Polychlorinated Biphenyls and 2,3,7,8-Tetrachlorodibenzo-p-dioxin Induce Intrachromosomal Recombination in Vitro and in Vivo

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ABSTRACT

Polychlorinated aromatic hydrocarbons such as polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are extremely stable and widely distributed environmental pollutants. These chemicals are animal carcinogens and probable human carcinogens, and TCDD is possibly one of the most potent toxins ever evaluated by the United States Environmental Protection Agency. Polychlorinated aromatic hydrocarbons score negatively in most genotoxicity assays, including the Ames (Salmonella) assay. Although their mechanism of toxicity is not well understood, they induce aryl hydrocarbons (AH) hydroxylases and bind to the AH receptor, which is believed to mediate toxicity. Here, we determine effects of polychlorinated aromatic hydrocarbons in genotoxicity assays that score for DNA deletions by intrachromosomal recombination in vivo and in vitro. In this study, TCDD, Aroclor 1221, and Aroclor 1260 induced deletions in vivo in the mouse embryo; Aroclor 1221 and Aroclor 1260 induced deletions in yeast. We also show that the induced deletion events did not correlate with induction of AH hydroxylases. None of the tested compounds induced CYP1A-associated ethoxyresorufin-O-deethylase activity in mouse embryos or in vitro. These results clearly demonstrate a genotoxic activity of polychlorinated aromatic hydrocarbons in vitro and in vivo, which is independent of induction of cytochrome P450 activity. Because genetic instability and deletions may be mechanistically involved in carcinogenesis, these results may encourage further research to determine whether such genotoxic mechanisms may be useful for cancer risk assessment of polychlorinated aromatic hydrocarbons.

INTRODUCTION

Polychlorinated aromatic hydrocarbons such as PCBs and TCDD have caused much concern, debate, and controversy. TCDD has been labeled the most potent carcinogen ever evaluated by the United States Environmental Protection Agency, and it is feared that exposure to even small amounts of PCBs may lead to serious, adverse health effects (1). Public concern has been fueled by several high-profile environmental releases of TCDD (2).

PCBs, a family of 209 different congeners (3), have been widely used since the early 1930s in paints, printing solutions, electrical capacitors, and transformers and were believed to be chemically inert (4). Widespread use resulted in global distribution and an estimated environmental load of several hundred million kilograms (4), and PCBs are found in the fatty tissues of almost all living organisms.

PCBs are stable and highly fat-soluble compounds, and their half-life ranges from days to years, the more highly chlorinated species being more persistent (5). The body metabolizes and eliminates PCBs very slowly, hence its tendency to retain the more toxic and more highly chlorinated isomers in adipose tissue. Despite voluntary restrictions on the use of PCBs, beginning in 1970, recent data suggest that environmental levels have not declined significantly over the past 25 years (4).

Epidemiological studies show a correlation between an increased risk of malignant neoplasm and other detrimental health effects and occupational or accidental PCB exposure (6—8). Carcinogenicity of PCBs and TCDD in animals has been well documented in medium as well as in standard, long-term animal assays (for reviews see Refs. 3, 4, and 9). However, PCBs and TCDD score negatively in most genotoxicity assays (for reviews see Refs. 3 and 4). PCBs can bind to protein, but their interaction with DNA is low or nonexistent, which supports the classification of PCBs as nongenotoxic carcinogens (10, 11).

On the other hand, PCBs and TCDD are powerful inducers of cytochrome P450 CYP1A and associated AHs (for review see Ref. 12). In fact, there seems to be almost a consensus that the initial event in the toxicity of PCBs and TCDD is their binding to the AH receptor (4, 12, 13).

PCBs and TCDD do not stand alone in their lack of activity in short-term assays. About 50% of all carcinogens do not induce point mutations in Salmonella (14—16), suggesting that alternative mechanisms of toxicity may account for the biological activity of many carcinogens. There are two lines of evidence suggesting that genome rearrangements, in addition to point mutations, are involved in carcinogenesis. First, tumor cells frequently contain genome rearrangements such as DELs (17—20). Second, an elevated frequency of recombination and genome rearrangements is found in normal, nontransformed cells from patients suffering from cancer-prone diseases such as ataxia telangiectasia (21), Li-Fraumeni syndrome (22), Bloom’s syndrome (23), and Werner’s syndrome (24). In fact, both the BLM gene and the WRN gene, mutations that cause Bloom’s syndrome and Werner’s syndrome, respectively, encode proteins that are highly homologous to bacterial RecQ helicases, which are central in the maintenance of genomic stability (25). These findings imply that recombination may play a role in carcinogenesis.

