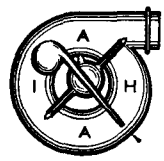
VOLUME 30, NUMBER 1

January-February, 1969

American Industrial Hygiene Association Journal



American Industrial Hygiene Conference
Denver Hilton, Denver, Colorado
May 12-16, 1969

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VOL. 30, NO. 1

Chronic Toxic Effects of Polyphenyl Mixtures

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Children's Hospital, Winnipeg 3, Manitoba, Canada

At a daily dietary intake of 350 mg/kg or more, Santowax OM, a mixture of terphenyls used as a coolant, causes severe and to some extent irreversible chemical nephrosis and interstitial nephritis in rats following prolonged ingestion. At a daily dietary intake of 33 mg/kg or more, an O.M.R.E. High Boiler sample, consisting of terphenyl and radiolytic and pyrolytic products thereof, induces a marked reticuloendothelial hyperplasia in rats following lengthy oral administration. This process is most marked in the liver and leads to an irreversible monolobular cirrhosis.

Introduction

CERTAIN POLYPHENYL COMPOUNDS have been used as moderator coolant in nuclear reactors for some years and some information has been gathered on the acute oral and inhalation toxicity of these compounds.[1,2,3,4] A recent investigation has indicated that prolonged ingestion of polyphenyl mixtures causes significant renal disease in experimental rats.[5] The same experiment has indicated that ingestion of the pyrolytic and radiolytic products of these polyphenyl compounds over a period of time gives rise to severe hepatic cirrhosis.

This paper reports a more detailed examination of the histopathology ensuing upon ingestion of Santowax OM and an O.M.R.E. High Boiler sample.

Methods and Materials

Santowax OM is a commercial organic coolant manufactured by the Monsanto Chemical Corporation. The O.M.R.E. High Boiler sample was an extract of the polyphenyl mixture used as a moderator and coolant in the Organic Moderator Reactor Experiment (O.M.R.E.) reactor at the National Reactor Testing Station (N.R.T.S.), Idaho Falls, Idaho and had been withdrawn from the reactor on October 9, 1961 having been irradiated for 152.7 megawatt days. The radioactive components were Co^{60}, Mn^{54}, and Cs^{137} in a concentration of less than 10^{-5} μci/gm of the sample. In mixing the High Boiler sample with rat chow, a dilution factor of 20 or greater was introduced. The concentrations of the isotopes in the made-up diets were therefore at least several orders of magnitude below the maximum permissible concentrations in water, $(MPC)_w$, given for these radionuclides in Table I of ICRP Publication 2.[6] Chemically this sample was composed almost completely of polyphenyls, triphenylenes and phenanthrenes together with alkylation, phenylation and hydrogenation products of these three types of aromatic compounds.[7] The compositions of the mixtures used in this study are detailed in Table I.

Two month old black hooded rats were used in the experiment and were fed on Purina rat chow. The rats were divided into eight groups, each containing nine male and nine female rats with the exception of the first group (Group A) which was composed

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of five male and six female rats. Groups A and B were control groups. Group A was fed chow pellets only and Group B was fed chow pellets and corn oil, equivalent to that consumed by the remaining groups. The composition of the diets together with the daily amount of polyphenyl ingested by the rats are outlined in Table II. Groups C, D and E were fed the diet B with the addition of 0.01, 0.1 and 1.0% by weight of Santowax OM, respectively. Groups F, G and H were fed diet B with the addition of 0.1, 1.0, and 5.0% by weight of O.M.R.E. sample, respectively. Three rats of each sex from the test groups were sacrificed on the 130th, 188th and 235th days, with the exception that the last three males and three females on diet E were put back on diet A from the 189th day until their sacrifice on the 235th day. This was done in order to assess the degree of reversibility, if any, of the renal lesions already discovered in the test groups previously sacrificed. The brain, stomach, gastro-intestinal tract, mesentery, liver, spleen, heart, lung, kidney, urinary bladder, testis or ovary, and bone marrow were obtained for microscopic examination.

Results

Santowax OM

In general, Santowax OM inhibits weight gain and leads to anemia, and these findings together with the results of a paired feeding experiment have already been published by Petkau and Hoogstraten.[5] Other morphological changes involved the kidneys and livers of test groups. Renal disease was present in animals on diets D and E whil gross hepatic disease was present in an mals on diets F, G, and H. In the presen study, the renal and hepatic lesions are de scribed in more detail.

The ingestion of 3 mg/kg/day for a perio of 235 days (Test Group C) produced n micropathological changes in any of the t sues examined.

A mean ingestion of 31 mg/kg/day (Te Group D) led to the accumulation of fi yellow-brown granules in the cytoplasm epithelial cells of the renal convoluted tubul and loops of Henle, but no other patholog This aggregation of granules was appare in a small proportion of the epithelial ce and was seen in animals sacrificed at 1 days. Those animals sacrificed at 188 a 235 days showed a progressive increase the number of cells so involved (Figure 1

The mean ingestion of 350 mg/kg/d (Test Group E) led to a pronounced d gree of renal disease. Although th

TABLE I*
Composition of the Polyphenyl Mixtures

Nonirradiated Mixture	Weight %	Irradiated Mixture	Weigh %
Santowax OM: Biphenyl	4.7	OMRE High Boilers:	
		High Boilers	> 98
o-Terphenyl	64.1	Biphenyl	< 0.
m-Terphenyl	25.1	o-Terphenyl	< 0.
p-Terphenyl	6.1	m-Terphenyl	< 0.
		p-Terphenyl	< 0.
		Inorganic	< 1

*From: Chronic Toxicity of Polyphenyl Mixtures, A. Pe kau, and J. Hoogstraten, *Amer. Ind. Hyg. Assoc. J. 26:* 3 (July-August 1965).

