Toxicologic and Metabolic Studies on Pentachloronitrobenzene


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Toxicologic and Metabolic Studies on Pentachloronitrobenzene. BORZELLECA, JOSEPH F., LARSON, P. S., CRAWFORD, E. M., HENNIGAR, GORDON R., JR., KUCHAR, EDWARD J., and KLEIN, H. HARVEY (1971). Toxicol. Appl. Pharmacol. 18, 522–534. In studies on technical grade pentachloronitrobenzene (PCNB), in a 3-generation reproduction study on rats, no adverse effects on any parameter appeared from diet levels through 500 ppm. Two-year feeding to beagle dogs established 30 ppm as the highest no-effect level tested; cholestatic hepatosis with secondary bile nephrosis was found in minimal degree on 180 ppm and in moderate degree on 1080 ppm, but was considered to be a reversible lesion. Storage of PCNB did not occur in tissues of the rat, dog, or cow (the latter fed at levels up to 1000 ppm). Traces of apparent PCNB were found in milk from treated cows, but this was also common to milk from control cows (analysis of the feed used showed apparent PCNB). Pentachloroaniline and methyl pentachlorophenyl sulfide, metabolites of PCNB, were found in tissues of treated animals of all 3 species, which together with absence of PCNB indicates rapid metabolism of the latter. In contrast, tissue storage of hexachlorobenzene and pentachlorobenzene, contaminants of technical PCNB, was found in all 3 species, in degrees paralleling their contents in the PCNB, indicative of a slower rate of metabolism of these compounds. Since PCNB is proposed for use as a soil fungicide in combination with 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole (Terrazole®), the possibility of potentiation of toxicity was examined in acute oral tests on rats; no potentiation was found. In acute (24-hr) percutaneous toxicity tests in rabbits, no signs of intoxication resulted from application of 4 g/kg of PCNB as a 30% solution in corn oil.

Pentachloronitrobenzene (PCNB)² has established usefulness as a soil fungicide in the control of Rhizoctonia species and Sclerotium rolfsii.

In an earlier report from our laboratory (Finnegan et al., 1958) data were presented on acute oral toxicity (rats, rabbits, dogs), 3-mo and 2-yr feeding to rats, 1-yr feeding

¹ Supported by a grant from The Olin Corporation.
² Terraclor®, Quintozene.
to dogs, fat storage in rats, and skin irritation and sensitization potential in man. Findings as to a no-effect level in dogs were inconclusive, requiring further study, and no attempts were made to ascertain effects on reproduction in rats or its metabolic fate in dairy animals (subject to exposure in feedstuffs). These latter are subjects of the present report, together with further observations on tissue storage in rats and dogs. Since PCNB is proposed for use in combination with 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole (Terrazole®), the possibility of potentiation of toxicity was examined in acute oral tests on rats. Also included was an acute percutaneous toxicity determination of PCNB in rabbits.

**METHODS**

**Pentachloronitrobenzene (PCNB)**

Technical grade PCNB of the following analytically determined composition was used: pentachloronitrobenzene 97.8%, hexachlorobenzene 1.8%, 2,3,4,5-tetrachloronitrobenzene 0.4%, and pentachlorobenzene < 0.1%.

**PCNB-Terrazole® Potentiation**

Technical grade Terrazole® (95.2% purity) was used. Both materials were prepared for dosing as 10% (w/v) solutions in corn oil. Male albino rats (mean weight ± SD 162 ± 35 g) were fasted overnight prior to dosing by gastric intubation. LD50 values were initially determined for each material, based on 5 groups of 10 rats in each case, and were calculated by the minimum approximate chi-square normit method of Berkson (1955). Subsequently, in a test for potentiation, the two materials were administered simultaneously in the various combinations shown in Table I. The rats were observed for survival for 14 days.

