"Literature Update on Potential Health Effects From Exposures to Polychlorinated Biphenyls and Related Chlorinated Heterocycles"

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December 20, 1983

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Executive Summary

This addition to a previous study of the available biomedical literature on potential health effects of the polychlorinated biphenyls (PCBs) and related impurities is essentially an update from literature and other data retrieved during late 1981 and up through November 15, 1982. As in the first study, the review and conclusions were formulated by a Category I team from DFHL&S, Inc. and subjected to a second level review and critique by an independent Category II team. The comments and suggestions from the Category II review have been incorporated into the final revised text of this annex, for which DFHL&S, Inc. takes full responsibility. All new conclusions for each specific health effect in test animal systems and human populations are discussed in the context of both the new studies reviewed and conclusions from the previous report material based on the earlier literature.

A summary of the major new findings and conclusions with respect to specific health effects resulting from PCB exposure is given below. Also included is a summary of observations on health effects attributable to exposure to polychlorinated dibenzofurans (PCDFs) in test animal models and humans, for this same literature retrieval period.

1. Recent findings on the health status of individuals previously exposed to the mixtures of PCBs, PCDFs and PCQs

(polychlorinated quaterphenyls) in the contaminated rice bran cooking oils consumed during the Yusho episodes in Japan (1960's) and Taiwan (1970's) have served to emphasize and reinforce two points made in the 1982 report. First, it appears that elevated PCDF levels in the cooking oils may have been a principal cause of the enhanced toxicity of those oils over that of the parent commercial PCB mixtures originally charged into the heat exchangers. Second, because of the mixed PCB: PCDF: PCQ content of those oils, the Yusho episodes are not appropriate models for prediction of human health effects from exposure to commercial PCB mixtures.

2. New findings in test animal models and exposed human populations have served to emphasize distinct species specificity in biological processes of tissue metabolism, distribution and excretion. The residual concentrations of PCB congeners in fatty and other tissues are seen to be particularly dependent on ease of metabolism and subsequent excretion of polar metabolites, free or conjugated, from specific congeners in commercial PCB mixtures.

3. In the domain of acute toxicity of PCBs, recent attention has been focused on: (a) the substantial toxicity of 2,3,6-hexa CB (chlorinated biphenyl) in inhibition of growth of a mouse embryo cell line; and (b) the marked hepatotoxicity of the 3,4,5-hexa CB congener in young mice. The latter was a rare congener in commercial PCB mixtures.

These findings do not alter significantly the generalizations on structure vs. toxicity in the 1982 report.

4. Dermal effects in humans attributable to exposure to PCBs and related impurities are of continued interest, even though most of the rare cases of chloracne found in PCB-exposed industrial workers are not severe and not long-lasting after exposure has stopped. Such signs were particularly severe and long-lasting in certain of the Yusho poisoning patients. The most recent evidence reviewed by Kashimoto et al. (1981) for chloracne in Yusho victims points to the enhanced levels of contaminants such as the PCDFs in the rice oils as primary factors in severe chloracne.

5. Recent results on the potential impact of PCB exposure on liver structure or function have derived from an experimental study in the rat and an epidemiological study of capacitor workers. It appears that Aroclor 1254 does not induce benign liver lesions (hyperplastic foci, nodular hyperplasia) in the male Sprague-Dawley rat, and that extended exposure of people in the electrical industry setting to various Aroclors has not resulted in findings of significant liver dysfunction or disease.

6. In two studies, gastric lesions were observed in rats and monkeys receiving Aroclor 1254 orally; such lesions have not been seen in occupationally exposed human populations.

In the rat, at sites near the glandular stomach that were high in alkaline phosphatase, an increased frequency of occurrence of cystic intestinal metaplasia was observed. In the monkey, the principal finding in the stomach was hypertrophic and hyperplastic mucous gastrophy. These are non-cancerous lesions.

7. Studies in the rat using Aroclor 1254 and focused on potential carcinogenesis in chronically exposed rats at target organs such as liver, bladder, stomach and intestinal tract have proven essentially negative, in our opinion. The recent reevaluation of rat stomach tissues from an NCI bioassay (1978) by Morgan et al. (1981) did not result in a statistically significant, dose-related increase in the numbers of stomach adenocarcinomas observed. However, the finding by Preston et al. (1981) of a possible tumor-promoting effect of Aroclor 1254 in the Sprague-Dawley rat is judged as biologically significant and requiring further investigation.

8. A new study has evaluated the effect of Aroclor 1254 on reproduction, the compound being administered in concentrations of 25-900 ppm in the diet of female rats during days 6-15 of gestation. Noted were: (a) decrease in placental protein and glycogen; and (b) decreases in fetal weight and survival rate. Since decreases in food intake were also noted, resulting in reduced nutritional status and a decrease in weight gain, attribution of the placental and fetal effects

to either direct PCB action or to altered nutritional state cannot clearly be made.

9. Recent test results on mutagenic potential of PCBs in Ames tests with Salmonella systems *in vitro*, with or without metabolic activation, have been negative.

10. The ability of Aroclors to induce a variety of microsomal enzyme systems in test animal tissues has been widely documented. A new and unexpected finding in this area of biological activity has been negative: Aroclor 1254 leads to a decrease in microsomal enzyme content of P-450$_I$ in rabbit lung, rather than an increase, with no alteration in P-450$_{II}$ content. On the positive side, Aroclor 1254 causes the induction of glucuronyl transferases and inhibits the release of polymorphonuclear glucuronidase from leucocytes. These biological actions have not been related to human disease states or dysfunctions.

11. Recent experimental studies have shown that immunological effects in test animal systems exposed to PCBs and in Yusho victims exposed to mixtures of PCBs, PCQs and PCDFs can indeed occur. Humans exposed to Yusho oils and monkeys exposed to synthetic mixtures of PCBs, PCQs and PCDFs show signs of immuno-suppression or alterations in immunoglobulins and T-cells. Immunogenic alteration or immunotoxicity have also been observed in rabbits and mice dosed with PCBs. How-

ever, the evidence to date does not point to a direct, dose-related effect of PCBs alone in exposed human populations, expressible as a change in immunological competence.

12. The new epidemiological study of Lawton et al. (1981) on electrical industry workers, taken in conjunction with other recent studies on workers occupationally exposed to PCBs, leads to additional insights in the areas of skin problems, lipid metabolism, pulmonary function, blood glucose levels, blood uric acid levels, and general complaints and symptoms. The Lawton study found no signs of chloracne, past or present, and no dermatological findings specifically related to PCB exposure. Associations were found between blood levels of PCBs and serum triglyceride levels in exposed personnel. The Lawton study extends previous conclusions that PCB exposure under occupational conditions does not lead to signs or symptoms of clinical disease, despite the presence of normal incidence rates for symptoms associated with intercurrent disease and/or general stress. Further, the Lawton study did not reveal any abnormal findings with respect to pulmonary function in PCB exposed workers, or any changes in either fasting blood glucose levels or serum uric acid levels vs. those levels in control populations.

13. In the domain of potential behavioral effects attributable to PCB exposure, several recent studies are of interest. In offspring of rats given prenatal treatment with Kanechlor 300 and 500, and of monkeys dosed with Aroclor 1248

in the diet during pregnancy, signs of decreased learning ability were observed. Offspring of mice after prenatal and postnatal exposure to Aroclor 1254 showed a latency period in making responses, following training in an avoidance response pattern. These behavioral manifestations of PCB exposure in animal models have not been related to any potential behavioral changes in exposed human populations.

14. The PCDFs have been studied with increasing interest in recent years as realization grows that they are toxic derivatives of the PCBs and may be important contributors to the toxicity of mixtures in which they are present if their concentrations are at high enough levels. In recent animal studies they have been shown to be powerful aryl hydrocarbon hydroxylase (AHH) inducers, especially when the lateral 2,3,7,8-positions are fully substituted by chlorine atoms. They produce many toxic signs in test animal systems at doses in the ug/kg/day range, including severe skin changes, swollen eyelids, enlargement of meibomian glands, squamous metaplasia, hyperplasia of the gastric mucosa, thymic involution, hypoplasia of the bone marrow, and hepatotoxicity. Aside from continued analysis of residual health effects in Yusho patients, in which the opinion is growing that the PCDFs are principal contributors to the persistent toxic syndrome observed, no human PCDF exposure vs. effect information has been documented in the 1981-1982 interval.

I. INTRODUCTION

A study on the potential health effects documented in the literature through 1981 for human populations, from exposure to commercial polychlorinated biphenyl (PCB) mixtures under occupational or other environmental conditions, was completed by this firm in 1982. Entitled "Potential Health Effects in the Human from Exposure to Polychlorinated Biphenyls (PCBs) and Related Impurities", the study (hereafter labeled as the DFHLS report), was sponsored by the Edison Electric Institute (EEI) and the National Electrical Manufacturers Association (NEMA). The literature coverage in that study included important toxicological and epidemiological papers generally available through the middle of 1981. It was issued under a date of February 12, 1982.

Since many important PCB studies have appeared in publications or presentations since mid 1981, an update of the DFHLS study in the form of an annex was deemed advisable by both sponsors. The present document constitutes that updated annex to the 1982 DFHLS report. It covers literature retrieved from library and other sources up to a cutoff search date of November 15, 1982.

As in the original DFHLS study, the present annex was constructed in draft by a Category I team composed of members of this firm (Drs. Drill, Friess, Hays and Loomis), reviewed

for content and with suggestions for revision by two external peer reviewers (Drs. Standaert and Alvares), and finally revised by the Category I team which takes full responsibility for its content and conclusions. The annex is cast in a format which largely tracks the original report, section by section, in the sequence of health effects. For each effect, the results of new toxicological and epidemiological studies are considered, and then reviewed in the framework of results cited in the 1982 report to reach judgment on the possibility of occurrence of that effect in exposed human populations. Also included in the annex are sections dealing with recent publications on behavioral effects attributed to PCB exposure, and with new findings on potential health effects from exposures to polychlorinated dibenzofurans (PCDFs).

It is of considerable interest that modern receptor theory has been brought to bear on interpretation of health effects of of PCBs and related compounds. It is possible that manifestations of toxicity may result from a common initial pathway of interactions of certain PCBs, PCDFs or PCDDs with tissue elements in mammalian organisms. In particular, recent interpretations of experimental findings in test animal systems have centered on the possibility that one or more receptor proteins may be involved in initial binding of a suitably substituted compound leading to ultimate expression of a biological

activity such as chloracne or thymic involution. In this picture of events, under a theory proposed by Poland and Knudsen (1982) and Nebert et al. (1981), the effects stemming from exposure to a given polychlorinated molecule would be traceable to initial binding to an inherent receptor surface whose structure and location in tissues are genetically controlled. Such binding could occur broadly across mammalian species and strains, providing a unifying basis for correlating phenomena of many types in different species. The PCBs as a group are considered to be the least potent in this binding - mobilization process, the PCDFs orders of magnitude more potent, and the PCDDs the most potent of all.

The receptor substance itself is thought to be regulated genetically, and to be cytoplasmic in location. If the receptor is induced by a given stimulus and triggered by binding of the agonist, the mobilization of the endogenous transmitter substance could then account under this theory for hyperplasia at a variety of cutaneous and hepatic tissues, involution of the thymus, and metabolic derrangements leading to lesions of chloracne. The theory is presently quite useful, in the material that follows, in efforts to understand phenomena that extend across many species and are triggered by specific molecular structures in the PCB and PCDF families of compounds.

II. YUSHO EPISODE

Since the occurrence of the 1968 Yusho episode in Japan involving consumption of Kanemi rice bran cooking oil containing PCB, PCQ, and PCDF impurities which were accidentally leaked into the oil from heat exchanger coils originally charged with Kanechlor 400, the medical follow-up and history of recovery of these patients has been persistent and well documented. Yoshihara and Yoshimura (1981) have recently reviewed residual health effects from this intoxication episode, as seen in medical examinations made over the past decade. In general, most of the clinical symptoms from this bran oil intoxication have diminished gradually, accompanied by a decrease in blood PCB levels to a mean residual of 7 ppb (vs. a mean level of 3 ppb in control subjects). Since the estimated average total intakes of PCBs, PCQs and PCDFs in these patients during 1968-1969 amounted to 633, 596 and 3.4 mg respectively (Yoshihara and Yoshimura, 1981) the persistence of traces of markers of the original intoxicant load in the circulatory systems of these individuals after a 15 year period is striking. Perhaps stemming from residual body burdens in storage tissues, this occurrence of traces of marker substances in blood has been accompanied by the persistence of certain symptoms, e.g. subcutaneous cyst formation, pigmentation of the skin, eyelids and gingiva, eye discharge, and abnormal changes of the tarsal gland in a relatively small number of severely affected Japanese patients.

Considerable variance has been recorded in estimates of PCB, PCQ and PCDF intakes in patients and in oil samples from the Taiwanese episode. In a very recent analytical study, Masuda et al. (1982) have reported on analyses for PCB, PCQ, and PCDF congeners in the 1979 Taiwan Yusho oil, and in the blood of Taiwanese patients sampled several months after the onset of symptoms from that episode. The levels in oil and blood were compared with those from the Japanese Yusho episode of 1968, except that blood samples from Japanese patients were taken 5 years after that poisoning episode. It was found that: (1) the concentrations of PCBs in the Taiwan oil (60-100 ppm) were 10 percent or less of that in the Japanese oil (900 ppm), but the concentrations in the blood of Taiwanese patients were about ten or more times those of Japanese patients. These results follow from the ingestion of higher amounts of oil by the Taiwanese (about 12.3 kg per person vs. about 800 ml for Japanese consumers), the net ingestion of about equal amounts of PCBs by the two populations, the longer time interval for metabolism/elimination by the Japanese, and the fact that the Taiwanese oil had a higher proportion of more highly chlorinated congeners. (2) The Taiwan rice oil contained a much lower concentration (90-180 ppm) of PCQs than did the Japanese Yusho oil (800 ppm). (3) Both the Taiwanese and Japanese oils contained PCDFs, chiefly 2,3,4,7,8 - penta CDF and 2,3,4,6,7 - penta CDF, which were also detected in the blood of Taiwanese

patients. In view of previous findings by these investigators that 2,3,4,7,8 - penta CDF is one of the most accumulative congeners in the livers of humans, monkeys and rats, and is very potent in its biological actions (P-448 induction, thymic atrophy or liver hypertrophy), Masuda et al. (1982) conjecture that this congener may well be one of the most important etiologic agents for the Yusho poisonings in both countries.

Yoshihara and Yoshimura (1981) also take note of the increasing body of evidence implicating the PCDF component in the Japanese Yusho oil as an important contributor to the total toxic syndrome. Further, they cite supporting experimental data in the rat with a variety of PCDF congeners, given at single doses in the range 1-10 mg/kg, which included significant lowering of thymus weight, increase in liver weight, and retention in liver of large fractions (20-97%) of the administered PCDF congeners that contain 5 or 6 Chlorine atoms per molecule.

Nagayama et al. (1981) have also contributed to the data base on possible contributions of the PCDF component to the Japanese Yusho syndrome, from experiments on controlled heating of Kanechlor 400 which parallel the cumulative heating cycles characteristic of the use of this fluid in a metal heat exchanger. In the presence of metallic iron, nickel or stainless steel and a small amount of water contaminant as

an oxygen source, heating at 300°C for seven weeks leads to an increase of PCDF content in the Kanechlor to levels of 240-560 ppm; further heating at 350°C for four more weeks led to enhancement of PCDF content to 610-740 ppm.

The possible role of PCDFs as a principal agent in Yusho poisoning has been studied in detail by Kashimoto et al. (1981). This work was based on comparative health effects observations on a group of 56 patients in the Japanese episode, 15 Yusho patients in the Taiwanese event that occurred about ten years later, control populations, and groups of workers occupationally exposed to PCBs in Japan and Taiwan. Kashimoto et al. have presented analytical data on relative PCB: PCQ: PCDF residual levels in liver in Yusho patients from the Japanese episode, showing relatively high retention of PCDFs in that tissue (PCBs: PCQs: PCDFs = 100:76:20), and on concentrations of these components in several samples of the toxic Yusho oils obtained during the two separate but parallel intoxication episodes. From their data analyses, Kashimoto and colleagues concluded that the PCDFs in the Yusho oils were the major pathogenic compounds contributing to the development of the Yusho syndrome in the Taiwanese patients, despite their low levels in blood compared to those of the PCBs and PCQs (PCBs: PCQs: PCDFs = 500:160:1). This conclusion was based principally on the following points.

1. The clinical manifestations and duration of Yusho symptoms were disproportionately more severe and

persistent in consideration of the blood PCB levels observed in Taiwanese patients than in PCB - exposed populations of industrial workers with considerably higher blood PCB levels who had much more benign or mild clinical syndromes.

2. PCDFs show a marked tendency to accumulate in the liver, which could well explain the frequent initial finding of jaundice and other serious abdominal symptoms in Yusho patients and the absence of such changes in people occupationally exposed to PCBs.

3. From biological dose vs. response data for a series of toxic effects in many test animals, including the chick, monkey, mouse, and rat, it is apparent that the toxicities of PCBs and the derived PCQs are of the same order of magnitude, but that the toxic potencies of PCDFs are greater by factors ranging from 100 - 10,000.

Finally, Chen et al. (1981) have analyzed the toxic contaminants in a Taiwanese Yusho cooking oil that was ingested by approximately 2,000 people. These investigators studied six samples of the oil, both for total PCB and PCDF content and for the distribution of congeners in each family of compounds. They found that the distribution in the PCB fraction favored the more highly chlorinated structures

(penta-CB: tetra: hexa: hepta: tri: = 47:30:16:6:1), and that the PCDF congeners were mostly of the penta - CDF and tetra CDF variety with only minor amounts of hexa - CDF and tri - CDF. This distribution of components differs from that in Japanese Kanechlor 400, and would be in accord with more lasting Yusho symptoms by persistence in tissue storage of more highly chlorinated molecular species that are less active metabolically and less likely to be excreted as polar metabolites or their conjugates.

Discussion and Conclusions

These recent findings, especially as they have focused increasingly on the potential role of elevated levels of PCDFs in contaminated cooking oils as the principal contributor to the Yusho syndrome in the Japanese and Taiwanese intoxication episodes, serve to emphasize and reinforce points made in the DFHLS analysis of earlier studies. First, heat exchanger action on PCB fluids leading to elevated PCDF levels contributed to the enhanced toxicity of the Yusho cooking oils over that of the parent commercial PCB mixture. Second, because of the unique PCB:PCQ:PCDF distribution of the Yusho oils, it is not correct to use these intoxication episodes as models of acute or subchronic PCB poisoning in humans, or for prediction of human health effects from exposure to commercial PCB mixtures.

III. BODY BURDENS, METABOLISM AND KINETICS

A. Animal Experiments

In continuation of studies on the biological fate of individual PCB congeners in animal models following administration of a single dose of a radiolabeled compound, Sipes et al. (1982) have reported on the distribution, metabolism and excretion of 2,4,5, 2',4',5'- hexa-CB in the beagle dog and the cynomolgus monkey by following the levels of ^{14}C labeled material in excreta, blood and tissues for up to 15 days in the dog and 90 days in the monkey. These workers observed the rates of elimination of this persistent hexa-CB from these test animals. Elimination from blood occurred as a biphasic kinetic process. After the first rapid phase, the slower terminal elimination from blood was characterized by rate constants that were three times as great in the dog as in the monkey. The kinetics of excretion of the PCB in the two species also differ markedly: by 15 days the dog had excreted 66 percent of the dose, mainly in the feces, whereas the monkey in a 90 day interval had excreted only 18 percent of the dose. The remainder of the dose in both species was found largely as the parent compound in adipose tissue and skin as the major storage depot. Further study on biliary excretion of the hexa-CB in the two species again showed that excretion processes in the dog are faster than in the monkey, fourfold in this case during two hours, with 0.8 percent of the label appearing in the bile of anesthetized dogs during this period. However, the monkey excreted a

greater percentage of the dose as parent compound into the biliary flow than did the dog, reflecting a lower degree of metabolism of this hexa-CB in the liver of the primate.

From these and other obeervations, Sipes et al. reached a number of significant conclusions. First, the dog is clearly able to eliminate this hexa-CB from the body substantially faster than the monkey. Indeed, the dog is the only test species that has been shown to reduce its body burden of this persistent PCB substantially, probably because of its ability to metabolize the molecule despite the lack of vicinal unsubstituted ring position pairs. The monkey appears to be similar to other species in its pattern of elimination of this hexa-CB. The processes of tissue distribution in the dog are relatively rapid, beginning with early distribution into skin and adipose tissue. In the dog liver, metabolism is relativsly facile in that approximately 80 percent of the radiolabel present in that tissue at 24 hours was in the form of metabolites. In contrast, for both dog and monkey, the major molecular species present in muscle, skin and adipose tissue was in the form of unmetabolized parent compound. For both animal species, the major route of elimination of the hexa-CB and metabolites was via the feces.

