...y must be taken into consideration, there is little evidence to support it: administration of magnesium sulfate to rats under withdrawal had little apparent effect on behavior, weight, or excretion of magnesium in these rats; bone and muscle magnesium concentrations of morphine-treated rats were not statistically different from those of control animals. Nevertheless, it is not unusual to observe normal magnesium concentrations in tissues in the presence of either hypo or hypermagnesemia (Walser, 19...).

Since hypermagnesemia is often associated with an augmentation of magnesium clearance (McCance and Widdowson, 1939), the increase in magnesium excretion observed here could be explained on that basis. The augmentation in urinary magnesium excretion could also be due to the diuretic effect of chronic morphine treatment, which is associated with an increase in sodium, potassium, calcium, and urea excretion (Marchand and Denis, 1968); it is well known that an osmotic diuresis causes an augmentation in urinary magnesium excretion (Walser, 1967). Since morphine was administered in the form of the sulfate, the anion could be responsible for the increase in urine output (Walser and Browder, 1959). However, it has previously been shown that nalorphine ...cks the diuretic effect of chronic morphine administration (Marchand ... Denis, ...68).

ACKNOWLEDGMENTS

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Dermal Toxicity Studies of Technical Polychlorinated Biphenyls and Fractions Thereof in Rabbits

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Dermal Toxicity Studies of Technical Polychlorinated Biphenyls and Fractions Thereof in Rabbits. Vos, J. G., and Beems, R. B. (1971). *Toxicol. Appl. Pharmacol.* 19, 617–633. A significant difference in toxicity between 3 polychlorinated biphenyl (PCB) preparations was found in a prior study: Clophen A 60 and Phenoclor DP6 showing the highest, Aroclor 1260 the lowest, toxicity (Vos and Koeman, 1970). A subsequent study revealed the presence of tetra- and pentachlorodibenzofuran in Phenoclor and Clophen (Vos *et al.*, 1970). In the present study, application of 118 mg of the 3 PCB's (5 times per wk, for 38 days) on the back skin of rabbits also resulted in differences in toxicity.

PCB-induced skin lesions were hyperplasia and hyperkeratosis of the epidermal and follicular epithelium. Histopathology of the liver included centrolobular degeneration, centrolobular liver cell atrophy, focal necrosis, and cytoplasmic hyalin degeneration. Definite hyperplasia and hyperkeratosis of the follicular epithelium of the ear skin were seen after the topical application of fractions of Phenoclor and Clophen eluted from chromatographic columns with 25% diethylether in hexane. The fraction from Aroclor caused a minimal hyperplasia and hyperkeratosis of the follicular epithelium. PCB-induced kidney lesions were hydropic degeneration of the convoluted tubules and tubular dilatation with the presence of casts. This dilatation was demonstrated also by a significantly increased relative percentage of the diameter which corresponds to the space of Bowman. Moreover, thymus atrophy and lymphopenia were found. Fecal coproporphyrin and protoporphyrin excretion was increased.

Polychlorinated biphenyls (PCB) have been identified in tissues of fish and wildlife in many countries (Jensen, 1966; Holmes *et al.*, 1967; Holden and Marsden, 1967; Koeman *et al.*, 1967; Risebrough *et al.*, 1968; Koeman *et al.*, 1969; Jensen *et al.*, 1969; Duke *et al.*, 1970; Prestt *et al.*, 1970).

PCB preparations are extremely stable, oily fluids with very low aqueous solubility. They are used as lubricants, as heat transfer media, in protective coatings for wood, metal, and concrete, and for many other applications. Which of these applications has contributed to the present environmental contamination has not yet been established.

Inhalation and feeding experiments in rats with 65% chlorinated PCB resulted in liver injury (Drinker *et al.*, 1937; Bennett *et al.*, 1938). Liver damage and skin lesions have been described after inhalation, ingestion, and application to the skin using rabbits, guinea pigs, and mice (von Wedel *et al.*, 1943). Hydropericardium occasionally accompanied by abdominal edema was found in chicks fed PCB (McCune *et al.*, 1962; Flick

Vos and Koeman, 1970). Epithelial and follicular hyperplasia and hyperre found after application of PCB on the skin of rabbits (Adams *et al.*, taneous injection of 42% chlorinated PCB in rats, guinea pigs, and rabbits iver injury and skin lesions, which were essentially those of chlor ne l). Follicular hyperkeratosis is an important feature of the occupat l n as chloracne, which is characterized by the appearance of papules, come-ysts. It may develop after exposure to some highly chlorinated aromatic Examples are chlorinated naphthalenes (Greenburg *et al.*, 1939; Hambrick, y and Kligman, 1957) and chlorinated phenols (Hofmann, 1957; Bauer *et* iberg *et al.*, 1964). The active compounds in chlorinated phenols have been chlorinated dibenzodioxins (chlorinated diphenylene dioxides) which as contaminants in the synthesis of chlorophenols (Bauer *et al.*, 1961). (acute yellow atrophy), whether accompanied by chloracne lesions or not, bed in people engaged in the manufacture of the chlorinated hydrocarbons 14; Braun, 1955; Behrbohm, 1959). Also for the PCB's there are early re-patio chloracne (Jones and Alden, 1936; Schwartz, 1936). The present-CB does not seem to have led to this kind of difficulty.

