# Toxicological Studies of Three Polychlorinated Biphenyls

Otis E. Fancher[1], M. L. Keplinger, E. P. Wheeler[2],

and J. C. Calandra

Industrial BIO-TEST Laboratories, Inc.

Northbrook, Illinois 60062

Running Title: Toxicological Studies of 3 PCB's

[1] Present Address: 624 N. Abrego Drive, Green Valley, Ariz. 85614, to whom proofs are to be sent.

[2] Monsanto Company, St. Louis, Missouri,

MONS 208571

INDEX TERMS

Aroclor 1242

Aroclor 1254

Aroclor 1260

Polychlorinated biphenyls

Ecological effects of

Toxicology of

MONS 208572

ABSTRACT

Toxicological Studies of Three Polychlorinated Biphenyls, Fancher, Otis. E., Koplinger M. L., Wheeler, E. P., and Calandra. J. C. (0000). Toxicol. Appl. Pharmacol 00. 00 - 00.

The toxicology and reported ecological effects of the polychlorinated biphenyls are selectively reviewed as a background for toxicological studies with Aroclors 1242, 1254 and 1260 which are reported in papers which follow.

MONS 208573

# Toxicological Studies of Three Polychlorinated Biphenyls

Otis E. Fancher[1], M. L. Keplinger, E. P. Wheeler[2], and J. C. Calandra

Industrial BIO-TEST Laboritories, Inc.

Northbrook, Illinois 60062

Toxicological Studies of Three Polychlorinated Biphenyls. Fancher, Otis E., Keplinger, M. L., Wheeler, E. P. and Calandra, J. C. (0000). Toxicol. Appl. Pharmacol, 00, 00 - 00.

The toxicology and reported ecological effects of the polychlorinated biphenyls are selectively reviewed as a background for toxicological studies with Aroclors 1242, 1254 and 1260 which are reported in papers which follow.

Aroclors 1242, 1254 and 1260 are members of the family of polychlorinated biphenyls (PCB's), the last two digits of the numerical designations indicating the chlorine content in percent. Since the chlorine atoms are randomly distributed among the 10 ring positions available for substitution, each material is a mixture of isomers.

The PCB's are highly stable toward hydrolysis under either acidic or alkaline conditions and toward heat and oxidation. They do not support combustion, have high resistivity and dielectric strength, are non-drying, and retain adhesiveness and thermoplasticity under conditions of repeated stress.

These properties have led to extensive use of the materials for a variety of dielectric applications, as insulation impregnants, as

MONS 208574

diffusion pump oils and liquid sealants, as hydaulic fluids and heat exchange media, as plasticizers for specialty inks, resins, rubbers, and paints and as adjuvants in pesticide formulations. Commercialization of the PCB's began in the early 1930's.

Questions were early raised regarding the systemic toxicity and dermatological effects which might be associated with industrial exposure to these materials. Jones and Alden (1936) reported a high incidence of acneform dermatitis among employees engaged in the manufacture of PCB's. Drinker et al. (1937) exposed rats to PCB's at a chamber concentration of 1 $mg/m^3$ for 16 weeks and produced liver damage. On the basis of these results and some additional observations, Drinker (1939) suggested a permissible concentration of PCB's in the air of workrooms of 0.5 $mg/m^3$. Bennett et al. (1938) reported morphological changes in the liver of rats fed or injected with chlorinated biphenyls.

Greenberg et al. (1939) attributed the death of 3 young workers to exposure to chlorinated napthalenes and biphenyls. Adams et al. (1941) demonstrated chloracneform responses in rabbits exposed to materials, including chlorinated biphenyls, which had been reported to cause acneform dermatitis in man. Miller (1944) reported on animal studies with a trichlorobiphenyl administered topically, by subcutaneous injection or by feeding to guinea pigs, rats and rabbits. The conspicuous pathologic finding consisted of liver damage characterized by degeneration of the centrilobular cells and by fatty degeneration. The greatest liver damage

was found in the guinea pig and the least in the rat.

