Ploidy and Specific Karyotypic Changes during Promotion with Phenobarbital, 2,5,2',5'-Tetrachlorobiphenyl, and/or 3,4,3',4'-Tetrachlorobiphenyl in Rat Liver

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ABSTRACT

Polychlorinated biphenyls are a group of industrial chemicals that are widely distributed in the environment. Since these compounds occur as mixtures, studies of their possible interactive effects are important. In order to determine whether an interaction of 2,5,2',5'-tetrachlorobiphenyl (TCB) with 3,4,3',4'-TCB occurs during multistage hepatocarcinogenesis in vivo, like that previously observed in lymphocyes in vitro (L. M. Sargent et al., Mutat. Res., 224: 79-88, 1989), we exposed rats to a single initiating dose of diethylnitrosamine (DEN), 10 mg/kg after a 70% partial hepatectomy, and subsequently to 0.1 ppm 3,4,3',4'-TCB and/or 10 ppm 2,5,2',5'-TCB in the diet for 1 year. Administration of each of the TCBs alone after DEN initiation resulted in a low incidence of chromosomal damage in hepatocytes; but when the two were given together after DEN initiation, there was no more additive effect on this parameter at both 7 and 12 months which was highly significant. Administration of the TCBs alone or in combination in the absence of DEN initiation also resulted in chromosomal damage, approaching that seen in livers of animals initiated with DEN when sacrificed at 12 months. In animals receiving 0.05% phenobarbital for a 12-month period after initiation with DEN, a significant degree of chromosomal breakage and fragment formation occurred both in hepatocytes expressing the ectoenzyme γ-glutamyltranspeptidase (GGT) and in those that were GGT negative. However, the GGT-negative cells showed a significantly lower incidence of chromosomal damage than the GGT-positive hepatocytes. Exposure to phenobarbital for 7 months after DEN initiation resulted in no significant chromosomal damage in hepatocytes, whether GGT positive or GGT negative. Some degree of specificity in chromosomal alterations was seen in hepatocytes of animals initiated with DEN and promoted either with a combination of TCBs or with phenobarbital. The most frequent alterations seen were a trisomy of chromosome 1 or of its long arm and a monosomy of chromosome 3 or its short arm. Some chromosome 7 aberrations were also seen. The highest frequency of specific aberrations occurred in hepatocytes from rats that also bore hepatocellular carcinomas, suggestive of the hypothesis that genes involved in the development of hepatic carcinoma may reside in chromosome 1 and/or 3 of the rat.

INTRODUCTION

PCBs are found in a number of industrial chemicals that have been widely distributed in the environment. PCB mixtures used in industry contain many PCB congeners, a number of which exhibit both toxicity and carcinogenicity in mammalian systems (1, 2). Their toxicity is characterized by increased mortality, edema, hyperkeratosis, thymic involution, and hepatotoxicity in several rodent species (1, 3-5). The most toxic of the PCB congeners are planar, bind with high affinity to the Ah receptor, and induce some of the P-448- and P-450-dependent mixed function oxidases (5, 6). The planar congener, 3,4,3',4'-TCB, and the nonplanar isomer, 2,5,2',5'-TCB, which are found in the major Aroclor mixtures 1254, 1242, and 1248, were chosen to examine the individual and the combined effects of a planar and a nonplanar PCB in vitro and in vivo. The two TCB congeners differ in toxicity and binding affinity for the Ah receptor and in their potencies as carcinogens (5-10); however, the systemic clearance and the volume of distribution of 3,4,3',4'-TCB and 2,5,2',5'-TCB are essentially the same (10).

The development of neoplasia is a highly complex, multistage process that has been examined in several model systems (11-14). The liver contains the primary target cell population for carcinogenesis induced by PCBs. Previous studies have indicated that PCBs exhibit promoting activity during hepatocarcinogenesis in the rat (5, 7-9). The potency of TCBs as promoting agents correlates with their affinity for the Ah receptor (8, 9). Since PCBs usually occur in the environment as components of mixtures, we thought it important to determine the combined effects of 3,4,3',4'-TCB and 2,5,2',5'-TCB on the stages of promotion and progression in multistage hepatocarcinogenesis in the rat. We have already reported on the effects of these agents singly and in combination on the development of AHF and neoplasms during hepatocarcinogenesis in the rat (15). In view of the acknowledged importance of chromosomal alterations as a hallmark of the stage of progression in multistage carcinogenesis and the indication that PCB combinations are clastogenic for lymphocytes in vitro (16), the studies described herein were undertaken.