nt difference in toxicity between 3 samples of commercial PCB preparations a previous study (Vos and Koeman, 1970), despite the marked resemblance romatograms and the mass spectra (Koeman *et al.*, 1969). One hundred per ty, centrolobular liver necrosis, and sc and abdominal edema were found s fed the samples of Clophen A60 and Phenoclor DP6. Hydropericardium d in nearly all chicks fed these PCB's, and was only occasionally seen in ith Aroclor 1260. Porphyria was found as a general PCB effect (Vos and 70).

thesis that in some technical PCB mixtures toxic factor(s) responsible emalike lesions and the liver necrosis were present has been the subject of . By means of column fractionation, gas chromatography, and mass spec-hniques, tetra- and pentachlorodibenzofuran were identified as the toxic e samples of Clophen A60 and Phenoclor DP6 (Vos *et al.*, 1970).

ults suggest that PCB's contaminated with polychlorodibenzofurans may chlo e and associated lesions when applied to the skin of experimental test the possibility the present study was undertaken.

METHODS

samples, which contain an average of 60% chlorine, were obtained m France (Phenoclor DP6), Bayer in Germany (Clophen A60 Lot No. 912434) nto in the United States (Aroclor 1260 Lot No. AK-3).

ale New Zealand rabbits, 5 mo old and weighing 2500–3050 g, were used in They received pelleted food (Cunicon 1, Trouw and Co., Amsterdam) and itum. The animals were distributed at random into 4 groups of 4 animals, eighed weekly.

of approximately 10 × 15 cm on the backs was clipped, the remaining hair with a shaving powder (90 g barium sulfide, 105 g zinc oxide, and 180 g the resulting small skin abrasions were allowed to heal for 3 days.

The 3 PCB's were dissolved in isopropanol (118 mg PCB/ml). One ml of PCB solution r of isopropanol as a control was dropped daily, 5 times a wk, on an area of 5 × 10 cm y means of a calibrated syringe. In order to minimize eventual ingestion of the PCB, e animals were held for 7 hr in restraining boxes, after which time they were returned o their wire cages (2 animals per cage).

In order to study the porphyrogenic action of the PCB preparations in the same abbits, pooled samples of the feces of the 4 groups were analyzed weekly for copro-orphyrin and protoporphyrin contents (Rimington, 1961), using a Beckman spectro-hotometer.

The animals which survived the 38-day test period (27 applications of 118 mg PCB) ere killed. At necropsy, performed on all animals that died or were killed, a gross athologic examination was carried out, including macroscopic examination in ultra-olet light using red fluorescence as an indication of porphyria. In order to get infor-ation on the fecal excretion of coproporphyrin and protoporphyrin of the individual imal, feces collected from the cecum were analyzed.

Hematologic examination included hemoglobin and hematocrit determinations, ukocyte and differential leukocyte counts. Clinical chemical determinations were ade of serum glutamic–oxaloacetic transaminase and glutamic–pyruvic transaminase eitman and Frankel, 1957) using a Beckman/Spinco 151 spectrocolorimeter.

The weights of body, liver, kidneys, spleen, heart, and adrenals were recorded. The in, liver, spleen, kidney, pancreas, ovary, heart, lung, thymus, mesenteric lymph ode, stomach, small intestine, cecum, large intestine, and skeletal muscle were fixed in % buffered formalin and embedded in Paraplast. Sections (7 μ and 2 μ) were stained th hematoxylin-eosin. For detailed histology, selected sections were stained with erds, Azan, PAS, Ziehl-Neelsen, Congo red, and thioflavine. Moreover, cryostat ctions of formalin-fixed livers and kidneys were stained with Sudan black. Thioflavine-ained sections, unstained Paraplast-embedded sections, and unmounted cryostat tions of liver, kidney and small intestine were examined in a fluorescence croscope.

Prompted by the observation of tubular dilatation in the kidneys in previous studies r PCB ingestion (McCune *et al.*, 1962; Vos and Koeman, 1970), measurements e made on random samples of capsules of Bowman and glomeruli in 2-μ kidney ctions. Measurements of one axis of the greatest diameter of both capsule and omerulus were made of 25 renal corpuscles in a straight traverse across the cortex of ch kidney. The relative percentage of the diameter which corresponds to the space of owman, as an index of dilatation of the nephron, was calculated from the measure-nts of each renal corpuscle.

