

# Toxicity and Fetotoxicity of TCDD, TCDF and PCB Isomers in Rhesus Macaques (*Macaca mulatta*)

by Wilbur P. McNulty\*

In rhesus macaques (*Macaca mulatta*), consumption of food containing commercial polychlorinated biphenyl (PCB) mixtures, some pure polychlorobiphenyl congeners, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) caused the same clinical toxic manifestations and histopathologic lesions, although the potencies of the toxicants covered a range of five orders of magnitude. Recovery from poisoning by 3,4,3',4'-tetrachlorobiphenyl (34TCB) or TCDF was rapid, whereas recovery from poisoning by Aroclor 1242, 3,4,5,3',4',5'-hexachlorobiphenyl (345HCB), or TCDD was protracted, if it occurred at all. 34TCB did not appreciably accumulate in body fat, but the level of 345HCB in fat rose steadily during ingestion. In one monkey, 25% of TCDD stored in fat after a single dose was still present after 2 years.

Among the symmetrical tetra- and hexachlorobiphenyl isomers tested, subacute oral toxicity could be demonstrated only for those without *ortho* chlorine substitutions. 34TCB and 345HCB were toxic at dietary levels of less than 1 ppm, but ingestion of food containing 2,5,2',5'-tetrachlorobiphenyl at 5 ppm, or 2,4,5,2',4',5'-, 2,4,6,2',4',6'-, or 2,3,6,2',3',6'-hexachlorobiphenyl at 15 or 65 ppm, caused no discernible deleterious effects.

The principal demonstrable histopathological lesions, bone marrow excepted, were metaplasias in some specialized epithelial structures, such as sebaceous glands, nail beds, gastric mucosa, ameloblast, and thymic corpuscles. These changes were interpreted as toxicant-induced, reversible redirection of differentiation. This aberration was wholly reversible. TCDD and 34TCB caused abortions when given in one or a few oral doses early in pregnancy. At the total doses used (1 or 5  $\mu\text{g}/\text{kg}$  of body weight for TCDD, 3 or 0.6 mg/kg of body weight for 34TCB), maternal toxicity was frequently apparent subsequent to the abortion.

## Introduction

Although toxicological research with nonhuman primates can be formidable because of the expense, scarcity, and complexity of husbandry of the animals, the use of monkeys for investigations with polychlorinated biphenyls and other polyhalogenated polyaromatic compounds offers certain attractive features. Since the sensitivities to and the manifestations of toxicity from these chemicals vary widely in different species of laboratory animals, at least one animal model that is biologically closely related to human beings is highly desirable—with the caveat that close biological relationship is not a guarantee of close toxicological similarity.

The large size and long life of monkeys permit studies of long-term exposure, uncomplicated by aging, during which repeated sampling of blood and tissue from individual animals can easily be done. On the other

hand, the long lifespan (for rhesus macaques, about 40 years) renders experimental carcinogenesis impractical.

Laboratory primates, at most a few generations from the wild, are outbred and individually variable; this attribute on the one hand reduces the statistical confidence in quantitative studies, while on the other provides the potential for investigations of genetically determined mechanisms of individual variation in toxicological response.

I will summarize here the published findings of the effects of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans in rhesus macaques (*Macaca mulatta*), and I will provide some unpublished data and a hypothesis that may suggest directions for future research.

## Similarities of Action and Relative Potencies of Individual Compounds

In rhesus macaques, the clinical and pathological findings (reviewed below) are indistinguishable after

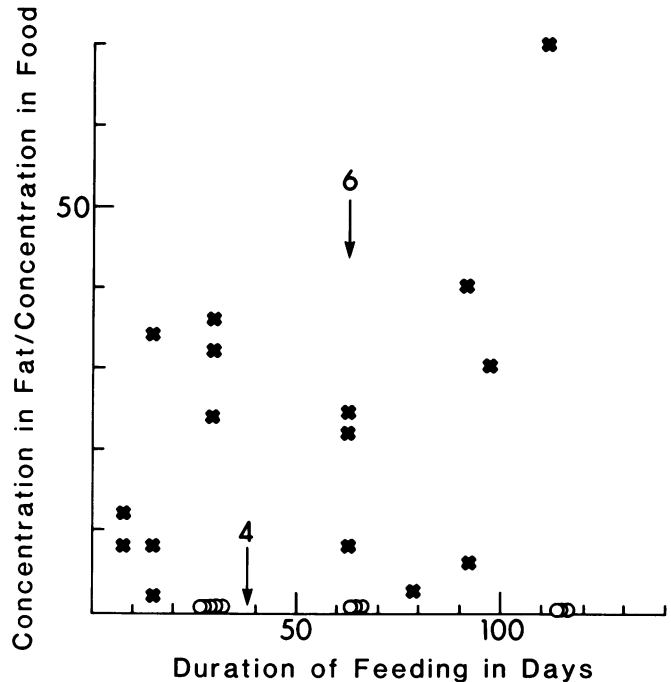
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**Table 1. Equitoxic levels of polyhalogenated polyaromatic compounds in the food of rhesus macaques.**

Compound	Polyhalogenated polyaromatic, ng/g food	Reference
Aroclor 1242	100,000	Unpublished
Aroclor 1248	25,000	(1)
Firemaster FF-1	25,000	(2)
3,4,3',4'-Tetrachlorobiphenyl	1,000	(3)
3,4,5,3',4',5'-Hexachlorobiphenyl	1,000	Unpublished
2,3,7,8-Tetrachlorodibenzofuran	50	(7)
2,3,4,6,7,8-Hexachlorodibenzo- <i>p</i> -dioxin	5	(6)
2,3,6,7-Tetrachlorodibenzo- <i>p</i> -dioxin	0.5-2	(4,5)

continued consumption of food contaminated with the polychlorinated biphenyl (PCB) mixtures Aroclor 1242 and 1248, the polybrominated biphenyl mixture Firemaster FF-1, 3,4,3',4'-tetrachlorobiphenyl (34TCB), 3,4,5,3',4',5'-hexachlorobiphenyl (345HCB), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,6,7,8-hexachlorodibenzo-*p*-dioxin, and 2,3,7,8-tetrachlorodibenzofuran (TCDF) (1-7). For convenience, I will lump these toxicants together as toxic halogenated aromatic compounds (THACs). The potencies of those THACs tested differ widely. Dietary levels that are "equitoxic" are shown in Table 1; the criterion for comparable toxicity is unequivocal clinical signs (red, swollen eyelids and elevated fingernails) after 1 month and severe morbidity or death after 2 months. The numbers shown are order-of-magnitude approximations based on observations in only a few animals; however, they merit some confidence, since feeding most of the compounds at 10-fold lower levels elicited less rapid and severe toxic signs that did not meet the criterion, even though toxicity at the lower level eventually was obvious. Aroclors 1242 and 1248 contain about 0.24 and 0.34% 34TCB (8), and hence this congener can account for some of the toxicity of the commercial mixtures. On the other hand, tetra-, penta-, and hexachlorodibenzofurans, present in commercial PCB mixtures at less than 1 ppm (9,10), cannot come close to accounting for the toxicity of Aroclors 1242 and 1248 for rhesus macaques.

However, comparisons of the chronic toxicities of THACs for monkeys is confounded by differences in pharmacokinetics and, presumably, metabolism. Remarkable differences in the rates of recovery were seen after oral intakes of different compounds were stopped. Rhesus macaques made severely toxic by food containing 34TCB or TCDF recovered after 2 months of normal diet. Therefore, these compounds cannot alone account for the toxicity of Aroclor 1242, because a monkey poisoned by continuous intake of this mixture at 400 ppm for 40 days died 13 months later, still showing all the characteristic lesions. None of six monkeys that ingested 3,4,5,3',4',5'-hexachlorobiphenyl recovered after intake stopped, and some pregnant rhesus females given an intragastric dose of TCDD (1  $\mu$ g/kg of body weight) showed toxic signs, and a few later died, 4 to 10 months after the dose (Table 2).



**FIGURE 1.** Ratio of concentration of PCB cogener in fat of rhesus macaques to concentration in food during continued ingestion: (○) 3,4,3',4'-tetrachlorobiphenyl (34TCB), dietary level 1 ppm; (×) 3,4,5,3',4',5'-hexachlorobiphenyl (345HCB), dietary level 0.5 ppm; (4) (with arrow) normal diet resumed after 38 days consumption of 34TCB-contaminated food; (6) (with arrow) normal diet resumed after 63 days consumption of 345HCB-contaminated food. Biopsy samples of abdominal subcutaneous fat were hexane-extracted and dried for lipid content; lipid samples were treated with sulfuric acid, washed, and extracted with hexane. The final extract was concentrated for PCB estimation by gas chromatography-mass spectrometry, selected ion monitoring (Dr. H. T. Cory).