**TABLE 1**

**ACUTE ORAL TOXICITY OF COMBINATIONS OF PCNB AND TERRAZOLE® IN MALE ALBINO RATS**

<table>
<thead>
<tr>
<th>Ratio of doses PCNB: Terrazole®</th>
<th>Solutions tested (total mg/ml)</th>
<th>Volume admin. (ml/kg)</th>
<th>Number of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD50: LD2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1740:495</td>
<td>100 + 28</td>
<td>17.4</td>
</tr>
<tr>
<td>LD50: LD6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1740:600</td>
<td>100 + 35</td>
<td>17.4</td>
</tr>
<tr>
<td>LD50: LD16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1740:730</td>
<td>100 + 42</td>
<td>17.4</td>
</tr>
<tr>
<td>LD50: LD31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1740:890</td>
<td>100 + 51</td>
<td>17.4</td>
</tr>
<tr>
<td>LD50: LD50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1740:1080</td>
<td>100 + 62</td>
<td>17.4</td>
</tr>
<tr>
<td>LD50: 1/2LD50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1740:540</td>
<td>100 + 31</td>
<td>17.4</td>
</tr>
<tr>
<td>1/2LD50: 1/2LD50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>870:540</td>
<td>100 + 62</td>
<td>8.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on calculated doses for the indicated LDs, a procedure kindly suggested to us by Dr. Frederick Sperling.

<sup>b</sup> Based on calculated fractions of the LD50 dose.

**Acute Percutaneous Toxicity**

Test animals were male albino New Zealand rabbits (mean weight ± SD 1.96 ± 0.37 kg). Animals with intact and abraded skin were used. The hair was removed from the

<sup>3</sup> Mazola®.

<sup>4</sup> CD strain, Charles River Laboratories.
trunk with an electric clipper. Abrasions, made with a metal grid, were sufficiently deep to penetrate the stratum corneum without causing bleeding. The rabbits were restrained in stocks for 24 hr. A double-layered plastic grid was placed around the trunk and secured at each end with adhesive tape. PCNB was dissolved in dimethyl phthalate in concentration of 300 mg/ml (w/v as principal ingredient), introduced under the girdle and distributed over the trunk. Ten rabbits were used per dose level; doses administered were 10.0 and 13.3 ml/kg to rabbits with intact skin and 13.3 ml/kg to those with abraded skin. Some initial pooling occurred in the dependent part of the girdle but at 24 hr absorption appeared to be virtually complete. The rabbits were observed for 14 days post-dosing.

Reproduction Study

CD-strain rats were used as in the potentiation study above. At 28 days of age, littermates, within but not between sexes, were separated into 4 groups each containing 25 males and 25 females and individually caged to constitute the F/O generation. One group was placed on each of the following dietary concentrations of PCNB: 0, 5, 50, or 500 ppm of the principal ingredient. Finely ground laboratory chow served as the basic diet, into which the PCNB dissolved in a small amount of corn oil was thoroughly mixed. After 11 wk on these diets, 20 males and 20 females in each group were mated for the F/1a generation, females being rotated to a different male for 3 successive weeks if this became necessary. Records were kept of mating, numbers of pregnancies and litters, young in the litter at 1, 4, and 21 days, and weaning weight at 21 days. Litters containing more than 10 were reduced to this size on day 4. Indices calculated were (1) fertility = (pregnancies/matings) × 100; (2) gestation = (litters cast/pregnancies) × 100; (3) viability = (live at 4 days/live born) × 100; (4) lactation = (weaned/live minus discards at day 4) × 100. Ten days after weaning of the last litter, F/0 rats were remated as above to produce (F/1b) litters.

Twenty-five male and 25 female F/1b rats from each diet level were continued on the respective parental diets and at about 105 days of age 20 of each sex within each group were mated using the same procedures followed with the F/0 generation through production of two litters (F/2a and F/2b). F/2b rats were continued through the same procedures as with the F/1b generation through production and weaning of two litters (F/3a and F/3b). Histopathologic studies were performed on 10 male and 10 female F/3b offspring at about 2 months of age from each diet level, tissues examined being heart, lung, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, cecum, lymph node, bone marrow, skeletal muscle, skin, brain, pituitary, thymus, thyroid, adrenal, pancreas, and gonad.

Two-Year Study in Dogs

Four purebred beagle dogs of each sex about 4.5 mo of age were placed on each of the following dietary levels of PCNB: 0, 5, 30, 180, or 1080 ppm of principal ingredient.

5 Saran Wrap®.
7 Purina Mills.
8 Hazleton Research Animals, Inc., Falls Church, Virginia.
Prior to this, each dog had received appropriate inoculations against distemper, infectious hepatitis and leptospirosis; a complete physical examination, including the eyes; and any needed treatment for intestinal parasites.

