

diographic trac-
tions of the gall
nal tract, and
percentage of
sence of symp-
ent may not be
the findings are
institution of
basis for early
develop. Sig-
indispensable,
olypsis alone
se as a routine

ical examina-
establishment
ons in whom
oratory stud-
sent when it
.3% of those
d.
and electro-
luable when
ngle abnor-
d with cau-
out verifica-

this exami-
ntly most
for occult
acid and

is a valu-
ly in dis-
yps." -
ll bladder,
colon re-
ptomatic

ry, O. T.,
minations,
55.

ation of
lucose, J.

mination,

Chronic Oral Toxicity of 2-Ethylhexyl Phthalate in Rats and Dogs

ROBERT S. HARRIS, Ph.D., Cambridge, Mass.

HAROLD C. HODGE, Ph.D.

ELLIOTT A. MAYNARD, Ph.D.

and

HARVEY J. BLANCHET JR., M.D., Rochester, N. Y.

INTRODUCTION

Among the useful and versatile plasticiz-
ers in the manufacture of films and tubing
from synthetic plastics, few are more widely
used than 2-ethylhexyl phthalate, known in
the plastics industry as DOP, dioctyl phthal-
ate. Large volumes of this material are being
handled in manufacturing and industrial ap-
plications. Of special interest is the use of
DOP as a plasticizer in films for wrapping
nonfatty foods and in the manufacture of
intravenous vinyl tubing. The low toxicity
and low leachability by aqueous media are
important properties justifying these uses.

In 1943 Hodge¹ characterized the acute
toxicity of 2-ethylhexyl phthalate in rats and
mice as "very low." Shaffer and associates²
confirmed and extended these observations in
other species and by other routes of adminis-
tration. From metabolic studies these authors
concluded that "such injurious action as
it (DOP) does exert within the body appears
to be due to the alkyl part of the molecule
rather than to the phthalate portion." Later

Received for publication Sept. 19, 1955.

This work was supported in part by a grant from
the Dewey and Almy Chemical Company.

From the Department of Food Technology,
Massachusetts Institute of Technology (Dr. Harris)
and the Division of Pharmacology and Toxicology,
University of Rochester School of Medicine and
Dentistry (Dr. Hodge, Dr. Maynard, and Dr.
Blanchet).

chronic feeding studies in rats, guinea pigs,
and dogs were reported by Carpenter and
associates³ and "no effect" levels of 0.06
gm/kg/day (or somewhat higher for rats)
were established. The current studies con-
firm these results and add data for higher
daily doses.

For completeness the available information
on the acute toxicity of DOP is summarized
in Table 1. The low acute toxicity in several
species by two routes is evident.

RAT FEEDING STUDIES

Chronic Oral Toxicity.—From a large group of
weanling albino male and female Wistar-strain rats
three groups, each comprising 43 males and 43 fe-
males, were assembled. The basal diet⁴ contained
82% whole wheat, 16% full-fat milk powder, and
1.6% sodium chloride; 600 I. U. of vitamin A was
added per 100 gm. of diet. Diets and distilled water
were supplied ad libitum. Record was kept of the food
intake of each group. DOP was added to the diets
in the following percentages: 0% (control groups);
0.1%, and 0.5%. Each group of 43 male and 43
female rats was further subdivided in a serial killing
program: (a) 4 rats were killed after 3 months;
(b) 4 rats were killed after 6 months; (c) 10 rats
were killed after 12 months, and (d) 24 rats were
to be maintained on the diets for 24 months. At each
killing period the body weights, food consumptions,
and organ weights of liver, testes, kidneys, lungs,
brains, stomach, heart, and spleen were recorded. A
number of tissues were sampled for histological
study; these included heart, lungs, spleen, esophagus,
stomach, small and large intestine, liver, kidney,
testes, brain, and bone marrow. In addition, any
pathological tissue was sampled and prepared for
histological study.

