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E I DUPONT DENEMOURS & CO INC -  
ALKYL PHTHALATES

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OFFICE OF TOXIC SUBSTANCES  
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REV. 7/27/82

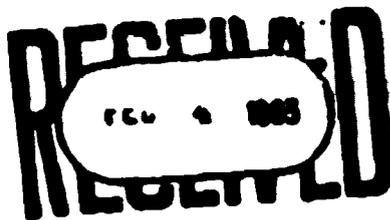
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Chemical Name (300 per name)			CAS No. (10)	24
ALKYL PHTHALATES			999999994	
BENZENEDICARBOXYLIC ACID, DIBUTYL ESTER			84-74-2	
BENZENEDICARBOXYLIC ACID, DICYCLOHEXYL ESTER			84-61-7	

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IB





**E. I. DU PONT DE NEMOURS & COMPANY**  
WILMINGTON, DELAWARE 19888



ENVIRONMENTAL QUALITY COMMITTEE

February 4, 1983

**CERTIFIED MAIL**  
**RETURN RECEIPT REQUESTED**

U.S. Environmental Protection Agency  
TSCA-8D1  
P. O. Box 2060  
Rockville, MD 20852

Gentlemen:

Health and Safety Data Reporting

On behalf of E. I. du Pont de Nemours and Company (Du Pont), I am pleased to submit the enclosed health and safety studies under the Toxic Substances Control Act Section 8(d), 15 U.S.C. 2607(d), and the regulation published at 47 Federal Register 38780 (1982).

This submission supplements Du Pont's December 3, 1982 submission for chemical substances listed in the above Federal Register notice. The determination of reportability was made after Du Pont consulted with and received guidance from the Agency. Moreover, Du Pont notes that certain portions of the enclosed studies contain confidential business information for which Du Pont asserts a claim of business confidentiality under 40 CFR 2.201 et seq. As required by 40 CFR 716.16, Du Pont has submitted both confidential and public copies of each health and safety study. On the confidential copy, the information claimed as confidential business information is circled in red ink. The confidential information has been excised from the public copy.

Very truly yours,

Robert R. Bonczek.  
Director of Safety, Health  
and Environmental Affairs

RRB:mdm  
Encs.

1 D

ALKYL PHTHALATES

CAS Nos. 84-74-2, 84-61-7, 84-66-2, 3648-20-2

KALLE AKTIEGESSELLSCHAFT

*Di-n-butyl phthalat* ①  
Di-n-butyl phthalat  
878211706

E.I. DU PONT  
Haskell Laboratory for  
Toxicology and Industrial Medicine

Wilmington 2<sup>nd</sup>, Delaware  
USA

Gentlemen:

We refer to our letter of 12/9/58, in which we informed you that a comprehensive investigation of the toxicity and cancer producing potential of dibutyl phthalate was being carried out in the scientific laboratories of a well-known German manufacturer. These investigations have been concluded. They have shown that the cancer producing effect of dibutyl phthalate is not greater than that of olive oil. The German state health authority in Berlin (Bundesgesundheitsamt) has recognized these investigations and based on them has permitted dibutyl phthalate as a plasticizer in moisture-proof cellophane without limitation. The corresponding publications by the state health authority will probably follow toward the end of the year.

We greet you.

With highest regard

KALLE AKTIEGESSELLSCHAFT

/s/

(signature illegible but  
probably W. Mauss)

Translated by: JAF  
6/27/60

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BENZENEDICARBOXYLIC ACID, DIBUTYL ESTER		84-74-2		
BENZENEDICARBOXYLIC ACID, DICYCLOHEXYL ESTER		84-61-7		

mark  
7/2/83  
1A



Concealed copy to

2

Dr. Robert de Righi, Foreign Relations Dept.  
Dr. George W. Rigby, Development Department  
Dr. G. H. Cahrman, Medical Division

878211707-B

June 30, 1949

99

*Handwritten signature/initials*

Svenska Institutet för Konserveringsforskning  
Göteborg,  
Sweden

RECEIVED  
JUL 4 1949

Attention: N. Erickson

Gentlemen:

Re: Your letter: Trn/S dated May 30, 1949

Your letter of the above date has been referred to us for reply.

Published literature on dibutyl phthalate is concerned with industrial inhalation of vapors or skin contact with the liquid, and therefore is not pertinent to the problem of use of this compound in chewing gum. We know of no published data on the oral toxicity of dibutyl phthalate, but we quote below data obtained in 1931 in an investigation made for the Du Pont Company by Dr. Henry Field Smyth of Philadelphia:

Smallest Dose Killing Any Animal  
Milligrams per Kilogram Body Weight  
(M = by mouth; I.P. = intraperitoneal)

Guinea Pigs		Rabbits		Rats		Mice		Chickens	
M.	I.P.	M.	I.P.	I.P.	I.P.	M.	I.P.	M.	I.P.
2,000	1,000	3,000	2,000	1,000	3,000	over	15,000	"	"
						20,000			

The low toxicity of polyvinyl acetate is indicated by the attached results of experiments made in this Laboratory in 1946.

We hope that the above will be of assistance to you.

Yours truly,

John H. Foulgor, M. D.  
Director

JHF:AMCB

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OFFICE OF TOXIC SUBSTANCES  
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ALKYL PHTHALATES			999999994	
BENZENE DICARBOXYLIC ACID, DIBUTYL ESTER			84-74-2	
BENZENE DICARBOXYLIC ACID, DICYCLOHEXYL ESTER			84-61-7	

IA



**HENRY FIELD SMYTH, M. D., DR. P. H.**  
**HENRY F. SMYTH, JR., B. S. IN CH. E., M. S.**  
CONSULTATION AND INVESTIGATION IN PUBLIC HEALTH, INDUSTRIAL HYGIENE, TOXIC  
HAZARDS IN INDUSTRY, BIO-CHEMICAL PROBLEMS, BACTERIOLOGICAL PROCEDURES,  
SPILLS, AND BACTERIOLOGICAL TROUBLE.  
307 N. WAYNE AVE.  
WAYNE, PA.

③

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*2.*

**INVESTIGATION OF TOXICITY OF CERTAIN PESTICIDES**

**Report 1.**

**Acute Toxicity to Small Animals**

September 8, 1931

**RECEIVED**  
FEB 4 1983  
**RECEIVED**

*K. H. ...*

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cont.

*as shown*

*Ethyl alcohol  
of Johnson  
Sulfonamide.  
(out  
Pena  
Drel a)*

## Scope of Report

This report covers only acute toxicity of plasticizers to small experimental animals.

## Materials

The plasticizer samples were received identified only by numbers. Our interpretation of these numbers is based on verbal information only, but was checked up fairly successfully by a determination of the specific gravity of the samples. The samples received, and their chemical names, to the best of our knowledge, are listed below.