TABLE II
Composition of Control and Polyphenyl Test Diets

Group			Mean Amount of Polyphenyl Ingested (mg/kg/day)	
Control Diet	Test Diet	Diet Description	Male	Female
B		Pellets of Purina rat chow	0	0
A		Powdered Purina rat chow mixed with corn oil in the ratio of 2/1 (w/w)	0	0
	C	Diet B with 0.01 wt.% Santowax OM	3.0	3.5
	D	Diet B with 0.1 wt.% Santowax OM	31	37
	E	Diet B with 1.0 wt.% Santowax OM	350	409
	F	Diet B with 0.1 wt.% OMRE High Boilers	33	38
	G	Diet B with 1.0 wt.% OMRE High Boilers	331	386
	H	Diet B with 5.0 wt.% OMRE High Boilers	1832	2140

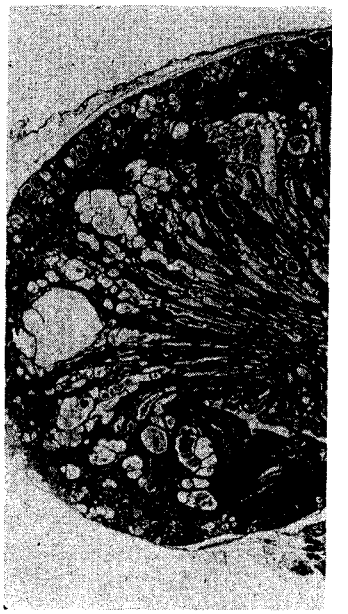

FIGURE 1. K mg Santowax/k dilatation of tul

FIGURE 2. K mg Santowax/l marked cystic d

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p-Terphenyl	6.1	m-Terphenyl	<
		p-Terphenyl	<
		Inorganic	<

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5 days showed a progressive increase
number of cells so involved (Figure 1
The mean ingestion of 350 mg/kg/d
'est Group E) led to a pronounced d
e of renal disease. Although th

Polyphenyl Test Diets

scription	Mean Amount of Polyphenyl Ingested (mg/kg/day) Male	Female
chow	0	0
chow mixed with io of 2/1 (w/w)	0	0
.% Santowax OM	3.0	3.5
% Santowax OM	31	37
% Santowax OM	350	409
% OMRE High Boilers	33	38
% OMRE High Boilers	331	386
OMRE High Boilers	1832	2140

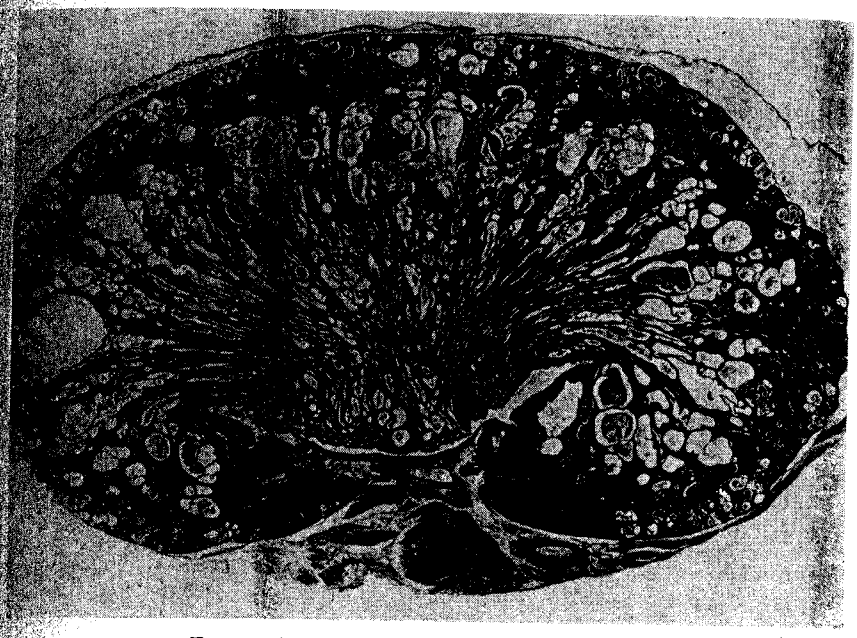

FIGURE 1. Kidney from animal on diet E (350 mg Santowax/kg/day) for 130 days showing cystic dilatation of tubules. × 15 H & E

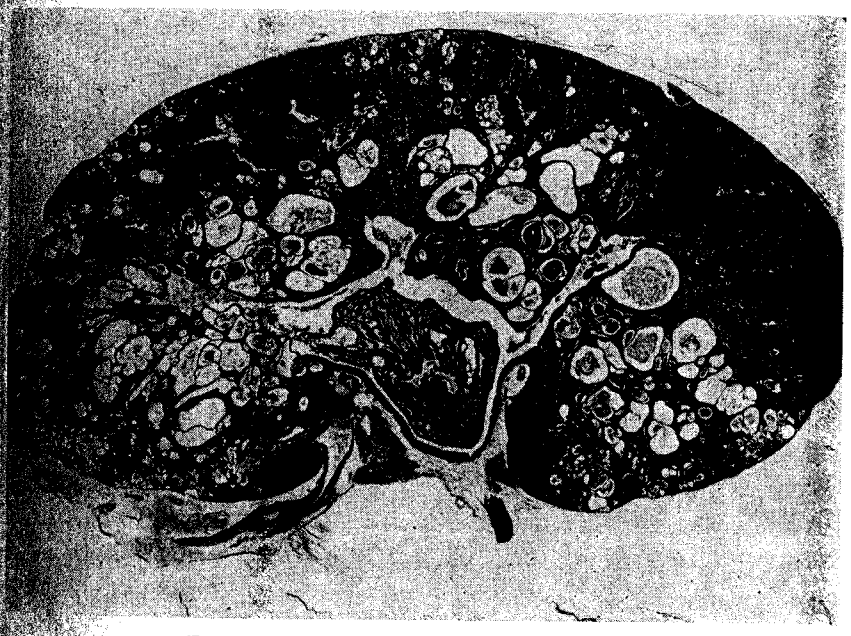

FIGURE 2. Kidney from animal on diet E (350 mg Santowax/kg/day) for 188 days with more marked cystic distortion of renal tissue. × 15 H&E