An additional experiment was made with the chromatographic fraction (column ted with 25% diethylether in hexane) of the 3 PCB's, containing tetra- and penta-hlorodibenzofuran in the case of Phenoclor DP6 and Clophen A60 (Vos *et al.*, 1970), determine the dermal toxicity of this fraction. Aliquots of 600 mg of the 3 PCB mix-res were fractionated (Vos *et al.*, 1970). The 25% diethylether-hexane fractions were aporated and dissolved in 0.6 ml of ethanol. Four female albino rabbits (3 test animals d 1 control), 2 mo of age and weighing 1550–1750 g, were used as the experimental imals. A 0.2-ml portion of the ethanol solution was applied weekly, for 3 wk, to an rea of 4 cm^2 on the inside of the rabbit ear. The opposite ear served as a control. The

TABLE 1

COPROPORPHYRIN AND PROTOPORPHYRIN CONTENTS (μG/G DRY WEIGHT) OF POOLED SAMPLES OF FECES FROM RABBITS TREATED WITH PCB FOR 38 DAYS, AND OF CONTROL ANIMALS

Time of sample (days)	Coproporphyrin				Protoporphyrin			
	Phenoclor[a]	Clophen[a]	Aroclor[a]	Control	Phenoclor[a]	Clophen[a]	Aroclor[a]	Control
0	0.6	0.5	0.3	0.4	11.3	5.4	5.0	7.7
7	2.8	4.2	2.9	0.8	24.3	28.5	17.0	6.7
14	2.0	1.6	1.1	1.0	26.3	10.2	13.4	7.1
22	3.3	17.2	11.2	1.0	13.5	18.3	29.4	4.7
29	3.1	4.5	3.2	1.4	10.1	19.2	21.2	7.5
36	8.7	9.8	10.0	1.7	24.8	19.5	27.7	8.8

[a] Significantly different from controls, $P < 0.05$, according to the sign test.

TABLE 2

COPROPORPHYRIN AND PROTOPORPHYRIN CONTENTS (μG/G DRY WEIGHT) OF FECES OF KILLED RABBITS TREATED WITH PCB FOR 38 DAYS, AND OF CONTROL ANIMALS[a]

	Coproporphyrin				Protoporphyrin			
	Phenoclor	Clophen	Aroclor	Control	Phenoclor	Clophen	Aroclor	Control
	8.1	74.0[b]	4.7	2.9	28.8	39.0[b]	25.0	21.4
	144.1	33.2	31.5	2.5	126.9	30.4	23.3	7.5
	44.5[b]	30.5[b]	33.2	2.9	38.2[b]	49.5[b]	12.5	9.7
	58.0	90.0[b]	21.7	4.9	38.2	94.0[b]	32.4	11.7
Mean:	63.7[d]	56.9[d]	22.8[c]	3.3	58.0[d]	53.2[d]	23.3[c]	12.6

[a] Figures are the contents of feces, collected from the cecum, of the individual animals.
[b] Animals that died during the experiment.
[c] Significantly different from controls, $P < 0.05$.
[d] Significantly different from controls, $P < 0.01$.

animals were killed after 3 wk. Body and liver weights were recorded ear and the liver were stained with hematoxylin-eosin. Cryostat secti were also stained with Sudan black.

Statistical significance, on a one-tail significance level, was deter Wilcoxon test for 2 unrelated samples (van der Waerden, 1957).

RESULTS

Experiment with Commercial PCB Preparations in Rabbits

The skin, especially of the Clophen- and Phenoclor-treated a reddened after 2 days of PCB treatment. This redness was more prono of treatment, together with clear desquamation of the external epide reduced regrowth of hair. Thickening of the skin and prominent tra of the treated skin developed during the experiment.

FIG. 1. Fluorescence of porphyrins under ultraviolet light in livers from PCB-tr *1*, Aroclor; *2*, Clophen; *3*, Phenoclor; and *4*, control liver.

The results of the spectrophotometric determinations of fecal copr protoporphyrin are given in Table 1.

The test animals showed, in general, a gradual loss of weight. One I Clophen-treated animals died, with severe weight loss, after 10, 31, respectively.

At necropsy, carried out on all animals, it was found that 1 anim 3 test groups showed definite edema formation, with fluid in the abdom cavity, subcutis, and pericardium.

All test animals, except for 1 rabbit of the Clophen group that died treatment, showed fluorescence in ultraviolet light. Liver (Fig. 1) and h

TABLE 3

RESULTS OF THE HEMATOLOGIC EXAMINATIONS MADE IN KILLED RABBITS TREATED WITH PCB FOR 38 DAYS, AND IN CONTROL ANIMALS[a]

	N	Hemoglobin (g/100 ml)	Hematocrit (%)	Leukocytes (10^3/mm^3)	SGOT (U)	SGPT (U)
Treated animals	8	9.2 (6.1–12.7)	27.0 (20–44)	7.2 (3.3–11.1)[b]	10.7 (7.0–14.5)	47.1 (18–98)
Controls	4	9.0 (8.2–10.0)	33.3 (27–38)	15.0 (9.5–26.2)	8.2 (6.5–10.0)	23.8 (17–27)

[a] Mean values; the range is given in parentheses.
[b] Significantly different from controls, $P < 0.025$.