About 25% of the human genome consists of repetitive DNA sequences in either tandem repeats or interspersed repetitive elements (26). A large number of repetitive sequences scattered throughout the human genome create the substrates for intrachromosomal recombination events between direct repeats (e.g., Ref. 27) and may lead to various genetic disorders upon deletion or disruption of a gene involved in disease.

Because of the association of recombination and genome rearrangements with cancer, recombination systems were constructed and used to detect the genotoxic effects of carcinogens in the yeast Saccharomyces cerevisiae (28), in human cells (29), and in vivo in the mouse (30). In general terms, a gene duplication is arranged so that the first allele contains a deletion at the 3’ end and the second allele contains a deletion at the 5’ end to generate a gene disruption (Fig. 1). Because the two inactive gene copies contain a region of homology, they can undergo homologous recombination with each other to produce a deletion of one copy of the gene duplication and reversion of the gene disruption to the wild-type copy (DEL recombination; Fig. 1).

The yeast DEL recombination substrate has been constructed by the
Previously, to determine frequencies of DEL recombination in human cells, we used the human lymphoblast cell line GM6804. These cells contain a 13.7-kb duplication of a region containing exons 2 and 3 in the human cell line GM6804 (29), and exons 6–18 of the p-gene in the mouse (60). A, intrachromatid crossing-over occurs after pairing of the two copies of the gene duplication (RK) in a looped configuration (28). Crossing-over results in DEL of one of the two copies, giving rise to reversion to the wild-type (MARKER) gene. B, single-strand annealing is initiated by a double-strand break between the duplicated fragment (54, 61). DNA ends are degraded by a 5′ → 3′ single-strand specific exonuclease to expose the flanking homologous sequences. 

DEL effects that are induced by these compounds and the induction of cytochrome P450 in the mouse embryo and in the human cells.

MATERIALS AND METHODS

Chemicals. MMS was obtained from Sigma Chemical Co. DMSO and acetone were obtained from Fisher Scientific. Aroclor 1221 and Aroclor 1260 were purchased from Chem Service (West Chester, PA). TCDD was purchased from AccuStandard (New Haven, CT).

Determination of the Frequency of DEL Recombination in the Mouse.

Homozygous CS7BL/6J p"/p" mice were obtained from the Jackson Laboratory and bred at our facility. An increase in the frequency of reversion events in the premelanocytes in the embryo gives rise to an increase in the number of offspring showing dark patches on their fur. The protocol used in this test was similar to the "mouse spot test" that has been used to determine effects of radiation and chemicals on the frequency of mutations (for review see Refs. 38 and 39). These forward mutations can occur in the wild-type alleles of any one of several heterozygous coat color markers, giving rise to mutant spots on black fur. The main differences in our p" reversion assay were that we selected for the reverse genetic event, going from mutant gray coat color to black wild-type spots, and that p" reversion events were due to DNA deletions of 70 kb of DNA (see Fig. 1) rather than forward mutations.

Matings were set up between p" mice, and pregnancy was timed from the discovery of a vaginal plug. First and second litters were used and gave similar spotting frequencies. Sperm entry into the egg was assumed to have occurred in the early morning hours of the day on which the plug was found and 12:00 pm that day was defined as 0.5 days postconception. The animals were exposed to an acute dose of the chemicals by i.p. injection at 10.5 days postconception. The carcinogens were dissolved in corn oil, and 0.2 ml of the solution was injected. Solvent-only controls were carried out. Offspring were examined for spots at 12–14 days of age, when spots were most easily visible.

Fig. 1. Recombination substrate structure and possible mechanisms of intrachromosomal recombination resulting in DELs (according to Refs. 28, 29, 37, and 54). Center, generic DEL recombination construct with an internal duplication of a DNA fragment (RK), which represents an internal fragment of the HIS3 gene in yeast (28), exons 2 and 3 in the human cell line GM6804 (29), and exons 6–18 of the p-gene in the mouse (60). A, intrachromatid crossing-over occurs after pairing of the two copies of the gene duplication (RK) in a looped configuration (28). Crossing-over results in DEL of one of the two copies, giving rise to reversion to the wild-type (MARKER) gene. B, single-strand annealing is initiated by a double-strand break between the duplicated fragment (54, 61). DNA ends are degraded by a 5′ → 3′ single-strand specific exonuclease to expose the flanking homologous sequences.
Two subsequent examinations were performed, the last one being at 4–5 weeks. Control values were obtained from mice bred at the same time as the experimental mice. Animal care and experiments were carried out according to institutional guidelines.