Sample 1	"Santicizer"
2	Methyl cellosolve phthalate
3	Mono lauryl phthalate
4	Tributyl phosphate
5	Diethyl phthalate
7	Dibutyl phthalate
8	Diethyl phthalate
9	"Santicizer No. 8"
10	"Ethox"
11	Tributyl phosphate
12	Methyl cellosolve phthalate

Samples 1 to 5 were received in such small amounts that they were used only for a few guinea pig doses. The results of these doses are included in this report with the results of doses of samples 7 to 12, when our information led us to believe the two numbers represented identical materials. That is, tabular material headed "sample 12" may include guinea pig doses of sample 2 and sample 12.

Early in this investigation we were told to do no work on Mono lauryl phthalate, as it was not obtainable in commercial quantities. Later, as results of tests showed other materials so toxic as to seem useless, such materials would

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be dropped, and no further work done on them. Thus, by no means all of the samples were carried through the entire program of tests.

Animals used.

This report covers results with only the smaller experimental animals. Larger animals, such as dogs and monkeys, have not been used, but it may seem advisable to test some of the materials on them later.

Albino mice and rats, guinea pigs, rabbits, and chickens were used. All groups included both male and female animals.

The mice weighed from 15 to 25 grams each. The rats weighed from 75 to 100 grams each. The guinea pigs were of mixed colors, and weighed from 130 to 375 grams. The rabbits were of mixed colors, and weighed from 350 to 2,360 grams. The chickens were all barred Plymouth Rocks, and weighed from 1,050 to 2,130 grams. All of these weights represent young growing animals.

No difference in sensitivity connected with the color of the rabbits and guinea pigs was seen, but the colors of all animals dosed are recorded in the original records.

According to almost universal custom, the doses are all reduced to terms of milligrams material per kilo body weight of animal, tabulated as "mgs. per kilo". This method of expressing results corrects for variations in weight between different animals of the same species.

### Methods of dosing

In general the toxicity of non-volatile industrial materials should be tested by administering doses by mouth, since this is the most probable mode of entry into the human body. There is often doubt of the value of results of toxicity studies based on injection of the materials. Since injection is not a natural path of entry into the body, results of such studies may have no relation to the actual dangers from the materials in commerce or industry.

However, injection of the materials was used in this study, along with feeding by mouth. This was done because much of the literature of toxicology contains data based on injection, and also because this method of administering doses enabled us to include animal species not easily fed by force.

Guinea pigs, rabbits, and chickens received the materials of this study both by mouth, and by intraperitoneal injection. Albino rats and mice received the materials only by intraperitoneal injection, because it was found impossible to force these two species to eat enough of the materials to injure them.

Rabbits and chickens were fed by passing a stomach tube down their throats and running the proper dose in from a syringe. The tube was lubricated with paraffine oil before inserting it. Guinea pigs were held in the hand, upside down, and the materials introduced into their mouths from a pipette, a little at a time. With patience it is possible to force guinea pigs to swallow tremendous doses of materials a man would not wish even to taste.

Observations on treated animals

Animals to be treated were drawn from stock colonies of animals, all kept under identical conditions. These colonies served as controls, without any definite animals being set aside for this purpose. There was no disease found during the tests, and untreated animals appeared normal in every way.

All animals were kept under observation 14 days after administration of the dose, after which any which had not already died were discarded, and that particular dose recorded as having no effect.

In doubtful cases, and in the case of animals dying from borderline doses, autopsies were performed to be sure the death had not been from disease rather than from the dose. In a few cases tuberculosis of the lungs was found, and in several cases a general peritonitis had resulted from infection during the injection. In these cases the dose was not blamed for death.

Number of animals used

The table below gives the number of animals reported upon for each material. (M = by mouth; I.P. = intraperitoneal)

	Guinea Pigs		Rabbits		Rats	Mice	Chickens		Total
	M.	I.P.	M.	I.P.	I.P.	I.P.	M.	I.P.	
1. "Santicizer"	6	6							12
2. Monolauryl phthalate	3								3
7. Dibutyl phthalate	31	26	26	24	20	24	13	12	176
8. Diethyl phthalate	31	34	26	20	21	25	13	10	180
9. "Santicizer no. 8"	24	23	24	21	24				116
10. "Ethox"	21	24	20	20	23	26	12	10	156
11. Tributyl phosphate	22	22	10	8	7				69
12. Methyl cellosolve phthalate	20	20	20	20	27	19	14	12	152
Total	158	155	126	113	122	94	52	44	864

Tabulation of Results

Smallest Dose Killing Any Animal

(M = by mouth; I.P. = intraperitoneal)

	Guinea Pigs		Rabbits		Rats	Mice	Chickens	
	M.	I.P.	M.	I.P.	I.P.	I.P.	M.	I.P.
7. Dibutyl phthalate	1,000	1,000	3,000	2,000	1,000	3,000	over 20,000	15,000
8. Diethyl phthalate	5,000	1,000	4,000	4,000	1,000	3,000	15,000	5,000
9. "Santicizer No. 8"	1,500	250	under 600	250	under 100			
10. "Ethox"	1,000	2,000	3,000	2,000	2,000	3,000	15,000	3,000
11. Tributyl phosphate	1,000	200	2,000	under 250	under 400			
12. Methyl cellosolve phthalate	5,000	3,000	1,500	2,000	1,000	4,000	5,000	3,000

*Methyl Cellosolve*

800000

The term "Minimum Lethal Dose" has not been used in this report because it is usually taken to mean the smallest dose certain to kill. We have thought it better to consider the smallest dose killing any animal, rather than the smallest dose killing every animal. Accordingly, the dose we report as "Smallest Fatal Dose" are considerably lower than what would properly be reported as "Minimum Lethal Dose" from the same data.

The tabulation on Page 5 shows that different species have different sensitivities to the materials, which was to have been expected. It is impossible to say which species gives a result closest to what man would give, since there is no general rule for this. As would be expected, the smallest fatal dose for intraperitoneal injection was usually smaller than that for feeding by mouth, for the same species.

Smith (The Pharmacological Action of Certain Phenol Esters, etc.; H.I. Smith, L. Elvove, and W.H. Prazier; Public Health Reports, 45, 2509-24, 1930) reports the Minimum Lethal Dose of triorthocresyl phosphate to rabbits to be 100 mgms. per kilo and to chickens to be from one-half to one gram, with marked symptoms from much smaller doses in chickens. From his data, we would report Smallest Fatal Dose to rabbits to be 50 mgms. per kilo, on the same basis as our other figures in this report. Comparing this with our figures in the table on Page 5, it is seen that none of the materials tested even approaches this toxicity to rabbits. The nearest approach to this is methyl cellosolve phthalate, requiring 30 times as much to kill one of our rabbits.