Some preliminary observations on accumulation of these compounds in adipose tissue were in accord with the clinical observations. The ratios of the concentration of 34TCB and 345HCB in fat biopsy samples to the level in the diet during continuous feeding is shown in Figure 1. The half-life of 34TCB in adipose tissue of a young rhesus male following a single dose of 1 mg/kg of body weight, measured in serial biopsies over 1 month, was about 7 days. These data indicate that 34TCB was rapidly excreted while 345HCB was not. In one adult non-pregnant rhesus female given 1  $\mu$ g TCDD/kg of body weight in a single dose, about one-fourth of the TCDD initially stored in fat was still present 2 years later (11). I have made no measurement of TCDF tissue levels during and after chronic oral exposure, but TCDF given in a single dose to rhesus macaques had a half-life of 8 days (12). Thus it seems likely that, though the intracellular biochemical lesion or lesions may be similar or identical for all THACs, the effective whole-animal toxicity is strongly dependent on the pharmacokinetics of a given compound in a given species. A single dose of a slowly excreted compound probably provides a chronic exposure, since blood levels would presumably be maintained through contact with stored compound in sequestering tissues, most probably adipose tissue. Or,

**Table 2. Abortions and maternal toxicity after doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or 3,4,3',4'-tetrachlorobiphenyl (34TCB) to rhesus macaques in early pregnancy.**

Dose, $\mu\text{g}/\text{kg}$ body weight	Abortions <sup>a</sup>	Time to abortion after dose, days <sup>b</sup>	Maternal toxicity	Time to toxicity after dose, days	Maternal deaths	Time to death after dose, days
Vehicle O <sup>c</sup>	3/12	118 (1)	0/12	—	0/12	—
TCDD						
0.2 <sup>c</sup>	1/4	? (0)	0/4	—	0/4	—
1.0 <sup>d</sup>	13/16	20–60 (9)	8/16	67–207	3/16	130–297
5.0 <sup>c</sup>	2/2	27–28 (2)	2/2	30–61	2/2	65–116
34 TCB						
3150 <sup>c</sup>	2/2	17–29 (2)	2/2	41–49	0/2	—
630 <sup>c</sup>	4/4	20–34 (2)	4/4	31–38	0/4	—

<sup>a</sup>Pregnancy confirmed by products of conception or serum progesterone on days 20–22.

<sup>b</sup>Numbers of recoveries of products of conception in parentheses.

<sup>c</sup>Divided in nine intragastric doses in corn oil, days 20 to 40 postconception.

<sup>d</sup>Four animals given nine divided doses, 12 given a single dose, days 20 to 40 postconception.

in case of continued intakes at the same dietary level of compounds of differing degrees of excretability, tissue fluid levels would reach higher steady-state levels for the more persistent compounds.

## Structure–Activity Relationships

To the extent that they have been tested in monkeys, isomers of PCB show the same pattern of differing toxicities that have been reported in other species (13–15). The effects of long-term feeding rhesus macaques isomers of tetra- and hexachlorobiphenyls are shown in Table 3. The rule that “planar” isomers, without chlorine substitution *ortho* to the biphenyl bridge, are the most toxic, is followed strictly. Indeed, no toxicity could be demonstrated, either by clinical appearance or by histopathological examination, for isomers with two or four *ortho* substitutions at the listed durations of intake and dietary levels. Even though the levels of 2,4,5,2',4',5'- and 2,4,6,2',4',6'-hexachlorobiphenyls in adipose tissue reached 0.1 to 0.2% after 2 months of food containing 65 ppm of these isomers, the monkeys were clinically robust and active and showed none of the characteristic lesions at autopsy.

Furthermore, only the planar isomers induced elevated levels of aryl hydrocarbon hydroxylase, measured in liver needle biopsies (Fig. 2). Asymmetric isomers with only one *ortho* substitution, which have shown toxicity and 3-methylcholanthrene-type enzyme induction (16,17), have not been tested in nonhuman primates.

## Histopathologic Lesions

The histopathological changes found in rhesus macaques after acute and long-term oral intake of TCDD (4,18), long-term intake of Aroclor 1248 (1) and 34TCB (3), and long-term intake of TCDF (?) have been described in detail. Briefly, these have consisted of squamous metaplasia of sebaceous glands, elevation and loss of the fingernails, mucous metaplasia and growth disorder of the fundic gastric mucosa, atrophy of the thymus gland, metaplasia of the epithelium of the gallbladder and biliary ducts, dilatation without obstruction of the extrahepatic biliary tree, hyperplasia of the mucosa of the urinary bladder, and hypocellularity of the bone marrow.

I have found the same lesions in rhesus macaques continuously ingesting Aroclor 1242 or 3,4,5,3',4',5'-hexachlorobiphenyl. Indeed, it has not been possible

**Table 3. Toxicity of isomers of tetra- and hexachlorobiphenyls fed to rhesus macaques.**

Isomer	Dietary level, $\mu\text{g}/\text{kg}$	Number of animals	Duration of feeding, days	Time toxicity first noted, day	Deaths
3,4,3',4'-Tetrachlorobiphenyl	3 → 1 <sup>a</sup>	3	215	14–21	3
	1	5	38	27	1
2,5,2',5'-Tetrachlorobiphenyl	3 → 1 <sup>a</sup>	3	215	—	0
	1 → 5 <sup>b</sup>	5	200	—	0
3,4,5,3',4',5'-Hexachlorobiphenyl	0.1	1	127	117	1
	0.5	4	63	28–30	4
	1	1	57	20	1
2,4,5,2',4',5'-Hexachlorobiphenyl	15	4	122	—	0
	65	1	63	—	0
2,4,6,2',4',6'-Hexachlorobiphenyl	15	4	122	—	0
	65	1	64	—	0
2,3,6,2',3',6'-Hexachlorobiphenyl	15	4	122	—	0

<sup>a</sup>Dietary level reduced from 3 to 1  $\mu\text{g}/\text{kg}$  after 23 days.

<sup>b</sup>Dietary level raised from 1 to 5  $\mu\text{g}/\text{kg}$  after 133 days.

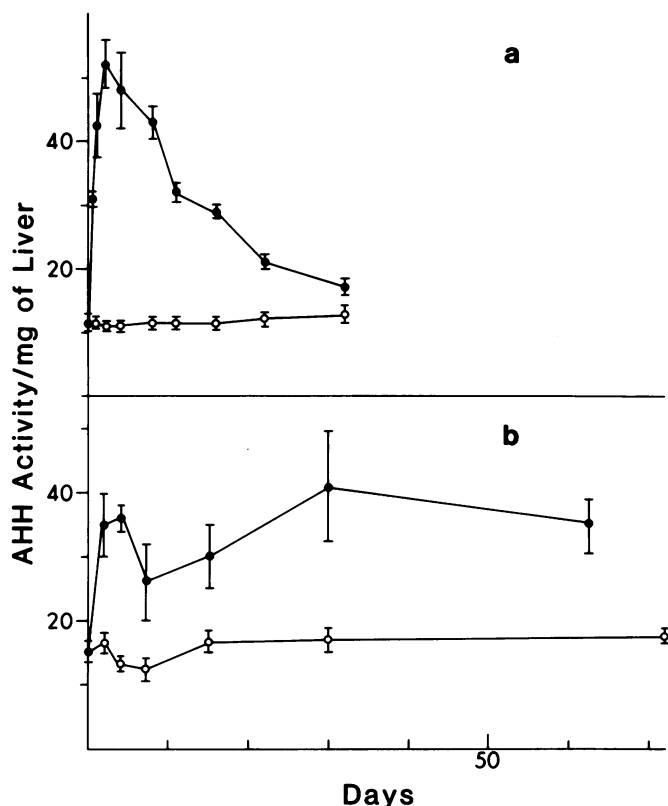


FIGURE 2. Hepatic enzyme induction by isomers of PCB. Hepatic aryl hydrocarbon hydroxylase, measured in liver needle biopsies (a) after a single oral dose, 1 mg/kg of body weight, of 3,4,3',4'-tetrachlorobiphenyl to three young male rhesus macaques, and (b) during continued feeding of food containing 0.5 ppm of 3,4,5,3',4',5'-hexachlorobiphenyl to three young male rhesus macaques.

at the autopsy table or the microscope to identify which THAC was the toxicant responsible for the lesions found.

