A REVIEW AND EVALUATION OF CARCINOGENICITY STUDIES IN MICE AND RATS AND MUTAGENICITY STUDIES WITH POLYCHLORINATED BIPHENYLS

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INTRODUCTION

This is a review and evaluation of studies which deal with the potential carcinogenicity and mutagenicity of polychlorinated biphenyls (PCBs). It is subdivided into 4 sections: Chronic Rodent Studies, Metabolism Studies, Co-Carcinogenesis Studies and Mutagenicity Studies. A brief summary of Epidemiology Studies is added to complete coverage of the issue of carcinogenicity.

This review does not discuss the effects of impurities or contaminants, particularly polychlorinated dibenzofurans (PCDF), which are reported to be present in some PCB mixtures (Brinkman and deKok, 1980). The presence and amounts of such impurities have not been specified in the materials used in many studies. Thus, attempts to apportion the observed biological effects between impurities and PCBs would only add further conjecture to a subject which currently is rife with speculation.

By contrast, specific chemical and trade names have been used to identify materials that were studied instead of the all encompassing term PCBs. This was deemed necessary because there are too many one-sided generalizations in the literature. Every adverse finding is, by implication at least, extrapolated to the entire class of materials designated as PCBs. Conversely, every report which has failed to find an adverse effect is careful to stipulate that it relates only to the substance studied. Even more difficult to contend with are misleading references to adverse findings which are not substantiated by the actual publications referred to. An example of this occurred in a discussion of <u>in vitro</u> metabolism studies with liver microsomal enzymes which stated that the formation of PCB-macromolecular adducts had been demonstrated. Among the references

cited was a paper entitled "The in vitro binding of 2,2',5,5'-tetrachlorobiphenyl metabolites to rat liver microsomal proteins". The latter paper clearly states "There is no clear evidence in the data obtained from the work reported here to substantiate covalent binding; however, it is also not possible to exclude the nondialyzable radioactivity as being covalently bound". (Hargraves and Allen, 1979).

To emphasize the point that dose is important in evaluating safety, test exposure conditions have been described so that the reader can compare them to ambient exposure levels encountered in the literature and elsewhere. Hoopingarner, et al. (1972) in their studies with Aroclor 1254 on cultured human lymphocytes are among the few authors who acknowledged that "The toxic dose of the chemical was several times greater than is usually found biologically". They used concentrations of 100 ppm of Aroclor 1254 in their studies discussed under Mutagenicity.

Chronic Rodent Studies

Difficulties in comparing and assessing different studies are compounded not only by problems of histopathologic diagnosis but also by variations in experimental design and animal strain differences. Animal feeding studies cited in the literature as evidence for the carcinogenicity of PCBs and related studies of similar duration have been tabulated for mice in Table 1 and for rats in Table 2. Studies have been grouped by the approximate weight percent of chlorine in the materials tested to facilitate comparisons between products from different manufacturers with the same chlorine content.

Mouse studies in Table 1 were conducted with high dietary levels of PCBs. Kimbrough and Linder (1974) reported a 52% mortality for mice fed 300 ppm of Aroclor 1254 for 6 months and then held an additional 5 months. During that interval, control mice had a 32% mortality. This appears to be quite high for control mice about 1 year of age. In a report discussed under Co-carcinogenesis, Koller (1977) studied mice injected with Aroclor products and Moloney leukemia virus. Some data from his study, although not shown in Table 1, are quite similar to those of Kimbrough and Linder (1974). Koller (1977) fed diets containing Aroclor 1221, 1242, or 1254 to groups of 25 Balb/c male mice for 6 months. After that time, some mice were sacrificed for examination and others were returned to a control diet for another 3 months and then sacrificed. Dietary levels of each Aroclor were 375, 37.5, or 3.75 ppm. The only feeding regimen that was toxic was 375 ppm of Aroclor 1254. Only 8 mice of that group survived for 6 months, giving a mortality of 68%. Other authors cited in Table 1 (Ito, et al., 1973a,b and Nagasaki, et al., 1972, 1974, 1975) did not indicate how many of the mice in their studies died when fed 500 ppm of Kanechlor 500, which has a chlorine content similar to Aroclor 1254. Koller (1977) reported that "PCBs did not produce hepatic neoplasia". There was marked liver injury in mice fed 375 ppm of Aroclor 1254, mild liver injury which persisted through the 3-month recovery period at 37.5 ppm, and no hepatic lesions at 3.75 ppm. Moderate liver injury was produced by 375 ppm of Aroclor 1242, but this regressed and no hepatic lesions were seen 3 months after mice were returned to a control diet. Other dietary levels of Aroclor 1242 and all levels of 1221 did not produce liver changes. Highly significant mean liver weight increases after 6 months of feeding occurred in all Aroclor 1254 groups and in the group fed 375 ppm of Aroclor 1242. Three months after mice were placed on a

control diet, mean liver weights of each group had decreased. The decreases were highly significant for the 37.5 ppm Aroclor 1254 and the 375 ppm Aroclor 1242 groups. These observations are generally consistent with those of other investigators which show regression of lesions when PCB dosing is ended, the degree of regression depending upon the duration of the recovery period.

Data for mice reported earlier by Nagasaki, et al. (1972) appear to have been included in the later publications of Ito, et al. (1973a,b). It is interesting to note that the tumors were called hepatomas in the former publication and were labeled well-differentiated hepatocellular carcinomas in the latter. Kimbrough and Linder (1974) apparently also felt that these same data on mice were reported in these 2 papers as they reference the production of hepatomas in male dd mice to the later publication by Ito, et al. (1973b). Similar terminology troubles beset the 1974 and 1975 Nagasaki publications. Data from the later publication list 9/17 males and 4/17 females fed Kanechlor 500 as having liver tumors. Table 1 shows data from the earlier publication; among males, 9/17 had nodular hyperplasia and 7/17 had hepatocellular carcinoma while the female incidence of 4/17 was described as nodular hyperplasia. The hepatomas described by Kimbrough and Linder (1974) were later referred to as "neoplastic nodules (hematomas [sic], hyperplastic nodules)" by Kimbrough, et al. (1978). The use of the term hepatoma has created confusion. With respect to terminology of tumors in the mouse liver, "Hepatoma is a collective term used to describe the progressive stages of tumour development from the lesion called "hyperplastic nodule" or "simple hyperplastic growth" to the morphologically and biologically malignant neoplasms" (Turusov and Takayama, 1979). Nevertheless, whatever

terminology is used to describe them, tumors were attributed only to PCBs containing 52-54 percent chlorine. Lower doses of the 52-54 percent chlorine materials, as well as lower chlorinated materials, were not described as tumorigenic.

Table 2 summarizes results of rat studies with PCBs. As in the mouse studies, only some studies with materials having a chlorine content of 52-54 percent, or higher, were reported to produce carcinomas in the livers of rats, and even for those materials this was not a consistent, reproducible observation.

Odashima (1976) reported the results from a series of different tests used to evaluate potential carcinogenicity of several compounds, including PCBs. Two tests are of sufficient interest to mention here. One is the transplacental method in which rats were treated for 3 days during pregnancy. These were days 15, 17, and 19 or 14, 16, and 18, depending on the strain used. The total dose was approximately the maximum one that did not cause abortion or early death of the weanlings. In the other, the newborn method, pups were dosed subcutaneously on days 1, 8, 15, and 22 after birth. The maximum dose was one that did not cause early death of over 20% of the animals. The subsequent observation period in each test was limited to one year after birth. For both Kanechlor 300 and Kanechlor 500, the transplacental test was scored negative and the newborn one as equivocal. Both of these tests gave positive results with some known carcinogens such as 4-aminobiphenyl, benzo[α]pyrene, butyl-nitrosourea and N-methyl-N-nitrosourea.

Objective appraisal of chronic rodent feeding studies is difficult. Some of the rodent studies in Table 1 and Table 2 are of the type used to detect potent carcinogens. They use relatively few animals, high doses of the test material, and have a relatively short duration. Studies of that nature have been included in Tables 1 and 2 to illustrate the various diagnoses of the rodent hepatic lesions observed after dosing with PCBs and to aid in the stepwise analysis of the results of all the reported carcinogenicity studies.

In any short-term test with a few animals, the chance occurrence of some tumors is a possibility which must be considered. The probability that an event is a chance occurrence is decreased if it is repeatable. Many of the studies in Table 1 did not produce tumors. Those which were reported to produce hepatocellular carcinoma apparently were also reported in other publications as producing nodular hyperplasia, or tumors, or hepatomas. Since terminology of tumors and the use of those terms have subjective, i.e., judgemental, elements it may not be valid to make direct comparisons between different studies on the basis of terminology alone.

The rodent studies which were of less than lifetime duration raise a question as to whether or not cancers would have occurred had the feeding periods been extended. That question is speculative, and it cannot be answered from the present data. Nevertheless, negative studies even if they are of relatively short duration are part of the overall evidence which has to be considered. As a first step in the analysis of the data, the evidence shows that at a minimum, PCBs are not potent carcinogens to rodents because they do not produce cancers when tested in studies designed to detect potent carcinogens.

