SUBSTITUTE CHEMICAL PROGRAM

INITIAL SCIENTIFIC REVIEW OF PCNB

APRIL 1976

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
CRITERIA AND EVALUATION DIVISION
WASHINGTON, D.C. 20460

EPA-540/1-75-016

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PREFACE

The Alternative (Substitute) Chemicals Program was initiated under Public Law 93-135 of October 24, 1973, to "provide research on, and testing of, substitute chemicals." The legislative intent is to avoid the use of substitute chemicals that would be even more deleterious to man and his environment than a pesticide that is cancelled or suspended for causing "unreasonable adverse effects to man or his environment." The major objective of the program is to determine whether potential substitute chemicals are suitable replacements for cancelled or suspended pesticides or for pesticides that are under litigation or are candidates for Rebuttable Presumption Against Registration (RPAR).

The review of the substitute chemical considers its chemistry, toxicology, and pharmacology as well as its use patterns, efficacy, and environmental fate and movement. EPA realizes that, even though a compound is registered, it still may not be a practical substitute for certain uses of a problem pesticide. Therefore, the utilitarian value of the "substitute" must be established by reviewing its biological and economic data.

The reviews of substitute chemicals are carried out in two phases. Phase I Initial Scientific Review evaluates the "safety and efficacy" of the substitute chemical based on data readily accessible at the present time. The Phase II Integrated Use Analysis examines the effects of possible regulatory action against a hazardous pesticide for each of its major and critical uses. The examination considers the suitable substitutes in conjunction with alternative agricultural management practices. Current and projected environmental, health, and economic impacts are also evaluated.

This report contains the Phase I Initial Scientific Review of PCNB. PCNB was identified as a registered substitute chemical for certain problematic uses of ethylenebisdithiocarbamate (EBDC) fungicides which are under EPA review for suspected adverse effects. The report covers all uses of PCNB and is intended to be adaptable to future needs. Should PCNB be identified as a substitute for a problem pesticide other than the EBDC fungicides, the review can be updated and made readily available for use. The data searches ended in January, 1976. The report summarizes rather than interprets scientific data reviewed during the course of the studies. Data from different sources is not correlated, nor are opinions presented on contradictory findings.

A team of EPA scientists in the Criteria and Evaluation Division of the Office of Pesticide Programs coordinated the review; the team leader provided guidance and direction and technically reviewed information retrieved during the course of the study. The following EPA scientists comprised the review team: E. Neil Pelletier, Ph.D. (Team Leader); Padma Datta, Ph.D. and Stewart Colten (Chemistry); William Burnam and Elsie Kelly (Pharmacology and Toxicology); John Bowser and Thomas Frietag (Fate and Significance in the Environment); E. Neil Pelletier, Ph.D. (Registered Uses); and Gary Ballard and Jeff Conopask, Ph. D. (Economics).

Data research, abstracting, and collection were primarily performed by Arthur D. Little, Inc. (ADL), Cambridge, Mass. (EPA Contract #68-01-2489) under the direction of Joan B. Berkowitz, Ph.D. The following A. D. Little scientists were principal contributors to the report: Janet Stevens; Douglas Arnold, Ph.D.; Muriel Goyer; Donald Senechal; Joan Harrison; Robert Ludwig; and John Neumeyer, Ph.D. (Consultant from Northeastern University).

The scientific staffs of EPA's Environmental Research Laboratories reviewed draft copies of the report. Comments and supplemental material provided by the following laboratories were greatly appreciated and have been incorporated into this report: Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida and Southeast Environmental Research Laboratory, Athens, Georgia. The Olin Corporation, a manufacturer of PCNB, also reviewed the draft of this report and made certain comments and additions.

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PART I. SUMMARY

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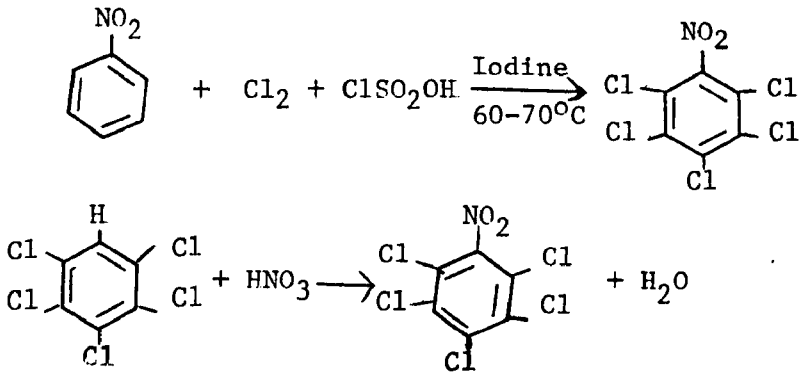
This section is a brief summary of the Initial Scientific Review of PCNB. The section summarizes rather than interprets data reviewed.

Production and Use

Pentachloronitrobenzene (PCNB) is registered primarily for use as a soil fungicide and as a seed treatment. In the United States, PCNB is currently manufactured by Olin Corporation in McIntosh, Alabama, under the trade name Terraclor®. The following are common international names: quintozene (International Standards Organization and British Standards Organization), PKhNB (USSR), and terrachlor (Turkey). PCNB is manufactured abroad under various trade names: Avicol®, Botrilex®, Brassicol®, Folosan®, Tilcarex®, Tri-PCNB, and Tritisan®. Primary foreign manufacturers are as follows: Farbwerke Hoechst AG (West Germany) and Fabriek van Chemische Produken Vondel ingenplicet N.V. (Netherlands).

PCNB was developed by I. G. Farbenindustrie and introduced in the late 1930's. The generic term PCNB refers to several products which vary in contents and quantities of impurities according to the manufacturing source. Domestically produced PCNB is approximately 97.8% PCNB, 1.8% hexachlorobenzene (HCB), 0.4% 2,3,4,5-tetrachloronitrobenzene (2,3,4,5-TCNB), and less than 0.1% pentachlorobenzene (PCB).

The fungicide is manufactured by a synthesis process involving the chlorination of nitrobenzene or the nitration of chlorinated benzenes (Olin method):



Various formulations of PCNB are commercially available, including emulsifiable concentrates, wettable powders, dusts and granulars.

Domestically, PCNB is primarily used on cotton (77%) and on peanuts (19%); there are no current industrial uses. Geographic usage distribution is mainly concentrated west of the Mississippi River. Surveys can account for only approximately 295,000 lb of PCNB used as a soil fungicide and 7,000 lb used as a seed treatment.

Chemistry and Methodology

PCNB has the form of colorless crystalline needles when chemically pure. The melting point is 146°C and the density is 1.718. The technical material has the form of pale yellow crystals with a melting point of 142 to 145°C. Technical PCNB is 98 to 99% pure (Olin method). It is practically insoluble in water. PCNB is soluble in carbon disulfide, benzene, and chloroform and is soluble to about 2 mg/l in ethyl alcohol at 25°C. It has a vapor pressure of 11.3×10^{-5}mm Hg at 25°C. Typical impurities of PCNB are HCB and 2,3,4,5-TCNB. PCNB is highly stable in soil and compatible with other pesticides at pH 7 or less. Analytical methods available for PCNB include spectrophotometry, polarography and gas chromatography. The gas chromatographic method is preferable because it can separately detect PCNB, its impurities and important metabolites.

Pharmacology and Toxicology

Toxicological studies were performed with technical PCNB containing HCB and 2,3,4,5-TCNB as impurities. The only studies in which the test material was analyzed showed its composition to be 97.8% PCNB, 1.8% HCB and 0.4% 2,3,4,5-TCNB (Olin technical PCNB).

Metabolism - The metabolism of PCNB has been studied in rats, dogs, cows, and rabbits. Pentachloroaniline (PCA) and methyl pentachlorophenyl sulfide (MPCPS) are the major metabolites of PCNB in both plants and animals. Absorption of PCNB varied from 50% in rabbits to 80% in dogs; it was rarely detected in body tissues. Tissue retention of PCNB metabolites and impurities is found primarily in body fat with minimal concentrations found in muscle. The impurity, HCB, is the major product stored in animal fat.

Toxicity - Oral LD_{50} values for PCNB include 2.5 g/kg for dogs and 1.2 to 1.743 g/kg for rats. A dermal LD_{50} of 4.0 g/kg was found for rabbits. A potentiation study of PCNB with Terrazole® administered to rats showed only an additive effect. A 3-month feeding study with rats showed significant increases in liver-to-body weight ratios at all dose levels, except in the females given 63.5 mg/kg. The body weights of males and females fed 5.0 g/kg and males fed 2.5 g/kg were decreased significantly. Another 90-day study of rats fed 0, 1,000, 5,000, and 10,000 ppm PCNB in the diet showed that rats given the 5,000-ppm diet grew slightly less than controls and the 10,000-ppm group grew markedly less than controls. A 1-yr feeding study of dogs given diets containing 25, 200, or 1,000 ppm PCNB showed no significant growth inhibition, hematological effects, or histopathology.

Rats fed diets containing 0, 25, 100, 300, 1,000, or 2,500 ppm PCNB for 2 yr had suppressed growth rates at dose levels of 100 ppm or higher among females and 2,500 ppm in males.

A 2-yr feeding study of dogs given diets containing 0, 5, 30, 180, and 1,080 ppm showed no effects at the levels of 0, 5, and 30 ppm. Dogs fed 180 ppm had a minimum degree of cholestatic hepatosis with secondary bile nephrosis. The group fed 1,080 ppm had increased liver weight and serum alkaline phosphatase levels as well as a moderate degree of cholestatic hepatosis with secondary bile nephrosis.

Reproductive Effects - A 3-generation study with groups of rats fed diets containing 0, 5, 50, or 500 ppm (Olin technical PCNB) showed no significant effects on fertility, gestation, viability, lactation, rats born per litter, or rats weaned per litter and their average weaning weights.

Teratogenicity - Teratogenic effects were produced when 500 mg/kg PCNB (containing 11% HCB) was given orally to C57BL/6 mice from day 7 through day 11 of gestation. Eighty percent of the litters had inhibited kidney formation.

In a screening study using C57BL/6 mice, significant increases in malformations (cystic kidneys) were observed when 464 mg/kg of PCNB was administered from days 6 to 14 and 6 to 10 of gestation. No significant effects were noted at 215 mg/kg. The PCNB used in this study contained 11% HCB.

PCNB (Olin Product) was administered to pregnant rats by intubation on days 6 to 15 of gestation at dosages from 100 to 1,563 ppm. Fetuses were examined for gross malformations. No significant effects were observed at any dose level on the number of corpora lutea, the position and number of dead or resorbed fetuses, or the fetal weights and sex ratios. No significant skeletal or soft tissue anomalies were reported in the fetuses.

Oncogenic Studies - Two mouse hydrids (C57BL/6 x C3H/AnfF_1 and C57BL/6 x AKRF_1) from 7 to 28 days of age were administered 464 mg/kg PCNB (87% PCNB, 11% HCB, and 2% salts) by stomach tube. After weaning at 28 days, they were given a diet containing 1,206 ppm of the PCNB material for 18 months. Of the 14 male and 18 female mice (C57BL/6 x C3H/Anf strain), 2 males and 4 females had hepatomas, 2 males and 1 female had pulmonary tumors, and 2 males had lymphomas. Among the 16 male and 17 female mice (C57BL/6 x AKR strain), 10 males and one female had hepatomas, 1 female had pulmonary tumors, and 1 of each sex had lymphomas. Control mice of the C57BL/6 x C3H/Anf group had no tumors. In the C57BL/6 x AKR control group, 3 out of 18 males and 2 out of 15 females had tumors. The doses given in this experiment were considered to be maximum tolerated doses.

Outbred stock albino mice between 6 and 8 weeks of age had their clipped backs exposed to 0.2 ml PCNB and 3 isomers of TCNB twice a week for 12 weeks. The mice were also treated with croton oil for 20 weeks. These studies showed that all 4 compounds act as tumor initiators in mouse skin when croton oil is used as the promoting agent. The investigators considered that their experiments gave no evidence that the chloronitrobenzenes induced tumors in the absence of a promoting agent.

Mutagenicity - PCNB was found to be mutagenic in the hcr$^-$ strain of *Escherichia coli* B/r ochre but not in another *E. coli* strain. In the host-mediated assay in mice, no significant increase in mutation rates in *Salmonella typhimurium* and *Serratia morcescens* was observed after subcutaneous injection of PCNB. The compound also gave negative results in spot tests.

Human Toxicity and Epidemiology - No accidents involving the use of PCNB have been reported to the Pesticide Episode Reporting System (PERS).

Among the subjects tested, patch tests with a 75% wettable powder on the volar surface of the right forearm showed no evidence of irritation. Two weeks later the same tests were done on the left arms of the subjects. Forty-six of the 50 subjects showed no signs of irritation after 48 hr. Three of the 4 subjects showed erythema, edema and small vesicle formation with marked itching; the fourth subject had only erythema and itching. Nine of the 46 subjects who were negative when the second patch was removed developed delayed reactions occurring from 8 hr to several days after treatment. In 2 of the cases, the reaction developed at the site of the first and second treatment applications. The reactions peaked during the first few days after appearance and subsided in time with some scaling of skin.

Environmental Effects

PCNB studies focus on the short-term laboratory effects in the environment.

Fish - In static, acute toxicity bioassays using various PCNB formulations, bluegill (*Lepomis macrochirus*) had 96-hr median lethal concentration (LC_{50}) values ranging from 0.29 to 0.38 ppm. Rainbow trout (*Salmo gairdneri*) had a 96-hr LC_{50} value of 0.31 ppm.

Wildlife - In an 8-day dietary toxicity study using a 98% PCNB formulation, an LC_{50} value for bobwhite (*Colinus virginianus*) was determined as $>$ 5,000 ppm. The 8-day dietary LC_{50} value of the same PCNB formulation to mallard (*Anas platyrhynchos*) was $>$ 5,000 ppm. No field studies were found on the effects of PCNB on wildlife.