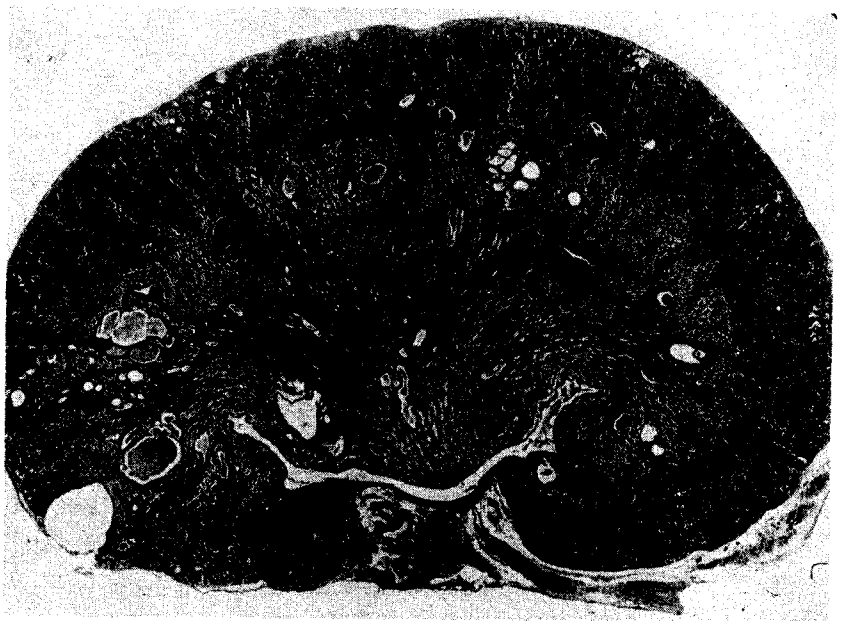

FIGURE 3. Kidney from animal on diet E (350 mg Santowax/kg/day) for 188 days then control diet A for 47 days. There has been regression of the cystic change although this is not complete, and considerable scarring is apparent in the cortico-medullary zone and pyramids. × 15 H & E

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weight did not differ appreciably from the weight of controls, the kidneys of the test animals had a finely granular cortical surface, an adherent capsule and a firm consistency. The cortex was diminished in depth, and small cysts in the region of the corticomedullary junction could be seen by the naked eye. These changes increased with continued ingestion of this concentration of Santowax OM to 188 days. In these kidneys, areas of cystic dilatation were seen extending through the full thickness of the organ from the subcapsular region to the apices of the pyramids. Dilatation was maximal in the region of the boundary zone. (Figures 1, 2 and 3.)

The lumen of the distal convoluted tubules, loops of Henle, and collecting tubules contained an amorphous eosinophilic material often possessing a yellow tinge. Histiocytes or effete remnants of neutrophil leukocytes were often embedded within the intratubular amorphous material which contained occasional colourless acicular crystals. (Figures 4 and 5.)

The epithelium of the dilated tubules w
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FIGURE
FIGURE 4

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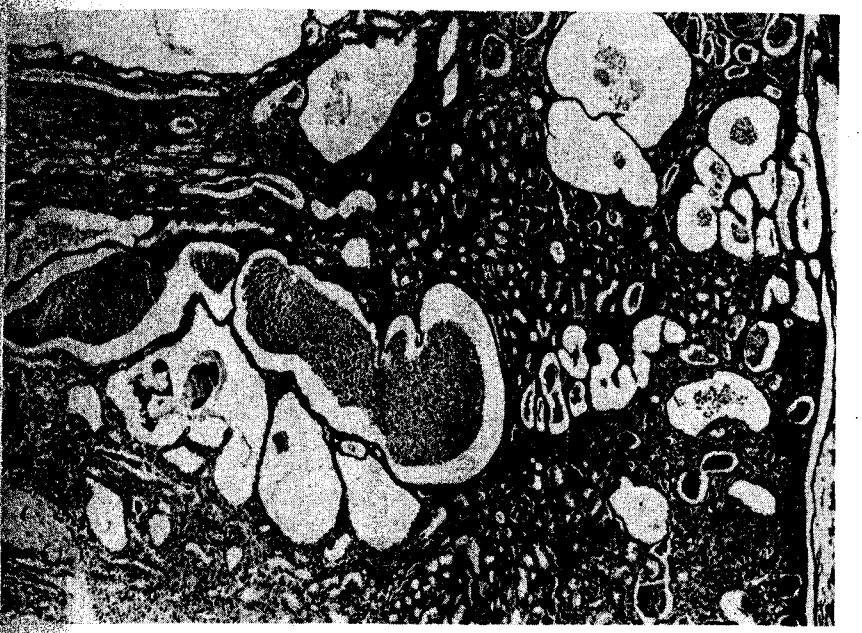

FIGURE 4. Kidney from animals on diet E (350 mg Santowax OM/kg/day) for 130 days. The dilated convoluted tubules, loops of Henle and collecting tubules contain masses of amorphous granular debris with remnants of histiocytes and polymorphonuclear leucocytes. × 100 H & E