These results of Sipes et al. with 2,4,5,2',4',5'- hexa-CB can be integrated with findings from previous studies by Matthews and his colleagues (e.g. Matthews and Anderson, 1975)

1975) to afford a brief survey of the pharmacokinetic behavior of three PCBs in three species, dogs, monkeys and rats. For 4,4' di-CB, data from the rat afford reasonable predictive power for distribution, metabolism and elimination in the dog, but not in the monkey. For 2,3,6- hexaCB, the dog and the rat eliminate the compound at similar rates which were, however, faster than the observed rate in the monkey. Finally, in the present study by Sipes et al., the 2,4,5 - hexaCB data from the monkey are quite similar to previous findings in the rat, but the dog is decidedly different in that its metebolism and elimination retes are much faster. Clearly, species specificity operetes markedly and not always predictably in the pharmacokinetics of PCBs in test animal models. The reason behind the ability of the dog to metabolize the persistent 2,4,5 - hexaCB, presumably by direct biochemical action at unsubstituted ring meta positions, is presently unknown.

A point bearing on the direction of PCB metabolism in liver by monooxygenases induced by Aroclor 1254 or by 3-methycholanthrene (3-MC) was demonstrated in a recent paper by Halpaap-Wood et al. (1981). These workers studied the actions of ensymes contained in 9000g fractions derived from the PCB induced Sprague-Dawley rat and the Ha/ICR mouse on unsubstituted biphenyls. When a single polar group is already in place, as in the monohydroxylated biphenyls, it appears that induction of the 3-MC type (P-448 requiring

enzymes) leads to further monohydroxylation in the ring that already bears the first hydroxyl group. The diols produced in this metabolic step result from ortho-para direction by the initial hydroxyl group, leading to 2,5 - and 3,4 -diols. These workers also demonstrated a species difference in the effects of PCBs on the hydroxylation of biphenyl in mice. They showed that Aroclor 1254 induction of biphenyl metabolism differed in the rat and in the mouse. In the rat, PCBs induced hydroxylation both in the 2- and 4- positions. In the mouse, Aroclor 1254 resulted in an increase in 2- OH biphenyl formation, but not in the 4- hydroxylation.

Work on metabolic hydroxylation of commercial Aroclor mixtures in test animal systems has recently been extended to the terphenyl series. Voss (1981) has observed that the mixture of polychlorinated terphenyls (PCTs) in Aroclor 5460 and a representative hexaCT congener (2,2',2" 5,5',5'' -hexa-CT) undergo metabolism in the Sprague-Dawley rat to yield monohydroxylated metabolites, which as yet have not been fully characterized. The rates of excretion of these hexa-CT congeners and their metabolites in feces and urine were quite low: within 7-14 days only about 10 percent of the radiolabel in the administered dose was recovered in excreta, largely in the feces.

B. Human Observations

Previous studies by Jensen and Sundstrom (1974), who determined the fate of 40 PCB congeners in human adipose had shown that PCBs with no chlorines at the 3,4- position of one ring (e.g. 2,5- diCl-) constituted about 20% of PCBs in human adipose tissue. Two compounds, 2,4,5,2',4',5'- and 2,3,4,2',4',5'- hexachlorobiphenyl accounted for 36% of the PCBs is tissue. Similar findings were reported by Kuroki and Masuda (1977).

In an effort to assess exposure among workers in capacitor manufacture, Wolff et al. (1982) determined PCB concentrations in plasma (290) and adipose tissue (61). The highly chlorinated congeners appeared to be present mostly in tissues of workers who had been exposed to the higher chlorine-containing Aroclors in the past. The less highly chlorinated PCBs, di-, tri-, and tetrachlorobiphenyls, were found in workers who were recently employed and had thus been exposed to the lower chlorine-containing PCBs mixtures, such as Aroclors 1016 and 1221 which were used from 1971 to 1976. Further they found that higher exposure occurred among persons with direct contact with PCBs, in jobs such as capacitor filling. Adipose tissue concentrations were proportional to those in plasma.

Wolff et al. (1982) have reported recently on epidemiological findings in a limited cohort of 26 workers employed

over a period of years in the manufacture of electrical capacitors. They presented analytical data on the content of 37 different PCB congeners in paired samples of adipose tissue and plasma in these workers. It was noted in general that the concentrations of PCBs in tissues were related to the duration and relative intensity of exposures to these chemicals in the workplace, with a median range of 0.1-9 ug/g for individual congeners in adipose tissue and 0.3 - 27 ng/ml in plasma. As a rule, the PCB concentration in adipose tissue was proportional to that observed in plasma, with partitioning between adipose and plasma in an approximate ratio 190:1. Congeners with chlorine atoms in 4 and 4'positions, such as 2,4,4'-tri, 2,4,2',4'-, 2,4,5,4'- and 2,4, 3',4' -tetra-CB, were major components in plasma and adipose tissue. These workers had been exposed primarily to Aroclors 1016, 1242, and 1254 (20-54% chlorine). Congeners with unsubstituted 3,4 positions (e.g. 2,5-dichloro substituted) were observed at lower tissue concentrations and lower adipose:plasma ratios than other congeners, probably reflecting metabolism to polar derivatives and excretion by virtue of vicinal positions unsubstituted in either ring. Conversely, compounds with 2,4 and/ or 3,4 positions substituted by Cl on both rings were present in much higher proportions in plasma or adipose tissue than in the PCB mixtures used to fill capacitors, probably because of diminished capability for hydroxylation and elimination, and were characterized by much higher adipose:plasma partition ratios than those for

congeners with unsubstituted 3,4-vicinal ring positions. Also, as expected on the basis of increased PCB tissue storage when metabolic possibilities are limited, those congeners with 2,4-substitution on both rings had higher adipose:plasma partition ratios than congeners with 3,4-substitution on at least one of the rings. Finally, metabolic oxidation and excretion of polar metabolites in human subjects of those PCB congeners with two vicinal unsubatituted carbons correlate rather well with lower adipose:plasma partition ratios previously observed by Matthews and Tuey (1980).

Bickel and Muehlebach (1980) seem to agree with Matthews that by blocking metabolically vulnerable groups (such as 2 unsubstituted carbons) a persistent compound may become an unmetabolizable one. They cite the "key work" of Matthews and Anderson (1975) that the hexa isomer, 2,4,5,2',4',5'CB has no unsubstituted pair and was practically unmetabolized. They also state that in addition to degree of chlorination, the para positions (4,4') are most metabolically vulnerable positions. Thus 2,4,2',4'- tetra-CB is more persistent than the 2,5,2',5'- isomer. Finally, they speculate that the general structure-persistence relationships apply to other polyhalogenated hydrocarbons, such as PBBs, and polychlorinated naphthalenes.

Bickel and Muehlebach noted, for correlation purposes between congener distributions in tissues and in commercial

Aroclor mixtures, that the distribution of PCB congeners in human adipose tissues sampled in their survey centered about a mean degree of chlorination near that of hexa-CB and therefore was closer to the composition of Aroclor 1254 (mean degree of chlorination, 5.1) than to Aroclor 1242 (mean, 3.1) or Aroclor 1221 (mean, 1.2). It is clear, however, that the distribution of congeners in human tissues after extended Aroclor exposure intervals, and a description of their mean degree of chlorination, do not necessarily constitute quantitative indices of identity or relationship to any single Aroclor source. Failure to establish this firm linkage is due, at least in part, to the shifts in residual retention in adipose tissue that can accompany structure-specific metabolism and excretion of suitably substituted congeners from a complex exposure mixture.

Discussion and Summary

The current findings on tissue levels and pharmacokinetic handling of PCBs in test animal models and exposed human populations have not revealed any significant new insights on the mechanisms employed by mammalian systems in coping with the presence of these molecules in tissues. Nearly all studies reviewed suggest strongly that in most species, including humans, the higher chlorine containing isomers are more biopersistent and resistant to metabolism to polar metabolites. The total data base on PCB handling

mechanisms including tissue distribution, metabolism excretion, points to distinct species specificity in these processes, with rate and tissue distribution constants that are also linked to molecular structure of the individual PCB congeners. The residual concentrations of PCB congeners in fatty and other tissues are seen to be particularly dependent on the ease of metabolism and subsequent excretion of polar metabolites of specific components of the commercial PCB mixtures to which humans and other animal species are exposed.

IV. GENERAL TOXICITY

In a recent study by Norback et al. (1981) a cell culture from a mouse embryo cell line was used to examine the toxicity of two hexachlorobiphenyls. This method involves measurement of growth rate and plating efficiencies of the cell line. Evaluation of two hexachlorobiphenyls (2,4,5,2',4',5' and 2,3,6,2',3',6') demonstrates that the 2,3,6,2',3',6'-isomer was the most toxic and the order of toxicity paralleled the long-term whole animal studies. Not all cases showed a linear dose (concentration)-response related effect on the cell numbers after 10 days exposure.

Biocca et al. (1981) fed hexachlorobiphenyl isomers in the diet of five week old mice. Hexachlorobiphenyl residues were determined in adipose tissue and liver. The 3,4,5,3',4',5' -isomer (not commonly encountered in commercial mixtures) was the most toxic of those studied and was the most concentrated in the fat and liver. It also produced the greatest mortality as well as hepatocellular markings, hepatocellular swelling and necrosis, subcutaneous edema and changes in body and organ weights at doses ranging from 0.3 to 300 ppm in the feed.

In a review article by McConnell and Moore (1979) on the general toxicities of halogenated aromatics, including the dioxins, biphenyls, dibenzofurans and naphthalenes, they noted that the structure-toxicity relationships are similar

for all the compounds in non-human primates but the absolute doses to produce toxic effects are quite different. The effects include loss of body weight, alopecia, chloracne, blepharitis, waxy ear canals, dry-scaly-skin and nails and progressive weakness. Fetal deaths often occur and offspring may die early. The signs are less conspicuous in rodents than in non-human primates. Rabbits specifically show chloracne. There are other effects, such as, testicular degeneration, hyperplasia of the epithelial tissue in the urinary tract, leucopenia, pancytopenia and altered serum enzyme systems which may be reflective of organ damage.

Behavioral Effects

The use of behavioral effects in the assessment of the toxicity of xenobiotics, particularly those affecting the nervous systems, is a new field of toxicology which may eventually provide sensitive biological endpoints. There are some who believe that behavioral changes in offspring as well as reduced growth rates should be considered as teratological effects, but until more is known about behavioral lesions and the underlying mechanisms we prefer to adhere to the original concept that teratology refers to abnormal structural changes during embryonic development.

Several animal species have been used to detect subtle behavioral effects of PCBs in offspring of mothers exposed to

the environmental pollutant during various stages of gestation. Shiota (1976) using the Japanese brand of PCBs, Kanechlors 300 and 500, administered by gavage on 8-14 and 15-21 days of gestation to rats, showed that the offspring had lower birth weights than controls and learning impairments were observed in maze testing. However, other field activities and swimming ability of the treated and control offspring were similar. Storm et al. (1981) administered Aroclor 1254 to female mice prior to mating and during gestation. There was no effect on the ability of the mice to learn an avoidance response; but they took longer to learn the response.

The data obtained with primates appear to be more definitive. Bowman et al. (1978) observed that feeding of Rhesus monkeys at 2.5 ppm of Aroclor 1248 in the diet resulted in disruption of discrimination learning. A dose-effect relationship between PCB body burdens early in life and later behavioral effects were observed. Surviving monkeys were hyperactive in locomotor tests and deficient in learning various types of discrimination problems at 6 and 12 months of age. These same monkeys became significantly hypoactive, when compared to controls, at 44 months of age (Bowman and Heironimus (1981). These data may indicate that primates may have a greater sensitivity to in utero PCB exposure than do mice. In other studies, Bowman et al. (1981) also observed hyperactivity in the early stages of development of monkeys whose mothers had received the PCBs. However, in

these studies the hyperactivity did not correlate with PCB dosage, leading to the desirability of further study.

More extensive studies are required to determine which particular period of exposure (gestation or lactation) contributes to the observed behavioral effects. It would also be important to correlate these effects with physiological or biochemical tissue markers, in particular at the central nervous system (CNS) level.

Discussion and Conclusions

Recent activity in the study of the acute toxicity of commercial Aroclors and synthetic or purified congeners has tended to focus on evaluation of specific changes induced in target organs and cell lines. Findings of interest that complement previous literature include: (1) substantial toxicity of 2,3,6-HCB in inhibition of growth of a mouse embryo cell line; (2) marked hepatocellular toxicity of the 3,4,5-HCB congener in young mice; and (3) an explanation for the inability of 4-chloromethylbiphenyl to induce in vitro genotoxic effects in terms of its ready metabolic conversion to the corresponding hydroxy compound. These findings do not alter the generalizations on PCB toxicity, acute or chronic, pointed out in the 1982 report.

The relevance of the behavioral studies in animals to man can only be a matter of speculation, for little or nothing is

known about any structural or metabolic changes that might have taken place during the period of gestation and the ingestion of PCBs in the animal testa. In animal studies, it is well known that housing conditions and malnutrition may alter the development of offspring, leading to some abnormal behavioral pattern. It is our view that one cannot draw any general conclusion from these studies in relation to behavioral changes in man and that much more work will be required to understand the mechanisms involved in altered behavioral activity. Further, the current findings do not cast additional light on the problem of whether or not lesions leading to behavioral aberrations are the precursors of pathologic changes in tissues in animal models or humans.

V. DERMAL EFFECTS

In the previous report on potential human health effects from exposure to PCBs, it was pointed out that one of the clinical manifestations of Yusho disease was the appearance of chloracne in about 82% of the patients who had consumed contaminated rice oil containing PCBs, PCDFs and PCQs. Fischbein, et al. (1979) reported on the clinical findings among PCB-exposed capacitor manufacturing workers, and noted a high incidence (38-41%) of dermatologic problems in his study population which included about 5% of cases with acne-form eruptions. Other investigators (Inoue et al., 1975; Ouw et al., 1976; Chase et al., 1981; Maroni et al., 1981; Baker et al., 1980; Smith et al., 1981) have found low to zero incidence rates for chloracne in populations occupationally exposed to commercial PCBs and derived products.

In a later paper (1982), Fishbein et al. made a more detailed study of dermatological findings among capacitor workers and noted a high prevalence of dermatological effects such as erythema, swelling, dryness, thickening of the skin, rash, hyperpigmentation, comedones and acne. However, acne was far less prevalent in these industrial workers than among patients with Yusho disease, despite high concentrations of PCBs found in the blood of some of the subjects in the Fischbein survey.

Discussion and Conclusions

It seems clear that prolonged exposure to certain Aroclor or Kanechlor type PCB mixtures in the occupational setting, sufficient to establish high blood levels of PCBs, could lead to the development of signs of chloracne in sensitive individuals, but this effect has been seen only rarely. It has generally been observed that an industrial outbreak of dermatitis, including chloracne cases, coincided with the production or use of a poor or oxidized grade of PCB containing enhanced levels of contaminants, presumably the PCDFs, and PCQs. Such contaminants, which have been identified as major components in samples of the rice oils from the Yusho events in Japan and Taiwan, in our opinion, have led to the high incidence of chloracne in Yusho patients. The recent findings of Kashimoto et al. (1981) also point to this conclusion. Compatible with these observations is the finding that most of the signs of chloracne found in industrial workers are not severe and not long-lasting after exposure has stopped, and are not at all comparable in severity or duration to the symptoms seen in some Yusho disease patients.

VI. EFFECTS OF PCBs ON LIVER

A. Hyperplastic Foci and Nodular Hyperplasia in the Liver

In a recent study, Preston et al. (1981) reported that the oral administration of Aroclor 1254 alone or with PCDF impurities did not induce foci of cellular alteration, hyperplastic nodules or cholangiomas in the livers of male Sprague-Dawley rats. Further, pretreatment of the animals with diethylnitrosamine (DENA) followed by administration of Aroclor 1254 did not significantly affect the incidence of foci of cellular alteration or other benign hepatic lesions. These results are summarized in Table I. The tumor-promoting activity of Aroclor 1254 recorded in the Table is discussed in Section VII of this annex. These results are of particular interest from the standpoint that the studies of Calandra (1975), the National Cancer Institute bioassay (1978) and Preston et al. (1981) with Aroclor 1254 all fail to confirm the increase in benign hepatic lesions reported by Kimbrough et al. (1975) for Aroclor 1260 in the rat.

B. Liver Function and Liver Disease in PCB-Exposed Workers

In an unpublished, comprehensive epidemiologic study of General Electric Co. employees, Lawton et al. (1981) studied liver function in 172 capacitor workers previously exposed to PCBs. Serum PCB levels for the workers averaged 276 ppb for

Aroclor 1242, 58 ppm for Aroclor 1254, and 34 ppb for Aroclor 1260. The hepatic studies showed that the mean serum values ± 2 standard deviations (SD) were within the normal range for SGOT, SGPT, alkaline phosphatase, total bilirubin and direct reacting bilirubin analyses. The mean serum SGPT value of 34.5 IU was within the normal range (1-55 IU), but the upper level of +2 SD exceeded the normal range. As a part of this study, Lawton et al. conducted an evaluation of the history of 194 PCB-exposed capacitor workers. There was no relationship between the exposure and episodes of jaundice or hepatitis, most of which occurred prior to employment.

Summary and Conclusions

These recent studies add important findings on the potential impact of PCB exposure on liver structure or function in the rat and in human populations. Clearly, Aroclor 1254 does not lead to benign liver lesions in the male Sprague-Dawley rat, and extended exposure to Aroclors in the industrial setting has not resulted in findings of significant liver dysfunction or disease in capacitor workers surveyed in the Lawton study.

TABLE I

Incidence of Liver Lesions In Male Sprague-Dawley Rats
Treated with Aroclor 1254
(Data from Preston et al., 1981)

Number of:	Treatment* Control	Ar 1254	Ar 1254 + PCDF	DENA + DENA	DENA + AR 1254	DENA + AR 1254 + PCDF
Animals necropsied	72	34	34	32	33	32
Foci of cellular alteration	0	0	0	10(31)	0	1(3)
Neoplastic nodules	0	0	0	6(19)	10(30)	5(16)
Hepatocellular carcinoma	0	0	0	5(16)	21(64)	27(84)
Cholangioma	0	0	0	1(3)	1(3)	2(6)
Cholangiocarcinoma	0	0	0	0	1(3)	1(3)

*Numbers in parentheses are % incidence.

VII. GASTRIC LESIONS

Morgan et al. (1981) examined histologically the stomachs of 144 rats treated with Aroclor 1254. (These preserved tissues were from the NCI, 1978 bioassay study). All stomachs were examined (a) by routine section through the junction of the pyloric region and the duodenum and (b) by section of any areas that gave a positive staining reaction for alkaline phosphatase. Although the phosphatase stain may aid in detecting areas with cellular change, the authors state that most of the stomachs showing positive alkaline phosphatase activity were histologically normal.

The authors reported an increased frequency of cystic intestinal metaplasia in the treated animals; comparative incidences were 0/47 in control rats, 3/48 in low-dose, 3/48 in medium-dose, and 13/48 in the high-dose treated animals. Treatment did not change the frequency of "crypt type" intestinal metaplasia or diffuse intestinal metaplasia. The total number of rats with all types of intestinal metaplasia was 3/47 in control rats, 4/48 in low-dose, 5/48 in medium-dose, and 15/48 in the high-dose treated group. Thus, the high-dose group, but not the low or medium-dose groups, showed a significant increase in intestinal metaplasia.

Metaplasia is characterized by an adaptive substitution by one type of adult or fully differentiated cell for another

type of adult cell. Although intestinal metaplasia and stomach cancer may co-exist in the pyloric region of the rat stomach, it does not mean that the changes are related or that metaplasia is a premalignant change. Findings in this study with respect to stomach or intestinal tract cancer are discussed elsewhere in this review.

An unusual form of gastric lesion has been observed (Geistfeld et al., 1982) in monkeys housed in new facilities which resembled the morphological changes that have been described for animals receiving PCBs in their diet. Forty-five of 259 male monkeys died of chronic progressive disease. The principal necropsy findings were hypertrophic and hyperplastic mucous gastrophy. The gastric lesion was characterized by hyperplasia of the gastric epithelium, hypertrophy of mucus-secreting glands, cyst formation and penetration of glands into the submucosa. PCBs were implicated in this finding because of the presence of Aroclor 1254 in the adipose tissue of the animals and in samples of concrete from pens in which the monkeys were housed. The source of the PCBs appeared to be the concrete sealer used in the construction of the pens. Removal of successive layers of concrete greatly reduced the level of PCB exposure, with a corresponding reduction in animal deaths. Quantitative information on levels of PCBs in the concrete and on dose transfer to the monkeys was not obtained.

Summary and Conclusions

It seems clear that relatively high doses of commercial Aroclors delivered chronically to the stomachs of rats or monkeys can produce pathologic changes in stomach or nearby intestinal tissues. In the rat the major change is seen in an increase in the frequency of cystic intestinal metaplasia in tissue loci otherwise characterized by elevated alkaline phosphatase levels. In the monkey the principal pathologic findings in the stomach are hypertrophic and hyperplastic mucous changes. These recent findings amplify and extend indications of target action on the stomach seen previously in the monkey and rat.