These lesions are distinct from those seen in the laboratory rat, mouse and rabbit, in which hepatocytic damage (multinucleated cells, necrosis, adenofibrosis, proliferative nodules and neoplasms) have been described (19), and in which changes in sebaceous glands and stomach have not been reported, with the exception of one account of gastric metaplasia and neoplasia in rats chronically ingesting Aroclor 1254 (20).

Chloracne is a hallmark clinical sign of PCB and TCDD toxicity in people (21); it is characterized by comedones and small cysts. Monkey "chloracne" differs somewhat clinically, in that comedones are not seen and pustules are uncommon, but microscopically the lesions are identical in man and monkeys (Fig. 3), namely squamous atrophy of or squamous cyst formation from sebaceous glands. The usual presenting sign of toxicity in monkeys—reddening and thickening of the eyelids—is an expression of the same process; the luxuriant racemose sebaceous (meibomian) glands in the tarsus of the eyelid become converted to tubular squamous cysts, with occasional rupture and attendant inflammatory reaction. Similar clinical affectation of the eyelids was

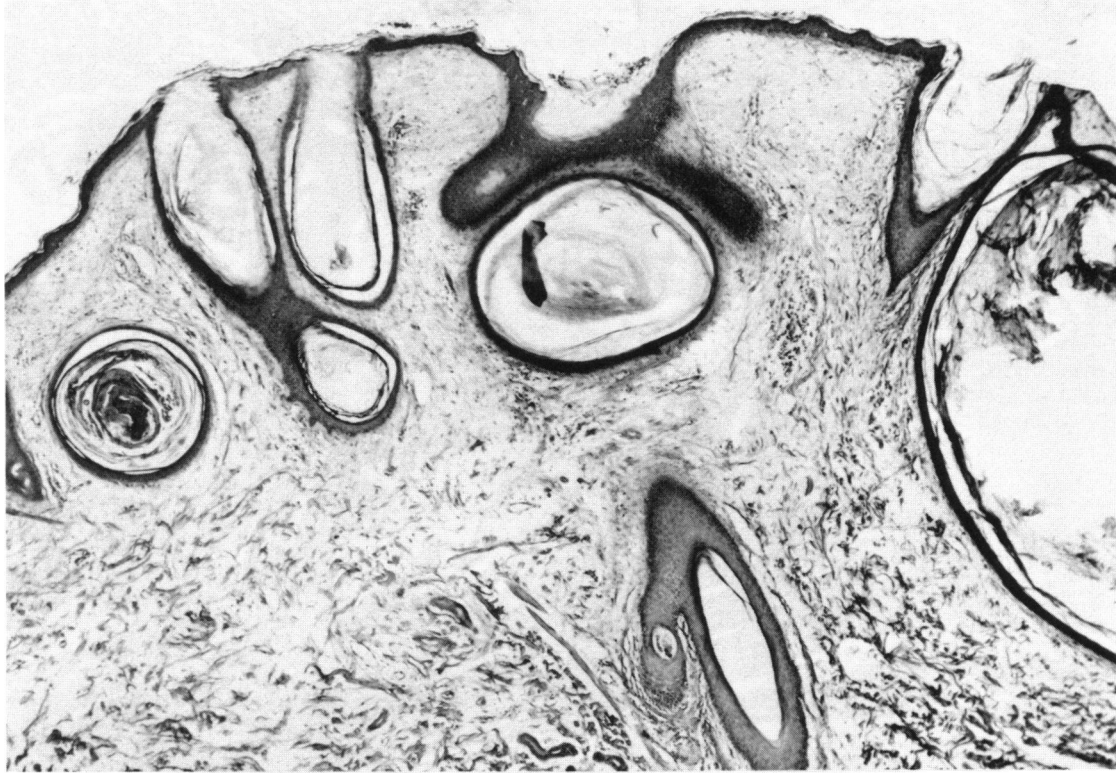
described in the victims of PCB poisoning in Japan (22) and Taiwan (23), and the resemblance of the eyelids in published pictures of Japanese victims to those observed in infant monkeys dying from a then unknown poison in 1967 led me to the suspect, and later verify, that the unknown poison was PCB (24).

The lesion in the gastric mucosa can be characterized as an interruption in the orderly process of replenishment of the parietal or acid-secreting cells and the chief or zymogenic cells of the gastric glands. Both the mucous cells lining the foveolae in the upper mucosa and the secretory cells in the deep glands are supplied from undifferentiated cells in the generative zone in the midportion of the mucosa; only here does DNA synthesis and mitosis occur, and the new daughter cells pass up or down, differentiating into the several types. During exposure to 34TCB, this process is deranged; preformed parietal cells degenerate, and mucous vacuoles appear among the zymogen granules of the chief cells (25). No new secretory cells are formed and DNA synthesis, assessed by radiothymidine pulse, appears throughout the mucosa, instead of being restricted to the midzone (26). Differentiation to mucous cells continues, and in fact mucus production is exaggerated; the cells are tall and mucous goblets exuberantly large. The normal architectural controls are lost, and mucous glands invade the submucosa and sometimes grow deep into the muscularis (Fig. 4). New antigens—carcino-embryonic antigen, large intestinal mucin antigen, and small intestinal antigen—become immunohistochemically demonstrable in the altered gastric mucosal cells with antibodies raised against human antigens (de Boer and McNulty, unpublished). In humans, these antigens appear in gastric cells only in neoplasms or in lesions considered to be preneoplastic (27).

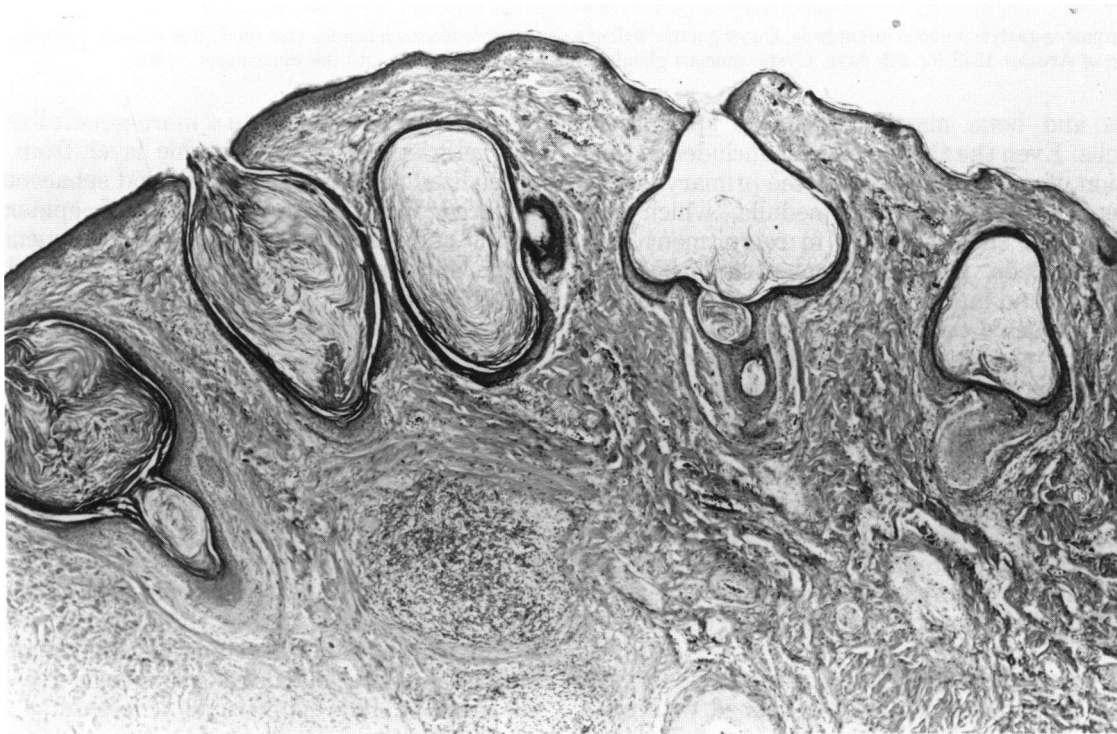
The fused keratinous substance of the fingernail is produced by keratinizing stratified squamous epithelium in the nail matrix—the half moon area at the base of the nail. The nail plate then slides forward over the nail bed without further contribution to its substance. The stratified epidermis of the bed is capped by a single layer of nonkeratinizing columnar cells that provide a substrate for the nail plate that is at once adherent and permissive of lateral sliding. The epidermis of the nail bed in rhesus macaques poisoned with 34TCB or TCDF becomes converted to desquamating, keratinizing epidermis, with the result that the nail plate is lifted away (and eventually mechanically broken off) by the keratin flakes accumulating beneath the plate (Fig. 5).