### Summary and Conclusions

When fed by mouth, the smallest fatal dose of any of the materials tested for any of the species fed was about 600 mgms. per kilo. The smallest fatal dose of dibutyl phthalate for any of the species was 2000 mgms. per kilo and of diethyl phthalate was 4000 mgms. per kilo. In fact, in most of the tests dibutyl phthalate and diethyl phthalate were less toxic than the other materials. Accordingly these two materials have been selected for further work, which will include an investigation of chronic toxicity by the feeding of small daily doses for periods of several months.

It seems reasonable to conclude, from the work done so far, that any of the materials tested would be safe to use as plasticizers in products such as the waterproof coating on cellophane. Any of them apparently would have a much greater margin of safety than triorthocresyl phosphate. Dibutyl phthalate and diethyl phthalate are the best two materials of the group tested.

Appendix

Data on all Doses Administered

Sample 1

"Santicizer" administered as a 50% solution in 95% alcohol

Guinea Pigs - by Mouth

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
51	241	2.2	5,000	Died within 24 hours
1	206	1.0	2,000	Died in 5 days - emaciated, weak
2	2.11	0.5	1,000	None
17	300	0.4	600	None
16	223	0.2	400	None
3	256	0.1	200	None

Guinea Pigs - Intraperitoneal

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result
20	196	0.4	900	Died in 3 hours, preceded by prostration, then coma
19	210	0.2	400	Died within 24 hours
38	240	0.15	300	Died within 24 hours
36	196	0.1	250	Died within 24 hours
18	215	0.1	200	Died within 24 hours
37	162	0.05	150	Died within 24 hours

Both the small number of animals used, and the use of alcohol as a solvent detract from the value of these results. Further tests of the material were discontinued before trials with a non-toxic solvent were possible.

Sample 3  
Mono lauryl phthalate

Guinea Pigs - by Mouth

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
7	208	1.0	5,000	No death - in coma 24 hours after dose, recovered
8	210	0.5	3,000	None
9	229	0.1	500	None

Results inconclusive, due to small number of animals used

Sample 7  
Dibutyl phthalate

Guinea Pigs - by Mouth

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
91	277	2.66	10,000	Died in 2 days
97	270	2.58	10,000	Died in 2 days
98	375	3.60	10,000	Died in 2 days
164	185	1.79	10,000	Died in 24 hours
99	220	1.68	6,000	Died in 6 days
165	290	2.23	8,000	Died in 2 days
166	228	1.53	7,000	Died in 2 days
232	323	2.1	7,000	Died in 2 days
167	217	1.24	6,000	Died in 7 days
234	248	1.5	6,000	None
237	270	1.55	6,000	Died in 24 hours
92	307	1.48	5,000	None
100	145	0.70	5,000	None
168	224	1.07	5,000	None
236	195	0.94	5,000	None
238	205	0.98	5,000	Died in 2 days
169	171	0.65	4,000	None
170	195	0.56	3,000	None
175	205	0.59	3,000	None
93	220	0.42	2,000	None
101	230	0.44	2,000	Died in 4 days
171	200	0.38	2,000	None
94	245	0.26	1,000	None
102	272	0.26	1,000	None
103	258	0.25	1,000	None
172	241	0.23	1,000	None
95	280	0.14	500	None
173	257	0.13	500	None
96	248	0.05	200	None
174	231	0.05	200	None

Smallest Fatal Dose -  
 2,000 mgms. per kilo

000014

10

Sample 7  
Dibutyl phthalate

Guinea Pigs - Intraperitoneal

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgs. per kilo	Result ("None" = none seen within 14 days)
104	227	1.09	5,000	Died in 2 days
105	253	1.25	5,000	Died in 24 hours
156	238	1.14	5,000	Died in 24 hours
161	226	1.08	5,000	Died in 24 hours
119	206	0.90	4,000	Died in 24 hours
157	221	0.91	4,000	Died in 11 days
162	177	0.88	4,000	None
158	208	0.90	3,000	None
163	320	0.92	3,000	None
106	208	0.50	2,500	None
107	173	0.43	2,500	Died in 3 days
120	216	0.43	2,000	None
159	267	0.51	2,000	Died in 3 days
233	278	0.53	2,000	None
108	302	0.30	1,000	Died in 12 days
109	244	0.23	1,000	None
118	208	0.20	1,000	Died in 3 days
160	316	0.30	1,000	None
235	347	0.33	1,000	None
110	269	0.20	750	None
111	224	0.16	750	None
112	273	0.13	500	None
113	305	0.15	500	None
114	190	0.09	500	None
115	196	0.05	250	None
116	205	0.02	100	Died in 24 hours from peritonitis (Not due to dibutyl phthalate)

Smallest Fatal Dose - 1,000 mgs. per kilo

000015

Sample 7  
Dibutyl Phthalate

Rabbits - by mouth

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ( "None" = none seen within 14 days "
19	1.28	24.4	20,000	Died within 24 hours
28	1.42	27.0	20,000	Died within 24 hours
20	1.14	10.9	10,000	Died in 2 days
21	1.14	13.8	10,000	Died within 24 hours
39	1.67	15.0	10,000	None
48	1.07	20.0	10,000	Died within 24 hours
40	1.42	10.4	7,500	None
22	1.08	5.0	5,000	None
23	1.08	5.0	5,000	Died within 24 hours
41	1.24	5.9	5,000	Died within 24 hours
42	1.19	5.7	5,000	None
43	1.50	5.7	4,000	None
111	1.05	4.1	4,000	Died in 9 days, prostrated on 8th day
112	0.96	3.2	3,500	Died in 8 days, prostrated in 24 hours, apparent recovery
49	1.63	5.2	3,000	Died in 24 hours
113	1.36	3.9	3,000	None
114	1.08	3.1	3,000	Died within 24 hours
24	1.21	2.9	2,500	None
25	1.39	3.3	2,500	None
115	1.13	2.7	2,500	None
116	0.96	2.3	2,500	None
45	1.31	2.5	2,000	None
46	1.31	2.5	2,000	None
26	1.02	1.0	1,000	None
27	1.19	1.0	1,000	None
47	2.86	2.7	1,000	None

Smallest Fatal Dose - 3,000 mgms. per kilo

000016

Sample 7  
Dibutyl Phthalate

Rabbits - Intraperitoneal

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ( "None" = none seen within 14 days )
30	1.10	4.8	5,000	Died within 24 hours
31	1.13	6.5	6,000	Died within 24 hours
32	0.68	4.2	5,000	Died within 24 hours
33	1.19	3.4	3,000	Died within 24 hours
117	1.08	2.60	2,500	None
24	1.28	2.4	2,000	Died within 24 hours
50	0.71	1.36	2,000	None
51	0.74	1.4	2,000	None
118	1.05	2.00	2,000	Died in 12 days
58	1.37	1.96	1,500	None
59	1.71	3.45	1,500	None
119	1.28	1.85	1,500	None
35	1.11	1.1	1,000	None
37	1.45	1.4	1,000	None
38	3.07	2.9	1,000	Died in 8 days - early tuberculosis, not killed by dibutyl phthalate alone
44	1.50	1.4	1,000	None
52	0.74	0.71	1,000	None
120	1.79	1.72	1,000	None
53	0.88	0.63	750	None
54	0.71	0.51	750	None
36	1.16	0.5	500	None
55	0.68	0.33	500	None
56	0.65	0.31	500	None
57	0.91	0.26	300	None

