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

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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 $P\text{-}450_I$ in rabbit lung, rather than an increase, with no alteration in $P\text{-}450_{II}$ 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 manifestetions 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 observations, 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 relatively 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 metabolism and elimination rates are much faster. Clearly, species specificity operates 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 enzymes 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 in 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 unsubstituted 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 syatems 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 embroyonic 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 tests. 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* Ar Control	Ar 1254	Ar + 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 dona, 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 (4ClMB) 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 4ClMB 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 dermetological 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 wes above the normal range. Deteiled 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 discontinuetion 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

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. Glucosuria 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 records 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 were 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 (57Bl/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 subatituted 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. Hutzinger 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 lending 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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