MATERIALS AND METHODS

Chemicals. DEN was purchased from the Eastman Kodak Co. (Rochester, NY). The purified diet was obtained from Teklad (Madison, WI). The 2,5,2',5'-tetrachlorobiphenyl was a gift from Dr. James A. Miller of the McArdle Laboratory; the supplier of the 3,4,3',4'-tetrachlorobiphenyl was Chemical Scientific (Lenexa, KS). Gurr’s buffer tablets and Giemsa stain R-66 were purchased from Biomedical Specialties (Santa Monica, CA). The Colcemid was obtained from Gibco Corp. (Grand Island, NY).

Animals and Treatment. Female Sprague-Dawley rats (90 g; Harlan Sprague Dawley, Madison, WI) were housed in wire mesh cages and fed a powdered Pariza diet (18). The animals were initiated and promoted according to a protocol previously described (17, 18). After a 70% partial hepatectomy, one-half of the rats were intubated with diethylnitrosamine in trioctanoin (10 mg/kg). One week later, animals were randomly assigned to the groups outlined in Fig. 1 (15). TCBs were dissolved in methylene chloride and added to the dry feed. After thorough mixing, the solvent was evaporated in a hood for 24 h. Owing to the high vapor pressure of methylene chloride, all of the solvent had disappeared by 24 h. After evaporation, 1 kg of the TCB stock was mixed with 10 kg of control feed, further diluting any residual methylene chloride. Animals were placed on diets containing various concentrations of 2,5,2',5'-TCB, 0.05% phenobarbital, and 3,4,3',4'-TCB.
perfusion was increased by 5 ml/min to allow a more thorough disso-
association of liver nodules. Cells isolated from the perfusion were purified
by Percoll isodensity centrifugation and cultured at a density of 2.6 ×
10⁶ cells/cm² on collagen-coated plates, as outlined by Xu et al. (19).
The hepatocytes isolated from the DEN plus PB-treated animals were
separated into GGT+ and GGT- populations by the methods of
Hanigan and Pitot (20), with modifications by Xu et al. (19). Cultures
were randomly selected for the examination of chromosomes by elec-
tron microscopy (21) or by phase contrast light microscopy.

Chromosome Analysis. After 47 h of culture, 0.1 μg Colcemid/ml
was added to each dish, and the cells were incubated for 5 h longer.
The hepatocytes were then removed from the dish with 6 ml of 0.1%
collagenase for 10 min at 37°C. Cells were then harvested by 0.075 m
KCl hypotonic fixation for 8.5 min, followed by two 3:1 methanol/acetic
acid washes. After 24 h storage in a −20°C freezer, slides were prepared
by standard methods (22), and metaphase spreads of good morphology
were chosen for cytogenetic analysis. The perfusions and the metaphase
analyses were also performed on all groups after 12 months of treatment
(n = 5), and both banded and unbanded slides were prepared as
described above.

The frozen liver slices were sectioned at 5 μm in a cryostat and
stained by the methods described by Hendrich et al. (23) for GGT,
placental isozymes of glutathione S-transferase, canalicular ATPase,
and glucose-6-phosphatase. The AHF were quantitated by the proce-
dure of Campbell et al. (24, 25).

Statistics. The data were analyzed by nonparametric χ² statistics.
For additivity testing, Steel and Torrie’s (26) χ² test for additivity was
used.

RESULTS

Distribution of Ploidy at 7 Months. The hepatocytes isolated
from control rat livers were predominantly tetraploid, with only
a 4% level of aneuploidy. As shown in Fig. 2, GGT-negative
hepatocytes isolated from rats treated with DEN plus PB for 7
months according to the protocol first described in our labora-
tory (27) were also predominantly tetraploid. By contrast, the
GGT-positive hepatocytes isolated from AHF at 7 months had
a diploid karyotype. These hepatocytes exhibited a low level of
aneuploidy, statistically the same as that of the control.