Smallest Fatal Dose - 2,000 mgms. per kilo

000017

Sample 7  
Dibutyl phthalate

Rats - intraperitoneal

Rat No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result (None" = none seen within 14 days)
21	107	0.82	8,000	Died within 24 hours
22	130	0.87	7,000	Died in 2 days (coma in 24 hours)
23	126	0.73	6,000	Died in 2 days (coma in 24 hours)
11	110	0.53	5,000	None
24	105	0.50	5,000	None
25	90	0.43	5,000	Died in 2 days
12	106	0.41	4,000	Died in 3 days
26	100	0.38	4,000	None
27	113	0.43	4,000	Died in 3 days (coma in 24 hours)
13	75	0.22	3,000	None
28	91	0.26	3,000	None (coma in 24 hours, recovered)
29	88	0.25	3,000	None (coma in 24 hours, recovered)
59	97	0.28	3,000	Died within 24 hours
14	112	0.22	2,000	None
60	125	0.24	2,000	None
61	115	0.22	2,000	None
15	107	0.10	1,000	None
62	125	0.12	1,000	None
63	111	0.11	1,000	Died in 3 days
16	124	0.06	500	None

Smallest Fatal Dose - 1,000 mgms. per kilo

000018

41

Sample 7  
Dibutyl Adipate

Mice - intraperitoneal

Mouse No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
23	20.0	.390	20,000	Died within 24 hours
24	20.0	.300	15,000	Died in 5 days
25	17.5	.170	10,000	Died in 5 days
26	21.0	.160	8,000	Died within 24 hours
27	20.0	.115	6,000	Died within 24 hours
1	21.0	.120	5,000	Died in 2 days
28	17.5	.080	5,000	None
42	19.0	.090	5,000	Died in 2 days
16	17.5	.085	5,000	Died within 24 hours
2	21.0	.090	4,000	Died in 2 days
43	14.0	.055	4,000	Died in 2 days
47	20.0	.075	4,000	None
49	19.0	.075	4,000	Died within 24 hours
3	18.0	.050	3,000	None
44	20.0	.055	3,000	None
48	21.0	.060	3,000	Died in 2 days
50	19.5	.060	3,000	None
85	20.0	.060	3,000	None
4	18.5	.035	2,000	None
45	16.5	.030	2,000	None
86	23	.045	2,000	Died in 4 days from peritonitis (not due to sample)
87	26.0	.050	2,000	None
5	15.5	.015	1,000	None
88	20.0	.020	1,000	None

Smallest Fatal Dose - 3,000 mgms. per kilo

000019

Sample 7  
Dibutyl Phthalate

Chickens - by mouth

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
64	1.39	30.6	20,000	None
65	1.39	26.8	20,000	None
85	1.22	23.4	20,000	None
86	1.30	29.0	20,000	None
13	1.17	14.1	10,000	None
14	1.47	11.4	8,000	None
1	1.35	9.5	6,000	None
15	1.47	5.5	6,000	None
2	1.53	7.3	5,000	None
3	1.30	5.0	4,000	None
4	1.28	3.7	3,000	None
5	1.59	3.0	2,000	None
6	1.25	1.2	1,000	None

Smallest Fatal Dose - over 20,000 mgms. per kilo

Sample 7  
Dibutyl Phthalate

Chickens - intraperitoneal

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
98	1.84	35.4	20,000	Died within 24 hours
102	1.87	36.0	20,000	None
99	1.79	25.8	15,000	Died within 24 hours
101	1.70	24.4	15,000	None
74	1.76	16.8	10,000	None
100	1.76	16.8	10,000	None
33	1.08	5.2	5,000	None
75	1.10	5.3	5,000	None
34	1.28	4.9	4,000	None
35	1.68	4.8	3,000	None
36	1.33	2.6	2,000	None
37	1.73	1.7	1,000	None

Smallest Fatal Dose - 15,000 mgms. per kilo

Sample 8  
Diethyl phthalate

Guinea Pigs - by mouth

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("none" = none seen within 14 days)
65	175	1.53	10,000	None
72	261	2.35	10,000	Died within 24 hours
239	267	2.56	10,000	Died in 8 hours
73	236	1.71	8,000	Died within 24 hours
240	293	2.24	8,000	Died within 24 hours
241	176	1.16	7,000	Died within 24 hours
282	237	1.50	7,000	Died in 2 days
74	216	1.16	6,000	Died within 24 hours
242	287	1.65	6,000	None
243	323	1.65	6,000	Died in 14 days
283	314	1.68	6,000	Died within 24 hours
13	238	1.0	5,000	None
62	273	1.23	5,000	None
176	200	0.90	5,000	None
177	210	1.15	5,000	None
284	273	1.22	5,000	Died within 24 hours
285	242	1.10	5,000	None
75	241	0.87	4,000	None
178	256	0.92	4,000	None
179	311	1.12	4,000	None
180	202	0.55	3,000	None
181	182	0.49	3,000	None
14	310	0.50	2,000	None
32	246	0.40	2,000	None
182	144	0.26	2,000	None
183	262	0.45	2,000	None
184	145	0.13	1,000	None
185	180	0.16	1,000	None
31	318	0.20	700	None
15	200	0.10	500	None
186	212	0.10	500	None

Smallest Fatal Dose - 5,000 mgms. per kilo

000022

Sample 8  
Diethyl phthalate

Guinea pigs - intraperitoneal

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
66	218	0.96	5,000	Died within 24 hours
186	184	0.83	5,000	Died within 24 hours
48	162	0.60	4,000	Died within 24 hours
49	221	0.80	4,000	None
63	195	0.70	4,000	Died within 24 hours
76	245	0.88	4,000	None
187	175	0.64	4,000	Died within 24 hours
222	200	0.72	4,000	None
286	242	0.87	4,000	Died within 24 hours
50	298	1.0	3,000	Died within 24 hours
77	247	0.67	3,000	None
188	293	0.79	3,000	None
189	190	0.51	3,000	None
223	164	0.44	3,000	None
244	312	0.90	3,000	Died within 24 hours
287	237	0.64	3,000	Died within 24 hours
288	276	0.74	3,000	None
35	172	0.4	2,500	None
64	224	0.41	2,000	Died within 24 hours
78	273	0.49	2,000	None
190	168	0.30	2,000	None
191	170	0.30	2,000	Died in 3 days
224	220	0.39	2,000	None
245	291	0.56	2,000	Died in 7 days
246	284	0.54	2,000	Died in 2 days
289	290	0.52	2,000	Died within 24 hours
34	232	0.20	1,000	Died in 14 days
79	281	0.25	1,000	None
80	260	0.24	1,000	None
192	242	0.22	1,000	None
193	260	0.12	1,000	None
247	335	0.30	1,000	None
248	288	0.28	1,000	None
33	225	0.10	500	None