Ground dog meal\(^9\) (9% fat content) was used as the basic diet, into which the PCNB dissolved in corn oil was thoroughly mixed; all diets were adjusted to contain the same amount of fat (11%). Prior to feeding, an equal weight of water was added to the food and thoroughly mixed. The amount of food presented to each animal was based on the recommendations of the National Academy of Sciences—National Research Council (Publication 989, Nutritional Requirements of Dogs) and was adjusted weekly based on the weight of the animal. Food consumptions were determined daily and body weights weekly.

Hematologic studies (hematocrit, hemoglobin, total white cell, differential white cell) and urinalyses (reducing substances, protein, specific gravity, microscopic) were made on all dogs at the start of the test and at 3, 6, 12, 18, and 24 months. BUN, SGOT, SAP, serum ChE, prothrombin time, and BSP retention determinations were made on all dogs at the start of the test and prior to sacrifice, and on control and 1080 ppm dogs at 3, 6, 12, and 18 months. External lymph nodes were palpated on all dogs at start and end of the study and weekly during its course on control and 1080 ppm animals. Females were observed for onset, duration, and frequency of estrus.

One dog of each sex on each diet was sacrificed for histopathologic study at 12 mo and the rest at 2 years. Organ weights and organ-to-body weight ratios were obtained at sacrifice for heart, spleen, liver, kidneys, and testes. Tissues submitted for histopathologic study were the same as listed for rats above, plus aorta and eye.

**Metabolic Studies**

All specimens were frozen and shipped to the Analytical Department of Olin, New Haven, Connecticut, for analysis. The analytical method used, gas chromatography employing electron-capture detection, has been described by Kuchar et al. (1969). Determinations included analysis for PCNB, the impurity ingredients hexachlorobenzene (HCB) and pentachlorobenzene (PCB), and two metabolites of PCNB, pentachloroaniline (PCA), and methyl pentachlorophenyl sulfide (MPCPS). All reported values were corrected for recovery standards.

**Rat.** Animals used were F/2b rats from the reproduction study following weaning of their second litters. Individual tissues or excreta were pooled from 3 rats of the same sex and diet level while still on diet (at about 33 weeks) and again on rats that had been returned to control diet for 2 months (withdrawal).

**Dog.** Analyses were largely confined to specimens from the male dogs in the study; such analyses as were made on specimens from females gave similar values.

**Cow.** Holstein cows were used in this study, during which they were continuously confined to stanchions in a dairy barn. The diet fed consisted of milking chow.\(^{10}\) Weighed amounts were offered to the cows twice daily at milking times, and amounts consumed were determined by weighing portions not eaten. Milking was done twice daily (about 6:30 AM and 6:30 PM) by milking machine and final stripping by hand. Milk collected was weighed and recorded, and specimens for analyses were taken from the morning.

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\(^9\) Purina Mills.

\(^{10}\) Purina BIR.
collections. Test materials were dissolved in corn oil and administered orally each evening in gelatin capsules (No. 10 or No. 11, Michigan Capsule Co., Detroit) by means of a bailing gun. Doses for each cow for any given week were based on the average food consumption for the preceding week in an attempt to relate the levels to ppm had the material(s) been incorporated in the diet. Doses used in the main study (over 12 and 16 wk) were equivalent to 0, 0.1, 1.0, and 10 ppm in the diet with 3 cows on each dosage. Fat biopsies for analyses during the course of the study were collected from the brisket area under local anesthesia. Additional tissues were obtained at autopsy.

RESULTS

PCNB-Terrazole® Potentiation

LD50 values (mg/kg ± SD of principal ingredient) obtained were: PCNB, 1743 ± 183; Terrazole®, 1077 ± 78. Combinations administered and resulting mortality are summarized in Table 1. The data suggest additive effects only.

Acute Percutaneous Toxicity

No deaths resulted from exposures to 4.0 g/kg (13.3 ml/kg of a 30% solution), the highest level tested. Neither skin irritation nor signs suggestive of intoxication were observed.