After 7 months of treatment, the hepatocytes isolated from the
DEN plus 2,5,2',5'-TCB group (group 4) or from the DEN
plus 3,4,3',4'-TCB (group 6) were predominantly tetraploid
with 10% aneuploidy (Fig. 3). The level of aneuploidy with the
single congeners was elevated but was not statistically different

Sacrifices were performed after 7 and 12 months of treatment. At the
time of sacrifice rats were anesthetized with Nembutal. Part of the
triangulate lobe of the liver was tied off, and two slices of this lobe were
frozen immediately on dry ice. Other sections of this lobe were pre-
served in 10% buffered formalin for histopathological study. The re-
mainder of the liver was perfused according to the methods
described by Xu et al. (19), with the exception that the flow rate of the
from that of the control. When hepatocytes were isolated from animals treated concurrently with both TCBs (group 8), the hepatocytes were mostly diploid. The 30% aneuploidy of this group was statistically greater than additive when compared with the sum of such changes in hepatocytes of rats in groups 4, 6, and 8, as illustrated in the histogram in Fig. 3. The increase in aneuploidy was greatest in the subtetraploid and hyperdiploid (45–54) populations of group 8 compared with other groups.

Chromosomal Breakage at 7 Months. The levels of chromosomal breakage of groups 4, 6, and 12 were statistically the same as that of the untreated control group (Table 1). When both PCBs were administered after DEN, however, the level of chromosomal breakage was dramatically elevated to a level much greater than would be predicted by an additive model ($P < 0.001$). A significant number of random translocations was observed in hepatocytes from rats in groups 4, 6, and 8 ($P < 0.001$). The highest percentage of translocations was in group 8.

Electron micrographs of mitotic figures in cultures from rats treated with both TCBs demonstrated an abnormally high number of metaphases with chromosomes that had failed to align at the metaphase plate during mitosis (Fig. 4).

Distribution of Ploidy at 12 Months. After 12 months of treatment, the hepatocytes from the 2,5,2',5'-TCB and the 3,4,3',4'-TCB groups (groups 3 and 5 in Fig. 1) remained predominantly tetraploid, with 10% aneuploidy (Fig. 5). When initiation with DEN was included in the protocol (groups 4 and 6), the aneuploidy was significantly increased ($P < 0.01$) (Fig. 6). When both TCBs were administered concurrently with (group 8) or without (group 7) the inclusion of DEN in the protocol, the chromosome number was reduced, the aneuploidy was increased to 50%, and a large subtetraploid population emerged. The hepatocytes cultured from the livers of the DEN plus PB (group 12) animals also had an overall decrease in ploidy from that of hepatocytes treated with DEN only (Fig. 6), with an increase in the subtetraploid population as seen in Fig. 7.

Chromosomal Breakage at 12 Months. Although lower than at 7 months, the chromosomal breakage of hepatocytes from the DEN plus both TCBs (group 8 in Fig. 1) remained elevated above control levels at 12 months of treatment, as seen in Table 2. The numbers of chromosomal breakages and fragments were significantly elevated in the DEN plus PB group (group 12) compared with animals treated on this protocol for 7 months. All of the control groups exhibited essentially no differences in the level of breakage compared with normal adult rat hepatocytes.

### Table 1 Chromosome damage after 7-month exposure to polychlorinated biphenyls

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chromosome damage</th>
<th>Fragments</th>
<th>Translocations</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEN + 3,4,3',4'-TCB and 2,5,2',5'-TCB (8)</td>
<td>45.5 ± 3.0*</td>
<td>11.0 ± 3.0</td>
<td>16.0 ± 2.0</td>
</tr>
<tr>
<td>DEN + 3,4,3',4'-TCB only (6)</td>
<td>6.0 ± 2.0</td>
<td>2.0 ± 1.0</td>
<td>6.0 ± 3.0</td>
</tr>
<tr>
<td>DEN + 2,5,2',5'-TCB only (4)</td>
<td>6.0 ± 2.0</td>
<td>2.0 ± 1.0</td>
<td>3.0 ± 2.0</td>
</tr>
<tr>
<td>DEN + phenobarbital (12)</td>
<td>4.0 ± 2.0</td>
<td>2.0 ± 1.0</td>
<td>3.0 ± 2.0</td>
</tr>
<tr>
<td>Control (1)</td>
<td>4.0 ± 2.0</td>
<td>1.0 ± 0.0</td>
<td>4.0 ± 2.0</td>
</tr>
</tbody>
</table>