Smallest Fatal Dose - 1,000 mgms. per kilo

000023

19

Sample 8  
Diethyl phthalate

Rabbits - by mouth

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
7	1.28	23.0	20,000	Died within 24 hours
8	1.47	13.2	10,000	Died in 2 days
9	0.54	4.8	10,000	None
66	1.06	9.7	10,000	Died within 24 hours
67	0.99	8.8	10,000	Died within 24 hours
68	1.22	9.9	9,000	Died within 24 hours
121	1.25	10.8	9,000	Died in 8 days
69	1.13	8.1	8,000	Died within 24 hours
122	1.26	9.8	8,000	Died within 24 hours
123	1.05	8.1	8,000	Died within 24 hours
70	1.19	8.0	7,500	None
126	1.53	10.3	7,500	Died within 24 hours
71	1.10	6.9	7,000	None
125	1.30	8.8	7,000	Died within 24 hours
128	1.16	7.3	7,000	Died within 24 hours
72	1.08	5.8	6,000	None
166	1.22	6.6	6,000	Died within 24 hours
167	1.10	5.9	6,000	Died within 24 hours
16	1.21	5.4	5,000	None
73	1.10	4.9	5,000	None
168	1.13	5.1	5,000	Died in 7 days
169	1.16	5.2	5,000	None
170	1.13	4.1	4,000	None
171	1.19	4.3	4,000	Died in 24 hours
17	1.27	3.3	2,500	None
18	1.05	1.1	1,000	None

Smallest Fatal Dose - 4,000 mgms. per kilo.

Sample 8  
Diethyl phthalate

Rabbits - intraperitoneal

Rabbit No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
124	1.02	9.8	10,000	Died within 24 hours
127	0.85	6.1	8,000	Died within 24 hours
130	1.13	6.1	6,000	Died within 24 hours
172	1.16	6.3	6,000	Died in 2 days
173	1.10	4.9	6,000	Died in 2 days
60	1.10	5.0	5,000	None
131	1.10	4.9	5,000	Died in 10 days
174	1.50	6.7	5,000	Died in 2 days
175	1.19	5.4	5,000	Died in 2 days
214	1.42	6.4	5,000	Died in 24 hours
61	0.91	3.3	4,000	None
176	1.02	3.7	4,000	Died in 2 days
177	1.05	3.8	4,000	Died in 2 days
215	1.08	3.9	4,000	None
62	0.85	2.3	3,000	None
216	1.02	2.8	3,000	None
63	1.05	2.0	2,000	None
217	1.13	2.0	2,000	None
64	0.99	0.89	1,000	None
65	0.99	0.45	500	None

Smallest Fatal Dose - 4,000 mgms. per kilo

000025

Sample 8  
Diethyl phthalate

Rats - intraperitoneal

Rat No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
30	95	0.85	10,000	Died within 24 hours
31	80	0.57	8,000	Died within 6 hours (prostrated in 1 hour)
32	82	0.44	6,000	Died within 6 hours (prostrated in 1 hour)
34	121	0.65	6,000	Died in 7 days
17	112	0.50	5,000	Died in 4 hours
33	112	0.50	5,000	Died in 4 hours (prostrated in 1 hour)
35	128	0.57	5,000	Died in 4 days
64	119	0.53	5,000	Died within 24 hours
18	98	0.35	4,000	Died in 4 days
65	95	0.34	4,000	Died within 24 hours
66	106	0.38	4,000	Died within 24 hours
19	97	0.27	3,000	Died in 3 days
67	136	0.37	3,000	Died within 24 hours
68	98	0.27	3,000	Died within 24 hours
20	108	0.19	2,000	None
69	129	0.23	2,000	Died in 7 days
70	103	0.20	2,000	None
21	113	0.10	1,000	None
71	100	0.90	1,000	Died in 24 hours
72	131	0.12	1,000	Died in 10 days
22	117	0.05	500	None

Smallest Fatal Dose - 1,000 mgms. per kilo

000026

Sample 8  
Diethyl phthalate

Mice - intraperitoneal

Mice No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
29	19.0	.170	10,000	Died within 24 hours
30	17.0	.130	8,000	Died within 24 hours
31	18.0	.100	6,000	Died in 5 days
32	18.0	.085	5,000	Died within 24 hours
51	23.0	.105	5,000	Died within 24 hours
52	17.5	.080	5,000	None
6	23.0	.090	4,000	Died in 2 days
33	20.5	.105	4,000	Died within 24 hours
53	15.0	.055	4,000	Died within 24 hours
60	18.0	.050	4,000	Died in 2 days
61	19.0	.050	4,000	Died in 4 days
7	22.0	.060	3,000	None
56	18.0	.050	3,000	Died within 24 hours
57	16.0	.045	3,000	Died within 24 hours
8	26.0	.050	2,000	None
55	16.0	.030	2,000	None
59	20.0	.035	2,000	None
89	23.0	.040	2,000	None
90	19.0	.035	2,000	None
9	23.0	.020	1,000	None
91	22.0	.020	1,000	None
92	21.0	.020	1,000	None
10	26.5	.020	800	None
11	23.0	.015	600	None
12	24.0	.015	500	None

Smallest Fatal Dose - 3,000 mgms. per kilo

000027

Sample 8  
Diethyl phthalate

Chickens - by mouth

Chick No.	Weight in Kilos	Dose in c.c.	Dosage in mgm. per kilo	Result ("None" = none seen within 14 days)
16	1.36	24.4	20,000	Died in 3 days
66	1.62	29.2	20,000	Died in 3 days
87	1.30	23.4	20,000	Died in 2 days
67	1.76	31.7	20,000	Died in 8 days
17	1.39	18.8	15,000	None
88	1.84	24.7	15,000	Died in 6 days
8	1.13	19.1	12,000	None
7	1.52	14.5	10,000	None
18	1.33	11.9	10,000	None
9	1.30	7.0	6,000	None
10	1.19	5.4	5,000	None
11	1.28	3.2	3,000	None
12	1.23	4.6	4,000	None

Smallest Fatal Dose - 15,000 mgms. per kilo

000028

Sample 8  
Diethyl Phthalate

Chickens - intraperitoneal

Chick No.	Weight in Kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
76	1.56	14.0	10,000	Died within 24 hours
103	2.13	15.3	8,000	None
38	1.39	6.2	5,000	None
77	1.13	5.1	5,000	Died within 24 hours
105	1.42	6.4	5,000	None
39	1.56	5.6	4,000	None
104	1.65	5.9	4,000	None
40	1.53	4.1	3,000	None
41	1.87	3.4	3,000	None
42	1.39	1.3	1,000	None