Reproduction Study

Indices (mean ± SD) resulting from the six matings of rats on the four dietary levels (0, 5, 50, and 500 ppm) were, respectively: fertility 95 ± 5, 97 ± 5, 99 ± 2, 99 ± 2;

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Diet (ppm)</th>
<th>Days on control dieta</th>
<th>Concentrations found (ppm)</th>
<th>Sex</th>
<th>PCNB</th>
<th>PCA</th>
<th>MPCPS</th>
<th>PCB</th>
<th>HCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sk Musc</td>
<td>500</td>
<td>0</td>
<td>M</td>
<td>NDb</td>
<td>0.117</td>
<td>8.13</td>
<td>0.042</td>
<td>29.7</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>500</td>
<td>0</td>
<td>M</td>
<td>ND</td>
<td>0.069</td>
<td>0.306</td>
<td>0.010</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>500</td>
<td>0</td>
<td>M</td>
<td>ND</td>
<td>0.084</td>
<td>0.269</td>
<td>0.131</td>
<td>6.43</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
<td>0</td>
<td>M</td>
<td>ND</td>
<td>0.019</td>
<td>0.46</td>
<td>0.019</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0</td>
<td>M</td>
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<td>0.345</td>
<td>0.011</td>
<td>4.73</td>
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</tr>
<tr>
<td></td>
<td>500</td>
<td>0</td>
<td>M</td>
<td>ND</td>
<td>1.11</td>
<td>4.74</td>
<td>0.304</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0</td>
<td>F</td>
<td>ND</td>
<td>0.238</td>
<td>3.82</td>
<td>0.176</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>60</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>60</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.67</td>
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</tr>
<tr>
<td></td>
<td>500</td>
<td>60</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>5</td>
<td>60</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>60</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.137</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>60</td>
<td>M</td>
<td>ND</td>
<td>0.027</td>
<td>0.101</td>
<td>ND</td>
<td>1.95</td>
<td></td>
</tr>
</tbody>
</table>