With respect to further analysis of the mouse studies, greater significance can be attached to the data of Kimbrough and Linder (1974) because their study had a larger number of animals, a longer duration, and a relatively high dietary level of PCB. In those respects, it more closely resembles conventional cancer studies. They did not observe any hepatocellular carcinomas. Thus, it can reasonably be concluded that PCBs are not carcinogenic to mice.

Review of the rat data in Table 2 shows that there are 2 studies on PCBs with an average chlorine content of 40% (Levinskas, 1981 and Weltman and Norback, 1979) and 3 on PCBs with an average chlorine content of 52-54% (Levinskas, 1981; NCI, 1978; Wasserman, et al., 1978). Results of those studies are consistent with respect to the absence of hepatocellular carcinomas. This consistency reasonably suggests a conclusion that PCBs with those chlorine contents are not carcinogenic.

Of the 3 studies with PCBs having an average chlorine content of 60%, one reported hepatocellular carcinomas (Kimbrough, et al. 1975) and 2 did not (Levinskas, 1981 and Weltman and Norback, 1978). Since Kimbrough, et al. (1975) and Levinskas (1981) both used Lot No. AK-3 of Aroclor 1260, the different conclusions they reached are not related to differences in the test material. In addition to the use of a different strain of rat, Kimbrough, et al. (1975) used a different histologic diagnostic criteria. Kimbrough, et al. (1975) used the criteria for classification of specific hepatocellular lesions in rats developed at a National Cancer Institute Workshop (Squire and Levitt, 1975). That workshop recommended that the term "neoplastic nodules" replace so-called "hyperplastic nodules" because "Such nodules are proliferative lesions and

are known to be induced by carcinogens and, at the least, they indicate an increased probability for the development of hepatocellular carcinoma" (Squire and Levitt, 1975). However, Pitot, et al. (1973) in a discussion of stages in the progression of hepatocarcinogenesis in rat liver have noted that "The demonstration of carcinoma cells that appeared to arise within the nodules was also reported (13)[1], suggesting the nodule was a precursor to the malignant neoplasm. On the other hand, as was shown by Farber and others, the vast majority of the regenerating or hyperplastic nodules disappeared on removing the animals from the carcinogenic diet. Thus, despite the more recent suggestion that these nodules be termed "neoplastic nodules" (14)[2], it is difficult to understand a precursor relationship of the nodule to carcinomas if the existence of the putative precursor is so transient." Further, the use of those criteria for classifying experimental hepatic lesions was considered and rejected by Kimura, et al. (1976) in their studies on the co-carcinogenesis of Kanechlor 400 and 3'-methyl-4-dimethylaminoazobenzene.

There is concern that a presently benign lesion might at some future date or under other circumstances be transformed into a malignant lesion. Kimbrough (1979) apparently had that concern when she stated "Although it has been shown many times that the neoplastic nodules are part of the carcinogenic response they are not always included in the statistical evaluation of bioassays, which may lead to erroneously interpreted results, particularly when they are classified as hyperplastic nodules or "nodular hyperplasia" and when the number of animals studied was small (Carcinogenesis Testing Program, 1977)".[3] A similar concern appears to have been behind the statement in a recent review (Anon., 1981) that "hexachlorobiphenyl administered to groups of 50 male and 50 female

Sprague Dawley rats at dietary levels of 0 and 100 ppm for 105 weeks was carcinogenic in female rats, producing an increased incidence of liver hepatocellular carcinomas among the dosed animals (Norback and Weltman, 1980. Personal communication)". Contact with Dr. Norback (Ribelin, 1981) revealed that her data were available only as an abstract (Weltman and Norback, 1979), and that the abstract and her oral presentation of the data referred to the lesions as neoplastic nodules, i.e., not distinctly tumorous. These 2 examples illustrate the difficulty in establishing that cancer is not present, and they strongly suggest that the different findings reported by various researchers are a reflection of their orientation, training, and philosophical perspective. The latter is defined as the difficulty in separating what is actually being observed under the microscope, from a concern over what it might have become if the animals had lived longer.

Since only an abstract has been published, details on the studies by Weltman and Norback (1979) are limited . Their studies are of particular interest because they observed the sequential development of liver changes over a 2-year period. They noted that hexachlorobiphenyl was more toxic than tetrachlorobiphenyl, and that the more toxic, more highly chlorinated hexachlorobiphenyl produced neoplastic nodules only. Again, the weight of the evidence leads to a reasonable conclusion that the carcinogenicity of biphenyls with an average chlorine content of ~60% has not been established.

Overall, one can surmise that the inability to resolve the crucial issue of whether or not a lesion is neoplastic in character was one of the factors which led to the following statement from a recent Surgeon General's report: "Science and society have not yet arrived at a final consensus on the definition of a carcinogen either in the human population or in experimental animals" (DHHS, 1980).

Metabolism Studies

Several reviews (Goldstein, 1980; IARC, 1978; Matthews and Kato, 1979; Roberts, et al., 1978; and Safe, 1980) discuss the metabolism of specific isomers as well as mixtures of PCBs. While there are some exceptions, the following general conclusions can be drawn. Both the degree of chlorination and the positions of the chlorine substituents determine the ease with which PCBs are metabolized. In general, the lower chlorinated ones are metabolized and excreted more readily while the more highly chlorinated materials are stored in fat. The process of metabolism converts the fat soluble PCB into a water soluble hydroxylated derivative which can be excreted in the urine. This metabolism and excretion appears to occur via arene oxide intermediates, and the carcinogenicity of some compounds has been attributed to formation of arene oxide intermediates and their binding to subcellular macromolecules. Chlorination at the 4,4' position blocks the metabolism and excretion of PCBs as illustrated below.

Studies in rats (Hansell, et al., 1977) and in mice (Morales and Matthews, 1978, 1979) are of interest because they report the metabolism of isomers which were fed to rats for 2 years by Weltman and Norback (1979). Hansell, et al. (1977) gave groups of male rats single intraperitoneal injections of 0.2 nmoles/kg of 2,2',5,5'-tetra- or 2,2',4,4',5,5'-hexachlorobiphenyl (TCB and HCB, respectively). They measured changes in hepatic mixed function oxidases and the persistence of the PCB in the livers of animals killed at selected intervals for 35 days after dosing. TCB produced a transient, but significant increase in O-demethylase activity only at day 3 while HCB produced significant increases in O-demethylase and aniline hydroxylase activities within 24-48 hours. Induced enzyme activities by HCB peaked at 4-6 times control activity during days 7-14 and were about 3 times control activity at the end of 35 days. Even though equimolar amounts of each isomer were given, liver residues of HCB one day after dosing were about 7 times higher than those of TCB. HCB residues decreased relatively slowly with time while TCB residues were more rapidly eliminated and had almost returned to control values by 35 days. Livers from HCB treated rats had centrolobular necrosis at day 35. They also were significantly increased in size, showed increased amounts of smooth endoplasmic reticulum (SER), and appeared to contain increased numbers of microbodies and reduced amounts of rough endoplasmic reticulum throughout the 35 day interval. By contrast, TCB treated livers were similar to control ones except for occasionally increased aggregates of SER on days 3 and 4. Hansell, et al. (1977) stated "It would be interesting to speculate that...2,4,5,2',4',5'-hexachlorobiphenyl undergoes direct hydroxylation whereas 2,5,2',5'-tetrachlorobiphenyl undergoes biotransformation via the arene oxide intermediate, thereby accounting for the different slope for the

elimination curve of the latter compound." Thus, the metabolism (Hansell, et al. 1977) and feeding (Weltman and Norback, 1979) study results lead to somewhat different conclusions. The more potent enzyme inducing, less readily excreted HCB which appears to resist hydroxylation produces neoplastic nodules in rat livers. By contrast, the more readily excreted TCB, which apparently is excreted via an arene oxide intermediate, does not produce tumors when fed to rats for 2 years.

Covalent binding to cellular macromolecules also appears to be greater for the more readily metabolized PCB isomers. Morales and Matthews (1978, 1979) compared the covalent binding of 2 hexachlorobiphenyls; the more readily metabolized 2,2',3,3',6,6'-hexachlorobiphenyl (2,3,6-isomer) and the more slowly metabolized 2,2',4,4',5,5'-hexachlorobiphenyl (2,4,5-isomer). Each PCB, with a radiocarbon label, was given orally to groups of mice at a dosage of 7.28 mg/kg on each of five successive days. Animals were killed 1, 5, and 8 days later. The concentration of each PCB was determined in liver, muscle, and kidney. All tissues had consistently higher concentrations of the less readily metabolized 2,4,5-isomer. The more readily metabolized 2,3,6-isomer showed a consistently greater binding to purified macromolecules. The binding was at least one order of magnitude greater than that seen with the 2,4,5-isomer. Results of animal feeding studies with the 2,3,6-isomer would be of particular interest to determine the degree of correlation, if any, between the carcinogenicity of this isomer and its covalent binding to cellular macromolecules.

Taken as a whole, metabolism studies suggest that if PCBs are carcinogenic, it should be those PCBs which are more readily metabolized and excreted, i.e., the lower chlorinated materials. This is in sharp

contrast to results on animal studies in which questions of carcinogenicity arise regarding higher chlorinated materials only. On balance, metabolism studies on the formation of arene oxide intermediates would lead one to expect lower chlorinated materials to show a more pronounced carcinogenic response in animals. Conclusions drawn from metabolism studies are not supported by animal feeding studies.