During succeeding years little information was reported concerning the toxicology of the PCB's until McLaughlin et al. (1963) reported Aroclor 1242 to be toxic in the chick embryo test at levels of 10 or 25 mg/egg. Hatched chicks, which later died, showed some weak deformities and growth retardation.

Questions have been raised as to the causative toxic constituent of the PCB's. Vos et al. (1970) studied three samples of PCB's containing 60 percent chlorine: Clophen A60 (Bayer AG, Leverkusen, Ger.); Phenoclor DP6 (Prodelec S. A., France); Aroclor 1260 (Monsanto Co., St. Louis, Mo.). Using the chick embryo test method and a dose level of 3.5 mg (in 0.05 ml of ethanol) /egg, it was demonstrated that Clophen A60 and Phenoclor DP6 were more toxic than Aroclor 1260, causing higher mortality and producing subcutaneous and abdominal edema, centrilobular liver necrosis, and hydropericardium in almost all chicks. Only an occasional hydropericardium was observed following treatment with Aroclor 1260.

Tetra and pentachlorodibenzofurans were analytically demonstrated to be present in Clophen A60 and Phenoclor DP6 but were not detected in Aroclor 1260. The chlorinated dibenzofurans are known to be potent chloracnegens, being somewhat weaker in this regard than the chlorinated dibenzodioxins (Bauer et al., 1961).

The PCB's remained inconspicuous as possible environmental contaminants until Jensen (1966) assigned a series hitherto unidentified

peaks in chromatograms of wildlife residues to these materials, Holmes et al. (1967) confirmed the presence of PCB's in the eggs and livers of birds in Britain, occurring most frequently and in the highest concentrations in samples from terrestrial predators and marine feeders. Hickey and Anderson (1968) related the drastic decline in populations of peregrine falcons, bald eagles and ospreys to increases in chlorinated hydrocarbon residues. Reproductive failures were associated with effects on calcium metabolism and decreases in eggshell thickness. It was suggested that derangement of calcium metabolism might result from induction by the chorinated hydrocarbons of the microsomal enzymes involved in the metabolism of steroidal hormones. The samples in this study were analyzed for chlorinated hydrocarbons but not for PCB's.

Risebrough et al. (1968) determined that PCB residues were present in significant quantities in samples from a variety of raptorial and fish-eating birds and also related population declines to derangements in calcium and steroid metabolism induced by the chlorinated hydrocarbons. They showed that a PCB (Aroclor 1262) was more active than DDE or DDT in increasing polar metabolites of oestradiol by pigeon liver homogenates.

Koeman et al. (1969) reported the presence of PCB's in fish and birds from the Rhine and the Netherlands coastal area. They observed that the lower chlorinated PCB's occurred more frequently in fish than in sea birds. This suggested that the lower chlorinated compounds are

metabolizable and less persistent than compounds of higher chlorine content.

Many additional reports relating to the ecological impact of the PCB's have continued to appear. Most of these include literature reviews which need not be redetailed here. Pertinent references include Pressl et al. (1970), Duke et al. (1970), Peakall and Lincer (1970), Heath et al. (1970), Hansen et al. (1971) and Edwards (1971).

Biological magnification of PCB concentrations by an early element of the food chain was shown to occur by Keil et al. (1971) who demonstrated magnification of Aroclor 1242 concentrations by a factor of 900 to 1000 by the marine diatom, Cylindrotheca closterium, which can also concentrate and metabolize DDT. A concentration of 0.1 ppm in water sharply retarded growth and RNA synthesis.

Biochemical studies relating to possible mechanisms of action of the PCB's have appeared. Using the 18-hour glycogen response of the immature rat uterus as the assay procedure, Bitman and Cecil (1970) demonstrated a low order of estrogenic activity for the lower chlorinated Aroclors. The minimum effective dose of Aroclors containing 48 percent chlorine or less was approximately twice that of p,p'-DDT whose activity in turn was less than that of diethylstilbesterol by a factor of $4 \times 10^4$. No activity was demonstrated for Aroclors containing 54 percent chlorine or more.