In a study with the PCNB impurity, hexachlorobenzene (HCB), Japanese quail (*Coturnix coturnix japonica*) were fed 0, 1, 5, 20, and 80 ppm HCB for 90 days. Birds fed 80 ppm HCB had tremors, liver damage, erythrophagocytosis in the spleen, ceroid granules in the kidney tubules, reduced reproduction and egg production, and some mortality. One ppm was considered to be the no-effect level.

Absorption and Degradation by Microorganisms and Plants - In the laboratory, mycelium of fungi accumulated a concentration of PCNB 7 times that of the surrounding soil. Some species of actinomycetes and fungi were found to be capable of degrading PCNB to PCA.

Effects on Soil Microorganisms - Actinomycetes are inhibited by PCNB and, to a lesser extent, PCA. Although total fungal populations in treated soils were not reported to vary substantially at recommended rates of application, changes in subpopulations such as increases in *Pythium* and *Fusarium* species have been observed. Soil respiration and nitrification were reportedly unaffected; nitrogen mineralization was observed to decrease in treated soil.

Environmental Transport Mechanism - No information was found on leaching of PCNB or its metabolites. Volatilization has been reported to be a major factor in loss of PCNB from soil, particularly from water-saturated soils.

Residues in Soil - Fate of PCNB in the soil has been studied by a number of investigators. Breakdown occurs more slowly in soils with high organic content and occurs more rapidly in water-saturated soils.

In laboratory studies the half-time for PCNB for various soil types has been calculated to range from 4.7 to 9.7 months. In discussions of their work, researchers theorize that under field conditions persistence would be even less when factors such as leaching, photodegradation, and plant uptake are considered.

Microbial activity apparently plays a significant role in the reduction of PCNB to its major soil metabolite, PCA, which is more persistent than PCNB. No quantitative data on the persistence of PCA has been reported.

Efficacy and Performance

PCNB is estimated to have been used as a soil fungicide in 1971 on approximately 2% of total U. S. cotton acreage and on approximately 17% of the cotton crop in California. The fungicide was also used as a soil fungicide on approximately 0.4% of the total U. S. peanut acreage.

The recommended dosage for use as a cotton soil fungicide is 1 to 2 lb/acre. PCNB can also be applied when mixed with the seed in the planter box at 0.6 to 1.0 lb/acre. When used either as a seed or soil treatment, PCNB has been demonstrated to increase seedling survival.

The recommended application rate on peanuts is 14 to 28 lb/acre. In tests which were reviewed, PCNB effectively controlled southern blight of peanuts as determined by increase in yield and quality.

PCNB is effective for control of wheat smut; control as high as 99% has been reported.

PART II. INITIAL SCIENTIFIC REVIEW

SUBPART A. Chemistry

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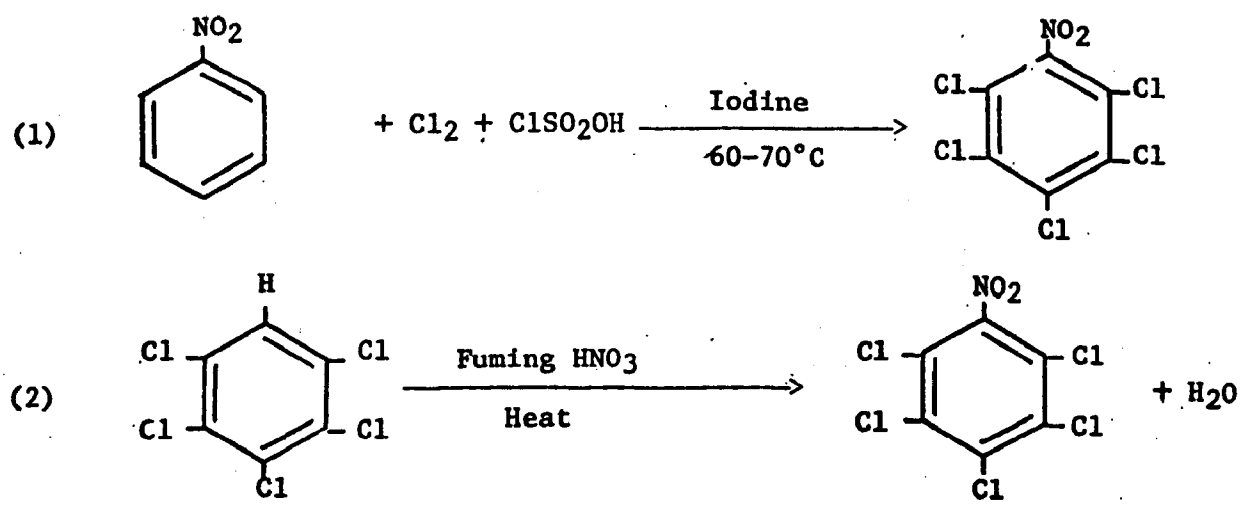
This section reviews available data on the chemistry of PCNB and its presence in foods. Nine subject areas have been examined: Synthesis and Production Technology; Nomenclature; Physical Properties; Analytical Methods; Composition and Formulation; Chemical Properties; Occurrence of Residues in Food and Feed Commodities; Acceptable Daily Intake; and Tolerances.

Synthesis and Production Technology

PCNB (pentachloronitrobenzene) was first synthesized by Jungfleisch in 1868 (Merck Index 1968). It was introduced as a fungicide in the late 1930's. In 1953 its effectiveness in control of wheat bunt was discovered. However, widespread use of the compound did not begin until its potential for control of certain soil-borne diseases was discovered after 1954.

PCNB can be prepared by a chlorination (1) or nitration (2) reaction (Olin method), illustrated as follows:



Both reactions are used commercially. Technical grade PCNB typically contains a number of impurities, such as hexachlorobenzene (HCB), 2,3,4,5-tetrachloronitrobenzene (TCNB), and pentachlorobenzene (PCB). The amounts of these impurities vary with the manufacturer. The domestic technical material (Olin) contains approximately 97.8% PCNB, 1.8% HCB, 0.4% TCNB and less than 0.1% PCB.

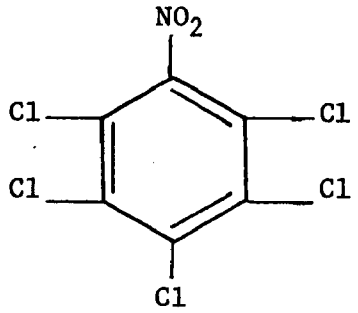
Nomenclature

Chemical Name: Pentachloronitrobenzene

Common Names : PCNB, quintozene (International Standards Organization, British Standards Institution), PKhNB (Soviet Union), terrachlor (Turkey)

Trade Names: Avicol®, Botrilex®, Brassicol®, Folosan®, Terraclor®, Tilcarex®, Tri-PCNB®, and Tritisan®

Structural Formula:



Pesticide Class: Fungicide

Physical Properties

State: Solid

Color: Pale yellow to white, depending on purity

Odor: Musty

Molecular Weight: 295.4

Melting Point: 142° - 146° C

Boiling Point: 328° C at 760 mm Hg (some decomposition occurs)

Vapor Pressure: 1.61×10^{-5} mm Hg at 10° C
5.0×10^{-5} mm Hg at 20° C
11.3×10^{-5} mm Hg at 25° C

Density: 1.718 at 25° C

Solubility: Freely soluble in carbon disulfide, benzene, chloroform, ketones, and aromatic and chlorinated hydrocarbons; slightly soluble in alkanols; water, 0.44 mg/l at 20° C; ethanol, 2 mg/l at 25° C.

Analytical Methods

Several methods are currently available for analyzing the formulations and residues of PCNB. The selection of a method depends on the purpose and complexity of the objective.

Formulation Analysis - Hanks and Engdohl (1972) reported a method for analyzing PCNB in fertilizers. PCNB is extracted with chloroform and compared to an internal standard (aldrin) after gas chromatography on an OV-17/QF-1 column with flame ionization detection.

Other methods of analyzing PCNB include the Association of Official Analytical Chemists (AOAC) Parr Bomb-boric anhydride method, the AOAC sodium biphenyl reduction method (AOAC 1975), and the total chloride method of Beckman et al. (1958).

Residue Analysis - Analytical methods for residues of PCNB include the colorimetric, polarographic, and gas chromatographic. The first 2 methods require the presence of a nitro group and, therefore, are specific for PCNB, metabolites, and impurities that have a nitro group. The gas chromatographic method, on the other hand, is capable of detecting PCNB and HCB, PCB, PCA, MPCPS and 2,3,4,5-TCNB (see Figure 1).

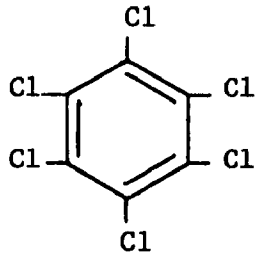
Colorimetric Method - This method determines only PCNB; it does not detect impurities or metabolites. The Ackerman method, as improved (Ackerman 1963), is applicable for PCNB in the 0 to 5 ppm range. It is, however, the slowest of the 3 analytical methods. There may also be interference from tetrachloronitrobenzene. The method, as originally developed by Ackerman, is described in the Pesticide Analytical Manual (PAM, Vol II 1973).

In this method, the residue is hydrolyzed with hot ethanolic KOH. Acidification produces nitrous acid which then reacts with procaine hydrochloride forming a diazonium compound, which couples with 1-naphthylamine forming an azo dye. The absorbance of the dye solution is measured at 525 mm (Ackerman et al. 1958).

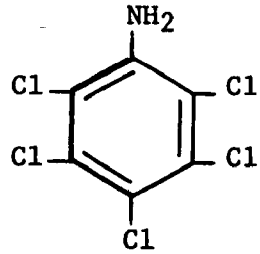
Polarographic Method - This is the most rapid method due to less stringent cleanup requirements. Since it is specifically for the aromatic nitro group which is reduced in an acetic acid-sodium acetate electrolytic solution (Klein and Gajan 1961), the method does not measure impurities and metabolites unless they have a nitro group.

Gas Chromatographic Methods - PCNB can be determined microcoulometrically after pyrolysis to the chloride using instrumentation described by Coulson et al. (1960). A lower limit of detection of 0.01 ppm can be achieved using the gas liquid chromatography (GLC) column described by Burke and Holswade (1964).

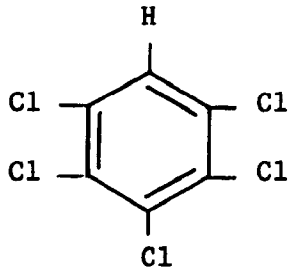
Electron capture gas chromatography is also used to determine PCNB after cleanup of the sample extract on a silicic acid column (Methatta et al. 1967). A recent modification of the method described by Kuchar et al. (1969) can also be used. This latter method has been used in support of PCNB tolerances.



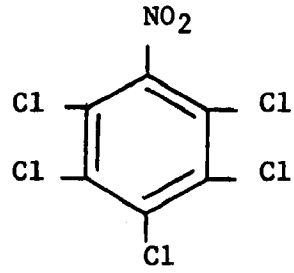
hexachlorobenzene
(HCB)



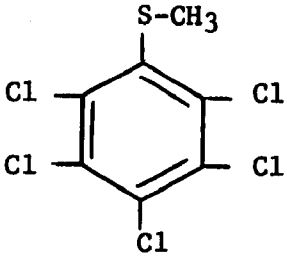
2,3,4,5,6-pentachloroaniline
(PCA)



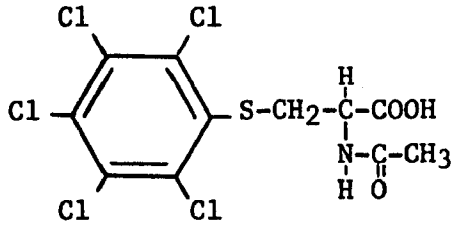
2,3,4,5,6-pentachlorobenzene
(PCB)



2,3,4,5-tetrachloronitrobenzene
(2,3,4,5-TCNB)



S-methyl pentachlorophenyl sulfide
(MPCPS)



N-acetyl-S-pentachlorophenyl-L-cysteine

Figure 1. Chemical structures of PCNB impurities and various PCNB metabolites

The Food and Drug Administration (FDA) multi-residue methods 211.1/231.1 and 212.1/232.1 are used for fatty and non-fatty foods. Recovery of PCNB from Florisis cleanup columns is essentially complete (PAM, Vol. 1 1973).

Confirmatory Methods - Thin layer chromatography (TLC) is the generally accepted method for confirmation of PCNB (Gorbach and Wagner 1967; PAM, Vol. 1 1973).

Composition and Formulation

Typical formulations of PCNB are available as wettable powders, dusts, emulsifiable concentrates, granulars, and combination products (see Table 1).

Chemical Properties

Crosby and Hamadmad (1971) reported the formulation of 2,3,4,5-tetrachloronitrobenzene and 2,3,4,6-tetrachloronitrobenzene as a result of irradiation of PCNB inorganic solvents with ultraviolet light at 253.7 nm.

Occurrence of Residues in Food and Feed Commodities

FDA residue studies from 1964 to 1969 found PCNB residues in 0.7% of the leaf and stem type vegetables sampled. Residue levels ranged from 0.005 ppm to .42 ppm, with an average of 0.01 ppm. No residues were found in total diet samples, such as ready-to-eat foods (Duggan et al. 1971). Additional residue studies have been published by the World Health Organization (WHO 1974).

Acceptable Daily Intake

The acceptable daily intake (ADI) is defined as the daily intake which, during an entire lifetime, appears to be without appreciable risk on the basis of all known facts at the time of evaluation (Lu 1973). It is expressed in milligrams of the chemical per kilogram of body weight.

The Food and Agricultural Organization/World Health Organization (FAO/WHO) has not yet determined an ADI for PCNB.

Tolerances

Tolerances for PCNB are within the purview of tolerance procedures for pesticide chemicals established under the Food, Drug, and Cosmetic Act, as amended. Cottonseed is the only raw agricultural commodity for which a formal tolerance for PCNB has been established. All other tolerances are interim tolerances. Table 2 summarizes the current U.S. tolerances for PCNB.