VIII. CARCINOGENESIS: EXPERIMENTAL

A. Hepatocellular Carcinoma

Preston et al. (1981) reported that administration of Aroclor 1254 alone or with added PCDF content for 23 weeks in male Sprague-Dawley rats did not induce hepatocellular carcinoma or cholangiocarcinoma in the treated animals (see Table I, Section VI). They found, however that the number of hepatocellular carcinomas induced by diethylnitrosamine (DENA) was increased when the DENA-treated rats also received Aroclor 1254, either with or without PCDF, indicating a tumor promoting effect of the Aroclor (Table I).

B. Bladder Cancer

Bladder tumors were not observed in rats given Aroclor 1254 for 23 weeks (Preston et al., 1981).

C. Stomach, Intestinal Tract Cancer

Using fixed tissues from an earlier bioassay, Morgan et al. (1981) conducted a reexamination of the gross morphology and histology of the stomachs of 191 animals preserved from the 1978 NCI bioassay of Aroclor 1254 in F344 rats. The stomachs of these animals were stained for alkaline phosphatase activity, and the alkaline phosphatase positive areas were used to select areas of possible pathological significance. By this means, they detected histological changes in an additional three animals which they characterized as early adenocarcinoma; one lesion was in the medium-dose group and two in the

high-dose group. Sex of the animals was not stated. They also noted two regions of severe dysplasia in the high-dose group. The lesions which were observed in the glandular stomach and at the junction between the pyloric region of the stomach and the duodenum by Morgan et al. included intestinal metaplasia, cystic lesions, and three new adenocarcinomas not seen in the original examination. The total incidence of adenocarcinomas observed in a total dosed population of 144 animals was therefore one cancer at the low-dose, three at the medium-dose and two at the high-dose of Aroclor 1254, for a total of six adenocarcinomas. The authors concluded that since the incidence of stomach adenocarcinomas was less than two percent (no tumors in 47 animals) in simultaneous controls, the observation of six cancers in 144 treated rats constituted a finding in which the likelihood of occurrence at random is less than 0.05. Further, they referred to this cancer incidence rate relative to the incidence of stomach adenocarcinomas in historical controls (cited to be one case in 3548 animals), and concluded anew that the occurrence of six adenocarcinomas in 144 Aroclor-treated rats is highly significant ($p < 0.001$).

These conclusions as to the statistical significance of the adenocarcinoma findings in the stomach tissues reexamined by Morgan et al. should be subjected to scientific scrutiny and challenged on several grounds, detailed below.

1. First, Morgan et al. do not specify what statistical

methodology they employed to reach their conclusions about the significance of the cancer incidence in Aroclor-treated animals vs. either the concurrent controls or the historical controls. The comparisons are further confounded in that the cancer incidence is not separated by both sex and dose for test animals, and proper comparisons are not made between cancer incidences for each sex at each dose level and the corresponding control group values.

2. Since the rate of occurrence of cancers by sex group was not recorded, the only valid test that one can apply to these experimental results is the simultaneous set of comparisons for three dose groups and controls. When this is done, for example, by chi-square analysis with three degrees of freedom, the chi-square statistic is 3.39 for the Morgan data. A value of 7.815 would be required for significance at a level of 0.05. Therefore, the rates of occurence of stomach adenocarcinomas among the dosed and concurrent control groups as presented in the paper could represent just a random drawing from the same populations.

3. Attribution of possible biological significance to the adenocarcinoma incidence rates in the three treated groups is further compromised by the fact that there is no clear Aroclor dosage vs. response relationship among the results. Indeed, fewer cancers were observed at the high-dose than at the the medium-dose. The authors do not discuss this point.

4. Finally, the reference by Morgan et al. to cancer rates in historical controls is inappropriate and possibly misleading, since the tissues of historical controls were not examined in the same painstaking manner and by the same personnel who performed the Aroclor animal reexamination. Bearing in mind that Morgan et al. picked out cancers in their tissue reexamination that had not been recorded by the pathologists in the 1978 bioassay procedures, it could well be that cancers in the large number of historical controls went unrecorded. These points constitute serious objections to any possible conclusion that the 1978 NCI bioassay provided consistent evidence for the occurrence of stomach adenocarcinomas in F344 rats treated with Aroclor 1254.

Another aspect of this general problem of potential stomach tumors in test animals dosed with Aroclor mixtures is provided by the findings from studies in monkeys discussed in our previous health effects document. Workers in the laboratory of Dr. J. R. Allen at the University of Wisconsin had performed experiments with Aroclor 1248 administered in the diet of the Rhesus monkey, leading to findings of gastric lesions in this primate species. These findings included hypertrophy and hyperplasia of the gastric mucosa, edema of the stomach submucosa, and mucous conversion of the gastric epithelium at doses as low as 100 ppm in the feed. No such gastric changes were documented in monkeys fed at 2.5 or 5 ppm in the diet. Additionally, Aroclor 1248 in the diet as low as 2.5 ppm

also produced reproductive problems in monkeys and some fetotoxicity in their offspring in the experiments conducted by Dr. Allen and his colleagues during the period 1974-1980.

The work of Allen et al. (1973) on gastric lesions in monkeys received some confirmation in the work of Becker et al. (1979) who produced gastric lesions in Rhesus monkeys receiving dosages of Aroclor 1242 in the mg/kg range. However, Iatropoulos et al. (1977) working with the PCB mixture Clophen A-30 and the monkey as test animal did not observe the production of gastric lesions. The question of whether low doses of PCB mixtures of a sufficient degree of chlorination can indeed produce gastric lesions in a primate model, possibly as the precursors of the carcinogenesis which Morgan et al. claim to have seen in Aroclor 1254-treated rats, is an important health effects issue that requires further experimental study.

Finally, in the recent study of Preston et al. (1981) it was found that treatment of male Sprague-Dawley rats with Aroclor 1254 (100 ppm in the diet) for 23 weeks did not induce gastrointestinal carcinomas.

Summary and Conclusion

The recent evidence on carcinogenic potential of Aroclor 1254 in the rat, as cited above in two experimental studies

focused on the liver, bladder, stomach and intestinal tract as target organs, must be viewed as essentially negative in our opinion. However, the finding by Preston et al. (1981) of a possible tumor promoting effect of Aroclor 1254 in the Sprague-Dawley rat must be judged as biologically significant. Further studies to probe possible promoter activity of PCB mixtures in mammalian systems seem worthy of early attention.

IX. REPRODUCTIVE EFFECTS

Spencer (1982) has made an assessment of the fetotoxic effects of Aroclor 1254 in rats by adding the compound to the diet of pregnant and pseudopregnant Sprague-Dawley rats; doses ranged from 25 to 900 ppm administered on days 6 through 15. There was a decrease in ovarian protein in pseudopregnant rats and a decrease in placental protein and glycogen in pregnant rats. Embryonic resorption did not occur up to exposure levels of 900 ppm, but there was a decrease in fetal survival rate and fetal weight at concentrations of 300 ppm and 100 ppm respectively. There was also a significant decrease in food intake and maternal body weight at doses of 300 ppm and above, and nutritional factors alone may account for the reduced fetal survival and fetal weights observed at these high concentrations.

Summary and Conclusion

This new study on the impact of Aroclor 1254 on reproduction in female rats fed 25 to 900 ppm in the diet at a critical period during gestation confirms previous reports that high doses of the PCBs may be fetotoxic. Although it is known that high doses of PCB may affect food intake and weight gain, the degree to which the fetotoxic effects are directly caused by PCBs or are a secondary consequence of altered nutritional state in the dosed animals is presently unknown.

X. MUTAGENESIS

Although this annex deals principally with polychlorinated biphenyls, it is of interest to note results from a study by Ashby et al. (1981) on mutagenic properties of substructural elements of the PCBs in the in vitro Ames test systems. Starting with single aryl rings, these workers found that aniline and benzyl chloride give a negative response in the Ames test for mutagenicity, using a number of strains of S.typhimurium. The addition, however, of a benzene ring to form 4-aminobiphenyl (4AB) and 4-chloromethylbiphenyl (4CMB) greatly enhances the biological activity above that of the parent compounds. Positive responses for 4AB were observed in strains TA 1537, TA 1538, TA 100 and TA 98. A negative response was observed in TA 1535. Positive responses for 4CMB were obtained in strains TA 98, TA 1538 and TA 100 but weak and non-reproducible positive effects in strains TA 1535 and TA 1537.

On the basis of these observations one might expect that certain PCBs would also be mutagenic in the same Ames assay systems. Schoeny (1982) tested 2,4, 2',4'-tetrachlorobiphenyl, 3,4,3',4'-tetrachlorobiphenyl, 4-chlorobiphenyl, 2,4,6, 2',4',6'hexachlorobiphenyl, and several chlorinated dibenzofurans. All the compounds were found to be non-mutagenic for strains TA 98 and TA 100 when tested over a 3-log dose range. They were also non-mutagenic when tested with either induced or non-induced microsomal extracts as

activators. These results are reminiscent of the recent finding on the lack of mutagenic potency, with or without S9 activation, of the important PCDD congener 2,3,7,8 dibenzo-p-dioxin in the Ames systems.

Summary and Conclusion

These recent test results on mutagenic potential of PCBs and their analogs in the Ames test with Salmonella test strains add to a body of previously available data pointing to a lack of potency with regard to this important biological effect. Although the biphenyl nucleus substituted with a single polar or reactive group (NH_2 or CH_2Cl) yields positive results in Ames systems, single or multiple substitution of Cl groups on the biphenyl rings generally does not yield mutagenic activity, with or without metabolic activation by S9 fractions. It is of further interest that this lack of mutagenic activity also extends to several polychlorinated dibenzo-p-dioxins.

XI. OTHER HEALTH EFFECTS

A. Effects on Enzyme Systems

Many reports have appeared in the past two or three years which dealt mainly with effects of the PCBs and related halogenated compounds on liver microsomal enzyme systems. Most of these reports simply confirm what is known of the effects of PCBs on the liver and its enzymes. Gartoff et al. (1981) showed that single or repeated doses of various Aroclors in rats produced decreased hexabarbital sleeping-time and increased liver weight. Shull et al. (1982) studied the inductive capacity of certain Aroclors in mink and ferrets and found that at 20 ppm in the diet, Aroclor 1016 and 1242 produced only weak induction of mixed function oxidases (MFO). Related polychlorinated compounds such as the polychlorinated naphthalenes induce mixed hepatic microsomal enzymes in immature male rats, as shown in a study by Cockerline et al. (1981). They induced both the phenobarbital and methylcholanthrene types of enzymes. Pereira et al. (1982) compared single doses of Aroclor 1254 to single doses of diethylnitrosamine (DENA) as inducers of foci in the liver which produce gamma glutamyl transpeptidase and found that Aroclor was a much weaker initiator of those foci than DENA.

The rate of progression of the effect of single doses of Aroclor 1254 on the different liver forms of cytochrome P-450s (a,b,c,) and epoxide hydrolase (EH) in rat liver microsomes was reported by Parkinson et al. (1982) to vary

in regard to rate of induction and duration of effect. Ryan et al. (1982) isolated a new hemoprotein from rat liver called P-450$_e$ and found that it could be induced by Aroclor 1254 and phenobarbital, but not by 3methylcholanthrene. Nilsen and Tuftgard (1981) reported that various forms of cytochrome P-450 were inducible in rat livers by paraffins. Ryan and Levin (1981) have purified and characterized the spectral peaks of three different cytochrome P-450 enzymes (a,b,c) from rats given Aroclor 1254.

Lilienblum et al. (1982) showed that Aroclor 1254 stimulated glucuronidation systems, that is, the glucuronyl tranferases, GT_1 and GT_2. It was also found that GT_1 is induced by methylcholanthrene, whereas GT_2 is like the phenobarbital induced enzyme.

In connection with the effects of PCBs on glucuronidase release when polymorphonuclear leucocytes are treated with certain releasers, such as human blood serum plus zymogen, Lee (1981) showed that Aroclor inhibited release of polymorphonuclear glucuronidase. The possible significance of this finding is not clear. A clearly protective mechanism has been shown to result when the microsomal system is induced by the PCBs in Aroclor 1254 since this induction partially protects mice from benzeneinduced leucopenia (Wierda et al., al., 1981; and Greenlee et al., 1979). In chicks, Hansen et al. (1981) found that Aroclor 1254 was less potent as an

enzyme inducer than was the same amount of Aroclor 1254 in animal fat obtained from swine which was subsequently fed to the chicks. High-dose levels (i.e.; 9mg/kg) of the Aroclor, were required, but the chicks did not show any other toxic effects from the PCBs. The effects of the pesticides mirex, photomirex and kepone were found by Chu et al. (1980) to be additive, but they did not potentiate the induction of microsomal enzymes produced by polybrominated and polychlorinated biphenyls in rats.

Ueng and Alvares (1981), Alvares et al. (1982), Alvares (undated workshop report, 1981) and Serabjit-Singh et al. (1982) have shown that in the rabbit lung, microsomal enzyme content as well as cytochrome P-450 were decreased, rather than induced, by administration of Aroclor 1254. The decrease was due to a decrease in $P\text{-}450_{I}$ with no alteration in $P\text{-}450_{II}$. At the doses of the Aroclor used by Alvares et al. (1982) the PCBs were poor inducers of arylhydrocarbon hydroxylase activity in the livers of mice as well as in the kidney of rabbits, but resulted in suppression of this enzyme in rabbit lung. The Serabjit-Singh et al. study (1982) showed that the effect on the rabbit lung enzyme system was dose related and that 200 mg/kg of Aroclor 1260 in one or two doses produced no change in lung cytochrome P-450.

The effect of topically applied Aroclor 1254 on the same enzyme systems in the skin has been investigated by Bickers et al. (1982) who showed induction in whole skin, epidermis and

dermis of arylhydrocarbon hydroxylase, O-deethylase and epoxide hydrolase. They emphasized the importance of the skin as an organ for metabolizing xenobiotics.

Summary and Conclusions

The ability of Aroclors to induce a variety of microsomal enzyme systems in tissues of test animals has been widely documented. The recent results add to the body of information on the specificity of the induction process in terms of the types of enzyme systems induced and of the specific tissues in which the process occurs. Notable new findings include: (1) the surprising observation that Aroclor 1254 leads to a decrease in microsomal enzyme content of rabbit lung rather than an increase, due to a decrease in $P\text{-}450_I$ with no alteration in $P\text{-}450_{II}$ content; (2) the finding of a new induced hemoprotein in rat liver designated as $P\text{-}450_e$; (3) the induction of glucuronyl transferases by Aroclor 1254; (4) Aroclor 1254 inhibition of release of polymorphonuclear glucuronidase from leucocytes; and (5) the demonstration that PCB induction in skin leads to an important contribution to metabolism of xenobiotics.

B. Immunologic Effects

Chang et al. (1981) followed 30 patients who had "Yu-Cheng" disease, due to ingestion of rice bran oil contaminated with PCBs, PCDFs and PCQs, in 1979, and found differences in these subjects compared to controls in regard to the serum immunoglobulins. They found that serum IgA and IgM but not IgG globulins were lower in the patients than in controls. Also total T-cells, active T-cells and T_u-cells were decreased in the patients but the percentages of B-cells and T-cells were not different in the patients. Hori et al. (1981) succeeded in simulating the Yusho disease in monkeys by feeding them daily doses of a mixture of 5.0 mg of Kanechlor 400, a polychlorinated dibenzofuran (5 ug) plus a polychlorinated quaterphenyl (5.0 mg). The animals exhibited the usual signs and symptoms of Yusho, such as edema, liver necrosis, and immune suppression. These results strongly suggest that PCDFs and/or PCQs play a major role in the etiology of Yusho.

Rabbits which were fed Aroclor 1248 for 4 weeks prior to mating, during gestation, and nursing of the young, were tested for immunogenic alterations (Thomas and Hinsdill, 1980). No general toxicity was seen in the mothers. Only the offspring from the mothers fed 250 ppm (the highest dose in the study) showed some impaired contact sensitivity but these offspring also showed impaired body weight gains. It was questioned whether the effect observed on contact sensitiv-

ity was a direct effect rather than an indirect effect due to the general action on body weight gain.

Silkworth and Grabstein (1982) showed that PCB-produced immunotoxicity was mediated through the Ah (aryl hydrocarbon) gene locus in mice and that only the "planar" structure of the PCBs (planar being 2,3,3',4'- versus non-planar 2,5,2',5'-isomers of tetrachlorobiphenyl) was active.

Summary and Conclusions

Continuing work on immunological effects in humans exposed to PCBs mixed with other chlorinated substances in contaminated rice bran oil (PCBs, PCDFs, PCQs), and in monkeys exposed to synthetic mixtures of these three components, has shown signs of immunosuppression or alteration in immunoglobulins and T-cells. Causality with respect to PCBs alone has yet to be established in these species. Additionally, signs of immunogenic alteration or immunotoxicity in rabbits and mice dosed with PCBs have been observed.

Despite the growing numbers of studies on this health effect, the body of available evidence does not suggest that PCBs alone can produce a dose related effect on immunological competence in the human. In human PCB workers there is no reported effect on food intake and body weight, an effect seen only at relatively high-dose levels in animals.

XII. Epidemiology

A. Skin Problems

Lawton et al. (1981) made a review of medical records of workers at several General Electric plants; the records from a five year period (1970-1975) were reviewed at which time the employee census varied between 1,500 and 1,800. Skin irritations and dermatitis were reported among these employees.

Medical records for a 15 year period of individuals occupationally exposed to PCBs were reviewed. During this period, 49 individuals presented with "allergic contact dermatitis" to PCBs or other constituents of the compounded dielectric fluid; the dermatitis cleared rapidly, usually without medication, upon removal from exposure. No cases of chloracne were observed.

Lawton et al. (1981) also examined the dermatological history of 194 PCB-exposed capacitor workers; 75 employees had 100 dermatological findings of various types in their medical history. Many of the tabulated cases had dermatological findings that would not be considered work-related, since employees recognized the contact nature of most rashes (fiberglass, dirty gloves, watch straps, etc.). Two cases of rash were related to epoxy exposure and not to dielectric fluid. Physical examination of these employees was unremark-

able and no signs of chloracne past or present were discovered. Pigmentation, thickening of the skin or discoloration of the fingernails were not noted. Many of the dermatological episodes were noted to antedate employment at General Electric.

Most employees had multiple industrial exposures arising from job mobility (maintenance men, use of other chemicals such as solvents, etc.). The authors conclude that it was difficult to relate any dermatological findings specifically to PCB exposure.

B. Lipid Metabolism

The recent epidemiologic study of Lawton et al. (1981) also focused on workers with elevated serum PCBs. The average serum levels were: Aroclor 1242, 276 ppb; Aroclor 1254, 58 ppb; Aroclor 1260, 34 ppb. The mean serum triglyceride level for 172 PCB-exposed workers was 144.5 mg/dl, which is within the normal range of 50-200 mg/dl, but the upper level of 2 SD was above the normal range. Detailed statistical analysis demonstrated:

1. A significant positive association between log serum triglycerides and log serum PCB levels, either as Aroclor 1242, Aroclor 1260 or total PCB;

2. When PCB and DDE levels were considered as

distributed in a serum lipid fraction (sum of triglycerides and cholesterol), the log serum triglyceride level was not significantly dependent on log serum PCBs plus DDE;

3. When PCBs and DDE were considered as body burden, the log serum triglyceride level was significantly dependent on burden only when the percent body fat was removed as an independent variable in the case of Aroclor 1242;

4. That the log serum triglycerides were positively related to percent body fat but negatively related to sex.

Following discontinuation of the use of PCBs at the General Electric plants on 6/30/77, Lawton et al. (1981) found that the serum cholesterol level for each sex of PCB-exposed workers showed a statistically significant decline through 1979. The 1979 determinations showed that the range ± 2SD) in the exposed group was above the upper limit for the standard range. The serum cholesterol had a significant positive association with log serum PCBs but not when stated as PCB in adipose tissue or PCB body burdens. The serum cholesterol level was service and/or age dependent.

C. General Complaints & Symptomatology MONS 015837

It should be recognized that any survey data on sympto-

matology are subjective in nature and that the "normal" background incidences of various symptoms can vary widely in different studies, depending in part on how questions are asked and on the type of questions used. Thus, symptomatology reported by workers is the least reliable of the data available for analysis.

Fischbein et al. (1979), studying volunteers from General Electric plants, did not find a significant correlation between reported symptoms and plasma PCB level or between symptoms and duration of employment. Lawton et al. (1981), studying symptoms reported by 194 PCB exposed capacitor workers at General Electric plants, found reported symptoms (headache, dizziness, depression, memory loss, fatigue, nervousness, sleeplessness, somnolence) to be less frequent than reported by Fischbein et al. (1979). The analysis of Lawton et al. (1981) suggests that the symptoms reported can be reasonably associated with intercurrent disease and/or the general situations of life stress.

Smith et al. (1981), summarizing data from combined work sites, reported that coughing at work and eye irritation were related to both L-PCB and H-PCB levels in serum whereas the symptoms of loss of appetite and peripheral "tingling" were associated only with L-PCB. Many of these differences were not observed when individual work site data were analyzed separately; the lack of a difference in each site may

result because jobs were included as a confounder variable. Most studies have not reported any major symptoms associated with exposure to PCBs (Smith et al., 1981; Kreiss et al. 1981).

