The effects of a technical polychlorinated biphenyl (PCB) mixture, Aroclor 1254, on the transplantability and growth of the Walker 256 carcinosarcoma were studied in rats. The animals received PCB either as a constituent of their diet, at levels ranging from 5 to 800 ppm, or as repeated intraperitoneal injections, at levels ranging from 50 to 400 mg/kg. Following the intramuscular injection of variable numbers of tumor cells, several parameters of solid tumor growth were examined, including the tumor growth rate, latent period, incidence of takes and regressions, as well as host survival time. An in vivo assessment of the direct effects of PCB on the tumor cells was also investigated, utilizing the ascitic variant of the Walker tumor.

Exposure of the host to PCB resulted in a dose-dependent inhibition of tumor growth, as measured by the weight of the tumor after a specified growth period. The magni-
tude of tumor weight inhibition appeared to be independent of the number of tumor cells injected and independent of the route of PCB exposure. With the implantation of small numbers of tumor cells \((10^3)\), the number of tumor takes was dose-dependently decreased, and the latent period of the tumors that did develop was significantly increased. With the implantation of large numbers of tumor cells \((10^7)\), a significant increase in host survival time was observed, which was accompanied by an increased incidence of tumor regression. Preconditioning of the host with PCB for five days prior to tumor inoculation was equally effective in inhibiting tumor growth as was treatment with PCB during the first five days after tumor injection. If PCB exposure was delayed until after the tumor was established, it was less effective in inhibiting the growth of the tumor. There was some evidence for a direct cytotoxic effect of PCB on the tumor cells, although it was not considered sufficient to fully account for the observed inhibition of tumor growth. Two additional hypotheses are considered, relative to the antigluconeogenic and immunosuppressive activities of PCB, which may account for the antitumor effect of PCB exposure.
THE TRANSPLANTABILITY AND GROWTH OF THE WALKER 256 CARCINOSARCOMA IN RATS EXPOSED TO A POLYCHLORINATED BIPHENYL, AROCLOR 1254

by

Nancy Isaacson Kerkvliet

A THESIS submitted to Oregon State University in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY June 1977
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THE TRANSPLANTABILITY AND GROWTH
OF THE WALKER 256 CARCINOSARCOMA IN RATS
EXPOSED TO A POLYCHLORINATED BIPHENYL, AROCLOR 1254

INTRODUCTION

General Remarks

Polychlorinated biphenyls (PCBs) comprise a class of chlorinated organic compounds that have experienced wide industrial usage since production first began in 1929. Owing to their extreme stability under a variety of conditions - their nonflammability, resistance to oxidation and hydrolysis, and water insolubility - PCBs have been amenable to broad application in industry as lubricants and hydraulic fluids and as heat transfer agents in electrical capacitors and transformers. They have also been used in such diverse products as paints, plastics, asphalt, rubber, printer's ink and pesticide formulations, lending flexibility, stability and/or adhesiveness to these products (Gustafson, 1970; Peakall and Lincer, 1970; Hammond, 1972; Nisbet and Sarofim, 1972). However, the properties that make PCBs so useful industrially are the same properties that have caused PCBs to become ubiquitous components of the global environment (Risebrough et al., 1968). Their overall inertness, water insolubility and lipophilic tendencies together contribute to their persistence in the
environment as well as their accumulation in animal tissue and magnification in food chains. Since the inevitable last link in the food chain is man herself, the possible hazardous effects of PCBs has recently attracted the concern and attention of scientists throughout the world.

Chemistry of PCBs

PCBs are manufactured in North America solely by the Monsanto Company under the trade name Aroclor. PCBs are also manufactured in Japan, Germany and France under the trade names Kanechlor, Phenoclor and Chlophen. The manufacturing process involves the uncontrolled chlorination of the biphenyl with anhydrous chlorine, using ferric chloride or iron filings as catalyst (Gustafson, 1970). The resulting technical products are complicated mixtures of chlorobiphenyls varying in degree of chlorination. The degree of chlorination is determined by measuring the specific gravity of the mixture, or, when the product is more viscous, the ball-and-ring softening point test is used (Gustafson, 1970). For Aroclor products, each mixture is identified by a four-digit number; the first two digits, 12, specify the compound as a polychlorinated biphenyl, and the last two digits, ranging from 21 to 68, the approximate percentage of chlorine in the mixture, by weight.
Although there are theoretically 209 different chlorinated biphenyls capable of being formed during the manufacturing process (as shown in Figure 1), the actual number that appear in any given technical mixture is much smaller. A near-complete identification of the isomers present in the various Aroclors has recently been accomplished by using a combination of gas-liquid chromatography, nuclear magnetic resonance and mass spectroscopy (Sissons and Welti, 1971; Stalling and Huckins, 1971; Hirwe et al., 1974). As might be expected, as the weight percentage of chlorine in the mixture increases, the proportion of more highly chlorinated biphenyl species also increases (Table 1).

No major components other than chlorinated biphenyls have been reported to be present in commercial PCB products. However, trace quantities of polychlorinated dibenzofurans, highly toxic in minute amounts, have been found in some PCB mixtures, particularly in those produced in foreign countries (Vos and Koeman, 1970; Vos et al., 1970; Curley et al., 1975). The presence of such highly toxic impurities, as well as the isomeric mixture of the commercial products, complicates the process of analyzing both the chemistry and toxicology of PCBs.
Figure 1. Structural formula for biphenyl and number of chlorinated biphenyl molecules that may theoretically be formed from the ten positions where chlorine can add. (From Cook, 1972)
Table 1. Empirical formula, percent chlorine and molecular composition of some Aroclor products. (Adapted from Hutzinger et al., 1974)

<table>
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<tr>
<th>Chlorobiphenyl Composition</th>
<th>Empirical Formula</th>
<th>Percent Chlorine</th>
<th>Presence (%) in Aroclor</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>( C_{12}H_{10} )</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_9Cl )</td>
<td>18.79</td>
<td>51, 3</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_8Cl_2 )</td>
<td>31.77</td>
<td>32, 13, 2</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_7Cl_3 )</td>
<td>41.30</td>
<td>4, 28, 18</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_6Cl_4 )</td>
<td>48.56</td>
<td>2, 30, 40, 11</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_5Cl_5 )</td>
<td>54.30</td>
<td>22, 36, 49, 12</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_4Cl_6 )</td>
<td>58.93</td>
<td>4, 4, 34, 38</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_3Cl_7 )</td>
<td>62.77</td>
<td>6, 41</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_2Cl_8 )</td>
<td>65.98</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>( C_{12}HCl_9 )</td>
<td>68.73</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( C_{12}H_{10} )</td>
<td>71.18</td>
<td>8</td>
</tr>
</tbody>
</table>
PCBs in the Environment

Despite the fact that PCBs have been in commercial use for over 40 years, it was not until the late 1960s that significant environmental contamination by these compounds was recognized. Although it is probable that PCB residues were widely present in samples analyzed for chlorinated hydrocarbon pesticides in the 1950s and 1960s, they were disregarded as "unknown interfering compounds" until they were identified as PCBs, independently, by Jensen and Widmark in 1966. Since that time, however, residues of PCBs have been detected, often at alarmingly high levels, in marine, fresh-water and terrestrial wildlife throughout the world, as well as in domestic livestock and poultry, and even in man himself (Risebrough et al., 1968; Jensen et al., 1969; Koeman et al., 1969; Biros et al., 1970; Duke et al., 1970; Fries, 1972; Kolbye, 1972; Price and Welch, 1972; Risebrough and deLappe, 1972; Veith, 1972; Yobs, 1972; Zitko and Choi, 1972; Greichus et al., 1973; Harvey et al., 1973; Clausen et al., 1974; Musial and Hutzinger, 1974; Smith et al., 1974; Johnson et al., 1975).

Once into the environment, PCBs persist for a very long time. The highly chlorinated species (pentachlorobiphenyls and above) are resistant to nearly every degra-
dative reaction that occurs in the environment, including oxidation, hydrolysis and microbial decomposition (Nisbet and Sarofim, 1972). Furthermore, being fat-soluble compounds, PCBs tend to accumulate in food chains and biological magnification can result in levels in top predators up to $10^9$ times higher than the levels found in ambient water (Risebrough et al., 1968; Jensen et al., 1969; Hansen et al., 1971; Nisbet and Sarofim, 1972; Stalling and Mayer, 1972). A typical food chain magnification scheme is shown in Table 2.

**Human Exposure**

Man's exposure to PCBs, aside from occupational exposure, occurs mainly through food, either directly as topmost predator or indirectly by accidental contamination of his food during processing or packaging. Data from two surveys of PCB residues in human adipose tissue in the United States indicated that 21-45% of the general population have PCB levels of 1.0 to 2.0 ppm. Approximately 9% of the general population have levels exceeding 2.0 ppm (Price and Welch, 1972; Yobs, 1972). Occasionally, however, samples with much higher levels are found. One autopsy case in Michigan, for example, contained approximately 175 ppm PCB in the fat, and no occupational exposure could be traced.
Table 2. PCB levels in Swedish marine organisms, 1965-1968. Figures given are the mean (ppm in extractable fat), range and sample size. (from Jensen et al., 1969)

<table>
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<th></th>
<th>Baltic Sea</th>
<th>Stockholm Archipelago</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Mussel</td>
<td>4.3</td>
<td>1.9-8.6</td>
</tr>
<tr>
<td>Herring</td>
<td>6.8</td>
<td>0.5-23</td>
</tr>
<tr>
<td>Seal</td>
<td>35.</td>
<td>16-44</td>
</tr>
<tr>
<td>Guillemot eggs</td>
<td>250.</td>
<td>140-360</td>
</tr>
<tr>
<td>Heron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White-tailed eagle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>breast muscle</td>
<td>14000</td>
<td>8400-17000</td>
</tr>
<tr>
<td>brain</td>
<td>910.</td>
<td>490-1500</td>
</tr>
<tr>
<td>eggs</td>
<td>540.</td>
<td>250-800</td>
</tr>
</tbody>
</table>
(Price and Welch, 1972). Two other samples contained 200 and 600 ppm PCB (Biros et al., 1970). Samples were collected from pathologists in over 18 states, and positive samples came from every hospital, city and state sampled (Yobs, 1972). It therefore appears that PCBs are widely found in the population.

Except for sporadic, localized incidents of food contamination, most of man's exposure to PCBs occurs through the consumption of fish (Kolbye, 1972). A Food and Drug Administration (FDA)-conducted survey in 1970 and 1971 of PCB residues in fresh-water fish reported detectable levels in 54% of the 670 samples examined, with a high of 35.29 ppm and a mean of 1.87 ppm (Kolbye, 1972). Fish taken from the waters of highly industrialized areas such as the Great Lakes routinely contained even higher levels, with residues as high as 213 ppm reported (Stalling and Mayer, 1972). Thus persons who habitually consume large quantities of fish, or, individuals who eat fish from highly polluted areas, could consume significant amounts of PCBs.

Isolated incidents of PCB contamination of other foodstuffs have also been reported (Kolbye, 1972). In a survey conducted by the FDA in 1970 and 1971, 69 samples of milk contained an average of 2.27 ppm PCB; one sample contained 27.8 ppm. The contamination apparently resulted from the
use of PCB-containing silo sealants (Skrentney et al., 1971; Fries, 1972). Cereal products packaged in PCB-contaminated paperboard have been reported to contain up to 27 ppm, and eggs up to 3.7 ppm (Kolbye, 1972).

By far, however, the most severe incident involving human exposure to PCBs occurred in Japan in 1968. Over 1000 people were afflicted with a disease termed Yusho, or rice-oil disease, based on the discovery that it was caused by the consumption of a brand of rice oil contaminated with 2000-3000 ppm PCB (Kuratsune et al., 1972). The contamination was ultimately traced to pinhole-sized leaks in a heating pipe which used PCB as a heat exchanger during processing of the oil. This incident will be discussed later in relation to the toxicological effects of PCBs.

In 1972, the FDA established temporary tolerance levels for PCB residues in human food and food packaging material in an attempt to minimize dietary exposure to PCBs (Kolbye, 1972). The limits for food were set at 5 ppm in the edible portion for fish, 5 ppm on a separate fat basis for poultry and 0.2 ppm in whole milk (5 ppm in the fat). Routine monitoring of products in interstate commerce under the pesticide surveillance program has permitted the FDA to seize products exceeding the designated limits, and, in many cases, to trace the source of contamination. Further-
more, the Monsanto Company in 1970 voluntarily restricted their sales of PCBs to closed system uses, and, in particular, to capacitor and transformer operations (Trout, 1972). However, monitoring data presented at the National Conference on Polychlorinated Biphenyls, November 19-21, 1975, in Chicago, suggested that these measures have fallen far short of their intended goal. Although the percentage of foods contaminated with PCBs has decreased, residues in fresh-water fish have continued to increase. PCB concentrations of 5 to 20 ppm are now routine in both salmon and striped bass from the upper Hudson River and Lake Ontario, well above the newly adopted limit of 2 ppm for edible fish. Furthermore, several investigators believe that if a limit of 1 ppm were adopted, none of the fish in the Great Lakes would be edible (Maugh, 1975). The Environmental Protection Agency, on the other hand, contends that without a Toxic Substances Control Act, it does not have the power to restrict or halt the production or importation of PCBs or to prevent manufacturing plants from releasing PCBs into waterways, all of which may be necessary to halt the contamination (Maugh, 1975). Passage of a Toxic Substance Control Act has been awaiting congressional action for five years.
Toxicological Effects of PCBs

Toxicity in Man

The earliest reports of human toxicity due to chlorobiphenyls concerned industrial exposure of electrical workers in the 1930s and 1940s. The disease, referred to as chloracne, manifested itself in the development of small dermal cysts, most commonly on the face and ears but also on other parts of the body (Schwartz, 1936; Jones, 1941). Systemic effects were reported in severe cases, which included nausea, lassitude and anorexia (Jones, 1941), digestive disturbances, impotence and hematuria (Schwartz, 1936). The onset of symptoms was generally quite slow, but, once developed, they persisted for several months after removal from the source (Jones, 1941).

By far the most well-documented data involving human toxicity to PCBs were obtained from the previously mentioned rice-oil incident in Japan which involved 1057 patients (Kuratsune et al., 1972). The most common initial symptoms were increased eye discharge and swelling of the upper eyelids, followed by acne-like skin eruptions, accentuation of hair follicles, and pigmentation of the skin and nails. Clinical symptoms included elevated urinary 17-ketosteroid excretion, elevated blood serum triglycerides and a hema-
tological picture suggestive of acute or chronic inflammation. Newborn infants of mothers exposed to PCB during pregnancy had skin discoloration and increased eye discharge due to placental transfer of the chemical.

The latency between exposure and development of symptoms was about five or six months. Symptoms generally cleared when exposure to PCB ceased, although several patients remained afflicted with chloracne for several years. It was estimated that the average Yusho patient consumed approximately 15 grams of oil per day, resulting in an average PCB intake of 30 milligrams per day. The minimum dose of PCB eliciting Yusho symptoms was estimated at three milligrams per day over several months (Fishbein, 1974).