Table 1. Formulations of PCNB marketed by Olin Corporation

Product	% Active ingredient
Terraclor® Dust Concentrate	80.0
Terraclor® 75% Wettable Powder	75.0
Terraclor® 40% Dust	40.0
Terraclor® 20% Dust	20.0
Terraclor® 10% Dust	10.0
Terraclor® 10% Granular	10.0
Terraclor® 2 lb Emulsifiable	24.0
Terra-Coat® LT-2	24.0
Terra-Coat® 2-LF	24.0
Turfcide® Emulsifiable	24.0
Turfcide® 10% Granular	10.0
Combination products	
Terraclor® 10% + Di-Syston® 1% Granular (PCNB 10%, disulfoton 1%)	
Terraclor Super-X® Granular (PCNB 10%, ETCMTD 1/ 2.5%)	
Terraclor® Super-X® with Di-Syston® Granular (PCNB 6.5%, ETCMTD 6.5% and disulfuton 1.63)	
Terraclor Super-X® with Thimet® Systemic Insecticide (PCNB 6.5%, ETCMTD 6.5% and phorate 1.63%)	
Terraclor Super-X® 20-5 Dust with graphite (PCNB 20.0%, ETCMTD 5.0%)	
Terraclor Super-X® Emulsifiable (PCNB 23.2%, ETCMTD 5.8%)	
Terraclor Super-X® with moly for soybeans (PCNB 10%, ETCMTD 2.5%)	
Terraclor Super-X® with graphite for soybeans (PCNB 10%, ETCMTD 2.5%)	
Terra-Coat® L-21 (PCNB 22.8% ETCMTD 11.4%)	
Terra-Coat® L-205 (PCNB 23.2%, ETCMTD 5.8%)	
Terra-Coat® SD-205 (PCNB 20.0%, ETCMTD 5.0%)	

1/ ETCMTD = 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole.

Source: Olin (1974).

Table 2. U.S. tolerances* for PCNB on raw agricultural commodities

Crop	ppm	Crop	ppm
Bananas	0.1	Cottonseed	0.1
Beans	0.1	Garlic	0.1
Broccoli	0.1	Peanuts	1.0
Brussel Sprouts	0.1	Peppers	0.1
Cabbage	0.1	Potatoes	0.1
Cauliflower	0.1	Tomatoes	0.1

* All tolerances except for cottonseed are interim tolerances.

Source: Code of Federal Regulations, Title 40, 180-219; 180-291, July 1, 1975.

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PART II. INITIAL SCIENTIFIC REVIEW

SUBPART B. PHARMACOLOGY AND TOXICOLOGY

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This section reviews pharmacological and toxicological data on PCNB. Acute, subacute, and chronic toxicity data is discussed for a number of species by various routes of administration. Data is presented on reproduction, metabolism, and on teratogenic, oncogenic and mutagenic effects. Data is also included on human toxicity and epidemiology. This section summarizes rather than interprets scientific data reviewed.

Acute, Subacute, and Chronic Toxicity

Acute Oral Toxicity - Rats - The oral LD_{50} for technical grade PCNB (Olin product) as a 10% solution in corn oil was found to be 1.71 ± 0.20 g/kg for males and 1.65 ± 0.17 g/kg for females (Finnegan et al. 1958). The oral LD_{50} of a 75% wettable powder commercial formulation (Olin product) administered as a 40% aqueous suspension to male rats was found to be greater than 12 g/kg (Borzelleca et al. 1971).

Since PCNB is used as a soil fungicide in combination with 5-ethoxy-3-(trichloromethyl)-1,2,4,-thiodiazole (Terrazole®, 95.2% purity; impurities not identified), the compound and PCNB (Olin product) were prepared for oral dosing as a 10% solution in corn oil. Male rats (CD strain, Charles River Laboratories) were fasted overnight prior to dosing by gastric intubation. According to Borzelleca et al., the results indicated that the toxic effects were additive and that there were no indications of potentiation.

Acute Oral Toxicity - Dogs - Groups of 10 mongrel dogs were administered PCNB (Olin product) via gavage as a 10% solution in warm corn oil. Doses up to 2.5 g/kg did not produce any deaths, although half of the dogs on this dosage level vomited after PCNB administration (Finnegan et al. 1958).

Acute Dermal Toxicity - Rabbits - Percutaneous toxicity was tested in male albino New Zealand rabbits weighing an average of 1.96 kg (Borzelleca et al. 1971). Animals with intact and abraded skin were used. The hair was removed from the trunk with an electric clipper. Abrasions made with a metal grid were sufficiently deep to penetrate the stratum corneum without drawing blood. The rabbits were restrained in stocks for 24 hr. PCNB (Olin product) was dissolved in dimethyl phthalate as a 30% solution and distributed over the trunk under the restraining device. Ten rabbits were used per dose level. Doses administered were 10.0 ml/kg and 13.3 ml/kg to rabbits with intact skin and 13.3 ml/kg to the rabbits with abraded skin. The rabbits were then observed for 14 days post-dosing, during which time there were no indications of toxicity or skin irritation.

Subacute Oral Toxicity - Rats - Five groups of 7 male and 7 female albino rats of weaning age were divided into separate groups for a 3-month feeding study. Each group was fed a diet containing 1 of 5 levels of PCNB (Olin Product): 0, 63.5, 635.0, 1,250, 2,500, or 5,000 ppm. The animals were weighed at weekly

intervals and a hematologic study was done upon termination of the study. Body weights for the males were significantly depressed at the 2,500 ppm level. The males and females on the 5,000 ppm level were killed at the end of 2 weeks because of continuous weight loss from the beginning of treatment. The kidney-to-body weight ratios showed a significant increase for male rats at the 1,250 and 2,500 ppm levels. No significant changes were noted in the kidneys of the female rats. No significant increases in the testes-to-body weight ratio were found. Significant increases in the liver-to-body weight ratio were found at all levels except in the females fed 63.5 ppm (Finnegan et al. 1958).

Rats (10 per sex per dose) were fed technical PCNB (purity and source unknown) in their diets at rates of 0, 1,000, 5,000 or 10,000 ppm for 90 days. The growth rate of rats fed 5,000 ppm PCNB was slightly less than that of controls. Rats on the 10,000 ppm had an even lower growth rate (FAO/WHO 1970).

Subacute Oral Toxicity - Dogs - Groups of 3 mongrel dogs were placed on diets containing either 25, 200, or 1,000 ppm PCNB for 1 yr. The PCNB (Olin product) was added as a dust formulation consisting of 20% PCNB, 77% Pyrax ABB (a pyrophyllite carrier) and 3% Armour "Sticker" (an adherence aid in field use). The dogs were weighed at weekly intervals and hematologic studies were made at the start, mid-point, and termination of the study. The PCNB did not inhibit growth or result in any significant hematological or histopathological changes (Finnegan et al. 1958).

Chronic Oral Toxicity - Rats - Finnegan et al. (1958) conducted a 2-yr study in which rats in groups of 10 males and 10 females were fed a diet containing 0, 25, 100, 300, 1,000 or 2,500 ppm PCNB. The PCNB (Olin product) was added to the diet as a dust formulation consisting of 20% PCNB, 77% Pyrax ABB, and 3% Armour "Sticker." The rats were housed individually and weighed weekly. They were given hematologic examinations during the eleventh and twenty-fourth months of the experiment.

Deaths during the 2-yr study did not correlate with dietary levels of PCNB. The body weight data indicated that the female rat showed a slight growth suppression at 100 ppm and above. PCNB accelerated the growth of male rats, especially at the lower level (25 ppm). Hematological values (not given) were reported to be within normal ranges. Histopathological changes, limited to occasional lung abscesses and the occurrence of fatty changes in liver, did not correlate with dietary levels of PCNB.

Chronic Toxicity Studies - Dogs - In a chronic toxicity study conducted by Farbwerke Hoechst AG (cited in FAO/WHO 1970), groups of 6 dogs, 3 males and 3 females, were fed diets containing 0, 500, 1,000 or 5,000 ppm of PCNB (purity not specified) for 2 yr. Liver changes occurred in all groups, the degree of damage was dose-related. The 5,000 ppm level produced fibrosis, narrowing of hepatic cells, thick leucocyte infiltration, and increased size of the periportal areas. At the 500 and 1,000 ppm levels, the changes were similar but to a lesser degree. The highest dose level also produced atrophy of bone marrow and reduced hematopoiesis.

In another study (Borzelleca et al. 1971), purebred beagles, approximately 4.5 months old (4 per sex per dose), were fed a diet containing PCNB (Olin product) at 0, 5, 30, 180 or 1,080 ppm for 2 yr. Hematocrit values showed a significant decrease at 18 months for males receiving dosages of 30 and 180 ppm. There was no significant change in males receiving a 1,080 ppm dosage. No parallel differences were found for hemoglobin. There were no dose-related effects on urine analysis, blood chemistry, mortality, body weight, food consumption or the estrous cycle.

A ratio of organ-to-body weight data showed a barely significant greater value for livers of the dogs on 1,080 ppm PCNB. The histologic examination of tissues from dogs sacrificed at 1 yr showed no treatment-related lesions. For dogs sacrificed at 2 yr, cholestatic hepatosis with secondary bile nephrosis was found in minimal degree in dogs fed 180 ppm and in moderate degree in dogs fed 1,080 ppm. Although these finding correlate with dosing, the authors considered the lesions reversible (Borzelleca et al. 1971).

Metabolism

Animals -

Distribution and Excretion - Rabbits - In a study conducted by Betts et al. (1955), 12 rabbits (unspecified size and strain) received either 1, 2, or 3 g of PCNB (recrystallized sample of Bayer Agri. Ltd.) via stomach tube. Urine and feces were collected for 72 hr. An average of 62% of the administered dose was found in the feces from the 2 g dose and was considered unabsorbed, although no differentiation was made between unabsorbed and biliary excretion. PCNB did not appear to undergo significant reduction to PCA in the alimentary canal. Another 11% of the dose could be accounted for as PCA in the urine, probably in part as a complex. N-acetyl-S-(pentachlorophenyl)-L-cysteine was also isolated from the urine at a level representing 14% of the initial dose. This product involves a direct replacement of the nitro group of PCNB by an acetyl cysteinyl group. Chemical analysis of urine after dosing with PCNB showed an increased level of glucuronic acid. Unlike findings with other chlorinated benzenes, the values did not return to near baseline levels over a 2 to 3 day period. This was interpreted to mean that the increase is not wholly associated with phenolic glucosiduronic acid conjugates. Pentachlorophenol, a predictable metabolite, was formed only in trace amounts.

To determine the metabolic pathways of 2,3,4,5-tetrachloronitrobenzene (2,3,4,5-TCNB), a PCNB impurity, 0.7 g was administered to rabbits as an aqueous suspension via stomach tube. This dose resulted in slight anorexia, sometimes lasting 2 days. The 2,3,4,5-TCNB was poorly absorbed; 33% was found in the feces after 48 hr. Furthermore, metabolic reduction of 2,3,4,5-TCNB in the rabbit resulted in 11% of the dose being excreted in the urine as 2,3,4,5-tetrachloroaniline (2,3,4,5-TCA). The excretion of the glucuronide (41%) and the ethereal sulfate (6%) of 6-amino-2,3,4,5-tetrachlorophenol indicated that ring hydroxylation had occurred (Bray et al. 1953).

Distribution and Storage - Dogs - Purebred beagles, approximately 4.5 months old (4 per sex per dose), were fed 0, 5, 30, 180 or 1,080 ppm PCNB in their diets for 2 yr (Borzelleca et al. 1971). An Olin technical grade PCNB (97.8%), containing 1.8% HCB, 0.4% 2,3,4,5-TCNB and 0.1% PCB, was used in the study. Toxicological aspects are reported in the subsection entitled Chronic Toxicity Studies - Dogs.

Kidney, brain, skeletal muscle, liver, spleen, fat, bile, blood, urine and feces samples were collected for analysis after 2 yr. Electron-capture gas chromatography was used to detect the presence of PCNB with its impurities (PCB and HCB) and its metabolites, PCA and MPCPS. Results for liver, kidney, fat, and feces at dose levels of 0, 5, 30, and 180 ppm dietary exposures are summarized in Table 3.

Table 3. Dog tissues and excreta concentrations of PCNB, its metabolites (PCA, MPCPS), and related impurities[a] after 2 yr on diet

Tissue or specimen	Level of PCNB in diet	PCNB	PCA	MPCPS	PCB	HCB
	0	ND	<.06	<.05	<.02	ND
Liver	5	ND	<.06	<.05	<.02	.039
	30	ND	<.06	<.10	<.02	.125
	180	ND	<.06	<.05	.54±.61	.74
	0	ND	<.08	<.03	.005	.005
Fat	5	ND	<.08	<.03	.093	.452
	30	ND	<.08	<.03	.163	1.11
	180	ND	<.08	ND[b]	.767	6.12
	0	ND	ND	ND	<.03	ND
Kidney	5	ND	ND	ND	<.03	.035
	30	ND	ND	ND	<.03	.099
	180	ND	.018	.021	<.10	.568
	0	.004	.005	.014	ND	<.1
Feces	5	.059	.188	.134	<.03	<.1
	30	.343	.450	.675	.03	<.1
	180	1.56	1.96	.192	<.03	<.1

[a](PCNB, 97.8%; HCB, 1.8%; PCB, 0.1%; 2,3,4,5-TCNB, 0.4%).
[b]2 of 3 samples.

Source: Adapted from J. F. Borzelleca et al. 1971. Toxicologic and metabolic studies on pentachloronitrobenzene. Toxicol. Appl. Pharmacol. 18:522-534. Reprinted by permission of the Society of Toxicology.

MPCPS, on the other hand, appeared to have an equal propensity to store in either the fat or skeletal muscle of the male rat. At the method's sensitivity level (sensitivity unstated), a 60-day withdrawal period from the treated diet resulted in non-detectable residues of PCA, MPCPS and PCB in adipose tissue (fat) at all exposure levels. Substantial residues of HCB remained at all levels of initial dietary exposure.