TABLE 4

ORGAN WEIGHTS AND ORGAN: BODY WEIGHT RATIOS OF KILLED RABBITS TREATED WITH PCB FOR 38 DAYS, AND OF CONTROLS[a]

Compound	N	Body (g)	Organ Weight (g)				
			Heart	Liver	Spleen	Kidneys	Adrenals
Phenoclor	3	2300 (1900–2900)	4.74 (4.16–5.78)	133 (85–169)	0.72 (0.21–1.55)	14.0 (13.1–14.6)	0.31 (0.24–0.34)
Clophen	1	2900	5.95	137	1.18	14.5	0.27
Aroclor	4	2312 (1950–2600)[b]	4.31 (3.88–5.00)	138 (106–215)	0.61 (0.33–0.92)	14.3 (12.7–16.5)	0.27 (0.23–0.37)
Control	4	2912 (2700–3100)	5.09 (4.60–5.95)	124 (112–152)	0.64 (0.52–0.76)	13.5 (12.0–15.1)	0.37 (0.28–0.43)
			Organ: body weight ratio (g/1000 g body weight)				
Phenoclor	3		2.07 (1.98–2.25)	58.5 (44.8–80.5)	0.28 (0.10–0.53)	6.2 (5.0–6.9)	0.13 (0.11–0.16)
Clophen	1		2.05	47.1	0.41	5.0	0.09
Aroclor	4		1.88 (1.63–2.22)	59.0 (45.0–82.6)	0.26 (0.17–0.42)	6.2 (5.2–6.7)[b]	0.12 (0.09–0.15)
Control	4		1.74 (1.55–2.02)	43.0 (37.8–56.2)	0.22 (0.18–0.25)	4.7 (3.8–5.1)	0.13 (0.09–0.15)

[a] Mean values; the range is shown in parentheses.
[b] Significantly different from controls, $P < 0.05$.

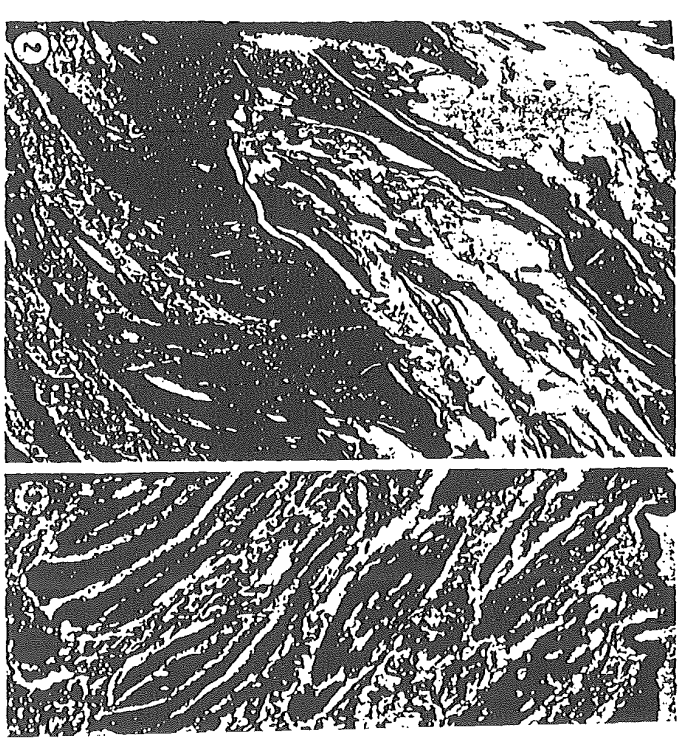

FIG. 2. Back skin of a killed rabbit from the Phenoclor-treated group. Hyperplas of the epidermal and follicular epithelium. Follicular plugging is prominent. Ne the cytoplasm of epidermal cells (*1*), and the resting (telogen) phase of the hair folli and eosin. ×46.

FIG. 3. Normal skin of back of a control animal treated with the solvent isopr dermis at *1* and dermis at *2*. Several groups of hair follicles, cut somewhat tangentia are seen at *3*; groups of hair bulbs are seen at *4*. Hematoxylin and eosin. ×46.

Coproporphyrin and protoporphyrin contents of feces collected fr necropsy are given in Table 2. The degree of excretion was not related porphyrin fluorescence: for example, the Clophen- and Aroclor-treat coproporphyrin excretion of 30.5 and 4.7 μg/g (Table 2), showed, resp re.cence at all, and fluorescence of liver, bone, incisors, kidneys, sm su xcutis.

The results of the hematologic determinations are given in Table 3. animals of the 3 groups have been taken together since there were n tween the groups. The total number of leukocytes was signific

was observed most frequently; fluorescence of incisors, kidneys, subcutis was found in about half of the test animals. There was no bone marrow. Fluorescent microscopic examination of cryostat s revealed that porphyric animals had porphyrin diffusely distribu whole lobule. Small foci inside parenchymal cells, probably nucle more intense red fluorescence. Kidney sections showed, when pr fluorescence, which was found in the cytoplasm of the tubules in r Fluorescence of granules (perhaps nuclei) in glomeruli was also n

animals. Mean SGOT and SGPT values were increased, but the large variation in individual values and the small number of animals made the apparent differences statistically indistinguishable. The differential count of leukocytes was not significantly altered.