Villaneuve et al. (1971) demonstrated induction of carboxylester-

ase, aniline hydroxylase and aminopyrine N-demethylase in homogenates of liver from female rabbits treated through the gestation period with Aroclor 1254 at a level of 10 mg/kg/day. Reproduction was not affected, however, and no enzyme induction was demonstrated with Aroclor 1221.

Vos and Koeman (1970) found that PCB could induce chemical porphyria in chickens, Japanese quail and rats and Vos et al. (1971) later showed that porphyria in Japanese quail was associated with induction of liver mitochondrial ALA-synthetase, leading to increased production of $\delta$-aminolevulinic acid, an obligatory precursor for porphyrin synthesis. This induction was brought about by Aroclor 1260 in which chlorinated dibenzofurans were not detected.

Westoo et al. (1970) reported the occurrence of PCB residues in all of 22 samples of human milk analyzed and Biros et al. (1970) demonstrated the presence of PCB residues in human adipose tissue. Nishizumi (1970) cited the occurrence of poisoning among the general population of western Japan as a result of contamination of rice bran oil by leakage of a chlorinated biphenyl (Kanechlor 400) used as a heat exchange medium in the manufacturing process.

In order to define the toxicological profile of the PCB's in terms of systems which have been traditionally used for assessment of the hazard of materials, the studies reported in papers which follow were conducted. Based on the permissible ambient concentration of the PCB's of 0.5 mg/m$^3$

suggested by Drinker (1939) and assuming that total absorption of the PCB's contained in an estimated daily inhalation volume of 15 $m^3$ for man, the permissible human exposure would correspond to 7.5 mg/day or to approximately 0.1 mg/kg/day. A high dietary level of 100 ppm, or approximately 10 mg/kg for rats, was selected for all chronic studies since this would correspond to a 100-fold safety factor for man.

MONS 208580

FOOTNOTES

1 Aroclors 1242, 1254 and 1260. Monsanto Company, St. Louis, Missouri.

2 Present Address: 624 North Abrego Drive, Green Valley, Arizona 85614.

3 Monsanto Company, St. Louis, Missouri, 63166, 800 North Lindbergh Boulevard,

## REFERENCES


Adams, E.M., Irish D. D., Spencer, H. C. and Rowe, V. K. (1941). The response of rabbit skin to compounds reported to have caused acneform dermatitis. Ind. Med. 10, Ind. Hyg. Sect. 2. 1-4.

Bauer, H., Schulz, K. H., and Spiegelberg, U. (1961). Occupational intoxications in manufacturing chlorophenol compounds. Arch. Gewerbepath. Gewerbehyg. 18, 538-555.

Bennett, G. A., Drinker, C. K. and Warren, M. F. (1938). Morphological changes in the livers of rats resulting from exposure to certain chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 20, 97-123.

Biros, F. J., Walker, A. C., and Medberry, A. (1970). Polychlorinated biphenyls in human adipose tissue. Bull. Env. Contam. Toxicol. 5, 317-323.

Bitman, J., and Cecil, H. C. (1970). Estrogenic activity of DDT analogs and polychlorinated biphenyls. J. Agr. Food Chem. 18, 1108-1112.

Drinker, C. K., Warren, M. F. and Bennett, G. A. (1937). The problem of possible systemic effects from certain chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 19, 283-311.

Drinker, C. K. (1939). Further observations on the possible systemic toxicity of certain chlorinated hydrocarbons with suggestions for permissible concentrations in the air of workrooms. J. Ind. Hyg. Toxicol. 21. 155-159.

Duke, T. W., Lowe, J. I., and Wilson, A. J., Jr. (1970). A polychlorinated biphenyl (Aroclor 1254) in the water, sediment and biota of Escambia Bay, Fla. Bull. Environ. Contam. and Toxicol. 5, 171-180.

Edwards, R. (1971). The polychlorinated biphenyls, their occurrence and significance; a review, Chem. and Ind. 1340-1348.