Distribution and Storage - Rats - Finnegan et al. (1958) conducted an initial study to determine the amount of PCNB stored in the fat of male and female albino rats. The diets contained 0, 63.5, 1,250 or 2,500 ppm of PCNB (Olin product) as a 25% dust. The animals were fed the diet for 3 months and then sacrificed. The results showed a dose-related increase in content of PCNB in adipose tissue. Subcutaneous and perirenal fat samples were taken for PCNB analysis using neutron activation of chlorine. The average concentration for the males of chlorine (expressed as PCNB) in ether extracts of fat was 42.4, 392, 571 and 1,151 μg/g of fat; for the female rats, it was 43.7, 470, 875 and 1,316 μg/g of fat for respective dietary levels.

However, as stated by Kuchar et al. (1969), the neutron activation procedure was later found to be inadequate for initial studies to determine residues of PCNB and its metabolites in body tissues, fluids and excrements. Neutron activation cannot distinguish individual chlorinated metabolites from one another.

A subsequent study by Borzelleca et al. (1971) stated that the residue in the fat originally identified as PCNB consists primarily of the impurities PCB and HCB. These impurities were present in a degree paralleling their concentration in the diet. Olin technical grace PCNB (97.9%) containing 1.8% HCB, 0.1% PCB and 0.4% 2,3,4,5-TCNB was used.

As part of a reproduction study (Borzelleca et al. 1971), a portion of F_{2b} generation rats were fed diets containing 0, 5, 50 or 500 ppm PCNB (Olin product) for approximately 33 weeks following weaning. The animals were sacrificed after producing 2 litters. Analyses were made of composites of tissue and excreta from 3 rats of the same sex and dietary level. Those animals that were not sacrificed at this time were returned to control diets for 2 months. Residues of PCNB, its impurities (HCB and PCB), and 2 metabolites (PCA and MPCPS) were in skeletal muscle, liver, kidney, fat, and feces at the end of the 33 week feeding period and 2 months after withdrawal from treated diets (see Table 4).

The major tissue of storage appeared to be adipose tissue (fat) where both PCB and HCB displayed dose-related storage levels. This storage trend continued to the 1,080 ppm exposure level where respective storage levels of PCB and HCB increased to 5.15 and 194 ppm, respectively. No significant amounts of any product, including PCNB, were found in the urine.

Kuchar et al. (1969) also analyzed the tissues from the above-mentioned studies and confirmed the presence of PCA and MPCPS by infrared and mass spectrometric techniques.

Table 4. Rat tissue and excreta concentrations of PCNB, its metabolites, (PCA, MPCPS) and related impurities (PCB, HCB)

Specimen	Diet (ppm)	Days on control diet[a]	sex	Concentrations found (ppm) PCNB	PCA	MPCPS	PCB	HCB
Skeletal Muscle	500	0	M	ND[b]	0.117	8.13	0.042	29.7
Liver	500	0	M	ND	0.069	0.306	0.010	1.93
Kidney	500	0	M	ND	0.084	0.269	0.131	6.43
Fat	5	0	M	ND	ND	0.060	<0.001	0.587
	5	0	F	ND	ND	0.055	<0.001	0.824
	50	0	M	ND	0.019	0.46	0.019	10.8
	50	0	F	ND	0.095	0.345	0.011	4.73
	500	0	M	ND	1.11	4.74	0.304	117.
	500	0	F	ND	0.238	3.82	0.176	49.
	5	60	M	ND	ND	ND	ND	1.07
	50	60	M	ND	ND	ND	ND	3.67
	500	60	M	ND	ND	ND	ND	22.3
Feces	5	60	M	ND	ND	ND	ND	0.023
	50	60	M	ND	ND	ND	ND	0.137
	500	60	M	ND	0.027	0.101	ND	1.95

[a]After 33 weeks on test diets.
[b]ND = none detected.

Source: J. F. Borzelleca et al. 1971. Toxicologic and metabolic studies on pentachloronitrobenzene. Toxicol. Appl. Pharmacol. 18:522-534. Reprinted by permission of the Society of Toxicology.

Decreased elimination of HCB from animal tissues over an extended time period was indicated by dose-related levels of HCB in fecal matter at the end of the 60-day withdrawal period.

Kuchar et al. (1969) also indicated the presence of conjugated products of PCA in liver and urine samples from the beagle study conducted by Borzelleca et al. (1971). The authors suggested that these products are probably the glucuronic and sulfuric acid conjugates. Also, the presence of 2,3,4,5-TCA, an established metabolic reduction product of 2,3,4,5-TCNB in the rabbit (Bray et al. 1953), was observed in hydrolyzed samples but not in unhydrolyzed samples.

Distribution and Storage - Cows - PCNB (Olin technical grade) was dissolved in corn oil and administered to cows orally in gelatin capsules (Borzelleca et al. 1971). Doses for each cow were dependent upon the animal's consumption of feed the previous week. Three cows per dose level received 0, 0.1, 1.0 or 10 ppm PCNB for 12 to 16 weeks. Fat biopsies for analyses during the course of the study were collected from the brisket area; additional tissues were obtained at autopsy. The cows were milked twice daily. An aliquot was removed and

analyzed using gas chromatography equipped with electron capture. Levels of detectability for control and treated samples differed from week to week; consequently, the results are difficult to assess accurately. Tissue samples included skeletal muscle, liver, kidney, and abdominal and subcutaneous fat.

Considerable variation in residue levels was noted when individual samples were analyzed. Levels of HCB in milk from cows fed a diet of 1 ppm PCNB ranged from .001 to .003 ppm between the twenty-first and fifty-sixth day of continuous exposure. The average value was .002 ppm. At a dietary level of 10 ppm PCNB, the HCB residue ranged from non-detectable to .015 ppm, with an average value of 0.01 ppm. Levels of PCNB, PCA, MPCPS and PCB showed sporadic variation but were generally .005 ppm or less. There was little indication of dose-related level changes.

Samples of subcutaneous fat biopsied at 0, 1, 2, 4, 7 and 8 weeks after exposure to dietary levels of 10 ppm suggested an 0.5 ppm plateau of HCB residue beginning in the fourth week and continuing through the eighth week. PCA appeared to stabilize at a level of approximately 0.1 ppm between weeks 1 and 8 at the 10 ppm dietary exposure level. The remaining residues (PCA, PCNB and MPCPS) appeared to be negligible or non-detectable at the limit of sensitivity of the procedure.

Plants - Kuchar et al. (1969) studied the metabolism of PCNB in immature cotton plants. Cotton plants grown in soil containing 300 ppm of PCNB were monitored for PCNB plus its impurities and metabolites. Results at the end of 2 weeks growth (roots excluded) are shown below:

Component	ppm found after 2 weeks
PCNB	155.0
PCB (PCNB impurity)	3.96
2,3,4,5-TCNB (PCNB impurity)	0.018
HCB (PCNB impurity)	4.61
PCA	1.11
MPCPS	2.95

PCA was also found in young corn and soybean plants grown in PCNB-treated soil (Kuchar et al. 1969).

Gorbach and Wagner (1967) have also cited PCA as a metabolite in potatoes along with 2 unidentified metabolites which were capable of being gas chromatographed. These metabolites were later described by Kuchar et al. (1969) as having identical gas chromatographic retention times to HCB and MPCPS.

Effects on Reproduction

Rats from Charles River Laboratories (CD strain) were used in a 3-generation study. At 28 days old, litter mates were individually caged to form the F_0 generation. Each group received a diet containing 0, 5, 50 or 500 ppm PCNB (Olin product) dissolved in corn oil. After 11 weeks on these diets, 20 males and 20 females in each group were mated for the F_{1a} generation. At approximately 105 days of age, 20 rats of each sex within each group were mated using the same procedures, followed with the F_2 generation through production of 2 litters (F_{3a} and F_{3b}).

Histopathologic studies were performed on 10 male and 10 female F_{3b} offspring when the rats were approximately 2 months old. Findings were negative. Each rat was grossly examined, but no structural defects were observed. The indices studies resulting from the 6 matings of the rats on the 4 dietary levels --fertility, gestation, viability, lactation, rats born per litter, rats weaned per litter, and average weaning weights--showed that the rats were not affected by PCNB ingestion (Borzelleca et al. 1971).

Teratogenic Effects

Mice - When 500 mg/kg PCNB (noncommercial product containing 87% PCNB, 11% HCB and 2% salts) was orally administered in corn oil to C57BL/6 mice from days 7 to 11 of gestation, 80% of the litters had inhibited kidney formation; for example, there was at least 1 abnormal fetus in 80% of the litters. Renal agenesis occurred unilaterally about twice as often as it occurred bilaterally (Courtney 1973).

Bionetics Research Laboratories reported that 464 mg/kg of PCNB (containing 11% HCB) caused an increase in renal agenesis between litters and within litters when administered orally to pregnant C57BL/6 mice on days 6 to 14 or days 6 to 10 of gestation. No teratogenic effects were observed in a second strain of mice (AKR). At a dose level of 216 mg/kg there were no significant effects in either strain of mice (U.S. Department of Health, Education and Welfare 1969).

Rats - PCNB (Olin product), dissolved in corn oil, was administered to pregnant Charles River Laboratories strain albino rats by oral intubation on days 6 and 15 of gestation. Dosages ranged from 100 to 1,563 ppm. On day 20, dams were sacrificed, and fetuses were removed and examined for gross malformations. The number of corpora lutea, the position and number of dead and/or resorbed fetuses, the fetal weights, and the sex ratios were recorded. None of these indices differed significantly from control values at any level of PCNB administration. Examination of fetal skeletons revealed little or no difference between controls and treated groups in either the number or type of skeletal malformation or in the incidence of minor skeletal variations, such as rudimentary accessory ribs. The number of soft tissue anomalies detected during sectioning of PCNB-treated fetuses did not differ significantly from that of controls. The most common defects--dilated renal pelvis, hydronephrosis, and hydroureter--

were found in control groups and reportedly appeared to be unrelated to treatment (Jordan and Borzelleca 1973).

The above studies indicate that a PCNB-containing mixture is teratogenic for at least 1 strain of mice but not for rats. The possible effects of unknown amounts of HCB or other contaminants of technical grade PCNB were not investigated. Teratogenic studies with HCB and PCNB are currently being conducted to determine the significance of HCB impurities in PCNB.

Oncogenic Effects

Oral Administration - Mice - Two hybrid mouse strains, (C57BL/6 x C3H/Anf)F_1 and (C57BL/6 x AKR)F_1, were given a "maximum tolerated dose" of PCNB (87% PCNB, 11% HCB and 2% salts) at 464 mg/kg in 0.5% gelatin daily via stomach tube, beginning when the mice were 7 days of age and continuing until they reached 28 days of age. Since the dosage was not adjusted for weight gain during this period, the same absolute amount was given daily (Innes et al. 1969). After the mice were weaned at 4 weeks, 1,206 ppm PCNB (a non-commercial mixture containing 87% PCNB, 11% HCB, and 2% salts), calculated to be equivalent to the maximum tolerated dose, was mixed directly with the diet which was provided *ad libitum* for the 18-month duration of the study.

A significantly elevated incidence of tumors occurred in both strains. Fourteen males and 18 females of the (C57BL/6 x C3H/Anf)F_1 strain were surviving at 78 weeks. Two males and 4 females had hepatomas, 2 males and 1 female had pulmonary tumors, and 2 males had lymphomas, for a total of 5 males and 5 females with tumors.

Of the 17 males and 17 females in the (C57BL/6 x AKR)F_1 strain which were necropsied, 10 males and 1 female had hepatomas, 1 male had pulmonary tumors and each sex had 1 lymphoma with a total of 11 males and 2 females with tumors.

For the gelatin control group, the (C57BL/6 x C3H/Anf)F_1 animals did not have any tumors upon sacrifice at 82 weeks. For the (C57BL/6 x AKR)F_1, 3 out of 18 males and 2 out of 17 females had tumors at 83 weeks.

According to Innes et al. (1969), estimates of the hazards posed by these compounds should take into account experiments performed in other laboratories. Furthermore, it should be stressed that the dosage received by the mice was far greater than that likely to be consumed by humans. The fact that this was a screening study should also be noted. Further definitive studies on tumorogenicity of PCNB are being conducted by the National Cancer Institute.

Dermal Administration - Mice - PCNB (recrystallized product, Plant Production Ltd.) and one of its impurities, TCNB, have been found to cause multiple papilloma formation during subsequent application of a promoting agent (croton oil). A study by Searle (1966) tested 20 outbred stock albino mice per compound (10 of each sex) between 6 and 8 weeks of age. They were fed a commercial diet. Applications of 0.2 ml of PCNB and 3 isomers of TCNB were made twice a week for 12 weeks to the clipped backs of the mice. All the mice were treated with croton oil for 20 weeks; the survivors were killed after an additional 20 weeks.

Tests showed that all 4 compounds act as tumor initiators on the skin of mice when croton oil is used as the promoting agent. The experiments gave no evidence that the chloronitrobenzenes induce tumors in the absence of a promoting agent.

Mutagenic Effects

PCNB (Murphy Chemical Company) has been shown to give a positive mutagenic response in hcr$^-$(host cell reactivation deficient) strain of *Escherichia coli* B/r ochre (Clarke 1971). PCNB (unknown analysis) has a negative mutagenic response as a concentration of 2 mg/ml on *E. coli* 343 (Mohn 1971).

In a host-mediated assay in mice, no significant increase in mutation rates in *Salmonella typhimurium* G46 His$^-$ and *Serratia marcescens* a21 leu$^-$ were observed after subcutaneous injection of PCNB (unknown analysis). PCNB also gave negative results in spot tests against the same strains of *Salmonella typhimurium* and *Serratia marcescens* (Buselmaier et al. 1973).

Human Toxicity and Epidemiology

No accidents due to the use of PCNB have been reported to the Pesticide Episode Reporting System (PERS) of Operations Division, Office of Pesticide Programs, EPA.