* Values are given as the average percentage of cells with aberrations from each of five rats on the protocol for 7 months. A minimum of 100 metaphases were prepared and counted as described in "Materials and Methods." In the DEN plus phenobarbital group (group 12), GGT+ and GGT− cells were separated by the method of Hanigan and Pilat (20). Statistical significance was determined by $x^2$ analysis.

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**Fig. 4.** Electron micrograph of a dividing hepatocyte prepared from a Sprague-Dawley rat following 10 mg DEN/kg and 7 months of treatment with 0.1 ppm 3,4,3',4'-TCB and 10 ppm 2,5,2',5'-TCB (group 8, Fig. 1). This cell in metaphase demonstrates chromatin that has not aligned (arrow) at the metaphase plate during mitosis. × 3900.

**Fig. 5.** Histogram of the percentage of hepatocytes in each ploidy group, analyzed from five animals in each group treated with TCBs as outlined in Fig. 1. One week after a 70% partial hepatectomy, animals were treated with 0.05% phenobarbital (group 11), 0.1 ppm 3,4,3',4'-TCB (group 5), 10 ppm 2,5,2',5'-TCB (group 3), or a combination of 0.1 ppm 3,4,3',4' and 10 ppm 2,5,2',5' (group 7).

**Fig. 6.** Histogram of the percentage of hepatocytes in each ploidy group, analyzed from five animals in each group treated with TCBs as outlined in Fig. 1. After a 70% partial hepatectomy, 50% of the animals received 10 mg DEN/kg. After 1 week, animals were treated with 0.1 ppm 3,4,3',4'-TCB (group 6), or with 10 ppm 2,5,2',5'-TCB (group 4), or with 0.1 ppm 3,4,3',4'-TCB and 10 ppm 2,5,2',5'-TCB (group 8) for 12 months. One hundred karyotypes were analyzed from each animal by procedures described in "Materials and Methods."
When banded slides were analyzed, the GGT-positive hepatocytes from the DEN plus PB group (group 12), as well as the hepatocytes from rats treated with DEN plus both TCBs (group 8), demonstrated a number of consistent chromosomal aberrations, as shown in Table 3. There was a consistent increase in the length of the long arm of chromosome number 1, multiple copies of the first chromosome, missing No. 3 chromosomes, and a deletion of the short arm of the third chromosome (Figs. 8, 9, and 10). Sixty % of the No. 1 chromosomes had an extra dark band between band q12 and band q31, while the others had an extended light band at band q12 to q43. The smallest region that was observed to be enhanced was between band q33 and band q43 of chromosome 1 (Fig. 11). In addition, chromosomes 4 and 7 were often rearranged. Examples of these changes can be seen in Figs. 8 and 9.

The most frequent chromosome changes seen in rats treated for 12 months with DEN plus both TCBs or DEN plus PB were from groups that had demonstrated numerous neoplastic nodules with cellular atypia (15). Five of five animals from the DEN plus PB group (group 12) had hepatocellular carcinoma as well as numerous neoplastic nodules with cellular atypia. Only one of the DEN plus both TCBs group (group 8) developed hepatocellular carcinoma. These results contrast with the control group, which did not develop neoplastic nodules with cellular atypia nor hepatocellular carcinoma.

DISCUSSION

The data described herein indicate that chronic administration of both 2,5,2',5'- and 3,4,3',4'-TCB for 7 months results in superadditive chromosomal damage (Table 1), aneuploidy, and a marked increase of diploid hepatocytes (Fig. 3) compared with chronic administration of either TCB alone. These findings give evidence for the synergistic clastogenic activity of these two congeners. The elevated percentage of hepatocytes that were diploid compared with normal liver indicates that a significant level of promotion had occurred (28–30) even after only 7 months of promotion. These results contrast with the low level of chromosomal damage, aneuploidy, and number of diploid hepatocytes in livers from the animals treated with the basal diet, phenobarbital only, or a single TCB congener.