Greenberg, L., Mayers, M. R., and Smith, A. R. (1939). The systemic effects resulting from exposure to certain chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 21, 29-38.

Hansen, D. J., Parrish, P. R., Lowe, J. I., Wilson, A. J., Jr., and Wilson, P. D. (1971). Chronic toxicity, uptake and retention of Aroclor 1254 in two estuarine fishes. Bull. Env. Contam. Toxicol. 6, 113-119.

Heath, R. G., Spann, J. W. Kreitzer, J. F., and Vance, C. (1970). Effects of polychlorinated biphenyls on birds. Proc. XV Int. Ornithol. Cong.

Hickey, J. J., and Anderson, D. W. (1968). Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 162, 271-273.

Holmes, D. C., Simmons, J. H., and Tatton, J. O'G. (1967). Chlorinated hydrocarbons in British wildlife. Nature 216, 227-229.

Jensen, S. (1966). Report of a new chemical hazard. New Scientist 32, 612.

MONS 208583

Jones, J. W., and Alden, H. S. (1936). An acneform dermatergosis. Arch. Dermatol. Syphilol. 33, 1022-1034.

Keil, J. E., Priester, L. E., and Sandifer, S. H. (1971). Polychlorinated biphenyl (Aroclor 1242): effects of uptake on growth, nucleic acids and chlorophyll of a marine diatom. Bull. Env. Contam. Toxicol. 6, 156-159.

Koeman, J. H., and Ten Noever De Brauw, M. C. (1969). Chlorinated biphenyls in fish, mussels and birds from the river Rhine and the Netherlands coastal area. Nature 221, 1126-1128.

McLaughlin, J., Jr., Marliac, J. P., Verrett, M. J., Mutchler, M. K., and Fitzhugh, O. G. (1963). The injection of chemicals into the yolk sac of fertile eggs prior to incubation as a toxicity test. Toxicol. Appl. Pharmacol. 5, 760-771.

Miller, J. W. (1944). Pathologic changes in animals exposed to a commercial chlorinated diphenyl. Public Health Reports 59, 1085-1093.

Nishizumi, M. (1970). Light and electron microscope study of chlorobiphenyl poisoning. Arch Environ. Health 21, 620-631.

Peakall, D. B., and Lincer, J. L. (1970). Polychlorinated biphenyls-another long life widespread chemical in the environment. Bio. Science 20, 958-964.

Presst, Ian, Jeffries, D. J., and Moore, N. W. (1970). Polychlorinated biphenyls in wild birds in Britain and their avian toxicity. Environ. Pollut. 1, 3-26.

MONS 208584

Risebrough, R. W., Peakall, D. B., Herman, S. G., and Kiruen, M. N. (1968). Polychlorinated biphenyls in the global ecosystem. Nature 220, 1098-1102.

Villeneuve, D. C., Grant, D. L., Phillips, W. E. J., Clark, M. L., and Clegg, D. J. (1971). Effects of PCB administration on microsomal enzyme activity in pregnant rabbits. Bull. Env. Contom. Toxicol. 6, 120-128.

Vos, J. G., Koeman, J. H., and Van Der Maas, H. L. (1970). Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated napthalene in two commercial polychlorinated biphenyls. Fd. Cosmet. Toxicol. 8, 625-633.

Vos, J. G., and Koeman, J. H. (1970). Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis and tissue residues. Toxicol. Appl. Pharmacol. 17, 656-668.

Vos, J. G., Strik, J. J. T. W., Van Holsteijn, C. W. M., and Pennings, J. H. (1971). Polychlorinated biphenyls as inducers of hepatic porphyria in Japanese quail, with special reference to δ-amino-levulinic acid synthetase activity, fluorescence and residues in the liver. Toxicol. Appl. Pharmacol. 20, 232-240.

Westoo, G., Noren, K., and Andersson, M. (1970). Levels of chlorinated pesticides and polychlorinated biphenyls in margarine, vegetable oils, and some foods of animal origin on the Swedish market in 1967-1969. Var Foeda 22, 9-31.

MONS 208585