A hitherto undescribed change occurs in the ameloblast, the specialized enamel-secreting epithelium investing the crowns of unerupted teeth. This single layer of columnar cells, and its associated underlying stratum intermedium, become converted to nonkeratinizing desquamating oral epidermis. The accumulating cast-off squamous cells create cysts around the unerupted teeth, which can cause severe deformation of the jaws (Fig. 6).

All of these changes, with the exception of those in



(a)



(b)

FIGURE 3. Facial chloracne: (a) human (courtesy of Dr. K. D. Crow; exposure unknown); (b) young male rhesus macaque, after 32 days consumption of food containing 10 ppm 3,4,3',4'-tetrachlorobiphenyl. In both, sebaceous glands are not identifiable, and numerous keratin cysts are present in the dermis, some associated with hair follicles.  $\times 50$ .

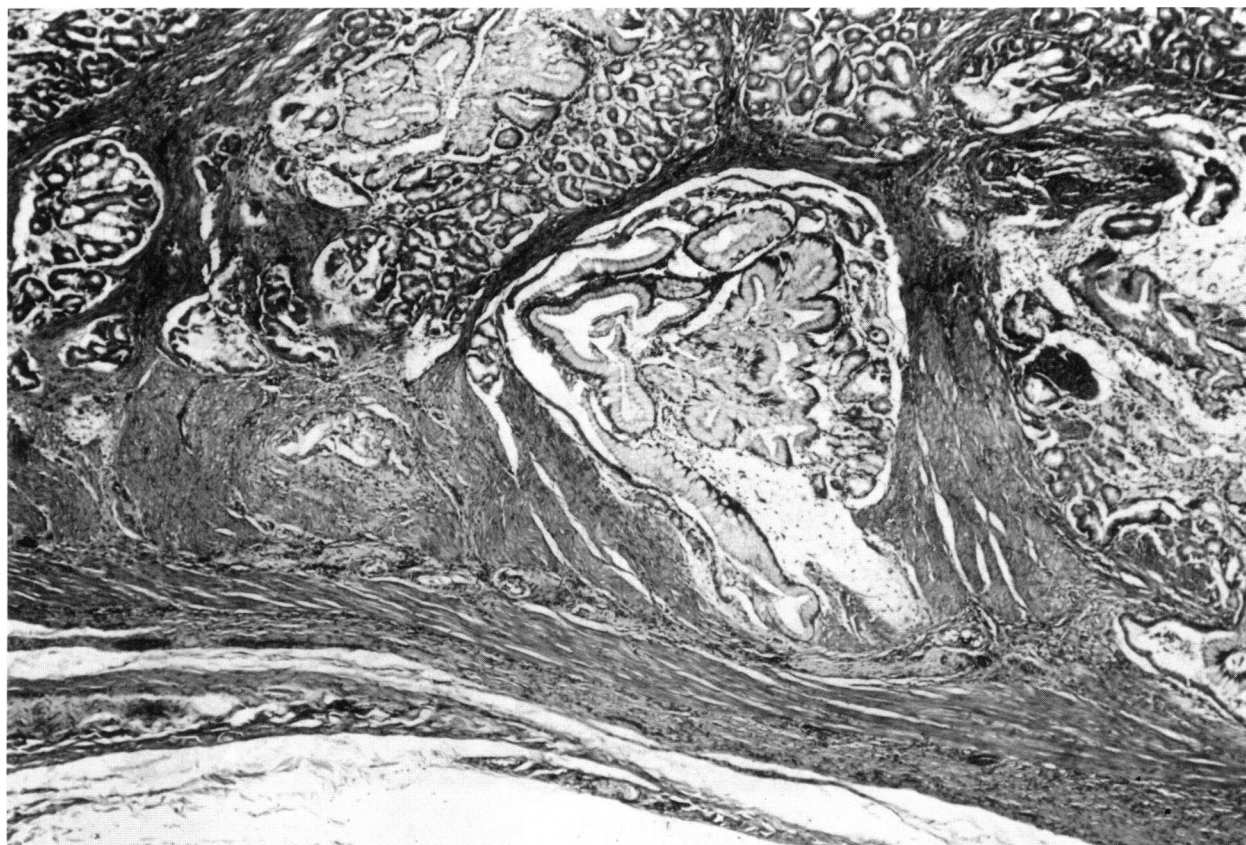


FIGURE 4. Invasive gastric mucous metaplasia. Outer gastric wall of a young male rhesus macaque that died after consuming food contaminated with 3 ppm of Aroclor 1242 for 245 days. Cystic mucous glands have penetrated deep into the muscularis.  $\times 50$ .

the thymus and bone marrow, occur in specialized epithelial cells. Even the thymus may be included in this generalization, if one speculates that the primary lesion is in the epithelial cells of the medulla, which are believed to play a secretory role in recruitment and processing of T cells. Hassall's corpuscles do become large and cystic, and mucous goblets can sometimes be found in the epithelial cells of the corpuscles (Fig. 7).

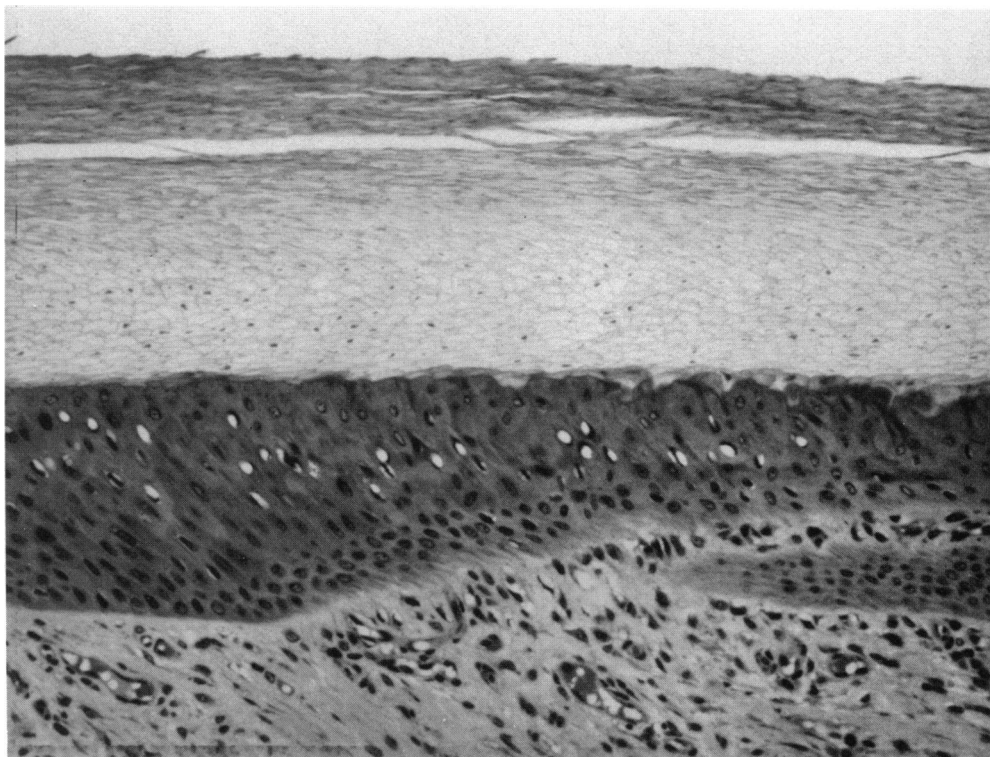
The epithelial changes are reversible. Histological examination of the stomach, skin, nailbeds, and thymus in those animals that recover from overt toxicity from 34TCB or TCDF discloses no residuals of the above lesions. It has been possible to document that lesions do disappear from the skin and nails of individual animals by serial biopsies and gross examination. This proof has not been provided for stomach and thymus, but no animal that has been examined at necropsy during overt toxicity has failed to show the typical changes in these organs.