The nonplanar congeners of PCBs have a relatively low affinity for the Ah receptor and induce cytochrome P-450 b and e (2B1, 2B2). PCB toxicity is currently evaluated as either total PCB level or as total Ah receptor equivalents (7). Ah receptor equivalents are based on the amount of competitive displacement of [3H]2,3,7,8-tetrachlorodibenzo-p-dioxin from the rat Ah receptor protein. The toxicity of Aroclor mixtures is thought to be due to the three most toxic congeners with the highest affinity for the Ah receptor: 3,4,3',4'-tetrachlorobiphenyl, 3,4,5,3',4',5'-hexachlorobiphenyl, and 3,4,5,3',4'-pentachlorobiphenyl (5, 7). The two TCB congeners used in this study differ in toxicity and binding affinity for the Ah receptor (5). The planar 3,4,3',4'-TCB was chosen for this study because of

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**Table 2 Percentage of cells with chromosome damage after a 12-month exposure to polychlorinated biphenyls or phenobarbital**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chromosome breakage</th>
<th>Fragments</th>
<th>Translocations and other chromosomal aberrations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEN + PB (12)</td>
<td>15.8 ± 5.0</td>
<td>3.0 ± 3.0</td>
<td>25.0 ± 3.0</td>
</tr>
<tr>
<td>GGT+</td>
<td>22.0 ± 2.0</td>
<td>7.3 ± 2.5</td>
<td>28.0 ± 2.0</td>
</tr>
<tr>
<td>GGT–</td>
<td>9.0 ± 2.0</td>
<td>5.0 ± 1.0</td>
<td>10.0 ± 2.0</td>
</tr>
<tr>
<td>DEN + 3,4,3',4'-TCB only (6)</td>
<td>7.0 ± 2.0</td>
<td>2.0 ± 1.0</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>DEN + 2,5,2',5'-TCB only (4)</td>
<td>7.0 ± 2.0</td>
<td>2.0 ± 1.0</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>DEN + 3,4,3',4'-TCB and 2,5,2',5'-TCB (8)</td>
<td>22.7 ± 7.0</td>
<td>6.5 ± 2.0</td>
<td>14.0 ± 3.0</td>
</tr>
<tr>
<td>DEN + 3,4,3',4'-TCB and 2,5,2',5'-TCB (7)</td>
<td>16.0 ± 1.0</td>
<td>2.5 ± 1.0</td>
<td>7.5 ± 2.5</td>
</tr>
<tr>
<td>DEN (2)</td>
<td>6.0 ± 5.0</td>
<td>1.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Control (1)</td>
<td>5.0 ± 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEN + 3,4,3',4'-TCB only (6)</td>
<td>7.0 ± 4.0</td>
<td>1.0 ± 1.0</td>
<td>7.0 ± 1.0</td>
</tr>
</tbody>
</table>

* Based on percentage of affected cells from studying 100 cells from each of 5 animals treated as shown.

* Values are given as the average percentage of cells with aberrations after 12 months of treatment according to various protocols. The diets for the protocols and groups are outlined in Fig. 1. Five animals were sacrificed per group, and at least 100 karyotypes were analyzed per animal. GGT+ and GGT- hepatocytes were separated by the method of Hanigan and Pitot (20), modified by Xu et al. (19). Statistical significance was determined by x² analysis.

* Numbers in parentheses, groups in Fig. 1.

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**Table 3 Specific chromosome damage in hepatocytes from rats subjected to 12 months of treatment with DEN plus phenobarbital or DEN plus 3,4,3',4'- and 2,5,2',5'-tetrachlorobiphenyl**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of cells with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chromosome aberrations*</td>
</tr>
<tr>
<td>DEN + 3,4,3',4'+ and 2,5,2',5'-TCBs (8)*</td>
<td>4.4 ± 2.0</td>
</tr>
<tr>
<td>DEN + phenobarbital (12)</td>
<td>2.0 ± 1.0</td>
</tr>
</tbody>
</table>

* Values are the percentage of all hepatocytes examined that demonstrated these aberrations. Five animals were sacrificed per group, and at least 50 banded metaphase spreads were analyzed from each animal. See legend of Table 2 for further details.