Except for Yusho disease, which is a special situation (see Section II), and an occasional report of chloracne (section XII), there has been no reported clinical disease in PCB-exposed populations (Karppanen and Kolho, 1972; Humphrey, 1980; Smith et al. 1981; Chase et al. 1981; Kreiss et al. 1981; Baker et al., 1980; Lawton et al., 1981). The common but not consistent findings of abnormalities of lipid metabolism, especially blood triglyceride levels, and minor abnormalities of liver enzymes, especially SGOT and GGTP, were not associated with symptoms or clinical disease.

D. Pulmonary Function

The work of Warshaw et al. (1979) of pulmonary function in workers in a capacitor plant, has been reviewed previously. In contrast, the study of Lawton et al. (1981) did not demonstrate that PCB exposure, as measured by serum PCB levels, is correlated with spirometric abnormalities. Their data are calculated on the basis of Knudson et al. (1976), using a normal range to >70% for both vital capacity and FEV_1/FVC as recommended by the American Thoracic Society. Abnormal findings occurred primarily in smokers and ex-smokers,

as shown in Table II.

E. Fasting Blood Glucose Levels

In the epidemiologic study of capacitor workers (Lawton et al., 1981), the mean fasting blood glucose level and range for PCB exposed workers were within normal limits. Comparison of 1976 and 1979 values showed an apparent decline in 1979 that was statistically significant. It was also observed that fasting blood glucose levels increased with age, which is in agreement with the literature. Glucoauria was not observed.

F. Uric Acid Levels

Normal serum uric acid levels were obtained in PCB-exposed workers (Lawton et al. 1981).

Summary and Conclusions

The epidemiological review of medical racords of General Electric workers by Lawton et al. (1981), focused on employees with records of PCB exposure in electrical capacitor manufacture provides additional clinical data to assess the possible hazards of PCBs. For workers occupationally exposed to PCBs over many years, it is important to note that dermatological findings were relatively minor, physical examinations ware unremarkable, and no signs of chloracne past or present

were discovered. Some relationships indicative of altered triglycerides in the circulating serum, were observed; they were not associated with symptoms of clinical disease. Other reported symptoms, e.g. headache, dizziness and depression are reasonably associated with general life stress, not PCB exposure. Complaints of coughing and eye irritation have been reported by Smith et al. (1981) as related to serum PCB levels, but this has not been reported by other authors.

The study by Lawton demonstrates that extensive exposure to PCBs in the occupational setting, sufficient to have established blood levels in excess of 200 ppb, has not led to the production of clinical disease states in General Electric capacitor workers.

TABLE II

Prevalence of spirometric abnormalities in 187 PCB-exposed capacitor workers. Criteria for abnormality of FEV_1/FVC given as percent of predicted using standards of Knudson et al. (<70% compared to the older standard (<75%) used by Warshaw et al.).

	MALE			FEMALE		
	Non-Smokers	Current & Ex-Smokers	All	Non-Smokers	Current & Ex-Smokers	All
FVC*	0/41	5/105 (4.8%)	5/146 (3.4%)	1/15 (7.0%)	4/26 (15.4%)	5/41 (12.2%)
FEV_1*	0/41	11/105 (10.5%)	11/146 (7.5%)	0/15	8/26 (30.8%)	8/41 (19.5%)
FEV_1/FVC+	0/41	21/105 (20.0%)	21/146 (14.4%)	1/15 (7.0%)	11/26 (42.3%)	12/41 (29.3%)
++	12/41 (29.3%)	36/105 (34.3%)	48/146 (32.9%)	4/15 (26.7%)	20/26 (76.9%)	24/41 (58.5%)

* < 79.5%

\+ < 70.0%

++ < 75.0%

XIII. Recent Findings on Health Effects of Polychlorinated Dibenzofurans

1. Introduction

With increasing realization that the relatively potent polychlorinated dibenzofurans (PCDFs) occur as trace constituents in commercial PCB mixtures, and are also formed in small yields at temperatures above 270°C (Choudhry and Hutzinger, 1982) by oxidation/cyclization of suitably substituted PCB congeners, attention has turned to evaluation of the contributions that these tricyclic compounds may make to the toxicity syndrome elicited by exposure to PCB/PCDF mixtures or to individual PCB congeners.

A workshop on chlorinated aromatic heterocyclic compounds was held in Rome in October, 1980. The papers presented at that workshop represent summaries of important studies performed to that date and form a useful framework for integration with work on the PCDFs that has appeared in the past two years. Specifically, major workshop papers included: (1) a presentation (Jansson and Sundstrom, 1980) which described the formation of PCDFs during a fire in electrical capacitors containing PCBs; and (2) a paper by Mc Nulty et al. (1980) which presented results on the chronic toxicity of 2,3,7,8-tetrachlorodibenzofuran in Rhesus macaques.

Since the biological potencies of PCDFs are of the order of 10^2 - 10^4 greater than the toxicities of corresponding substituted PCBs with respect to production of such health effects as chloracne and P-448 oxidase induction, it is clearly important to sort out the contributions to total toxicity patterns made by PCDFs even when they are minor constituents (at a few ppm) in PCB mixtures. Indeed, Kashimoto et al. (1981) have already high-lighted this point in their analysis of the progress of the Yusho disease syndrome in Japanese patients. These patients were exposed to contaminated rice oil in which the PCDF content was only 5 ppm (on a rice oil basis) but may have been almost entirely responsible for the persistence of chloracne and skin pigmentation over many years in some of the more severely affected patients. Therefore, the following sections are devoted to a brief review of some recent advances toward understanding of the structure vs. activity relationships for this important class of halogenated polycyclic compounds.

2. Animal Studies

Recently, Hori et al. (1982) reported on an interesting study in which the PCB and PCDF fractions isolated from a batch of the original Japanese Yusho rice oil were administered orally as the PCB fraction alone (15.4 ug/mouse/day) or mixed PCB + PCDF fractions (15.4 ug +

1.0 ug/mouse/day) for seven days to male ICR strain mice, followed by analysis of the retention of individual PCB congeners in liver and adipose tissue for the next 50 days. Overall, results were essentially the same for the two experimental groups in the following respects: (1) by day 50 in both liver and adipose tissue the relative content of hexa-and hepta-CBs retained was nearly 90 percent of the PCBs in those tissues, probably because of metabolic elimination of lower chlorinated congeners; and (2) residual amounts of PCBs in tissue with the meta and para positions unsubstituted by Cl atoms (i.e. the 3,4-, 4,5-, 3',4'-, or 4', 5'-positions) were small, presumably because of low original concentrations and ready metabolism via vicinal unsubstituted ring positions. The principal effect of the PCDFs on PCB retention in tissues was to lower the half-lives of three marker PCB congeners in each tissue by a factor of about two. This effect was presumed to originate from selective metabolic activation/elimination of these congeners by P-448 oxidases induced by the PCDFs.

Yoshihara et al. (1981) have reported on an important study of the acute actions of 13 different PCDF congeners in male Wistar rats, with single intraperitoneal injections of congeners in corn oil vehicle at levels near 1 mg/kg leading to enzyme induction of the 3-methylcholanthrene (P-448) type and to variable accumulations

in liver tissue. A major finding of the study concerned the controlling influence of ring substitution by Cl atoms on the course of 3-MC type enzyme induction in liver tissue; all 9 PCDF congeners having at least 3 Cl atoms in the critical lateral positions (2,3,7,8 on the PCDF rings) exhibited typical 3-MC type induction. The most potent congeners for this effect, active at a single dose near 1 mg/kg, were those with the lateral positions completely filled, i.e. both 2,3,7,8 - tetra CDF and 2,3,4,7,8 - penta CDF. Congeners with two or less Cl atoms in the lateral positions did not show any enzyme inductive effect at a single dose as high as 5-10 mg/kg.

In this study, the PCDF congeners active as 3-MC type inducers also produced marked atrophy of the thymus and hypertrophy of the liver in rats; congeners inactive as inducers failed to elicit these effects on thymus and liver. The ability of congeners to concentrate in liver and produce acute hepatotoxicity turned out to be quite variable and to bear no clear relationship to PCDF structure.

The comparative acute and subchronic toxicities of two of these highly substituted PCDFs with lateral positions completely filled, the 2,3,7,8 - tetra CDF and 2,3,4,7,8 penta CDF congeners, were studied earlier by

Moore et al. (1979) in single strains of mice and guinea pigs and also in the Rhesus monkey. All animals were dosed by gastric intubation in corn oil, and showed toxic signs ranging through the set: body weight loss, decrease in body weight gain, thymic atrophy, liver hypertrophy, anemia, edema of face and lips, loss of facial and other hair, loss of fat, hyperkeratosis, squamous metaplasia, etc. Curiously, both the guinea pig and the monkey were quite sensitive to the acute toxicities of these PCDFs 5-10 ug/kg (12-15 days) and 1000 ug/kg, respectively, but the (57B1/6fh(J67) strain of mouse was relatively insensitive to acute toxic actions of these PCDFs, showing no clinical signs of toxicity over 30 days at doses up to 6600 ug/kg. These acute lethality data could also be compared with acute data on the correspondingly substituted dibenzo-p-dioxin, the 2,3,7,8 - tetra CDD congener, to give an estimate of the relative lethal potencies of fully laterally substituted PCDFs vs. PCDDs. In the guinea pig the PCDFs were only 2-4 fold weaker than the tetra CDD; in the monkey the factor by which the PCDFs was weaker was about 20 fold; and in the resistant mouse the factor difference in 30 day LD50s was approximately 23 fold. These differences amount to one to two orders of magnitude in lethal strength of fully laterally substituted PCDDs over PCDFs.

The PCDFs are not uncommon contaminants in the eco-

sphere. They are formed in the course of synthesis of polychlorinated phenols. Rappe et al. (1979) have noted that they are found in fly ash and flue gases, resulting from pyrolysis of PCB residues by at least three cyclization reactions involving either loss of two ortho Cl atoms, loss of ortho H and Cl atoms, or migration of Cl atoms on rings followed by cyclization. Cavallaro et al. (1980) have also measured the amounts of PCDF congeners in effluents from incinerated solid urban wastes. Rappe et al. (1981) have found many PCDF congeners stored in the fat of animal species as widely separated as the snapping turtle (U.S.) and the grey seal (Sweden), using mass fragmentograms as the detection/identification tool.

PCDF congeners have been studied in recent pharmacokinetic and toxicologic experiments with a variety of animal models. Decad et al. (1981) studied the distribution and excretion of the fully laterally substituted 2,3,7,8 - tetra CDF in two strains of mice, with the findings that the liver was the major site of initial accumulation, redistribution to adipose tissue then occurred, 55-80 percent was excreted as polar metabolites in the feces within 10 days, and the whole body half life for total elimination of parent compound and metabolites was of the order of 2-4 days. Veerkamp et al. (1981) also studied PCDF metabolism, using the Wistar rat. These workers found that monohydroxy and dihydroxy

derivatives were in various tissues and excreta (fat, liver, urine, feces), involving, at least in part, the occurrence of Cl atom shifts plus hydroxylation. Lower degrees of PCDF chlorination led to a variety of hydroxylated derivatives, plus some containing the -SH sulfhydryl group isolated as the methylthioether, but the higher PCDFs (e.g. the fully substituted octa CDF) resisted metabolism. Much unchanged PCDF was excreted in the feces, presumably before intestinal reabsorption had occurred. Mc Nulty et al. (1981) extended the study of the actions of the potent congener 2,3,7,8 - tetra CDF to Rhesus monkeys. Dosing in the food at 50 ug/kg/day for 2 months or 5 ug/kg/day for 6 months yielded severe signs of toxicity (and early deaths) in these primates, including reduction in physical activity, severe skin changes, swollen eyelids and enlargement of the meibomian glands, hyperkeratonic nail bed, atrophy or squamous metaplasia of sebaceous glands, mucous metaplasia and hyperplasia of the gastric mucosa, involution of the thymus and hypoplasia of the bone marrow. In animals that survived, recovery was apparently complete after 3 months on control diet.

Because of the biological potencies of suitably substituted PCDFs, the ability to detect and quantitate small amounts of these compounds in tissues and other samples becomes very important. Hutsinger et al. (1981)

have reported on a bioanalytical approach to this problem which makes use of an initial step involving reversible binding to a specific receptor protein of the hepatic cytosol of rodents or in cultured mammalian cells. Essentially, this technique measures the ability of a compound in a sample to displace radiolabeled 2,3,7,8-TCDD from its binding to the receptor system, and quantitates the molar amount of that compound in reference to that concentration of unlabeled TCDD which can effect half-displacement of the label. From extracts of air samples containing PCDFs or PCDDs in the ng/m^3 concentration range, this method is able to afford estimates of the amounts of tetra-octa chloro-substituted congeners in either family of compounds.

A recent report (Silkworth et al., 1982) on a 1981 fire in the State Office Building in Binghamton, New York involved fire products from a transformer containing as dielectric fluid 65 percent of a PCB mixture and 35 percent of chlorinated benzenes. The fire resulted in release of large quantities of soot contaminated with PCBs, PCDDs and PCDFs. Congeners present included the fully laterally substituted 2,3,7,8-TCDD (two samples: 2.8, 2.9 ppm) and 2,3,7,8-TCDF (272, 124 ppm). Both the soot and equivalent sample of its benzene extract were tested for acute oral toxicity and lethality in guinea pigs and dermal toxicity in rabbits. In guinea pigs,

the spectrum of toxic signs expected for PCDD-PCDF mixtures (weight decreases, effect on thymus and kidney, hyperplasia and metaplasia, triglyceride elevation, etc.) was about equivalent for either carrier mode, soot vs. extract, as were the respective LD50 values (410 mg soot/kg, 327 mg soot equivalent/kg). In rabbits, dermal application of the soot or the extract resulted in liver pathology, but local skin serous inflammation occurred only with the benzene extract, indicating that the soot matrix prevented local inflammatory reaction from particle-bound PCDDs and PCDFs.

Finally, in a comprehensive recent review, Parkinson and Safe (1981) have attempted correlations of PCDF toxicities with aryl hydrocarbon hydroxylase (AHH) induction, as a key biological action of these chemicals. The animal toxicities sort into the following major health categories: (1) wasting syndrome, involving progressive weight loss that is not simply related to decreased food consumption; (2) a variety of skin disorders including chloracne, alopecia, etc.; (3) lymphoid involution, with thymic and splenic atrophy and immunosuppression; porphyria, involving an overproduction of uroporphyrinogen; (5) endocrine and reproduction dysfunction; (6) teratogenesis; (7) carcinogenesis. These authors tested correlations between specific health effects

in these categories and the ability to induce AHH, reasoning from the basis that corresponding structure vs. activity relationships in each chemical class should influence or govern toxic potency for a given effect, receptor binding affinity to the specific cytosol receptor, and the ability to induce P-448 requiring AHH. Taking account of age dependent, sex-dependent and organ-specific differences in susceptibility seen in different test species and strains, and effects dependent on the presence or absence of a genetic Ah locus controlling AHH synthesis, some positive correlations were indeed noted. Good correspondence was found between molecular structure leading to AHH induction and that producing significant binding affinity to the cytosol receptor. However, it was noted that AHH induction may correlate with one toxic effect but not another as structure is varied, and that in some cases the relationship between AHH induction and toxicity is not established unequivocally. No compelling evidence was found that AHH per se mediates the lethal effects of these compounds, even though AHH induction correlates with toxicity for certain specific health effects. Parkinson and Safe also pointed out that other factors impinge on the ability to sort out clear correlations between AHH induction and specific toxic effects. For example, these polyhalogenated compounds alter the vitamin status of

animal models (vitamin A, etc.), which may in itself have great impact on biological response patterns, and iron deficiency in a test animal model may be able to negate or perturb the ability of a compound to initiate porphyria.

3. Human Observations

No new information bearing on PCDF-induced health effects in exposed human populations has appeared in the past two years, aside from the increasing emphasis placed on potential causation of persistent Yusho disease symptoms in Japanese and Taiwanese patients by these chemicals. It is possible that PCDF effects such as chloracne and enzyme induction might also occur in other sufficiently exposed human populations, but detection would depend on being able to sort out the specific contributions of these agents alone from the total spectrum of chemical mixtures (e.g. PCBs and chlorinated phenols) in which they are generally encountered as trace contaminants. To date, the identification of unique PCDF effects in humans has not been documented.

Summary and Conclusions

The PCDFs have been studied with increasing interest in recent years as realization grows that they are important contributors to the toxicity of mixtures in which they may be present at only tiny mole fractions. In

recent animal studies they have been shown to be powerful AHH inducers, especially when the lateral 2,3,7,8-positions are fully substituted by chlorine atoms. They produce many toxic signs in test animal systems including severe skin changes, swollen eyelids, enlargement of meibomian glands, squamous metaplasia, hyperplasia of the gastric mucosa, thymic involution, hypoplasia of the bone marrow, and hepatotoxicity. They undergo metabolic hydroxylation and excretion when the pattern of chlorine substitution is suitable, with major excretion of polar metabolites in the feces. Aside from continued analysis of residual health effects in Yusho patients, in which the opinion is growing that PCDFs are major contributors to the persistent toxic syndrome observed, little in the way of human PCDF exposure vs. effect information has been documented.

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CM-143

SUMMARY OF THE HEALTH EFFECTS OF PCBs

November 1981

Prepared for:

CHEMICAL MANUFACTURERS ASSOCIATION

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PREFACE

The technical review concerned with the effects of PCBs, as provided on the following pages, summarizes the findings reported in the following documents:

- "The Epidemiology of PCBs" by William Gaffey, a Monsanto publication, (1981).

- "A Review and Evaluation of Carcinogenicity Studies in Mice and Rats and Mutagenicity Studies with Polychlorinated Biphenyls" by George Levinskas, a Monsanto publication, (1981).

- "The Toxicity of Aroclor Products 1242, 1254, and 1260 to the Liver of Albino Rats" by George Levinskas, a Monsanto publication, (1981).

- "Human Health Effects of Electrical-Grade PCBs" by J.F. Brown, Jr., J.T. Coe, and H.D. Pocock, Jr., a General Electric publication, (1981).

- "Technical Review of the Health Effects of PCBs" by Robert James, Morris Cranmer, and Raymond Harbison, a New England Gas Association publication, (1981).

- "Assessment of Carcinogenic Risks From PCBs in Food" by Kenny S. Grump and Marjory Masterman, prepared for the United States Congress Office of Technology, contract # 933.1350.0, (1979).

The reader will note that, with the exception of the section on risk assessment, the summaries in this report contain no specific references to other documents. Since the documents listed above, which review the pertinent literature on PCB health effects to date, give specific references for further study, it was not thought necessary to repeat these same citations in the body of this report. For a more in-depth review of the pertinent PCB literature and specific citations, it is suggested that the reader refer to the documents listed above.

EXECUTIVE SUMMARY

In this paper we examined the human health risks associated with exposure to PCBs. To accomplish this purpose, we reviewed all significant published or readily available studies of the effects of PCBs as well as the pertinent current theories of carcinogenesis, the scientific body of knowledge which supports these theories; and have provided an analysis of the chemical carcinogenesis testing performed with commercial PCBs. As a result of this review and evaluation, it is our independent professional opinion that "any exposure to PCBs" does not pose a significant health risk to humans. These conclusions are based on data of the primary exposure to commercial grade PCBs.

After reviewing the carcinogenesis test data, we have concluded that commercial PCBs do not represent a carcinogenic (genotoxic/initiator) risk and that, even if they possess oncogenic (epigenetic/promoting) activity, the risk at low exposures is insignificant. This mechanistic distinction is a very important basis from which to address the cancer risk. To paraphrase a succinct summary of this distinction by Weisburger and Williams, promoters (oncogens) share the characteristic of being active only at high, sustained doses, and up to a certain point, the lesion may be reversible. Thus, these types of carcinogens represent only quantitative hazards to humans, and safe levels of exposure may be established by carrying out proper dose-response studies.

Attempts to estimate the potential risk of cancer to man caused by PCB exposure using data developed in rodents represents a controversial but crucial dilemma. Numerous studies involving both mice and

rats have been reported, but only one study exists to date that suggests PCBs may cause an increase in hepatocellular carcinoma. When the paucity of convincing data is compared to the large number of negative tests, and when other important contributing factors are considered (e.g., the spontaneous rate of liver injury and liver regeneration, thereby promoting aberrant hepatocellular growth; etc.), it is difficult to conclude that PCBs represent a significant cancer risk in man.

Analysis of published animal data leads to the conclusion that, in animals, commercial PCBs represent a low acute exposure hazard; that mutagenic, teratogenic, and reproductive risks are minimal; and that the carcinogenic potential of this compound has not been convincingly demonstrated in an animal model relevant to man. This lack of suggestive risk based on animal data has been further borne out by the fact that neither liver nor other cancers in man have been positively linked to PCB exposure in recent epidemiologic studies utilizing large study populations.

At worst, PCBs at high or elevated doses meet some of the criteria for a chemical promoter, i.e., they enhance tumor growth through epigenetic mechanisms but do not induce new tumor growths. This distinction is of extreme importance and can be made based upon the known mechanisms for genotoxic carcinogens and those for promoting agents. PCBs demonstrate many characteristics of the latter and none of the former. A further importance of this distinction is that PCBs have only been shown to possess a weak promoting activity at most. Therefore, the thresholds both proposed in theory and observed in animal studies suggest that there are safe exposure levels for PCBs.