Smallest Fatal Dose - 5,000 mgms. per kilo

000029

54

Sample 9  
"Senticizer No. 8"

Guinea Pigs - by mouth

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgs. per kilo	Result ("None" = none seen within 14 days)
194	205	1.72	10,000	Died within 24 hours
195	210	1.42	8,000	Died within 24 hours
250	157	1.05	8,000	Died within 24 hours (prostrated in 1 hour)
196	200	1.00	6,000	None
251	259	1.50	6,000	Died in 5 days (in coma for 3 days)
203	245	1.02	5,000	None (in coma for 24 hours)
252	256	1.08	5,000	Died in 2 days (in coma for 24 hours)
197	165	0.55	4,000	None
225	257	0.87	4,000	Died within 24 hours
253	202	0.62	4,000	None (in coma for 24 hours)
198	190	0.48	3,000	Died within 24 hours
226	300	0.75	3,000	None
254	308	0.78	3,000	None
255	186	0.47	3,000	None (in coma for 24 hours)
228	195	0.42	2,500	None (very weak for days)
199	200	0.34	2,000	None
227	160	0.27	2,000	None
256	283	0.48	2,000	None
229	320	0.41	1,500	Died in 9 days
230	180	0.15	1,000	None
200	190	0.16	1,000	None
231	215	0.18	1,000	None
201	190	0.08	500	None
202	215	0.04	200	None

Smallest Fatal Dose - 1,500 mgms. per kilo

000030

Sample 9  
"Sartimizer No. 8"

Guinea Pigs - Intraperitoneal

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
216	188	0.79	5,000	Died in 2 hours (prostrated in 10 minutes)
217	128	0.42	4,000	Died in 2 hours (prostrated in 10 minutes)
218	214	0.54	3,000	Died within 24 hours (Prostrated in 2 hours)
219	195	0.33	2,000	Died within 24 hours (Prostrated in 2 hours)
220	255	0.22	1,000	Died within 24 hours (Prostrated in 2 hours)
257	263	0.22	1,000	Died in 6 hours (Prostrated in one hour)
258	274	0.21	800	No death - prostrated in 2 hours, recovered
259	244	0.16	750	Died within 24 hours (prostrated in 2 hours)
221	210	0.09	500	None - prostrated in 2 hours, recovered
222	226	0.09	500	Died within 24 hours
260	275	0.12	500	None - prostrated in 2 hours, recovered
261	244	0.08	400	None - prostrated in 2 hours, recovered
262	189	0.06	400	None
263	240	0.06	300	None - prostrated in 2 hours, recovered
264	295	0.07	300	None - prostrated in 2 hours, recovered
223	222	0.05	250	Died within 24 hours
224	188	0.04	250	None
265	267	0.05	200	None - prostrated in 2 hours, recovered
266	271	0.05	200	None
225	138	0.01	100	None
226	175	0.015	100	None
267	353	0.03	100	None
268	283	0.03	100	None

Smallest Fatal Dose - 250 mgms. per kilo

000031

22

Sample 9  
"Santicizer No. 8"

Rabbits - by mouth

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
74	0.88	7.4	10,000	Died in 2 hours
75	0.95	6.5	8,000	Died in 24 hours
76	1.10	5.5	6,000	Died in 2 hours
77	1.08	4.6	5,000	None
82	1.16	4.9	5,000	Died in 24 hours
78	1.10	3.7	4,000	Died in 2 hours
83	1.70	5.7	4,000	Died in 7 hours
132	1.68	5.7	4,000	Died in 24 hours
79	1.02	2.6	3,000	Died in 24 hours
84	1.28	3.2	3,000	Died in 24 hours
85	1.22	3.2	3,000	Died in 24 hours
133	1.22	3.1	3,000	Died in 24 hours
80	0.96	1.6	2,000	Died in 24 hours
86	1.70	2.9	2,000	Died in 2 days
87	1.79	3.0	2,000	None
134	1.10	1.9	2,000	Died in 24 hours
135	1.25	2.1	2,000	Died in 24 hours
81	1.05	0.89	1,000	None
136	1.59	1.3	1,000	None
137	1.68	1.4	1,000	None
90	1.25	0.84	800	Died in 11 days
91	1.25	0.84	800	None
92	1.05	0.53	600	Died in 12 days
93	1.05	0.53	600	Died in 11 days

Trials to find a smallest fatal dose were not continued for the material seemed too toxic to be of use.

000032

22

Sample 9  
"Santicizer No. 8"

Rabbits - Intraperitoneal

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
94	0.91	3.8	5,000	Died in one hour
95	1.45	4.9	4,000	Died in 6 hours
96	1.22	3.1	3,000	Died in one hour
138	1.36	3.4	3,000	Died in 24 hours
139	1.39	2.9	2,500	Died within 24 hours
140	1.47	3.1	2,500	Died within 24 hours
97	1.25	2.1	2,000	None
141	1.62	2.6	2,000	Died within 24 hours
142	1.10	1.9	2,000	Died within 24 hours
89	1.16	0.97	1,000	None
98	0.85	0.72	1,000	Died within 24 hours
143	1.02	0.85	1,000	None
144	1.25	1.04	1,000	None
145	1.45	0.91	750	Died within 24 hours of peritonitis (Not due to Santicizer No. 8)
146	1.10	0.69	750	None
149	1.10	0.47	500	None
147	1.30	0.55	500	None
148	1.13	0.24	250	None
150	1.28	0.27	250	Died in 8 days
151	1.47	0.12	100	None
152	1.39	0.12	100	None

Smallest fatal dose - 250 mgms. per kilo

000033

## Sample 9

"Senticizer No. 8"Rats - intraperitoneal

Rat No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
37	102	0.17	2,000	Died in one hour
50	135	0.23	2,000	Died in 5 hours
38	112	0.14	1,500	Died in one hour
39	128	0.15	1,500	Died in one hour
40	117	0.10	1,000	Died in 5 hours
41	163	0.14	1,000	Died in one hour
42	155	0.10	750	None
43	115	0.07	750	Died in 5 hours
73	81	0.05	750	Died within 24 hours
74	98	0.06	750	Died within 24 hours
75	110	0.06	600	None
76	134	0.07	600	Died within 24 hours
44	124	0.05	500	None
45	154	0.07	500	Died in 5 hours
77	106	0.04	500	None
78	126	0.05	500	Died in 8 days
79	102	0.03	400	Died in 11 days
80	119	0.04	400	Died in 8 days
81	140	0.04	300	Died in 12 days
82	137	0.04	300	Died in 9 days
46	130	0.03	250	Died in 4 days
47	130	0.03	250	None
48	134	0.01	100	Died in 7 days
49	140	0.01	100	Died in 8 days