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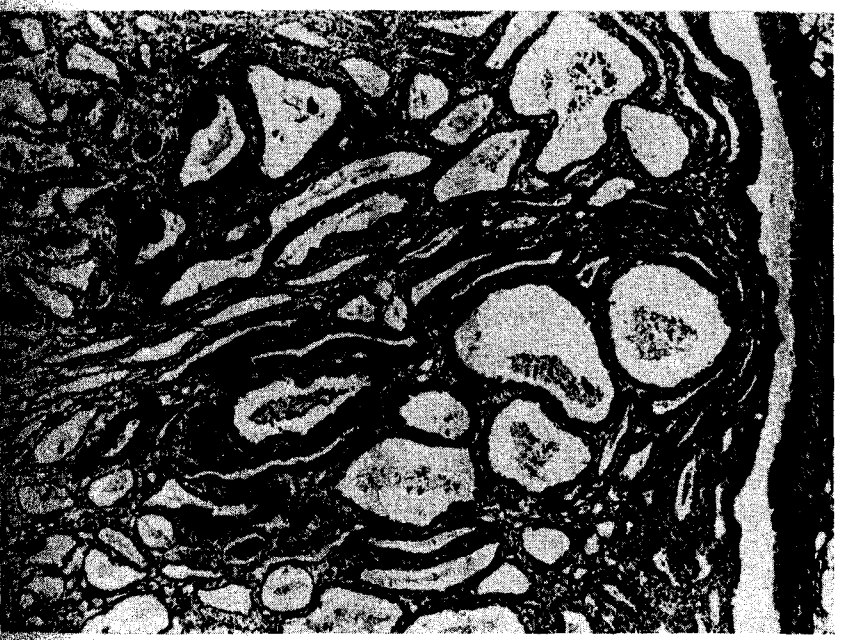

FIGURE 5. Same exposure and pathology as in FIGURE 4.

FIGURE 6. Diet E (350 mg Santowax OM/kg/day) for 188 days. The epithelium of the collecting tubules shows squamous metaplasia. Kidney × 290

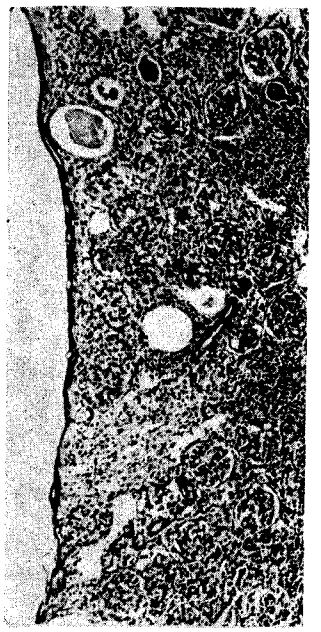

FIGURE 7. day) for 188 days. The in ring and infl

FIGURE 8. day) for 18 days. Accun scarred renal

histiocytes were intensely fuchsinophilic with the periodic acid Schiff (PAS) stain. This affinity was abolished by prior acetylation and restored following deacetylation. The pigment did not stain with Sudan IV in frozen sections and no hemosiderin was demonstrable.

Similar but more severe histopathological changes were apparent after the ingestion of 350 mg/kg/day over a period of 188 days. The animals of Group E who received Santowax OM 350 mg/kg/day for 188 days and then were fed diet A until their sacrifice on the 235th day, established that the renal histopathology was to some extent reversible. Relatively few tubules exhibited cystic dilatation after the test animals had been returned to the Control Diet for 47 days. However, the renal parenchyma exhibited a considerable degree of scarring and the interstitial tissue was heavily infiltrated with lymphocytes and histiocytes containing the PAS positive pigment granules. (Figures 7, 8, 9 and 10.)

A benzene extract of an homogenate was made of a sample of kidney from this last group. Thin layer chromatography of th extract showed the presence of ortho- an meta-terphenyl. The meta-terphenyl wa present in trace quantities only. The contr kidneys were examined similarly and n terphenyl was detected.

OMRE High Boiler Sample

A mean ingestion of 33 mg/kg/day f 130 days (Test Group F) led to a perceptibl increase in the number of histiocytes with the hepatic sinusoids. These cells were lar with abundant finely granular eosinophil cytoplasm and were multinucleated on o casion. The cells occurred either singly o in small groups and increased in numb with increasing length of exposure, to 1 and 235 days. Nevertheless, there was no d tortion of hepatic lobular architecture this rate of dosage. (Figures 11 and 1 The cord cells of the liver showed ani nucleosis and an undue prominence of t nucleoli. Bile duct proliferation was not se

The ingestion of 331 mg/kg/day (T Group G) led to the appearance of lar

Santowax OM/kg/
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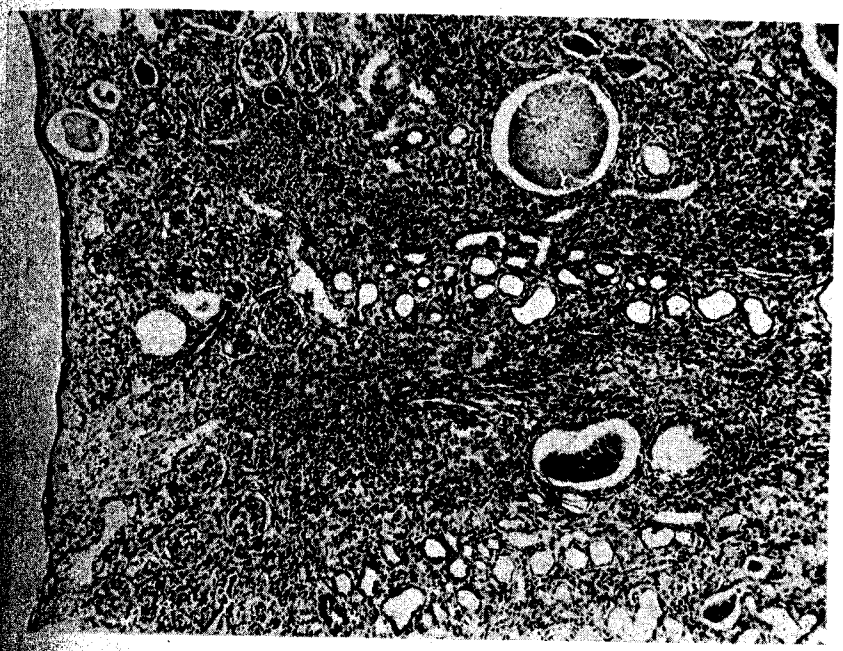

FIGURE 7. Diet E (490 mg Santowax OM/kg/day) for 188 days—then control diet A for 47 days. The interstitium of the kidney shows scarring and inflammation.