In acute and subacute toxicity studies, a quarter-inch square of cotton cloth was moistened with water and dipped in a 75% PCNB wettable powder (Olin formulation) and then placed on the volar surface of the right forearms of 50 human subjects. The patches were then covered with a 1 in square of aluminum foil held in place by a 2 in square of adhesive tape. After 48 hr the patches were removed. No evidence of irritation was seen in any of the subjects.

Two weeks later the same test was repeated on the left arms of the same 50 subjects. After 48 hr, 46 of the 50 subjects showed no signs of irritation. In 3 of the 4 reported reactions, a 1 in square area showed erythema, edema and small vesicle formation with marked itching; the fourth subject had only erythema and itching. Of the 46 subjects who were negative when the second patch was removed, 9 developed a delayed reaction. Time of onset varied from approximately 8 hr to several days. In 2 of these, the reaction included erythema, edema, small vesicle formation and itching. The skin reached a peak during the first few days after appearance and subsided with time, with some scaling of the skin (Finnegan et al. 1958).

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PART II. INITIAL SCIENTIFIC REVIEW

SUBPART C. FATE AND SIGNIFICANCE IN THE ENVIRONMENT

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This section contains data on the environmental effects of PCNB on fish, wildlife, and nontarget plants. Residues in soil, interactions with lower terrestrial organisms, bioaccumulation and biomagnification, and environmental transport mechansims are also considered. The section summarizes rather than interprets scientific data reviewed.

Effects on Fish

Olin (1974) reported LC_{50} values of Terraclor® for bluegill (Lepomis macrochirus) and rainbow trout (Salmo gairdneri). The formulation tested contained 98% PCNB and, as impurities, 0.9% HCB and 1.1% other compounds. Aerated well water with a pH of 7.1 and 47.0 ppm hardness (calcium carbonate) was used in all tests; the water was exchanged at a rate of 4 liters/min. Bluegill and rainbow trout were maintained at a temperature of 20 $\pm$ 1°C and 10 $\pm$ 1°C, respectively. Average weights were 1.0 g for bluegill and 1.3 g for rainbow trout. Thirty fish were used in each of the 7 concentrations tested and for the control. The 24- and 96-hr LC_{50} values for bluegill were, respectively, 0.42 and 0.29 ppm (95% confidence limit; 0.21 to 0.39 ppm). The LC_{50} values for rainbow trout for 24 and 96 hr were, respectively, 0.42 and 0.31 ppm (95% confidence limit; 0.23 to 0.42 ppm).

Bluegill exposed to 0.13 ppm Terraclor® showed hemorrhaging of the caudal area. Rainbow trout had partial loss of equilibrium at all Terraclor® concentrations. Before death both species became dark, lethargic, and lost equilibrium.

McCann (1967) conducted a study to determine the toxicity to bluegill (Lepomis macrochirus) of Terraclor®, a formulation of PCNB containing 75% AI. The specimens were in healthy condition with an average length of 39.3 mm and an average weight of 0.849 g. Ten fish were placed in each 15 liter vessel of water. The test water was demineralized to 1 million ohms and reconstituted, and then maintained at a temperature of 65°F and a pH of 7. Observations were made after 3/4, 1 1/2, 3, 6, 12, and 24 hr, and at 24-hr intervals thereafter. Twenty fish were tested at each concentration. From these results it was calculated that the LC_{50} after 24 hr was 7.4 ppm; after 48 hr, 2.43 ppm; and after 96 hr, 0.88 ppm.

Effects on Wildlife

In 8-day dietary tests, Olin (1973a, 1973b) reported the median lethal concentrations (LC_{50}) of Terraclor® to mallard ducks (Anas platyrhynchos) and bobwhite quail (Colinus virginianus). A group of 10 pen-reared, 10- to 15-day-old birds were used. The LC_{50} for this PCNB formulation for both species was greater than 5,000 ppm (the only concentration tested). No mortality occurred at this concentration. No abnormal behavior was observed and no gross abnormalities were noted in either species on autopsy at the conclusion of the experiment.

Vos et al. (1971) studied the effects of a PCNB impurity (HCB) on Japanese quail (Coturnix coturnix japonica). Seventy-five 10-week-old quail were used in the experiment. Twelve females and 3 males received each of the following concentrations of 99.5% pure HCB: 0, 1, 5, 20, and 80 ppm. Feed and water were available ad libitum. Weekly weights of the birds and production of eggs were recorded throughout the 90-day study. Hatchability was determined on days 37 to 46, 64 to 73, and 81 to 90. Upon sacrifice, the following organs and tissues were examined: spleen, kidney, pancreas, ovary/testes, oviduct, heart, lung, thyroid, crop, glandular stomach, muscular stomach, small intestine, cecum, large intestine, skin, skeletal muscle and spinal cord.

The results showed that at a concentration of 80 ppm many effects were evident, including tremors, red fluorescence of tissue, liver damage, erythrophagocytosis in the spleen, ceroid granules in the kidney tubules, reduced reproduction and reduced egg production, and some mortality. Higher brain residues were found in males than in females. One ppm was considered to be the no-effect level.

Effects on Lower Terrestrial Organisms

In laboratory studies, Ko and Farley (1969) examined the effects of PCNB and its metabolite, PCA, on soil microorganisms. In one experiment, nutrient agar plates containing 1, 10, or 50 ppm of PCNB or PCA were seeded with representative soil microorganisms. Among these organisms, more species of fungi and actinomycetes were inhibited by PCNB than by PCA. PCNB was also inhibitory at lower concentrations than PCA. The authors concluded that conversion of PCNB to PCA may be a detoxification step. None of the 10 bacterial species tested was noticeably inhibited by the compounds. Results are summarized in Table 5.

In a companion study, Conover loam soil amended with a carbon source and either 50 ppm PCNB or 50 ppm PCA was incubated for 7 days and then plated on selective media. Greater numbers of bacteria and fungi, but fewer numbers of actinomycetes, were recovered from the PCNB-amended soil than from the controls. In the PCA-amended soil actinomycete populations declined and fungal populations increased, but no statistically significant effect was observed on the bacterial populations. Results are presented in Table 6. In their discussion the authors noted that both PCNB and PCA were inhibitory to Rhizoctonia solani and that the conversion of PCNB to PCA in the soil may be partially responsible for the long-term control of the organism in PCNB-treated soils.

Farley and Lockwood (1968) demonstrated that numbers of actinomycetes on chitin agar were reduced by 65, 90, and 99% by PCNB at 1, 10, and 25 to 100 ppm of PCNB, respectively. Numbers of bacteria on soil extract agar were not reduced at concentrations of PCNB up to 200 ppm. The researchers also demonstrated that numbers of bacterial colonies increased, while actinomycete colonies decreased, when soil samples amended with 25 to 50 ppm of PCNB were plated on Thornton's agar, sodium albuminate agar and soil extract agar.

Table 5. Inhibitory effect of PCNB and PCA on microorganisms in nutrient agar

Microorganism	Control	PCNB, ppm 1	PCNB, ppm 10	PCNB, ppm 50	PCA, ppm 1	PCA, ppm 10	PCA, ppm 50
Actinomycetes							
Actinoplanes philippinensis	-[a]	-	-	++	-	-	-
Micromonospora sp.	-	+	++	++	+	++	++
Nocardia erythropolis	-	-	-	+	-	-	-
Streptomyces aureofaciens	-	-	-	-	-	-	-
Streptomyce griseus	-	-	-	++	-	+	+
Streptomyce lavendulae	-	-	-	-	-	-	-
Streptomyce scabies	-	-	-	++	-	-	-
Streptomyce venezuelae	-	-	-	++	-	+	+
Streptomyce viridochromogenes	-	-	-	-	-	-	-
Streptosporangium roseum	-	-	-	+	-	-	-
Fungi							
Aphanomyces euteiches	-	-	+	+	-	-	-
Aspergillus terreus	-	-	-	-	-	-	-
Fusarium solani f. species *phaseoli*	-	-	-	-	-	-	-
Glomerella cingulata	-	-	+	+	-	+	+
Helminthosporium victoriae	-	-	++	++	-	-	-
Mucor ramannianus	-	-	-	+	-	-	-
Pythium ultimum	-	-	+	+	-	-	-
Rhizoctonia solani (R-47)	-	+	+	-	-	+	+
R. solani (R-52)	-	+	++	++	-	+	++
Thielaviopsis basicola	-	-	-	-	-	-	-
Trichoderma viride	-	-	-	+	-	-	-
Verticillium albo-atrum	-	-	-	+	-	-	-

a- No inhibition of growth; +, partial inhibition; ++, complete inhibition.

Source: W. H. Ko and J. D. Farley. 1969. Conversion of pentachloronitrobenzene to pentachloroaniline in soil and the effect of these compounds on soil microorganisms. Phytopathology 59:64-67. Reprinted by permission of The American Phytopathological Society.

Table 6. Effect of 50 ppm PCNB or PCA on microorganisms in nutrient-amended soils.

Microorganism[a]	Colonies ($X 10^5$)/g soil[b] Control	PCNB	PCA
Bacteria	1500 x	2180 y	1160 x
Actinomycetes	345 x	125 y	316 z
Fungi	3.3 x	4.6 y	4.2 y

a/ Numbers of actinomycetes were estimated from chitin-amended soil; bacteria and fungi were estimated from glucose-amended soil.

b/ For each category of microorganisms, values followed by the same letter (x, y, or z) are not significantly different (5% level).

Source: Adapted from W. H. Ko and J. D. Farley. 1969. Conversion of pentachloronitrobenzene to pentachloroaniline in soil and the effect of these compounds on soil microorganisms. Phytopathology 59:64-67. Reprinted by permission of The American Phytopathological Society.

Oxygen uptake, as a measure of respiration of soil microorganisms, is used as an indicator of overall biological activity. Caseley and Broadlent (1968) treated a fine loamy sand soil (amended with 1% alfalfa meal) with 10, 100, and 1,000 ppm technical grade PCNB. Over a 60-hr period, oxygen consumption in the 1,000 ppm sample and the control was 1,400 and 1,850 microliters, respectively. Soil samples treated with 10 and 100 ppm (concentrations closer to recommended rates of application) demonstrated no substantial decrease in oxygen consumption. Nitrification was not significantly affected by 100 or 1,000 ppm of PCNB, but 10 and 100 ppm of PCNB decreased nitrogen mineralization by 36% over a 35-day period.

The effect of PCNB on saprophytic colonization of organic substrates in soil was studied by Katan and Lockwood (1970), who used alfalfa hay as a substrate for colonization by soil organisms. PCNB, at a concentration of 10 μg/g (10 ppm) soil, was found to produce both quantitative and qualitative changes in the fungal and streptomycete populations which colonize particles of alfalfa hay in natural soil. Table 7 illustrates the effect of PCNB on fungi-colonizing alfalfa residues in soil; soil without PCNB served as the control. The following observations are based on the tests conducted:

1. PCNB-treated soil showed a reduction of total numbers of fungi.
2. The proportion of PCNB-tolerant fungi was greater in soils containing PCNB than in those soils without PCNB.
3. In PCNB-treated soil, populations of *Pythium ultimum* and *Fusarium* species increased, while those of *Rhizopus stolonifer*, *Fusarium axalicum*, and streptomycetes decreased.
4. Alfalfa accumulated PCNB to levels 7- to 11-fold higher than the concentration in soil.

In a later report, Lockwood (1971) suggested that PCNB has the potential of promoting the population expansion of nontarget disease organisms. Under such circumstances a formerly unimportant disease may become more prevalent than the one controlled by the fungicide. Katan and Lockwood (1970) stated that the presence of plant residues appears to enhance the effectiveness of PCNB by providing a site for concentration of PCNB and for colonization by microbial communities. Sorption of PCNB to organic residue may reduce the compound's availability for biological activity against microbes elsewhere in the soil.

In a preliminary investigation, Tews (1971) treated an area of a cattail marsh with PCNB at an equivalent recommended rate (2.29 mg/2,000 sq cm). Subsequent examination of the treated area revealed no substantial decrease in propagules of the dominant fungi: *Mucor hiemalis*, *Penicillium stipitatum*, and *Trichoderma viridae*. The author indicated that the results must be considered as inconclusive.

Bhaskaran (1973) studied the effects of PCNB on rhizosphere organisms of cotton plants. Concentrations ranging from 100 to 2,000 ppm AI (concentrations in excess of recommended rates) caused little change in population levels of bacteria, but resulted in significant reductions in actinomycete populations. Fungal populations were suppressed at concentrations of 500 ppm and above; at 100 ppm the number of fungal propagules recovered exceeded those recovered from

Table 7. Effect of PCNB on fungi-colonizing alfalfa residues in soil

Fungi	PCNB in soil[a]	Fungal populations at different days of incubation						
		1	2	4	8	18	32	Mean[b]
Total fungi[c]	+	1.2	27	46	374	1,142	144	289
	-	5.6	68	61	523	1,542	176	396
Penicillium oxalicum[c]	+	0	0	0	8	11	5	8
	-	0	0	0	23	39	10	24
Rhizopus stonlonifer[c]	+	0	6	21	37	20	12	19
	-	0	19	41	44	44	25	35
Fusarium sp.[c]	+	0.5	10	18	152	280	110	95
	-	0.1	5	6	106	185	81	64
Pythium ultimum[d]	+	20.0	66	36	42	16	14	32
	-	6.6	33	18	25	8	7	16
PCNB-tolerant	+		38	58	26	21	60	41
(large) colonies[e]	-		4	18	19	14	45	20
PCNB-sensitive	+		59	19	57	61	10	41
(small) colonies[e]	-		87	39	63	73	14	55

[a]+ = soil with 10 μg PCNB/g.
- = soil without PCNB.
[b]Means for soil with PCNB differed significantly ($P < .05$) from those for soil without PCNB for each fungal group.
[c]Propagules/g dry alfalfa residue, x 10^4.
[d]Percentage of alfalfa particles colonized.
[e]Percentage of total fungi. Colonies with diameter > 7-8 mm were classed as tolerant; colonies with diameter < 2-3 mm were classed as sensitive (as determined by growth on an agar medium supplemented with 100 μg PCNB/ml).