Smallest Fatal Dose - 100 mgms. per kilo, or less

Sample 10  
"Ethox"

Guinea Pigs - by Mouth

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days )
121	225	3.06	10,000	Died within 24 hours
122	279	1.82	7,500	Died within 24 hours
290	266	1.81	7,500	Died within 24 hours
123	205	0.94	5,000	Died within 24 hours
269	247	1.11	5,000	None
291	231	1.05	5,000	None
292	265	0.95	4,000	None
293	207	0.74	4,000	None
124	261	0.64	2,500	Died in 8 days
270	244	0.55	2,500	None
271	259	0.58	2,500	None
125	175	0.16	1,000	Died in 3 days
272	206	0.19	1,000	None
273	299	0.27	1,000	None
274	288	0.20	750	None
126	230	0.11	500	None
275	275	0.12	500	None
127	248	0.056	250	None
276	286	0.065	250	None
128	238	0.020	100	None
277	297	0.030	100	None

Smallest Fatal Dose - 1,000 mgms. per kilo

000035

Sample 10  
"Ethox"

Guinea Pigs - Intraperitoneal

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
129	231	1.05	5,000	Died within 24 hours
130	258	0.95	4,000	Died within 24 hours
204	196	0.71	4,000	Died within 24 hours
206	226	0.71	3,500	Died within 24 hours
205	213	0.58	3,000	Died within 24 hours
278	289	0.78	3,000	Died in 6 hours
207	245	0.56	2,500	None
279	234	0.53	2,500	Died in 6 hours
280	298	0.67	2,500	Died in 7 days
131	256	0.46	2,000	Died in 9 days
208	261	0.47	2,000	None
281	147	0.26	2,000	None
209	206	0.28	1,500	None
132	165	0.15	1,000	None
210	192	0.17	1,000	None
211	303	0.25	900	None
212	275	0.20	800	None
133	348	0.24	750	None
213	222	0.14	700	None
214	332	0.18	600	None
134	172	0.08	500	Died in 3 days from peritonitis (Not due to Ethox)
215	302	0.14	500	None
135	310	0.07	250	None
136	278	0.03	100	None

Smallest Fatal Dose - 2,000 mgms. per kilo

000036

Sample 10  
"Stox"

Rabbits - by Mouth

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
99	1.25	11.4	10,000	Died in 24 hours
100	1.02	6.9	7,500	Died in 24 hours
153	0.91	4.9	6,000	Died in 24 hours
101	1.53	6.9	5,000	Died in 24 hours
154	0.93	4.2	5,000	Died in 24 hours
155	1.13	5.1	5,000	Died in 24 hours
178	0.71	3.2	5,000	Died in 24 hours
179	1.08	5.0	5,000	Died in 24 hours
102	1.10	4.0	4,000	Died in 6 days
156	1.53	5.5	4,000	None
157	1.05	3.8	4,000	None
180	0.76	2.7	4,000	Died in 8 days
181	1.42	5.1	4,000	Died in 3 days
103	1.05	2.9	3,000	None
158	1.10	3.0	3,000	None
159	1.10	3.0	3,000	None
183	0.96	2.6	3,000	Died in 6 days
104	1.08	2.0	2,000	None
160	1.42	2.6	2,000	None
105	1.30	0.93	1,000	None

Smallest Fatal Dose - 3,000 mgms. per kilo

000037

Sample 10

"Stroox"

Rabbits - Intraperitoneal

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
106	1.08	4.9	5,000	Died within 24 hours
163	1.10	5.0	5,000	Died within 24 hours
184	1.28	5.8	5,000	Died within 24 hours
107	1.28	4.6	4,000	None
162	1.47	5.3	4,000	Died within 24 hours
185	0.93	3.4	4,000	Died within 24 hours
186	0.88	3.2	4,000	Died within 24 hours
108	1.13	3.1	3,000	Died within 24 hours
161	1.33	3.6	3,000	None
164	1.08	2.9	3,000	Died in 3 days
182	0.82	2.2	3,000	Died within 24 hours
187	1.05	2.8	3,000	None
109	1.19	2.2	2,000	Died in 9 days
165	1.08	2.0	2,000	Died in 4 days
166	1.36	2.5	2,000	None
188	1.19	2.2	2,000	Died in 12 days
189	1.28	2.3	2,000	None
110	0.96	0.87	1,000	None
190	1.33	1.2	1,000	None
191	1.42	1.3	1,000	None

Smallest Fatal Dose - 2,000 mgms. per kilo

Sample 10  
"Strox"

Rats - intraperitoneal

Rat No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
1	152	1.4	10,000	Died in 4 hours
2	136	0.98	8,000	Died in 4 hours
3	105	0.57	6,000	Died in 4 hours
51	93	0.50	6,000	None
52	108	0.58	6,000	Died in 5 hours
4	130	0.59	5,000	Died in 4 hours
53	117	0.53	5,000	Died in 5 hours
54	108	0.50	5,000	Died within 24 hours
5	124	0.45	4,000	None
55	98	0.36	4,000	Died within 24 hours
56	84	0.30	4,000	Died in 3 days
83	152	0.55	4,000	Died within 24 hours
6	111	0.30	3,000	None
57	79	0.21	3,000	None
58	111	0.30	3,000	None
84	88	0.24	3,000	Died within 24 hours
85	95	0.26	3,000	Died in 3 days
7	98	0.18	2,000	Died in 13 days
86	107	0.19	2,000	None
87	99	0.18	2,000	Died in 11 days
8	115	0.11	1,000	None
9	132	0.20	1,000	None
10	174	0.08	500	None

Smallest Fatal Dose - 2,000 mgms. per kilo

000039

Sample 10  
"Ethox"

Mice- intraperitoneal

House No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
34	18.5	.165	10,000	Died within 24 hours
35	18.5	.105	8,000	Died within 24 hours
36	16.5	.090	6,000	Died within 24 hours
62	16.0	.085	6,000	Died within 24 hours
69	18.0	.095	6,000	Died in 2 days
13	18.0	.085	5,000	None
37	18.0	.085	5,000	Died in 2 days
63	20.0	.090	5,000	Died within 24 hours
64	20.0	.090	5,000	Died within 24 hours
70	19.5	.090	5,000	Died within 24 hours
71	19.0	.085	5,000	Died within 24 hours
14	25.0	.090	4,000	Died in 4 days
65	19.5	.090	4,000	Died within 24 hours
66	17.0	.060	4,000	Died within 24 hours
72	19.0	.070	4,000	Died within 24 hours
15	18.0	.050	3,000	None
67	17.5	.050	3,000	None
68	17.0	.045	3,000	None
73	21.0	.055	3,000	Died within 24 hours
16	20.0	.035	2,000	None
93	22.0	.040	2,000	None
94	19.0	.040	2,000	None
95	22.0	.040	2,000	None
17	24.0	.020	1,000	None
96	19.0	.020	1,000	None
97	20.0	.020	1,000	None