The Yeast Recombination Assay. The diploid strain RS112 (MATa/ura3–52/ura3–52 leu2–3,112/leu2–Δ98 trp5–279/trp5–279 arg4–3/ARG4 ade2–40/ade2–101 itvl–92/ILV1 HIS3::pRS6his–Δ200 LYS2/lys2–801; Ref. 31) was used and contained the DEL system on one homologue (HIS3::pRS6) and a deletion of the entire open reading frame of HIS3 on the other homologue (his3–Δ200). Yeast extract, peptone, adenine, and dextrose synthetic complete (YPD) and SC were described previously (31). The Aroclor 1254-induced S9 fraction from rat liver was obtained from Microbiological Associates Inc. (Rockville, MD). The biological activity of this batch was tested by the company using the Salmonella test. The S9 mix was prepared as described by Maron and Ames (40), and MMS was used as a direct-acting mutagenic positive control in each experiment.

The DEL recombination substrate was constructed as described previously (28) and contained an integration of plasmid pRS6 containing an internal fragment of the HIS3 gene at the genomic HIS3 site of the yeast strain. This resulted in two copies of the his3 gene, one with a terminal deletion at the 3' end and the other with a terminal deletion at the 5' end (see Fig. 1). The diploid strain RS112 carries the his3Δ23'–his3Δ5' (HIS3::pRS6) recombination substrate on one homologue and a deletion of the entire region of homology to the recombination substrate on the other homologue (his3–Δ200). About 99% of HIS3 recombinants lose the integrated plasmid, including the LEU2 gene (28); therefore, the culture used to select for HIS3 recombinants were pregrown on medium lacking leucine, and following treatment with the appropriate agent, they were plated onto medium lacking histidine. Growth and accumulation of HIS3 recombination does not occur in the preculture, and therefore, the HIS3 frequency is a measure of the recombination rate. The spontaneous rates are highly reproducible. Strain RS112 is also heteroallelic for ade2-40 and ade2-101, such that the ICR between homologues can be independently measured.

The influence of Aroclor 1221 on the frequency of DELs and ICR was determined according to the standard yeast DEL assay, as described previously (33). Single colonies were picked from YPAD medium and inoculated into 5–25 ml of SC lacking leucine and grown for 24 h at 30°C with constant shaking to reach stationary phase. Cells were counted, and cell density was adjusted to 2 × 10⁶ cells/ml in fresh SC lacking leucine but containing 30% S9 mix or phosphate buffer. The medium containing the cells was distributed in aliquots of 5 ml each into disposable 15-ml tubes. The agent to be tested was added, and the cells were incubated for 17 h at 30°C under constant shaking. Cells were pelleted in a clinical table-top centrifuge and then washed twice with sterile, distilled water. Finally, the pellets were resuspended in 0.5–1 ml of sterile distilled water, transferred to glass tubes, and sonicated to disperse any clumps. Cells were counted, and appropriate numbers were plated onto two plates of SC for the number of survivors, onto two plates of SC lacking histidine for DEL recombination events, and onto two plates of SC lacking adenosine for ICR events. Colonies were counted after 2–3 days of incubation at 30°C and the numbers of recombinants were calculated per 10⁶ survivors for DEL recombination and per 10⁶ survivors for ICR recombination.

Data derived from less than five colonies were not included. A minimum increase of 2-fold over the spontaneous frequency in a dose-dependent manner has been regarded as evidence for inducibility. Each experiment was performed three times. The trends observed in the data were reproducible.

### RESULTS

PCBs and TCDD Induce Deletions in Vivo and in Vitro. We used mice that were homozygous for the DEL recombination substrate p<sup>p</sup>/p<sup>p</sup> to determine the frequency of spontaneous and Aroclor 1221-, Aroclor 1260-, and TCDD-induced DEL recombination via the spotting frequency of the fur of offspring mice. Thirteen % of the untreated mice in the breeding colony and about 4% of the mice injected with the solvent control corn oil developed spots (Table 1). The results from the breeding colony were obtained at the same time that the exposures were performed (concurrent controls). The current spontaneous rate was higher and the solvent control rate was lower than the previously observed control values of 5.6–11% (30, 37). However, the difference between these two control values was not significant using χ² distribution values. Animals were exposed to carcinogens by single i.p. doses, which were based on published fetotoxicity data and our own acute toxicity data. These doses, in many cases, approached the maximal acute exposure levels tolerated by the dams. We did not observe toxic effects on the female adult mice after these treatments. Carcinogen exposure decreased the litter size for the Aroclors; however, only 1000 mg/kg Aroclor 1221 showed a significant decrease

