversion was G:C → C:G, followed by G:C → T:A. This is different from the findings in the liver of these rats, and in the livers and lungs of 3-MC-exposed rats, where G:C → T:A was the most common type. Taken together these findings strongly suggest that PCB3 is a mutagen in the lung of rats after IP application. This raises the question whether inhalation exposure, the most likely route of exposure to this lower chlorinated PCB, may pose an even larger risk.

There was a 2.1-fold increase in mutant frequency after treatment with 4-OH-PCB3. Although this increase was larger than the significant increase in the livers (1.9-fold), this increase was not statistically significant in the lung due to the smaller sample size and variation within the groups. In treatment group IV, the most common type of mutation was transitions (42%), followed by transversions (33%) and deletions (25%), a small shift from the spontaneous mutation spectrum of 50%, 25% and 25% transitions, transversions and deletions, respectively, towards more transversions. This shift was more pronounced than the one observed in the livers of 4-OH-PCB3-treated animals, where only 22% of mutations were transversions (Lehmann et al., 2007). In addition, only 75% of these mutations were within the first 400 bp of the lacI gene, compared to 100% in the control group. Thus, even though these changes individually are small and not significant, taken together they form a trend or pattern that indicates that 4-

OH-PCB3 may be a mutagen in the lung, and may have stronger mutagenic activity in the lung than in the liver of animals with this IP route of exposure

It can be speculated that any mutagenic and initiating activity of PCBs is most likely due to metabolic activation. The first step in biotransformation by cytochromes P450 (CYPs) is the oxidation to an arene oxide with an epoxide group. Arene oxides can undergo further biotransformation to a mono-hydroxylated form and further to di-hydroxylated metabolites, which may be oxidized to quinones (McLean et al., 1996a). The arene oxide and quinone metabolites are electrophiles and can bind to nucleophilic sites in amino acids, proteins, and DNA (Amaro et al., 1996; Arif et al., 2003; McLean et al., 1996b; Pereg et al., 2002; Pereg et al., 2001; Srinivasan et al., 2001; Srinivasan et al., 2002; Tampal et al., 2003). Also, during this oxidative metabolism reactive oxygen species (ROS) can be generated, which produce 8-oxo-dG and DNA strand breaks (McLean et al., 1998; Oakley et al., 1996; Srinivasan et al., 2001). The shift in the mutation spectra towards transversions could be caused by bulky adducts, for example of a PCB3 arene oxide or quinone metabolite, or by ROS. Although the effects of 4-OH-PCB3 in the lung were very small, this does not imply that a secondary arene oxide or quinone metabolite are not mutagenic, since the exposure occurred via ip injection and a lower dose of the metabolite was used compared to PCB3 to prevent possible general toxicity. More experiments are needed to identify the ultimate mutagen.

Conclusions

All 3 test compounds of this study, 3-MC, PCB3, and 4-OH-PCB3, had previously been shown to statistically significantly increase the mutant frequency in the liver of rats (Lehmann et al., 2007). In this study 3-MC was even more mutagenic in the lung than in the liver, which is in agreement with its carcinogenicity in the lung. Here, we also provide evidence, based primarily on changes in the mutation spectra, that PCB3 may generate mutations in the lung following ip exposure. The evidence is much weaker for its metabolite 4-OH-PCB3. Considering recent reports about high levels of low chlorinated biphenyls and specifically PCB3 in indoor (Davis et al., 2002) and outdoor air (Ishikawa et al., 2007) these are troubling results. In this study ip exposure was chosen to mimic previous Solt-Farber experiments that had provided evidence of initiating activity of the 2 PCBs in the liver, and to better control for the dose that the animals received. Inhalation experiments with PCBs are technically extremely difficult, but are urgently needed to provide greater certainty of the mutagenic effect of PCB3 and 4-OH-PCB3 in lung and the risk of human exposure to airborne PCBs.

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Table 1

Lung weights, body weight, and lung to b.w. ratio for each animal and average and standard deviation for each treatment group.