Animal Experiments

Following the rice-oil incident in Japan, as well as the discovery of global contamination by PCBs, interest in the toxicological effects of PCBs heightened, and a vast amount of research on various species of animals has been published in the last five years. For this reason, the following discussion will be limited to the toxicological effects seen in mammals, and, in particular, laboratory rodents and monkeys. Invertebrates, birds and fish exhibit considerably different symptoms of PCB intoxication, and
the interested reader is referred to Environmental Health Perspectives, Volume 1, 1972, and Environmental Research, Volume 5, 1972, for further information.

**Acute Toxicity**

Although there is a considerable amount of variation in reported LD$_{50}$ values for PCBs when administered as a single dose, all available information indicates that they are of a low order of toxicity. In rats, it appears that oral toxicity may decrease with increasing degree of chlorination. The FDA reported an oral LD$_{50}$ of 4 g/kg for Aroclor 1221 and 10 g/kg for Aroclor 1260 (Fishbein, 1974). The same trend, however, was not seen in dermally exposed rabbits which exhibited mean lethal doses of 1 to 4 g/kg for all Aroclors tested, which were topically administered in corn oil (Fishbein, 1974). Kimbrough and coworkers (1972) indicated that it was not possible to calculate an acute oral LD$_{50}$ for Aroclors 1254 and 1260 due to variation in response. However, they indicated a range of 4 to 10 g/kg for both mixtures in male and female rats. Bruckner et al. (1973) reported that the minimum lethal dose of Aroclor 1254 was 2.5 g/kg, a dose which killed one out of six rats in 72 hours. Their reported 14-day oral LD$_{50}$ value was 4.25 g/kg, with a 95% confidence interval of 2.8 - 6.4 g/kg. Finally,
Grant and Phillips (1974) reported the 5-day oral LD$_{50}$ of Aroclor 1254 as 1.4 g/kg for 30 and 60 day old rats and 2.0 g/kg for 120 day old rats. Therefore it appears that some of the variation in reported LD$_{50}$ values may be due to the age of the animal studied. Also it has been suggested that the solute concentration of the injected solution (Lewin et al., 1972), as well as variation between different batches of PCB mixtures (i.e., presence of contaminants) (Vos and Koeman, 1970) may influence the toxicity of the PCB under study.

Death due to acute PCB poisoning is preceded by diarrhea, decreased response to pain stimuli and unusual stance and gait. Eventual ataxia, coma and death follow (Bruckner et al., 1973). Autopsies of dead animals reveal hemorrhage into the lung, stomach and pancreas, with foci of ulceration, surrounded by a severe inflammatory reaction, in the duodenum and occasionally the glandular stomach (Kimbrough et al., 1972).

Subacute and Chronic Toxicity

Curley et al. (1971) reported that Aroclor 1254 added to the diet at a concentration of 1000 ppm resulted in the death of five out of ten male rats and eight out of ten female rats fed the contaminated diet for 100 days. Death
occurred between day 50 and 98 for the male rats and between day 35 and 89 for the female rats. The animals surviving to day 100 appeared normal except for decreased body weights. The authors cited a similar study in which the feeding of 1000 ppm Aroclor 1254 was lethal to six of six male rats in 53 days. Food consumption measurements indicated that the lethal dose level under these conditions was 500-2000 mg/kg. Kimbrough et al. (1972) reported that feeding Aroclor 1260 at levels up to 1000 ppm was not lethal to any of the ten male rats fed the diets for eight months, however, eight out of ten females died on the 1000 ppm diet and two out of ten females died on the 500 ppm diet. Aroclor 1254 at a level of 1000 ppm, on the other hand, caused the death of five out of ten male rats and eight out of ten female rats, similar to the results of Curley et al. (1971). In mice, the feeding of Aroclor 1254 at a level of 4000 ppm was lethal to five of five animals in seven days. Three of five mice receiving 1000 ppm PCB also died, two on day 12 and one on day 15. No mice fed 250 ppm PCB died (Sanders et al., 1974).

Pathological lesions associated with death due to chronic PCB exposure in rats have been noted only in the liver and include cellular hypertrophy, fatty infiltration, necrosis, vacuolization and hyalinization (Bruckner et al., 1973;
Kimbrough et al., 1972). Liver changes were more pronounced in animals exposed to Aroclor 1254 as compared to Aroclor 1260 (Kimbrough et al., 1972). In addition to liver damage, severe hyperplastic gastritis and bone marrow hypoplasia have been observed in monkeys following death due to PCB intoxication (Allen et al., 1974).

Sublethal Toxicological Effects

Liver Effects

By far the most dramatic changes seen in animals exposed to sublethal levels of PCB occur in the liver. Most mammalian species, including rats, mice, rabbits, dogs and monkeys, respond to PCB exposure with liver hypertrophy as indicated by increased liver weight and increased liver to body weight ratios, and by an increase in liver microsomal enzyme activity (Vos and Koeman, 1970; Kimbrough et al., 1972; Litterst et al., 1972; Vos and Notenboom-Ram, 1972; Allen et al., 1973; Alvares et al., 1973; Bruckner et al., 1973; Koller and Zinkl, 1973; Bruckner et al., 1974a; Grant et al., 1974; Grant and Phillips, 1974; Kiriyama et al., 1974a; Sanders et al., 1974; Goldstein et al., 1975; Iversen et al., 1975). The amount of increase in liver weight and microsomal enzyme activity have been shown to be directly correlated with the degree of PCB chlorination (Keplinger
1971; Litterst et al., 1972; Chen and DuBois, 1973; Eco-
bichon and Comeau, 1974; Hansell and Ecobichon, 1974;
Schmoldt et al., 1974; Ecobichon and Comeau, 1975). The
induction and enhancement of activity of this enzyme system
is considered important since it could alter the body's
metabolism of a variety of normal substrates, including
steroids, vitamins and hormones, as well as the metabolism
and detoxification of many drugs and several polycyclic
hydrocarbon carcinogens such as benzopyrene and 3-methyl-
cholanthrene (Fouts, 1972; Alvares et al., 1973; Kappas and
Alvares, 1975). Specific studies suggestive of abnormal
metabolism of sex steroids, vitamins and drugs in PCB-ex-
posed animals have been reported (Platanow et al., 1972;
Koller and Zinkl, 1973; Orberg and Kihlstrom, 1973; Innami
et al., 1974; Jones, D., et al., 1974; Nagayama et al., 1974;
Orberg and Lundberg, 1974; Sanders et al., 1974).

Structural changes observed in the liver associated
with sublethal PCB exposure are similar to those mentioned
previously following exposure to lethal levels of PCB. How-
ever, the severity and extent of the changes will reflect
both the dose level of PCB as well as the duration of expo-
sure. Furthermore, the changes are considered reversible
following discontinuance of PCB exposure (Kimbrough et al.,
1972).
The most prominent ultrastructural change observed in the liver is a proliferation of smooth endoplasmic reticulum (SER) with a concomitant decrease in rough ER. This is considered to be a structural reflection of the enhanced microsomal enzyme activity (Vos and Notenboom-Ram, 1972; Bruckner et al., 1974b; Hansell and Ecobichon, 1974). The hyalinization seen in light microscopy has been shown to consist of tightly packed tubules of proliferated SER (Vos and Notenboom-Ram, 1972). In addition, lipid droplets, cytoplasmic vacuolization and nuclear degeneration of hepatocytes are seen in more severely affected areas (Vos and Notenboom-Ram, 1972; Hansell and Ecobichon, 1974).

Immunosuppressive Activity of PCB

Effects of exposure to PCB on the lymphoid system has been noted in some studies. Lymphopenia, atrophy of the cortex of the thymus and a reduction in the number of germinal centers in the spleen and lymph nodes in rabbits were reported to be suggestive of immunosuppressive activity (Vos and Beems, 1971). Suppression of the humoral immune response in guinea pigs was confirmed with the observation that ten ppm Aroclor 1260 in the diet for eight weeks significantly decreased the number of antibody-forming cells in the popliteal lymph node after stimulation with tetanus toxoid
(Vos and de Roij, 1972). In addition, Koller and Thigpen (1973) reported a reduction in antibody production to pseudorabies virus in rabbits given 300 mg of PCB once a week for 14 weeks. Three Aroclor formulations were tested, 1221, 1242 and 1254, and all were effective in reducing antibody titers. Aroclor 1242, however, produced the lowest titers, succeeded by Aroclor 1254 and then by 1221. Vos (1972) inferred an effect of PCB on the cell-mediated immune response from the observation of decreased numbers of circulating lymphocytes following exposure of guinea pigs to 50 ppm Aroclor 1260 for six weeks. However, Street and Sharma (1975) reported no significant effect of Aroclor 1254 on rabbit skin reactivity to tuberculin (a measure of cell-mediated immunity) despite an observed thymic atrophy. The PCB was fed to rabbits at levels as high as 170 ppm for four weeks prior to antigen administration.

Carcinogenic Activity of PCB

Liver tumorigenesis has been reported to occur in mice treated with high levels of PCB for extended periods of exposure. Ito et al. (1973) described the formation of nodular hyperplasia and well-differentiated hepatocellular carcinomas in the liver of mice fed 500 ppm Kanechlor 500
for 32 weeks. Similar neoplastic changes were not seen in mice exposed to Kanechlor 300 or 400, indicating an effect of chlorination. Similarly, Kimbrough and Linder (1974) reported the induction of adenofibrosis and hepatomas in the liver of mice fed 300 ppm Aroclor 1254 for 11 months. Although the authors described the hepatomas as well-differentiated, they indicated the potential for the development of true malignant lesions. No metastatic growths, however, were seen to accompany the development of the primary lesions, nor were transplantation attempts performed, two primary criteria of malignancy.

Rats, on the other hand, appear to be more resistant to the tumorigenic action of PCBs. Ito et al. (1974) reported that no hepatocellular carcinomas were observed in male rats following 52 weeks of exposure to 1000 ppm Kanechlor 500, 400 or 300. Nodular hyperplasia, however, which may represent a preneoplastic lesion, was present and most severe in the group given Kanechlor 500. Kimbrough et al. (1972) similarly reported no neoplastic lesions present in the livers of rats fed up to 1000 ppm Aroclors 1254 or 1260 for eight months. Areas classified as adenofibrosis were described and were considered to represent possible preneoplastic change.

Allen and Norback (1973) reported the induction of
hyperplasia and dysplasia in the gastric mucosa of monkeys fed a diet containing 300 ppm Aroclor 1248 for three months. The increased cellularity, abnormal growth pattern and invasion of adjacent tissue was believed by the authors to be suggestive of eventual neoplastic transformation.

The co-carcinogenic potential of PCB has also received some attention. Ito et al. (1973) indicated that 250 ppm Kanechlor 500, when given simultaneously with α- or β- benzene hexachloride (BHC), a known hepatocarcinogen, for 24 weeks, promoted the formation of liver tumors in mice. Among groups fed BHC alone, only animals receiving 250 ppm of the α isomer developed tumors. However, among groups receiving PCB and BHC, tumors were found in animals fed 100 or 50 ppm α-BHC and in animals fed 250 or 100 ppm β-BHC. On the other hand, in rats, cotreatment with PCB (500 ppm Kanechlor 500 for 20 weeks) significantly inhibited the production of liver neoplasms by three different potent hepatocarcinogens, 3'-methyl-4-dimethyl-aminoazo-benzene (3'-Me-DAB), N-2-fluorenylacetamide (2-FAA), and diethylnitrosamine (DEN) (Makiura et al., 1974). For example, DEN alone produced liver tumors in 12 of 13 rats; when PCB was given concomitantly, no liver tumors were discovered. The authors suggested that the induction of
liver microsomal enzymes may have resulted in a more rapid metabolism of the carcinogens. The promotive effect of PCB on BHC-induced tumors in mice, on the other hand, might suggest that metabolite(s) of BHC are more carcinogenic than the parent compound. Finally, Uchiyama and Chiba (1974) reported that feeding PCB (Kanechlor 400) at a level of 100 ppm for eight weeks did not promote the formation of cervical carcinoma induced in mice by the uterine implantation of a methylcholanthrene-impregnated thread. It therefore appears that not only the species of animal studied, but also the carcinogen and the chlorine content of the PCB formulation, are important factors in determining the co-carcinogenic effects of polychlorinated biphenyls.

Miscellaneous Effects

In a study cited by Fishbein (1974), PCBs were shown to affect reproduction in rats. Feeding 100 ppm Aroclor 1254 for 76 days prior to breeding resulted in fewer offspring, decreased survival to weaning, and a decreased body weight at weaning. Keplinger et al. (1971) reported similar findings in a study in which Aroclors 1242, 1254 and 1260 were fed to rats at levels of 1, 10 and 100 ppm. Low mating indices were observed for animals receiving
100 ppm Aroclor 1242, and decreased survival of pups occurred with both Aroclor 1242 and Aroclor 1254 at the 100 ppm level. No reproductive effects were seen with Aroclor 1260 at any dose level, nor with Aroclors 1242 and 1254 at 1 or 10 ppm. It would suggest that mammalian reproductive effects decrease with increasing chlorination. Orberg and Kihlstrom (1973) showed that feeding 0.025 mg PCB (Clophen A60) for 62 days resulted in a lengthening of the estrus cycle and a decrease in the number of implanted ova in mice. Since both phenomena are regulated by sex steroids, the authors suggested that PCBs affect reproduction by affecting the metabolism of sex hormones. Atrophy of the uteri of Aroclor 1254-treated rabbits has also been reported (Koller and Zinkl, 1973).

Kidney damage has been occasionally reported to occur in rats and rabbits exposed to PCB. Kidneys of rats that received intraperitoneal (IP) injections of 100 mg/kg Aroclor 1242 for ten weeks exhibited dilation and degeneration of proximal convoluted and collecting tubules, with diffuse areas of sudanophilic vacuolation evident within the renal epithelium (Bruckner et al., 1974). Similar changes have been seen in rabbits dermally exposed to PCB formulations containing 60% chlorine (Vos and Beems, 1971).
Metabolism, Distribution and Excretion of PCBs

Although PCBs are extremely inert chemicals and very resistant to degradation, evidence is accumulating that indicates that certain PCBs can be altered metabolically. Metabolic alteration is an important factor in toxicology because of the possible conversion of the original chemical to more or less toxic metabolites in the body.

In general, it appears that the relative ease of metabolic processing of PCBs decreases as the chlorine content increases. Initially, circumstantial evidence supporting this conclusion was obtained from chromatograms of PCB residues in wildlife which were reported to resemble Aroclor 1254 or 1260, but that the lesser chlorinated isomers were decreased or entirely absent (Jensen et al., 1969; Koeman et al., 1969; Risebrough and deLappe, 1972; Zitko et al., 1972). Also, early laboratory studies implied that the lesser chlorinated species (di-, tri- and tetrachlorobiphenyls) were taken up and/or retained less efficiently, based on the observation that the proportion of lower isomers in the tissue was less than the proportion in the mixture to which the animal was exposed (Grant et al., 1971; Curley et al., 1971). It was not clear, however, whether the difference resulted from metabolism or
from differential absorption or excretion. Metabolism by
the liver was implicated, however, since rats with livers
damaged by exposure to carbon tetrachloride had higher
tissue residues and the chromatograms more closely resem-
bled the standard Aroclor 1254 mixture which was fed to
the rats (Grant et al., 1971). Also, small amounts of
PCB-derived material recovered from the urine of PCB-ex-
posed rats had an entirely different chromatogram compared
to the standard, indicating metabolism to more polar com-
 pounds (Curley et al., 1971). Albro and Fishbein (1972)
more conclusively demonstrated that metabolism, as opposed
to differential excretion, was the critical factor, with
the observation that more than 90% of all PCBs, having one
to six chlorine atoms per molecule, were absorbed from the
gut following dietary exposure, and, further, that the per-
centage retained was independent of the dose administered.