Source: J. Katan and J. L. Lockwood. 1970. Effect on pentachloronitrobenzene on colonization of alfalfa residues by fungi and streptomycetes in soil. Phytopathology 60:1578-1582. Reprinted by permission of The American Phytopathological Society.

the nontreated areas. Dehydrogenase activity was unaffected in any of the samples, but indole acetic acid production was inhibited in all treated samples.

Residues in Soil

Ko and Farley (1969) attempted to confirm the transformation of PCNB to PCA in soil. Fifty mg of PCNB or PCA were dissolved in 20 ml acetone, mixed with the soils, and incubated at 25°C. Five g of the resultant soil was placed in a screw cap culture tube and moistened with 0.5 ml water, or submerged in 3 ml of distilled water. Seventy to 100% recovery was reported from the extraction procedure. The analyses of PCNB or PCA were done by gas chromatography. In tests with soils containing no pesticides, no peaks were reported corresponding to PCA or PCNB.

The results which appear in Figure 2 show that the PCNB disappeared rapidly from nonsterilized submerged soil; only 1% of the original PCNB was left after 3 weeks. In the nonsterilized moist soil, 82% of the PCNB remained. The difference between the sterilized soils was also significant. No change was observed in the moist sterilized soil, but the PCNB in the submerged sterilized soil had been reduced to 56% of the original amount after 3 weeks. No contamination was observed in the sterilized soils at any time. Figure 3 shows that an increase of PCA accompanied the decrease in PCNB in submerged soil; a similar transformation occurred in moist soil. No PCA was found in sterilized submerged soil. The addition of 1% alfalfa residue to the soil did not affect the conversion of PCNB in any of the tests.

Ko and Farley (1969) also reported that PCA seemed to be much more persistent in the soil than PCNB. The PCA did not decrease after 2 weeks incubation in submerged or moist soil even with the addition of 1% alfalfa. The authors concluded that the decomposition of PCNB is increased under anaerobic conditions. This is supported by the results that show increased degradation in submerged soil. They also concluded that the conversion of PCNB to PCA is biological in nature. The prevention of the conversion of PCNB to PCA by soil sterilization supports this conclusion. However, only 56% of the total PCNB was present as PCA after 3 weeks. The authors suggest that the remaining PCNB was lost in a nonbiological process since the same amount disappeared in the sterilized submerged soil.

Caseley (1968) conducted a study to trace the disappearance of PCNB and 2 other fungicides from the soil. After 10 months, 80% of the PCNB was lost either through metabolic action or physical means. Five mg of PCNB was applied to 50 g of sterilized soil and 50 g of unsterilized soil contained in 125 ml flasks. A stream of air saturated with water flowed through each flask at approximately 2 ml per min. The rate of PCNB loss was found to be proportional to the moisture content of the soil. The author partially explained this observation by the fact that water, as a highly polar molecule, becomes absorbed more easily than the PCNB. Decreased absorption means more mobility of PCNB in the soil and increased loss by such processes as volatilization.

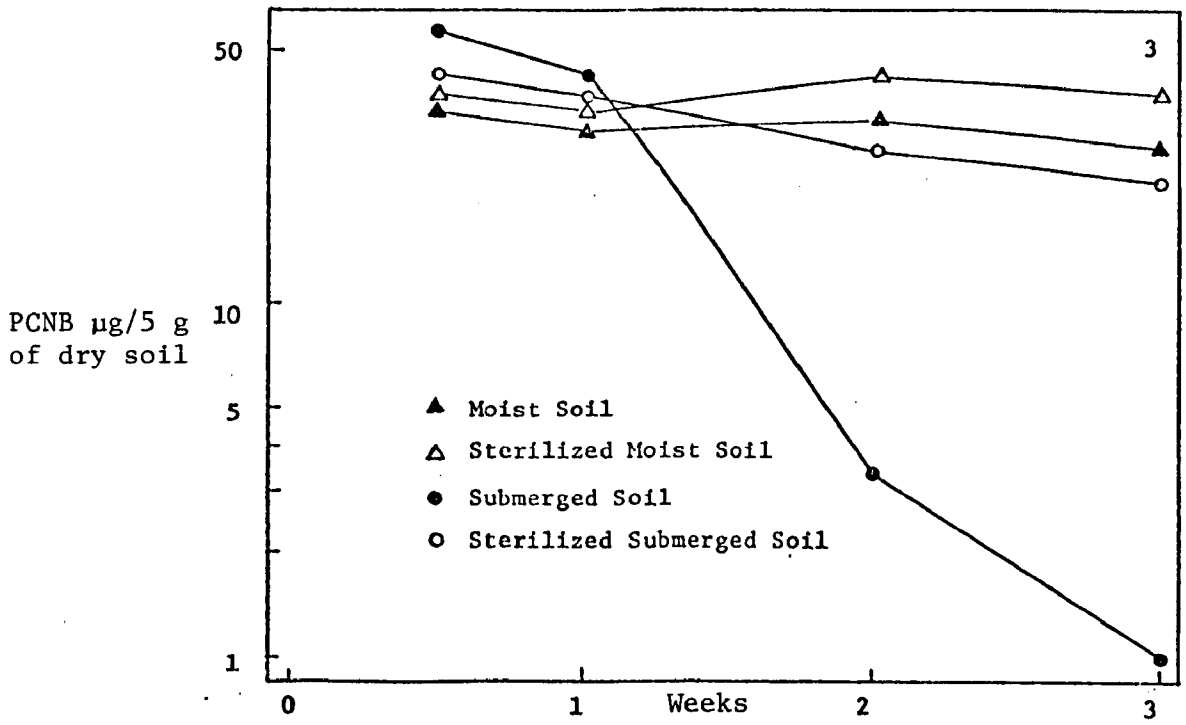

Figure 2. Loss of PCNB from nonsterilized or sterilized, moist or submerged soil.

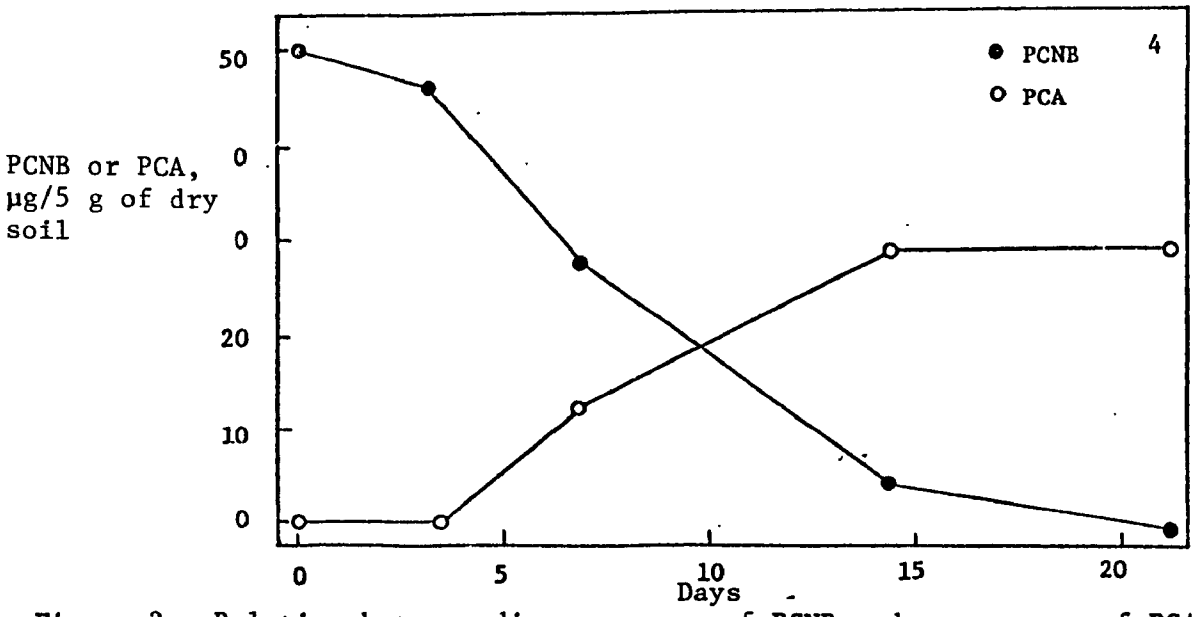

Figure 3. Relation between disappearance of PCNB and appearance of PCA in nonsterilized submerged soil.

Source: W. H. Ko and J. D. Farley. 1969. Conversion of pentachloronitrobenzene to pentachloroaniline in soil and the effect of these compounds on soil microorganisms. Phytopathology 59:64-67. Reprinted by permission of The American Phytopathological Society.

In a laboratory experiment, Wang and Broadbent (1972) measured the rate of loss of PCNB from 3 California soils: Colombia fine sandy loam (0.34% organic matter), Sacramento clay (1.83% organic matter), and Staten peaty muck (22.43% organic matter). In the experiment, 25 g of air-dried soil was treated with 2.5 mg of fungicide in 0.5 ml of hexane acetone (8:2). Water was added to approximate upland moisture conditions, and moisture and temperature (25°C) were controlled during the incubation time. The samples were analysed at 0, 1, 2, 3, 4, 6, 9 and 12 month durations. A half-life designation was used to indicate the stability or persistence of the fungicides. PCNB was found to have a half-life of 4.7, 7.6, and 9.7 months in sandy loam, clay, and muck, respectively. The authors noted that actual persistence of the fungicide could be less than that reported in their data since plant uptake, leaching, and photodecomposition losses were not considered in their experiments. PCNB loss was slowed by increasing the amount of organic matter in the soils. The authors concluded that absorption of PCNB by organic matter markedly retards its loss and that volatilization was the major cause of loss of PCNB.

Nakanishi and Oku (1969) investigated the metabolism of PCNB by *Fusarium oxysporum* f. *lycopersici*. The metabolites of PCNB were determined by thin layer chromatography (TLC). The fungus was cultured on a mechanical shaker at 26°C for 72 hr in a liquid medium containing 25 ppm PCNB with cyclohexane as the solvent. TLC was used to isolate PCNB and the 2 metabolites. By use of UV, IR, and nuclear magnetic resonance (NMR) spectra, the authors concluded that the 2 metabolites were PCA and pentachlorothioanisole (PCTA).

In vitro tests employing selected actinomycetes and fungi demonstrated that the organisms were possible agents of PCNB degradation. In a study done by Chacko et al. (1966), all 8 species of fungi and 9 species of actinomycetes tested degraded PCNB. PCA was the only metabolite identified in this study.

Kaufman (1970), in a review of pesticide metabolism in soil, stated that the conversion of PCNB to PCA was enhanced by submergence of the soil in water. In laboratory experiments, Kaufman also observed PCA in soils treated with PCNB. He isolated methylthiopentachlorophenol as a possible degradation product, but postulated that this product might arise through a displacement type reaction.

Several reports (Caseley 1968, Ware and Roan 1970, and Chacko et al. 1966) have stated that degradation of PCNB occurs only with microorganisms in an active growth phase, indicating that the mechanism is biological. Addition of 1% sucrose was found to rapidly increase the breakdown of PCNB, possibly because the organic material stimulates microbial growth (Caseley 1968).

Residue Uptake by Plants in PCNB-Treated Soils

Casanova and Dubroca (1973) studied the residues of PCNB found in lettuce after treatment of the soil with PCNB. In the first set of experiments (conducted in 1970 to 1971), PCNB was applied to the soil before planting at rates of 15 and 30 kg AI/ha. Residues were determined by gas chromatography after approximately 12 weeks of growth. Table 8 gives the results of the residue tests.

Table 8. Residues of PCNB in lettuce leaves (in ppm) from plants grown in treated soils

Study location (areas of France)	Soil Treatment rates, kg AI/ha		
	0 kg	15 kg	30 kg
Nantes	0.51	0.63	1.10
Rennes	0.17	0.52	1.05
Nantes	0.05	0.47	1.15
Rennes	0.07	0.61	1.10
Rennes	0.10	0.16	0.43
Average	0.18	0.48	0.96

Source: Casanova and Dubroca (1973).

The residues after the application of 15 kg AI/ha were approximately 0.5 ppm, while the residues after an application of 30 kg approached 1.0 ppm, the established tolerance for PCNB in France. About 0.2 ppm of PCNB was found in lettuce grown in soil not treated with PCNB. The authors suggest that this was due to PCNB applications prior to the study.

In the second set of experiments (1971 to 1972), the same application and test methods were used, except that analyses for HCB were included and residues were determined not only for lettuce but also for soil before treatment and at the end of the growing season. The results are given in Table 9. The results of the first test were confirmed in the second test which revealed the presence of PCNB residues in lettuce (approximately 1.0 ppm PCNB after an application of 30 kg AI/ha). Residues of HCB for the same application were considerably lower than these values, and ranged from 0.02 in lettuce from untreated soils to 0.04 ppm in lettuce from treated soils.

Residues in soil before treatment varied considerably with the study location. HCB residues also varied considerably, but they were consistently lower than the PCNB residues.

Casanova and Dubroca (1973) attempted to show that residues found in lettuce were a result of absorption from soil through roots rather than from surface contamination. In a laboratory study the soil was treated with the equivalent of 15 and 45 kg PCNB (source unknown) AI/ha. Residue measurements made by GLC were taken 8 weeks later. Table 10 shows the residues of PCNB and HCB in soil and lettuce samples.

Additional residue studies concerned with uptake in cotton plants, corn, soybeans, and potatoes are discussed in the section on Plant Metabolism.

Bioaccumulation and Biomagnification

Field Studies - Goursaud et al. (1972) studied the relationship of HCB residues in endive roots to residue levels of HCB in the milk of cows eating the roots.