Co-Carcinogenesis Studies

The position and degree of chlorination of PCBs also are important in inducing microsomal mixed function monooxygenases (Goldstein, 1980 and Yoshimura, et al., 1979). Such induction of microsomal monooxygenases could alter the metabolism of exogenous and endogenous substances in the body, as reported in a variety of studies which have been conducted to determine whether PCBs might be cocarcinogens. These are summarized in Table 3 and discussed in greater detail below.

Uchiyama and Chiba (1974) inserted 20-methylcholanthrene impregnated threads into the uteri of virgin mice and fed them diets containing up to 100 ppm of Kanechlor 400 or DDT. Animals were killed at various times and the cervical epithelium was examined for cancerous changes. Kanechlor 400 dosed mice showed no significant changes as compared to the controls, but those receiving 100 ppm of DDT "showed a remarkable tendency towards the induction of cancer."

Diets containing benzene hexachloride (BHC) isomers and Kanechlor 500 separately and in various combinations were fed to male mice by Ito, et al. (1973a,b). Concentrations of the BHC isomers ranged from 250 ppm to

50 ppm. The amount of Kanechlor 500 was 250 ppm or 100 ppm. Test groups consisted of 20 to 30 animals. When fed alone, only α-BHC at 250 ppm produced a high incidence of nodular hyperplasia and a moderate incidence of hepatocellular carcinoma. A diet containing 250 ppm each of α-BHC and Kanechlor 500 increased the number of hepatocellular carcinomas. In comparison, combinations of 100 or 50 ppm of α-BHC and 250 ppm of Kanechlor 500 yielded a moderate incidence of nodular hyperplasia and produced only a few hepatocellular carcinomas. There were a few nodular hyperplasias in mice fed 100 ppm each of α-BHC and Kanechlor 500. A mixture of 50 ppm α-BHC and 100 ppm of Kanechlor 500 was without effect. Diets containing 250 ppm or 100 ppm of β-BHC and 250 ppm of Kanechlor 500 produced both nodular hyperplasia and hepatocellular carcinomas at a lower incidence than that seen with comparable diets of α-BHC. No liver nodules were seen with 50 ppm of β-BHC and 250 ppm of Kanechlor 500 or with 100 ppm of each. γ-BHC and Kanechlor 500 did not produce liver nodules either singly or in combination. Nagasaki, et al. (1974, 1975) reported a similar series of experiments except that Kanechlor 400 was included. The same concentrations of the PCBs, 250 and 100 ppm, were used. Their test groups consisted of 20 to 38 male mice. For α-BHC and Kanechlor 500, they presented essentially the same data as Ito, et al. (1973a,b). While Kanechlor 400 did not produce liver nodules when fed alone at 250 ppm, there was an increase in the incidence of hepatocellular carcinomas when it was fed in combination with 250 ppm of α-BHC. The increase was similar to that reported by Ito, et al. (1973a,b) for α-BHC and Kanechlor 500. A combination of 100 ppm of α-BHC and 250 ppm of Kanechlor 400 produced only a few nodular hyperplasias and 100 ppm of each in the diet did not induce any liver nodules.

The effects of PCBs on tumor induction by diethylnitrosamine (DEN) have been studied extensively. Male rats were given 25 ppm of DEN in their drinking water and 500 ppm of Kanechlor 500 in their diet concurrently for 20 weeks, returned to a control diet for 4 weeks, and then killed (Makiura, et al. 1974). Neither liver tumors nor nodular hyperplasia were seen, even though rats receiving the same DEN treatment alone had a 92% incidence of liver cancer. The livers of rats treated with Kanechlor 500 only also had no tumors. When administered after DEN, Kanechlor 500 markedly enhanced liver tumor production as reported in a series of papers by Nishizumi (1976, 1979a, 1979b). He described studies in which male rats were given 50 ppm DEN in drinking water for 2 to 10 weeks, after which they were intubated twice weekly with a corn oil solution of Kanechlor 500 for 6 to 12 weeks. Kanechlor 500 doses were either 0.2 ml of a 5% or 0.1 ml of a 10% solution. Since experiments were terminated at 20 or 52 weeks after dosing, some animals received untreated diets prior to sacrifice. Only one paper (Nishizumi, 1976) contained results for animals dosed only with Kanechlor 500 alone. That treatment produced enlargement of the livers of rats but no neoplastic lesions. After pre-treatment with DEN, however, hepatocarcinogenesis was enhanced as evidenced by the earlier appearance and a significant increase in the number of tumors. A similar dosing pattern was used by Preston, et al. (1981). Male rats were given drinking water containing 66 µg DEN/ml (66 ppm) for 5 weeks after which they were fed for 18 weeks on a control diet or one containing 100 ppm of either of 2 Aroclor 1254 diets. One was Aroclor 1254 as received. The other was an Aroclor 1254 which the authors claimed to have purified by removing polychlorinated dibenzofuran (PCDF) impurities by adsorption onto and subsequent elution from activated Florisil. No analysis was made of Aroclor 1254 as received for

PCDF. The authors reported recovery of PCDF at a level comparable to 30 μg of tetrachlorodibenzofuran (TCDF) per 10 g of Aroclor 1254. TCDF was used as a positive control and a standard for quantification for the purification process. Other animals received one of the 2 Aroclor 1254 diets only during the latter 18-week interval. No evidence of hepatic tumor formation was seen in control animals or those receiving 100 ppm of either Aroclor 1254 diet. However, using the diagnostic criteria of Squire and Levitt (1975), they reported that both Aroclor 1254 diets resulted in significantly greater incidences of hepatocellular carcinomas in rats pretreated with DEN as compared to those dosed with DEN alone. Thus, they concluded that the hepatic tumor-promoting ability appears to reside in Aroclor 1254 itself. As the authors of this paper pointed out, it cannot be concluded that PCDFs are not promoters of hepatocarcinogenesis since appropriate studies have not been done on PCDFs alone. Similarly, their findings do not invalidate the hypothesis that some of the other effects reported for some PCBs may have been due to PCDF impurities.

The dependence of the inhibition or promotion effect of PCBs on DEN-induced tumors on the dosing sequence was illustrated in a different manner by Nishizumi (1980). Pregnant female rats were given oral doses of 200 mg/kg or 50 mg/kg Kanechlor 500 on days 5, 10, and 15 of gestation and were allowed to deliver their pups. At 28 days of age, pups were given 50 ppm of DEN in their drinking water for 5 weeks. Groups of these pups were sacrificed at 16, 20, and 24 weeks after the start of DEN dosing and their livers were examined. The number of liver tumors in the offspring from Kanechlor 500 treated dams was significantly decreased as compared to the controls. The decreases were more pronounced in males. This decrease in DEN-induced tumors apparently resulted from induction of

microsomal enzymes in the livers of the pups. Those livers contained Kanechlor 500 residues and electron microscopy showed an increase in the smooth endoplasmic reticulum of hepatic cells.

Rainbow trout were fed 6 ppb of aflatoxin B_1 (AFB_1), or 100 ppm of Aroclor 1254, or a combination of both for a year (Hendricks, et al., 1977). At intervals during the year, some fish were killed. The remainder were killed at the end of that time. All livers were examined grossly and microscopically. There were no tumors in those fed a control diet or Aroclor 1254 alone, and growth was not affected by 100 ppm of Aroclor 1254. The number of tumor-bearing fish in the group fed AFB_1, plus Aroclor 1254 was significantly reduced (to less than one-half) when compared to those fed AFB_1, alone. There also were fewer tumors per liver and the tumors were smaller in those fed the mixture.

In the studies cited earlier, Makiura, et al. (1974) also fed combinations of 500 ppm Kanechlor 500 with 300 ppm of 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) or 150 ppm of N-2-fluorenylacetamide (2-FAA). Results similar to those when PCBs were fed concurrently with DEN were obtained. Kanechlor 500 reduced the incidence of liver tumors to zero from 65% for those treated with 3'-Me-DAB and from 54% for those treated with 2-FAA. However, the effect of PCBs on the tumorigenicity of 3'-Me-DAB appears to depend on the dosing sequence as shown for DEN. Kimura, et al. (1976) used different sequences to feed groups of rats diets containing 400 ppm of Kanechlor 400 for six months and 600 ppm of 3'-Me-DAB for 2 months. Some received Kanechlor 400 alone and some only 3'-Me-DAB. Others received one diet, then the other, after a 2-month interval on control diet. A final group received Kanechlor 400 for

4 months and both materials for another 2 months. No hepatocarcinomas were produced by Kanechlor 400 alone or when it was given before or during the overlap with 3'-Me-DAB. No hepatocarcinomas occurred in control animals. The incidence of liver cancer rose from 13% in those receiving 3'-Me-DAB alone to 64% in the group which received Kanechlor 400 after 3'-Me-DAB.

The inhibitory effect of Kanechlor 500 on rat liver tumors was evident when rats were dosed with two carcinogens (Makiura, et al., 1974). Combinations of 3'-Me-DAB and DEN or 2-FAA and DEN were administered with and without Kanechlor 500 at the concentrations stated earlier. The liver cancer incidence fell from 92% to 8% when Kanechlor 500 was given to rats dosed with 3'-Me-DAB and DEN. It went to zero from 82% for those receiving 2-FAA and DEN.