More recent work with pure isomers of PCB has revealed
the presence of hydroxylated metabolites in the feces and
urine of animals exposed to PCBs. The results of Hutzinger
and coworkers (1972) showed the conversion of 4-chloro-, 
4,4'-dichloro-, and 2,2',5,5',-tetrachlorobiphenyl isomers
into monohydroxylated derivatives by the rat. No hydroxy-
lated products were detected following exposure to 2,2', 
4,4',5,5'-hexachlorobiphenyl. Dihydroxylated metabolites
and phenolic derivatives of tri- and tetrachlorobiphenyl isomers have also been reported in rabbits, rats and monkeys (Gardener et al., 1973; Yoshimura and Yamamoto, 1973; Greb et al., 1975).

The tissue distribution and storage of polychlorinated biphenyls following oral or intravenous exposure has also been studied. Grant et al. (1974) measured the accumulation and rate of decline of Aroclor 1254 residues in the tissues of male rats fed dietary levels of 0, 2, 20 or 100 ppm for 246 days, followed by a PCB-free diet for an additional 182 days. Their results revealed that residues of Aroclor 1254 were dose-related in all tissues, with the highest concentration in the fat and the lowest concentration in the brain. The residue levels appeared to reach a plateau in 60 days. After 182 days on a PCB-free diet, the residues present in the adipose tissue were decreased by 49%. Curley et al. (1971), on the other hand, reported a continuous increase in residues of Aroclor 1254 in all tissues of rats fed 100 ppm PCB for 240 days. Furthermore, the residue levels in the fat after 240 days were almost four times higher than the levels reported in the previously cited study after 246 days on a 100 ppm diet (1101 ppm versus 348 ppm). After 71 days of feeding an Aroclor-free diet, residues in the fat were reduced by 20%.
Burse et al. (1974) reported that a steady-state of storage was approached in two months and essentially achieved in four months after feeding Aroclor 1242 to rats at a level of 100 ppm for up to ten months. The highest concentration of PCB was found in adipose tissue (133 ppm), with much lower levels in brain, kidney and liver. The concentration in plasma was always less than one ppm. Following a recovery period of six months, the residue levels in adipose tissue were reduced to 21.8 % of the level observed after a six month feeding period. These results further indicate that the storage level is lower and elimination more rapid with the lesser chlorinated PCBs.

Recently, the distribution and excretion of several pure isomers of PCB labelled with carbon-14 has been described (Matthews and Anderson, 1975a; Matthews and Anderson, 1975b). More than 90% of a single intravenous dose was removed from the blood within ten minutes after administration. Most of the radioactivity was initially deposited in the liver and muscle and then translocated to the skin and adipose tissues, which remained as long-term storage sites. The corpora luteum, adrenal cortex and gastric mucosa have also been reported to show a strong initial uptake of carbon-14 labelled PCB (Brandt, 1975). The percentage of total radioactivity accounted for by
metabolites of PCB decreased as the chlorination of PCB increased, but storage of metabolites did not account for a significant portion of any of the PCB doses. Biliary excretion accounted for more than 90% of the dose of mono- and dichlorobiphenyls and 30% of the dose of pentachlorobiphenyl during the 42 days of study. Extrapolation of the hexachlorobiphenyl data indicated that less than 20% of the dose would ever be excreted.

**Purpose of This Study**

Man is continually being assaulted by the potentially hazardous effects of new chemicals introduced into the environment and inadvertently resulting in human exposure. Unfortunately, unless a chemical has a high degree of toxicity, it is usually not until widespread environmental contamination has occurred before any degree of concern is registered. Pursuant to this concern, one of the primary questions asked by scientists investigating the effects of a new contaminant is, "Is it carcinogenic?". Usually, when given long enough and at high enough doses, the majority have been found to be. However, in addition to the direct carcinogenic potential of such a chemical, the host's ability to counteract the inception and growth of tumors may play a key role in the eventual outcome. If a contaminant
has the ability to suppress this protective reaction of the host and, consequently, leads to conditions permitting or promoting tumor growth, then the hazards of the chemical are even greater.

In the present study, utilizing a transplantable tumor in rats, the following question was asked: "Is PCB, a newly discovered environmental contaminant that can accumulate in the body, capable of modifying conditions within the animal which results in a change in the growth of the tumor, either by acting directly on the tumor cells, or by affecting mechanism(s) that control tumor growth?". Based primarily on the reported immunosuppressive activity of PCB, it was felt that exposure to this chemical might constitute a health hazard with respect to tumors that has not been previously recognized.
Tumor Transplantation as a Tool in Cancer Research

Tumor transplantation, as the name implies, involves the actual transfer of living tumor cells from one animal to another; the new tumors develop solely from the implanted cells (Stewart et al., 1959). The technique offers several advantages over the use of spontaneous or carcinogen-induced tumors. It provides the only practical method of studying reproducible malignant growths under controllable conditions during a reasonably short time period, yet still retains all the salient biological and biochemical characteristics of malignant tissue (Klein, 1959; Liebelt and Liebelt, 1967). Furthermore, transplantable tumors allow for the natural interplay of the host's physiological systems and the growing tumor, a critical factor absent in tissue culture studies.

The Walker 256 carcinosarcoma, chosen for use in this study, is one of the most extensively studied transplantable rat tumors, having been utilized over the past forty-plus years in numerous laboratories throughout the world. It is also one of the primary tumor systems used for the chemotherapeutic evaluation of anticancer drugs at the U.S.
National Cancer Institute, the Chester Beatty Research Institute and the Sloan-Kettering Institute for Cancer Research (Schmid et al., 1966; DeWys et al., 1968). The tumor readily grows in a variety of strains of rats following intramuscular or subcutaneous inoculation of tumor cell suspensions or fragments. It is also readily converted to an ascitic form, characterized by the proliferation of free tumor cells in the peritoneal exudate, following intraperitoneal injection. With intravenous inoculation, tumor metastases to specific organs can be achieved.

In the Walker tumor system, the effectiveness of the experimental treatment is generally based on the extent of tumor growth alteration, as measured in the size of the tumor after a given time period (Devik et al., 1950; Talalay et al., 1952; Hoffman et al., 1962; Strawitz and Glaser, 1962; Grollman and Crass, 1966; Rosenoer et al., 1966; Dowlowy et al., 1968; DeWys et al., 1970; McQuitty et al., 1970; MacBeth and Fischer, 1971; Cho-Chung and Gullino, 1974; Gershbein et al., 1974). The survival time of the host bearing either an ascitic or solid tumor, as well as the incidence and size of metastases, have also been used as indicators of effect (Fisher and Fisher, 1959; Fisher and Fisher, 1960; Fisher et al., 1965; Agostino et al., 1966; Hart and Adamson, 1971; Adamson, 1972; Torpie, 1974).
History and Description of Original Tumor

The history and description of the original Walker 256 tumor has been previously reviewed (Earle, 1935; Stewart et al., 1959). Briefly, the tumor developed spontaneously in the region of the mammary gland of a ten-month old pregnant albino rat in the laboratory of George Walker at the John Hopkins School of Medicine in 1928. The mass, about the size of a large pecan, regressed during lactation but recurred after weaning and grew rapidly thereafter. When the animal was killed, the tumor was about the size of a small egg, incompletely encapsulated and with no accompanying metastatic growths. On microscopic examination, the tumor was diagnosed as an adenocarcinoma, consisting of both simple and complex acini, with proliferated glandular epithelia often several cells thick. The stroma of the tumor consisted primarily of loose fibrous connective tissue which was quite cellular, with the major cell type resembling a fibroblast.

Of 16 rats of both sexes injected with fragments of the parent tumor, 9 developed tumors. Thereafter, the tumor was transplanted approximately every two weeks, and, following the first few generations, the percentage of tumor takes was nearly 100. Histological examination of
the transplanted tumors revealed a loss of the glandular architecture, however, the epithelial cell remained as the dominant cell type, with a stroma similar to that seen in the original tumor. Although the Doure-Doure strain of rat was generally employed for transplantation, other strains were also used with nearly 100% success. The tumor was highly virulent and usually killed the animal within five weeks. During the period of study by Dr. Walker from 1928 to 1932, only four instances of tumor regression were observed.

**Description and Growth Characteristics of Current Tumor**

A well-established intramuscular transplant of the Walker tumor, weighing about five grams, can be described as firm, round to oblate, and partially- or non-encapsulated. On section, there is generally a large area of yellowish-white necrotic material surrounded by a rind of solid pinkish-white viable tumor tissue. Metastases are routinely found in the lumbar and renal lymph nodes, and less frequently in the axial nodes of the armpits, the thoracic nodes and duct, and the intestinal lymph nodes (nomenclature from Greene, 1955). Tumor metastases have also been observed in the lung and kidney, and, very occa-
sionally, in the liver.

On microscopic examination, the Walker tumor is seen to consist of two architectural patterns, carcinoma and sarcoma (see Figure 2). The carcinomatous variant is composed of broad sheets or clumps of so-called "principal cells" supported by a small amount of connective tissue stroma (Stewart et al., 1959). The principal cell is more or less round and has an indistinct cell membrane. The cytoplasm is granular, basophilic and may be vacuolated. There is usually one centrally-located nucleus, surrounded by a deeply-stained nuclear membrane and containing one or two large eosinophilic nucleoli. Occasionally, two or three nuclei are present.

The sarcomatous element of the tumor consists of interlacing bundles of spindle-shaped and stellate cells with long processes (Stewart et al., 1959). Numerous reticular and collagenic fibers surround each cell. The sarcomatous component of the tumor is most often found bordering areas of necrosis and surrounding blood vessels. The capsule, if present, also reflects a sarcomatous histology.

Interspersed among the cells of the carcinomatous and sarcomatous portions of the tumor are the so-called "undifferentiated cells", which are small and deeply stained.
Figure 2. Photomicrograph of the Walker 256 carcinosarcoma transplant tumor showing the two architectural patterns, sarcoma (left) and carcinoma (right). Hematoxylin and eosin stain. 48 x
Their shape varies from round to elongate, and they may appear singly, in groups, or in a linear arrangement within the stroma. Their predilection for areas of necrosis as well as electron microscopic observations have led some investigators to suggest that the cells represent degenerating cells rather than ones capable of differentiation (Pool, 1962).

At present, it is not known whether there exists in the Walker tumor a multipotential cell from which all cell types arise or whether the tumor originated as a mixed histologic type (Stewart et al., 1959). Several investigators, however, consider the Walker tumor to be simply a carcinoma, with the spindle-shaped cells representing only a morphological variation of the basic epithelial cell type (Earle, 1935; Fisher and Fisher, 1961). It is interesting to note that when the tumor is grown in the ascitic form, the only cell type seen resembles the principal cell of the carcinomatous tissue (Agostino and Cliffton, 1968). Also, the metastatic growths of the Walker tumor consist solely of the epithelial cell type, regardless of the tissue to which they have metastasized.

The growth characteristics of the solid Walker tumor in the living host are highly dependent on the concentration of tumor cells in the initiating inoculum. Because the
tumor is not genetically identical to the host, antigenic histocompatibility reactions may occur associated with the implantation of foreign tissue (Bale et al., 1955; Klein, 1959; Boroff, 1961; Dufour, 1961; Walker, 1966; Liebelt and Liebelt, 1967; Dinh and Brassard, 1968). Depending on the number of tumor cells injected, host response may vary from no tumor development (nontake), to limited tumor growth followed by regression, to progressive tumor growth leading to host death. Experience in our laboratory over the past five years has indicated that at least $10^6$ tumor cells are required to cause progressive tumor growth in 100% of the animals. The tumor becomes palpable within two or three days after injection and kills the host within three weeks. When $10^5$ tumor cells are injected, all tumors show an initial period of growth (100% takes) with a latent period of about five days. However, after approximately nine or ten days, tumor regression occurs in a variable fraction of the animals. If the initial inoculum is less than $10^5$ cells, the number of tumor takes is proportionally reduced and the latent period is proportionally increased. In general, all the tumors that do develop, later regress. With the ascitic variant of the Walker tumor, the inoculation of $10^5$ tumor cells results in the death of all animals within seven days with little varia-
Numerous intrinsic and extrinsic factors have also been reported to influence the growth of transplanted tumors in general. For example, the age of the host has been shown to alter both the incidence of takes and the growth rate of the Walker tumor (Kerkvliet and Kimeldorf, 1973). Also, physical factors such as light, temperature and humidity have been shown to influence the growth of tumors (Liebelt and Liebelt, 1967), and even the fondling of some animals and not of others by "overly maternal technicians" has resulted in quantitative differences in tumor growth (Fisher and Fisher, 1967). The standardization and rigid control of such factors must obviously be employed in any experimental situation involving tumor transplantation.

**Tumor Maintenance and Transplantation Techniques**

The Walker tumor used in these studies was originally obtained from the Arthur D. Little, Inc., Cambridge, Massachusetts, in October, 1971. The tumor line has been maintained since that time in this laboratory by weekly intraperitoneal injection of 0.1 ml of ascitic tumor fluid diluted with 0.9% sterile saline to contain $1 \times 10^5$ cells.
All tumor line carriers were four to eight week old male Sprague-Dawley rats.

For the preparation of tumor cell suspensions used for inoculation of experimental animals, approximately 5 ml of fresh ascitic tumor fluid were withdrawn by syringe from a donor animal bearing a six- or seven-day ascitic tumor. The fluid was diluted 1:100 with 0.9% sterile saline, and the number of viable cells was determined in a hemacytometer utilizing the trypan blue dye exclusion technique (Eaton et al., 1959). Viability was most often greater than 99%, and never less than 95%. The original undiluted fluid was then diluted with the appropriate amount of saline to yield the desired concentration of cells. The cell suspension was placed in an ice bath to optimize viability and used within one hour of preparation.

For the study of effects of PCB exposure on solid tumor growth, recipient animals were injected with 0.1 or 0.2 ml of the cell suspension in the flexor muscle mass of each hind limb. A 22 gauge needle was used for inoculation. At the specified sacrifice date, the animals were killed by ether asphyxiation and the tumors were removed, dissected free of surrounding connective tissue and weighed on an analytical balance to the nearest hundredth of a gram. For survival time studies, the animals were allowed to die a
natural death.

Animals and Animal Maintenance

Random-bred Sprague-Dawley albino rats, approximately one month of age, were used in all experiments. Except for the dietary exposure study, all rats were obtained from Simonsen Laboratories, Inc., Gilroy, California. The animals used in the dietary study were the first generation offspring of animals obtained from Hilltop Laboratory Animals, Inc., Scottsdale, Pennsylvania. Previous experience in our laboratory indicated no difference in susceptibility to or growth of the Walker tumor in the animals from the two breeders. Except for the dietary study, only male rats were utilized to avoid any possible complications introduced by estrus cycling in females.