* Numbers in parentheses, groups in Fig. 1.
its high toxicity and strong affinity for the Ah receptor. The 2,5,2',5' congener has a low affinity for the Ah receptor, is nontoxic, and is a phenobarbital type inducer. The concentrations of the two TCB congeners studied here were based on the ratio in which they exist in Aroclor 1254. In addition, the doses administered were low, environmentally relevant concentrations to allow for a more realistic estimation of the potency of the combination in hepatocarcinogenesis.

At 12 months of treatment with the combination of 2,5,2',5'-TCB and 3,4,3',4'-TCB, chromosome aberrations including chromosome damage and aneuploidy, as well as focal malignant neoplasia, were evident. In addition, neoplastic nodules were found in livers of rats on the 12-month DEN plus both TCBs regimen (group 8 in Fig. 1) (15). Therefore, after 12 months of treatment, the combination of 2,5,2',5'-TCB and 3,4,3',4'-TCB may act synergistically in the production of chromosome damage and aneuploidy as well as in the induction of malignant neoplasia ($P < 0.001$).

The interaction of the 3,4,3',4' and the 2,5,2',5' congeners observed in rat hepatocytes in vivo may be due in part to the metabolic activation of the 2,5,2',5' congener. Since a low background of induction of the P-450 enzymes was exhibited by livers from animals maintained on the purified Pariza diet, we chose to use this diet when PCBs were administered (23). The 3,4,3',4' congener induces the cytochrome P-450 c and d forms (1A1, 1A2) (6, 7), which in turn may increase the formation of the epoxide of the 2,5,2',5' congener. Congeners with two unsubstituted vicinal positions (at least one of which is in the para position) are metabolized through an epoxide intermediate (5, 8). The epoxide intermediate is more toxic than the parent compound when administered to preweanling mice or to V-79 and HeLa cells (31–34). In addition, epoxides of PCBs can alkylate DNA and other tissue macromolecules (35).

The most interesting changes seen in karyotypes of hepatocytes in this study were the specific chromosome aberrations that are common to the 12-month DEN plus PB and the DEN plus both TCBs regimen (groups 12 and 8, Fig. 1, respectively). The enhanced light band and the extra dark band that were observed on chromosome 1 (q33 to q43, Fig. 11) are near the site assigned to the Ha-ras gene (36). Experiments are in progress to examine a possible changed expression of Ha-ras.

Aberrations of rat chromosome 1 have been reported in a number of transformed cell lines (37–42). Extra copies or structural abnormalities of chromosome 1 have been reported in rat tracheal epithelial cells and in rat sarcomas and carcinomas transformed in vitro by carcinogens (38–40). The Ha-ras gene has been mapped to band q41 to q42 on chromosome 1. Trisomy of the chromosomes bearing the Ha-ras has been shown in other experimental carcinogenesis models (43, 44). The frequencies of the chromosome 1 aberrations and of the
EFFECT OF PCBs ON PLOIDY AND KARYOTYPE IN RAT LIVER

RATTUS NORVEGICUS

Fig. 9. Karyotype of a GGT+ hepatocyte from the liver of a rat subjected to 10 mg DEN/kg followed by 12 months of treatment with phenobarbital. The karyotype demonstrates the deletion of the short arm of chromosome 3; this deletion was observed with this treatment at a relatively high frequency.

Fig. 10. A summary of the most frequent deletions of chromosome 3 in hepatocytes isolated from rats initiated with DEN and promoted with phenobarbital (group 12) or both tetrachlorobiphenyls (group 8) for 12 months.

Fig. 11. A summary of the most frequent duplications seen with chromosome number 1 in hepatocytes isolated from rats initiated with DEN and promoted with phenobarbital (group 12) or both tetrachlorobiphenyls (group 8) for 12 months.
multiple copies of chromosome 1 described in this study were found to correlate with the loss of chromosome 3 or with the deletion of the short arm of chromosome 3.