The occupational exposures are certainly the most extensive and longest-term human PCB exposures that we are currently aware of, and are therefore probably most representative of what adverse effects might be expected. A comparative review of these occupational-exposure studies reveals that, like other chemicals, PCBs can cause adverse health effects, but in many respects these have been minimal. While dermatitis and chloracne, which were reversible after discontinuing the exposure, have been noted in some cases, no other significant findings were routinely made. Even though several studies incorporated clinical chemistry analysis as indications of organ

dysfunction or other physical disorders, no remarkable clinical findings have been uncovered. Furthermore, several investigators have commented to the effect that there is a "paucity of abnormal results" and that "there is no evidence of physical harm resulting from working with PCBs." In spite of over 50 years of use, no causal relationship has been established for any specific type of cancer, nor has it been proved that the incidence of cancer mortality has increased. The largest study, by the National Institute for Occupational Safety and Health (NIOSH), which involved over 2,500 persons, did not detect any statistically significant excess in the cancer mortality. Since this study failed to demonstrate an excess cancer rate in a high exposure population, it provides some reassurance that it is unlikely that future studies will show any increased risk of cancer from PCBs.

In summary, epidemiologic studies have demonstrated that PCBs are not remarkably toxic chemicals after acute exposure, and that when excess exposure does occur, the usual consequences are dermatologic and not of a serious or permanent nature. Chronic exposures have added little or no additional adverse effects of note to this picture. The preponderance of studies has not identified a clinical disease associated with exposure to PCBs, nor has it provided persuasive evidence of health impairment. There is no evidence of an excess in total mortality or in mortality due to cancer, cardiovascular disease, or nervous system disease associated with occupational exposure to PCBs. PCBs have not been linked to any human cancer, and studies to date indicate it is highly unlikely future studies will establish such a link. Therefore, it appears that PCBs are not a remarkable toxicant, but a chemical which requires high doses to produce harmful effects.

The chemical analyses of PCBs have shown that they often contain polychlorinated dibenzofurans (PCDFs) at low levels. The concentrations of these toxic contaminants are generally in the parts-per-million range in pure PCB mixtures, but percent of contamination can be substantially increased as a result of industrial use. Since levels of PCBs generally found in the environment are in the parts-per-million range or lower, the concomitant concentrations of PCDFs would be expected to be unmeasurable. For this reason and because the toxicity data of all PCB exposures have probably included this

contaminant, the concern for low level exposures to PCDFs is still expected to be minimal. However, the conclusions reached in this report apply to exposure to commercial PCBs and cannot be applied to all environmental PCB exposures, which may include exposure to concentrated contaminated waste mixtures.

1. MAMMALIAN TOXICOLOGY OF PCBs

Any assessment of hazards posed by exposure to a chemical must consider the doses required to elicit each toxic response as well as the spectrum of toxicities the chemical is capable of inducing. All of these factors must be carefully incorporated into the final estimation of risk. Many toxicity tests depend upon clearly observable effects, such as organ injury or tissue damage. For these tests, detection of the effects in question is clear, and the results can directly be extrapolated to determine the potential for a similar adverse effect in man. However, a complete safety assessment and evaluation must also include tests designed to measure mutagenic or carcinogenic potential. While the biological manifestations for these tests may be easily measured and reproduced, what the experimental results represent with respect to the expected human response is less clear. Therefore, results from the latter tests should not necessarily overshadow findings in the former tests. Instead, the results from all tests must be evaluated for internal consistency. As new data emerges, this information should likewise be incorporated and, if necessary, the risk reassessed.

Polychlorinated biphenyls (PCBs) are not capable of causing immediate life-threatening responses in animals except at very high doses. When given as a single oral dose to rats, mice, or rabbits, the dose lethal to 50% of the test species lies in the 1,000 to 16,000 mg/kg of body weight dosage range. According to the scheme proposed by the American Industrial Hygiene Association, this dosage range for acute or immediate toxicity classifies PCBs as only a slightly toxic to

practically nontoxic chemical. Ethyl alcohol is in the same toxicity class. The most consistent pathologic findings associated with the lethality of short-term tests are fatty infiltration of the liver, liver injury, and centrilobular necrosis of the liver. This is not an unexpected finding. Many chlorinated organic chemicals produce liver or kidney injury in mammals. Other effects observed in acute studies include depression and lethargy, decreased pain response, anorexia (loss of appetite), ataxia (unsteady gait), and diarrhea. These are signs of chemical intoxication that are also commonly seen with many other organic chemicals.

PCBs applied directly to the skin of rabbits produce dermal responses such as erythema, hyperkeratosis, blisters, and desquamation. These effects are not a particularly remarkable finding for any organic chemical. In monkeys, the epidermal disorders from oral dosages of 250 to 400 mg/kg are facial edema, hair loss, and acne. While chloracne has been reproduced in humans after high occupational exposures, it can also be produced by exposure to other chlorinated benzene derivatives. Other than discomfort and some scarring in severe cases, it is a reversible effect.

Subchronic studies have revealed other effects. Weight loss and liver injury become a more consistent finding in the rodent species, with liver enlargement and an induction of liver metabolism occurring at lower doses. PCBs are potent inducers of the mixed function oxidase system, more potent in fact than either phenobarbital or DDT, which have been used extensively as experimental tools for this purpose. PCB mixtures are also uniquely capable of inducing both cytochrome P-450 and cytochrome P-448.

The other systemic effects reported are porphyria; increased thyroid metabolism; gastric hyperplasia; increases in triglycerides, cholesterol, and phospholipids; and atrophy of the spleen and thymus. Estrogenic activity for PCBs has been reported in rats, and prolonged menstrual cycles and increased bleeding have been observed in monkeys. Of the above effects, the thymic change and the immunosuppressive effects of PCBs are probably of most concern. Changes in the immune system could lead to increases in the susceptibility to and severity of infectious and neoplastic diseases. However, attempts to verify that the immunosuppressive effects of high doses of PCBs might

increase the cancer risk from other causative agents have produced inconsistent results. PCBs have been reported to inhibit the tumor growth of Walker 256 carcinosarcoma cells, to enhance the growth rate of MCB cells derived from the moloney sarcoma virus, and to be without any effect on the moloney leukemia virus.

Test procedures designed to indicate serious long-term or chronic effects have been somewhat variable, but these too have provided fairly unremarkable effects when compared to the potential harm caused by other useful industrial chemicals that have been similarly tested. Mammalian studies indicate that there is little reproductive risk associated with moderate to high doses of PCBs. Studies using up to 100 mg/kg/day did not affect reproduction in the rat, and similar results have been reported from tests in mice and monkeys. PCBs do, however, cross the "placental barrier." Primates born to exposed mothers have shown symptoms of intoxication. Also, those doses high enough to disrupt the estrus cycle have been shown to decrease the implantation rate. Yet PCBs did not produce any histopathological changes in the reproductive tracts of either sex of rats, caused no chromosomal damage or arrested spermatogenesis, and were negative in the dominant lethal test for inheritable damage to germ cells. More importantly, PCBs have not significantly altered embryo or fetal development in either rodents or nonhuman primates at doses that were not maternally toxic; thus, it can be concluded that there appears to be little or no teratogenic or reproductive risk associated with exposure to PCBs.

The overall mutagenic potential of PCBs is inconsequential. Direct mutagenic activity as measured by point mutations in bacterial tester strains has been recently reviewed by Levinskas (1981). According to the results of seven different studies, a group of chlorinated biphenyl compounds did not demonstrate any mutagenic activity. Although there is one report in the literature that 4-chlorobiphenyl might have mutagenic activity, other researchers have not been able to reproduce this observation. Additionally, Aroclor 1254 was found not to stimulate unscheduled DNA synthesis in the rat hepatocyte. In tests designed to observe chromosomal aberrations or alterations in number, the results were also overwhelmingly negative. PCBs were

negative when tested in the Drosophila melanogaster, negative in cytogenetic analysis of in vitro human lymphocytes, negative in cytogenetic preparations of rat sperm and bone marrow cells, and negative in the dominant lethal assay. Thus, chlorinated biphenyls have not demonstrated any mutagenic potential when tested with the majority of commonly used and well-substantiated mutagenicity procedures.

Attempts to estimate the potential risk of cancer to man caused by PCB exposure using data developed in rodents represents a controversial but crucial dilemma. Numerous studies involving both mice and rats have been reported, but only one study exists to date that suggests PCBs may cause an increase in hepatocellular carcinoma. When the paucity of convincing data is compared to the large number of negative tests, and when other important contributing factors are considered (e.g., the spontaneous rate of liver cancer in rodents; the inductive and cellular growth-promoting potential of PCBs; the potential for PCBs to induce recurrent liver injury and liver regeneration, thereby promoting aberrant hepatocellular growth; etc.), it is difficult to conclude that PCBs represent a significant cancer risk in man. This lack of suggestive risk based on animal data has been further borne out by the fact that neither liver cancer nor other cancers in man have been positively linked to PCB exposure in recent epidemiologic studies utilizing large test populations.

Industrial-grade PCBs may not be just a mixture of chlorinated biphenyls but a mixture that may also contain chemical contaminants, the polychlorinated dibenzofurans (PCDFs). Therefore, when considering all of the data summarized in preceding paragraphs, an assessment of the toxicity and risk to PCB exposure may be complicated by the fairly recent recognition and acknowledgement of PCDF contamination. Tetra-, penta-, and hexachlorodibenzofurans have been measured in Aroclors 1248, 1254, and 1260 in concentrations on the order of 0.1 ppm for each isomer. Studies of Japanese brands of PCBs indicate that they contained concentrations which were several times higher.

Of even more concern is the possibility that dibenzofurans may be formed in significant amounts when PCBs are exposed to high heat and some reactable form of oxygen. This concern was first expressed after the Yusho incident when it was discovered that the PCDF content was

approximately 1,000 times higher than expected for commercial Japanese PCB mixtures. Studies have revealed that the oxidation of PCBs to PCDFs is 2% to 3% for Aroclor 1254 at 550° to 600°C and that, during combustion, the rate of formation is time and temperature dependent. However, these conditions do not exist in the normal manufacture or use of PCBs nor in the normal use of PCBs in capacitors or transformers. These conditions might have occurred, however, in some heat exchange equipment. So, at present, the conditions for and the extent of PCDF contamination in PCB wastes is unknown.

While it should always be considered that PCDFs may increase the toxicity of commercial PCB mixtures, the variety of acute and chronic tests reviewed on the preceding pages are tests in which the contribution of PCDF toxicity was, no doubt, unconsciously measured. Therefore, concern for the greater toxicity of this contaminant, when it is in concentrations approximating those previously measured in commercial PCB mixtures (i.e., about 1 ppm), should be tempered by the possibility that its risk is that reflected by the animal test data gathered for what was previously considered to be a PCB mixture alone.

To reiterate the original premise, evaluating the hazard of any chemical on the basis of animal data requires careful consideration of (1) the spectrum and types of toxic responses induced; (2) the degree of species variation or consistency of the effects monitored; (3) the relevancy and validity of the test performed; (4) the dosage required to elicit the response; and (5) the level of relevance of the toxic effects observed, based upon the expected level of human exposure.

Thus, many of the PCB-induced adverse effects would be expected for a chlorinated organic compound of the PCB type, or at least these effects are consistently produced by many chlorinated organics. These effects include liver injury, liver enlargement, liver enzyme induction, irritation when applied to the skin, chloracne, and possibly a disruption of the estrogenic balance in females because of increased liver metabolism. Although these may indeed be considered adverse effects, all chemicals are capable of altering some physiologic function at a high enough dose. In the more relevant reproductive, teratogenic, mutagenic, and carcinogenic tests, chlorinated biphenyls were consistently negative with only a few exceptions. These exceptions

can be discounted because of inadequate study design, disputable interpretations, and unusual tests. Thus, it seems unnecessary to display a disproportionately high concern for those few controversial studies, especially when their findings were not reproduced in subsequent studies under similar or identical conditions. In the vast majority of experiments, the effects were produced by PCBs at high doses. Since the expected and likely human exposure to PCBs is low, the results of the tests using high-exposure conditions are not relevant to the expected human exposure level.

Therefore, it appears that PCBs are not a remarkable toxicant, but a chemical which requires high doses to produce harmful effects and one which clearly represents minimal or no risk to humans because of the limited nature of anticipated human exposure. While PCBs can be toxic, and their toxicity can be dramatically increased by high levels of dibenzofuran contamination, the mammalian toxicity data suggest that, in general, PCB mixtures can be tolerated under most real-world conditions.

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2. THE ROLE OF PCBs IN PRODUCING CANCER

Chemically induced carcinogenesis is a special aspect of toxicology, for the toxicants may or may not observe traditional toxicologic principles. For example, although carcinogens show dose-response relationships, may undergo biotransformation to active or inactive metabolites, and demonstrate specific structure activity relationships as other toxicants do, they may also be unlike other toxicants: they may not demonstrate thresholds and there may exist a long and indefinite latency period between the critical biochemical event and any cellular or physiologic expression.

In recent years, the scientific community has concluded that even among chemical carcinogens there exist vast differences in the mechanisms by which they induce cancer. One can separate chemical carcinogens into two groups. One group obeys traditional toxicologic principles while the other does not. This classification of carcinogens has been described by Weisburger and Williams in Casarett and Doulls' "Toxicology: The Basic Science of Poisons." These authors separate carcinogens into genotoxic (initiator) and epigenetic (promoter) carcinogens, the distinction being that genotoxic carcinogens induce cancer by initiating a permanent change in DNA. Epigenetic chemicals comprise those chemicals which alter the manifestation of cancer by other than genetic means and include hormones, immunosuppressants, cocarcinogens, and promoters. This distinction is extremely important because genotoxic carcinogens are irreversible and may not have thresholds; therefore, some risk is present at any dose. Promoting

agents (epigenetic chemicals), on the other hand, do have demonstrable threshold levels; thus, there are exposures for which the risk is minimal or the risk exists only for some finite interval after exposure.

For our discussion of the carcinogenic potential of PCBs, we reviewed the data for evidence to determine to which of these two classes of tumorogenic chemicals PCBs belong, and in our discussion we refer to the epigenetic class as promoters (i.e., chemicals that only increase the manifestation of cancer cells) and to the genotoxic class as initiators.

After reviewing the carcinogenic test data, we have concluded that PCBs do not represent a genotoxic (initiator) risk and that, while they may possess some promoting activity, the risk at low exposures is insignificant. This mechanistic distinction is a very important basis from which to address the cancer risk. To paraphrase a succinct summary of this distinction by Weisburger and Williams, promoters share the characteristic of being active only at high, sustained doses, and up to a certain point, the lesion may be reversible. Thus, these types of carcinogens represent only quantitative hazards to humans, and safe levels of exposure may be established by carrying out proper dose-response studies. The following paragraphs summarize the data supporting this important distinction and address their significance for PCBs.

There has been great controversy about the relevance and nomenclature of rodent hepatic lesions. Most of this controversy surrounds the criteria used to diagnose cancer. The National Cancer Institute (NCI) has recommended a scheme for the classification of hepatocellular tumors and related lesions in rats. These recommendations have not been universally accepted, but are utilized in many of the reports of PCB carcinogenesis. The NCI classification scheme is extensive and specific in defining neoplastic lesions. It was concluded by NCI that benign hepatic cell tumors, i.e., without potential for malignant behavior, could not be diagnosed consistently. Therefore, terms such as "adenoma," which refers to a benign tumor, were not recommended. It was also determined that the term "hepatoma," used to denote a benign liver tumor, was imprecise in its usage and was not recommended for the description of any of the lesions under discussion. In contrast

to tradition, detection of vascular invasion or metastases was not considered by NCI to be essential for the diagnosis of hepatocellular carcinoma. Instead, the utilization of inclusive cytological criteria describing changes which may or may not have the potential to become the disease process we call cancer, including many responses which will never progress to cancer, was accepted at the expense of the more definitive criteria of malignant or invasive neoplasms.

The first report of PCB-induced tumors was that of an increased incidence of hepatomas. A later publication of the first study, which appears to have included the animals from the original report, terms the previously described hepatomas as well-differentiated hepatocellular carcinomas. Similarly, another study describes the production of hepatomas by PCBs in mice, but later the hepatomas are described as neoplastic nodules. Changes in liver cellular morphology were observed in mice only when the PCB chlorine content was 52% to 54%, and then only at exposure levels of 300 to 500 ppm. Lower doses did not produce tumors, and those PCBs with a chlorine content below 52% to 54% did not induce tumors. Further, the reported study of PCB-induced cancer in mice included only 12 mice studied for 32 weeks. This is not an acceptable bioassay procedure because of the small number of experimental animals used and the short time of exposure. Also, there was an inconsistency in reporting terminology. The liver changes were reported as hepatocellular carcinoma and also as hepatomas or tumors or nodular hyperplasia. A better bioassay was performed later that included larger numbers of test animals exposed for longer periods of time, and no increased incidence of tumors was reported. Subsequent studies were consistent with this larger study, and the majority of scientific literature supports the claim that PCBs are not carcinogenic in mice.

Similarly, only PCBs with a chlorine content of 60% produced those cellular morphological changes in rat liver that were classified using NCI guidelines as hepatocellular carcinoma. However, this same PCB mixture was tested in another strain of rat and did not significantly increase the incidence of tumor formation. Moreover, four other studies also failed to show any PCB-induced tumors. With one exception, there is a consistent absence of reports finding PCB-induced hepatocellular carcinomas. Again, those PCBs with a chlorine

content below 52% to 54% did not produce tumors in rats. These results are similar to those for mice. Neither were tumors induced when the average PCB chlorine content was 52% to 54%. The two studies using an average chlorine content of 60% reported no induction of hepatocellular carcinomas.

There is only one report of PCB-induced hepatocellular carcinoma in rats. Since this report used the criteria developed by NCI for classifying liver pathology, we must weigh the importance of this report against the majority whose data are negative. As previously stated, utilization of these inclusive cytological criteria to define cancer will define some hepatocellular changes as cancer that will never progress to cancer. Furthermore, investigations have shown that the vast majority of regenerating or hyperplastic nodules reported as cancer disappear upon removing the animals from the chemical exposure. While some investigators have considered the use of the inclusive cytological criteria for classifying hepatocellular lesions, many have rejected this classification scheme because of its lack of diagnostic discrimination. Thus, the single report of PCB-induced cancer in rats uses a pathological description in opposition to the usual terminology for describing a benign tumor.

Chemical carcinogens are those substances capable of initiating cancer. Cancer is defined as the production of life-threatening malignant tumors of potentially unlimited growth that expand locally by invasion and systemically by metastasis. Chemical carcinogens irreversibly initiate and ultimately produce cancer. This is an important distinction and requires careful and accurate evaluation of the kind of tumors produced. Chemicals that promote benign tumors are not carcinogens. Therefore, tumor type is critical to determining whether a compound is a carcinogen. Failure to distinguish between tumor types will place equal importance on test results regardless of whether a malignant or benign tumor is produced as a result of exposure to a chemical. Therefore, it is absolutely essential to define the tumor type associated with chemical exposure to be able to evaluate the outcome and apply the data to humans.

Equally important, it is essential to determine the mechanism by which tumors are produced. Failure to distinguish the mechanism by which tumors are produced will place equal importance on chemicals

which are initiators (genotoxic) and on those which are promoters (epigenetic). The following discussion of cancer will make the importance of this point clear.

Initiation of cancer is the process of producing damage to DNA of a magnitude sufficient to be sustained, but of a nature which permits cell survival and thereby predisposes the capacity of uncontrolled growth. Those cells containing damaged DNA then produce clones containing an inheritable mutation. Mutagenicity is defined as production of an inheritable alteration in the genome of a cell. An altered genome will increase the probability of cellular dysfunction. Therefore, inheritable mutations or initiation of genes responsible for cellular regulation increase the probability of producing cancer, a condition of regulatory dysfunction expressed as uncontrolled growth. Since most mammalian genes exist in allelic pairs and the malignant phenotype is probably recessive for nonviral induced cancers, a recessive mutation would not normally express itself as cancer until a second mutation occurred in the other allele. While there is normally a low probability of this occurring spontaneously by chance, given enough mutations in enough cells and a prior inheritance of susceptible genes through the germ cell line, the chance of having a successful mutation within both alleles of a critical gene, thereby producing a dysfunction in cellular regulation like cancer, becomes probable.

Thus, we can easily see how genotoxic agents (initiators) can apply selective pressure upon the cell toward the cancerous state. However, in our most often used test species, the mouse and the rat, there is a normal background incidence of the initiation process leading to cancer in the liver. Thus, where there is a history of spontaneous cancer incidence, there is little doubt that cancer will occur; only the rate and the time required to develop cancer (latency) is unknown. In this situation, the risk of cancer increases with time and the magnitude of the initiated response is a cumulative probability. In rodents, several factors have been shown to alter their background cancer incidence including total dietary calories, fat content, vitamin E, stress, crowding, and gender. Therefore, to define a chemical as a carcinogen (initiator) rather than another promoting factor in these species by using changes in liver cytoarchitecture requires proof that the chemical is an initiator and not a promoter of the

spontaneously occurring disease state. To do this, it must be shown that the compound initiates sustained damage to DNA in a dose-dependent manner.