The animals were caged singly in well-ventilated isolated animal quarters with a 12-hours on, 12-hours off light cycle. Except for the dietary study, all animals were provided with OSU Laboratory Rat Chow and water ad libitum.

PCB Formulation

The polychlorinated biphenyl mixture used in this study, Aroclor 1254, Lot Number KA-625, was kindly supplied
by the Monsanto Chemical Company, St. Louis, Missouri, to the Department of Agricultural Chemistry, OSU. Since both environmental persistence and relative biological effectiveness appear to be directly correlated with the degree of chlorination, Aroclor 1254 was chosen as a representative PCB mixture containing a greater proportion of these more highly chlorinated species.

**Dietary Exposure to PCB**

Animals exposed to PCB via the diet were fed a specially prepared powdered chow (OSU Department of Agricultural Chemistry) to which the PCB was incorporated by dissolution in corn oil prior to mixing. The diets were available to the animals ad libitum, and food consumption measurements were recorded for each animal three times per week during the experiment. This was accomplished by placing a weighed container of food into the cage and re-weighing the container before filling on the next day. The amount of food consumed was equal to the difference in weight between the filled and partially filled container.

The animals were fed the PCB-contaminated diets for 30 days prior to tumor implantation and remained on their respective diets during the tumor growth period. Control rats were fed the same diet without added PCB.
The body weights of the animals were recorded weekly. The basal powdered diet appeared to be adequate for growth and maintenance of the animals. No difference was noted in the growth rate of animals on the powdered diets compared to animals fed the standard laboratory rat chow. Table 3 indicates the basic constituents of the powdered chow.

**Intraperitoneal Exposure to PCB**

For studies utilizing the intraperitoneal (IP) route of exposure to PCB, the chemical was dissolved in Planters® peanut oil and maintained at a temperature of approximately 100°F to facilitate injection of the viscous fluid. The PCB solutions of varying concentration were prepared in a manner to standardize the volume of fluid injected per animal at 1 ml/kg of body weight. The PCB injections were, except as noted, initiated on the day of tumor inoculation and repeated every other day for a specified time period or until the death of the animal. Control animals were similarly injected with an equivalent volume of peanut oil only.
Table 3. Composition of basal powdered diet (2000 g).
(OSU Department of Agricultural Chemistry)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Cerelose</td>
<td>1340 g</td>
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<tr>
<td>Casein</td>
<td>440 g</td>
</tr>
<tr>
<td>HMW salt mix (Hubbard et al., 1937)</td>
<td>80 g</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>20 g</td>
</tr>
<tr>
<td>1066.0 g corn starch</td>
<td></td>
</tr>
<tr>
<td>840.0 g dihydrogen citrate</td>
<td></td>
</tr>
<tr>
<td>40.0 g inositol</td>
<td></td>
</tr>
<tr>
<td>20.0 g vitamin B12 mix (1 g in 99 g corn starch)</td>
<td></td>
</tr>
<tr>
<td>8.0 g niacin</td>
<td></td>
</tr>
<tr>
<td>8.0 g calcium pantothenate</td>
<td></td>
</tr>
<tr>
<td>6.0 g biotin mix (1 g in 500 g corn starch)</td>
<td></td>
</tr>
<tr>
<td>1.6 g riboflavin</td>
<td></td>
</tr>
<tr>
<td>1.0 g pyridoxine hydrochloride (B6)</td>
<td></td>
</tr>
<tr>
<td>0.8 g thiamine hydrochloride</td>
<td></td>
</tr>
<tr>
<td>0.8 g menadione</td>
<td></td>
</tr>
<tr>
<td>0.8 g folic acid</td>
<td></td>
</tr>
<tr>
<td>Zinc mix</td>
<td>20 g</td>
</tr>
<tr>
<td>1.67 g zinc acetate in 1000 g cerelose</td>
<td></td>
</tr>
<tr>
<td>Vitamins A, D and E</td>
<td>4 ml</td>
</tr>
<tr>
<td>1.82 g D-tocopherol</td>
<td></td>
</tr>
<tr>
<td>0.15 g vitamin A acetate</td>
<td></td>
</tr>
<tr>
<td>0.075 g calciferol</td>
<td></td>
</tr>
<tr>
<td>100 ml ethanol</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>100 g</td>
</tr>
</tbody>
</table>
In several experiments, the rate of growth of the Walker tumor was estimated *in vivo* during tumor progression. Once the tumor was palpable, the diameters of the tumors were measured every one or two days using a vernier calipers. As shown in Figure 3, the animal was restrained by tightly wrapping the fore limbs and chest in an elasticized bandage; a procedure the animals tolerated quite amicably. The hair over the tumor was moistened with warm water to more clearly reveal the tumor shape, and two diameter measurements were taken, at perpendicular axes, one parallel to the leg bone and one around the leg. The mean tumor diameter (an arithmetical average of the two measurements) is related to the tumor weight by the formula: tumor weight (g) = $0.5435 \ d^3$, where \( d \) represents the mean tumor diameter in centimeters. The mathematical relationship was derived by Shrek (1935) from the formula for the volume of a sphere ($V = 0.5236$) multiplied by the specific gravity of the tumor (1.038).

**Histology**

Tissue samples were rapidly excised after animal sacrifice and placed in Bouin's Picrol Formol solution or 10%
Figure 3. Technique for measuring tumor size in situ.
buffered formalin for fixation. Following routine dehydration, the samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopic examination. Liver specimens were also stained for lipid with Oil red O. Dr. Loren Koller, pathologist, Department of Veterinary Medicine, OSU, was consulted for assessment of PCB-induced liver damage.

**PCB Tissue Residue Analysis**

All tissue samples were submitted to the Analytical Section, OSU Department of Agricultural Chemistry, for gas chromatographic analysis of PCB residues. The procedure, as described by A. Marin, laboratory technician, was as follows. All samples were dropped, whole, while still frozen into a cooled mortar of liquid nitrogen, ground until finely powdered, and weighed into a tared beaker containing sodium sulfate. The sample, with sodium sulfate as filler, was then transferred to a chromatographic column and eluted with hexane. An aliquot of each sample extract (equivalent to 0.1 g of sample) was loaded onto an alumina clean-up column and eluted with hexane. Samples were then made up to appropriate volumes and analyzed by electron-capture gas chromatography (Hewlett-Packard 5700A). Samples were analyzed against a 1.0 µg/ml Aroclor 1254 stand-
A solvent blank and spiked control were run for contamination and recovery data.
RESULTS

Effects of Dietary Exposure to PCB on Tumor Growth

In order to reflect man's primary route of exposure to PCBs, initial studies were concerned with the influence of dietary PCB on the growth of the Walker tumor. Animals were exposed to diets containing 0, 5, 25, 100, 400 or 800 ppm PCB for 30 days prior to the injection of $10^5$ tumor cells. The animals remained on their respective diets during the period of tumor growth. The assessment of PCB-induced tumor growth modification was based on the weight of the tumors after a nine-day growth period.

As shown in Table 4, the mean tumor weights of all PCB-fed groups were significantly smaller than the mean tumor weight of their sex-matched control after the nine-day tumor growth period. In both male and female rats, the magnitude of tumor weight inhibition directly correlated with the concentration of PCB in the diet. Although female rats consistently showed smaller tumor weights than their male counterparts, the degree of tumor weight inhibition observed at each PCB dose level was comparable in both sexes. Figure 4 indicates the functional relationship between total PCB intake and the magnitude of tumor weight inhibition.
Table 4. Effect of dietary exposure to PCB (Aroclor 1254) on the weight of the Walker tumor at nine days after injection in male and female rats.\(^1\)

<table>
<thead>
<tr>
<th>PPM PCB in feed</th>
<th>PCB Intake(^2)</th>
<th>Sex</th>
<th>No. of Animals</th>
<th>No. of Tumors</th>
<th>Tumor Weight (g) (\bar{X} \pm \text{s.e.})</th>
<th>Tumor Weight % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>--</td>
<td>M</td>
<td>28</td>
<td>52</td>
<td>4.91 ± 0.33</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>M</td>
<td>20</td>
<td>38</td>
<td>4.12 ± 0.33(^a)</td>
<td>84.1</td>
</tr>
<tr>
<td>25</td>
<td>113</td>
<td>M</td>
<td>20</td>
<td>40</td>
<td>3.90 ± 0.33(^b)</td>
<td>79.4</td>
</tr>
<tr>
<td>100</td>
<td>300</td>
<td>M</td>
<td>8</td>
<td>16</td>
<td>3.67 ± 0.35(^b)</td>
<td>74.7</td>
</tr>
<tr>
<td>400</td>
<td>1112</td>
<td>M</td>
<td>8</td>
<td>14</td>
<td>2.90 ± 0.74(^b)</td>
<td>59.1</td>
</tr>
<tr>
<td>800</td>
<td>2063</td>
<td>M</td>
<td>8</td>
<td>16</td>
<td>2.01 ± 0.30(^c)</td>
<td>40.9</td>
</tr>
<tr>
<td>0</td>
<td>--</td>
<td>F</td>
<td>8</td>
<td>16</td>
<td>3.99 ± 0.23</td>
<td>--</td>
</tr>
<tr>
<td>100</td>
<td>320</td>
<td>F</td>
<td>8</td>
<td>16</td>
<td>3.02 ± 0.21(^c)</td>
<td>75.7</td>
</tr>
<tr>
<td>400</td>
<td>1102</td>
<td>F</td>
<td>8</td>
<td>16</td>
<td>2.27 ± 0.32(^c)</td>
<td>56.9</td>
</tr>
<tr>
<td>800</td>
<td>2126</td>
<td>F</td>
<td>8</td>
<td>16</td>
<td>1.81 ± 0.14(^c)</td>
<td>45.4</td>
</tr>
</tbody>
</table>

\(^1\)All rats injected with \(1.0 \times 10^5\) tumor cells in each hind leg after 30 days on experimental diets; animals remained on respective diets during tumor growth.

\(^2\)Total PCB intake, day 0 to 39, calculated from food consumption measurements.

\(^a, b, c\)Significantly different from control; \(p < 0.05, 0.025, \text{and } 0.01\) respectively; Student's t test.
Figure 4. Quantitative relationship between total PCB intake and magnitude of tumor weight inhibition measured at nine days post-tumor inoculation. Male (●) and female (○) rats exposed, ad lib, to PCB diets for 30 days prior to and during tumor growth. Each point represents the mean of 8-20 animals.
In an attempt to see if the effectiveness of PCB exposure could be enhanced with slower growing tumors, two additional groups of animals were placed on diets containing 0 or 250 ppm PCB. One thousand ($10^3$) tumor cells were used as initiating inoculum, and the tumors were allowed to grow for 13 days. The mean tumor weight of the control and PCB-fed group after the 13-day tumor growth period was $3.28 \pm 0.40$ g and $2.02 \pm 0.21$ g, respectively. The reduction in tumor weight in the PCB-treated animals was statistically significant ($p<0.025$).

By referring to Figure 4 in which tumor weight (as percent of control tumor weight) is plotted as a function of total PCB intake (in mg/kg) for $10^5$ tumor cell inocula, it is possible to predict the amount of tumor weight inhibition anticipated following exposure to a diet containing 250 ppm PCB. Based on food consumption measurements, the animals on the 250 ppm diet consumed an average of 900 mg/kg PCB. The predicted tumor weight for this dosage, from Figure 4, would be approximately 63% of the control tumor weight. The actual observed tumor weight (as percent of control) of 61.6% is essentially the same as the predicted value. This would indicate that the number of tumor cells used as initiating inoculum, and consequently, the growth rate of the tumor, does not influence the re-
response of the tumor to PCB exposure when the inoculum ranges from $10^3$ to $10^5$ tumor cells.

As shown in Table 5, male rats fed diets containing 100 ppm or less PCB showed no significant alteration in body growth rate during the experiment. At PCB levels of 250 ppm or more, however, there was a significant reduction in body weight gain. Female rats appeared to be more sensitive to this parameter of PCB intoxication, showing reduced body weight gain at the 100 ppm dietary level. The observed alteration in body growth rate could, in part, be accounted for by a significant decrease in food consumption by the PCB-fed rats, as shown in Table 5. It is possible that an adverse taste was associated with the higher dietary levels of PCB as the animals showed decreased food consumption immediately after being placed on the experimental diets.

Effects of Intraperitoneal Exposure to PCB on Tumor Growth

Based on the results of the dietary exposure study demonstrating a tumor-inhibitory effect of PCB exposure, the problem appeared to become one less associated with the "environmentally hazardous" concept, and, consequently, the need to simulate environmental conditions diminished.
Table 5. Food consumption and body weight gain of male and female rats exposed to PCB-contaminated diets for 39 days.

<table>
<thead>
<tr>
<th>PPM PCB in feed</th>
<th>Sex</th>
<th>Food Consumption&lt;sup&gt;1&lt;/sup&gt; g/day ± S.D.</th>
<th>Body Weight (g) ± S.D.</th>
<th>Initial</th>
<th>Final</th>
<th>Net Change&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>M</td>
<td>18.3 ± 1.7</td>
<td>154 ± 12</td>
<td>329 ± 26</td>
<td>181 ± 21</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>18.9 ± 1.5</td>
<td>142 ± 15</td>
<td>330 ± 23</td>
<td>184 ± 19</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>18.9 ± 1.7</td>
<td>139 ± 19</td>
<td>334 ± 31</td>
<td>194 ± 27</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>M</td>
<td>19.2 ± 0.8</td>
<td>158 ± 12</td>
<td>345 ± 21</td>
<td>188 ± 25</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>M</td>
<td>15.5 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>153 ± 31</td>
<td>270 ± 30</td>
<td>117 ± 40&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>M</td>
<td>11.5 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>144 ± 26</td>
<td>203 ± 43</td>
<td>70 ± 23&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0&lt;sup&gt;3&lt;/sup&gt;</td>
<td>M</td>
<td>19.0 ± 2.2</td>
<td>138 ± 9</td>
<td>323 ± 23</td>
<td>137 ± 18</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>M</td>
<td>16.5 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>126 ± 11</td>
<td>275 ± 29</td>
<td>159 ± 28&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>F</td>
<td>14.5 ± 0.5</td>
<td>128 ± 8</td>
<td>217 ± 22</td>
<td>89 ± 15</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>F</td>
<td>14.0 ± 1.2</td>
<td>133 ± 6</td>
<td>203 ± 18</td>
<td>71 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>F</td>
<td>10.7 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134 ± 5</td>
<td>162 ± 35</td>
<td>35 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>F</td>
<td>9.5 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124 ± 18</td>
<td>146 ± 16</td>
<td>22 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Grand mean of daily means

<sup>2</sup>Net change in body weight calculated for individual animals and then averaged.

<sup>3</sup>Animals fed contaminated diet for a total of 43 days.

<sup>a</sup>Significantly different from control, p<0.01, Student's t test.
Therefore, in the remaining experiments, animals were exposed to PCB by repeated intraperitoneal injections of the chemical. This method not only allowed for the standardization of the dose of PCB per animal, but also permitted the administration of higher levels of the chemical during the period of tumor development. In addition, variable tumor inoculum levels were employed to allow for the assessment of the effect of PCB on inoculum-sensitive parameters of tumor growth.