Nishizumi (1979a,b) studied the effects of various combinations of DDT and sodium phenobarbital (SPB), in the absence and in the presence of Kanechlor 500, on the induction of rat liver hepatocellular carcinoma by DEN. Pretreatment of rats with DEN followed by DDT, by SPB, or by a combination of both produced low incidences of liver cancer while DEN pretreatment alone did not induce cancer. When Kanechlor 500 was included with each of the preceding dosing regimens, there was a marked increase in liver cancers. Increases resulting from joint administration of compounds after pretreatment with DEN were lower than those observed for DEN followed by Kanechlor 500 alone discussed earlier.

Ito, et al. (1978) fed diets containing 200 ppm of 2-FAA to male rats for two weeks and then a diet containing 1000 ppm of an unspecified Kanechlor

mixture for 8 weeks. Partial hepatectomies (PH) were performed on some rats during the third week of the study. Feeding of 2-FAA was without effect, but that diet plus PH produced a few hyperplastic nodules in the liver. Treatment with both diets caused an even greater incidence of hyperplastic nodules and both diets combined with PH produced a marked rise in the number of liver nodules.

DiGiovanni, et al. (1977) and Berry, et al. (1978, 1979) studied Aroclor 1254 in a two-stage mouse skin carcinogenesis assay to see if it was a tumor initiator or promoter. The shaved skin of female mice was dosed with Aroclor 1254 at a level of 100 µg or 625 µg per mouse. This was applied alone as an initiator or from 5 minutes to 72 hours before initiation with 7,12-dimethylbenz[α]anthracene (DMBA). One week later, mice received twice weekly applications of 5 µg of the phorbol diester promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) for 32 weeks. Animals were observed for both papillomas and carcinomas. Aroclor 1254 alone produced a few papillomas. The authors of these reports apparently faced a dilemma common to scientists, i.e., how much significance should be attached to the observation of a low incidence finding which may or may not have a causal relationship to treatment. On the basis of these few papillomas, Aroclor 1254 was called a "weak tumor initiator" (DiGiovanni, 1977). Later, it was described as possessing "little or no tumor-initiating properties" (Berry, et al., 1979). While Aroclor 1254 had a negligible effect on tumor induction when given 5 minutes before an initiating dose of DMBA, it markedly inhibited tumor induction when given 18 to 72 hours before DMBA. In other studies, an initiating dose of 200 nmole of DMBA was applied to the shaved backs of mice. After one week, 100 µg of Aroclor 1254 was applied twice weekly for 30 weeks. Aroclor 1254 failed to

promote tumors while 0.2 μg doses of TPA applied in a similar manner induced an average of 8 papillomas per mouse. The authors concluded that Aroclor 1254 possessed little or no tumor-initiating or tumor-promoting properties.

The transplantability and growth of Walker 256 carcinosarcoma in rats was inhibited by Aroclor 1254 (Kerkvliet and Kimeldorf, 1977a,b). Diets containing up to 800 ppm of Aroclor 1254 were fed to rats of both sexes for 30 days, after which they were given intramuscular injections of Walker tumor cells. Nine days later, during which time they continued to receive Aroclor 1254 in their diets, animals were killed and the tumors were dissected out and weighed. Mean tumor weights of all Aroclor 1254 fed groups were significantly reduced as compared to their sex-matched controls, and the reductions were dose-related to the dietary level of Aroclor 1254. Body weight gain was depressed in males receiving 400 ppm or more of Aroclor 1254. Females showed reduced body weight at 100 ppm, the lowest level fed to that sex. Intraperitoneal injections of 50 to 200 mg/kg every other day for 14 days after injection of a small inoculum of Walker 256 cells (10^3) inhibited both the development and growth of the Walker tumor. The number of tumor takes and the size of the tumors were reduced and the tumor latency period was increased. Body weight gain was reduced only at the 200 mg/kg dosage. Alternate day intraperitoneal injections of 100 mg/kg and 200 mg/kg of Aroclor 1254 after a large inoculum of Walker 256 cells (10^7) were continued for 60 days. Control animals receiving tumor cells only had 100% mortality by day 20 with a mean survival time of 13.5 days. Mean survival times were significantly increased to 17.7 days and 18.9 days for animals dosed with 100 mg/kg and 200 mg/kg of Aroclor 1254. A few animals survived to 60

days, and four receiving the higher dosage of Aroclor 1254 showed total regression of their bilateral tumors. Tumor growth rates in Aroclor-treated animals were significantly reduced. Again, body weight gain was depressed only at the 200 mg/kg dosage. An experiment was undertaken to determine if there was an optimum time period for the antitumor effect of Aroclor 1254. Intraperitoneal injections of 100 mg/kg of Aroclor 1254 were given on 5 consecutive days before tumor inoculation, on 5 days after inoculation, and daily from 5 days before until 10 days after inoculation. This study was ended 14 days after the initiating tumor inoculum was given. While all dosing schedules reduced tumor growth, there were variations in the responses. The greatest inhibition of tumor growth resulted from dosing animals for 15 days. Almost equally effective were pretreatment and treatment starting with inoculation of the Walker cells. The delayed treatment starting after 5 days was least effective as the tumor had become established. While early treatment reduced tumor growth, it was less effective in preventing metastases and death of the animals.

Kerkvliet and Koller (1980) offered groups of male mice diets containing 10, 100, or 500 ppm of Aroclor 1254 for 15 weeks prior to injecting them with MSB, an established tissue culture cell line derived from Moloney sarcoma virus. They measured tumor growth and cell-mediated toxicity over the next 19 days. A dietary level of 500 ppm of Aroclor 1254 resulted in marked weight loss and deaths. This was reduced to 250 ppm at 11 weeks, and a few weeks later surviving animals were placed on a control diet. They reported that Aroclor 1254 pretreatment enhanced the growth rate of the MSB tumor and inhibited or delayed the development of cellular immunity against the tumor.

Koller (1977) fed groups of mice diets containing 3.75, 37.5, or 375 ppm of Aroclor 1221, Aroclor 1242, or Aroclor 1254 for 6 months. About 2 weeks after being placed on test diets, some mice in each group were inoculated intraperitoneally with Moloney leukemia virus. None of the dietary levels of any Aroclor affected, i.e., did not promote or induce, the oncogenesis of Moloney leukemia virus.

Since PCBs are enzyme inducers, their biological effects resemble those of other enzyme inducers. Peraino, et al. (1978) noted that phenobarbital had a specific tumorigenic enhancing effect that was restricted to the liver. With the exception of the mouse skin painting studies and those in which tumor cells or a virus were injected, all of the studies in Table 3 were concerned with liver tumors. Peraino, et al. (1978) also pointed out resemblances between phenobarbital and PCBs. Both substances "enhanced hepatic tumorigenesis" when given after DEN, and both "exerted a protective effect against hepatic tumorigenesis" when administered concurrently with AAF or DEN. The comparison between PCBs and phenobarbital is extended by the observation of Berry, et al. (1978, 1979) that PCBs did not promote skin tumors in mice pretreated with DMBA. Peraino, et al. (1978) refer to a study[4] in which skin tumorigenesis was not enhanced in mice fed phenobarbital after skin painting with DMBA.

Lichti, et al. (1978) described the induction of ornithine decarboxylase (ODC) in mouse epidermal cell cultures by TPA. Aroclor 1254, apparently at much higher concentrations than TPA, induced a low but reproducible stimulation of ODC in the same system. They also noted a report[5] that a high intraperitoneal dose of Aroclor 1254 induced ODC in the liver of rats. After commenting that PCBs "warrant further investigation into their

possible action as tumor promoters," they added that "These compounds are being tested on carcinogen-initiated mouse skin (T.J. Slaga, personal communication)". The series of publications by Berry, et al. (1978, 1979) and DiGiovanni, et al. (1977) show that PCBs are not promoters on mouse skin.

Authors of some of the mutagenicity studies to be discussed (Danz and Urban, 1980; Norback, et al., 1981; and Stadnicki, et al., 1979) have suggested that PCBs may act as promoters of carcinogenicity. In general, those comments appear to have been offered as hypotheses to aid in understanding the observations which had been made. This also appears to be the case for the NCI study on Aroclor 1254 which was reviewed by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens. That group, charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity, made and accepted a motion to add the following to the report summary: "Based on the liver proliferative lesions in the treated rats and published reports, it is suggested that Aroclor 1254 may be a tumor promoter" (NCI, 1978).

With respect to tumor promotion, Weisburger and Williams (1980) state that "certain inducers of liver metabolic enzyme systems, such as phenobarbital, DDT and BHT, when administered after minimal doses of primary hepatocarcinogens exerted a powerful promoting effect". As mentioned earlier, PCBs also induce liver metabolic enzyme systems. However, since the carcinogen alone produced tumors in animals in the co-carcinogenesis studies summarized in Table 3, it appears that the doses were greater than minimal. In addition, even though the co-carcinogenesis studies were of

shorter duration, several used much higher dietary levels of PCBs than those fed to rats for 2 years. The latter studies are the ones which gave rise to questions of carcinogenicity. Thus, while the evidence indicates that PCBs are not carcinogenic, their reported effects in rodent livers following prolonged exposure in conjunction with an initiator may be promotion or inhibition.