Organ weights and organ:body weight ratios, are given in Table 4. A significant decrease in body weight and a significant increase in the relative weight of the kidneys was found in the Aroclor-treated animals. Mean absolute and relative liver weights of the test animals were increased, but the variation was also very high.

At microscopic examination of the skin, thickening of the skin due to hyperplasia and hyperkeratosis of the epidermal epithelium was apparent (Fig. 2). A pronounced hyperplasia and hyperkeratosis of the follicular epithelium was also present, with the formation of comedolike structures (hair follicles which become dilated and filled with a keratinous plug) (Fig. 2). These changes were found, in general, in all treated animals but the Phenoclor-treated animal which died after 10 days showed less advanced alterations. Follicular changes in the Aroclor group were generally less deep; the hyperplasia and hyperkeratosis were more restricted to the upper part of the hair follicles. Thickening of the surface and follicular epithelium was accompanied by the presence of large quantities of keratohyalin granules in the stratum granulosum. In some PAS-positive, diastase-resistant hyalin foci were seen in the cytoplasm of epidermal cells, located above the cutis papilla (Fig. 2). Dedifferentiation of the sebaceous gland tissue was found in primarily the Phenoclor and the Clophen groups. Hair follicles in the resting phase (telogen phase) were predominantly seen in the same 2 experimental groups (Fig. 2). The differences in response between Aroclor-treated rabbits on the one hand and the Clophen- and Phenoclor-treated rabbits on the other are quantitative and qualitative. No differences in response were found between the two latter groups. In control rabbits treated with the solvent isopropanol, these changes did not occur (Fig. 3).

Histopathology of the livers of the treated animals showed a considerable diversity in lesions (Figs. 4 and 5), among them centrolobular degeneration (degenerated cells with nuclear pycnosis, rhexis, and lysis), focal hydropic degeneration (foci of swollen cells with a light cytoplasm that did not stain with Sudan black and PAS, suggesting a pronounced cellular edema), focal necrosis (foci of necrotic cells that were found in various portions of the lobules), centrolobular liver cell atrophy (atrophy of the cytoplasm of liver parenchymal cells in the centrolobular area), cytoplasmic hyalin degeneration (foci of intracellular eosinophilic hyalin material), and pigment in Kupffer and to a lesser degree in parenchymal cells. The pigment stained positive with Ziehl-Neelsen, PAS and Perls and showed a yellow-brownish fluorescence; these are strong indications for ceroid (Porta and Hartroft, 1959). The results of the microscopic examination are given in Table 5. In general, PCB-induced liver lesions were most pronounced in the Clophen group and least in the Aroclor group. Periportal fibrosis was a common finding in the treated animals, but it was not found in the controls. Since some coccidia were found in a bile duct in 1 liver, it is quite possible that the fibrosis has to be considered as a healed coccidiosis. Material positive to Sudan black was seen in some fibrotic portal triades.

Kidney damage was found in all PCB-treated animals, though with a wide individual variation. Hydropic degeneration (negative to PAS and Sudan black) of the convoluted