Effect of PCB Treatment on the Growth of Tumors Initiated by the Injection of $10^3$ Tumor Cells

The animals in this study were injected with 0, 50, 100, 200 or 400 mg/kg PCB, IP, every other day, beginning on the day of tumor inoculation. The experiment was terminated after 14 days.

As shown in Table 6, the body weight gain of the animals was dependent on the dose of PCB administered. Body weight gain was significantly inhibited at the 100, 200 and 400 mg/kg dose levels. In the 400 mg/kg group, lethality also occurred, with 9 out of 16 rats dead by day 14. The median time to death was 12 days. Death was preceded by a rapid loss in body weight (see Figure 5). Also, one animal injected with 200 mg/kg PCB exhibited severe toxic
Table 6. Body weight gain of animals intraperitoneally exposed to PCB (Aroclor 1254).

<table>
<thead>
<tr>
<th>PCB Dose mg/kg</th>
<th>No. of Animals</th>
<th>Body Weight (g) ± S.D.</th>
<th>Net Change²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>31</td>
<td>91 ± 11</td>
<td>179 ± 18</td>
</tr>
<tr>
<td>50</td>
<td>16</td>
<td>83 ± 6</td>
<td>167 ± 15</td>
</tr>
<tr>
<td>100</td>
<td>31</td>
<td>94 ± 13</td>
<td>168 ± 24</td>
</tr>
<tr>
<td>200</td>
<td>16</td>
<td>84 ± 8</td>
<td>124 ± 21</td>
</tr>
<tr>
<td>400</td>
<td>7</td>
<td>82 ± 5</td>
<td>108 ± 14</td>
</tr>
</tbody>
</table>

¹PCB injected every other day for 14 days.
²Net change in body weight, day 0 to 14, calculated for each animal individually and then averaged.
³Significantly different from control, p<0.01, Student's t test.

symptoms. Changes in his body weight during the experiment resembled that of the nonsurvivors given 400 mg/kg PCB, and, by day 14, he weighed only 75 grams. Tumor data from this animal, as well as the nonsurvivors, were not included in the following analysis of the effects of PCB on tumor growth.

As shown in Table 7, both the number of tumors that developed (the number of 'takes') and the tumor latent period (time to first palpable size) were affected by PCB exposure. The proportion of takes decreased with increasing PCB dose, and the latent period increased with increasing PCB dose, except at the highest dose level. The data at the 400 mg/kg dose level, however, may not be representative of
Figure 5. Body growth curves for nonsurvivors injected with 400 mg/kg PCB every other day. Each line represents one animal. Cessation of line indicates the death of the animal.
the dose, as only seven animals survived and only eight tumors developed in these animals. By analyzing the data from the survivors only, one is, in effect, selecting out the most resistant animals. If, in fact, the data from the three animals that died on day 14 are included in the calculations, the latent period is increased to 9.70 days and the number of tumor takes is reduced to 50%, as shown in brackets in Table 7.

Table 7. Effect of intraperitoneal exposure to PCB on the number of tumor takes and the length of the latent period of the Walker 256 tumor initiated by the injection of $10^3$ tumor cells.

<table>
<thead>
<tr>
<th>Dose of PCB$^1$ mg/kg</th>
<th>Proportion of Takes (%)</th>
<th>Tumor Latent Period$^2$ X ± s.e. (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50/62 (80.6)</td>
<td>8.44 ± 0.25</td>
</tr>
<tr>
<td>50</td>
<td>25/32 (78.1)</td>
<td>8.94 ± 0.46</td>
</tr>
<tr>
<td>100</td>
<td>42/62 (67.7)</td>
<td>9.64 ± 0.33$^a$</td>
</tr>
<tr>
<td>200</td>
<td>16/30 (53.3)</td>
<td>9.88 ± 0.46$^a$</td>
</tr>
<tr>
<td>400</td>
<td>8/14 (57.1)</td>
<td>9.00 ± 0.57</td>
</tr>
<tr>
<td>[400]$^3$</td>
<td>10/20 (50.0)</td>
<td>9.70 ± 0.52$^a$</td>
</tr>
</tbody>
</table>

$^1$PCB injected every other day for 14 days.

$^2$Average tumor latent period calculated by the formula: $TLP = \Sigma (td)/n$, where $t$ = the number of tumors first palpable on a given day, $d$ = the day the tumor is first palpable, and $n$ = the total number of tumors that developed.

$^3$See text for discussion.

$^a$Significantly different from control, $p<0.01$, Student's $t$ test.
The effect of PCB administration on the growth rate of the Walker tumor is shown graphically in Figure 6. Because of the few data points available on any given day in the 400 mg/kg group, it was felt that the calculation of a reliable growth curve for this group was not possible. This group was therefore omitted from the graph. The slope of the linear portion of the curves, from day 9 to day 13, indicates the maximum growth rate of the tumors in each group (Bertalanffy and Lau, 1962). Extrapolation of the data indicated that the growth rates of the tumors in each group were 550, 452, 318 and 268 mg/day, respectively, with increasing PCB dose. It can be concluded that the amount of inhibition in the growth rate of the Walker tumor initiated by the inoculation of $10^3$ tumor cells is dependent on the dose of PCB injected.

Referring to Table 8 for the data on dissected tumor weight at day 14, it can be seen that tumor weight following the administration of 50 mg/kg PCB, although smaller than the control tumor weight, is not significantly different statistically. All other PCB dose levels, however, caused a significant reduction in the weight of the tumors. The magnitude of tumor weight reduction was dose-related.
Figure 6. Effect of PCB treatment on the growth rate of the Walker 256 tumor initiated by the intramuscular injection of $10^3$ tumor cells. PCB (Aroclor 1254) injected IP every other day beginning on the day of tumor inoculation. Tumor weight calculated from in vivo tumor diameter measurements. Vertical bars indicate $\pm 1$ s.e. of the mean.
CALCULATED TUMOR WEIGHT (grams)

T.W. = 0.5435 \text{d}^3

- Control
- 50 mg
- 100 mg
- 200 mg

DAY POST-TUMOR INJECTION
Table 8. Effect of intraperitoneal injection of PCB (Aroclor 1254) on the weight of the Walker tumor 14 days after the injection of $10^3$ tumor cells.

<table>
<thead>
<tr>
<th>Dose of PCB (mg/kg)</th>
<th>Number of Tumors</th>
<th>Dissected Tumor Weight $X \pm$ s.e. (g)</th>
<th>t value</th>
<th>Tumor Weight % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>2.57 ± 0.23</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>50</td>
<td>25</td>
<td>2.09 ± 0.35</td>
<td>1.179</td>
<td>81.3</td>
</tr>
<tr>
<td>100</td>
<td>42</td>
<td>1.54 ± 0.16</td>
<td>3.515$^a$</td>
<td>59.9</td>
</tr>
<tr>
<td>200</td>
<td>16</td>
<td>1.15 ± 0.23</td>
<td>3.333$^a$</td>
<td>44.7</td>
</tr>
<tr>
<td>400</td>
<td>8</td>
<td>0.92 ± 0.14</td>
<td>2.850$^a$</td>
<td>35.7</td>
</tr>
</tbody>
</table>

$^1$PCB injected every other day for 14 days.

$^a$Significantly different from control, $p<0.01$, one-tailed $t$ test.
Effects of PCB Treatment on Tumor Growth and Host Survival Following the Injection of $10^7$ Tumor Cells

The injection of sufficiently large numbers of tumor cells results in progressive tumor growth leading to the death of the host animal. The following experiment was designed to test the antitumor effectiveness of PCB based on the survival time of the host. PCB was injected at a dose of 100 or 200 mg/kg, every other day, beginning on the day of tumor inoculation and repeated until the death of the animal. The experiment was terminated after 60 days.

Following the injection of $10^7$ tumor cells, the control animals exhibited, as expected, progressive tumor growth that resulted in the death of all animals within 20 days (see Table 9). Mean survival time (MST) was 13.5 days, calculated by the formula, $MST = \Sigma(t_f)/n$, where $t = \text{day of death}$, $f = \text{number of dead animals on a given day}$, and $n = \text{the total number of dead animals per group}$. For animals injected with 100 mg/kg PCB, mean survival time was increased to 17.7 days, representing a significant increase over the control group (Table 9), and one animal survived 60 days and was terminated. Mean survival time for the 200 mg/kg group was 18.9 days, also significantly increased over control survival time. Furthermore, four
Table 9. Effect of host exposure to PCB (Aroclor 1254) on mean survival time and number of tumor regressions following the intramuscular injection of $10^7$ tumor cells.

<table>
<thead>
<tr>
<th>PCB Dose mg/kg</th>
<th>Number of Takes</th>
<th>Number of Regressions</th>
<th>Dead by Day 60</th>
<th>MST in days (range)</th>
<th>Alive Day 60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>w/tumor</td>
<td>w/o tumor</td>
<td>w/tumor</td>
</tr>
<tr>
<td>0</td>
<td>32/32</td>
<td>0/32</td>
<td>16</td>
<td>0</td>
<td>13.5 (9-19)</td>
</tr>
<tr>
<td>100</td>
<td>32/32</td>
<td>0/32</td>
<td>15</td>
<td>0</td>
<td>17.7 (10-45)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>32/32</td>
<td>8/32</td>
<td>12</td>
<td>3</td>
<td>18.9 (9-53)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1PCB injected IP every other day until the death of the animal.

<sup>a</sup>Significantly different from control, p<0.01, Student's t test.
rats treated with 200 mg/kg PCB showed a total regression of their bilateral tumors. Of these four rats, one was still alive at day 60, whereas the remaining three animals died, one each on days 23, 25 and 53. The latter three apparently died of the toxic effects of the PCB treatment itself. Their total PCB intake ranged from 2400 to 5400 mg/kg, within the range of reported LD$_{50}$ values for Aroclor 1254 (Bruckner et al., 1973; Grant and Phillips, 1974). Moreover, it is not unlikely that an even greater increase in survival time would have been observed in the 200 mg/kg group had the PCB treatments been discontinued at some definitive time point rather than repeatedly administered until death. The fact that the animals treated with 200 mg/kg PCB died with smaller tumor burdens than control or 100 mg/kg PCB-treated animals (see Table 10) suggests that another factor, probably PCB toxicity, contributed to their deaths.

The effect of PCB exposure on the growth rate of the Walker tumor is shown in Figure 7. Since over 50% of the animals were dead by day 14, growth curves were terminated at this point. As shown in both Figure 7 and Table 11, the weight of the tumors at day 14 was inversely correlated with the dose of PCB injected. The magnitude of tumor weight inhibition at each PCB dose level was similar to that seen
Table 10. Total tumor burden at death in control and PCB-treated animals injected with $10^7$ tumor cells in both hind leg muscles.

<table>
<thead>
<tr>
<th>PCB Dose$^1$ mg/kg</th>
<th>Tumor Weight (g) ± s.e.</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Metastases</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>$35.5 \pm 3.9$</td>
<td>$5.0 \pm 0.8$</td>
<td>$41.7 \pm 4.8$</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>$34.3 \pm 3.0$</td>
<td>$4.1 \pm 0.8$</td>
<td>$38.4 \pm 3.1$</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>$21.9 \pm 3.6^a$</td>
<td>$2.5 \pm 0.5^a$</td>
<td>$24.4 \pm 4.0^a$</td>
<td></td>
</tr>
</tbody>
</table>

$^1$PCB injected IP every other day until the death of the animal.

$^a$Significantly different from control, $p<0.01$, Student's t test.
Figure 7. Effect of PCB treatment on the growth rate of the Walker 256 tumor initiated by the intramuscular injection of $10^7$ tumor cells. PCB (Aroclor 1254) injected IP every other day beginning on the day of tumor inoculation. Each point represents the mean of 32 tumors except where indicated in (). Vertical bars represent ±1 s.e. of the mean.
Table 11. Effect of intraperitoneally-injected PCB (Aroclor 1254) on the weight of the Walker tumor 14 days after the injection of $10^7$ tumor cells.

<table>
<thead>
<tr>
<th>Dose of PCB$^1$ (mg/kg)</th>
<th>Number of Tumors</th>
<th>Calculated Tumor Weight$^2$ ($\bar{X} \pm$ s.e. (g))</th>
<th>t value</th>
<th>Tumor Weight % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16</td>
<td>21.8 ± 1.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>100</td>
<td>18</td>
<td>13.9 ± 1.1</td>
<td>4.302$^a$</td>
<td>63.8</td>
</tr>
<tr>
<td>200</td>
<td>22</td>
<td>10.4 ± 1.2</td>
<td>6.230$^a$</td>
<td>47.7</td>
</tr>
</tbody>
</table>

$^1$PCB injected every other day beginning on the day of tumor inoculation.

$^2$Tumor weight (g) = 0.5435d$^3$, where d represents the mean diameter of the tumor measured with vernier calipers in vivo.

$^a$Significantly different from control, p<0.01, one-tailed t test.
following the injection of $10^3$ tumor cells (see Table 8).

Extrapolation of maximum tumor growth rates from the linear portion of the curves at the region of greatest slope (Figure 7) revealed that the difference in tumor weight was not simply a growth-rate-inhibition effect by PCB. Instead, it appeared that the length of time that a maximum growth rate was maintained was more important. Whereas control animals maintained a maximum tumor growth rate (1985 mg/day) for at least ten days (day 4-14), the PCB-treated animals were able to maintain maximum tumor growth rates (1700 mg/day for both groups) for only five days (day 4-9) and two days (day 4-6), respectively, with increasing PCB dose.

Under the conditions of the present experiment, only the 200 mg/kg dose level had a significant effect on the net body weight gain (gross body weight minus tumor weight) of the animals. During the first 14 days of the experiment, the animals gained 49.7, 47.2 and 30.0 grams, respectively, for control, 100 and 200 mg/kg PCB dose levels. The amount of weight gained by the 200 mg/kg group represented a 40% decrease with respect to the control.
The following experiment was designed to determine if a critical time period could be specified for the antitumor effect of PCB treatment. Three PCB injection time periods were selected, corresponding to various phases of tumor development and growth. PCB was injected, IP, at a dose of 100 mg/kg, daily for five days, during one of the following time periods: prior to tumor injection (day -5 to day 0); early, during the take and establishment phase (day 0 to day 5); or, delayed until after the tumor had become established and entered its linear growth phase (day 5 to day 10). Also included were a PCB-injected control which received PCB daily from day -5 to day 10, and a carrier-injected control which received peanut oil from day -5 to day 10. The initiating tumor inoculum was $10^6$ cells, injected on day 0. The experiment was terminated after 14 days.

The effects of specifically timed exposure to PCB on the growth rate of the Walker tumor is shown in Figure 8. The greatest amount of inhibition occurred with daily PCB exposure, prior to and during tumor growth (PCB, day -5 to 10). Also highly effective was treatment with PCB prior to tumor injection (PCB, day -5 to 0), and early treatment during the establishment of the tumor (PCB, day 0 to 5). Treat-
Figure 8. Effect of PCB exposure (100 mg/kg IP) at selected times prior to and/or during tumor growth on the growth rate of the Walker 256 tumor. Tumor growth was initiated by the intramuscular injection of $10^6$ tumor cells on day 0. Each point represents the mean of 20-30 tumors. Vertical bars indicate ±1 s.e. of the mean.
CALCULATED TUMOR WEIGHT (grams)

$T.W. = 0.5435 d_3$

- Oil, day -5 to 10
- PCB, day 5 to 10
- PCB, day 0 to 5
- PCB, day -5 to 0
- PCB, day -5 to 10

DAY POST-TUMOR INJECTION
ment of the animals with PCB after the tumor had become established (PCB, day 5 to 10) was least effective in slowing the growth of the tumor. The data for dissected tumor weights on day 14 are shown in Table 12.