When administered to animals together with a biological agent, PCBs show a promoting or inhibitory effect similar to that seen with chemical agents. Pretreatment with PCBs inhibited the growth of Walker 256 carcinosarcomas in rats and increased their survival time (Kerkvliet and Kimmeldorf, 1977a,b), enhanced the growth of MSB tumor in mice (Kerkvliet and Koller, 1978) and neither promoted nor induced the oncogenesis of Moloney leukemia virus in mice (Koller, 1977).

Mutagenicity Studies

Mutagenicity testing of PCBs has ranged from bacterial systems to intact animal studies. A series of studies with eleven bacterial test strains (Heddle and Bruce, 1977; Hsia, et al. 1978; McMahon, et al. 1979; Odashima, 1976; Probst, et al., 1981; Sugimura, et al., 1976; and Wyndham, et al., 1976) are summarized in Table 4. Hsia, et al. (1978) used both phenobarbital and Aroclor 1254 to induce liver enzymes in rats for preparation of S-9 activation systems. They also tested 4-hydroxy-2,2',5,5'-tetrachlorobiphenyl and 2,2',5,5'-tetrachlorobiphenyl-3,4-oxide, a known and a presumed metabolite of 2,2',5,5'-tetrachlorobiphenyl, respectively. All three materials gave negative responses with each activation system. Odashima (1976) presented data in tabular form from a series of screening tests. In addition to the results shown in Table 9,

bacterial strains WP2, TA100, TA98, H-17, M-45, W3110, and TA1978 are shown in groups in their Table 5 with a text notation that not all chemicals were tested with each strain. For Kanechlor 300 and 500, such of the preceding strains as were tested showed a negative response. The group consisting of H-17 & M-45, WP_2 try$^-$ (hcr$^+$ & hcr$^-$) shows a plus sign for Kanechlor 300. Presumably, one or more of those bacterial strains gave a positive response. Kanechlor 500 was listed as giving a negative response with the latter group.

With the exception of Wyndham, et al., (1976), all of the studies were negative with and without the addition of a microsomal enzyme activation system. It is somewhat difficult to reconcile the text and the graphs in the publication by the latter authors. They stated that their "results clearly showed that as the degree of chlorination decreased the mutagenicity increased, a concentration of 100 µg of 4-chlorobiphenyl in the test medium gave over 2000 revertant colonies per plate. The higher chlorinated biphenyls show very little activity as mutagens". While Figure 3 in their article shows their marked increase in revertants for 4-chlorobiphenyl, the same figure also shows results for Aroclor 1221, which averages 1.15 chlorine units per molecule. The mutagenicity of Aroclor 1221 is not discussed in the text, but Figure 3 shows that at a concentration of 100 µg per plate, it produced about one-tenth of the revertant colonies that 4-chlorobiphenyl did. Thus, a 15% increase in chlorine content produced a 10-fold decrease in the reported mutagenic activity. In light of that sharp reduction of mutagenic activity with a slight chlorine increase, it is difficult to understand the statement in their text that Aroclor 1254 "was only weakly mutagenic." Results from Aroclor 1254 (average of 4.96 chlorine per molecule) are not shown in their

Table 3, but 2,2',5,5'-tetrachlorobiphenyl (average of 4 chlorine per molecule) was presented. At 100 µg per plate, 2,2',5,5'-tetrachlorobiphenyl was virtually devoid of mutagenic activity. Overall, primarily because McMahon, et al. (1979) reported that 4-chlorobiphenyl was not mutagenic, it can only be concluded that the findings of Wyndham, et al. (1976) are aberrant. A similar conclusion that the findings reported by Wyndham, et al. (1976) are unfounded because attempts to repeat them have been unsuccessful was reached by the State of California (Anon. 1981).

In a variant of this test, Stott and Sinnhuber (1978) used Aroclor 1221, 1242, 1254, and 1260 to induce the mixed function oxidase system of the liver in trout. The submitochondrial fraction of those livers was used to activate the metabolism of AFB_1 which was assayed for mutagenicity using strain TA 1538 of S. typhimurium. The mutagenic response was decreased compared to the concurrent control using untreated trout liver. A general pattern of decreasing response with increasing degree of chlorination was observed, except for Aroclor 1260. Induction of mutagen detoxifying enzyme systems was suggested as a possible explanation for the apparent conflict between the reported induction of trout mixed function oxidases and the decrease in mutagenic activity. These results are consistent with those of Hendricks, et al. (1977) discussed earlier who reported that Aroclor 1254 reduced hepatic tumors in trout fed AFB_1.

On the basis of slower sedimentation rates in alkaline sucrose gradients, Stadnicki, et al. (1979) reported that 2,2',5,5'-tetrachlorobiphenyl (TCB), a mixture of the 3-hydroxy and 4-hydroxy derivatives of TCB and the 3,4-epoxide of TCB induced single strand breaks in DNA of L-929 cells.

At 100 µg/ml, each of the three materials caused all of the DNA to come out in fractions at the top of the gradient. The epoxide caused some breakage down to 1 µg/ml. The mixture of hydroxy derivatives caused significant breakage at 20 µg/ml and only slight breakage at 1 and 10 µg/ml. TCB, the least potent, caused lesser breakage at 20 µg/ml and was without effect at 1 and 10 µg/ml.

Nilsson and Ramel (1974) conducted genetic tests on adults and larvae of Drosophila melanogaster fed Clophen 30 and Clophen 50. These PCB mixtures were without effect on the loss of sex chromosomes used to measure chromosome breaking action and nondisjunction of the sex chromosomes. Tazima (1980) has described a specific locus test using the silkworm (Bombyx mori). Neither Kanechlor 300 nor Kanechlor 500 showed mutagenic activity in that system.

Hoopingarner, et al. (1972) induced mitosis in cultured human lymphocytes with phytohemagglutinin and treated them with 100 ppm of Aroclor 1254. There was no effect on the mitotic index, satellite association, chromatid gaps or chromatid breaks in comparison to control human lymphocytes.

Odashima (1976) also studied the incidence of chromosomal aberrations. Their in vitro test used Yoshida ascites sarcoma cells cultured for 6 to 72 hours in the presence of PCBs at concentrations producing a minimal or 50% growth inhibition. Kanechlor 300 gave a positive response and Kanechlor 500 a negative one. For an in vivo system, they examined bone marrow cells 6 to 48 hours after adult or newborn animals were given an approximately lethal dose of PCBs. This test gave the opposite result; Kanechlor 500 was positive and Kanechlor 300 was negative. They noted

that chromosomal aberrations frequently occurred in controls and that chemicals were called positive when they induced more than twice the aberrations in controls.

In a Syrian hamster cell transformation system (Pienta, 1980), Aroclor 1254 was one of several chemicals tested double-blind. It gave a negative result. Mouse embryo fibroblasts also have been exposed to different PCBs. Nesnow, et al. (1981) studied the cocarcinogenic action of agents which increase microsomal mixed-function oxidase activity in the C3H10T½CL8 transformation assay. After a 48-hour pretreatment with Aroclor 1254, cells were then treated with benzo(a)pyrene [B(a)P] and the agent for an additional 24 hours. Aroclor 1254 did not increase B(a)P-mediated transformation and no Type II or Type III foci were observed. Norback, et al. (1979, 1980, 1981) exposed C3H10T½ cells continuously for 6 weeks to 10 µg Aroclor 1254 per ml of medium. Treated cells developed Type III foci. Cells exposed to the same concentration of Aroclor 1254 for 24 hours or to 1 µg of Aroclor 1254 per ml of medium did not develop Type III foci. Continuous exposure of cells to Aroclor 1260 and 2,4,5,2',4',5'-hexachlorobiphenyl also caused formation of Type III foci. A clone from a focus transformed by Aroclor 1254 induced sarcoma formation when inoculated in irradiated mice. Foci from Aroclor 1260 and 2,4,5,2',4',5'-hexachlorobiphenyl had not been characterized further. Since transformation occurred only after continuous exposure, Norback, et al. (1981) suggested that the effects of PCBs in culture include promotion. When fed to rats, however, 2,4,5,2',4',5'-hexachlorobiphenyl produced neoplastic liver nodules, not hepatocarcinomas (Weltman and Norback, 1979).

Wong, et al. (1979) claimed that 4-chlorobiphenyl induced an increase in unscheduled DNA synthesis in Chinese hamster ovary cell cultures in the presence of hydroxyurea, a chemical agent which suppresses normal replicative DNA synthesis. Few details were presented regarding the validation and reliability of their test system. By contrast, Aroclor 1254 gave a negative response in an unscheduled DNA synthesis in primary cultures of adult rat hepatocytes (Probst, et al., 1981). The latter authors presented results of an extended series of compounds tested in their system.

Danz and Urban (1980) have proposed using the mitogenic response of the rat adrenal cortex after dosing the animals with a test substance as a short-term test for evaluating the promoting action of chemical compounds. Clophen A60, Delor 103[6], Delor 106[6], and Phenoclor DP6 all gave positive responses in their test system.