They first determined that, of all the pesticides used on endives, only PCNB was chemically related to HCB. By the use of gas chromatography they found that the roots of the endives were contaminated with both HCB and PCNB and that the milk of cows eating these endives was contaminated with HCB. The most contaminated milk (26 ppm HCB) was from cows receiving 25 to 30 kg of endive roots per day. The roots were contaminated with 0.11 ppm of HCB. Another milk sample containing 0.13 ppm of HCB came from a cow receiving endive roots contaminated with 0.006 ppm of HCB. No volume of feed per day was given for this test.

In a subsequent study (Goursaud et al. 1972), 2 cows received a feed mixture containing 12 kg of endive roots containing 0.16 ppm HCB and 2.16 ppm PCNB. The actual amount of the endives consumed was not given. The residues found in the milk are shown in Table 11. PCNB residues were negligible throughout the test period. The authors concluded that HCB contamination in milk is linked to the application of PCNB to endives. However, they speculated that the source of HCB is from contamination of the original formulation rather than from PCNB degradation.

Table 9. Residues of HCB and PCNB in lettuce from treated soil in ppm

Study location (areas of France)	Test procedure	Soil treatment rates, kg AI/ha					
		None		15 kg		30 kg	
		HCB	PCNB	HCB	PCNB	HCB	PCNB
Nantes	Lettuce	0.016	0.29	0.034	0.83	0.041	1.04
	Soil before planting	0.160	1.50	0.160	1.50	0.160	1.50
	Soil at end of growth season	0.170	1.57	0.620	20.90	2.07	82.41
Rennes	Lettuce	0.025	0.34	0.043	0.66	---	---
	Soil before planting	0.080	0.64	0.090	0.90	---	---
	Soil at end of growth season	0.087	0.67	0.660	10.27	---	---

Source: Adapted from Casanova and Duboca (1973).

Table 10. Residues of PCNB and HCB in soil and lettuce (ppm)

Nature of test	Soil treatment rates kg/ha					
	None		15 kg		45 kg	
	HCB	PCNB	HCB	PCNB	HCB	PCNB
Soil after treatment	N.D.*	0.14	0.50	20.40	1.86	69.10
Lettuce	N.D.	0.03	0.01	0.73	0.02	1.56

*N.D. = nondetected.

Source: Casanova and Dubroca (1973).

Table 11. Residues of HCB and PCNB (ppm) in milk of cows fed treated endive roots

Milk sampling day	HCB (ppm)	PCNB
0*	0.21	Negligible for all samples
1	0.57	
2	1.23	
3	1.34	
4	1.68	
5	1.83	
6	2.00	
7	2.02	

*Day 0 refers to the day before the endive root feed mixture was made available to the cows.

Source: Goursaud et al. (1972).

Laboratory Studies - Ko and Lockwood (1968) studied the accumulation and concentration of PCNB by microorganisms in soil. After 24 hr, PCNB uptake by the mycelium of fungi and actinomycetes equalled approximately the same concentration as that in the soil. Less than 1% of the PCNB in the soil had been accumulated in the fungi. After 48 hr, however, the mycelium of the fungi had accumulated a concentration of PCNB 7 times that of the surrounding soil. The authors compared the rate of uptake of PCNB by the microorganism to the rates of uptake of soil-persistent insecticides.

Environmental Transport Mechanisms

The relatively high volatility of PCNB (vapor pressure of 11.3×10^{-5} mm at 25°C) could account for its route of entry into the air. In a study done by

Caseley (1968), the loss of PCNB from soil in a partially enclosed flask via the slow-moving air stream was higher than could be expected from volatilization alone. The author accounted for the increased loss by an additional mechanism which he described as similar to "codistillation." PCNB has an affinity for the air-water interface and, therefore, may have been removed in this experiment by the stream of moist air passing over the soil. The tests showed that 62% of the PCNB applied to the unsterilized soil was lost in this manner.

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PART II. INITIAL SCIENTIFIC REVIEW

SUBPART D. PRODUCTION AND USE

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This section contains data on the registered uses and the production, domestic supply, and use patterns of PCNB. The section summarizes rather than interprets data reviewed.

Registered Uses of PCNB

PCNB is primarily registered as a soil fungicide for a wide variety of field crops and selected vegetables and horticultural crops; it is also used as a seed-treatment fungicide for a limited number of vegetables and field crop seeds, such as cotton, peanuts, soybeans, and grain. According to Andrilenas (1971), about 7,000 lb of PCNB were used for seed treatment in 1971. A summary of registered uses of PCNB appears in Table 12. There are no current nonagricultural uses of PCNB.

Supply of PCNB in the United States

Volume of Production - The Olin Corporation produces all the domestic supply of PCNB under the trade name Terraclor®. Midwest Research Institute (1972) estimates placed production at 3 million lb. PCNB is currently being produced at 100% of plant capacity. Expansion of production facilities scheduled for the beginning of 1975 is expected to increase capacity by 40 to 50% (Olin 1974). According to Olin, 60 to 70% of the PCNB produced is used domestically; the remainder is exported. Olin reports that the domestic market for PCNB is experiencing moderate growth and that export demand is increasing.

Exports and Imports - According to Olin (1974), significant quantities of PCNB are exported to markets in the Middle East, Far East, Europe, and South America. Export figures are not available. The United States imported the following quantities of PCNB in the 1966 to 1971 period: 40,000 lb (1966); 30,000 lb (1967); 20,000 lb (1968); 132,000 lb (1969). No imports were recorded for 1970 and 1971 (U. S. Department of Agriculture 1972).

Use Patterns of PCNB in the United States

Approximately 295,000 lb of PCNB were used in the United States as a soil fungicide (U. S. Department of Agriculture, 1971). Cotton and peanuts, the largest users of PCNB, were reported to account for 77% and 19%, respectively. Nursery and fruit crops, and vegetables accounted for the remaining 4%.

Despite PCNB's high percentage of use on cotton and peanuts, it is considered unlikely that PCNB is used to treat more than 12% of the total U.S. cotton acreage and 2 to 3% of U.S. peanut acreage (U.S. Department of Agriculture 1974; University of California 1974; Texas A&M University 1974; North Carolina State 1974; Mississippi State University 1974b).

Table 12. Summary of registered uses of PCNB

I. As a soil fungicide

Crop	Target disease	Fungus	Application rate as AI	Limitations	Tolerance
Beans	Root and Stem rot	Rhizoctonia solani	0.75 - 17.5 lb/14,500 ft	Apply at planting time	0.1 ppm
	White mold	Sclerotinia sclerotiorum	2.0 lb - 5.0 lb/14,500 ft	No application after first bloom, no feeding of vines	0.1 ppm
Broccoli Brussel sprouts Cabbage	Clubroot and Wire stem (black rot)	Plasmodiophora brassica and Corticum solani	30 - 60 lb/acre	60 lb/acre	0.1 ppm
Cauliflower	Clubroot	Plasmodiophora brassica	30 - 60 lb/acre	60 lb/acre	0.1 ppm
Garlic	White rot	Sclerotium cepivorum	15 - 22.5 lb/21,800 ft	Apply at planting time	0.1 ppm
Peppers	Southern blight	Sclerotium rolfsii	7 - 7.5 lb/14,500 ft	Apply at transplanting time	0.1 ppm
Potatoes	Black scurf, Stem canker, and Damping-off	Rhizoctonia solani	24 lb/acre	Preplanting use only	0.1 ppm
Tomatoes	Southern blight	Sclerotium rolfsii	7.0 - 7.5 lb/7,300 ft; 2,000 ppm/plant	7.5 lb/7,300 ft; At transplanting only	0.1 ppm

Source: Adapted from U.S. Environmental Protection Agency, EPA Compendium of Registered Pesticides, Vol. II.

Table 12. (continued)

Crop	Target disease	Fungus	Application rate as AI	Limitation	Tolerance
Cotton	Damping-off, seedling blight, and soreshin	*Rhizoctonia solani*	1 - 2 lb/12,400 ft or 0.6 - 1.0 lb/acre (planter lox)	2 lb/12,400 ft. Do not feed foliage; do not rotate with root crops not registered for PCNB	0.1 ppm
Peanuts	Southern blight	*Sclerotium rolfsii*	3.2 - 20 lb/12,400 ft	20 lb/12,400 ft. Do not use peanut hay as feed.	1.0 ppm
Southern pine seedlings	Needle blight	*Rhizoctonia* sp.	37 lb/acre	None	N.A.*
African violets	Stem rot	*Rhizoctonia solani*	2.0 - 2.5 lb/1,000 sq ft	None	N.A.
Azaleas	Petal blight	*Ovulinia azelea*	0.75 lb/150 sq ft	None	N.A.
Begonias	(see African violets)				
Calendula	Stem rot	*Sclerotinia sclerotiorum*	100 lb/acre	None	N.A.
Camellias	Petal blight	*Sclerotinia* sp.	200 lb/acre	None	N.A.
Carnations	(see African violets)				
Dicandra	Brown patch	*Rhizoctonia solani*	0.75 lb/1,000 sq ft	None	N.A.

*N.A. = Not Applicable.

Table 12. (continued)

Crop	Target disease	Fungus	Application rate	Limitation	Tolerance
Gladiolus	Neck and bulb dry rot	*Stromatinia* or *Sclerotinia gladioli*	120.0 lb/acre	Apply at planting time	N.A.*
Grasses	Brown patch	*Rhizoctonia solani*	0.2 - 0.25 lb/1,000 sq ft	Do not use on grazing areas.	N.A.
	Dollar spot	*Sclerotinia homeocarpa*	0.4 - 4.9 lb/1,000 sq ft		
	Leaf spots	*Helminthosporium* spp.	0.4 - 4.9 lb/1,000 sq ft		
	Rusts	*Puccinia* spp.	0.4 - 4.9 lb/1,000 sq ft		
	Snow mold	*Fusarium* sp.	0.4 - 4.9 lb/1,000 sq ft		
	Strip smut	*Ustilago striiformis*	0.91 - 4.9 lb/1,000 sq ft		
Hyacinth	Black rot, and Crown rot	*Sclerotinia bulborum* and *Sclerotinium* sp.	100 - 200 lb/acre	Preplanting or at planting	N.A.
Iris (bulbous)	(see under Hyacinth)				N.A.
Iris (Dutch)	Bulb and stem rot	*Sclerotium delphinii*	100 - 150 lb/acre	Preplanting or at planting	N.A.
Larkspur	(see under Calendula)				
Lillies	Root rot	*Rhizoctonia solani*	100 - 200 lb/acre	Preplanting or at planting	N.A.
Magnolia grandiflora	Leaf spot	*Phylloslicta cookeri*	1.5 lb/100 gal	None	N.A.

*N.A. = Not Applicable

Table 12. (continued)

Crop	Target disease	Fungus	Application rate	Limitation	Tolerance
Narcissus	(see under Iris bulbous)				
Poinsettias	Stem rot	*Rhizoctonia solani*	2.0 - 2.5 lb/1,000 sq ft	None	N.A.*
Snapdragons	(see under Calendula)				
Sweet peas	(see under Calendula)				
Tulips	(see under Iris bulbous)				
Chrysanthemums	Stem rot	*Rhizoctonia solani*	2.0 - 2.5 lb/1,000 sq ft	None	N.A.
		II. As a Post-harvest Treatment			
Bananas	Butt and stem end rot	*Thielaviopsis*, *Gloeosporium* and *Fusarium* spp.	0.75 - 1.63% paste applied to cut surface	Do not contaminate skins of fruit.	0.1 ppm
Roses	Storage rot	*Botrytis cinerea*	0.75 - 1.5 lb/100 gal	For plants in storage	N.A.
Barley	Covered smut	*Ustilago hordei*	0.5 - 1.0 oz/bu seed	Do not use treated seed for feed.	N.A.

*N.A. = Not Applicable.

Table 12. (continued)

Crop	Target disease	Fungus	Application rate	Limitation	Tolerance
Beans	Seedling disease	*Fusarium*, *Rhizoctonia*, *Pythium*, and *Thielaviapsis* spp.	0.5 oz/bu seed	Do not use treated seed for feed.	N.A.*
Corn	Seedling disease	*Fusarium*, *Rhizoctonia*, *Pythium* and *Thielaviapsis* spp.	0.5 oz/bu seed	Do not use treated seed for feed.	N.A.
Cotton	Seedling disease	*Fusarium* *Rhizoctonia*, *Pythium* and *Thielaviapsis* spp.	4 - 6 oz/100 lb seed	Not for fuzzy cotton seed. Do not use treated seed for feed.	N.A.
Garlic	White rot	*Sclerotium cepirorum*	10.2 lb/1,000 lb cloves	Do not use treated cloves for feed.	N.A.
Oats	Smut	*Ustilago* sp.	0.5 - 1.0 lb/bu seed	Do not use treated seed for feed.	N.A.
Peas	Seedling disease	Complex of species	0.5 - 1.0 oz/bu seed	Do not use treated seed for feed.	N.A.

*N.A. = Not Applicable.

Table 12. (continued)

Crops	Target disease	Fungus	Application rate	Limitation	Tolerance
Peanuts	Seedling disease	Complex of species	0.5 - 1.0 oz/bu seed	Do not use treated seed for feed.	N.A.*
Rice	Seedling disease	Complex of species	0.5 - 1. 0 oz/bu seed	Do not use treated seed for feed.	N.A.
Safflower	Seed borne rust	*Puccinia carthami*	0.5 - 1 lb/100 lb seed	N.A.*	N.A.
	Seedling disease	Complex of species			
Sorghum	Seedling disease	Complex of species	0.5 oz/100 lb seed	N.A	N.A.
	Covered kernel smut	*Sphacelotheca sorghi*	1.4 - 2.4 oz/100 lb seed	N.A.	N.A.
Soybeans	Seedling disease	Complex of species	0.5 - 1 oz/bu seed	N.A.	N.A.
Sugar beet	Damping-off	*Aphanomyces*, and *Rhizoctonia* spp.	1.4 - 3.0 oz/100 lb seed	N.A.	N.A.
Wheat	Common smut	*Tilletia caries*	0.5 - 0.75 oz/bu seed	N.A.	N.A.
	Seedling diesase	Complex of species	0.5 - 0.75 oz/bu seed	N.A.	N.A.