Host survival was also influenced by the timing of PCB exposure, as shown in Table 12. Early host death appeared to be associated with the formation of tumor metastases in critical organs. All animals that died prior to day 14 had extensive metastatic involvement of the lungs and/or kidneys. None of the animals that survived to day 14 exhibited visible metastases to these critical organs, except two control animals that had tumorous lungs. It would appear, therefore, that although treatment with PCB prior to tumor injection was highly effective in inhibiting the growth of the primary tumors, it was least effective in preventing the establishment of critical metastases. On the other hand, whereas late treatment with PCB was not as effective in retarding primary tumor growth, it appeared to be highly active against critical metastases formation.

The effect of PCB treatment on the body weight gain of the animals is shown in Table 13. Only daily treatment with PCB from day -5 to day 10 significantly altered the body weight gain of the animals compared to the carrier-injected controls.
Table 12. Effect of PCB (Aroclor 1254) treatment during selected time periods of tumor growth on host survival and the weight of tumors 14 days after the injection of $10^6$ Walker tumor cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Tumors</th>
<th>Dissected Tumor Weight $\bar{X} \pm $ s.e. (g)</th>
<th>Tumor Weight % of control</th>
<th>Deaths Prior to Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier-injected</td>
<td>20</td>
<td>13.1 ± 1.4</td>
<td>--</td>
<td>5/15</td>
</tr>
<tr>
<td>PCB, day -5 to 0</td>
<td>22</td>
<td>10.4 ± 1.2$^b$</td>
<td>57.5</td>
<td>4/15</td>
</tr>
<tr>
<td>PCB, day 0 to 5</td>
<td>26</td>
<td>10.5 ± 1.1$^b$</td>
<td>58.0</td>
<td>2/15</td>
</tr>
<tr>
<td>PCB, day 5 to 10</td>
<td>30</td>
<td>14.4 ± 1.3$^a$</td>
<td>79.6</td>
<td>0/15</td>
</tr>
<tr>
<td>PCB, day -5 to 10</td>
<td>30</td>
<td>8.1 ± 1.0$^b$</td>
<td>44.8</td>
<td>0/15</td>
</tr>
</tbody>
</table>

1100 mg/kg PCB injected IP daily during treatment period; carrier-injected controls injected with peanut oil from day -5 to day 10.

$^a,b$Significantly different from carrier-injected control, $p<0.05$ and 0.01 respectively. Student's t test.
Table 13. Body weight gain of animals intraperitoneally injected with PCB (Aroclor 1254) during selected time periods of tumor growth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Animals</th>
<th>Body Weight (g) ± S.D.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier-injected</td>
<td>10</td>
<td>97 ± 10</td>
<td>170 ± 35</td>
</tr>
<tr>
<td>PCB, day -5 to 0</td>
<td>11</td>
<td>101 ± 6</td>
<td>178 ± 26</td>
</tr>
<tr>
<td>PCB, day 0 to 5</td>
<td>13</td>
<td>93 ± 9</td>
<td>173 ± 25</td>
</tr>
<tr>
<td>PCB, day 5 to 10</td>
<td>15</td>
<td>90 ± 7</td>
<td>164 ± 23</td>
</tr>
<tr>
<td>PCB, day -5 to 10</td>
<td>15</td>
<td>102 ± 7</td>
<td>148 ± 20</td>
</tr>
</tbody>
</table>

100 mg/kg PCB injected daily during treatment period; carrier-injected controls injected with peanut oil from day -5 to day 10.

2Final gross body weight minus dissected tumor weight.

3Net change in body weight, day -5 to day 14, calculated for each animal individually and then averaged.

aSignificantly different from carrier-injected control, p<0.01, Student's t test.
Effect of PCB Treatment on the Survival Time of Animals Bearing the Ascitic Form of the Walker Tumor

The following study was designed to assess the cytotoxic effects of PCB on the tumor cells. Since the ascitic form of the Walker tumor is confined entirely to the peritoneal cavity and its membranes, the administration of PCB intraperitoneally should allow for intimate contact between the dividing tumor cells and the chemical. Based on the assumption that the survival time of the host bearing an ascitic tumor is dependent on the formation of a critical number of tumor cells, alteration in the mean survival time of the animals could reflect a cytotoxic action by PCB. Therefore, PCB was injected IP at a dosage of 100 mg/kg either daily or every other day beginning on the day of tumor inoculation ($10^5$ tumor cells) and repeated until the death of the host. Control groups included both non-injected and carrier-injected tumor-bearing animals.

All control animals, both non-injected and carrier-injected, were dead within 180 hours (7.5 days) after tumor inoculation. Only 50% (15/30) of the PCB-treated animals were dead in the same time interval. The mean survival time for each group is shown in Table 14. The injection of peanut oil (carrier) caused no significant deviation in mean
Table 14. Effect of PCB exposure on mean survival time (MST) of animals bearing ascitic Walker 256 tumor.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Animals</th>
<th>MST in hours ± s.e. (^3) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjected control</td>
<td>14</td>
<td>166 ± 2 (152-180)</td>
</tr>
<tr>
<td>Control(^1)</td>
<td>15</td>
<td>167 ± 3 (152-180)</td>
</tr>
<tr>
<td>PCB-treated(^1)</td>
<td>15</td>
<td>193 ± 10 (168-324)(^a)</td>
</tr>
<tr>
<td>Control(^2)</td>
<td>10</td>
<td>164 ± 2 (156-176)</td>
</tr>
<tr>
<td>PCB-treated(^2)</td>
<td>15</td>
<td>185 ± 7 (160-244)(^a)</td>
</tr>
</tbody>
</table>

\(^1\)Oil or PCB (100 mg/kg) injected IP every other day beginning on the day of tumor inoculation.

\(^2\)Oil or PCB (100 mg/kg) injected IP every day beginning on the day of tumor inoculation.

\(^3\)Animals checked every four hours for survival time.

\(^a\)Significantly different from respective carrier-injected control, p<0.01, Student's t test.

The injection of PCB, however, caused a significant increase in mean survival time. Animals injected with PCB every other day showed a 15.6% (26 hour) increase in mean survival time compared to animals injected with peanut oil every other day. Animals injected with PCB every day showed a 12.8% (21 hour) increase in mean survival time compared to animals injected with oil every day. The difference in survival time between the two PCB-treated groups was not statistically significant (p<0.05), suggesting the absence of a dosage effect on host survival time. It is
unlikely that PCB toxicity in the animals injected with PCB every day accounted for the absence of a dosage effect, as there was no indication of PCB toxicity reflected in the body growth rates of the animals (Table 15).

Table 15. Body weight gain of animals injected with PCB (Aroclor 1254) and bearing ascitic Walker 256 tumors.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight (g) ± S.D.</th>
<th>Day 0</th>
<th>Day 6</th>
<th>Net Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjected control</td>
<td>100 ± 8</td>
<td>141 ± 10</td>
<td>+41</td>
<td></td>
</tr>
<tr>
<td>Control¹</td>
<td>100 ± 7</td>
<td>140 ± 11</td>
<td>+40</td>
<td></td>
</tr>
<tr>
<td>PCB-treated¹</td>
<td>100 ± 7</td>
<td>141 ± 10</td>
<td>+41</td>
<td></td>
</tr>
<tr>
<td>Control²</td>
<td>68 ± 6</td>
<td>99 ± 9</td>
<td>+31</td>
<td></td>
</tr>
<tr>
<td>PCB-treated²</td>
<td>71 ± 7</td>
<td>100 ± 10</td>
<td>+29</td>
<td></td>
</tr>
</tbody>
</table>

1 and 2 as in Table 14

As shown in Table 16, treatment with PCB did not alter the number of tumor cells per volume of ascitic fluid present at death, nor did it alter the volume of ascites produced. This finding supports the previous assumption that a critical number of tumor cells is required to cause death. However, because mesenteric implantation of tumor cells regularly occurs during ascitic tumor growth, and, in some cases, a small solid tumor developed at the injection site,
Table 16. Effect of PCB treatment on the number of free tumor cells in the peritoneal fluid and the volume of ascitic fluid produced in animals bearing ascitic Walker 256 tumors. Figures given are the mean and standard deviation of the mean.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor Cells per ml $\times 10^6$</th>
<th>Volume (ml) of Ascitic Fluid</th>
<th>Total No. of Free Tumor Cells $\times 10^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113 ± 18</td>
<td>8.0 ± 2.4</td>
<td>9.0 ± 2.9</td>
</tr>
<tr>
<td>PCB every other day 2</td>
<td>115 ± 21</td>
<td>7.9 ± 2.1</td>
<td>8.9 ± 1.6</td>
</tr>
<tr>
<td>PCB every day 2</td>
<td>115 ± 28</td>
<td>7.7 ± 2.8</td>
<td>8.8 ± 1.6</td>
</tr>
</tbody>
</table>

1 Measured in five randomly selected animals from each treatment group.
2 100 mg/kg Aroclor 1254 injected intraperitoneally.
it was not possible to determine the absolute number of tumor cells which proliferated from the initial inoculum.
DISCUSSION

This study was undertaken to assess the effects of host exposure to polychlorinated biphenyls, a major environmental contaminant, on the transplantability and growth of the Walker 256 tumor in rats. Although the direct carcinogenic potential of this contaminant has been investigated, little attention has been directed toward the assessment of possible effects on tumor growth per se. Based primarily on reports that PCBs can act as immunosuppressive agents, it was felt that exposure to this chemical might constitute a health hazard with respect to tumor growth that had not been previously recognized.

It was indeed interesting, as well as unexpected, to observe the marked protective effect that PCB exposure conferred on the host in response to the implantation and growth of inoculated tumor cells. In animals exposed to PCB, not only was the tumor growth rate retarded, but, under appropriate transplantation conditions, PCB administration was observed to increase the tumor latent period, increase host resistance to the take of the tumor, increase host survival time, and increase the incidence of tumor regression. All are suggestive of a heightened rather than a suppressed immune response.

In many cases, no notable side effects of the PCB
treatment accompanied the inhibition of tumor growth, although body weight inhibition and liver damage (see Appendix B) were seen to occur with relatively high levels of PCB. It was also remarkable that a PCB level as low as 5 ppm in the diet for 30 days was sufficient to induce significant tumor growth inhibition.

**Dietary versus Intraperitoneal Exposure**

If one plots the percent inhibition of tumor weight as a function of total PCB intake as in Figure 9, it appears that neither the route of PCB exposure (oral vs IP) nor the number of tumor cells used as initiating inoculum ($10^3 - 10^7$) was important in determining the magnitude of tumor weight inhibition. Rather, the total PCB intake appears to be the critical determinant. Since almost complete absorption of PCB from the GI tract has been reported to occur following oral exposure to PCB (Albro and Fishbein, 1972; Kiriyama et al., 1974b), it is, perhaps, not unusual that the same total dose of PCB given orally or IP resulted in the same tumor weight inhibition. What might be considered unusual is that the same tumor weight inhibition occurred even though the exposure time periods were so different. Whereas the IP-treated animals, in most cases, received PCB only during the period of tumor growth, the animals exposed
Figure 9. Reduction in tumor weight as a function of total PCB intake, independent of the route of PCB exposure or number of tumor cells used as initiating inoculum. (●) $10^5$ cells, dietary exposure prior to and during tumor growth, (▲) $10^3$ cells, dietary exposure prior to and during tumor growth, (□) $10^6$ cells, IP exposure prior to and during tumor growth, (▲) $10^3$ cells, IP exposure during tumor growth only, (○) $10^7$ cells, IP exposure during tumor growth only.
to PCB via the diet consumed their greatest dose of PCB prior to tumor injection. Since the same total PCB intake resulted in the same degree of tumor inhibition, it suggests that the PCB consumed prior to tumor injection was at least as effective in inhibiting tumor growth as was the PCB given during the actual period of tumor development.

That treatment with PCB prior to tumor injection was effective was confirmed in the experiment in which animals were injected with PCB (100 mg/kg) for five days prior to tumor inoculation but received no PCB during the period of tumor growth. The mean tumor weight of the animals pretreated with PCB was reduced to 57% of the control tumor weight, indicating that pretreatment is highly effective in inhibiting tumor growth.

It is possible that the phenomenon of preprotection is related to the storage dynamics of PCB in the body. Since the more highly chlorinated isomers are absorbed and retained with little metabolism or excretion occurring, internal exposure to PCB can continue long after direct external exposure has ceased. Grant et al. (1974), for example, reported that hepatic microsomal enzyme activity remains significantly elevated in rats for at least 182 days after removal of a PCB-containing diet. Also several Yusho patients displayed chloracne symptoms for as long as three
years after the exposure episode occurred (Fishbein, 1974). This has been attributed to the persistence of PCBs in the body.

**Influence of Host Body Growth Rate on Tumor Growth Rate**

Nonspecific host inanition, whether it be drug-induced or calorie restriction-induced, can significantly inhibit the growth of many transplantable and carcinogen-induced tumors (Laster et al., 1961; White, 1961). Considerable inhibition of body weight gain occurred in the present study following exposure to diets containing 400 or 800 ppm PCB and following intraperitoneal injection of 200 or 400 mg/kg PCB. Food consumption measurements indicated that the decrease in body weight gain was due, at least in part, to decreased food intake, i.e., host inanition. However, without specific pair-feeding data available, it is difficult to assess the significance of this inanition.

Most reports in the literature concerning the effects of food restriction of the growth of the Walker tumor have involved extreme calorie restriction which was accompanied by a substantial loss in host body weight. As shown in Table 17, the reported degree of tumor inhibition varied greatly but was generally in accordance with the degree of
Table 17. Literature review of the effects of calorie restriction and the accompanying body weight loss on the growth of the Walker 256 carcinosarcoma in rats.