RESULTS

Mortality.—The over-all mortality during
the two-year period was high. During the
two-year test period 85%-96% of the rats
died. There was no adverse effect on mor-

tality with increasing percentages of DOP in the diets. This confirms the observation of Carpenter and associates.³

Body Weights.—During the initial weeks growths of the several groups were roughly equivalent. The rats given the 0.1% diet grew a little more rapidly than the control groups. The addition of 0.5% DOP to the diet ultimately depressed growth somewhat. For example, at the end of a year the body weights of the control and the 0.1% group were practically identical, whereas the average for the 0.5% group was approximately 50 gm. less. In the second year, as the groups grew smaller, there was a general trend for the average body weights of all three groups to become more alike, and just before the

of the group given the 0.5% diet had fallen to about 75% that of the control group. The DOP intake calculated for the group given the 0.1% diet ranged between 0.05 and 0.08 gm/kg/day and for the group given the 0.5% diet between 0.3 and 0.4 gm/kg/day. The higher figures were observed during the earlier part of the experiment, i. e., in the third to the sixth month. In the study of Carpenter and associates³ the group given the diet containing 0.4% DOP ingested about 0.2 gm/kg/day and exhibited no retardation of growth. The rats in the current study ingested nearly twice as much DOP daily and exhibited a growth reduction. The difference may well be due to the higher dietary intake.

TABLE 1.—Acute Toxicity of Di(2-Ethylhexyl) Phthalate

Reference	Species	Sex	Strain	Body Wt., Gm.	Observation Period, Days	Comments
Oral Administration						
2	Rats	Male	Wistar	90-120	14	30.8 gm./kg.—L.D. ₅₀
1	Rats	Male	Wistar	120-214	..	None died with 34 gm./kg.
	Rats	Male	Wistar	168	2	None died with 61 cc./kg.
2	Rabbits	Male	Albino	2,500-3,800	14	33.9 gm./kg.—L.D. ₅₀
2	Dogs	Male		2,300-10,800	3	2 gm./kg./day without effect
2	Humans	Male		...	..	5 gm. single dose, no effect 10 gm. single dose, mild catharsis
Intraperitoneal Administration						
1	Mice		Albino	19-22	..	125 gm./kg. gave 5% mortality
	Mice			...	2	None died with 100 cc./kg.
2	Rats	Male	Wistar	...	..	30.7 gm./kg.—L.D. ₅₀
1	Rats			149-198	..	None died with 34 gm./kg.
	Rats			281-281	2	None died with 71 cc./kg.
	Rabbits	Male		2,300-4,000	21	51 cc./kg. tolerated
		Female				

end of the second year the averages for the three groups were numerically practically identical. This phenomenon is attributed to the small number of rats surviving in each group.

Food Consumption.—Records of food disappearance maintained to the third month and again to the sixth month showed practically no differences between the control group and the two experimental groups receiving DOP. This confirms the observation of Carpenter and associates³ that food utilization was the same in all their groups. By the end of the first year, however, the evidence indicated that the food consumption

Organ Weights.—Only in the case of the liver and kidney was there any question of a systematic variation in average organ weights, either fresh weight or calculated on the basis of body weight. At three months and six months, for both the males and the females, fresh weights of liver and kidney^{*} and weights calculated on the basis of body weights were elevated for the 0.5% group (Table 2). These data confirm the finding by Carpenter and associates³ that livers and kidneys were enlarged when the diet contained 0.4% DOP. At 12 months and 24 months, however, the fresh weights of the

* Except the male kidney weights at six months.

Cont
0.1%
0.5%

Cont
0.1%
0.5%

organs
basis o
cantly
there
end of

Path
killed
above.
ination
various
round
from t
groups
sional

Organ
Liver

Kidney

Testis

Lungs

Brain
Heart

Spleen

GI Tra

Bladder

TABLE 2.—Organ Weights of Rats Fed DOP
(Milligram/Gram Body Weight)

Diet Groups	3 Mo.		6 Mo.		12 Mo.		24 Mo.	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Males								
Control.....	30.2	7.4	25.4	7.0	32.4	7.1	41.9	8.1
0.1%.....	33.9	7.7	30.4	6.9	30.0	7.0	34.4	7.7
0.5%.....	43.3	7.9	32.3	6.7	28.7	7.0	41.9	8.1
Females								
Control.....	27.0	7.3	27.2	7.0	31.8	7.4	42.8	8.5
0.1%.....	33.9	8.1	29.9	7.5	33.0	7.4	35.0	8.0
0.5%.....	40.4	8.1	35.3	7.9	27.3	7.6	37.6	8.0

organs and the weights calculated on the basis of body weights did not differ significantly from group to group, mainly because there were only a few rats surviving at the end of the second year.