At 3-6 days of incubation, embryos from ring doves (Streptopelia risoria) fed a control diet or one containing 10 ppm of Aroclor 1254 were examined for cytogenetic changes (Peakall, et al., 1972). The relative frequencies of chromosome aberrations in the 8 largest chromosome pairs in metaphase cells of allantoic sac and limb bud origin were scored. The 6 control embryos had a mean aberration rate of 0.8% (0-2.0). The aberration rate in 17 embryos from Aroclor 1254 treated birds was 1.8% (0-9.4). In the latter group, there was one chromosome rearrangement, 13 embryos with aberration rates exceeding the mean control rate, and 4 embryos which exeeded the highest control rate. Aroclor 1242 was injected into fertile White Leghorn eggs to give estimated final concentrations of 10 or 20 ppm (Blazak and Marcun, 1975). After 4 or 5 days of incubation, eggs were

injected with colcemide, incubated an additional 45-60 minutes, and embryos were harvested for examination. There was a high degree of early embryonic death, and a few live embryos showed drastically retarded development without malformation. The first five pairs of chromosomes, constituting over 50% of the chromatin material per cell, were examined for detection of clastogenesis. There were no chromosomal aberrations.

Heddle and Bruce (1977) injected mice with Aroclor 1254 for five consecutive days and then examined bone marrow preparations for chromosomal breakage and sperm cell preparations for sperm with abnormally shaped heads. While nonspecific factors can induce sperm abnormalities, the authors believe the latter also can result from point mutations or small deletions. Aroclor 1254 was judged to be neither carcinogenic or mutagenic.

Dikshith, et al. (1975) gave male rats oral dosages of 50 mg/kg of Aroclor 1254 on each of 7 consecutive days. Animals were killed at intervals over the next 3 days. Those scheduled for cytogenetic analysis were injected with colchicine 2 hours before killing, after which time the seminiferous tubules were prepared for such study. At autopsy of the other animals, testis, epididymis, and liver weight were recorded and sections were prepared for microscopic study and histochemical determinations. Livers were markedly enlarged and showed a significant increase in weight compared to controls. There were no differences in body weight or weight and appearance of testis, epididymis, or vas deferens between control and treated rats. Aroclor 1254 treated rats showed a few metaphase figures with abnormal chromosomes whch appeared to be sporadic and not specific to any one type. Histological examination showed testis

and epididymis from Aroclor 1254 treated rats were comparable to controls although interstitial cells were increased in Aroclor 1254 dosed rats. These cells showed an increase in acid phosphatase activity. The authors concluded that they "found no evidence to suggest that Aroclor 1254 causes significant chromosome damage or histopathological changes in the rat testis."

Similar studies were conducted by Green, et al. (1973, 1975a) who gave male rats single oral dosages of 5000 mg/kg, 2500 mg/kg or 1250 mg/kg or four successive daily dosages of 500 mg/kg of Aroclor 1242. Other rats received five consecutive daily dosages of 300 mg/kg, 150 mg/kg or 75 mg/kg of Aroclor 1254. Animals were injected with colcemide 3 or 4 hours before sacrifice which occurred about 24 hours after the single dose or the series of doses. There were some body weight losses and some deaths occurred. Bone marrow from rats dosed with both PCB mixtures and spermatogonial preparations from Aroclor 1242 treated rats were prepared for cytogenetic study. Repeated dosages of 150 mg/kg and 300 mg/kg of Aroclor 1254 produced decreases in the number of mitoses in bone marrow cells. Repeated dosages of 75 mg/kg of Aroclor 1254 and all dosages of Aroclor 1242 were without effect. Neither PCB mixture at any dosage produced a significant number of chromosomal abnormalities in bone marrow cells. At the lower dosages, Aroclor 1242 did not affect mitoses of spermatogonial cells, but 5000 mg/kg or 4 dosages of 500 mg/kg significantly reduced the rate of cell division. No dosage of Aroclor 1242 produced cytogenetic abnormalities in spermatogonial cells. The authors concluded from their studies that Aroclor 1242 and Aroclor 1254 did not possess mutagenic potential. Garthoff, et al. (1977) fed male rats diets containing 5, 50, or 500 ppm of Aroclor 1254 for 5 weeks, after which they

examined bone marrow and testis samples. There was no significant difference between test and control animals with respect to the incidence of chromosomal abnormalities and the number of cells in mitosis from bone marrow and spermatogonial cells.

In a dominant lethal study (Keplinger, et al., 1972 and Calandra, 1976), albino mice were given single intraperitoneal dosages of 500 or 1000 mg/kg of Aroclor 1242, Aroclor 1254, or Aroclor 1260 and mated on successive weeks to virgin females. There was no evidence of mutagenic effects. Although details on their studies are limited, their results are in general agreement with those from another dominant lethal study in rats conducted by Green, et al. (1975b) with Aroclor 1242 and Aroclor 1254. The former was administered orally at a single dosage of 625, 1250, or 2500 mg/kg or in five daily dosages of 125 or 250 mg/kg. Aroclor 1254 was given orally in five daily dosages of 75, 150, or 300 mg/kg. After dosing, they were mated with untreated females for 10-11 weeks. Another group of rats was given 150 mg/kg of Aroclor 1254 for five successive days and starved overnight before admittance to females. Other male rats were offered diets containing 25 or 100 ppm of Aroclor 1254 for 70 days, then mated with untreated females for one week. TEM (triethylenemelamine) was used as a positive control. There was a body weight loss and some deaths occurred in a few groups. Neither the oral dosing nor the dietary feeding of Aroclor 1242 or Aroclor 1254 had any effect on the number of implantations or the number of dead implantations per pregnant female while TEM produced significant postimplantation losses in weeks 2, 3, and 4. These authors noted that the reduction in the number of dividing spermatogonial cells reported earlier for Aroclor 1242 (Green, et al., 1973, 1975a) did not impair the reproductive performance of male animals.

Review of the various mutagenicity tests and their results prompts three comments. Chlorinated hydrocarbons do not generally give positive results in Salmonella strains. Therefore, the mutagenic responses with TA1538 (Wyndham, et al. 1976) are either aberrant or else the response is, indeed, limited to essentially monochlorobiphenyl. The degree of correlation between in vitro mutagenicity tests and carcinogenicity depends upon the initial classification of the test materials. Pienta (1980) classifies Aroclor 1254 as a non-carcinogen. Rinkus and Legator (1980) regard Aroclor 1254 and Kanechlor 500 as having known or suspected carcinogenic activity. Odashima (1976) and Sugimura, et al. (1976, 1977) consider Kanechlor 500 to be carcinogenic and Kanechlor 300 to be non-carcinogenic. Apart from the difficulties they present for correlation, these different opinions about the carcinogenicity of PCBs are a reflection of what data these individuals considered and how they evaluated those data. Finally, in light of the large number of tests conducted to evaluate various mutagenic parameters, it is not surprising that an occasional suspicious or positive finding resulted, simply on a statistical basis. Those occurred in in vitro systems. In vivo tests gave negative results and provide a basis for concluding that ambient levels of PCBs do not present a mutagenic risk.

Epidemiology Studies

With respect to epidemiologic studies, it should be noted that there are frequent references in the literature to an epidemiologic study of workers exposed to Aroclor 1254 (Bahn, et al., 1976, 1977). Those articles are cited in two recent reviews. One states "The epidemiological data provide suggestive evidence of a relationship between exposure to polychlorinated biphenyls and the development of malignant melanoma. ...for practical

purposes, polychlorinated biphenyls should be regarded if they were carcinogenic to humans" (IARC, 1978). The other states "No conclusive evidence has thus far been reported which demonstrates that occupational exposure to PCBs has caused an increased incidence of cancer" (Kimbrough, 1980). The latter author then proceeds to discuss the study by Bahn, et al. (1976). Among the references for these 2 reviews are NIOSH (1978) which has the following comment on the Bahn study, "PCB exposure histories were based on recollections of two company employees. Exposures to other chemicals could not be ascertained.----To correct these deficiencies in the preliminary study, a more intensive investigation is being conducted (B.N. Kightlinger, written communication, November 1976). A substantial change has occurred in the cohort since release of the preliminary report by Bahn and her coworkers, and it seems likely that the findings on this new cohort will differ significantly from those of the preliminary study. The final report is not yet available."

Recently, Gaffey (1981) reviewed and evaluated existing reports concerning health effects and exposure to PCBs. The reports he discussed dealt with diverse potential health effects. With respect to liver effects, he concluded that "Alterations of liver function and fat metabolism associated with PCB exposure have been observed in several studies, but are characterized by investigators as mild and of no clinical significance."

He also concluded that "Mortality studies concerned primarily with cancer present problems of interpretation due to the small sample size of some of the studies, and to the confounding effect of other exposures. However, they do exhibit a pattern, which is that none of the studies agree on the cancer sites at which an excess mortality was found, and the excesses that

were found are in general not statistically significant. One must conclude that the findings of the mortality studies reflect a sporadic pattern of excess mortality at different sites which is not consistent with a carcinogenic effect of PCBs. In addition, where an examination of duration and latency of exposure was possible, no association with these variables was found [32][7].

"Taken as a whole, the epidemiologic studies find that high occupational exposures to PCBs may cause dermatitis of various kinds, but that there are no other clinically observable effects, including the occurrence of cancer."