During the recovery phase, the squamous cysts formed from the meibomian glands are first relined with conjunctival epithelium, and then by sebaceous epithelium (Figure 8).

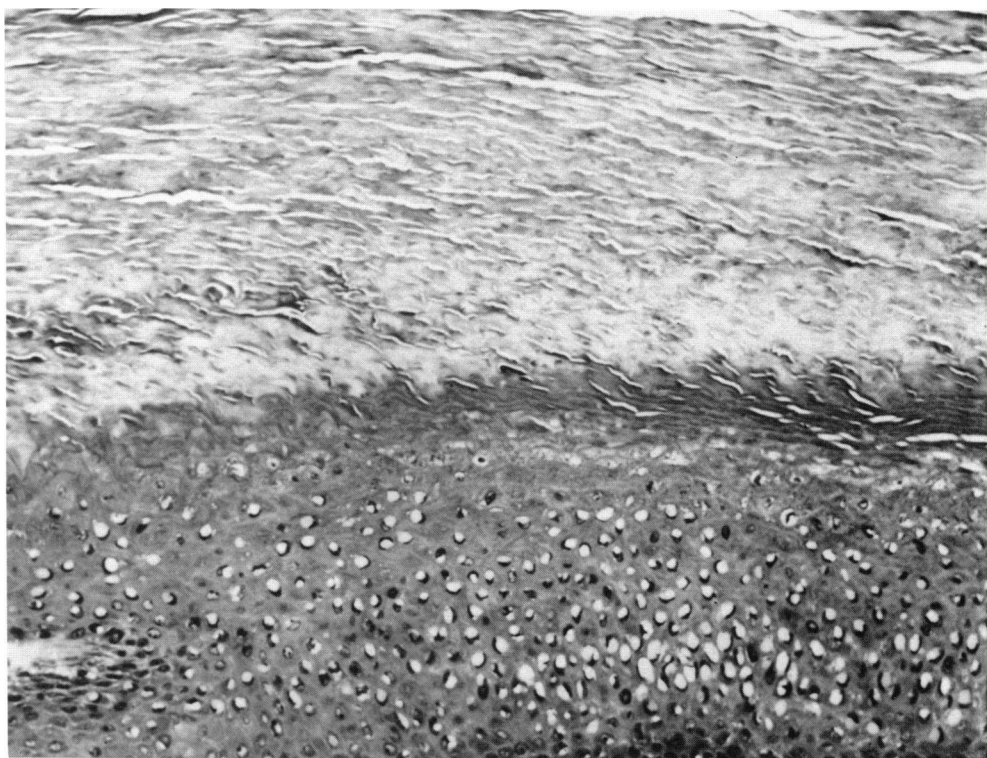
I therefore hypothesize that a principal effect of the toxic chlorinated polyaromatic compounds is a redirection of differentiation in certain specialized epithelia—

in each case a reversion to a more generalized cell type characteristic of the embryonic layer from which the specialized cells arose. Nailbed and sebaceous cells are replaced by keratinizing stratified epidermal cells, thymic and gastric cells are replaced by mucus-secreting cells, and ameloblast is replaced by oral stratified epithelium. Not all specialized cells appear to be affected, however. Sweat glands and ducts show no changes, and neither do hair follicles, although most pass into telogen phase. The odontoblast is not morphologically altered, although epidermal pearls and cysts appear throughout the bone of the jaws and in the periodontal tissue around erupted teeth; possibly these represent metaplastic activation of remnants of the dental lamina, which, during early embryological development, grows down into the bony jaws from the oral cavity to form the tooth buds. The mucosa of the intestine is not affected at the light microscopic level, except for Brunner's glands in the duodenum. The epithelium lining bile ducts, pancreatic ducts and bronchi seems to show hypertrophy of individual cells and an increase in the number and size of mucous goblets, but the change is qualitative and somewhat subjective.

These morphologically discernible effects on differentiation surely are not the only action of THACs. They cannot explain why monkeys lose weight and die. The

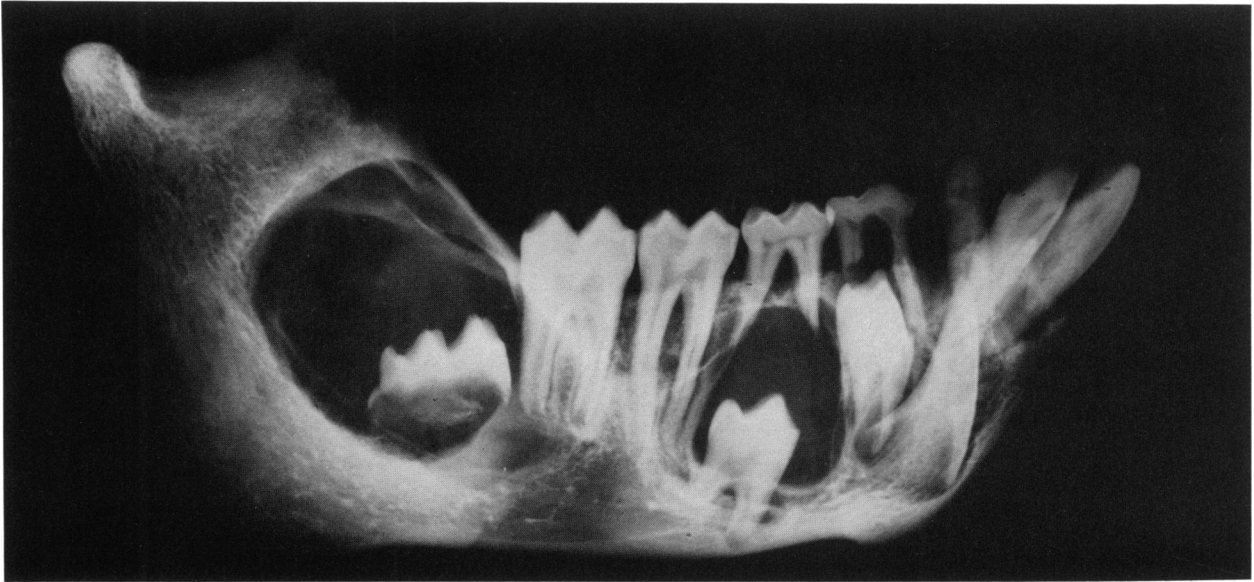


(a)



(b)

FIGURE 5. Nail beds of normal and PCB-poisoned young male rhesus macaques: (a) normal; stratified epithelium capped by "resting" cylindrical cells underlying nail plate; (b) after consumption for 62 days of food containing 1 ppm of 3,4,3',4'-tetrachlorobiphenyl; stratified epithelium is keratinizing, and accumulating keratin flakes are elevating nail plate.  $\times 200$ .



(a)



(b)

FIGURE 6. Cystic periodontal lesions in Aroclor 1242 poisoning: (a) radiograph of mandible, showing large radiolucencies surrounding unerupted second premolar and third molar, 13 months after consumption of food containing 400 ppm of Aroclor 1242 for 40 days; (b) squamous metaplasia of ameloblast surrounding unerupted tooth. The enamel has been dissolved away in decalcification; the amorphous material over the crown of the tooth is a packed mass of shed squamous cells.  $\times 10$ .