Pathology.—Three Months: Rats were killed by decapitation at the times indicated above. The results of the histological examinations will be reported separately for the various feeding periods. Scattered foci of round cells were seen in the liver sections from the control group and also from both groups fed DOP. In the control rats occasional dilated renal collecting tubules were

seen. One rat on the 0.1% DOP diet showed some tubular atrophy in one kidney section, and one rat on the 0.5% DOP diet exhibited occasional casts. One 0.5% DOP-fed rat showed marked tubular atrophy in one section of testis, and another showed mild changes.

Six Months: In the livers of rats from the three groups there was an occasional focus of round cells. One rat of the 0.1% DOP group showed slight changes in some of the cord cells of the liver. In the kidneys of certain rats in each group there were occasional dilated tubules and casts. Calculi

TABLE 3.—Pathological Findings: Twelve Months

Organ	Diet					
	Control		0.1% DOP		0.5% DOP	
	Males (8)	Females (8)	Males (8)	Females (8)	Males (8)	Females (8)
Liver		Occasional focus of round cells, 1		Occasional round-cell focus, 1	Slight foamy cytoplasm, 1	
Kidneys	Casts, 1 Nephritis, 1	Small areas of tubular dilation, 2; pigment in tubule epithelium, 1	Few hyaline casts, 2	Occasional round-cell focus, 1; casts, 2	Casts, 3	Cyst, 1; chronic inflammation, 1
Testis	Slight tubular atrophy, 2					
Lungs		Broncho-pneumonia, 3	Bronchiectasis, 3	Broncho-pneumonia, 3	Bronchiectasis, 2; broncho-pneumonia w/abscess, 1	
Brain						
Heart	Polyarteritis of a coronary artery, 1					
Spleen			Red cell phagocytosis, 1; excessive pigment, 1	Cyst, 1	Excessive pigment, 1; red cell phagocytosis, 1	
GI Tract	Round-cell foci in submucosa of stomach, 1				Round-cell foci in submucosa of large intestine, 1	
Bladder	Debris, 1		Debris, 1	Parasite, 1	Thin, homogeneous eosinophilic coat over epithelium, 2	

were observed in the bladders of one of the male rats on the 0.1% diet and two of the male rats on the 0.5% diet; such calculi are found from time to time in control rats of our colony. One rat of the 0.5% group exhibited a small focus of round cells in the heart muscle.

Twelve Months: The pathological findings at 12 months are presented in Table 3. Tissues from eight males and eight females from each of the three diet groups were ex-

and excessive pigment were seen occasionally in the spleens, suggesting that there had been at some time an infection by Salmonella.

Twenty-Four Months: A thorough histological examination of selected tissues from the relatively few animals that survived the full 24-month period revealed no evidence of any pathological change that was attributed to the administration of DOP. Among the unrelated pathological findings listed in Table 4, the commonest were (a) lung

TABLE 4.—Pathological Findings: Twenty-Four Months

Organ	Diet					
	Control		0.1% DOP		0.5% DOP	
Tumor	Males (1)	Females (1)	Males (2)	Females (2)	Males (8)	Females (4)
Liver	Warts on foot	Warts on foot			Warts on foot	Warts on foot
Kidneys	Casts	Casts	Chronic nephritis, 2; casts, 2	Chronic nephritis, 1; cortical scarring, 1; casts, 2	Nephritis, 1; casts, 2	Nephritis, 3; cortical scarring, 1; casts, 2
Gonads	Arterial changes	Acute and chronic endometritis		Acute and chronic endometritis, 1	Atrophy, 1; focal necrosis, 1	Vascular mass in fallopian tube, 1
Lungs	Bronchiectasis	Bronchiectasis	Minimal bronchitis and bronchiectasis, 2	Acute bronchitis, 1; minimal bronchiectasis, 2	Bronchiectasis, 1; acute bronchitis, 1; bronchopneumonia, 1	Bronchiectasis, 1; acute chronic bronchitis, 1; bronchopneumonia, 1; arteriosclerosis, 1
Brain						Foci of hyaline thickening of meninges, 1
Heart	Slight myocardial fibrosis				Myocardial fibrosis, 1	
Spleen						Excessive pigment, 1
GI Tract		Foci of edema in submucosa	Foci of eosinophiles in submucosa of stomach, 1			Foci of eosinophiles in submucosa of small bowel, 1
Bladder			Parasite, 1			
Marrow	Moderate increase polys					