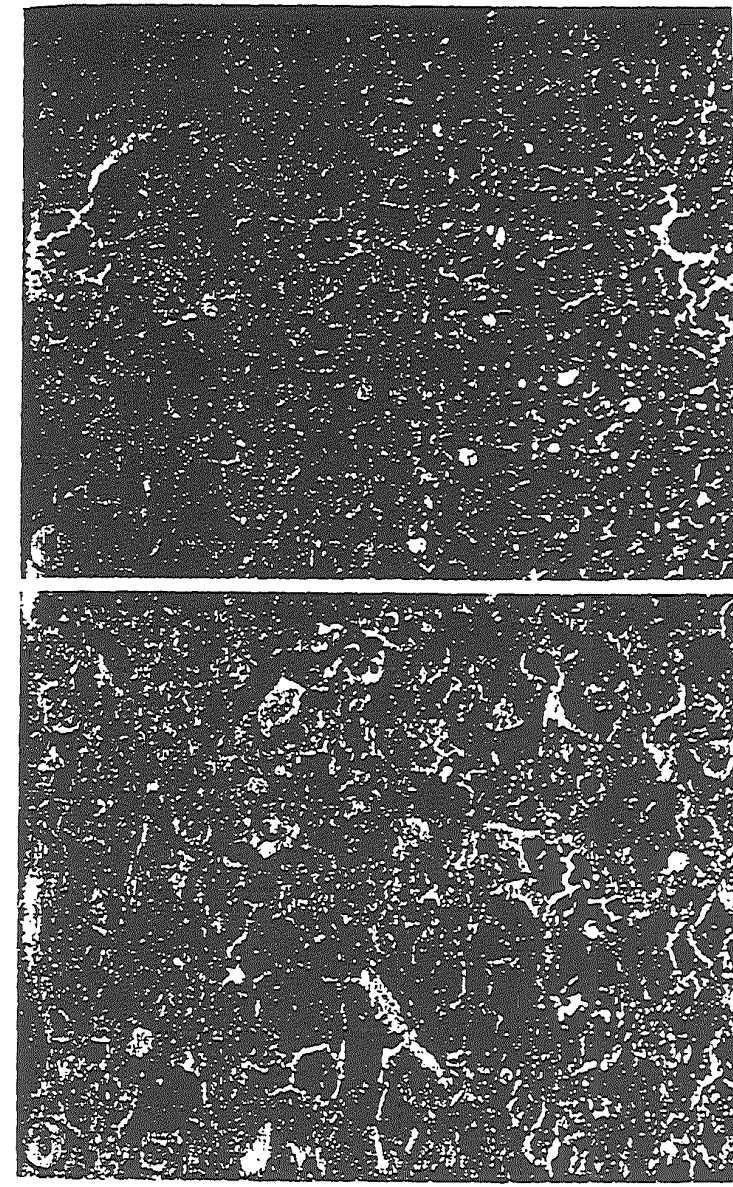

FIG. 4. Liver of a Clophen-treated rabbit that died after 31 days of treatment sho triad at *1*, and degeneration and atrophy of the cytoplasm of the hepatocytes in the ce at *2*. Hematoxylin and eosin. ×150.

FIG. 5. Higher magnification of the liver shown in Fig. 4. *1*: central vein; *2*: hydrop cytoplasmic hyalin degeneration. Note also the enlarged nuclei and the karyopycnos and eosin. ×375.

TABLE 5

PATHOLOGIC FINDINGS IN LIVERS OF RABBITS TREATED WITH PCB FOR 38 DAYS, AND OF CONTROL ANIMALS[a]

	Phenoclor				Clophen			Aroclor			Control
			b			b	b				
Fatty degeneration	2	2	1	1	1	1	—	—	—	1	2	—	—	—
Centrolobular loss of glycogen	—	1	2	1	2	1	2	—	—	1	—	—	—	—
Centrolobular degeneration	1	2	—	1	1	2	1	2	—	1	1	1	—	—	—
Focal hydropic degeneration	—	1	—	—	—	2	2	—	—	1	—	2	—	—	—
Focal necrosis	—	1	—	—	2	2	2	2	—	—	1	2	—	—	—
Centrolobular liver cell atrophy	2	1	1	2	2	1	2	1	—	—	—	1	—	—	—
Enlargement of nuclei	2	1	—	1	2	2	2	2	1	—	1	2	—	—	1
Cytoplasmic hyalin degeneration	1	—	1	2	2	1	2	2	—	—	—	1	—	—	—
Ceroid pigment in Kupffer cells	—	1	—	2	1	1	1	1	—	2	1	2	—	—	—
Periportal fibrosis	2	—	2	1	1	1	2	2	1	—	1	1	—	—	—

[a] Severity of lesion: 1 = slight, 2 = marked.
[b] Animals that died during the experiment.

tubules (Fig. 6) was found in half of the animals. Nuclear pycnosis, rhexis
the tubular epithelial cells were seen in all animals. Tubular dilatation, acc
the presence of casts of necrotic epithelial cells (Fig. 7), was found in half of

FIG. 6. Kidney of a killed rabbit from the Aroclor-treated group showing hydropic de
nuclear pycnosis of the convoluted tubules (*1*). A slightly distended space of Bowman
(*2*). Hematoxylin and eosin. ×375.

FIG. 7. Kidney section of the same animal as shown in Fig. 6, showing

TABLE 6

DIAMETER MEASUREMENTS (μ) OF RENAL CORPUSCLES OF KILLED RABBITS TREATED WITH PCB FOR 38 DAYS, AND OF CONTROL ANIMALS[a]

	N	Capsule of Bowman	Glomerulus	Relative % of the diameter corresponding to the space of Bowman
Treated animals	8	94.5 (76.4–105.8)	82.2 (69.4–96.2)	12.5 (8.1–22.1)[b]
Controls	4	91.5 (86.0– 96.5)	85.2 (79.4–90.4)	6.5 (5.0– 7.9)

[a] Mean values; the range is shown in parentheses.
[b] Significantly different from controls, $P < 0.005$.

The tubular lesions explain the results of the diameter measurements of the renal corpuscles (Table 6).

Hemosiderin was found in the splenic macrophages of the test animals in larger quantities than in the controls. Moreover, a reduction in the number of germinal centers in the spleens as well as in the lymph nodes was found in the PCB groups. Atrophy of the cortex of the thymus was a common finding in the PCB-treated animals. No evidence of PCB-induced damage was found in the sections of the other organs.

Experiment with the Diethylether-Hexane Fractions on the Ear Skin of Rabbits

The four animals did not differ in weight gain during the test period. After two weekly topical applications of the fraction from the 3 PCB samples, follicular keratosis was seen in both rabbits treated with the fraction of Phenoclor and Clophen. After 3 weeks, when the animals were killed, the changes were more apparent (Fig. 8). The polar fraction of Aroclor did not give rise to gross changes.