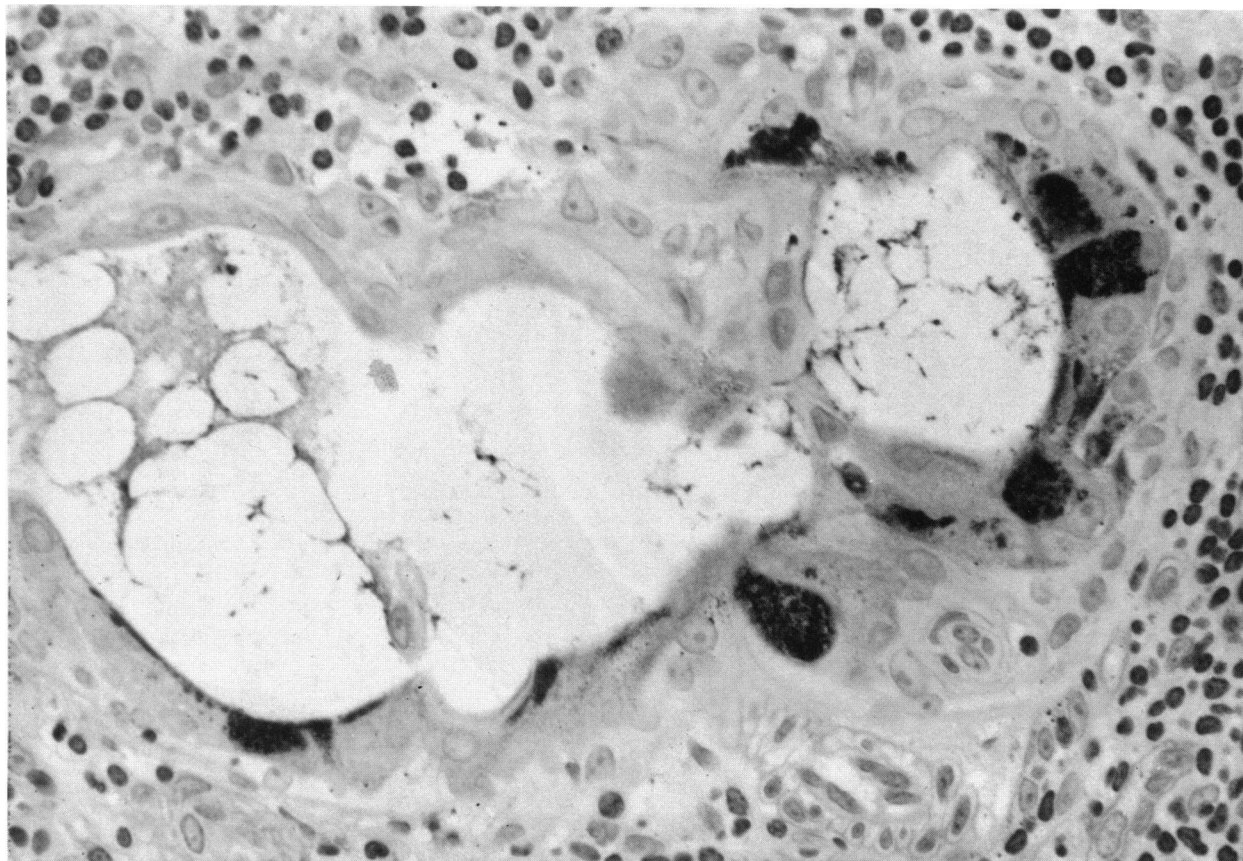


FIGURE 7. Mucous metaplasia in thymic corpuscles in PCB poisoning. Mucous goblets in epithelial cells of a cystic Hassall's corpuscle in the thymus of a young male rhesus macaque after consumption of food containing 0.5 ppm of 3,4,5,3',4',5'-hexachlorobiphenyl for 44 days. Periodic acid-Schiff stain,  $\times 500$ .

"epithelial redifferentiation" hypothesis does not encompass the bone marrow depression, which is the apparent immediate cause of death in some—but not all—monkeys. The severe systemic effects and ultimate lethality of THACs for monkeys remain a mystery; no vital organ (except the bone marrow) shows life-threatening morphologic alterations, and no serious physiologic dysfunctions of any organ have yet been indicated by significant derangements of blood chemical values.

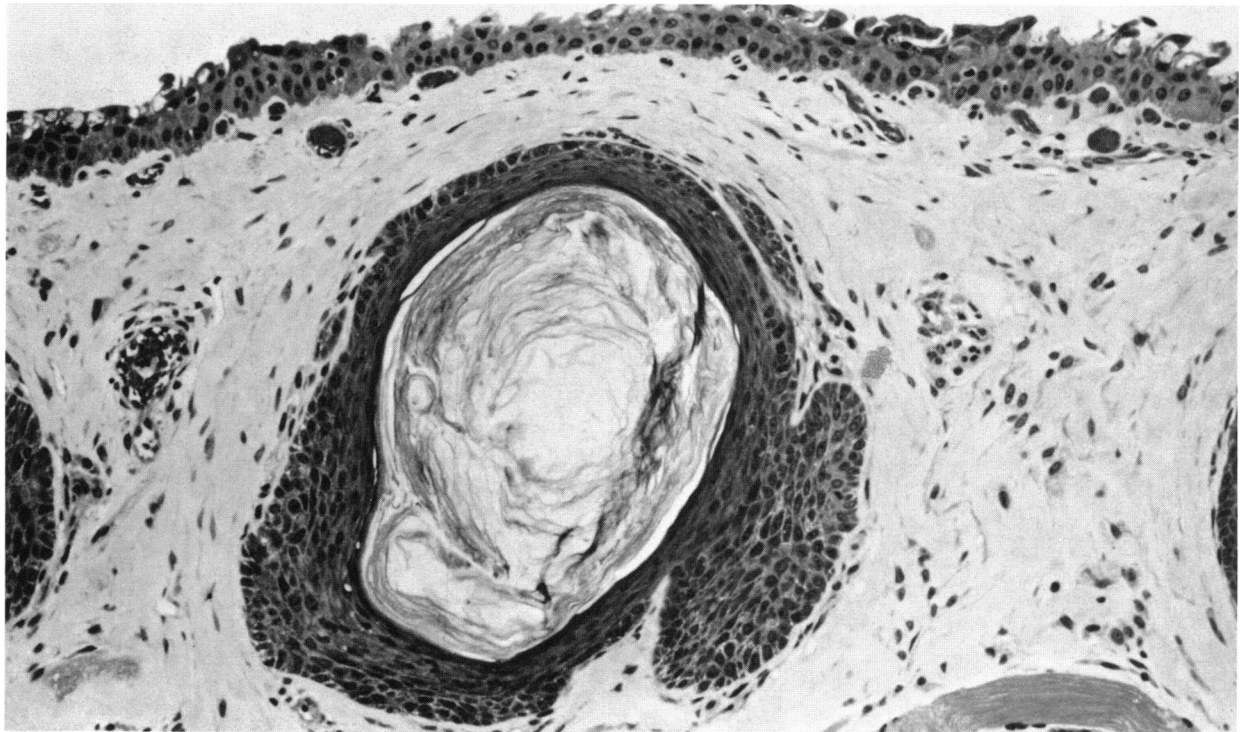
Nevertheless, the unique action of THACs on epithelial differentiation may provide a powerful probe into the very nature of differentiation itself, quite aside from potential threats to health from environmental dissemination of these compounds. From the point of view of cell biology, interest in THACs would not be diminished were they merely chemical curiosities on a laboratory shelf.

Some directions to be taken in future research are clear. Much more detailed morphologic studies, especially ultrastructural, should be made of the early events in both onset and regression of THAC-induced metaplasia. Fortunately, the largest single organ of the body—the skin—is a target for THACs in monkeys and is readily available for serial biopsies. Such biopsies can also provide material for biochemical studies. Cytosolic