### Table 1 Effects of Aroclor 1221, Aroclor 1260, and TCDD on the frequency of p<sup>p</sup> reversion in mice

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Dose</th>
<th>No. of mice treated</th>
<th>No. of live offspring</th>
<th>Average litter size</th>
<th>No. of spotted offspring</th>
<th>Frequency (%)</th>
<th>P&lt;sub&gt;compared to control&lt;/sub&gt; breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding control</td>
<td></td>
<td>0</td>
<td>78</td>
<td>512</td>
<td>6.6</td>
<td>66</td>
<td>13</td>
</tr>
<tr>
<td>Corn oil control</td>
<td>0.2 ml</td>
<td>10</td>
<td>51</td>
<td>5.1</td>
<td>2</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Aroclor 1221</td>
<td>300 mg/kg</td>
<td>13</td>
<td>71</td>
<td>5.5</td>
<td>10</td>
<td>14</td>
<td>NS&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000 mg/kg</td>
<td>10</td>
<td>40</td>
<td>4.0</td>
<td>20</td>
<td>20</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Aroclor 1260</td>
<td>75 mg/kg</td>
<td>8</td>
<td>54</td>
<td>6.8</td>
<td>6</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>500 mg/kg</td>
<td>32</td>
<td>140</td>
<td>4.4</td>
<td>36</td>
<td>26</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>TCDD</td>
<td>2.25 μg/kg</td>
<td>12</td>
<td>69</td>
<td>5.8</td>
<td>17</td>
<td>25</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

<sup>a</sup> NS, not significant.
of at least three separate experiments for each compound; two plates were used for each system and each concentration. Acetone (3—5%) was used as a solvent.

...obtained after 17 h of incubation, the survival, and DEL as well as ICR frequencies. Data derived from less than five colonies were not included. A minimum increase of 2-fold over the spontaneous frequency in a dose-dependent manner has been regarded as evidence for inducibility. Due to the narrow range in dose response with certain chemicals in the DEL assay, absolute values for survival and recombination frequencies at a given dose were often variable between experiments performed with different starter cultures on different days (e.g., the lowest dose giving an induction may occur one dose up or down in different experiments). However, qualitatively, the results were quite reproducible and are representative of at least three separate experiments for each compound; two plates were used for each system and each concentration. Acetone (3—5%) was used as a solvent.

Table 3 Effects of Aroclor 1221, Aroclor 1260, and TCDD on induction of EROD activity in liver, embryos, and the human cell line GM6804.

- Polychlorinated aromatic hydrocarbons such as PCBs and TCDD are significant environmental pollutants and animal carcinogens. They score negatively in most genotoxicity assays, including the Salmonella assay, and therefore, there is a need for in vitro methods to screen, rank, and identify toxic members of such classes of compounds (4). Here, we show that the PCBs Aroclor 1221 and Aroclor 1260 as well as TCDD induce DEL recombination events in vivo in the mouse; furthermore, we confirmed their genotoxic activity in vitro. The PCBs Aroclor 1221 and Aroclor 1260 induced DEL events in yeast; in addition, the former induced DEL events in the human lymphoblastoma cell line GM6804. We also show that PCB- and TCDD-induced DEL events did not correlate with induction of the AHH in embryos or in tissue culture.

**DISCUSSION**

Polychlorinated aromatic hydrocarbons such as PCBs and TCDD are significant environmental pollutants and animal carcinogens. They score negatively in most genotoxicity assays, including the Salmonella assay, and therefore, there is a need for in vitro methods to screen, rank, and identify toxic members of such classes of compounds (4). Here, we show that the PCBs Aroclor 1221 and Aroclor 1260 as well as TCDD induce DEL recombination events in vivo in the mouse; furthermore, we confirmed their genotoxic activity in vitro. The PCBs Aroclor 1221 and Aroclor 1260 induced DEL events in yeast; in addition, the former induced DEL events in the human lymphoblastoma cell line GM6804. We also show that PCB- and TCDD-induced DEL events did not correlate with induction of the AHH in embryos or in tissue culture.