Animal Number	Treatment Group	Lung Weight (g)	Final Body Weight (g)	Lung wt/100g b.w.
I-1	Corn Oil	1.190	257.3	0.46
I-2	Corn Oil	1.167	241.0	0.48
I-3	Corn Oil	1.062	241.5	0.44
I-4	Corn Oil	1.200	240.4	0.50
	Average:	1.155 ± 0.063	245.1±8.2	0.47±0.03
II-1	3-MC	1.206	178.2	0.68
II-2	3-MC	0.827	131.0	0.63
II-3	3-MC	1.038	162.6	0.64
II-4	3-MC	0.636	219.6	0.29
	Average:	0.927 ± 0.248	172.9±36.8	0.56±0.18
III-1	PCB3	0.600	222.2	0.27
III-2	PCB3	1.067	237.8	0.46
III-3	PCB3	0.900	208.7	0.42
III-4	PCB3	1.156	220.2	0.52
	Average:	0.931 ± 0.245	222.2±12.0	0.42±0.11
IV-1	4-OH-PCB3	1.232	240.3	0.51
IV-2	4-OH-PCB3	1.018	254.3	0.40
IV-3	4-OH-PCB3	1.168	233.3	0.50
IV-4	4-OH-PCB3	0.681	189.5	0.36
	Average:	1.025 ± 0.246	229.4±28.0	0.44±0.08

Table 2

Pathological changes in the lung of each animal in the exposure groups.

Animal Number	Pathological Changes
I-1	One focus of acute inflammation
I-2	Focal acute inflammation with focal increase in perivascular lymphocytes
I-3	Minimal perivascular lymphocytes
I-4	Minimal perivascular lymphocytes
II-1	No abnormality
II-2	Focal perivascular lymphocytes
II-3	Focal perivascular lymphocytes
II-4	Focal perivascular lymphocytes with increased intra-alveolar macrophages.
III-1	Increased perivascular lymphocytes
III-2	Peribronchial lymphocytes
III-3	Peribronchial lymphocytes with increased intra-alveolar macrophages.
III-4	Peribronchial lymphocytes
IV-1	No abnormality
IV-2	No abnormality
IV-3	Minimal perivascular lymphocytes
IV-4	Minimal perivascular lymphocytes

Table 3
The number of mutants and plaques plated, mutant frequency, number of mutations and mutation frequency for each treatment group.
** significantly different from control (I) $p < 0.005$

Treatment Group	Total Number of Mutants	Total Number of Plaques	Mutants per 100,000 Plaques	Number of Mutations (corrected)	Mutation Frequency (corrected)
I	5	455,840	1.096	4	0.88
II	43	342,570	12.550	45	13.13**
III	11	528,220	2.082	10	1.89
IV	14	660,420	2.119	12	1.81

Table 4

The number of independent mutations, and mutation type for each exposure group.

Types of mutations	Number of mutations (% total mutations)			
	Corn Oil (I)	3-MC (II)	PCB3 (III)	4-OH-PCB3 (IV)
Total	4	45	10	12
Transitions (total)	2 (50%)	8 (18%)	2 (20%)	5 (42%)
G:C → A:T	1 (25%)	3 (7%)	1 (10%)	3 (25%)
A:T → G:C	1 (25%)	5 (11%)	1 (10%)	2 (17%)
Transversions (total)	1 (25%)	31 (69%)	6 (60%)	4 (33%)
G:C → T:A	1 (25%)	20 (44%)	2 (20%)	1 (8%)
G:C → C:G	0	6 (13%)	3 (30%)	1 (8%)
A:T → C:G	0	3 (7%)	1 (10%)	0
A:T → T:A	0	2 (4%)	0	2 (17%)
Frameshifts (total)	1 (25%)	6 (13%)	2 (20%)	3 (25%)
- 1 Frameshift	0	6 (13%)	2 (20%)	2 (17%)
Other Deletions	1 (25%)	0	0	1 (8%)
Insertions	0	0	0	0