Summary

Some remarks by Cole and Merletti (1980), although written in a more general vein, appear to be particularly suited for ending a discussion on the potential carcinogenicity of PCBs. "In view of the difficulty of deciding whether a "suspect" carcinogen is in fact a weak carcinogen or in fact harmless, we point out one aspect of this scientific judgement which, though well known, is often lost sight of; namely, that in principle, it is more likely that a non-carcinogen will appear to be a weak carcinogen than the reverse. This asymmetry should be fully appreciated by legislators and regulators. It is summed-up succinctly, if not very accurately, in the oft-heard phrase "you can prove a positive but not a negative". This is clearly illustrated in the case of PCBs.

Review of the results of a large number and wide range of chronic feeding and metabolism studies in animals and of mutagenicity studies in various systems has failed to establish that PCBs are carcinogenic. However,

presently available rodent data raise some suspicions and lead to disputes about the carcinogenicity of PCBs. Attempts to compare and evaluate results of rodent studies reported in the literature are complicated by different uses of similar terminology and variations in the criteria employed for diagnosing hepatic tumors in rodents. Co-carcinogenesis studies have reported both promotion and inhibition of tumors in rodent livers following prolonged administration of PCBs in conjunction with an initiator. Thus, it is not likely that animal studies and other laboratory procedures will lead to a universal consensus on the carcinogenicity or non-carcinogenicity of PCBs.

While epidemiology studies of humans exposed to PCBs present problems of interpretation due to their small sample size and the confounding effect of other exposures, they present the best available evidence for assessing the carcinogenic potential of PCBs to humans. Epidemiology studies, including recent studies involving large cohorts with lengthy exposures to PCBs, demonstrate a pattern that is not consistent with a carcinogenic effect of PCBs.

Footnotes

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TABLE 1

PCBs: Tabulation of Liver Nodules Reported in Mice[1]

Approx. wt. % Cl[2]	Product	Strain	Dosing Duration	PCB in diet (ppm)	No. Animals	Nodular Hyperplasia	Hepatoma	Hepatocellular Carcinoma	Reference
52-54	Aroclor 1254	BALB/cJ	6 mos[3]	0	34	-	0	-	Kimbrough + Linder (1974)
				300	24	-	1	-	
			11 mos	0	24	-	0	-	
				300	22	-	9[4]	-	
	Kanechlor 500	dd	32 weeks	0	6	0	-	0	Nagasaki, et al. (1972) Ito, et al. (1973a,b)
				100	12	0	-	0	
				250	12	0	-	0	
				500	12	7	(-)[5]	5[5]	
	Kanechlor 500	dd	32 weeks	0	20	-	-	0	Nagasaki, et al. (1974, 1975)
				100	18	-	-	0	
				250	20	-	-	0	
				500	17	9	-	7[6]	
	Kanechlor 500	dd[7]	32 weeks	0	12	-	-	0	Nagasaki, et al. (1974, 1975)
				100	19	-	-	0	
				250	20	-	-	0	
				500	17	4	-	0[6]	
48	Kanechlor 400	dd	32 weeks	0	6	0	-	0	Nagasaki, et al. (1972) Ito, et al. (1973a,b)
				100	12	0	-	0	
				250	12	0	-	0	
				500	12	0	-	0	
	Kanechlor 400	dd	32 weeks	0	0	-	-	0	Nagasaki, et al. (1974, 1975)
				100	17	-	-	0	
				250	19	-	-	0	
				500	20	-	-	0	
	Kanechlor 400	dd[7]	32 weeks	0	12	-	-	0	Nagasaki, et al. (1974, 1975)
				100	20	-	-	0	
				250	20	-	-	0	
				500	17	-	-	0	

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TABLE 1-continued

PCBs: Tabulation of Liver Nodules Reported in Mice[1]

Approx. wt. % Cl[2]	Product	Strain	Dosing Duration	PCB in diet (ppm)	No. Animals	Nodular Hyperplasia	Hepatoma	Hepato-cellular Carcinoma	Reference
40-42	Kanechlor 300	dd	32 weeks	0	6	0	-	0	Nagasaki, et al.
				100	12	0	-	0	(1972)
				250	12	0	-	0	Ito, et al. (1973a,b
				500	12	0	-	0	
	Kanechlor 300	dd	32 weeks	0	20	-	-	0	Nagasaki, et al.
				100	19	-	-	0	(1974, 1975)
				250	19	-	-	0	
				500	20	-	-	0	
	Kanechlor 300	dd[7]	32 weeks	0	12	-	-	0	Nagasaki, et al.
				100	19	-	-	0	(1974, 1975)
				250	20	-	-	0	
				500	20	-	-	0	

[1] Males, except as noted.
[2] From Brinkman and deKok (1980).
[3] Held an additional 5 months before sacrifice.
[4] No. of animals. One had 2 hepatomas for a tumor total of 10.
[5] Apparently same data reported as hepatoma and hepatocellular carcinoma. See references and text.
[6] Apparently same data reported as nodular hyperplasi. and hepacellular carcinoma or tumor. See references text.
[7] Females.

TABLE 2

PCBs: Tabulation of Liver Nodules Reported in Rats

Approx. wt. % Cl[1]	Product	Strain	Sex	Dosing Duration	PCB in diet (ppm)	No. Animals	Adenomatous Nodules	Nodular Hyperplasia	Neoplastic Nodules	Adenoma	Hepatoma	Hepatocellular Carcinoma	Cholangio-Hepatoma	Reference
60	Aroclor 1260	Sherman	F	21 mos.[2]	0	173	-	-	0	-	-	1	-	Kimbrough,
					100	184	-	-	144	-	-	26	-	et al. (197
	Aroclor 1260	Charles River	M, F	24 mos.	0	23	-	1	-	-	0	-	0	Levinskas
					1	26	-	0	-	-	0	-	0	(1981)
					10	25	-	7	-	-	1	-	0	
					100	25	-	6	-	-	7	-	4	
	2,2',4,4',5,5'-Hexachlorobiphenyl[3]	-	-	24 mos.	100	-	-	-	+[4]	-	-	-	-	Weltman & Norback (197
52-54	Aroclor 1254	Charles River	M, F	24 mos.	0	23	-	1	-	-	0	-	0	Levinskas
					1	30	-	0	-	-	0	-	0	(1981)
					10	26	-	3	-	-	0	-	0	
					100	26	-	14	-	-	4	-	2	
	Aroclor 1254	Fischer 344	M	105 weeks	0	24	-	-	-	0	-	0	-	NCI (1978)
					25	24	-	-	-	0	-	0	-	
					50	24	-	-	-	0	-	1	-	
					100	24	-	-	-	1	-	2	-	
	Aroclor 1254	Fischer 344	F	105 weeks	0	23	-	-	-	0	-	-	-	NCI (1978)
					25	24	-	-	-	0	-	-	-	
					50	22	-	-	-	1	-	-	-	
					100	24	-	-	-	2	-	-	-	
	Aroclor 1254	Local	F	28 mos.[5]	0	4	-	-	-	0	-	-	-	Wasserman,
					200	6	-	-	-	+[4]	-	-	-	et al. (1978)
	Kanechlor 500	Wistar	M	28-52 wks	0	18	-	0	-	-	-	-	-	Ito, et al.
					100	25	-	3	-	-	-	-	-	(1974)
					500	16	-	5	-	-	-	-	-	
					1000	13	-	5	-	-	-	-	-	

TABLE 2-continued

PCBs: Tabulation of Liver Nodules Reported in Rats

Approx. wt. % Cl[1]	Product	Strain	Sex	Dosing Duration	PCB in diet (ppm)	No. Animals	Adenomatous Nodules	Nodular Hyperplasia	Neoplastic Nodules	Adenoma	Hepatoma	Hepato-cellular Carcinoma	Cho-langio-Hepatoma	Reference
48	Kanechlor 400	Donryu	M	23-62 wks	38.5-616	5	0	-	-	-	-	-	-	Kimura + Ba. (1973)
						10	0	-	-	-	-	-	-	
			F			5	0	-	-	-	-	-	-	
						10	6	-	-	-	-	-	-	
	Kanechlor	Wistar	M	28-52 wks	0	18	-	0	-	-	-	-	-	Ito, et al. (1974)
					100	16	-	2	-	-	-	-	-	
					500	8	-	0	-	-	-	-	-	
					1000	10	-	3	-	-	-	-	-	
40-42	Aroclor 1242	Charles River	M, F	24 mos.	0	23	-	1	-	-	0	-	0	Levinskas (1981)
					1	32	-	1	-	-	0	-	0	
					10	29	-	1	-	-	0	-	0	
					100	19	-	6	-	-	3	-	1	
	Kanechlor	Wistar	M	28-52 wks	0	18	-	0	-	-	-	-	-	Ito, et al. (1974)
					100	22	-	1	-	-	-	-	-	
					500	19	-	0	-	-	-	-	-	
					1000	15	-	0	-	-	-	-	-	
	2,2',5,5'-tetra-chlorobiphenyl[3]	-	-	24 mos.	100	-	-	-	-	-	-	-	-	Weltman & Norbath (197)

[1] From Brinkman and deKok (1980).
[2] Held an additional 2 months before sacrifice.
[3] Abstract. Details limited.
[4] Presence reported.
[5] Ethanol solution in drinking water.