*N.A. = Not Applicable.

Andrilenas (1971) reported the following regional distribution of PCNB use on cotton for 1971: Pacific, 57%, South Plains, 26%; Delta, 16%, other areas 1%. Peanut distribution was reported to be 95% for the South Plains and 5% for the Southwest.

Cotton Use and Application

As a soil fungicide for cotton, PCNB is used primarily to combat various seedling diseases, such as seedling blight, seedling rot, and shore-shin caused by the fungus, Rhizoctonia. Although most cotton fields do not have frequent severe problems with Rhizoctonia, the fungus may kill significant numbers of cotton seedlings and reduce the hardiness of survivors.

PCNB is considered to be highly effective in controlling Rhizoctonia (University of California at Riverside 1974; Texas A&M University 1974b; North Carolina State University at Raleigh 1974b; Mississippi State University 1974).

When significant loss of seedlings occurs, the grower usually replants the whole crop. In replanting, the farmer incurs added costs due to the extra labor, seed, and fertilizer required. In addition, the replanted crop may mature 2 to 4 weeks later than the normal harvest time which may make the crop more vulnerable to other pests.

Weather and soil conditions greatly influence the vulnerability of the cotton plants to Rhizoctonia. In an area where soil and climatic conditions predispose the crop to frequent problems with Rhizoctonia, the cotton grower may apply PCNB as a preventative measure. The soil and climatic conditions of the cotton-producing area of California are such that the growers in that region frequently use PCNB as a preventative measure (University of California 1974).

In Mississippi, particularly, Agricultural Extension Service personnel are recommending PCNB usage to more growers. For a grower with crops averaging 1 yr of sever Rhizoctonia in every 3 growing seasons, PCNB is recommended every year (Mississippi State University 1974).

Peanut Use and Application

As a soil fungicide for peanuts, PCNB is used to control southern blight caused by the fungus, Sclerotium rolfsii. A crop severely attacked by southern blight may produce only 85 to 95% of normal yields. In addition, 50% of the crop that is harvested may be of poorer quality than normal yields. Farmers who have frequent problems with southern blight apply PCNB yearly as a preventative measure.

One plant pathologist, however, has reported that application of PCNB has sometimes become counter-productive when used annually (North Carolina State University at Raleigh 1974b). He cited observations in which the first year's

application of PCNB increased peanut yields by approximately 20 to 30%; during the second year's application, yields were only 10 to 15% higher; in the third year's application, continuous use of PCNB may produce a shift in the population of soil pathogens, causing damping-off disease (*Pythium* sp.)

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PART III. EFFICACY AND PERFORMANCE REVIEW

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This section contains a general assessment of the efficacy and performance of PCNB. The section summarizes rather than interprets data reviewed.

Introduction

Assessment of PCNB efficacy is based on data obtained from replicated field test plots. Efficacy is affected by rainfall, fertilizer use, weather conditions, soil type, region of the country, pest infestation levels, and rate, frequency, and method of pesticide application. Consequently, test plot data has limited reliability since the tests are conducted under field conditions which may never be fully duplicated and may not be representative of general field use.

Several criteria, such as stand count or seedling survival, yield, and quality, are used to measure the efficacy of a soil fungicide. These criteria are, however, not directly comparable. Thus, the results of different tests measuring different effects cannot always be related to one another.

Since PCNB's first registration in 1955, data on efficacy has been reported for several crops, including cotton, peanuts, and wheat. Many of the tests reported in the available literature measure the efficacy of PCNB in combination with insecticides and other fungicides. For example, PCNB is commonly formulated with another fungicide, Terrazole (the registered trademark for Olin Corporation's 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole). When a combination of chemicals is applied in a test for control of one disease problem, it becomes impossible to attribute efficacy to a single component of the combination. Although it appears that PCNB is commonly applied in combination with other chemicals, only those test results for use of PCNB alone are reported here.

Efficacy and Performance - Wheat

PCNB is registered for use as a seed treatment on wheat for controlling common smut or bunt fungi and damping-off caused by Rhizoctonia. A seed treatment formulation is available which is applied at 0.5 oz/bu of seed.

Hansing (1973) artificially innoculated Red Chief wheat seed with bunt spores for trials in Kansas. The seed was treated with PCNB 24L at the rate of 0.5 oz/bu. Seeds were planted in 3 replicated 12-ft rows 2 and 5 days after treatment. The number of bunted spikes was determined after the heading stage. The sample planted 2 days after treatment produced less than 0.5% bunted spikes; the sample planted 5 days after treatment had no bunted spikes. Nontreated controls produced 84% and 52% bunted spikes, respectively.

Hoffman (1973) conducted trials for control of common bunt in Washington and Oregon in the fall of 1972. Seeds of Orin winter wheat artificially innoculated with spore of common bunt were treated with PCNB 24L at 0.5 oz/bu of seed. The seeds were planted in replicated 5-foot rows in bunt-infested and uninfested soil at the 2 sites. Evaluations based on counts of smutted spikes were recorded in the following summer. In uninfested soil the PCNB-treated

seed produced 0 and 1% smutted heads as compared to 96 and 99% in the checks. In the smut-infested soil, PCNB produced 5 and 10% smutted heads compared to 96 and 98% in the checks.

Line (1973) included PCNB 24L in trials for control of flag smut of wheat in Washington. Uninoculated Paha winter wheat seeds treated at 0.5 oz/bu with PCNB were planted in naturally infested fields. In 2 test plots, the treated seed produced 11 and 20% smutted tillers compared to 67 and 69% smutted tillers in the check. In an artificially infested plot 0.5 oz/bu of PCNB 24L reduced smutted tillers to 12% as compared to 15% in the check for inoculated seeds. In a field which had previously been free of flag smut, 0.5 oz/bu of PCNB 24L, when applied to inoculated seeds, reduced smutted tillers to 1% as compared to 6% in the check.

Hoffman (1974) treated artificially contaminated Orin winter wheat seeds with 0.5 oz/bu PCNB 24L. The seeds were planted in bunt-infested soil at Pullman, Washington, and under the same conditions at Pendleton, Oregon, in the fall of 1973. Effectiveness was based on counts of smutted spikes recorded in the summer of 1974. The PCNB-treated seed gave 100% control of common bunt. The check plots produced 60 to 80% bunted spikes for comparison.

Line and Hewitt (1974) conducted trials for control of flag smut on wheat at sites in Washington. Uninoculated seeds were treated with PCNB 24L at .25 and 0.5 oz/bu and planted in a field infested with flag smut (plot A). Inoculated seeds treated with PCNB 24L at the same rates were planted in soil which had been infested with flag smut in 1968, 1970, and 1972 (plot B). The same treatments were applied to inoculated seeds planted in soil which had been infested only once in 1972 (plot C). All treatments consisted of 4 replicated plots.

Efficacy was measured by the percentage of tillers with flag smut. The 0.25 oz/bu treatment produced 19, 65, and 38% smutted tillers; the 0.5 oz/bu treatment produced 15, 61, and 20% smutted tillers; while the check showed 34, 74, and 52% smutted tillers in plots A, B, and C, respectively. The statistical significance of the differences was not reported.

Efficacy and Performance - Peanuts

PCNB is registered for control of southern blight (*Sclerotium rolfsii*) on peanuts. Application rates vary from 3.2 to 20 lb/12,400 row ft when used as a soil fungicide. PCNB is also used as a seed treatment for peanuts at 0.5 to 1.0 oz/bu of seed.

Sturgeon and Shackelford (1972) evaluated PCNB as a soil fungicide treatment on irrigated land in Oklahoma in 1971. The land was known to have high yield potential and had a history of southern blight incidence. Applications of PCNB 30G were made as in-furrow treatments at planting, as banded treatments at pegging, and as banded treatments at mid-season. Results of various combinations of the treatments above were reported. Plots consisted of 2 rows 300 ft long, replicated and randomized. All PCNB-treated plots produced greater yields than did the check plots (see Table 13). The peanuts from the treated

plots received a grading of 72 to 75 while the peanuts from the check plot averaged a grade of 69. The grade is a factor in the price received for the crop.

Sturgeon and Jackson (1973) repeated the tests of Sturgeon and Shackleford (1972). The trials were conducted in Oklahoma in 1972 on a site with a past incidence of southern blight. Plots consisted of 2 replicated and randomized 300 ft rows. PCNB 30G applications were made in-furrow at planting, banded at pegging, and banded at mid-season. All PCNB-treated plots produced higher yields of peanuts than did the check plots. Except for one test in which 0.3 lb/acre of PCNB was applied at planting, all tests produced higher yields which were significantly different at the 0.05 level than the check. Results of the tests are summarized in Table 13.

Horne et al. (1972) included PCNB as a soil fungicide in demonstrations for control of peanut disease. PCNB 30G was applied as a single application at 2.1 lb/acre at planting and as 2 applications at 2.1 lb/acre in-furrow at planting and 9.6 lb/acre banded at pegging. All treatments increased the yields of peanuts, but the differences were not significant at the 0.05 level. There were no consistent differences in grades between the checks and the treated plots (see Table 13).

Table 13. Summary of PCNB tests against southern blight of peanuts

Application of 30G formulation			Increse in yield		
lb/acre	Number	Method[1/]	(lb/acre)	Grade	Source
3.0	1	F	541	75	Sturgeon and Shackleford 1972
3.0	1	P	476	72	Sturgeon and Shackleford 1972
3.0	2	F, P	436	74	Sturgeon and Shackleford 1972
3.0	1	B	371	72	Sturgeon and Shackleford 1972
1.8	2	P, B	113	74	Sturgeon and Shackleford 1972
3.0	3	F, P, B	728	73	Sturgeon and Jackson 1973
2.7	3	F, P, B	720	72	Sturgeon and Jackson 1973
1.5	2	F, P	568	72	Sturgeon and Jackson 1973
0.9	1	F	432	72	Sturgeon and Jackson 1973
0.6	1	F	392	71	Sturgeon and Jackson 1973
0.3	1	F	304	71	Sturgeon and Jackson 1973
11.7	2	F, P	309	74	Horne et al. 1972
2.1	1	F	85	74	Horne et al. 1972
2.1	1	F	249	71	Horne et al. 1972
11.7	2	F, P	227	69	Horne et al. 1972
11.7	2	F, P	239	71	Horne et al. 1972
2.1	1	F	211	71	Horne et al. 1972

1/ F = In furrow at planting.
P = Banded at pegging.
B = Banded at mid-season.

Efficacy and Performance - Cotton

PCNB is registered for control of postemergence damping-off disease (Rhizoctonia solani) on cotton. Soil fungicide application rates vary from 1 to 2 lb/acre. PCNB is also available in formulation for use as a seed treatment on cotton for control of the same disease. Seed treatment is made at 3 to 4 oz/100 lb of seed.

Baldwin (1972) conducted soil fungicide tests in Missouri in 1971 with acid-delinted cotton seeds which had been treated with PCNB at 4 oz/100 lb. There were 4 replications of each treatment in a complete, randomized, block design. PCNB 75WP was applied at 1.5 lb/acre both as an in-furrow spray and as a hopper box treatment. The in-furrow spray and the hopper box treatment increased seed cotton yield over the check by 241 and 568 lb/acre, respectively. The increases, however, were not statistically significant.

Sinclair et al. (1965) evaluated PCNB for control of seedling diseases of cotton at 2 sites in Louisiana. Tests were conducted on randomized, replicated plots. PCNB 10D applied at 2.5 lb/acre in the hopper box increased stand count by 36% (significant at 0.05 level), and increased seed cotton yield by 206 lb/acre over the check. PCNB 10G applied at 3 and 6 lb/acre in-furrow increased stand counts by 6.7 and 10%, respectively, over the check; however, the two rates produced declines in yield of 15 and 145 lb/acre of seed cotton. The 6 lb/acre rate of PCNB 10G at the second site increased seed cotton yield by 74 lb/acre in comparison with the check. None of the yield results reported were significant at the 0.05 level.

Maier (1965) evaluated PCNB in New Mexico for control of a cotton seedling disease complex which included Rhizoctonia solani. PCNB 10G was applied at 1.0 lb/acre in the furrow. Stand counts taken 4 weeks after planting showed that the treatment increased the stand by 13% (non-significant at 0.5 level). Yield was increased by 17.5%, but the actual yields were not reported.

The National Cotton Council (1961) reported the results of regional seed treatment trials conducted in 1960. Mechanically delinted seed treated with PCNB at 4 oz/100 lb had a higher percentage of seedling survival in 10 out of 12 trials. In 2 of the 10 trials, the survival rates were significantly higher at the 0.01 level. In the 2 tests in which the checks had a higher survival rate, the differences were not significant. In 12 additional trials, acid-delinted seeds were treated with PCNB at 4 oz/100 lb. In 6 of the trials, the PCNB-treated seeds produced higher seedling survival, but none of the differences was significant. Of the 6 trials in which the checks had higher seedling survival rates, differences for 2 of the trials were significant at the 0.01% level.

Olin Corporation (1964) conducted an experiment to determine the effect that soil placement of PCNB would have on cotton seedling survival. The test conducted at the U.S. Cotton Station in Shafter, California, consisted of treatments made on 2 row plots, each replicated 4 times in a randomized block. PCNB 24 EC and PCNB 10G were applied at planting at rates of 1 to 2 lb/acre. Control was measured in terms of postemergence loss of seedlings. PCNB 24 EC

at 1 lb/acre applied in the bottom of the furrow reduced loss to 2.8%; 1 lb/acre applied in the top of the furrow reduced seedling loss to 2.0%. PCNB 24 EC at 2 lb/acre applied both in the top and bottom of the furrow reduced the loss to 2.3%. PCNB 10G at 1 lb/acre placed in both the top and bottom of the furrow reduced seedling loss to 1.7%. PCNB 10G at 1 lb/acre placed in the bottom of the furrow held seedling loss to 1.6%. The untreated check suffered a seedling loss of 10.8%. All treatments significantly increased emergence and survival of seedlings.

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