On the other hand, promoters are chemicals that modify gene expression and cellular communication. Promoters can alter the ability to control various genes, thereby providing a selective growth advantage. Promotion is defined as enhancing the selective proliferation of previously initiated cells. By definition, promotion cannot occur until a critical gene is initiated. Wounding, growth stimuli, necrosis, inflammation, and certain chemicals can selectively and rapidly allow cells containing the mutated gene to form a clone of semiinitiated cells. Cell death, cell trauma, and cell hyperplasia are all produced by PCBs and can be expected to yield conditions favoring preferential growth of (promotion of) previously initiated cells. One can safely generalize that the tumorigenic properties of PCBs result, at least, in greatest part from alteration of the homeostasis of control mechanisms. Thus, changes seen in rodents with a high incidence of liver tumors must be interpreted carefully so as not to mistake toxic responses and promotion for initiation. Additionally, processes only promoting cancer in situ would be expected to regress upon removal of the promoter and not change the ratio of metastasizing to nonmetastasizing tumors. It is noteworthy that often such appears to be the case with liver cancer in rodents produced by cyclic chlorinated compounds.

In summary then, it has been documented that PCBs do not increase the incidence of tumors at any site except the rodent liver. Liver tumors are a common part of the natural disease process of rodents. Thus, changes seen in rodents with a natural history of a high incidence of liver tumors must be interpreted carefully so that toxic responses and promotion are not mistaken for initiation. Liver cell death, trauma, and hyperplasia are all produced by PCBs and can be expected to yield conditions favoring preferential growth of cells with dormant neoplastic phenotypes. Therefore, one can safely generalize that the tumorigenic properties of PCBs in the rodent liver result, at least, in greatest part from these alterations of homeostasis. The following summarizes the data delineating PCBs as a potential promoting agent (epigenetic) but not an initiator (genotoxic carcinogen).

Comparison of the Characteristics of Promoting Agents and Effects of PCBs

1. Promoters produce increases of spontaneous tumors at selected sites. PCBs produce only liver tumors.

2. Tumors produced by promoters usually do not increase the metastatic characteristics of spontaneous tumors of the same site. PCB-increased liver tumors do not metastasize.

3. Promoters do not produce transplantable tumors unless the naturally occurring tumor is capable of transplantation. PCB tumors do not transplant.

4. Tumors influenced by promoters are often affected by factors such as nutrition, stress, chronic injury, and sex of the animal. Liver tumors are affected by these factors.

5. Promoters need not be mutagenic. PCBs are not mutagenic.

6. Neoplastic lesions increased by promoters may be reversible. PCB-increased liver tumors regress.

7. Progression of differentiation of neoplastic lesions increased by promoters often ceases when the stimulus is removed. Continued presence of PCBs is required to sustain liver neoplasia.

8. Neoplastic effects of promoters are usually associated with the chronic dysfunction of the affected site. PCBs produce chronic hepatotoxic responses.

9. Promoters do not necessarily increase the effect of carcinogens acting at the target site. PCBs reduce the response of several other liver carcinogens.

10. Promoters affecting the liver often increase microsomal enzyme activity and eventually produce evidence of metabolic dysfunction. PCBs stimulate LME and produce metabolic dysfunction.

11. Promoters rarely, if ever, increase tumor incidence at exposure levels producing no toxic effects. PCBs are tumorogens only at toxic doses.

12. Promoter effects often require continued presence of the stimulus. Continued presence of PCB is required to sustain neoplastic alterations of the liver.

Comparison of the Characteristics of Carcinogens (Initiators/Genotoxic) and the Effects of PCBs

1. Carcinogens usually produce neoplastic lesions at multiple sites. PCBs increase lesions of the liver only.

2. Carcinogens often increase the malignancy of spontaneous tumors at the same site. PCB-increased liver tumors are of low malignancy.

3. Carcinomas irreversibly progress once a critical tissue mass is established. PCB-increased tumors can regress.

4. Carcinogens produce tumors which metastasize. PCB-increased liver tumors do not metastasize.

5. Carcinogens produce lethal tumors. PCB-increased liver tumors are not lethal.

6. Carcinogens produce transplantable tumors. PCB-increased liver tumors do not transplant.

7. Carcinogens are often effective at single exposures. PCBs increase liver tumors only after prolonged, continuous exposures.

8. Carcinogens are often active at nontoxic doses. PCBs only increase liver tumors at hepatotoxic doses.

9. Initiators are mutagens. PCBs are not mutagens and therefore are not initiators.

It is apparent that the neoplastic effects of PCBs match the definitions of a promoting agent and do not match the definitions of an initiator.

It might also be mentioned that Butler and Jones (1978) have previously stated that certain compounds, especially persistent chlorinated compounds including PCBs, increase the incidence of only liver tumors in rodents and that these tumors may not be malignant. In fact, halogenated chemicals are the most numerous class of chemicals to induce rat or mouse liver tumors alone, and most are known not to be mutagenic. These chemicals produce liver cell damage and regeneration and/or induction of hypertrophic and hyperplastic changes. Thus, it is likely that these nonmutagenic chemicals are causing rodent tumors by an epigenetic mechanism.

This distinction concerning the halogenated hydrocarbons has been noted by other scientists as well. For example, Weisburger and Williams have stated:

> Validation of this concept of a promoting rather than a genotoxic action of chlorinated hydrocarbons is not only an important distinction from a theoretic point of view, but is even more relevant in terms of regulatory actions indicated to prevent chronic disease.

They further reiterated this idea by stating:

> If, on the other hand, no convincing evidence for genotoxicity is obtained, but the chemical is carcinogenic in animal bioassays, then the possibility exists that the chemical is an epigenetic carcinogen. The strength of this conclusion depends on the relevance of the in vitro tests. For example, the finding that certain stable organochlorine pesticides do not display genotoxic effects in liver cell systems, which are identical to the in vivo target cell for these carcinogens, strongly supports the interpretation that these carcinogens may act by epigenetic mechanisms. The nature of these mechanisms is poorly understood at present and is probably quite different for different classes of carcinogens. They may involve chronic tissue injury, immunosuppressive effects, hormonal imbalances, blocks in differentiation, promotion of pre-existing altered cells, or processes not yet known.

In conclusion, we have raised the question, Does one experiment utilizing prolonged exposures at high levels, in a single sex of a

single strain, incriminate commercial PCBs as a carcinogen? To answer this question, we have reviewed the pertinent current theories of carcinogens, the scientific body of knowledge which supports these theories, and have provided an analysis of the chemical carcinogenesis testing performed with PCBs. On the basis of the data that have been reported in the last few years, PCBs do not appear to be carcinogens (initiators).

At worst, PCBs at high or elevated doses meet some of the criteria for a chemical promoter, i.e., they enhance tumor growth through epigenetic mechanisms but do not induce new tumor growths. This distinction is of extreme importance and can be made based upon the known mechanisms for initiators and those for promoting agents. PCBs demonstrate many characteristics of the latter and none of the former. A further importance of this distinction is that PCBs have only been shown to possess a weak promoting activity, at most. Therefore, the thresholds both proposed in theory and observed in animal studies suggest that there are safe exposure levels for PCBs.

3

3. RISK ASSESSMENT AS PART OF THE REGULATION OF PCBs: STATEMENT OF PURPOSE, NEED, AND LIMITATION

The increasing use of chemicals and our ever-growing realization of their impacts on human health necessitate a scientific basis from which to determine the largest quantity of a chemical, if any, that could be absorbed over an indefinite period without producing adverse health effects. For this purpose, the toxicologist has long relied on dose-response relationships to guide extrapolation downwards from the observed range to estimate a "safe" dose. A general premise of toxicology has been that the extent of induced adverse effects is closely related to the extent of exposure. Some interested parties have called for an exception to this established toxicological concept, suggesting that chemicals should be considered either as carcinogens or noncarcinogens, regardless of dosage involved. Many toxicologists continue to maintain that carcinogenesis, although complicated, is in fact consistent with classical toxicological concepts. National efforts have been made by several government agencies to develop a data base for evaluating test procedures used in the detection of cancer, as well as to develop methodologies for performing risk estimations for chemically induced cancer.

Undeniably, chemical technology has in a large measure contributed to the achievement of the present standard of living. It has produced many of the tools and resources needed to reduce human suffering and to modify selective pressures of the environment. Accompanying these benefits, however, is the possibility that toxic properties of certain chemicals have the potential to threaten man's health.

Therefore, a rational policy for chemical utilization is to produce the highest standard of living consistent with a quantitatively acceptable hazard-to-benefit ratio.

A product containing chemicals should be approved for use if there is an acceptable margin of safety between the anticipated exposure and the level estimated as hazardous to humans or the environment. Yet, to accomplish this task, the toxicologist must face the dilemma of estimating the risk to an enormous and variable human population by extrapolating the hazards determined from studies conducted on small numbers of environmentally controlled and genetically homogeneous experimental animals. With the limitations of present experimental and theoretical techniques, there is considerable potential for error in assessing both sides of the hazard-to-benefit ratio. For these reasons, the limitations of toxicological evaluations should be clearly stated. It never has been, and is not now possible to guarantee absolute safety. Small populations of experimental subjects, either animal or human, may provide an imprecise estimate of hazard for comparison to a large human population of variable genetic disease states, cultural backgrounds, and ages. Ways of overcoming these limitations are slowly being recognized, and techniques for extrapolation must make maximum use of available data.

Thresholds

As a prologue to the discussion, it must be stated that we should resist falling into the intellectual and experimental quagmire called "threshold" and the mischief of "value judgement." There is no mathematical reason to distinguish cancer from many other toxicological effects. For cancer or any measurable response that may theoretically lack a threshold, the no-effect dose merely becomes a sample-size-dependent phenomena. To argue threshold is to obfuscate. We will later develop the thesis that time is the critical point of consideration.

The concept of a threshold dose is based on the premise that unless a specific set of conditions exists, some smaller dose will not produce the measured effect. For carcinogens (i.e., initiators), since there always exists the possibility of a response, the threshold limits of an effectively measured response only depend upon the sample

size in a statistically significant evaluation. This does not mean that thresholds do not exist, nor does it mean that thresholds cannot be estimated in a practical manner. It does not mean that bounds cannot be established. It only means that thresholds are not absolute and cannot be defined with absolute certainty. In fact, if known, they might yield little practical relief to our current dilemma, since under the preceding limitations time, rather than dose, may become the critical and determining factor of risk. This stems from our perception that as the dose decreases the latency period increases. Therefore, time is the most appropriate medium to describe the magnitude of excess risk or reduced quality and quantity of life due to neoplastic lesions. Finally, we have demonstrated that PCBs are not genotoxic; therefore, the no-threshold arguments do not apply.

Germane to the modification of risk determinations by combining socially accepted values is the consideration of the hazard/benefit margin. Some chemicals may be considered essential insofar as there is no substitute process considered less hazardous, or for which the requirements of a replacement strategy outweigh or replace the original health concerns. For such chemicals, a relevant approach to controlling the risk is to permit only those uses for which the absence of hazardous exposures of the chemical can reasonably be assured. The cornerstone of this strategy requires that we define the hazard, then determine the amount of exposure which is not expected to be hazardous, which in turn defines the limits of the sensitivity of the analytical methodologies required.

Rephrased, the requirements of the analytical methodology and the limitations of chemical use would be defined toxicologically by the acceptable risk rather than as a function of the state of the art of analytical chemistry. The alternatives, zero-residue rules (such as found in the Delaney Clause) or risk analysis based upon an endless series of conservative presumptions, are inadequate and unusable in several respects. Such alternative approaches provide a false sense of security by ignoring the problem of "false negatives," which may result from chance or inadequate testing. In addition, they encourage poor and limited experimentation, since the less we do and the less precise we are, the less chance we have of determining anything at a statistically significant level. Moreover, these alternative

approaches capriciously eliminate from consideration the use of chemicals whose benefits might be documented to outweigh a worst-case calculation of the carcinogenic hazard.

Value Judgement and Emphasis

Ambiguities in the process of articulating an acceptable safety policy will automatically arise if proposed criteria require incorporation of the unobtainable goal of attempting to prove scientifically that no deleterious effect will take place, i.e., to prove the absence of the possibility of an occurrence in some future time. Examples have been described by Dr. Alvin Weinberg as "transcience." Such a policy postulates society's need to insure an absence of positive findings beyond the range of the methods available for data collection. Toxicologists' tools, however, are limited to experiments employing the scientific method. These experiments are usually designed to establish that phenomena resulting from repeated experimental manipulations are real, are not artifacts, and have not occurred simply by chance.

We are all aware that positive as well as negative findings may be artifacts and that in most scientific disciplines there is a positive reward for investing in adequate techniques and replication of experiments. Insistence on any desired degree of assurance against making a wrong conclusion is standard operating procedure. The quality of safety evaluations will suffer if we adopt ingrained unidirectional attitudes which demand that, once we find a positive carcinogenic effect, no number of negative studies provide an adequate counterbalance. Such attitudes inappropriately ignore false positives and discourage rigorous and repeated experimentation by even the most responsible sponsor. In addition, this emphasis places the sponsor and the conscientious regulator alike in the position of being bludgeoned with experiments carried out by third parties under conditions that often are not appropriate for safety evaluation or for which the results have not been validated. This dilemma ferments frustration, and inevitably intellectual problem-solving deteriorates into arguments of the wrong issue--for example, thresholds.

Why Are Traditional Models Inapplicable?

When direct measurements are impossible, assumptions must be made if we are to be able to extrapolate a cancer hazard from animal studies at relatively high dose rates to human population cancer hazards at intermittent and low dose rates. Thus, one set of assumptions we must face concerns the description of the function relating the real hazard measured at high dose rates to a theoretically estimated animal risk at low dose rates. The magnitude of low dose risk is highly dependent upon the assumed shape of the dose-response function, and for neoplastic disease we must consider time. Unfortunately, the shape of the curve at low dose rates for most models is much more sensitive to manipulating the extrapolation procedure than to the experimental data. This has led to the discouraging observation that, for most models advanced to date for regulatory purposes, extensive experimentation and quality assurance means less than arbitrary mathematical manipulation, e.g., worst-case confidence limits.

The extreme differences between models for extrapolating to low risks have been reviewed in detail elsewhere and a summary is presented in Table 3-1. The probit, logistic, and one-hit curves can all be shown to calculate that one-fourth of a dose producing a 50% tumor incidence will produce a 16% tumor response. The families of curves generated by these models are indistinguishable in the range usually described by most experiments, that is, the 8% to 92% tumor response. However, several thousand animals would have to be tested in order to distinguish with confidence between predictions by the probit and logistic models in the 2% to 4% response. Hence, extreme differences between the estimated doses generated by the three models are noted when extrapolating to an upper confidence of no more than a one in a million risk.

The "extreme value" curve, another possible model, would generally lie between the probit and logistic, depending on the slopes selected. The choice of an extrapolation model is critical, but the parameters to be adjusted in the use of the model, particularly the slope and upper confidence intervals, are even more critical.

The logistic analysis can also be rejected because the logistic function, as originally proposed by Reed and Berkson in 1929, is an empirical form of a series of at least six equations obtained by data-fitting and not by any form of derivation. In addition, the forms of

Table 3-1

DOSES REQUIRED TO PLACE UPPER LIMITS
ON ESTIMATED RISKS

Estimated Risk	Probit	Logistic	One-Hit
10^{-3}	1/67	1/323	1/714
10^{-6}	1/714	$1/9.8 \times 10^{5}$	$1/7.14 \times 10^{5}$
10^{-8}	1/2440	$1/6.3 \times 10^{6}$	$1/7.14 \times 10^{7}$

the logistic are uncertain since different investigators use different equations, and the different slopes and intercepts thus obtained are without physical meaning, even in well-defined chemical or biochemical systems. The logistic equation has a mathematical form similar to the Hill equation that is based on the mass-action law. Thus, there seems to be no reason to use the logistic function.

Even for a particular model, for example the probit, nondata factors influencing the model can produce widely different extrapolation results (Table 3-2). For comparative purposes, dose-reduction factors for a risk not to exceed one in a million are given in Table 3-3 using probit of slope of one.

It is obvious that application of the most refined methods of observation or changing diagnostic criteria can lower any observed threshold. For example, if we consider hyperplasia as the endpoint rather than neoplasia, we shift the threshold. Repeating the bioassay will demonstrate variability even among genetically equivalent individuals. Phenotypic heterogeneity of any population and microenvironments will be expressed and will influence the responses observed. Almost all toxicologists believe that for any compound and for given conditions a "biologically insignificant dose" exists. There is little doubt that this is true; however, so far no acceptable approach has emerged for cancer risk assessment since a consensus on the definition of "insignificant" has not been reached.

Low Dose Models

Most investigators grappling with low dose extrapolation problems agree that we must continue to develop mathematical models describing hazards from carcinogenesis. A more fundamental insight into the relationship which undoubtedly prevails between dose and excess response at low constant dose rates is of critical importance. Much effort has already been expended. Crump et al. (1), Guess et al. (4), and Peto (5) have invoked certain hypotheses of carcinogenesis to argue that the possibility of linear responses at low doses must be considered. For example, Crump et al. (1) and Guess et al. (4) offer the proposition that the probability, P(d), of response at dose rate is given by:

$$P(d) = 1-\exp[-(a_0 + a_1 d + a_2 d^2 + \ldots.)], \; a_i \geq 0.$$

Table 3-2

FRACTION OF EXPERIMENTAL DOSE YIELDING ZERO TUMORS
USING PROBIT EXTRAPOLATION WITH DIFFERENT SLOPES
FOR AN ESTIMATED RISK NOT TO EXCEED
ONE IN 100 MILLION

Tumors Observed at Dose	Fraction of Dose X Not Exceeding Risk		
	Slope = 1	Slope = 1.5	Slope = 2.0
0/50	X/18,000	X/690	X/135
0/100	X/ 8,300	X/410	X/ 91
0/500	X/ 1,800	X/150	X/ 42
0/1000	X/ 1,000	X/100	X/ 32

MONS 015896

Table 3-3

FRACTION OF EXPERIMENTAL DOSE USING PROBIT EXTRAPOLATION WITH A SLOPE OF ONE WHICH IS CALCULATED TO PRODUCE AN ESTIMATED UPPER LIMIT RISK NOT TO EXCEED ONE IN A MILLION

Tumors Observed at Dose X	Fraction of Experimental Dosage
0/50	X/2,500
0/100	X/1,140
0/500	X/ 250
0/1,000	X/ 140

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When P(d) is small, the excess risk P(d)-(0) is approximately

$$P(d)-P(0) = a_1 d + a_2 d^2 + \ldots,$$

and the pseudo-question of nonthreshold low-dose linearity for purposes of modeling is determined by whether or not the coefficient a_1 is zero. There are plausible assumptions, such as detoxification mechanisms, DNA repair, or the necessity for multiple molecular interactions, that lead to $a_1 = 0$. There are also plausible assumptions for some agents that can lead to positive values for a_1. We will almost never have the knowledge to completely discriminate between these alternatives. Acceptable approaches to extrapolation must make efficient use of what experimental data we have or can get. Injection of value judgements, such as selected worst-case confidence limits in place of central expected values for the coefficients a_1, incorporates a bias value judgement into the extrapolation procedures that almost always outweighs the information inherent in the data. When one applies selected worst-case confidence limits rather than the central expected value (i.e., the most likely value) for a_1 it is equivalent to accepting that a_1 is positive. At low dose rates, this value judgement (such as an arbitrary worst-case confidence limits) dominates the remaining terms. The requirement to use upper confidence limits rather than central expected values in fact guarantees that models will yield nonthreshold linearity at low dose rates. Masquerades should not be necessary. If a policy calls for the selection of nonthreshold linear models, let us state so clearly and forthrightly. The arguments associated with masked conservatism have become redundant, predictable, and intellectually unappealing.

The lack of mathematical logic supporting certain value judgements becomes apparent when they are applied to the procedures of Crump et al. (1) and Guess et al. (4). Techniques become untenable when they are insensitive to responses at experimental dosages and fail to distinguish between low levels of potent carcinogens and noncarcinogenic substances. Guess et al. (4) have documented this by constructing two sets of dose response data for a noncarcinogenic agent. One simulation contained 150 responses at each of 10 dose rates, while the second was made up of 300 responses at each of 5 dose

rates. In both cases, as was predicted, the 90% upper confidence limit on a_1 was positive. It then follows that the upper confidence limits on curves relating limits of possible extra response to dose rate will always appear to be linear at low dose rates. The predictability of false positive prediction of nonexistent risk supports the contention that the value judgement inherent in the use of worst-case confidence limits is not always appropriate. Value judgements should be delineated or completely separated from the quantitative use of mathematical models.