amined. Despite a number of findings of obscure pathogenesis, there were no changes in the tissues that were attributed to DOP. The findings are those observed customarily in rats of this age in our colony. Specifically, in the control rats there were typical examples of bronchiectasis and of chronic nephritis. A slight testicular atrophy was found in two males. A rare periarteritis of the coronary artery was observed. In the rats given DOP, a bladder concretion was seen in one male. Red blood cell phagocytosis

disease, frequently fairly well advanced, and (b) chronic nephritis. Three of the rats showed warty tumors of the hindfoot, described as fibroliposarcomata. A rare mild cardiofibrosis was observed in two rats. Acute and chronic endometritis was seen in two rats. An example of a rare arteritis of the testis and an occasional focal atrophy or necrosis of the testis were seen. Small granulomata were found in the liver of one animal presumably as a result of some infection.

DOP in
Diet, %
Control
0.04*
0.1
0.15*
0.4*
0.5

* Data from

In Table form a summary observed in the data associates increased in the diet. In the diets compared was a reduction to 0.5%. Food that for control and included pentar and tion in the diet compared even though comparable to 100% g/mal when including 0. were observed 0.4% or 0.

Dogs, No.

4
4

1

1

1

* Data from

TABLE 5.—Summary of Chronic Feeding Studies: Rats

DOP in Diet, %	Mortality	Body Wt.	Food Consumption	Daily DOP Intake, Gm./Kg.	Organ Wt.	Pathology
Control	70,* 96	Normal	Normal	0	Normal	Same as control rats
0.04*	Same as control	Same as control	Same as control	0.02	Normal	Same as control rats
0.1	Same as control	Same as control	Same as control	0.04-0.06	Normal	Same as control rats
0.13*	Same as control	Same as control	Same as control	0.06	Normal	Same as control rats
0.4*	Same as control	Lower	Same as control	0.80	Enlarged liver and kidney	Same as control rats
0.5	Same as control	Lower	Lower	0.8-0.4	Enlarged liver and kidney at 3 and 6 mo.	Same as control rats

* Data from Carpenter, Well, and Smyth.^a

SUMMARY OF RAT STUDIES

In Table 5 is presented in an abbreviated form a summary of the effects of DOP observed in chronic feeding studies of rats. The data from the work of Carpenter and associates^a are included. Mortality was not increased by feeding 0.5% DOP or less in the diet. Body weights were normal when the diets contained up to 0.13%, but there was a retardation in growth at 0.4% or 0.5%. Food consumption was the same as that of control rats when diets contained up to and including 0.4% in the study by Carpenter and associates,^a but food consumption in the current study was lower when the diet contained 0.5%. In the current study, even though the dietary percentages are comparable, the intake of DOP was 50% to 100% greater. Organ weights were normal when the diets contained up to and including 0.13%. Enlarged livers and kidneys were observed when the diets contained 0.4% or 0.5%. At the end of the current

study definitive comparisons of organ weights could not be made because of the very small number of rats that survived the two-year period. There were no specific histological lesions that were attributed to the administration of DOP.^a

DOG FEEDING STUDIES

Chronic Oral Toxicity.—Two dogs were maintained for a period of 14 weeks on daily doses of DOP as follows: One was given 5 gm/kg. daily by stomach tube; the second was given 0.1 gm/kg. mixed in the diet. The male dog given the larger dose lost little weight during the 14-week period (initial weight—27.1 kg.; final weight—25.5 kg.). The female dog given 0.1 gm/kg. gained a little weight (initial weight—10.4 kg.; final weight—11.3 kg.). Blood samples were taken for hematological examination prior to the study, at the midpoint, and at the end; all the values lay in the normal ranges. When the dogs were killed, samples of a number of