FIGURE 8. Diet E (409 mg Santowax OM/kg/day) for 188 days—then control diet A for 47 days. Accumulations of large histiocytes in the scarred renal interstitium. × 400

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MRE High Boiler Sample

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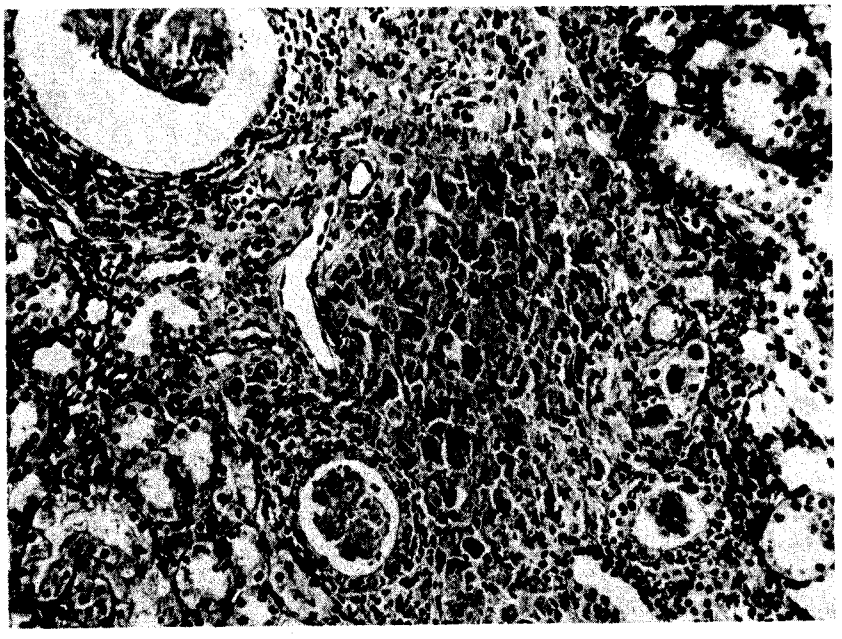


FIGURE 9. Diet E (409 mg Santowax OM/kg/day) for 188 days—then control diet A for 47 days. Accumulations of large histiocytes in the scarred renal interstitium. × 400 H & E

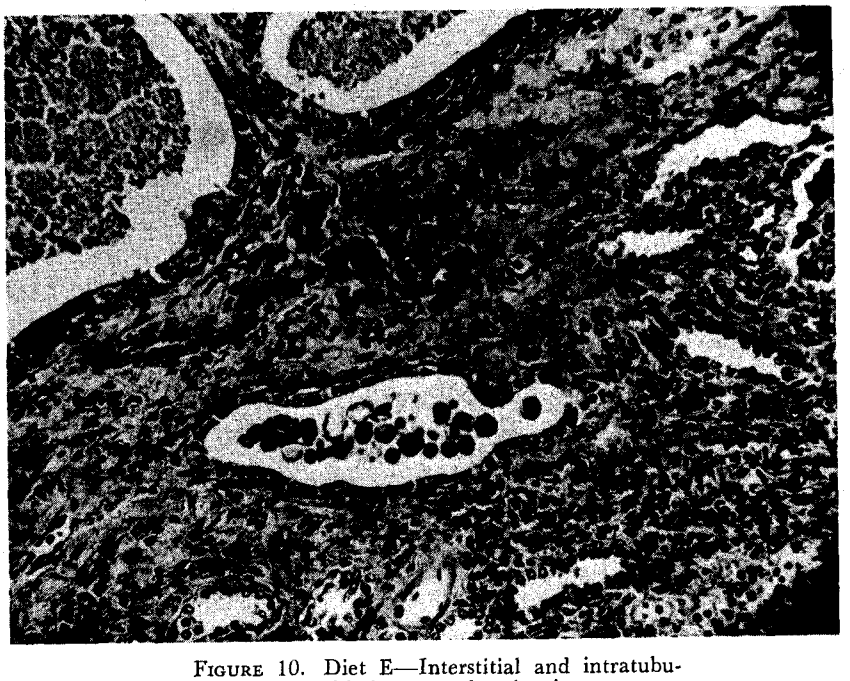
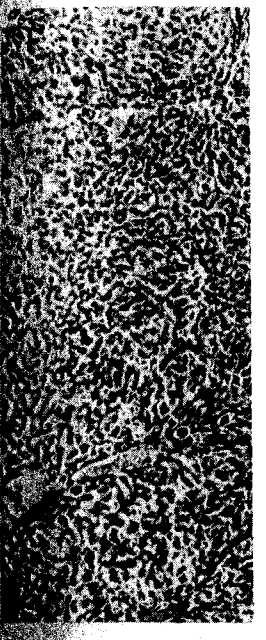


FIGURE 10. Diet E—Interstitial and intratubular histiocytes with intracytoplasmic pigment granules. × 400 H & E



FIGURE
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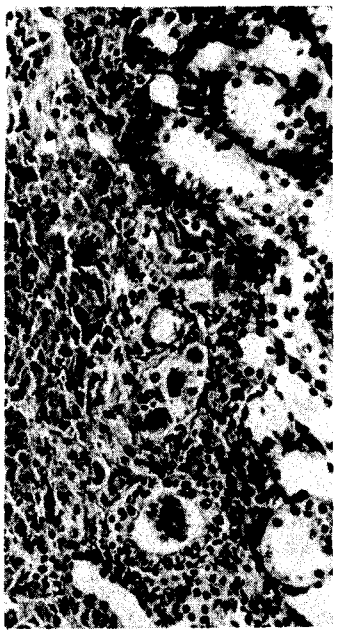