a After 33 wk on test diets.
b ND = none detected.
TABLE 3  

**DOG TISSUE AND EXCRETA CONCENTRATIONS OF PCNB, ITS METABOLITES (PCA, MPCPS), AND RELATED IMPURITIES (PCB, HCB) AT TWO YEARS ON DIET**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Diet</th>
<th>PCNB</th>
<th>PCA</th>
<th>MPCPS</th>
<th>PCB</th>
<th>HCB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.017 ± 0.007</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.012 ± 0.004</td>
<td>0.035 ± 0.045</td>
</tr>
<tr>
<td>30</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.022 ± 0.004</td>
<td>0.099 ± 0.021</td>
</tr>
<tr>
<td>180</td>
<td>ND</td>
<td>0.018e</td>
<td>0.021 ± 0.007</td>
<td>0.083 ± 0.009</td>
<td>0.568 ± 0.139</td>
<td></td>
</tr>
<tr>
<td>1080</td>
<td>ND</td>
<td>ND</td>
<td>1.08 ± 0.47</td>
<td>0.214 ± 0.065</td>
<td>6.41 ± 9.17</td>
<td></td>
</tr>
<tr>
<td><strong>Brain</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>0.018 ± 0.012</td>
<td>0.001e</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>0.017 ± 0.002</td>
<td>0.007 ± 0.001</td>
<td>0.025d</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>ND</td>
<td>ND</td>
<td>0.024e</td>
<td>0.019 ± 0.004</td>
<td>0.068 ± 0.024</td>
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</tr>
<tr>
<td>180</td>
<td>ND</td>
<td>ND</td>
<td>0.045 ± 0.017</td>
<td>0.102 ± 0.021</td>
<td>0.427 ± 0.082</td>
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<tr>
<td>1080</td>
<td>ND</td>
<td>ND</td>
<td>2.64 ± 4.15</td>
<td>0.561 ± 0.275</td>
<td>9.48 ± 15.0</td>
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<tr>
<td><strong>Sk Musc</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.009e</td>
<td>0.016 ± 0.023</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.004 ± 0.001</td>
<td>0.053 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.037 ± 0.044</td>
<td>0.281 ± 0.243</td>
</tr>
<tr>
<td>180</td>
<td>ND</td>
<td>ND</td>
<td>0.227 ± 0.080</td>
<td>0.234 ± 0.215</td>
<td>7.28 ± 10.53</td>
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</tr>
<tr>
<td>1080</td>
<td>ND</td>
<td>ND</td>
<td>0.049 ± 0.015</td>
<td>0.037 ± 0.003</td>
<td>0.016c</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>0.057 ± 0.012</td>
<td>0.039 ± 0.007</td>
<td>0.007 ± 0.001</td>
<td>0.039 ± 0.039</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>0.039 ± 0.004</td>
<td>0.087 ± 0.006</td>
<td>0.024 ± 0.006</td>
<td>0.125 ± 0.025</td>
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<tr>
<td>30</td>
<td>ND</td>
<td>0.045 ± 0.024</td>
<td>0.037 ± 0.003</td>
<td>0.542 ± 0.610</td>
<td>0.737 ± 0.141</td>
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</tr>
<tr>
<td>1080</td>
<td>ND</td>
<td>0.039 ± 0.007</td>
<td>0.369 ± 0.204</td>
<td>0.419 ± 0.206</td>
<td>1.25 ± 1.157</td>
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<tr>
<td><strong>Spleen</strong></td>
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</tr>
<tr>
<td>0</td>
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<tr>
<td>1080</td>
<td>ND</td>
<td>ND</td>
<td>0.128 ± 0.046</td>
<td>0.204 ± 0.110</td>
<td>2.86 ± 3.97</td>
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<td><strong>Bile</strong></td>
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</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>&lt;0.001</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>1080</td>
<td>ND</td>
<td>1.92 ± 1.30</td>
<td>4.53 ± 2.25</td>
<td>0.333 ± 0.061</td>
<td>5.98 ± 8.25</td>
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</tr>
<tr>
<td>Specimen</td>
<td>Diet</td>
<td>PCNB</td>
<td>PCA</td>
<td>MPCPS</td>
<td>PCB</td>
<td>HCB</td>
</tr>
<tr>
<td>----------</td>
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<td>----------</td>
<td>-----------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Fat</td>
<td>0</td>
<td>ND</td>
<td>0.075 ± 0.035</td>
<td>&lt;0.025&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.005 ± 0.002</td>
<td>0.005 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>ND</td>
<td>0.030&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.027 ± 0.006</td>
<td>0.093 ± 0.045</td>
<td>0.452 ± 0.535</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>ND</td>
<td>0.070&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.020&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.163 ± 0.114</td>
<td>1.11 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>ND</td>
<td>0.016&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.340&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.767 ± 0.342</td>
<td>6.12 ± 1.24</td>
</tr>
<tr>
<td></td>
<td>1080</td>
<td>ND</td>
<td>0.643 ± 0.880</td>
<td>2.50 ± 2.88</td>
<td>5.15 ± 3.12</td>
<td>194 ± 239</td>
</tr>
<tr>
<td>Blood</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>0.004 ± 0.001</td>
<td>&lt;0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>0.006 ± 0.001</td>
<td>0.001 ± 0.000</td>
<td>0.003&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>ND</td>
<td>0.001 ± 0.001</td>
<td>0.006 ± 0.001</td>
<td>0.002 ± 0.000</td>
<td>0.007 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>ND</td>
<td>0.004&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.018 ± 0.003</td>
<td>0.010 ± 0.005</td>
<td>0.055 ± 0.031</td>
</tr>
<tr>
<td></td>
<td>1080</td>
<td>ND</td>
<td>0.008 ± 0.007</td>
<td>0.076 ± 0.091</td>
<td>0.036 ± 0.031</td>
<td>0.646 ± 0.993</td>
</tr>
<tr>
<td>Feces</td>
<td>0</td>
<td>0.004 ± 0.001</td>
<td>0.005&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.014&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>0.004&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.059 ± 0.027</td>
<td>0.188 ± 0.043</td>
<td>0.134 ± 0.051</td>
<td>0.007 ± 0.001</td>
<td>0.009 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.343 ± 0.085</td>
<td>0.450 ± 0.325</td>
<td>0.675 ± 0.526</td>
<td>0.031 ± 0.034</td>
<td>0.022 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>1.56 ± 0.47</td>
<td>1.96 ± 0.22</td>
<td>0.192 ± 0.064</td>
<td>0.020 ± 0.011</td>
<td>0.072 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>1080</td>
<td>14.1 ± 3.9</td>
<td>16.7 ± 5.8</td>
<td>3.64 ± 2.48</td>
<td>0.422 ± 0.089</td>
<td>1.37 ± 1.37</td>
</tr>
<tr>
<td>Urine</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>0.001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>0.002&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>&lt;0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.003&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>&lt;0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.011 ± 0.005</td>
<td>0.001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1080</td>
<td>0.004 ± 0.004</td>
<td>0.092 ± 0.138</td>
<td>0.001 ± 0.001</td>
<td>ND</td>
<td>0.0015&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values = mean ±SD for 3 males.
<sup>b</sup> ND = none detected.
<sup>c</sup> One dog, ND in two.
<sup>d</sup> Average two dogs, ND in one.
gestation 100, 100, 99 ± 2, 100; viability 93.8 ± 4.4, 92.8 ± 6.7, 94.8 ± 5.5, 92.8 ± 5.9; lactation 94.7 ± 4.1, 97.7 ± 2.1, 95.3 ± 3.4, 93.0 ± 5.3. Corresponding values for pups born per litter and weaned per litter were: born per litter 11.8 ± 3.0, 11.4 ± 2.7, 11.9 ± 2.9, 11.7 ± 2.9; weaned per litter 8.55 ± 2.66, 8.70 ± 2.60, 8.64 ± 2.4, 8.32 ± 2.94. Corresponding mean values for weaning weights(g) were: females 48.2, 49.8, 48.0, 48.4; males 50.8, 52.2, 50.6, 51.3. Apparent stillborn from the 6 matings were: 0 ppm 19, 5 ppm 33 (16 from one litter), 50 ppm 23, 500 ppm 11. Additionally, 4 litters were cannibalized, 2 on 0 ppm, 1 each on 50 and 500 ppm. No structural defect was noted in any pup. Histopathologic findings on F/3b offspring were entirely negative, except for minimal chronic lymphocytic infiltration of the bronchi in one control female. Thus, no adverse effects of PCNB were found in any of the above parameters.