**Table 4 Induction of DEL recombination in vitro in human GM6804 lymphoblasts after exposure to Aroclor 1221**

<table>
<thead>
<tr>
<th>Concentration of Aroclor 1221 (mg/ml)</th>
<th>0</th>
<th>0.005</th>
<th>0.01</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability, CFE&lt;sup&gt;a&lt;/sup&gt; (%)</td>
<td>100</td>
<td>28</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Reversion frequency (10&lt;sup&gt;5&lt;/sup&gt; viable cells)</td>
<td>7.9</td>
<td>42.9</td>
<td>129</td>
<td>321</td>
</tr>
<tr>
<td>Fold induction</td>
<td>1</td>
<td>5.2</td>
<td>13</td>
<td>38</td>
</tr>
</tbody>
</table>

<sup>a</sup> DEL recombination frequencies were previously determined in human GM6804 lymphoblast cells (29). A summary of the data are shown here for comparison only and are described in more detail in (29). Shown are the concentrations of Aroclor 1221, the CFE as viability parameter, and reversion frequencies. Averages of three experiments are shown.

<sup>b</sup> CFE, colony-forming efficiency.
TCDD is their binding to the AH receptor (4, 12, 13). Upon TCDD binding, the AH receptor translocates to the nucleus, where it dimerizes with the AH receptor nuclear translocator. This ligand-receptor-translocator complex enhances transcription of certain genes, such as genes encoding P450 CYP1A1, through recognition of specific xenobiotic response elements located upstream of target genes (43). The regulation of these target genes is thought to cause the toxic effects.

In an attempt to determine the importance of the AH receptor in TCDD toxicity, Fernandez-Salgueiro et al. (44) constructed AH receptor-deficient mice by homologous recombination. Although half of these mice died shortly after birth, the remaining mice showed fewer lymphocytes, a 50% reduction in sizes of livers, and a tolerance to TCDD doses 10-fold higher than the dose that is toxic to wild-type mice (45). These knockout mice, however, still showed some liver and lung damage that was induced by TCDD.

On the other hand, there are data that strongly argue against a universal involvement of the AH receptor and the AH locus in TCDD toxicity (46). First, 3-methylcholanthrene competes with TCDD for AH receptor binding and induces the same microsomal fraction, but it does not produce the same signs of toxicity. Second, the binding capacities and affinities for the AH receptor between different rodent species and between different strains of rats are fairly similar, despite a difference in toxicity of more than three orders of magnitude (46). Collectively, these results suggest that TCDD may affect multiple cellular pathways. In particular, initiation of carcinogenesis may be difficult to explain by AHH induction because PCBs and TCDD are metabolized to compounds that show a reduced toxicity (for reviews see Refs. 4 and 46).

The mouse strain C57BL/6J that we used in our study has been shown to be responsive to induction of CYP1A1 and associated AHH activity in tissues or cells that are exposed to polycyclic aromatic hydrocarbons (47). Thus, we measured levels of induction of AHH by the PCBs and TCDD in mouse embryos to determine whether this responsiveness is correlated with the induction of DEL recombination. TCDD and, to a lesser degree, Aroclor 1260, induced expression of the AHH in livers of mice. In the embryos, however, where induced DEL recombination occurred, no induction of the expression of the AHH was detected. Others found induction of AHH activity by TCDD in embryonic culture from 15–19-day-old C57BL/6J embryos (47) and induction of CYP1A-specific mRNA by 3-methylcholanthrene in the embryo proper at gestational days 12.5 and 14.5 but not at days 5–10.5 (48) and by TCDD at days 14 and 20 but not at day 7 (49). Thus, it does not seem surprising that we did not detect any measurable increase of CYP1A-associated EROD activity in embryos treated with TCDD at day 10 after conception. In addition, our dose of 2.25 μg/kg TCDD was 44-fold lower than the dose of 100 μg/kg used in the Kimura et al. study (49). In summary, our results suggest that the PCB- and TCDD-induced DEL events do not correlate with the induction of the AHH and that the two biological activities are independent of each other.