Another example of a mathematical procedure is provided by Gaylor and Kodell (3) in their study of 14 sets of toxicological dose response data previously described by the Scientific Committee of the Food Safety Council (2). The behavior of a gamma multihit model was purported to be compared to the Armitage-Doll multistage model for low dose extrapolation using the technique given by Crump et al. (1). The Gaylor and Kodell Armitage-Doll multistage model results did not exactly agree with the Scientific Committee of the Food Safety Council data because the limits calculated by Gaylor and Kodel did not assume a maximum degree of dose in the exponential term, whereas the limits calculated by the Food Safety Council took the degree of dose as fixed. Gaylor and Kodell (3) concluded that: "Conceptually linear extrapolation would (should) be more conservative, but this is not the case in actuality, apparently because more stringent mathematical assumptions and conditions are required" (by the gamma one-hit model). Selected examples are given in Table 3-4.

The examples given in Table 3-4 reinforce the recommendation made by the Scientific Committee of the Food Safety Council (2) calling for reason when mathematical interpretations affect real-life decisions:

> Although the value judgement involved in the use of conservative risk assessments may seem appropriate in the light of the many scientific unknowns involved, once formalized as a specific mathematical procedure it escapes the control of the decision-maker and can lead to undesirable and unsound results by distorting the balance between risk and benefit. We, therefore, recommend the separation of the mathematical and societal aspects of the problems, with the extrapolation procedure chosen to provide 'best estimates' of risk as well as their upper or lower limits.

The toxicological and mathematical uncertainties associated with extrapolating risks from relatively high experimental dosages in

Table 3-4

LOWER 97.5% CONFIDENCE LIMIT FOR DOSAGES PREDICTED
TO HAVE A RISK OF LESS THAN ONE MILLION IN RODENT POPULATIONS

Substance	Dose Unit	Sci. FSC Com. 1978	Linear	Armitage-Doll
Aflatoxin B_1	ppb	3.4×10^{-5}	7.9×10^{-6}	5.9×10^{-6}
Vinyl Chloride	ppm	1.6×10^{-3}	7.1×10^{-4}	5.2×10^{-4}
Ethylenethiourea	ppm	4.4×10^{-4}	1.0×10^{-4}	3.2×10^{-4}
Dieldrin	ppm	9.4×10^{-6}	5.7×10^{-6}	2.9×10^{-6}
DDT	ppm	2.0×10^{-2}	3.4×10^{-5}	2.6×10^{-5}

animals to low human exposure levels are often cited as a principal concern of those who call for complete prohibition when a chemical has been "demonstrated" to be a carcinogen. A slight modification of this approach is to use a series of conservative assumptions for data presentation followed by linear extrapolation or equivalent "model" use from upper confidence limits of the experimental results to a zero response at zero dosage. Since rodents, the principal bioassay species, have substantial rates of neoplastic disease at many sites, such an approach invites the option of declaring almost any thoroughly studied compound a carcinogen. PCBs are just such an example when one weighs all the negative studies against the single positive study of Kimbrough.

Evaluation of Criteria and Calculations of Risk from PCBs for Humans by Crump

We have now discussed the lack of mathematical logic supporting use of certain value judgements. In addition, Crump violated his own criteria for justifying the selected model for PCB risk analysis. This can be demonstrated with Crump's own words. Crump states in explaining his approach to risk analysis for PCBs:

> Tumors of so many different types arise in such a diversity of different tissues, their etiology is so little understood, and the agents that cause tumors affect a subject in such diverse ways, that it might seem that no general conclusions can be drawn. However, for a certain broad class of 'directly-acting' chemical carcinogens the range of uncertainty associated with the shape of the dose response curve at low doses can be greatly narrowed. As used in this paper, the term "directly-acting carcinogen" encompasses (Guess, Crump and Peto, 1977) carcinogenic agents for which either the agent itself or a metabolite acts directly at the cellular level and produces a hereditable change which eventually leads to the formation of a tumor. Carcinogens which are carcinogenic by reasons of their mutagenicity should fall into the category of 'directly-acting carcinogens.' Accordingly, carcinogens which test positively using the Ames mutagenicity screening test for carcinogenicity are very likely to be directly-acting.

The available data suggest that even if Crump's statement was true, the concept could not be appropriately applied to PCB risk analysis since PCBs are not direct-acting carcinogens as demonstrated by scientific literature. For example, the Ames assay utilizes

Salmonella typhimurium to detect reverse-point mutation at the histidine locus. Only 4-chlorobiphenyls have demonstrated any activity in the Ames 1538 tester strain. In this same study, PCBs such as 2, 2', 5, 5' tetrachlorobiphenyl, 1254, and 1260 were negative. In subsequent studies, not even the positive for monochlorobiphenyl could be reproduced.

It has been demonstrated that PCBs do not cause significant clastogenic effects in rat bone marrow or sperm cells even at high doses. Aroclor 1242 was given at a single dose of 1,250 to 5,000 mg/kg and at 500 mg/kg for four days (a regimen causing the condition of the animals to deteriorate), while Aroclor 1254 was given at doses of 75 to 300 mg/kg for five days. These findings are consistent with the lack of chromosomal aberrations observed in human lymphocyte cultures with doses of 100 mg/kg Aroclor 1254.

The possible mutagenicity of PCBs has also been studied using the dominant lethal test. There was no statistically significant increase in the number of dead implants, again at high dosages of Aroclor 1242 and 1254. This test, the dominant lethal assay, has been repeated and again the results were negative. PCBs do not have significant mutagenic potential.

Crump continues to try to justify his models and approach taken for risk estimation for PCBs by stating:

> A partial solution to the low-dose extrapolation problem for the case of directly-acting chemical carcinogens has been given in Peto (1977), Crump, Hoel, Langley and Peto (1976), and Guess et al. (1976). The key result is that, at least as long as background carcinogenesis is present, we should expect the dose response curve not to be absolutely flat at zero dose. What this means is simply that when risk is plotted against dose response on ordinary linear scales, the tangent line to the dose response curve at zero dose should have a positive slope. When a dose response function has this property we will say it is linear at low dose.

Crump also offers a possible explanation of why the dose response function should be linear at low dose when background is present (Crump, et al. 1976), as does Peto (1977), which will be briefly outlined here. Crump et al. and Peto argue that when background carcinogenesis is present, the cellular mechanisms through which the test agent produces cancer should already be operative in producing background tumors. When this is true, the effect of the test agent is to

add to any already ongoing process. The dose response curve is for all tumors produced through the mechanisms through which the test agents acts. Background carcinogenesis is allowed for by an effective background dose d_0. In this case, the added risk caused by a dose d of the test agent could be expected to increase approximately linearly near d = 0 (i.e., the tangent line at d = 0 will have a positive slope). Implicitly assumed is the fact that an added dose of a carcinogen acting through this mechanism does not produce a smaller risk. If background carcinogenesis is allowed for by positing an effective background dose d_0 estimated from the data, then the wide range of risks obtained using different models effectively disappears (Peto 1977) because they all approach a simple linear model.

The Crump and Peto argument does not apply to PCBs. That the liver cancer rate induced by direct-acting carcinogens is not added to by PCBs has been demonstrated on several occasions. The effects of PCBs on carcinogenicity of various chemicals have been investigated by numerous groups. Kanechlor-500 in combination with 3' methyl-4-dimethylaminoazobenzene, N-2-fluorenylacetamide, and diethylnitrosamine in the diets of rats markedly decreased the formation of hepatocarcinomas. Kimura et al. demonstrated that pretreatment with Kanechlor400 in diets of rats 4 months prior to and 2 months during treatment with 3' methyl-4-dimethyulaminoazobenzene protected the rats against the formation of hepatocarcinomas induced by this carcinogen.

The two-stage system of mouse skin tumorigenesis allows one to evaluate critically the initiation and promotion phases of carcinogenesis individually. This system allows one to study the effects of modifiers on initiation and promotion separately in a skin carcinogenesis assay. The results of Berry et al. demonstrate that PCBs possess the capacity to decrease tumor initiation in a mouse skin assay and that at the doses utilized, PCBs had no initiating or promoting properties. Their study tested PCBs for promoting activity in mouse skin with a high (200 nmol) initiating dose of DMBA. In a 30-week treatment period, the normal DMBA-inititated, TPA-promoted controls yielded approximately 8 papillomas per mouse. PCBs (at doses of 100 µg/mouse given twice weekly) did not promote the development of skin tumors.

When tested without DMBA initiation, PCBs did not demonstrate any carcinogenic activity. PCBs did not produce any observable skin lesions.

Crump himself invalidates the model's use with the linear low dose assumption for PCB risk analysis when he states:

> The evidence for low-dose-linearity given above applies mainly to directly-acting carcinogens. An indirectly acting carcinogen might be one which causes some gross physiological change such as suppression of ovulation which could predispose the subject to cancer. For such carcinogens the shape of the dose response curve at low dose is highly speculative. There could possibly be a threshold dose below which the agent has no carcinogenic effect at all on an individual.

The reader should be reminded that for the purposes of the discussion, promoter and indirect carcinogen can be used interchangeably. That PCBs are at worst an indirect carcinogen is supported by a large amount of data. PCBs induce microsomal mixed-function oxidases and cause hepatomegaly in rodents and other mammals. Heptomegaly has been interpreted by Kimbrough to be the result of the hypertrophy of individual hepatocytes. Hyperplasia also commonly occurs and increased mitotic activity can occasionally be noted. Hepatocytes enlarge and may accumulate lipid in their cytoplasm. At the ultrastructural level, enlarged hepatocytes show an increase in smooth endoplasmic reticulum and inclusions within the cytoplasm, which appear like concentric whorls, surrounding lipid vacuoles. Morphologic changes in the mitochondria have also been described (Kimbrough et al. 1972).

In addition to these alterations, PCBs induce experimental hepatic porphyria. In the rat, experimental hepatic porphyria only occurs in the Sherman strain female. This observation indicates a unique sensitivity for the Sherman female rat. On microscopic examination, an increase in macrophages and prominent Kupffer cells containing brown ceroid pigment and necrobiosis of liver cells is prominent. Lipid accumulates in the cytoplasma of hepatocytes, resulting at times in hepatocytes with foamy cytoplasm.

Acute as well as chronic toxicity of PCBs has been studied in rats, monkeys, mice, and cows. The organ consistently affected was

the liver. For example, when male Sprague-Dawley rats were fed a diet containing mixtures of PCB isomers (Aroclor 1248, 1254, and 1260) at a concentration of 100 ppm in the diet for 52 weeks, there was an increase in their serum lipids and cholesterol, and a transient increase in triglycerides accompanied by distinct morphological changes in the liver. Generalized liver hypertrophy and focal areas of hepatocellular degeneration were followed by a wide spectrum of repair processes. The tissue levels of PCB were greater in the animal receiving the high chlorine mixtures and high levels persisted after the PCB treatment had been discontinued.

Summary Supporting the Statement that PCBs Are at Worst Indirect Carcinogens (Promoters)

1. Promoters produce increases of spontaneous tumor at selected sites. PCBs only produce liver tumors in rodents for which there is a spontaneous occurrence.

2. Tumors produced by promoters usually do not increase the metastatic characteristics of spontaneous tumors of the same global (direct-acting) site. PCB increased liver tumors do not metastasize.

3. Promoters do not produce transplantable tumors unless the naturally occurring tumor is capable of transplantation. PCB tumors do not transplant.

4. Tumors influenced by promoters are often affected by factors such as nutrition, stress, chronic injury, and sex of the animal. Rodent liver tumors are affected by these factors.

5. Promoters need not be mutagenic. PCBs are not mutagenic.

6. Neoplastic lesions increased by promoters may be reversible. PCB increased liver tumors regress.

7. Progression of differentiation of neoplastic lesions increased by promoters often ceases when stimulus is removed. Continued presence of PCBs is required to sustain liver neoplasia.

8. Neoplastic effects of promoters are usually associated with the chronic dysfunction of the affected site. PCBs produce chronic hepatotoxic responses.

9. Promoters do not necessarily increase the effect of carcinogens acting at the target site. PCBs reduce the response of several other liver carcinogens.

10. Promoters affecting the liver often increase microsomal enzyme activity and eventually produce evidence of metabolic dysfunction. PCBs stimulate LME and produce metabolic dysfunction.

11. Promoters rarely if ever increase tumor incidence at exposure levels producing no toxic effects. PCBs are tumorogens only at toxic doses.

Characteristics of Initiators (Direct-Acting Carcinogens)

1. Initiators usually produce neoplastic lesions at multiple sites. PCBs only increase lesions of the liver.

2. Initiators often increase the malignancy of spontaneous tumors at the same site. PCB-increased liver tumors are of low malignancy.

3. Carcinomas irreversibly progress once a critical tissue mass is established. PCB-increased tumors can regress.

4. Initiators produce tumors which metastasize. PCB-increased liver tumors do not metastasize.

5. Initiators produce lethal tumors. PCB-increased liver tumors are not lethal.

6. Initiators produce transplantable tumors. PCB-increased liver tumors do not transplant.

7. Initiators are often effective at single exposures. PCBs increase liver tumors only after prolonged continuous exposures.

8. Initiators are often active at nontoxic doses. PCBs only increase liver tumors at hepatotoxic doses.

9. Initiators are mutagens. PCBs are not mutagens and therefore not initiators.

It is apparent that the neoplastic effects of PCBs match the definitions of a promoter and do not match the definitions of an initiator. We have established that the rationale for justifying the technique for analysis of PCBs by Crump is inappropriate when compared against Crump's own criteria.

Conclusions About the Application of Linear Extrapolation and the Equivalent Nonthreshold Models to Risk Analysis of PCBs

We will establish our thesis when we thoroughly analyze the risk analysis offered by Crump. When referring to his modeling efforts, Crump states, "The expected numbers of extra cases of hepatocellular carcinomas per year resulting from a nationwide exposure at a dietary level detected in the 1976 Total Diet Study (3.3 μg/day) would range between 220,000 (288,000 x 70) = 11.1 cases/year and 220,000,000/(764,000 x 70) = 4.1 cases/year."

Crump misleads the reader by suggesting that the mathematical model used to generate these numbers brings logic to the effort. For example, if one simply takes the ratio of animals with and without liver tumors at 100 ppm in the Kimbrough study, divides by the estimated human exposure (1 ppb), then multiplies by the size of the human population in the United States (220 million), and finally divides by the human lifespan of 70 years, one gets the same answer as Crump:

26 tumors/184 animals 1 ppb PCB/200 ppm PCB/70 years = 4.3 cancers/year.

Crump's analysis of risk to infants is markedly overstated. A rat consumes approximately 10% of its body weight per day. Feed with 100 ppm PCB equals a consumption of 100 mg/kg. Therefore, a rat consumes the equivalent of 10 mg PCB/kg/day. If the human milk sample was 2% fat, then the whole milk would contain 2% of the 4 ppm Crump considered, or .08 ppm. A liter of milk would contain .08 mg of PCB. A child weighing 6 kg would have to consume 750 liters of milk a day to equal the exposure of the rats. If the child truly only consumes 1.5 liters a day, then there is a 500-fold difference in exposure rate between rat and baby.

If the baby is exposed, as suggested by Crump, for only 1/140 of its lifespan, this has to be considered. The 500-fold difference times the 1/140 of the lifespan is equal to a 70,000 exposure differential. The linear risk as calculated by Crump's technique would be 2/1,000,000, not the 1/4,800 reported by Crump.

If we approach the problem another way, we obtain other interesting comparisons. The PCB dose to high exposure women averaged 1.7 ug/kg/day. A lactating female maintaining her weight would then have 1.7 ug x 50 kg, or 85 ug/day of PCB, available for transfer to the baby. If the baby weighs 6 kg, the dose is 14 ug/kg/day. This, when compared to the 10 mg/kg/day dose to the rats, yields an exposure ratio differential of 700 times. The exposure period of 1/140 times the exposure ratio yields a total exposure risk of 98,000 times less for the human than the rat. The linear risk as calculated by Crump's technique would be 1.4×10^{-4}, not the 1/4,800 reported by Crump. If we insist on <u>linear extrapolation</u>, what are the relative risks? Let us assign an average exposure resulting in 500 ppb blood level to capacitor workers, 70 ppb to the heavy fish eaters in Michigan, and 20 ppm to the general Michigan population. Let us assign an exposure of 12 ug/kg/day to the capacitor workers, 1.7 ug/kg/day to the fish eaters, and .5 ug/kg/day to the general population. These estimates were taken from the documents of Crump and the EPA water quality criteria for PCBs.

The rats in the Kimbrough study were exposed to 10,000 µg/kg/day. Therefore, the ratio of exposure between rats and man is:

Capacitor worker/rat = .0012
Fish eater/rat = .00017
General population/rat = .00005

The chances of dying from liver cancer can be expressed in several ways. For example, 816.3/100,000 Americans die each year from all causes. The incidence of liver cancer disease is 3/100,000. This translates to a .37% chance of liver cancer. Expressed another way, there will be about 2,000,000 deaths in 1981 and 9,400 are estimated to be from liver cancer. Therefore, 9,400/2,000,000 equals a .47% chance that the registered death will result from liver cancer. This estimate will be rounded off to a .5% chance for the purposes of our discussion.

If the risk were linear (and the risk to humans is the same as in rats, or .14), then the risk in man would be .00017 for capacitor workers, .00024 for fish eaters, and .000007 for the general population. Expressed another way, the risk to humans for liver cancer would increase to:

Capacitor worker .005 + .00517 = .01017
Fish eater .005 + .000024 = .005024
General population .005 + .000007 = .005007

Thus, the cancer mortality data suggest that there is only a slight possibility that the actual risk for capacitor workers would be higher; however, the data are far from conclusive. It is also obvious that it would be impossible to determine epidemiologically the tiny increases expected to occur either in the fish-eating population or in the general population.

Let us approach the problem from a different viewpoint. Let us suppose that our risk analysis should be conducted on a relative risk basis. If the relative risk to rats at 100 ppm is 26, and the same exposure ratios between rat and man hold, then the risks are as follows:

Capacitor worker	.005 + .0012 x 26 = .0362	(724% background)
Fish eater	.005 + .00017 x 26 = .0094	(188% background)
General population	.005 + .00005 x 26 = .00552	(110% background)

Again, this hypothesis cannot be rejected at the lower exposures because it is not possible to analyze it on the basis of information available. However, the capacitor workers aid in the evaluation of this hypothesis since the highly exposed individuals would demonstrate an incidence which is seven times the background incidence.

How Did EPA Analyze the Data?

The preceding discussion of human exposure makes clear the fact that a high percentage of the United States population has been and is exposed to low levels of PCBs in food, water, and air. Those groups at particular risk for PCB exposure include industrial workers exposed in the workplace and individuals consuming large amounts of contaminated fish, such as sport fishermen (42 FR 17487).

An assessment of carcinogenic risk was made by EPA performing extrapolation from animal data using a linearized multistage (non-threshold) model. The extrapolation used was reported to take into account the bioaccumulation of PCBs in fish and shellfish. It is assumed that an average of 2 l/day of water are consumed along with 6.5 g of fish taken from that water source. Exposures from other food sources, air, or occupational exposure are not included in the criterion level derived by this model.

Among the studies reviewed by EPA, only one was considered suitable for use in the cancer risk assessment. EPA made the remarkable statement that, "Of the rat studies, the only one involving long-term exposure and adequate numbers of animals is the study of Sherman rats by Kimbrough et al. (1975)." EPA goes on to state, "Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of PCBs in water for maximum protection of human health is zero." Since attaining a zero concentration level may be infeasible in some cases, and in order to assist the agency and states in the possible future development of water quality regulations, the concentration of PCBs corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides

an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5}, for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register Notice of Availability of Draft Ambient Water Quality Criteria, EPA stated that it is considering setting criteria at an interim target risk levels of 10^{-5}, 10^{-6}, and 10^{-7}, as shown in Table 3-5.

If such is the EPA's estimate, 80 µg/day, how does it compare with other EPA statements and those results gathered in the NIOSH and FDA studies? Even when one makes all the conservative assumptions utilized in official PCB cancer risk estimations, one arrives at answers which are so low as to escape our capability of detecting a positive effect via epidemiology. Therefore, one must conclude that mathematical risk estimation, even for the occupationally exposed, yields estimates which are vanishingly small. However, the overzealous use of mathematical models and the assumptions made by the EPA without comparing these results to reality are easily demonstrated. EPA states in the Federal Register Notice of Availability of Draft Ambient Water Quality Criteria that the following risk to cancer from PCBs exists, 10^{-5} cancer risk = 80 ng PCB/day.

Table 3-6 summarizes known human exposures to the risks calculated by an extrapolation of the values.

Using estimates performed on preceding pages calculated from actual cancer rates in the United States, 9,400/2,000,000 equals a .47% chance of dying from liver cancer. This estimate will be rounded off to a .5% chance for the purposes of our discussion. Using these comparisons, it can be seen from the table above that if the EPA estimates are correct then 10% of the liver cancer in the United States would be due to PCB exposure; the Michigan control population would expect a 100% increase in liver cancer incidence, the Michigan fish eaters would have a 3-fold increase, the average capacitor worker would have a 10% incidence of liver cancer or a 20-fold increase, and the highly exposed capacitor worker would have a 25% incidence of liver cancer or a 50-fold increase. No human evidence supports any of

Table 3-5

EPA INTERIM TARGET RISK LEVELS

Exposure Assumptions	Risk Levels and Corresponding Criteria[1] 10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 6.5 g fish and shellfish only[2]	0.0079 ng/l	0.079 ng/l	0.79 ng/l (80 ug/day)
Consumptions of fish and shellfish only	.0079 ng/l	0.079 ng/l	0.79 ng/l (80 ug/day)

1Calculated by applying a linearized multistage model as discussed in the Human Health Appendices to the October 1980 Federal Register notice which announced the availability of this document. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations shown in the table above vary by factors of 10, 100, 1,000, and so forth.