Two-Year Study in Dogs

All dogs survived to sacrifice periods. Statistically significant differences between control and treatment groups did not occur in body weight gain or in food consumption. A few male dogs lost weight during the terminal weeks, but their scatter through the groups, including control dogs, bore no clear relationship to treatment.

**TABLE 4**

**BIOPSIED FAT CONCENTRATIONS OF PCNB, ITS METABOLITES (PCA, MPCPS) AND RELATED IMPURITIES (PCB, HCB) IN COWS**

<table>
<thead>
<tr>
<th>Diet (ppm)</th>
<th>In fat a</th>
<th>Ppm at indicated week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>PCNB</td>
<td>ND b</td>
</tr>
<tr>
<td></td>
<td>PCA</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>MPCPS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>HCB</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>PCNB</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCA</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>MPCPS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>HCB</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>PCNB</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCA</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>MPCPS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>HCB</td>
<td>ND</td>
</tr>
<tr>
<td>0.1</td>
<td>PCNB</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCA</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>MPCPS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>HCB</td>
<td>ND</td>
</tr>
</tbody>
</table>

a From one cow on each diet at each time period.
b ND = none detected.
c Interfering peak calculated as MPCPS.
d Probably due to contamination in handling.
Statistically significant differences in hematologic findings were limited to lower hematocrit values at 18 mo in males on 30 and 180 ppm diets, but at this same period hematocrit values for males on 1080 ppm were entirely comparable to those of the control males, and no comparably parallel differences were found for hemoglobin values.

Urinalysis revealed nothing remarkable throughout the study period.

### TABLE 5

**Cow Tissue Concentrations of PCNB, Its Metabolites (PCA, MPCPS) and Related Impurities (PCB, HCB) at 12 and 16 Weeks**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Diet (ppm)</th>
<th>Duration (wks)</th>
<th>PCNB</th>
<th>PCA</th>
<th>MPCPS</th>
<th>PCB</th>
<th>HCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (abdom.)</td>
<td>0</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.499</td>
<td>ND</td>
<td>0.001</td>
<td>0.698</td>
</tr>
<tr>
<td></td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.264</td>
<td>ND</td>
<td>0.004</td>
<td>0.785</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.005</td>
<td>0.017&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.034</td>
<td>ND</td>
<td>ND</td>
<td>0.102</td>
</tr>
<tr>
<td>Fat (subcut.)</td>
<td>0</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.018&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.381</td>
<td>ND</td>
<td>0.001</td>
<td>0.537</td>
</tr>
<tr>
<td></td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.237</td>
<td>ND</td>
<td>0.004</td>
<td>0.722</td>
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<tr>
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<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.019</td>
<td>ND</td>
<td>ND</td>
<td>0.030</td>
</tr>
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<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.054</td>
<td>ND</td>
<td>ND</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.010</td>
</tr>
<tr>
<td>Sk. Musc.</td>
<td>0</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
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<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.018</td>
<td>ND</td>
<td>ND</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.021</td>
<td>ND</td>
<td>0.004</td>
<td>0.070</td>
</tr>
<tr>
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<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
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</tr>
<tr>
<td></td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.006</td>
</tr>
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<td>0.1</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.010</td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
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<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>ND</td>
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</tr>
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<td>1</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.005</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Kidney</td>
<td>0</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.043</td>
<td>0.020&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.070</td>
<td>ND</td>
<td>0.001</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<sup>a</sup> One cow.
<sup>b</sup> ND = none detected.
<sup>c</sup> Average of two cows.
<sup>d</sup> Interfering peak calculated as MPCPS.
TABLE 6
Milk Concentrations of PCNB, Its Metabolites (PCA, MPCPS) and Related Impurities (PCB, HCB) in Cows