<table>
<thead>
<tr>
<th>Restriction Parameters</th>
<th>Body Weight Change (g)</th>
<th>Tumor Weight</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>To give a body weight loss of 17.5 g in 15 days</td>
<td>Ad lib fed +34.3</td>
<td>Ad lib fed 24.4g</td>
<td>Bischoff et al., 1935; cited by White, 1961</td>
</tr>
<tr>
<td></td>
<td>Restricted -17.5</td>
<td>Restricted 22.8g</td>
<td></td>
</tr>
<tr>
<td>To maintain a constant body wt. for 11-13 days</td>
<td>Ad lib fed +26</td>
<td>7% inhibition of tumor weight</td>
<td>McEuen &amp; Thomson, 1933; cited by White, 1961</td>
</tr>
<tr>
<td></td>
<td>Restricted - 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic diet diluted 50% w/ nonnutritive bulk for 21 days</td>
<td>Ad lib fed +130</td>
<td>Ad lib fed 50.3g</td>
<td>Morrison, 1973</td>
</tr>
<tr>
<td></td>
<td>Dilute diet + 80</td>
<td>Dilute diet 53.5g</td>
<td></td>
</tr>
<tr>
<td>3 days after tumor injected, fast for 1 day, then given 4, 3 or 2 g of food per day for 3 days</td>
<td>Ad lib fed +29</td>
<td>Ad lib fed 4.6g</td>
<td>DeWys et al., 1968</td>
</tr>
<tr>
<td></td>
<td>Fast + 4 g -10</td>
<td>Fast + 4 g 2.3g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fast + 3 g -11</td>
<td>Fast + 3 g 1.5g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fast + 2 g -18</td>
<td>Fast + 2 g 1.5g</td>
<td></td>
</tr>
<tr>
<td>8, 6, 4 or 2 g of food per day</td>
<td>Ad lib fed +42</td>
<td>As % of ad lib fed:</td>
<td>Tarnowski et al., 1966</td>
</tr>
<tr>
<td></td>
<td>8 g/day +16</td>
<td>8 g/day 0.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 g/day + 1</td>
<td>6 g/day 0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 g/day -13</td>
<td>4 g/day 0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 g/day -29</td>
<td>2 g/day 0.21</td>
<td></td>
</tr>
</tbody>
</table>
restriction.

In the present study, no PCB-treated groups experienced body weight loss. Rather, the amount of weight gained by PCB-treated animals was less than the controls. This situation possibly most closely approximates the Morrison study (Table 17) in which the experimental group gained only 80 grams compared to a weight gain of 130 grams in the controls. The results of Morrison's study indicated that there was no effect on the growth of the Walker tumor under these conditions. Furthermore, in the present study, there did not appear to be any correlation between the individual amount of body weight gained by an animal and the size of his resultant tumors within any given group of animals, control or PCB-treated. Two examples are shown in Figures 10 and 11. Therefore it seems reasonable to conclude that the magnitude of body weight gain inhibition seen in the present study was not a significant factor in the observed inhibition of tumor growth.

Reliability of Measuring Tumor Size in situ

The measurement of the size of a tumor in the living animal for quantitative studies of tumor growth offers the chief advantage of not having to serially sacrifice animals at various times after tumor injection. If the size of the
Figure 10. Tumor size as a function of carcass growth rate (body weight minus tumor weight) in non-PCB injected rats. Animals injected with $10^3$ Walker tumor cells in each hind limb on day 0 and killed on day 14.
Figure 11. Tumor size as a function of carcass growth rate (body weight minus tumor weight) in PCB-injected (200 mg/kg) rats. Animals were injected with $10^3$ tumor cells in each hind limb on day 0 and killed on day 14. PCB injections began on day 0 and were repeated every other day.
NET BODY WEIGHT GAIN (grams)

TUMOR WEIGHT (grams)

DAY 0-14
tumor can be reliably estimated in situ, not only are animal lives saved, but also more accurate data are obtained by eliminating biological variability between the serially sacrificed animals. Shrek (1935) derived a reliable formula for the estimation of the volume and/or weight of the Walker tumor from the mean diameter of the tumor measured with vernier calipers. He showed that the cube root of the volume ($V$) is a linear function of the mean diameter ($d$), and that the calculated volume of the tumor closely approximates the actual volume, where $V = 0.5236d^3$, the formula for the volume of a sphere. Tumor weight could then be calculated by multiplying the volume of the tumor by its specific gravity (1.038), whence tumor weight (g) = 0.5435$d^3$ (cm). These formulae have been used by a number of investigators for the estimation of tumor size in situ (Migliarese and Bly, 1956; Morrison, 1968; Song and Levitt, 1971; Morrison, 1973).

The accuracy of this method was determined in the present study by comparing the calculated tumor weights to the dissected tumor weights, shown graphically in Figure 12. No correction was made for skin thickness, as has been done by some investigators (Grollman and Crass, 1966; Song and Levitt, 1971). The average error in calculation of individual tumor weights for nearly 300 tumors was 9.2% How-
Figure 12. Accuracy of determining the weight of the Walker tumor by caliper measurements of the tumor diameters \textit{in vivo}. 

\[
T.W. = 0.5435d^3
\]
ever, because the error for individual tumors was not always in the same direction (i.e., always heavy or always light), the error for the calculated mean tumor weight for groups of animals was much less than 9.2%, as shown in Table 18. There appeared to be a tendency to overcalculate the weight of small tumors and to undercalculate the weight of large tumors. For small tumors, the error may have been due to not using a skin correction factor. For large tumors, the specific gravity of the tumor may be different due to large areas of central necrosis. Error is also introduced when the tumors are not entirely spherical in shape. These errors, however, apply randomly to treatment as well as control groups, such that, for comparative purposes, the method is highly reliable.

Possible Mechanisms for PCB-Induced Host Resistance to Tumor Growth

The primary purpose of the present study was to determine if PCB intoxication could modify the growth of a tumor, using transplantation techniques. Secondarily, it was to explore situations that would maximize the observed inhibition of tumor growth. Although no intensive study was developed concerning mechanisms, some speculations consistent with the observations can be offered.
Table 18. Agreement between calculated and dissected tumor weights.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calculated Tumor Weight (g)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Dissected Tumor Weight (g)</th>
<th>Error&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10&lt;sup&gt;3&lt;/sup&gt; tumor cells injected:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>2.64 ± 0.23</td>
<td>2.57 ± 0.23</td>
<td>+2.7</td>
</tr>
<tr>
<td>50 mg/kg PCB</td>
<td>2.13 ± 0.35</td>
<td>2.09 ± 0.35</td>
<td>+1.9</td>
</tr>
<tr>
<td>100 mg/kg PCB</td>
<td>1.58 ± 0.17</td>
<td>1.54 ± 0.16</td>
<td>+2.6</td>
</tr>
<tr>
<td>200 mg/kg PCB</td>
<td>1.23 ± 0.25</td>
<td>1.15 ± 0.23</td>
<td>+7.0</td>
</tr>
<tr>
<td>400 mg/kg PCB</td>
<td>0.98 ± 0.15</td>
<td>0.92 ± 0.14</td>
<td>+6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10&lt;sup&gt;6&lt;/sup&gt; tumor cells injected:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>17.34 ± 1.29</td>
<td>18.12 ± 1.39</td>
<td>-4.3</td>
</tr>
<tr>
<td>PCB, day -5 to 0</td>
<td>10.05 ± 1.16</td>
<td>10.40 ± 1.23</td>
<td>-3.4</td>
</tr>
<tr>
<td>PCB, day 0 to 5</td>
<td>10.18 ± 1.06</td>
<td>10.54 ± 1.13</td>
<td>-3.4</td>
</tr>
<tr>
<td>PCB, day 5 to 10</td>
<td>14.07 ± 1.13</td>
<td>14.44 ± 1.26</td>
<td>-2.6</td>
</tr>
<tr>
<td>PCB, day -5 to 10</td>
<td>7.90 ± 0.92</td>
<td>8.13 ± 0.98</td>
<td>-2.8</td>
</tr>
</tbody>
</table>

<sup>1</sup>Calculated tumor weight (g) = 0.5435d<sup>3</sup>, where d represents the mean diameter of the tumor measure with vernier calipers in vivo.

<sup>2</sup>% Error = \( \frac{\text{Calculated tumor wt} - \text{Dissected tumor wt}}{\text{Calculated tumor wt}} \) x 100
Direct Cytotoxicity

It is possible that PCB inhibits the growth of the Walker tumor by directly killing the tumor cells injected into the host animal. The assessment of such cytotoxic activity is generally conducted on cells in tissue culture, which provides for direct analysis of the effects of the test compound on intact, dividing cells and eliminates the possible obscuration of effects by extrinsic factors present in in vivo test systems (Gabliks and Friedman, 1965a; Litterst et al., 1969). Cytotoxic effects are usually evaluated on the basis of growth inhibition, measured as decreases in cell number, DNA, RNA or protein synthesis, and/or cell destruction observed microscopically (Gabliks and Friedman, 1965b; Chung et al., 1967; Johnson and Weiss, 1967; Myhr, 1973).

Several investigators have reported on the cytotoxic potential of PCBs. Offner et al. (1973) described the cytotoxic action of Aroclor 1254 on rat cerebellum explants. They reported that, although the initial growth of the cells in the presence of 100 μM PCB was comparable to controls, morphological changes appeared after about 20 days, and, by the termination date (day 25-27), some neurons were distinctly degenerated. Degenerative changes included vacuolization and swelling of the cell body and destruction of cell pro-
cesses. Hoopingarner et al. (1972) more clearly demonstrated the cytotoxic action of various PCB mixtures on Chinese hamster (quasi-diploid epithelial) cells in culture. They found that 50 ppm Aroclor 1254 caused a 34.3% drop in cell number by 24 hours and a 75% decrease in cell number by 30 hours, compared to non-PCB treated controls. Of the Aroclor products tested, toxicity within cell cultures generally increased as the degree of chlorination decreased. Litterst and Lichtenstein (1971), utilizing cultures of human skin fibroblasts and HeLa cells, evaluated the toxicity of Aroclor 1254 by determining the concentration of chemical that produced a 50% decrease in the number of cells per culture (ID$_{50}$) after 48 hours of exposure. The ID$_{50}$ for HeLa cells was 63 ppm, whereas for fibroblasts it was 110 ppm. This would suggest that the malignant cells were nearly twice as sensitive to the cytotoxic action of PCB as were the non-malignant cells. This observation may be relevant to the present study in that the Walker tumor could be inhibited apparently without affecting the growth of other body tissues (as reflected in body growth rate). The mechanism of cytotoxic action by PCBs has not been determined.

In the present study, an attempt was made to assess the cytotoxic action of PCB on Walker tumor cells in vivo by determining the effects of intraperitoneally injected PCB
on the ascitic variant of the tumor. Since the ascitic form of the tumor is confined entirely to the peritoneal cavity and its membranes, the injection of PCB IP should have allowed for intimate contact between the dividing tumor cells and the chemical. The results of this study indicated that PCB may have some direct cytotoxicity against Walker tumor cells.

It has been shown for several ascitic tumors that host death ensues when the tumor cells in the peritoneal cavity have proliferated to a critical number (Klein and Revesz, 1953). Personal experience with the Walker tumor suggests that the phenomenon holds true for this tumor as well. Therefore, if a treatment causes an increase in the survival time of the host, one might assume that the treatment acted to reduce the number of dividing cells such that it took longer to reach the critical number of cells necessary to cause death.

Treatment of the host with PCB during ascitic tumor growth resulted in a significant increase in host survival time, suggesting a cytotoxic effect of PCB. However, when the dose of PCB was essentially doubled (injected every day rather than every other day), no further increase in survival time was observed. There are at least two possible explanations for the apparent absence of a dosage effect.
It is possible that the absence of a dosage effect was due to the rapid removal of PCB from the peritoneal cavity after injection, such that the residual amount of PCB remaining in the peritoneal cavity to which the cells were directly exposed was similar under both treatment regimes. The fact that the ascitic fluid, when examined after death, was not very oily suggests that the PCB-peanut oil solution had been efficiently absorbed from the peritoneal cavity.

It is also possible that the lower dose of PCB was a saturation dose. If, for instance, one type of cell composing the tumor was more sensitive to the cytotoxic action of PCB, and, if the lower dose of PCB was sufficient to kill the majority of the sensitive cells, then further increases in the dose of PCB administered would not be expected to further increase host survival time. Since the Walker tumor is composed of at least three types of cells (carcinoma, sarcoma and undifferentiated cells), it is possible that one cell type was more sensitive than the others.

Histological examination of several solid tumors from PCB-treated and control animals further indicated that it may have been the sarcoma element that was most sensitive to PCB. Although such observations are difficult to quantify, there were several tumors from animals exposed to high levels of PCB that appeared as solid masses of carci-
noma with no visible sarcomatous portions present. In control animals, the sarcoma elements were routinely found bordering blood vessels and areas of necrosis. Moreover, gas chromatographic analysis of PCB tissue residues indicated that the solid tumors were assimilating some PCB (see Appendix A), which would have provided the opportunity for direct cytotoxic activity by PCB.

There is, however, an additional observation that may argue against direct cytotoxicity as the primary mechanism for PCB tumor inhibitory activity. If cytotoxicity for the tumor cells is responsible for smaller tumors, then it should make no difference if the animals are exposed to PCB when the tumor cells are first injected or if exposure is delayed until after the tumor is established. One would expect that the same relative reduction in tumor size would occur after exposure to equal dose levels of PCB, independent of the timing of exposure. In the present study, however, this was not the case. In fact, if PCB treatment was delayed until after the tumor was established, PCB was only about half as effective in reducing the size of the tumor. It would appear, instead, that some condition must be established in the host either before the tumor is injected or early during tumor establishment which accounts for at least a part of the inhibitory activity of PCB.
PCB-Induced Inhibition of Critical Enzyme Systems

It is a well-documented fact that tumors derive most, if not all, of their energy for growth from glycolysis, utilizing glucose both anaerobically, producing lactate, and aerobically, producing pyruvate (Norman and Smith, 1956; Warburg, 1956a; Warburg, 1956b; Hiatt, 1957; Papaconstantinou and Colowick, 1961; Nyhan, 1966; Racker, 1972). Except for the net three moles of ATP produced at the substrate level during glycolysis, most of the ATP is produced by the coupling of oxidation and phosphorylation in the electron transport system (Lehninger, 1970). PCBs have been shown to inhibit the activity of six different enzymes that could result in a significant alteration of this generation of energy.

PCBs have been shown to inhibit the activity of NADH-oxidase and succinoxidase, studied in heavy beef heart mitochondria preparations (Pardini, 1971). All PCB mixtures tested (21-62% chlorine) were inhibitory, depressing the activity of both enzymes to below 20% of the activity of the untreated control. Since these two enzymes catalyze the initial transfer of electrons (to a flavoprotein) at the first step in the electron transport system (Lehninger, 1970), their inhibition by PCB could significantly reduce the production of energy (ATP) within the cells.
In addition, it has been reported that PCBs inhibit the activity of ATPase (Yap et al., 1971; Cutkomp et al., 1972; Desaiah et al., 1972; Koch, 1972). Yap et al. (1971) described the inhibition of both Mg$^{++}$ ATPase and Na$^+$K$^+$ ATPase in fish brain, kidney and liver preparations at PCB levels as low as 0.03 ppm. Similarly, Koch (1972) reported greater than 50% inhibition of the activity of both oligomycin-sensitive (mitochondrial) and oligomycin-insensitive (cytoplasmic) Mg$^{++}$ ATPase in fish muscle homogenates at PCB levels as low as 2 ppm. It has been postulated (Mitchell's Chemiosmotic Theory) that Mg$^{++}$ ATPase catalyzes the formation of ATP from ADP and inorganic phosphate (Lehninger, 1970). Therefore, the inhibition of Mg$^{++}$ ATPase by PCB could further add to the reduced production of energy, at both the substrate and electron transport system levels.