Potency of Carcinogenicity versus DEL Recombination in Mice. The mice in our experiments were treated with single acute doses of chemicals, and so, it is difficult to compare these doses, per se, with doses used in long-term animal bioassays in which mice are treated 5 days a week for 2 years; however, relative potencies may be compared. Our data indicate similar levels of induction of DEL recombination (about 25% of animals showing spots) with 500 mg/kg Aroclor 1260 and 2.25 μg/kg TCDD, which manifests as a difference of 2 × 10^5 in potency. This finding correlates with results for carcinogenesis in the rat, in which 1 mg/kg/day Aroclor 1260 and 6.67 × 10^{-6} mg/kg/day TCDD both cause 50% of rats to develop tumors, which is also a difference of about 2 × 10^5 in potency (9). The same approximate difference is true for Aroclor 1254 and TCDD in mice (9). In summary, the relative potencies between Aroclor 1260 and TCDD for induction of DNA DELs in our experiments is similar to that found for carcinogenicity.

Polychlorinated Aromatic Hydrocarbons Are Embryotoxic. Because our protocol involves the exposure of pregnant dams to chemicals and a genetic end point caused by DEL events in embryos, the results of this study are relevant to embryotoxicity. McNulty (50) has shown that pregnant monkeys are very sensitive to TCDD; a single 1-μg/kg dose caused abortion in 13 of 16 animals. In addition, TCDD is a potent teratogen in the mouse and causes cleft palate and kidney anomalies at levels of >1 μg/kg/day during days 6–15 (51). Because TCDD has a half-life of 11 days in C57BL16 mice (46), the accumulated dose of >1 μg/kg/day for 10 days is much higher than the single acute dose of 2.25 μg/kg, given at day 10 postconception, which elevated the frequency of DNA DELs in the embryos in our experiments.

In a review of seven different rat studies, PCB mixtures with 60% chlorination consistently resulted in a high incidence of liver tumors, whereas mixtures with 42% chlorination showed no statistically significant tumor induction (52). Therefore, Moore et al. (52) proposed that congeners of lower chlorination content may have much lower potency for carcinogenicity than do congeners of higher chlorination. Nevertheless, Aroclor 1016 (16% chlorination) induced neonatal toxicity in monkeys (53), indicating that newborns may be more sensitive to the toxicity of congeners with lower chlorination content. Our results show that Aroclor 1260 (60% chlorination), at 500 mg/kg, induced p<sub>50</sub> reversions in 26% of offspring and Aroclor 1221 (21% chlorination), at the same dose, induced reversions in only 20% of offspring. However, the 1000-mg/kg dose of Aroclor 1221 caused p<sub>50</sub> reversions in 25% of offspring. These results indicate that the less chlorinated congener induced p<sub>50</sub> reversions, although the more chlorinated congener was 2-fold more potent. Taking into account our results and embryotoxicity data, it seems likely that embryos may be sensitive to toxicity that is induced by congeners with lower chlorination content.

Potential Mechanism for Genotoxicity of Polychlorinated Aromatic Hydrocarbons. The most likely mechanism for induced DEL recombination in yeast is the single-strand annealing pathway, which uses a double strand break to initiate recombination (Fig. 1B; Ref. 54). In fact, it has been shown that DEL recombination can be induced by a specific DNA double-strand break in the sequence between a gene duplication (55, 56). Additionally, a yeast DNA ligase temperature-sensitive mutant, which has increased levels of DNA strand breaks, shows a 100-fold increased level of DEL recombination (57). The hypothesis that polychlorinated aromatic hydrocarbons may induce DNA strand breaks might also explain their status in the Salmonella assay. DNA strand breaks may induce DELs or forward mutations, but they are less likely to induce specific reversions in the Salmonella assay.

Oxidative stress caused by polychlorinated aromatic hydrocarbons may explain a possible accumulation of DNA strand breaks. DNA strand breaks may be induced by oxidative stress. Numerous studies of TCDD exemplify oxidation activities, such as enhanced lipid peroxidation (reviewed in Refs. 46 and 58), or inhibition of antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase [reviewed in Stohs (58)]. The main sources of TCDD-generated reactive oxygen species may be microsomes, mitochondria, and phagocytes (reviewed in Ref. 46). Our laboratory has previously shown that oxidative mutagens are powerful inducers of DEL recombination in yeast (34) and that cadmium, a DEL assay-positive but Salmonella assay-negative carcinogen, induces free radicals in yeast (59). Thus, it might be interesting to test whether oxidative stress plays any role in the genotoxic activity of polychlorinated aromatic hydrocarbons.

In summary, the results presented in this paper clearly demonstrate a genotoxic activity of PCBs in vitro and in vivo in three different
organisms. Because genetic instability and DEls may be mechanistically involved in carcinogenesis, these results may encourage further research to determine whether such genotoxic mechanisms may be useful for cancer risk assessment of polychlorinated aromatic hydrocarbons.

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