2Approximately 99 percent of the PCB exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 31,200-fold. The remaining 1 percent of PCB exposure results from drinking water.

Table 3-6

EXTRAPOLATIONS OF EPA RISK LEVELS
TO A HIGHER LEVEL OF HUMAN EXPOSURE

	Blood Exposure		
Group	Blood	Exposure	EPA Extrapolated Risk
Highly exposed capacitor workers	≥1,000 ppb	2 mg PCB/day	25%
Average capacitor worker	500 ppb	.84 mg PCB/day	10%
Michigan fish eater	70 ppb	.12 mg PCB/day	1.4%
Michigan control population	20 ppb	.035 mg PCB/day	.5%
General population	2 ppb	.0035 mg PCB/day	.05%

these estimates. On the contrary, it is obvious that the EPA standard is from 10 to 100 times more severe than can be justified on the basis of human experience, and from 10- to 20-fold more severe than can be excluded on the basis of human experience. As our data base grows, it is likely that the standard will be found to be at least another order of magnitude too severe.

In summary, the rationale that was used by Crump and Masterman in establishing the PCB cancer risk was based on a genotoxic theory of carcinogenesis and was compiled from a series of ultra-conservative assumptions. These assumptions ignored the fact that PCBs are not genotoxic; utilized only the positive data and ignored the negative data; applied constraints to mathematical models which transformed them to the equivalent of linear extrapolation; ignored all other competing causes of death; equated cancer risk in the last moment of life with the risk of a newborn; and exaggerated exposures, for example: assumed that mother's milk was 100% fat, equated all PCBs' toxicities as the most toxic results observed for any PCB, and ignored the reversibility of PCBs' effects and the marked improvement in human exposure levels that has occurred in the last five years.

Furthermore, using a mathematical model to extrapolate the risk at lower doses does not increase the reliability of animal data. The most important question still remains: "Does the animal data reflect the human situation and response?" With the addition of the NIOSH study on capacitor workers to the PCB literature it can be demonstrated that the risks estimated using Crump's model are unrealistically conservative by serveral orders of magnitude. In a practical sense, a substantial number of people have been chronically exposed to higher levels of PCBs for a longer period of time than that which occurs for the general population. Since no adverse health effects were causally demonstrated in this population, no measurable adverse effects would be expected for the general population.

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MONS 015916

4. SUMMARY AND CONCLUSIONS

In past decades, a combination of "open" systems and sloppy or illegal disposal practices resulted in the release of large quantities of chemicals into the surrounding environment. In the 1960s, when it became apparent that persistent chemicals like DDT and PCBs were ubiquitous environmental contaminants which took years to biodegrade, attention became focused on their potential bioaccumulation and the possible negative effects of this on animals and humans. Even though the principal United States manufacturer, Monsanto Industrial Chemical Company, had voluntarily restricted PCB sales to manufacturers of "closed" electrical equipment in 1971, Congress enacted the Toxic Substances Control Act in 1976, mandating a phaseout of PCB manufacturing and use. During this time, PCBs were considered as highly toxic and dangerous chemicals capable of causing permanent adverse health effects such as cancer and birth defects. In recent years, however, a substantial increase in scientific data has resulted in a better understanding of the actual effects of human exposure to PCBs. It was the purpose of this document to review the data regarding the health effects of PCBs to provide an updated perspective to the PCB issue.

While it is the consensus of this report that PCBs do not pose the health hazards once feared, this conclusion may be complicated by a contaminant, polychlorinated dibenzofurans (PCDFs), often found in commercial PCB mixtures, which in some special circumstances may pose an increased risk. Yet PCDFs are only a minor component of PCBs produced in America, probably in the range of only 0.1 to 2 parts per million (ppm) of commercial PCBs. In addition, such commercial PCB

mixtures were apparently the same as those extensively tested over the years. Therefore, the concern for this level of contamination is expected to be no greater than that predicted for what was previously considered to be "pure" PCBs.

It was not the intent of this document to determine the full impact of PCDFs with regard to the hazards associated with PCBs. However, the PCDF content of PCBs may be increased under conditions of high temperatures, and the possibility of increased toxicity by the presence of this contaminant or others should always be considered when dealing with PCB waste mixtures of uncertain origin. Since the consequences of commercial PCB exposure alone were feared and regulated in the past, it was this issue alone which was addressed in this document. Therefore, having brought the above qualifying considerations to the attention of the reader, this report focused only on those health hazards expected to arise from exposure to commercial PCBs alone.

For the benefit of readers whose background and understanding of the field of toxicology, and the principles upon which it is based, is minimal, the following short discussion on safety and hazards is provided to help elucidate the problems involved in extrapolating test data to human consequences.

A poison is an agent that can produce an adverse effect in a biological system. This adverse effect may be an alteration of normal function or the destruction of life. The definition of a poison is broad by necessity and would include most chemicals, if not all. That is, all agents are capable of altering some function or producing death in some biological organism. Thus, classifying a chemical as a poison does not describe the most important feature of poisons, which is those circumstances and conditions under which an adverse effect can be produced. An agent that is a poison produces harm only within prescribed conditions of usage.

The safety of an agent can be defined in terms of its probability for producing an alteration of normal function or causing destruction of life under specified conditions. To determine the safety of an agent, the toxicity of the substance must be determined under controlled circumstances. Assessment of the toxicity of the agent will define the limits and conditions necessary to produce any adverse

effects. The question then asked is, "What is considered safe and under what circumstances?" Critical to this determination is predicting the impact of the substance and the variations that are likely to occur within the exposed population. A critical evaluation of experimental evidence is necessary to define the probable hazard to man and the magnitude of the hazard. The accuracy of the prediction will depend on the types of experiments performed; the adequacy with which they have been performed; and, most importantly, the interpretation of the experimental results and the limits of the data.

The questions to be asked are, "Are PCBs poisons or are they safe? And if they are poisonous, when?" The problem is thus reduced to predicting an agent's safety based upon experimental evidence and a comparison of the results to the likely levels of human exposure. PCBs can be poisons. That is, like all other chemicals, PCBs are capable of altering normal function and can destroy life under specific circumstances and conditions. Alternatively, PCBs are safe under specific circumstances and conditions. The following summary of PCB properties and effects is meant to provide a perspective on their safety.

PCBs are chlorinated compounds of the biphenyl molecule. Theoretically, approximately 209 separate chlorinated biphenyls can be produced chemically. These compounds have unique physical and chemical properties that have made them useful and applicable to many commercial needs. The physical-chemical properties that make these compounds useful are their thermal stability; resistance to alteration by acids, bases, and other chemical agents; and their ability to provide excellent electrical insulation, fire resistance, and low volatility. PCBs are useful as lubricants, heat transfer liquids, and hydraulic fluids.

PCBs are ubiquitous to the human environment, as evidenced by their detection in the fat and blood of persons not occupationally exposed to them. These compounds have been identified in air, water, soils, wildlife, marine organisms, fish, and in our food supply. Thus, to minimize man's environmental exposure to PCBs, the United States Food and Drug Administration (FDA) has set tolerances of 0.5 to 5.0 ppm for the various components of our daily food supply. In the early 1970s, the daily consumption of PCBs was estimated by FDA to be

about 15 micrograms per day (µg/day). However, all evidence now indicates that the level of daily PCB intake by humans is subsiding. Yet these factors have resulted in a major concern for the potential long-term effects of PCBs on human health because many of us still contain low levels of PCBs.

Owing to their low solubility in water and low concentrations in air, PCBs do not present an acute toxicity threat to either birds or aquatic organisms. In other words, the quantity of PCBs necessary to cause death within a short period of time, such as 96 hours, is greater than the amount generally found in natural environments. PCBs can cause major problems for some species, however, because these chemicals are persistent, lipid soluble, and resistant to biodegration and/or excretion. Consequently, PCBs can bioaccumulate (build up in quantity within an organism) as well as biomagnify within food webs (accumulate in larger amounts per group of organisms at each subsequent trophic level). The greatest amounts of PCBs are present in the long-lived predatory fish (e.g., trout, sharks) and birds (e.g., eagles, hawks). Sufficient quantities of PCBs may build up such that physiology or function is disrupted. At high levels, PCBs may decrease resistance to diseases, interfere with reproduction, and disrupt defensive behavior. In the case of birds, increased mortality of embryos can result as a consequence of the thinning of eggshells. Therefore, it has been concluded that PCBs can cause major environmental disruptions, particularly within aquatic habitats. Responding to this problem, the United States Environmental Protection Agency (EPA) has attempted to curtail the release of PCBs into the environment by regulating their use and disposal.

Many aspects must be considered and weighed before the hazards to humans associated with exposure to PCBs can be estimated. The human hazard evaluation and subsequent risk estimation for any chemical must address the data base utilizing thorough comparative toxicologic assessments. This means that the animal and human data available must undergo a careful consideration of 1) the breadth and variety of the toxic responses manifested; 2) the degree of species variation or species consistency in the effects monitored; 3) the possible and/or proposed mechanisms of toxicity; 4) the validity of the tests performed and their relevance for extrapolations to man; 5) the dosage used in

animal tests versus the expected human exposures; and, finally, to the extent that the data are available, 6) the outcomes of serious poisonings and long-term occupational exposures. Only when a consistent pattern of toxicity in animals is consistent with the human experiences can safe guidelines be promulgated.

When assessing the hazards associated with human PCB exposure based upon the mammalian test data, we must consider all of the animal data. While concern is given to positive findings, our decisions must also address the negative data and those doses required to induce the toxicities seen in animals versus our expected human exposures.

A review of the PCB literature reveals that PCBs are not very toxic if the exposure is of short duration. The organ dysfunctions caused by longer but nonchronic exposures also occur only at relatively high doses. This suggests that while organ dysfunction such as liver injury or chloracne might occur in man, it should occur only after high or sustained exposures. This suggestion has, in fact, been supported by the available human data. Therefore, there is little risk that these effects will occur in persons exposed to the generally low levels of PCBs in today's environment, if exposure occurs at all.

Similarly, the animal data indicate that there is little reproductive risk and that PCBs do not cause birth defects even at moderately high exposures. Again the animal data reflect the findings in humans. The Yusho incident, a high PCB exposure combined with exposure to other toxic chemicals, failed to demonstrate a substantive concern for teratogenic effects. Considering the balance of the reproduction studies and the breadth of the mutagenic tests performed, it has been concluded that PCBs do not pose a significant mutagenic or reproductive hazard.

While positive carcinogenic activity has been reported in one study using rats, this study must be placed in perspective against a far greater number of studies involving rats and mice that were negative. When a positive test result is our only available data, then that result should be considered as an obvious basis of concern for human exposure. However, a lack of comparable results in subsequent or other similar studies surely decreases the concern for and the validity of focusing on only the positive data. Such is the situation for PCBs. It would seem more reasonable to place greater emphasis on

the large amount of negative data gathered, a suggestion that is becoming increasingly substantiated by human exposures. In the last few years, the reported epidemiologic evidence has not identified PCB exposure as the cause of any form of human cancer.

Therefore, even though there are animal data to demonstrate that PCBs are toxic at high doses, some toxicity at high doses is expected for any chemical. Additionally, while there is some evidence suggesting that the carcinogenic potential of PCBs be considered, the balance of the animal data also suggests that it is unlikely that this single response should become the focus of disproportionate concern usually given to potent carcinogens with definite initiator or genotoxic properties. On the contrary, one might propose that there exist enough animal data to suggest that this finding could be pertinent only to those specific test conditions and the particular mixture of PCBs tested. In summary, when all of the animal data are considered, they suggest that low levels of PCBs do not pose a significant health risk.

At this point in assessing the hazard associated with exposure to PCBs, a more thorough discussion of the carcinogenic potential of PCBs is needed because the risk of cancer from PCB exposure is the most serious concern of the PCB-induced adverse effects. This concern is surely of the utmost importance to the general public. This has been reflected by the stance taken by the EPA, which is manifested by EPA's recent (1980) publication summarizing its concern for PCBs. This ambient water quality criteria document indicates (and we paraphrase) that if PCBs are not carcinogenic, then an allowable daily intake of 210 μg of PCBs per day would be an estimated safe continual exposure. If, however, PCBs are carcinogens, while there may be no safe level, a level of risk approaching a lifetime risk for cancer of only one excess cancer in 100,000 persons exposed would be approximately 2 to 80 nanograms per day (ng/day). By comparison then, the distinction of whether or not PCBs have carcinogenic potential translates into a 2,500- to 100,000-fold difference for what is calculated as an allowable or safe level. Thus, the distinction between whether or not a chemical has carcinogenic potential has a profound impact on how the chemical is perceived by the public as well as how it might be regulated.

While we agree with the concern for and the distinction of chemicals which are carcinogens, it is the conclusion of this review that

there presently exist sufficient PCB test data to reevaluate those concerns for PCBs initiated by reported findings years ago. Further, the data now allow us to make the important distinction of whether or not PCBs are indeed initiating carcinogens.

Current theories of chemically-induced tumorogenesis differentiate tumorogens by mechanism. One class is the initiating carcinogens that alter DNA and produce permanent, inheritable, expressible changes in the initiated cell. These initiated cells may ultimately be transformed into the disease process we call cancer. The second class is the promoting agents, which do not directly alter DNA, do not produce inheritable changes, and are not effective with single or limited exposures. Chemicals producing tumors by a promoting mechanism have demonstrable threshold levels below which chemical exposure will not result in the expression of tumors. The risk caused by these chemicals therefore is insignificant in some for exposures that are below the threshold.

A review of animal testing data documents the case that PCBs, at worst, have some of the characteristics of a promoting agent and do not exhibit characteristics of an initiating carcinogen. A review of chronic cancer bioassay data further reveals that PCBs produced only a single species-, sex-, and mixture-specific positive response. The large number of negative tests indicate that they do not possess broad, potent, promoting activity. At the hepatotoxic doses tested, one reported PCB-induced increase in rodent liver tumor incidence after the many studies conducted should not be a totally unexpected finding. The animal testing data and biological characteristics of PCBs overwhelmingly indicate that if they produce an increase in tumors they do so by a promoting mechanism. Therefore, it can further be concluded that there are indeed safe levels of PCB exposure and that not all exposures carry a risk of producing cancer.

The occupational exposures are certainly the most extensive and longest-term human PCB exposures that we are currently aware of and are most representative of what adverse effects might be expected. A comparative review of these occupational-exposure studies reveals that, like other chemicals, PCBs can cause adverse health effects, but in many respects these have been minimal. While dermatitis and chloracne, which were reversible after discontinuing the exposure, have

been noted in many cases, no other significant findings were routinely found. Even though several studies incorporated clinical chemistry analysis as indications of organ dysfunction or other physical disorders, no remarkable clinical findings have been uncovered. Furthermore, several investigators have commented to the effect that there is a "paucity of abnormal results" and that "there is no evidence of physical harm resulting from working with PCBs." In spite of over 50 years of use, no causal relationship has been established for any specific type of cancer nor has the incidence of cancer mortality been proven to be increased. The largest study, by the National Institute for Occupational Safety and Health (NIOSH), which involved over 2,500 persons did not detect any statistically significant excess in the cancer mortality. Since this study failed to demonstrate any statistically significant excess in cancer in a high exposure population, it provides some reassurance that it is unlikely that future studies will find any cancer risk from PCB exposure.

There are four epidemiologic studies addressing the carcinogenetic potential of PCBs. However, the first two studies are being reconsidered and will not be addressed here. The two most recent studies have larger populations with better defined exposure records and therefore more accurately reflect PCB effects. A recently reported NIOSH study by Brown and Jones examined 2,567 workers from two capacitor plants with about half of the cohort having exposure periods of at least 20 years. There was a higher than expected incidence of rectal cancer among the workers, but the higher than normal rectal cancer rates in the geographical area in which the workers lived were not taken into consideration in the experimental design. There was also a statistically insignificant increase in liver cancer deaths. The death rates from all cancers was less than expected (39 as opposed to 43), as was the mortality from all causes. The last study by Bertazzi and coworkers, also just reported this year, examined 1,310 employees with at least six months' employment in a capacitor plant. An excess incidence of digestive cancer was observed, but there were no liver cancer deaths. Again, the total number of deaths was small. The study is continuing and these results may be considered preliminary.

It must be remembered that in any epidemiologic study it may be impossible to eliminate all of the unrelated but confounding variables in the observed populations. Additionally, it is not uncommon to see measurable increases in one type of cancer over what is mathematically expected because we cannot choose a subpopulation to observe that reflects exactly the baseline cancer rates of the total population. In fact, it is well known that different areas of the United States have different backgrounds for many of the specific types of cancer. All this must be considered carefully, and no conclusions should be attributed to any finding of excess cancer which is not statistically significant but above expected values, unless this same excess for that specific type of cancer is repeatedly demonstrated in subsequent studies.

A positive correlation between exposure to a chemical and a resultant adverse health effect relies on the following conditions: (1) a positive association must be seen in individuals with known exposures; (2) the positive association cannot be explained by bias in recording, detection, or experimental design; (3) the positive association must be statistically significant; (4) the positive association should show both dose and exposure period dependency; and (5) the positive association must be observed repeatedly in subsequent studies and cannot be a single, confounding, variable observation. To date, the mortality studies concerned with PCB exposures present several problems of interpretation, the most obvious and important of which is the different increases in cancer type reported. Taken as a whole, they provide no support for assertions that PCBs are a cancer-causing chemical. The variety of tumor sites found to be of most concern within each study largely differs from the animal data and is inconsistent with the selectivity of currently known promoting agents.

In summary, epidemiologic studies have demonstrated that commercial PCBs are not remarkably toxic chemicals after acute exposure and that when excess exposure does occur, the usual consequences are dermatologic and not of a serious or permanent nature. Chronic exposures have added little or no additional adverse effects of note to this picture. The preponderance of studies has not identified a clinical disease associated with exposure to PCBs nor has it provided persuasive evidence of health impairment. There is no evidence of an excess

in total mortality or in mortality due to cancer, cardiovascular disease, or nervous system disease associated with occupational exposure to PCBs. PCBs have not been linked to any human cancer, and studies to date indicate it is unlikely that future studies will establish such a link.

The NIOSH epidemiology study demonstrated the absence of any cancer excess among those exposed to far higher levels than the general population has received. The liver cancer rate in the United States is one of the lowest in the world and is not increasing over time. If one were observing a significant effect from PCBs, one would expect to detect a gross rate change, yet such is not the case.

Even when one makes all the conservative assumptions utilized in the official PCB cancer risk estimations, one arrives at answers which are so low as to escape our capability of detecting a positive effect via epidemiology. One must conclude that mathematical risk estimation, even for the occupationally exposed, yields estimates which are vanishingly small. The data provided by recent studies do not support the current assumptions by EPA. The EPA, in the Federal Register Notice of Availability of Draft Ambient Water Quality Criteria, estimates that the following risk to cancer from PCBs as: 10^{-5} cancer risk = 80 ng PCB/day. When this estimate is compared to the NIOSH and the FDA studies, one finds a large disparity between those results that would be predicted by this value and those that were actually measured.

The following table summarizes estimated exposures and the risk calculated based upon the EPA calculated risk at lower doses, as proposed in EPA's ambient water quality criteria document (45 FR 79320).

Group	Blood Levels of PCBs	Estimated Exposure	EPA Calculated Risks
Highly Exposed Capacitor Workers	≥1,000 ppb	2.0 mg PCB/day	25.0
Average Capacitor Worker	500 ppb	.84 mg PCB/day	10.0
Michigan Fish-Eater	70 ppb	.12 mg PCB/day	1.4
Michigan Control Population	20 ppb	.035 mg PCB/day	.5
General Population	2 ppb	.0035 mg PCB/day	.05

The chances of dying from liver cancer can be expressed in several ways. But simply speaking, there will be about 2,000,000 deaths in 1981. Of these, it is estimated that 9,400 will be from liver cancer. Therefore, 9,400/2,000,000 equals a .47% chance that those dying this year will be dying from liver cancer. Rounding off these estimates to a .5% chance for the purposes of our discussion, and using these comparisons, thus leads to the conclusion that for the general population, about 10% of the liver cancer in the United States would be due to PCB exposures; that the Michigan control population would expect a 100% increase in liver cancer incidence, that the Michigan fish-eaters would have a three-fold increase, and that the average capacitor worker would have a 20-fold increase, while the highly exposed worker would have a 25% incidence or a 50-fold increase. There is no human evidence to support any of this. The EPA's proposed water criteria standard is from 10 to 100 times higher than can be justified on the basis of recently published human experience, and from 10 to 20 times higher than can be concluded on the basis of the negative findings from the human experience.

It is our conclusion that commercial PCBs do not represent a significant health hazard. However, the presence and extent of contaminants (e.g., PCDFs) may alter the health concerns. Therefore, it is essential to determine the level of contaminants present when dealing with PCB wastes before applying the conclusions of this report to waste mixtures.