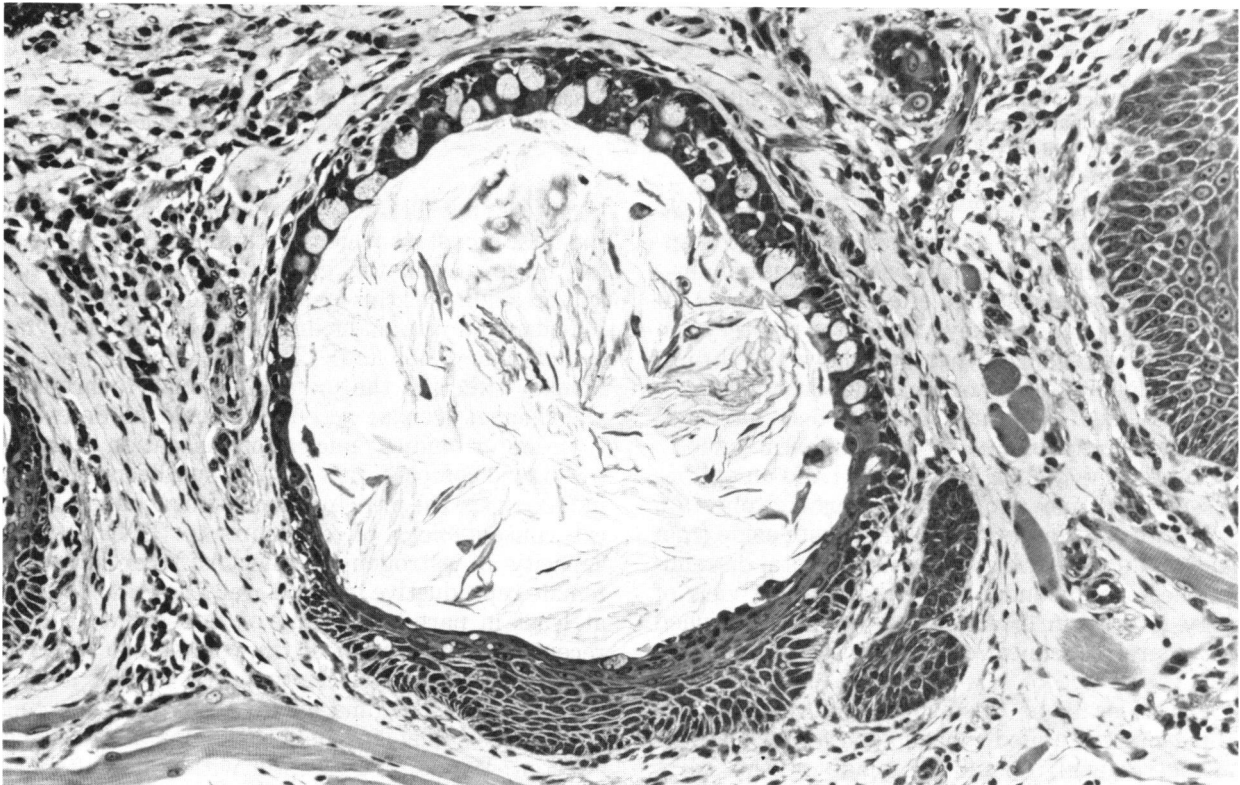
receptors for THACs are present in the tissues of rodents, receptors which on binding are translocated to the nucleus, and the genetically determined amount and/or avidity of this receptor is correlated both with toxicity and the inducibility of certain isozymes of cytochrome P-450 (28). The presence of receptor in monkey skin and the inducibility of cytochrome P-450 and monooxygenase activities should be determined.

Receptor binding, nuclear translocation of the receptor-ligand complex, new mRNA synthesis, and enzyme induction are the principal known intracellular biochemical consequences of exposure to TCDD (29). The capacity of estrogen to affect differentiation in the female reproductive tracts is well known to be mediated at least in part by binding of estrogen to a cytosolic receptor, translocation of the complex to the nucleus, and new mRNA transcription. If a similar mechanism obtains for TCDD (and all THACs), questions immediately arise: Why are only some kinds of epithelium affected? Why does a receptor with a high affinity for a xenobiotic exist, and what is its "natural" ligand? What new proteins, if any, besides isozymes of cytochrome P-450, are coded for by the new mRNAs, and what role do they play in differentiation?

Conceivably, the effects of THACs on differentiation

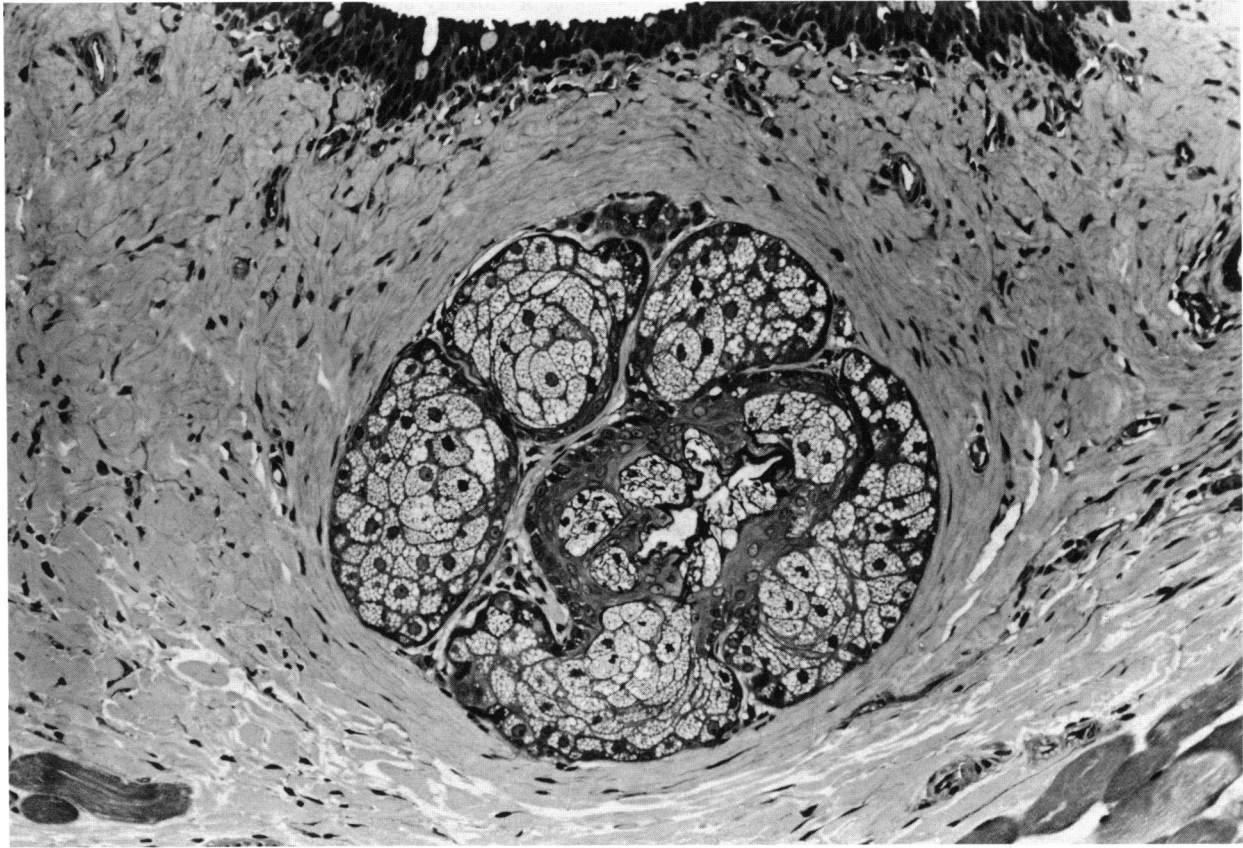


(a)



(b)

FIGURE 8. Resolution of PCB-induced squamous metaplasia of meibomian gland: (a) complete squamous metaplasia in young male rhesus macaque after consumption of food containing 1 ppm of 3,4,3',4'-tetrachlorobiphenyl for 33 days; (b) partial relining of meibomian gland with conjunctival epithelium after consumption of food containing 0.1 ppm of 3,4,5,3',4',5'-hexachlorobiphenyl for 127 days, then uncontaminated food for 133 days;



(c)

FIGURE 8 (con't). (c) complete restoration after consumption of food containing 1 ppm of 3,4,3',4'-tetrachlorobiphenyl for 38 days, then uncontaminated diet for 2 years.  $\times 200$ .

may be mediated solely through induction of isozymes of cytochromes P-450 for which there is little or no constitutive synthesis. Elevated levels of these isozymes might perturb the metabolism of substances known to play a part in differentiation, such as retinoids, prostaglandins, and steroids, or of substances not yet known.

### Fetotoxicity of TCDD and 34TCB

TCDD is fetotoxic and teratogenic for mice, rats, and rabbits (30-32), and is fetotoxic for rhesus monkeys (34) when given orally at 1  $\mu\text{g}/\text{kg}$  body weight in a single or divided doses between days 20 and 40 after conception (Table 2). None of 13 fetuses aborted in 16 pregnancies were recovered in a condition permitting dissection, and the proximate cause of fetal death was not determined. No malformations were found by radiography or complete dissection of the three infants that were not aborted. Eight of the 16 mothers that aborted showed clinical signs of TCDD toxicity, 67 to 207 days after the dose (or last dose) of TCDD, and 20 to 147 days after the abortion. Three of these eight animals showing toxicity subsequently died or were killed when moribund, 130 to

297 days after the last dose of TCDD. The findings at necropsy were characteristic of poisoning with THACs.

One abortion occurred among the four pregnant monkeys given 0.2  $\mu\text{g}/\text{kg}$  body weight, and none of the mothers showed signs of toxicity. The three infants were normal.