Previous studies have found the transformation of rat epithelial cells after transfection with Ha-ras and myc to be correlated with the deletion of the short arm of chromosome 3. The mechanism of this linkage is not known but may involve the deletion of a suppressor gene (45). Furthermore, Wolman et al. (46) observed the deletion of 3p in the differentiated Morris hepatoma cell line 7800. Monosomy of 3 was also reported in another Morris hepatoma, the 7288 (47). This same deletion has been noted in primary rat hepatocellular carcinoma that is differentiated (48). Loss of specific chromosome segments has been reported in human hepatocellular carcinoma, including the loss of 4q, 16q, lip, 17p, as well as 13q (49–51). The observation that several specific chromosome aberrations are common to three separate regimens in which neoplastic nodules formed may indicate that these chromosomal changes are important in the development of these lesions and in the progression to carcinogenesis. In studies reported here, the highest frequency of these specific aberrations was seen in hepatocytes isolated from livers with hepatocellular carcinoma as well as numerous neoplastic nodules.

An increased expression of myc and Ha-ras was observed in foci and neoplastic nodules in rat livers after 12 months of treatment with the protocol of 10 mg DEN/kg as initiator and the continuous administration of TCDD (0.1 µg/kg/day) (52). An increase in the expression of the p21-Ha-ras gene product has also been noted in human hepatocellular carcinomas (53). Amplification of both Ha-ras and myc has been observed in primary rat tumors induced with 2-amino-3-methylimidazo[4,5-f] quinoline (54), with 3′-methyl-4-dimethylaminoazobenzene (55) and with choline-deficient diet (56). However, Beer et al. (57) did not find Ha-ras to be elevated in GGT+ hepatocytes isolated from rats treated with the Pitot model for 6 or 11 months. By contrast, the animals in the experiment reported herein were treated for 12 months, similar to the investigation by Sills and Sleight (52). In addition, the increased flow rate of the perfusion increased the dissociation of the neoplastic nodules that were evident in the livers. This increased dissociation of the neoplastic nodules is important because these nodular cells may contain cells from foci-in-foci (18) that may express gene products not expressed in the GGT+ cells during early stages of promotion.

A significant number of aberrations of chromosome 7 were also observed in our studies. Although these aberrations were statistically significant, the frequency of chromosome 7 aberrations was lower than that of the aberrations of chromosomes 1 and 3. Similar aberrations of chromosome 7 have been observed in Morris hepatoma 7288 cells (47). These chromosome changes are interesting because the protooncogene myc has been mapped to chromosome 7 (58). The coexpression of c-Ha-ras and c-myc has been reported to be synergistic in the production of murine mammary tumors (59). Because nonmalignant tissue surrounding mammary epithelium also expresses c-myc and c-Ha-ras, it cannot be concluded that these two oncogenes are sufficient for the induction of mammary neoplasia. A third event may be required for the progression to neoplasia. Several genetic models of carcinogenesis (60–62) indicate that progression to carcinoma involves the activation of protooncogenes and an additional event involving the deletion or inactivation of a suppressor gene.

Rat chromosome number 1 has regions that are syntenic with human chromosome 11 (63). Rearrangements of human chromosome 11 have been reported in human hepatocellular carcinoma (49). Two suppressor genes have been mapped to human chromosome 11 (64–66). The inserted dark band on chromosome 1 that was observed in this investigation was near the recently mapped Ha-ras gene. It is possible that an insertion at this location could activate the nearby Ha-ras gene or modify the activity of the putative suppressor gene linked to the Inv gene that is also located in this same chromosome region (36, 44).

Our results demonstrate an in vivo interaction of low doses of two PCBs in the liver. The development of neoplastic nodules with this combination protocol, as well as the DEN plus PB regimen, correlated with specific chromosome rearrangements. These specific rearrangements increase in frequency with the development of carcinoma. This would indicate that the nodules with these specific chromosome rearrangements contain hepatocytes that progress to hepatocellular carcinoma.

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Ploidy and Specific Karyotypic Changes during Promotion with Phenobarbital, 2,5,2′,5′-Tetrachlorobiphenyl, and/or 3,4,3′,4′-Tetrachlorobiphenyl in Rat Liver

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