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TABLE 3

SUMMARY OF CO-CARCINOGENESIS STUDIES WITH PCBs

Product	Mode	Time	Other Treatment[1]	Mode	Species	Observations	Ref.
Kanechlor 400	Diet	With	20-MeCh	Uterine implant	dd mouse	Hepatocellular carcinoma with 20-MeCh. None with combination.	Uchiyama, and Chiba, 1974
Kanechlor 500	Diet	With	α-BHC	Diet	dd mouse	Combination increased hepatocellular carcinoma over α-BHC alone.	Ito, et al. 1973a,b Nagasaki, et al. 1974, 1975
Kanechlor 500	Diet	With	β-BHC	Diet	dd mouse	Combination produced hepatocellular carcinoma. Each alone negative.	Ito, et al. 1973a,b Nagasaki, et al. 1974, 1975
Kanechlor 400	Diet	With	α-BHC	Diet	dd mouse	Combination increased hepatocellular carcinoma over α-BHC alone.	Nagasaki, et al. 1974, 1975
Kanechlor 500	Diet	With	DEN	Water	Sprague-Dawley rat	Hepatocellular carcinoma with DEN. None with combination.	Makiura, et al. 1974
Kanechlor 500	Gavage	After	DEN	Water	Wistar rat	Combination increased hepatocellular carcinoma over DEN alone.	Nishizumi, 1976, 1979a, b
Aroclor 1254	Diet	After	DEN	Water	Sprague-Dawley rat	Combination increased hepatocellular carcinoma over DEN alone.	Preston, et al. 1981
Kanechlor 500[2]	Gavage	Before	DEN[2]	Water	Wistar rat	Combination decreased hepatocellular carcinoma in offspring.	Nishizumi, 1980
Aroclor 1254	Diet	With	AFB_1	Diet	Rainbow trout	Hepatocellular carcinoma with AFB_1. None with combination.	Hendricks, et al. 1977
Kanechlor 500	Diet	With	2-FAA	Diet	Sprague-Dawley rat	Hepatocellular carcinoma with 2-FAA. None with combination.	Makiura, et al. 1974
Kanechlor 500	Diet	With	3'-Me-DAB	Diet	Sprague-Dawley rat	Hepatocellular carcinoma with 3'-Me-DAB. None with combination.	Makiura, et al. 1974 Kimura, et al. 1976
Kanechlor 500	Diet	Before	3'-Me-DAB	Diet	Sprague-Dawley rat	Hepatocellular carcinoma with 3'-Me-DAB. None with combination.	Kimura, et al. 1976
Kanechlor 500	Diet	After	3'-Me-DAB	Diet	Sprague-Dawley rat	Combination increased hepatocellular carcinoma over 3'-Me-DAB alone.	Kimura, et al. 1976

TABLE 3-continued

SUMMARY OF CO-CARCINOGENESIS STUDIES WITH PCBs

Product	Mode	Time	Other Treatment[1]	Mode	Species	Observations	Ref.
Kanechlor 500	Diet	With	3'-Me-DAB + DEN	Diet	Sprague-Dawley rat	Combination decreased hepatocellular carcinoma over 3'-Me-DAB + DEN.	Makiura, et al. 1974
Kanechlor 500	Diet	With	2-FAA + DEN	Diet	Sprague-Dawley rat	Combination decreased hepatocellular carcinoma over 2-FAA + DEN.	Makiura, et al. 1974
Kanechlor 500	Gavage	After & With	DEN DDT	Water Gavage	Wistar rat	Combination increased hepatocellular carcinoma over DEN + DDT.	Nishizumi, 1979a,b
Kanechlor 500	Gavage	After & With	DEN SPB	Water Water	Wistar rat	Combination increased hepatocellular carcinoma over DEN + SPB.	Nishizumi, 1979a,b
Kanechlor 500	Gavage	After & With	DEN SPB, DDT	Water Water, Gavage	Wistar rat	Combination increased hepatocellular carcinoma over DEN, SPB, + DDT.	Nishizumi, 1979a,b
Unspecified Kanechlor	Diet	After & With	2-FAA Partial hepatectomy	Diet	Fischer rat	Marked increase in hyperplastic liver nodules.	Ito, et al. 1978
Aroclor 1254	Skin	After	7,12-DMBA	Skin	CD1 mouse	Not a promoter of skin tumors.	Berry, et al. 1978, 1979
Aroclor 1254	Skin	Before	TPA	Skin	CD1 mouse	Little or no skin tumor initiation.	Berry, et al. 1979 DiGiovanni, et al. 1977
Aroclor 1254	Skin	Before	7,12-DMBA + TPA	Skin	CD1 mouse	No difference or decrease in skin tumor induction over 7,12-DMBA & TPA, depending on pretreatment interval.	Berry, et al. 1979 DiGiovanni, et al. 1977
Aroclor 1254	Diet	Before	Walker 256 tumor cells	Intra-muscular	Sprague-Dawley rat	Tumor growth inhibited. Survival time increased.	Kerkvliet and Kimmeldorf, 1977a, b
Aroclor 1254	Diet	Before	MSB cells	Intra-muscular	C57BL/6 mouse	Enhanced growth of MSB tumor.	Kerkvliet and Koller, 1978
Aroclor 1254	Diet	Before	Moloney leukemia virus	Intra-peritoneal	Balb/c mouse	Did not affect oncogenesis of Moloney leukemia virus.	Koller, 1977

TABLE 3-continued

SUMMARY OF CO-CARCINOGENESIS STUDIES WITH PCBs

Product	Mode	Time	Other Treatment[1]	Mode	Species	Observations	Ref.
Aroclor 1242	Diet	Before	Moloney leukemia virus	Intra-peritoneal	Balb/c mouse	Did not affect oncogenesis of Moloney leukemia virus.	Koller, 1977
Aroclor 1221	Diet	Before	Moloney leukemia virus	Intra-peritoneal	Balb/c mouse	Did not affect oncogenesis of Moloney leukemia virus.	Koller, 1977

[1]Chemicals used:
20-MeCh = 20-methylcholanthrene
α-BHC = α-benzene hexachloride
β-BHC = β-benzene hexachloride
DEN = diethylnitrosamine
AFB_1 = aflatoxin B_1
2-FAA = N-2-fluorenylacetamide
3'-Me-DAB = 3'-methyl-4-dimethylaminoazobenzene
DDT = dichlorodiphenyltrichloroethane
SPB = sodium phenobarbital
7,12-DMA = 7,2-dimethylbenz[a]anthracene
TPA = 12-O-tetradecanoylphorbol-13-acetate

[2]Pregnant dams dosed with Kanechlor 400.
Offspring dosed with DEN.

[3]Established tissue culture cell line derived from Moloney sarcoma virus-induced tumors in B6 mice.

TABLE 4

PCBs: Tabulation of Microbial Mutagenicity Tests

Tester Strain	S. typhimurium C3076	D3052	G46	TA98	TA100	TA1000	TA1535	TA1536	TA1537	TA1538	E. coli WP2	WP2 uvrA⁻	Ref.
Product													
AROCLOR 1268	•	•	•	•	•	•	•	•	•	Neg.	•	•	Wyndham, et al. 1976
AROCLOR 1254	Neg.	Neg.	Neg.	Neg.	•	Neg.	Neg.	•	Neg.	Neg.	Neg.	Neg.	Probst, et al. 1981
AROCLOR 1254	•	•	•	Neg.	Neg.	•	Neg.	•	Neg.	•	•	•	Heddle and Bruce 1977
AROCLOR 1254	•	•	•	•	•	•	•	•	•	Pos[?]	•	•	Wyndham, et al. 1976
Kanechlor 500[2]	•	•	•	•	•	•	Neg.	Neg.	Neg.	Neg.	•	•	Odashima, 1976
Kanechlor 500	•	•	•	Neg.	Neg.	•	•	•	•	•	Neg.[o]	•	Sugimura, et al. 1976
2,2',5,5-tetra-chlorobiphenyl	•	•	•	•	•	•	•	•	•	Neg.	•	•	Wyndham, et al. 1976
2,2'5,5'-tetra-chlorobiphenyl	•	•	•	Neg[*]	Neg[*]	•	Neg[*]	•	Neg[*]	•	•	•	Hsia, et al. 1978
Kanechlor 300[2]	•	•	•	•	•	•	Neg.	Neg.	Neg.	Neg.	•	•	Odashima, 1976
Kanechlor 300	•	•	•	Neg.	Neg.	•	•	•	•	•	Neg.[o]	•	Sugimura, et al. 1976
AROCLOR 1221	•	•	•	•	•	•	•	•	•	Pos[*]	•	•	Wyndham, et al. 1976
4-chlorobiphenyl	•	•	•	•	•	•	•	•	•	Pos[*]	•	•	Wyndham, et al. 1976
4-chlorobiphenyl	Neg.	Neg.	Neg.	Neg.	Neg.	•	Neg.	•	Neg.	Neg.	Neg.	Neg.	McMahon, et al. 1979

1. All tests done with and without microsomal activation except as noted.

2. Other strains tested, see text and reference.

• = Not tested.

Neg. = Negative.

Neg[o] = Only tested without activation.

Neg[*] = Only tested with activation.

Pos[*] = Positive only with activation.

Pos[?] = Uncertain. See text.