Furthermore, it has been shown that PCBs inhibit the activity of several liver gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEP ck), important in the formation of glucose from pyruvate (Mehlman et al., 1975), and glucose-6-phosphatase, which acts to donate free glucose to the blood (Allen, et al., 1973; Allen et al., 1975; Litterst et al., 1972). The activity of PEP ck was reduced by 40% after Aroclor 1254 treatment (500 ppm in the diet for two weeks) (Mehlman et al., 1975), and a decidedly hypogly-
cemic state has been produced in rats fed a diet containing 400 ppm PCB (Aroclor 1254) for two weeks (Friend et al., 1973; Mehlman et al., 1974). Since hepatic gluconeogenesis supplies glucose for extrahepatic consumption, the inhibition of the synthesis of glucose (via PEP ck) and the induction of a hypoglycemic state by PCB could markedly influence the growth of tumors dependent on the catabolism of glucose for energy. That the inhibition of host gluconeogenesis is an effective means of cancer chemotherapy has been verified in tests at the Syracuse Cancer Research Institute (Gold, 1968). Hydrazine sulfate, an inhibitor of gluconeogenesis at the PEP ck reaction, has been demonstrated to be an effective in vivo inhibitor of several experimental animal tumors, including the Walker 256 carcinosarcoma (Gold, 1971; Gold, 1973), and has been used, with preliminary indications of success, against a wide array of advanced human cancers (Gold, 1974). It is interesting that hydrazine sulfate lacks direct cytotoxicity on cancer cells in culture (Gold, 1975).

In addition to inhibiting gluconeogenesis, however, PCBs could further act to limit the supply of glucose to tumors by inhibition of glucose-6-phosphate and Na\(^+\)K\(^+\) ATPase. Na\(^+\)K\(^+\) ATPase, as it applies to the present situation, is required for the active transport of glucose across the
cell membrane.

Thus, PCB exposure could induce a combination of enzyme inhibitions which may act to curtail significantly the energetic machinery of the tumor cells and result in inhibition of tumor growth. This hypothesis is further supported by observations preceding PCB-induced mortality. Animals exposed to lethal levels of PCB exhibit a marked, rapid decline in body weight, despite continued, albeit low level, food consumption. No overt signs of the cause of death are apparent at autopsy, except that the animals are extremely thin, with no visible signs of body fat. Liver damage, *per se*, is generally not considered sufficient to cause death, and other organs appear normal. It might suggest that the animals may not be able to utilize the food they consume, possibly due to the inhibition of the previously discussed enzyme systems, and literally "starve to death in the face of plenty". Furthermore, this hypothesis would tend to support the observed effectiveness of pretumor exposure to PCB as well as the lesser effectiveness of delaying exposure until after the tumor had become established, since repeated exposure to PCB would probable induce a progressively severe hypoglycemic and energy-deficient state.
Suppression of the Humoral Immune Response

One of the most important discoveries in the field of tumor biology in recent years is the conclusive demonstration that most tumors, spontaneous or induced, possess antigenicity capable of eliciting a specific immune response in the host directed against itself (Old and Boyse, 1964; Mikulska et al., 1966; Takeda et al., 1966; Deckers et al., 1971; Aoki et al., 1974; Bartholomaeus et al., 1974). This immune response is characterized primarily by the development of specifically sensitized, cytotoxic lymphocytes (T-cells), which may act directly to kill tumor cells, or may mediate their cytotoxic action through macrophages by the secretion of lymphokines, such as macrophage-arming factor (MAF) and migration-inhibition factor (MIF) (Klein and Sjogren, 1960; Cerottini and Brunner, 1974; Herberman, 1974; Marx, 1974; Ritts and Neel, 1974). The inability of the host, equipped with this cytotoxic defense mechanism, to rid itself of a tumor, has been attributed to the presence of serum "blocking factors" (Hellstrom and Hellstrom, 1969; Bansal et al., 1972; Feldman, 1972; Ran and Witz, 1972; Baldwin et al., 1973; Baldwin et al., 1974; Hellstrom and Hellstrom, 1974). Evidence is accumulating which indicates that these blocking factors consist of antibodies or antigen-antibody complexes that are able to interfere with...
specific T-cell mediated immunity, either by binding to the cytotoxic effector cells to inhibit their activity or by binding to antigenic sites on the tumor cells, protecting them from attack (Takasugi and Hildemann, 1969; Hellstrom and Hellstrom, 1970; Sjogren et al., 1971; Baldwin et al., 1972; Mitchell, 1972; Rowley et al., 1973).

Insofar as PCBs are concerned, there is ample evidence, both direct and indirect, to indicate that PCBs interfere with humoral immune responses (i.e., antibody formation) (Friend and Trainer, 1970; Vos and de Roij, 1972; Koller and Thigpen, 1973; Street and Sharma, 1975). No studies to date, however, have confirmed that PCBs interfere with cell-mediated (T-cell) immune reactions. Although an effect on the cell-mediated response has been suggested from the observation of thymic cortical atrophy and decreased numbers of circulating lymphocytes (Vos, 1972; Street and Sharma, 1975), a quantitative study involving skin reactivity to tuberculin in rabbits failed to show any significant alteration in response after PCB exposure (Street and Sharma, 1975). Furthermore, thymic atrophy was not seen in the present study following dietary exposures of up to 400 ppm PCB, nor was a decrease in the number of circulating lymphocytes observed (unpublished data). Therefore, it is possible that what appeared to be a heightened immune re-
response in the PCB-exposed tumor-bearing animals in this study was, in reality, due to an inhibition of the formation of serum blocking factor. If the cell-mediated response remained intact, but the formation of serum blocking factor was inhibited, then one could have expected to see slower tumor growth and an increased incidence of tumor regression in the PCB-treated animals. This explanation would also be consistent with the observation that delaying host exposure to PCB until after the tumor was established was not as effective as PCB treatment before or during the early stages of tumor growth, when antigen recognition and development of specific antibody-producing cells and sensitized T-cells would begin. Furthermore, when one examines the tumor growth curves following the injection of large numbers of tumor cells (see Figures 7 and 8), it can be seen that on approximately day 6-9, the tumor growth curves of the PCB-exposed animals began to deviate more severely from the control curves. Whereas control tumors continue to grow at a maximum rate, the growth rate of tumors from PCB-exposed animals diminishes. It is, perhaps, more than coincidental that it also takes approximately 6-10 days for serum blocking activity to be detected and for a significant cell-mediated immune response to become established (Henney et al., 1971; Oren et al.,
1971; Jurin, 1973; Jones et al., 1974; Jurin and Suit, 1974; Whitney et al., 1974).
SUMMARY AND CONCLUSIONS

This thesis provides experimental evidence to indicate that polychlorinated biphenyls (Aroclor 1254) have an inhibitory effect on the growth of at least one experimental cancer, the Walker 256 carcinosarcoma in rats. Both dietary and intraperitoneal treatment with PCB resulted in a dose-dependent inhibition of tumor growth, as measured in the weight of the tumor after a specified growth period. The magnitude of tumor weight inhibition was independent of the route of PCB exposure and independent of the number of tumor cells used as tumor-initiating inoculum. Following implantation of small numbers of tumor cells (10³), PCB exposure resulted in total inhibition of tumor growth in some animals, as reflected by a dose-dependent decrease in the number of tumor takes. In other animals, the tumors that did develop showed a significantly lengthened latent period. With the implantation of large numbers of tumor cells (10⁶), a significant increase in host survival time was observed, which was accompanied by an increased incidence of tumor regressions. Preconditioning of the host with PCB for five days prior to tumor inoculation was as effective in inhibiting tumor growth as was treatment with PCB during the first five days after tumor inoculation. If PCB exposure was delayed until after the tumor was estab-
lished, it was less effective in inhibiting the growth of the tumor. In animals bearing ascitic Walker tumors, intraperitoneal injection of PCB resulted in a significant lengthening of host survival time.

The results of the present study led to the evolution of three hypotheses which may account for the observed antitumor activity of PCBs. These include direct cytotoxicity by PCB, inhibition of gluconeogenesis, and inhibition of the formation of serum blocking factor. It is possible that any one of these mechanisms alone, or in combination, act to effectuate tumor inhibition. It is also possible that an, as yet, unidentified mechanism may be responsible for the observed reduction in tumor growth.

Although it is difficult to extrapolate from animal data to the human situation, it is reasonable to conclude that a potential exists for the use of PCBs as a chemotherapeutic agent. Further studies with other tumors, as well as more definitive investigations into the precise mechanism of action of PCBs on tumors are required.
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APPENDICES
Levels and Character of PCB Residues in Tissues of Rats Exposed to Aroclor 1254

The residue levels of PCB in various tissues of the rat following dietary consumption or intraperitoneal injection of Aroclor 1254 are shown in Table A1 and A2. Tissue storage levels directly correlated with the dose of PCB given orally or IP, with adipose tissue showing the highest concentration of residues. The limited tumor data indicate that the tumor, growing in the muscle, stored PCB residues to about the same extent as the liver. Since Curley and coworkers (1971) showed that liver and muscle tissues store PCB residues similarly, it would suggest that the tumor did not represent a preferential storage site for PCB.

PCB residues in the fat appeared to be much higher following intraperitoneal injection of PCB when compared to residue levels following a similar total dose of PCB taken in via the diet. Liver residues, on the other hand, appeared to correlate more closely with total dose, independent of the route of exposure. It is possible that the indicated fat levels in the IP-exposed groups reflect the fat sampling technique. In all cases, testicular fat was
Table A1. PCB residues in fat and liver of rats fed diets containing Aroclor 1254.

<table>
<thead>
<tr>
<th>PCB in Diet ppm</th>
<th>PCB Intake mg/kg</th>
<th>Fat Residues ppm</th>
<th>Liver Residues ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.3</td>
<td>0.02</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>26.</td>
<td>--</td>
</tr>
<tr>
<td>25</td>
<td>113</td>
<td>151.</td>
<td>--</td>
</tr>
<tr>
<td>100</td>
<td>300</td>
<td>418.</td>
<td>20.</td>
</tr>
<tr>
<td>250</td>
<td>900</td>
<td>1117.</td>
<td>--</td>
</tr>
<tr>
<td>400</td>
<td>1112</td>
<td>1715.</td>
<td>208.</td>
</tr>
<tr>
<td>800</td>
<td>2125</td>
<td>3677.</td>
<td>569.</td>
</tr>
</tbody>
</table>

1All animals consumed experimental diets for a total of 39 days, except for the 250 ppm group which was on the diet for 43 days.
2Single determination of pooled samples.

Table A2. PCB residues in fat, liver and tumor tissue of rats intraperitoneally injected with Aroclor 1254.

<table>
<thead>
<tr>
<th>PCB Dose mg/kg</th>
<th>PCB Intake mg/kg</th>
<th>Tissue Residues (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fat²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumor³</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>50</td>
<td>350</td>
<td>1239.</td>
</tr>
<tr>
<td>100</td>
<td>700</td>
<td>2487.</td>
</tr>
<tr>
<td>200</td>
<td>1400</td>
<td>7684.</td>
</tr>
<tr>
<td>400</td>
<td>2800</td>
<td>18189.</td>
</tr>
</tbody>
</table>

¹PCB injected every other day for 14 days.
²Mean of two pooled sample determinations.
³Tumors analyzed individually (n=4), range indicated.
utilized for analysis. Since this fat storage site is separated from the peritoneal cavity only by the peritoneum, it is possible that there was direct absorption of PCB through the peritoneal membrane into the fat. This could result in higher PCB residues in regional fat stores as compared to more uniform distribution of residues throughout the body by the blood following dietary exposure. That direct absorption into the fat occurred is supported by the presence of lower (tetra-) chlorinated isomers in chromatograms of adipose tissue from IP exposed rats (see Figure 1A). The chromatogram of adipose tissue from dietary-exposed animals, on the other hand, totally lacks these lesser chlorinated isomers, indicating metabolism by the liver prior to distribution and storage.

The chromatograms of liver tissue from both dietary- and IP-exposed animals showed traces (1%) of the tetra-chlorinated isomers. Since the animals were exposed to PCB until the time of sacrifice without any intervening recovery period, the presence of these residues in the liver may simply indicate that there was not enough time for the animals to metabolize the PCB before death intervened.

The tumor tissue from IP-exposed rats also showed traces (< 0.5%) of these lower chlorinated isomers. It is probable that the PCB was absorbed from the peritoneal...
Figure A1. Comparison of gas chromatograms of Aroclor 1254 residues in adipose tissue following feeding or intraperitoneal injection of PCB and Aroclor 1254 standard. Numbers above peaks indicate the number of chlorine atoms per molecule.
cavity into the circulatory system at various sites, some of which could then pass from the general circulation into the tissues without first passing through the liver and being metabolized.
PCB exposure has been reported to cause significant pathological changes in the livers of exposed animals, including increased liver weight, hepatomegacytosis, fatty infiltration, vacuolar degeneration and fibrosis. These findings were confirmed in the present study.

Liver hypertrophy, as evidenced by increased liver weights and increased liver to body weight ratios, was seen following dietary or intraperitoneal exposure to Aroclor 1254. The data are presented in Table B1. The increase in liver weight as percent body weight directly correlated with the intraperitoneal dose of PCB to which the animal was exposed. The limited data for animals exposed to PCB via the diet preclude any meaningful comparison between the two routes of exposure.

The extent and severity of microscopic changes in the liver reflected the cumulative dose of PCB and the duration of exposure. The earliest changes to appear were liver cell hypertrophy and fat infiltration, followed by vacuolar degeneration and fibrosis (see Figures B1 to B4).
Table B1. Changes in the liver weight of rats exposed to Aroclor 1254.

<table>
<thead>
<tr>
<th>PCB Intake mg/kg</th>
<th>Route of Exposure</th>
<th>Liver Weight g ± s.e.</th>
<th>Liver Wt./Body Wt. a x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>IP</td>
<td>9.75 ± 0.35</td>
<td>5.60</td>
</tr>
<tr>
<td>350</td>
<td>IP</td>
<td>12.30 ± 0.51</td>
<td>7.38</td>
</tr>
<tr>
<td>700</td>
<td>IP</td>
<td>13.31 ± 0.47</td>
<td>8.21</td>
</tr>
<tr>
<td>1400</td>
<td>IP</td>
<td>11.20 ± 0.71</td>
<td>8.73</td>
</tr>
<tr>
<td>2800</td>
<td>IP</td>
<td>10.08 ± 0.64</td>
<td>9.33</td>
</tr>
<tr>
<td>0</td>
<td>Diet</td>
<td>16.11 ± 0.53</td>
<td>4.47</td>
</tr>
<tr>
<td>900</td>
<td>Diet</td>
<td>23.26 ± 0.93</td>
<td>7.55</td>
</tr>
</tbody>
</table>

a All PCB-treated animals showed a significant (p<0.01) increase in liver weight as % body weight when compared to the respective control.
Figure B1. Control liver (upper print) and liver from rat treated with 100 mg/kg PCB every other day for 14 days (lower print). Note hepatocyte enlargement. Hematoxylin and eosin. x 48
Figure B2. Section from liver of control rat (upper print) and rat injected IP with 100 mg/kg PCB every other day for 14 days showing fat deposition in hepatocytes (lower print). Oil Red O. x 48
Figure B3. Area of vacuolar degeneration in liver of rat injected IP with 200 mg/kg PCB every other day for 22 days. The animal apparently died from PCB poisoning. Hematoxylin and eosin. x 48
Figure B4. Area of fibrotic degeneration in liver of rat injected with 100 mg/kg PCB every other day for 60 days. The animal was sacrificed. Hematoxylin and eosin. x 48