FIG. 8. Chloracne-like lesions on the inside of a rabbit's ear skin treated weekly, for 3 weeks, with the 25% diethylether in hexane fraction from Clophen. Note the follicular keratosis in the treated ear (1) as compared with the control (2).

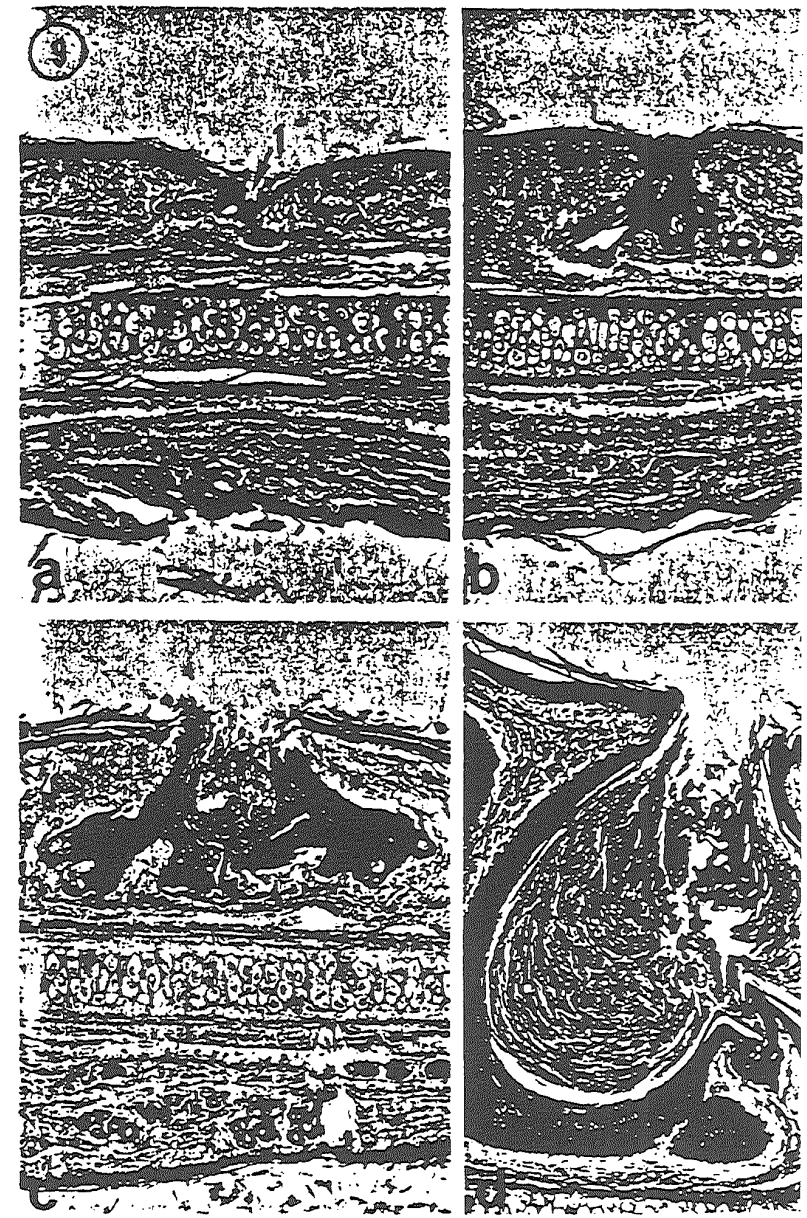

FIG. 9. Response of the inside of the rabbit's ear after topical application of the 25% c hexane fractions from technical polychlorinated biphenyls as seen in hematoxylin and eo ×60.

(a) Skin of control animal treated with ethanol. Note the hair follicle at 1, sebaceous glan and cartilage at 3. (b) Ear skin of the animal treated with the fraction from Aroclor. Some and hyperkeratosis of the follicular epithelium can be seen. (c) Ear skin of the rabbit trea fraction from Clophen. Considerable hyperplasia and hyperkeratosis of the follicular (d) Ear skin of the rabbit treated with the fraction from Phenoclor. Part of a section that sh severe lesion. The gravity of the response was in general the same as seen in Fig. 9c. Note t hair follicle with prominent hyperplasia and hyperkeratosis of the

Histologically, hyperplasia and hyperkeratosis of the follicular epithelium with presence of many keratohyalin granules (Fig. 10) were evident in the ear skin of rabbits treated with the Phenoclor and Clophen fractions (Fig. 9c and d). This comedone formation was topically most pronounced in the rabbit treated with the Phenoclor fraction (Fig. 9d). Sebaceous gland tissue was decreased by treatment with both fractions (Fig. 10). Only mild hyperplasia and hyperkeratosis of the follicular epithelium was noted in the animal treated with the fraction of Aroclor (Fig. 9b). Hyperplasia and hyperkeratosis of the epidermal epithelium were also seen in the rabbits treated with Phenoclor (Fig. 9d) and Clophen fractions but not in the animal treated with Aroclor fraction.