34TCB was also fetotoxic when given at 3150 or 630  $\mu\text{g}/\text{kg}$  of body weight in nine divided doses to six pregnant rhesus macaques between days 20 and 40 postconception (Table 2). As with TCDD, the aborted fetuses were either autolyzed or not recovered, and maternal toxicity appeared after the abortions. For purposes of comparison with continuous feeding experiments with 34TCB, doses of 3150 and 630  $\mu\text{g}/\text{kg}$  of body weight over 20 days correspond to dietary levels of about 10 and 2 ppm, respectively.

Thus, TCDD and 34TCB were fetotoxic at doses that caused significant morbidity and mortality in the mothers. However, these maternal effects became clinically manifest only after fetal death, and in some cases were never apparent. Since the rhesus fetus becomes independent of maternal hormonal (progesterone) support by 20 days after conception (35), it seems likely that TCDD and 34TCB had deleterious effects directly

on the fetus or placenta, but recovery of the products of conception before abortion will be required to establish this.

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## REFERENCES

- Allen, J. R. Response of the nonhuman primate to polychlorinated biphenyl exposure. *Fed. Proc.* 34: 1675-1679 (1975).
- Allen, J. R., Lambrecht, M. S., and Barsotti, B. A. Effects of polybrominated biphenyls in nonhuman primates. *J. Am. Vet. Assoc.* 173: 1485-1489 (1978).
- McNulty, W. P., Becker, G. M., and Cory, H. T. Chronic toxicity of 3,4,3',4'- and 2,5,2',5'-tetrachlorobiphenyls in rhesus macaques. *Toxicol. Appl. Pharmacol.* 56: 182-190 (1980).
- Allen, J. R., Barsotti, D. A., Van Miller, J. P., Abrahamson, L. J., and Lalich, J. J. Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Food Cosmet. Toxicol.* 15: 401-410 (1977).
- McNulty, W. P. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for rhesus monkeys: brief report. *Bull. Environ. Contam. Toxicol.* 18: 108-109 (1977).
- Allen, J. R., Van Miller, J. P., Barsotti, D. A., and Carstens, L. A. Toxicological responses of nonhuman primates to 2,3,4,6,7,8-hexachlorodibenzo-*p*-dioxin (HCDD). *Toxicol. Appl. Pharmacol.* 48: A179 (1979).
- McNulty, W. P., Pomerantz, I., and Farrell, T. Chronic toxicity of 2,3,7,8-tetrachlorodibenzofuran for rhesus macaques. *Food Cosmet. Toxicol.* 19: 57-65 (1981).
- Huckins, J. N., Stalling, D. L., and Petty, J. D. Carbon-foam chromatographic separation of non-*o-o'*-chlorine substituted PCBs from Aroclor mixtures. *J. Assoc. Off. Anal. Chem.* 63: 750-755 (1980).
- Bowes, G. W., Mulvihill, M. J., DeCamp, M. R., and Kende, A. S. Gas chromatographic characteristics of authentic chlorinated dibenzofurans; identification of two isomers in American and Japanese polychlorinated biphenyls. *Agr. Food Chem.* 23: 1222-1223 (1975).
- Albro, P. W., and Parker, C. E. Comparison of the compositions of Aroclor 1242 and Aroclor 1016. *J. Chromatogr.* 169: 161-166 (1979).
- McNulty, W. P., Nielsen-Smith, K. A., Lay, J. O., Jr., Lippstreu, D. L., Kangas, N. L., Lyon, P. A., and Gross, M. L. Letters to the Editor: Persistence of TCDD in monkey adipose tissue. *Food Chem. Toxicol.* 20: 985-987 (1982).
- Birnbaum, L. S., Decad, G. M., Matthews, H. B., and McConnell, E. E. Fate of 2,3,7,8-tetrachlorodibenzofuran in the monkey. *Toxicol. Appl. Pharmacol.* 57: 189-196 (1981).
- McKinney, J. D., Chae, K., Gupta, B. N., Moore, J. A., and Goldstein, J. A. Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. I. Relationship of chemical parameters. *Toxicol. Appl. Pharmacol.* 36: 65-80 (1976).
- Yoshimura, H., Yoshihara, S., Ozawa, N., and Miki, M. Possible correlation between induction modes of hepatic enzymes by PCBs and their toxicity in rats. *Ann. N.Y. Acad. Sci.* 320: 179-192 (1979).
- Biocca, M., Gupta, B. N., Chae, K., McKinney, J. D., and Moore, J. A. Toxicity of selected symmetrical hexachlorobiphenyl isomers in the mouse. *Toxicol. Appl. Pharmacol.* 58: 461-474 (1981).
- Parkinson, A., Robertson, L., Safe, L., and Safe, S. Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: Structure-activity rules. *Chem.-Biol. Interact.* 30: 271-285 (1980).
- Parkinson, A., Robertson, L., Uhlig, L., Campbell, M. A., and Safe, S. 2,3,4,4',5-Pentachlorobiphenyl: differential effects on C57BL/6J and DBA/2J inbred mice. *Biochem. Pharmacol.* 31: 2830-2833 (1982).
- McConnell, E. E., Moore, J. A., and Dalgard, D. W. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rhesus monkeys (*Macaca mulatta*) following a single oral dose. *Toxicol. Appl. Pharmacol.* 43: 175-187 (1978).
- Kimbrough, R., Buckley, J., Fishbein, L., Flamm, G., Kasza, L., Marcus, W., Shibko, S., and Teske, R. Animal toxicology. *Environ. Health Perspect.* 24: 173-184 (1978).
- Morgan, R. W., Ward, J. M., and Hartman, P. E. Aroclor 1254-induced intestinal metaplasia and adenocarcinoma in the glandular stomach of F344 rats. *Cancer Res.* 41: 5051-5059 (1981).
- Taylor, J. S. Environmental chloracne: update and review. *Ann. N.Y. Acad. Sci.* 320: 295-307 (1979).
- Urabe, H., and Koda, H. The dermal symptomatology of Yusho. In: *PCB Poisoning and Pollution* (K. Higuchi, Ed.), Academic Press, New York-San Francisco-London, 1976, pp. 105-123.
- Fu, Y.-A. Ocular manifestation of polychlorinated biphenyl (PCB) intoxication. *Arch. Ophthalmol.* 101: 379-381 (1983).
- McNulty, W. P., and Griffin, D. A. Possible polychlorinated biphenyl poisoning in rhesus monkeys (*Macaca mulatta*). *J. Med. Primatol.* 5: 237-246 (1976).
- Becker, G. M., McNulty, W. P., and Bell, M. Polychlorinated biphenyl-induced morphologic changes in the gastric mucosa of the rhesus monkey. *Lab. Invest.* 40: 373-383 (1979).
- Becker, G. M., and McNulty, W. P. Gastric epithelial cell proliferation in monkeys fed 3,4,3',4'-tetrachlorobiphenyl. *J. Pathol.* 143: 267-274 (1984).
- Ma, J., de Boer, W. G. R. M., and Nayman, J. Intestinal mucinous substances in gastric intestinal metaplasia and carcinoma studied by immunofluorescence. *Cancer* 49: 1664-1667 (1982).
- Poland, A., and Knutson, J. C. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Ann. Rev. Pharmacol. Toxicol.* 22: 517-554 (1982).
- Tukey, R. H., Hannah, R. R., Negishi, M., Nebert, D. W., and Eisen, H. J. The *Ah* locus: correlation of intranuclear appearance of inducer-receptor complex with induction of cytochrome P-450 mRNA. *Cell* 31: 275-284 (1982).
- Courtney, K. D., and Moore, J. A. Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 20: 396-403 (1971).
- Sparschu, G. L., Dunn, F. L., and Rowe, V. K. Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Food Cosmet. Toxicol.* 9: 405-412 (1971).
- Smith, F. A., Schwetz, B. A., and Nitschke, K. D. Teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in CF-1 mice. *Toxicol. Appl. Pharmacol.* 38: 517-523 (1976).
- Giavini, E., Prati, M., and Vismara, C. Rabbit teratology study with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ. Res.* 27: 74-78 (1982).
- McNulty, W. P. Fetotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for rhesus macaques (*Macaca mulatta*). *Am. J. Primatol.* 6: 41-47 (1984).
- Goodman, A. L., and Hodgen, G. D. Corpus luteum-conceptus-follicle relationships during the fertile cycle in rhesus monkeys: pregnancy maintenance despite early luteal removal. *J. Clin. Endocrinol. Metab.* 49: 469-471 (1979).