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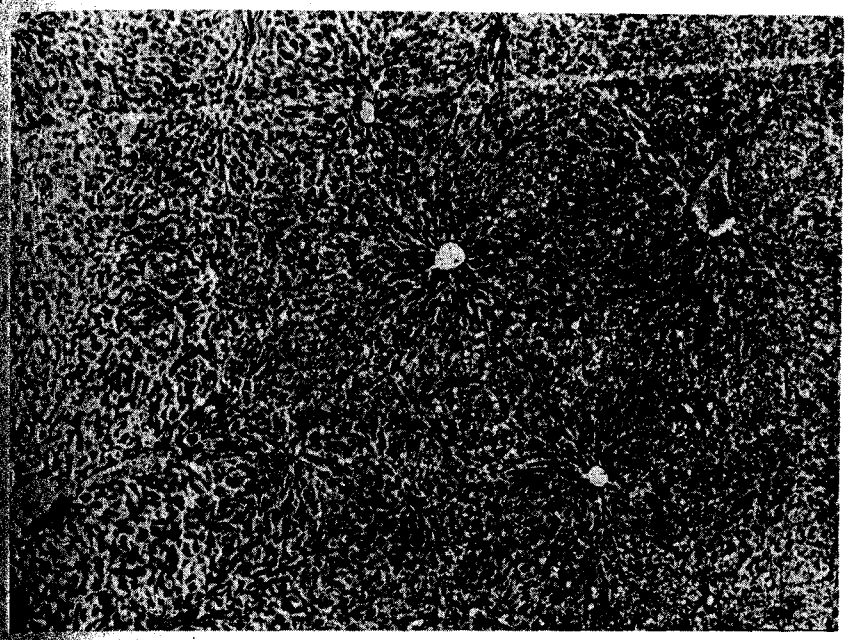


FIGURE 11. Diet F (33 mg O.M.R.E. High Boilers/kg/day) for 235 days. Liver × 100 P.A.S. Liver retains a normal achitectural pattern. Large histiocytes are present in the sinusoids.

the macrophages in the sinus- more pronounced distortion of nuclei. At 130 days, the general architecture with a central vein and portal spaces was preserved, but masses of macrophages transect- lobule in irregular meandering the normal architecture and pseudolobules. These small lob- consisted of irregular aggregates of hep- cells and did not show normal orienta- central vein and peripheral portal tiocytes were more closely packed zones where they formed encircling the central veins. cells in these regions were small, and, on occasion, necrotic. At the these bands of histiocytes elongated cells reminiscent of fibrocytes These features became more pro- at 235 days. (Figures 12 and 15.)

The ingestion of 1832 mg/kg/day (Test caused a further accentuation of histological features so that hepatic were seen by the naked eye. At this there was a greater tendency of the histiocytes at the margins of the transecting bands to form spindle-shaped cells resembling fibrocytes. (Figures 13 and 16.) Reticulin stain demonstrated delicate reticulin fibres laid down in the long axis of the plump fusiform cells.

Morphologically similar histiocytes were found in the spleens and bone marrows of all animals exposed to the O.M.R.E. High Boiler sample. The cells presented mainly in the lymphoid aggregates associated with the Malpighian corpuscles. The cells seen in the bone marrow tended to appear singly and to occur less frequently than those seen in the liver and the spleen.

The mucosa of the stomach, small bowel and colon, the intestinal lymphoid tissue and the sinuses of the adrenal cortex were devoid of these histiocytes.

In contrast to Santowax OM, the High Boiler sample produced no renal changes other than the accumulations of golden-yellow granules in the cytoplasm of the epithelial cells lining the convoluted tubules and loops of Henle. The cytoplasm of the anom-

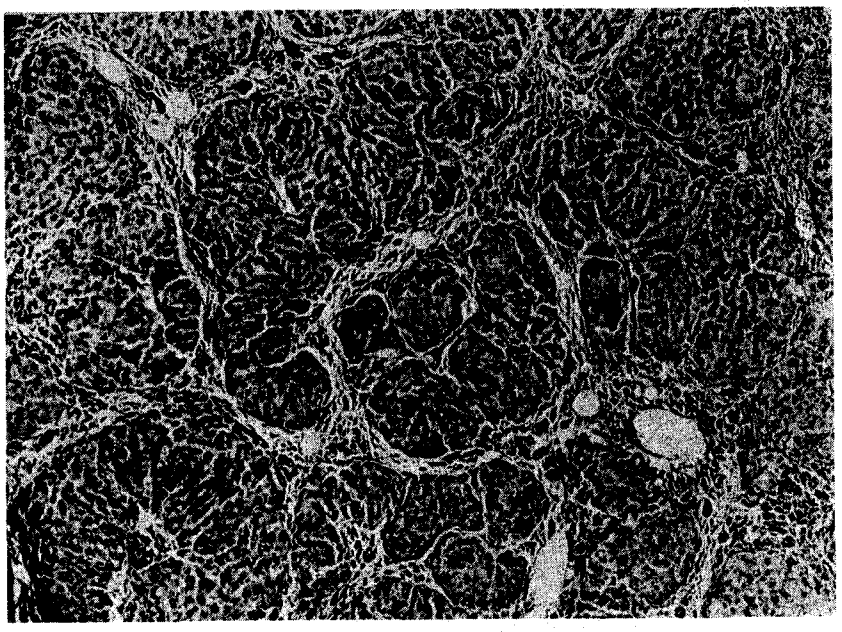


FIGURE 12. Diet G (331 mg O.M.R.E. High Boilers/kg/day) for 235 days. Liver P. A. S. × 100. Although the general lobular appearance is preserved, the lobules are transected by broad bands of histiocytes to form small pseudolobules.

FIGURE 13. Liver P. A. S. × 100. Diet H (1832 mg O.M.R.E. High Boilers/kg/day) for 235 days. There is disruption of the normal lobular pattern. The small pseudolobules are delineated by broad bands of histiocytes.