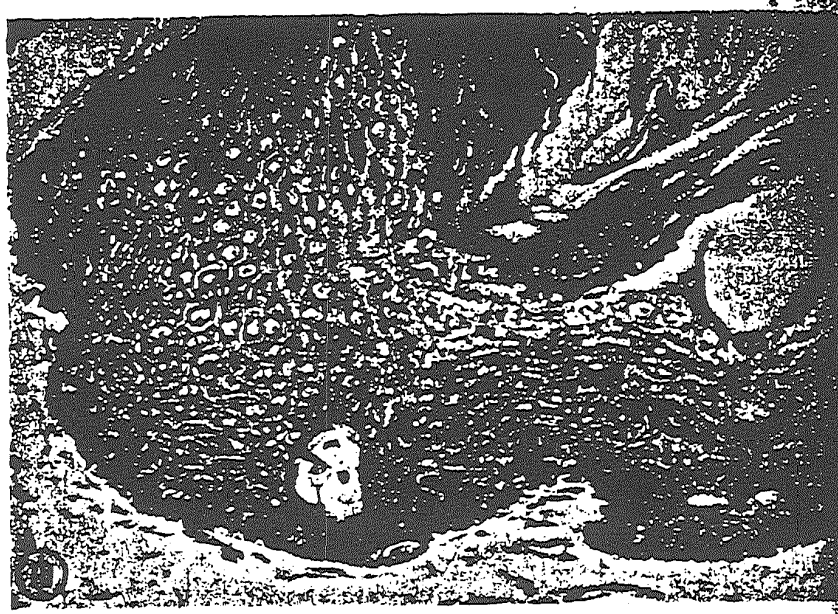

FIG. 10. Higher magnification of the ear skin shown in Fig. 9d. Hyperplasia and hyperkeratosis follicular epithelium with the presence of many keratohyalin granules (*1*). Note also a diminution differentiation) of the sebaceous gland tissue (2). Hematoxylin and eosin. ×375.

No red fluorescence was recorded at examination of the total body and of liver sections in ultraviolet light. Liver sections of the test animals showed centrolobular vacuolisation, which stained positive with Sudan black.

DISCUSSION

An increase in fecal excretion of coproporphyrin and protoporphyrin induced by PCB is as readily established in the rabbit (Table 1) as in the chicken (Vos and Koeman, 1970). Absence of porphyria in animals treated with the toxic fractions indicates that PCB itself is porphyrogenic.

Liver and kidney lesions, except the loss of glycogen, the enlargement of nuclei,

confirm the observations of Miller (1944). The lymphopenia (Table 3), the r number of germinal centers in the spleens and lymph nodes, the atrophy of the of the thymus, and the atrophy of the white pulp and lymphoid foci found in the of chicks fed PCB (Vos and Koeman, 1970) are strong indications for an immu pressive effect. This may need further study, particularly with regard to the ex which these observations are due to stress (release of glucocorticoids).

It can be concluded that dermal application of PCB mixtures causes, besides th on the skin, systemic lesions of liver, kidneys, and lymphoid tissue.

From the response of the back skin and the liver of the rabbit to the 3 PCB m and from the response of the ear to the 25% diethylether-hexane fractions, it concluded that there are definite quantitative differences in toxicity, at least betw samples that were used in the present and the prior studies. The extent to whic samples are representative of the normal commercial output has not been studi prior study (Vos *et al.*, 1970), it appeared that the fraction of Clophen A60 was sible for the toxicity of the PCB mixture, which also can be assumed to be tru case of our samples of Phenoclor and Aroclor. From the response of the ear ski fractions it can be concluded that the presence of tetra- and pentachlorodibenz in Clophen A60 and Phenoclor DP6, found by mass spectrometry (Vos *et al.*, 1 definitely proved. The presence of a smaller quantity of toxic impurity in Aroclo compared with the Clophen and Phenoclor samples, is likely, considering the pericardium found in chicks fed with Aroclor (Flick *et al.*, 1965) and also cons the present results. Preparation of pure PCB is therefore necessary for a comp study. Uncontaminated PCB should be available.

These findings suggest that chloracne lesions induced by chlorinated napht and chick-edema-like lesions in chickens (Pudelkiewicz *et al.*, 1959) may also b toxic impurities, as in the case of PCB's and chlorinated phenols (Bauer *et al.* Coal tar, which is an important source for naphthalene, may contain traces of d furan (Fieser and Fieser, 1956). Chlorination of technical naphthalene could th produce chlorinated dibenzofuran.

Since PCB is a porphyrogenic chemical, it is possible that the skin lesions in r to PCB are a combination of chloracne and acquired porphyria cutanea tarda. I *et al.* (1964) found an etiologic relationship between the chloracne in workers in the manufacture of dichlorophenol and trichlorophenol and acquired po cutanea tarda.

From the present and prior studies (Vos and Koeman, 1970; Vos *et al.*, 19 concluded that it is important to take into account, in the evaluation of toxic of PCB, the possibility that PCB samples may differ in an important respect: sence of toxic impurities. The interpretation of residue data in wildlife is very cated for this reason.

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