<table>
<thead>
<tr>
<th>Diet (ppm)</th>
<th>In milk</th>
<th>Ppm at indicated day(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>PCNB</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>PCA</td>
<td>ND(^b)</td>
</tr>
<tr>
<td></td>
<td>MPCPS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HCB</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>PCNB</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>PCA</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>MPCPS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HCB</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>PCNB</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>PCA</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>MPCPS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HCB</td>
<td>ND</td>
</tr>
<tr>
<td>0.1</td>
<td>PCNB</td>
<td>0.003</td>
</tr>
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<td>PCA</td>
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</tr>
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<td></td>
<td>MPCPS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PCB</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HCB</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) Averages from 3 cows.

\(^b\) ND = None Detected

\(^c\) Interfering peak calculated as MPCPS.
Blood chemical determinations showed significantly higher SGOT values in females on 1080 ppm at 3 mo compared with their controls (45.8 ± 5.7 vs 32.8 ± 5.8 Karmen units). At 12 mo, significantly elevated SAP levels as compared to controls were present in dogs on 1080 ppm in combined data for both sexes (2.10 ± 0.86 vs. 1.29 ± 0.18 Bessey-Lowry units), although not between data for the same sex. Statistically significant group differences were not found at 18 and 24 mo, but one male on 1080 ppm gave an SAP value of 11.5 units at 24 mo.

Estrus data for the study period revealed no treatment-related trends. Lymph node palpation findings were not remarkable.

In organ and organ-to-body-weight data, significantly (though barely so) higher values for livers of 1080 ppm dogs compared to controls constituted the most notable findings.

Histologic examination of tissues from dogs sacrificed at one year showed no treatment-relatable lesions. In dogs sacrificed at 2 years, cholestatic hepatosis with secondary bile nephrosis was found in minimal degree in dogs on 180 ppm and in moderate degree in dogs on 1080 ppm, and while relatable to the test material, it is considered to be a reversible phenomenon. Lower feeding levels were not involved.

Metabolic Studies

Apparent presence of traces of PCNB, its metabolites, and impurities were detected in some specimens from control animals. Possible sources are thought to be traces present in the feed and/or inadvertent contamination in handling.

Rats. Findings are summarized in Table 2. Although not shown, for each listed specimen, samples from control rats were also analyzed, with negative results except for occasional apparent traces of HCB and PCB.

Dogs. Findings are summarized in Table 3.

Cows. Findings are summarized in Tables 4-6. Additionally, no adverse effects of the test material were observed on the general health of the cows, food consumption, milk production, or necropsy findings.

DISCUSSION

As noted under Results, no potentiation of acute oral toxicity to rats resulted from combined administration of PCNB and Terrazole ®; the acute percutaneous LD50 of PCNB to rabbits was greater than 4 g/kg; and no adverse effects on any parameter of reproduction in rats appeared from diet concentrations of PCNB through 500 ppm.

Present findings on two-year feeding of PCNB to dogs establish 30 ppm as the highest no-effect level tested. As in our earlier study (Finnegan et al., 1958), the liver was the site of pathologic involvement: increased organ weight on 1080 ppm and cholestatic hepatosis with secondary bile nephrosis, in minimal degree on 180 ppm and in moderate degree on 1080 ppm, but was considered to be a reversible lesion.