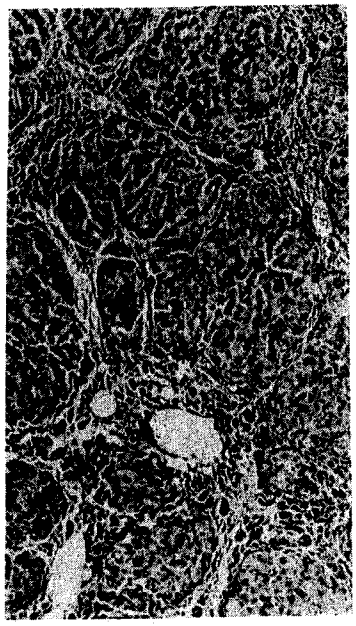
alous macrophages within the spleen contained translucent gc pigment granules. This material tensely PAS positive in paraffi Prior acetylation rendered the negative but this property was deacetylation. Digestion with ronidase and with diastase had n the PAS positive quality of thes The cytoplasm was not Sudar frozen section and selective stai showed negative results.

A benzene extract of an hom the liver of an animal given 5% High Boilers for 235 days was s thin layer chromatography. The tained a strongly fluorescent mat was not present in a similarly p tract of control liver. The nature terial has not been established. extract of a homogenate of the tal given Santowax OM conta dully fluorescent material which ht Rf value. Although these two have not been identified they a not the same material judging by ing fluorescent characteristics.

Discussion

The ingestion by rats of 3 mg c O.M./kg/day for 235 days produ rectable disease. The ingestion of day for a similar period led to t lation of a golden-yellow pigmen toplasm of some of the renal tu The accumulation of this pigme to be of little consequence to the the kidneys, for it was accompa other pathological alteration. Th this pigment was not established.

The ingestion of 350 mg Sant kg/day led to very significant irreversible renal disease. Pigmen similar to that observed with the centration of ingested material w but the major renal lesion consi accumulation of amorphous, crys cellular debris within portions of th with associated gross cystic di tubules, metaplasia of epithelium lecting tubules, and an inflammato



ıg O.M.R.E. High Liver P. A. S. × ıular appearance is ınsected by broad ll pseudolobules.

ɜ. × 100. Diet H lers/kg/day) for 235 the normal lobular les are delineated by

alous macrophages within the liver and spleen contained translucent golden-yellow pigment granules. This material stained intensely PAS positive in paraffin sections. Prior acetylation rendered the cells PAS negative but this property was restored by deacetylation. Digestion with beta-glucuronidase and with diastase had no effect on the PAS positive quality of these granules. The cytoplasm was not Sudanophilic on frozen section and selective stains for iron [illegible] negative results.

A benzene extract of an homogenate of the liver of an animal given 5% O.M.R.E. in rollers for 235 days was subjected to thin-layer chromatography. The extract contained a strongly fluorescent material which was not present in a similarly prepared extract of control liver. The nature of this material has not been established. A benzene extract of a homogenate of the liver of a rat given Santowax OM contained some similar fluorescent material which has a similar R_f value. Although these two compounds have not been identified they are probably not the same material judging by the different fluorescent characteristics.

[illegible]

The ingestion by rats of 3 mg of Santowax OM/kg/day for 235 days produced no detectable disease. The ingestion of 31 mg/kg/day for a similar period led to the accumulation of a golden-yellow pigment in the cytoplasm of some of the renal tubular cells. This accumulation of this pigment appears to be of little consequence to the integrity of the kidneys, for it was accompanied by no other pathological alteration. The nature of this pigment was not established.

The ingestion of 350 mg Santowax OM/kg/day led to very significant and largely irreversible renal disease. Pigment deposition similar to that observed with the lower concentration of ingested material was evident, but the major renal lesion consisted of the accumulation of amorphous, crystalline and cellular debris within portions of the nephrons, with associated gross cystic dilatation of tubules, metaplasia of epithelium of the collecting tubules, and an inflammatory infiltrate in the interstitium. The cystic dilatation of tubules was reversible and regressed when Santowax OM was eliminated from the diet, but scarring and inflammatory infiltration of the interstitium of the kidney persisted. The marked alterations in the renal tubules and interstitium were not accompanied by micropathological changes in the glomeruli or renal vasculature.

The histology of this lesion suggests that it is essentially a "chemical" nephrosis and interstitial nephritis induced by the accumulation of irritant material and debris within renal tubules leading to cystic dilatation and with associated inflammatory reaction and scarring of the interstitial tissue. The fact that dilatation of the tubules and accumulation of intratubular material regressed after ingestion of Santowax was discontinued favours the assumption that the dilatation of tubules is to a large extent the consequence of mechanical obstruction. Dilatation may possibly be accentuated by diminished tubular absorption of glomerular filtrate.

The exact nature of the contents of the obstructed tubules was not determined with any certainty, although one might speculate that some proportion of the contents consist of terphenyl, metabolic products of terphenyl, or conjugates of terphenyl.

There is at present no available method for the histochemical demonstration of these substances, but small amounts of terphenyl in the urine obtained directly from the bladder were detected by thin-layer chromatography.[8]

When an homogenate of the kidney of an animal given the highest dosage of Santowax OM was submitted to gas chromatography, ortho- and meta-terphenyl were found to be present, the latter in trace quantities only. Recent work has also demonstrated that unmetabolized terphenyl and increased amounts of glucuronic and galacturonic acids are excreted in the urine of rats exposed to orally administered Santowax OM.[8]

Some of the amorphous material within the lumen of tubules and within the cytoplasm of epithelial cells and histiocytes was intensely PAS positive. This staining quality

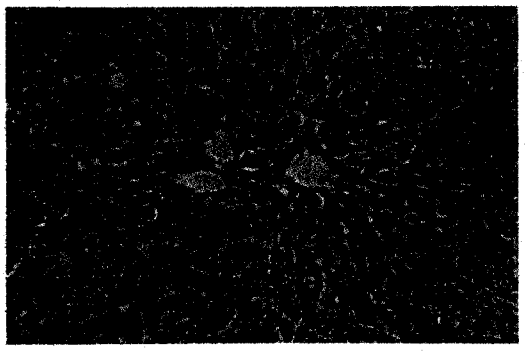


FIGURE 14. Diet F (33 mg O.M.R.E. High Boilers/kg/day) for 235 days. Liver P.A.S. Liver retains a normal architectural pattern. Large histiocytes with fuchsinophilic cytoplasm are present in the sinusoids.