TABLE 6.—Summary of Chronic Feeding Studies: Dogs

Dogs, No.	Daily Dose	Body Wt.	Hematology	Liver and Kidney Wt.	Pathology
4	Control*	3/4 gained	Normal	Normal	Normal
4	0.06 ml./kg. for 19 doses*	4/4 gained	Normal	Normal	Normal
	0.06 ml./kg. for 240 doses				
1	0.06 ml./kg. for 77 doses*	4/4 gained	Normal	Normal	Fatty changes in liver; cloudy swelling in kidney
	0.06 ml./kg. for 180 doses				
1	0.1 gm./kg. for 14 wk.	4/4 gained	Normal	Not recorded	Normal
1	5.0 gm./kg. for 14 wk.	Slight loss	Normal	Not recorded	Chronic cholecystitis; hemosiderosis of spleen

* Data from Carpenter, Well, and Smyth.^a

tissues were prepared for histological examination. These included the following organs: lung, spleen, stomach, small and large intestine, liver, gall bladder, pancreas, adrenal, kidney, urinary bladder, thyroid, gonads, brain, and bone marrow. The female dog given 0.1 gm/kg. daily had entirely normal tissues. The male dog given 5 gm/kg/day showed some chronic cholecystitis. There was also some hemosiderosis of the spleen, a condition not unusual in control dogs of our colony.

The data available on the effects of DOP on dogs have been compiled in Table 6, including the work of Carpenter and associates on dogs given up to 0.09 ml/kg. daily for a period of one year. Body weight gains were recorded, except for one control dog and for the dog given 5 gm/kg/day. Normal hematological findings were observed. Liver and kidney weights were normal where recorded. The only pathological changes related to treatment were in the liver and kidney of the dog given 0.09 ml/kg. for 169 doses and in the liver of the dog given 5 gm/kg. daily for three months. In the 14-week studies 0.1 gm/kg. was a "no effect" level, whereas in the study of Carpenter and associates 0.09 ml/kg/day for one year was associated with toxic changes in the liver and kidney.

SUMMARY

Feeding 2-ethylhexyl phthalate for two years to rats was without detectable effect

when the diets contained about 0.1% or less. No specific histological changes were found in rats maintained on diets containing 0.5%. Mild toxic changes occurred in three months when a dog was given 5 gm/kg. daily; 0.1 gm/kg. was without effect. The absence of serious toxic effects when relatively substantial amounts of DOP were administered for protracted periods to rats and dogs is good evidence that no serious injury will accrue to the workmen handling DOP in manufacturing or in plastics fabrication. The minute traces that may appear in non-fatty foods wrapped in plastics in which DOP is the plasticizer, on the basis of the animal studies, pose no threat of toxic hazard.

REFERENCES

1. Hodge, H. C.: Acute Toxicity for Rats and Mice of 2-Ethyl Hexanol and 2-Ethyl Hexyl Phthalate, *Proc. Soc. Exper. Biol. & Med.* **53**:20 (May) 1943.
2. Shaffer, C. B.; Carpenter, C. P., and Smyth, H. F., Jr.: Acute and Subacute Toxicity of Di(2-Ethylhexyl) Phthalate with Note upon Its Metabolism, *J. Indust. Hyg. & Toxicol.* **27**:130 (May) 1945.
3. Carpenter, C. P.; Weil, C. S., and Smyth, H. F., Jr.: Chronic Oral Toxicity of Di(2-Ethylhexyl) Phthalate for Rats, Guinea Pigs, and Dogs, *A. M. A. Arch. Indust. Hyg.* **8**:219 (Sept.) 1953.
4. Caldwell, A. B.; MacLeod, G., and Sherman, H. C.: Bodily Storage of Vitamin A in Relation to Diet and Age Studied by Means of the Antimony Trichloride Reaction Using a Photoelectric Colorimeter, *J. Nutrition* **30**:349 (Nov.) 1945.

Pu
D

L. Vent

HUR
REG
and
AAR

This
function
ers, 31
with t
ice in
try, w
to eva
conne
cause
roent
Int
which
posur
lated
osis i
repor
Vigli
in tw
expo
of ca
mace
from
prod

Ac
Fi
versit
Re
custr.
Annu
ation.
TI
Rese
Instit
Heal