A striking feature of the present metabolism studies is the absence of storage of PCNB in the tissues of the rat, dog, or cow (Tables 2–5), a few trace positives in the cow probably resulting from contamination in handling. As a further indication of this, no PCNB was found in abdominal or subcutaneous fat, kidney, liver, or muscle from an additional cow dosed at a level of 1000 ppm for one month. It is now clear that our earlier findings
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(Finnegan et al., 1958) of apparent storage in the fat of rats was due to the nonspecific nature of the analytical method used, namely, neutron activation of chlorine. On the other hand, the present data show storage of 2 impurities in the sample of PCNB used, pentachlorobenzene (PCB) and hexachlorobenzene (HCB), in degrees paralleling their concentrations in the diets. Their virtual absence from the urine of the dog (Table 3) and presence in bile and feces (Tables 2 and 3) indicate an enterohepatic circulation and excretion in the feces as the chief route of elimination of unmetabolized forms. Small amounts of PCNB are also excreted in the feces, virtually none via the urine, giving an overall picture of rapid metabolism.

In the cow, traces of apparent PCNB were found in the milk (Table 6), but this was also common to milk from the control cows. Also, in the cow dosed at 1000 ppm PCNB for one month the apparent concentration in the milk was no greater (ca. 0.002 ppm). It may be noted that analysis of the feed used showed apparent PCNB at levels of 0.002–0.006 ppm.

Data for the 10-ppm dosing level in Tables 4 and 6 indicate plateauing time for HCB in sc fat and milk to be at 4 weeks. In milk, HCB was concentrated in the fat portion. Withdrawal data for the rat (Table 2) indicate a decline in fat storage by about 80% in 60 days in animals that had been receiving 500 ppm in the diet.

Pentachloroaniline (PCA) has been identified as a metabolite of PCNB in the rabbit (Betts et al., 1955) and in the cow (St. John et al., 1965). That it is also a metabolite in the rat and dog has been established in the present study. Site of formation appears to be the liver with subsequent passage into the bile and excretion in the feces as the chief route of elimination (Tables 2 and 3). Small amounts were found in milk (0.006 ppm or less, Table 6) with plateauing time being virtually immediate. Acid hydrolysis of milk, kidney, and liver (cow) resulted in an increase in PCA, indicating the probability of conjugates, most likely with glucuronic and sulfuric acids (Williams, 1959).

During the course of this study, the presence of a new metabolic product of PCNB, methyl pentachlorophenyl sulfide (MPCPS), was established (Kuchar et al., 1969). For its presence and distribution in rat and dog tissues and excreta, see Tables 2 and 3. In the cow, apparent presence could be detected only in the one animal that was dosed with PCNB at 1000 ppm for one month, and then only in comparatively low degree: fat, 0.1 ppm; muscle, 0.01 ppm. One additional cow was dosed orally with MPCPS for 6 days at a level equivalent to 100 ppm in the diet. Milk collected on the fifth day contained 0.008 ppm. Subcutaneous and abdominal fat obtained at sacrifice on day 7 contained 0.011 and 0.012 ppm, respectively, and none was detected in the feces.

Lastly, the possibility that PCNB has tumor-initiating activity has been raised. Searle (1966) has reported multiple papilloma formation resulting from treatment of mouse skin with 0.2 ml of 0.3% PCNB in acetone twice weekly for 12 wk followed by 0.2 ml of 0.5% croton oil in acetone for 20 wk. He suggested that the tumor-initiating activity observed was due to hydroxylamine derivative formed as an intermediate in the metabolic reduction of the nitro group. Recently, Innes et al. (1969) reported an increase in hepatoma formation in mice receiving maximally tolerated amounts of PCNB (464 mg/kg by gavage from 7 to 28 days of age, followed by 1206 ppm in the diet), and sacrificed at 78 wk. Findings in the present study do not contribute pro or con to the matter of tumorigenicity. However, assuming adequacy of the above observations, the demonstrated rapid metabolism and lack of tissue accumulation of PCNB makes it
difficult to envision it as a tumor initiator per se. However, the possibility exists in the study of Innes et al. (1969) that critical insult to the liver occurred before detoxication mechanisms had fully developed. The possibility of a metabolite involvement has been raised by Searle. Also, as shown in the present study, impurities associated with PCNB production are less susceptible to rapid metabolism and elimination.

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REFERENCES