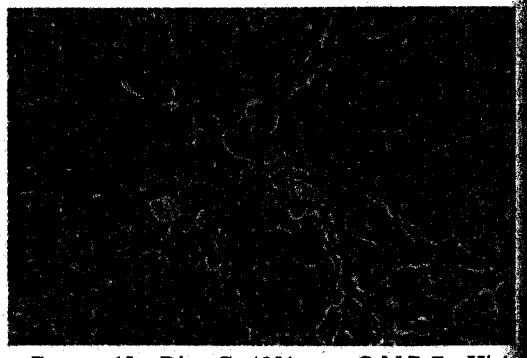


FIGURE 15. Diet G (331 mg O.M.R.E. High Boilers/kg/day) for 235 days. Liver P. A. S. The cord cells are large and show anisonucleosis. The macrophages are often multinucleated and contain intracytoplasmic P. A. S. positive material.

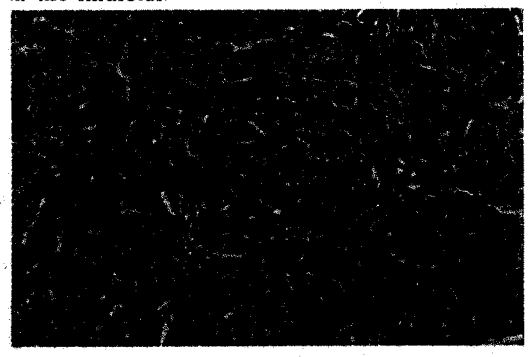


FIGURE 16. Liver P. A. S. Diet H (1832 mg O.M.R.E. High Boilers/kg/day) for 235 days. There is accentuation of the features seen in Figure 15. At the margins of the linear aggregates of histiocytes, spindle-shaped cells are seen.

FIGURE 17. Kidney H & E. Diet E (350 m Santowax OM/kg/day) for 188 days. Yellow brown, translucent pigment granules are present i the tubular epithelial cells.

was abolished by prior acetylation, but was restored by de-acetylation. Moreover, these reactions were evident in paraffin sections. It is therefore most likely that the PAS positive characteristic of this material is due to its carbohydrate content rather than an unsaturated lipid. It is tempting to suggest that this accumulated material may be a glycuronide or galacturonide of terphenyl, particularly in the light of investigations on the metabolism of biphenyls and terphenyl,[2,3,9] but this material may equally represent a carbohydrate-protein complex.

The renal lesions induced by oral administration of Santowax OM were accompanied by slight haematuria, glucosuria and polyuria. While the prolonged ingestion of Santowax OM in sufficient concentration led to marked renal disease changes, no histo pathological changes were observed in th liver or other viscera. Ingestion of O.M.R.E High Boiler produced marked hepatic path ology but no renal pathology other than th deposition of an unidentified yellow pigmen similar to that observed with Santowax OM administration.

It would appear that polymerized com ponents of the High Boiler sample after being absorbed by the gastro-intestinal tract, ac cumulate within the cytoplasm of histiocytes but do not pass the glomerular filter, hence they do not cause pathological changes in the kidney.

The accumulation of these products with in histiocytes produces lesions analogous to the storage reticuloses with prominent his

tiocytic infiltration of liver, spleen lesser extent in bone marrow. rally, although these products of H ingestion must be absorbed by t intestinal tract, no storage of anom terial was observed within histiocy gastro-intestinal lymphoid aggregat in the mesentery. The brunt of the accumulation is borne by the liver a

In the liver, the dense accumu histiocytes transected and distorted tic lobules, and in time were eit formed or replaced by fibrocytes, florid cirrhosis. Hepatic parench exhibited a concomitant hypertr little evidence of mitotic or prolife tivity. The cirrhosis was not pr accompanied by perceptible cellula or fatty metamorphosis.

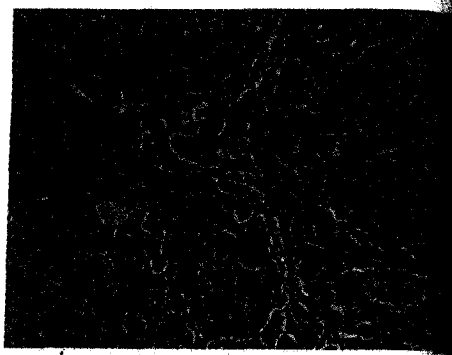
High Boiler ingestion at a dos mg/kg/day for 235 days led to p accumulation of storage histiocyte cirrhosis or distortion of hepatic ar

As the deposition of storage progressive and accumulative, likely that a longer period of in dosage may have led to the sar appearance achieved by the hi dosage.

Conclusions

The ingestion of 3 mg of Santo kg/day for 235 days produced n strable pathology in rats.

The ingestion of 31 mg of OM/kg/day for a similar peri the accumulation of an unidenti yellow pigment in the cyto some renal tubular cells, but detectable changes.



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Boiler ingestion at a dosage of 33
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Conclusions

1. The ingestion of 3 mg of Santowax OM/kg/day for 235 days produced no demonstrable pathology in rats.
2. The ingestion of 31 mg of Santowax OM/kg/day for a similar period led to the accumulation of an unidentified gold-yellow pigment in the cytoplasm of some renal tubular cells, but no other demonstrable changes.
3. The ingestion of 350 mg of Santowax OM/kg/day caused pathological changes in the kidney consisting of tubular obstruction by precipitated products of Santowax OM and an irreversible interstitial nephritis with scarring.
4. In contrast to Santowax OM, the ingestion of 33 mg O.M.R.E. High Boiler sample/kg/day led to an histiocytic storage disease with minimal or insignificant renal pathology. The sequence of events in the rat liver indicates that the transformation or replacement of accumulated histiocytes in the liver by fibrocytes leads to a florid monolobular cirrhosis consisting of "false" lobules. The cirrhosis was not preceded or accompanied by perceptible cellular necrosis or fatty metamorphosis.

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