Smallest Fatal Dose - 3,000 mgms. per kilo

00004G

36

Sample 10  
"Stock"

Chickens - by mouth

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
61	1.53	28.0	20,000	None
99	1.56	28.1	20,000	None
62	1.73	23.0	15,000	Died in 2 days
90	1.45	19.6	15,000	None
63	1.39	12.6	10,000	None
91	1.53	13.8	10,000	None
19	1.63	12.1	8,000	None
20	1.39	7.5	6,000	None
21	1.50	6.8	5,000	None
22	1.47	4.0	3,000	None
24	1.47	2.7	2,000	None
25	1.25	1.1	1,000	None

Smallest Fatal Dose - 15,000 mgms. per kilo

000041

Sample 10  
"Ethox"

Chickens - intraperitoneal

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
78	1.76	16.0	10,000	Died within 24 hours
107	1.53	13.7	10,000	Died within 24 hours
106	1.79	12.9	3,000	Died within 24 hours
108	1.68	12.1	3,000	Died in 2 days
56	1.47	6.6	5,000	None
79	1.56	7.1	5,000	None
57	1.05	3.8	4,000	None
53	1.79	3.2	3,000	None
59	1.50	2.7	2,000	None
60	1.45	1.3	1,000	None

Smallest Fatal Dose - .8,000 mgms. per kilo

000042

38

Sample 11  
Triobutyl phosphate

Guinea Pigs - by mouth

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms per kilo	Result ("None" = none seen within 14 days)
56	232	0.36	10,000	Died within 24 hours
10	195	1.0	5,000	Died in 6 days
57	302	1.54	5,000	None
61	335	1.71	5,000	Died within 24 hours
294	279	1.42	5,000	Died in 2 days
295	248	1.01	4,000	Died in 2 days
298	261	1.16	4,000	Died in 3 days
296	272	0.84	3,000	Died within 24 hours
297	337	1.03	3,000	None
46	241	0.50	2,000	None
58	223	0.45	2,000	None
82	245	0.56	2,000	None
299	230	0.47	2,000	None
300	295	0.60	2,000	None
11	285	0.50	1,700	Died in 2 days
27	239	0.40	1,500	None
47	306	0.3	1,000	Died within 24 hours
59	216	0.22	1,000	None
83	230	0.23	1,000	None
84	263	0.27	1,000	None
26	236	0.20	800	None
12	258	0.12	400	None

Smallest Fatal Dose - 1,000 mgms. per kilo

000043

Sample 11  
Tributyl phosphate

Guinea pigs - intraperitoneal

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
30	263	0.40	1,500	Died in one hour
60	300	0.31	1,000	Died within 24 hours
85	300	0.31	1,000	Died within 24 hours
29	242	0.20	600	Died in 2 hours
43	243	0.20	800	None
86	279	0.23	800	Died in 24 hours
301	250	0.20	800	Died in 24 hours
302	243	0.20	800	Died in 24 hours
87	246	0.15	600	Died within 24 hours
303	246	0.15	600	Died within 24 hours
304	287	0.12	600	Died within 24 hours
44	222	0.11	500	None
61	200	0.10	500	None
28	248	0.10	400	None
88	188	0.08	400	Died within 24 hours
305	231	0.09	400	Died within 24 hours
306	361	0.15	400	Died within 24 hours
307	281	0.09	300	None
308	290	0.09	300	Died in 6 days
45	242	0.05	200	Died in 3 days
89	220	0.05	200	None
90	262	0.03	100	None

Smallest Fatal Dose - 200 mgms. per kilo

000044

14

Sample 11  
Tributyl Phosphate

Rabbits - by Mouth

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
4	0.99	20.0	20,000	Died within 24 hours
5	1.42	7.3	5,000	Died within 24 hours
13	1.11	5.7	5,000	Died in 2 days
192	1.30	5.3	4,000	Died within 24 hours
193	1.13	3.5	3,000	Died within 24 hours
14	1.85	3.2	2,500	Died within 24 hours
194	1.35	2.5	2,000	Died within 24 hours
195	1.22	2.5	2,000	Died within 24 hours
15	1.22	1.25	1,000	None
196	1.42	1.4	1,000	None

Smallest Fatal Dose - 2,000 mgms. per kilo

000045

Sample 11  
Tributyl phosphate

Rabbits - Intraperitoneal

Rabbit No.	Weight in kilo's.	Dose in c.c.	Dosage in mgms. per kilo.	Result
197	1.22	1.3	1,000	Died in 24 hours
198	1.39	1.6	1,000	Died in 8 days
200	1.59	1.2	750	Died in 24 hours
201	1.38	1.3	750	Died in 24 hours
202	1.25	0.64	500	Died in 24 hours
203	1.05	0.54	500	Died in 24 hours
204	1.19	0.30	250	Died in 9 days
205	1.13	0.29	250	Died in 24 hours

Trials to find a smallest fatal dose were not continued because the material seemed too toxic to be of use.

000046

43

Sample 11  
Tricetyl phosphate

Rats - intraperitoneal

Rat No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
98	97	0.10	1,000	None
99	103	0.11	1,000	Died within 24 hours
100	116	0.09	800	Died within 24 hours
101	93	0.075	800	Died within 24 hours
102	111	0.07	600	Died within 24 hours
103	101	0.07	600	Died within 24 hours
104	118	0.05	400	Died within 24 hours

Smallest Fatal Dose below 400 mgms. per kilo

000047

44

Sample 12  
Methyl cellosolve phthalate

Guinea Pigs - by Mouth

Pig No.	Weight in grams	Dose in c.c.	dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
55	314	2.7	10,000	Died within 24 hours
137	313	1.8	10,000	Died within 24 hours
67	358	2.5	8,000	Died in 2 days
138	220	1.5	8,000	Died within 24 hours
139	175	1.2	8,000	Died in 2 days
141	231	1.7	7,000	Died in 4 days
68	305	1.6	6,000	None
69	244	1.3	6,000	None
140	207	1.1	6,000	None
4	246	1.0	5,000	Died in 2 days; stomach ulcers
39	228	0.9	5,000	None
40	180	0.8	5,000	Died in 9 days
52	221	0.95	5,000	Died in 14 days
70	232	1.2	5,000	None
142	202	0.86	5,000	None
71	209	0.72	4,000	None
5	218	0.5	3,000	None
22	208	0.4	2,000	None
21	163	0.2	1,500	None
6	235	0.1	500	None

Smallest Fatal Dose - 5,000 mgms. per kilo

000048

45

Sample 12  
Methyl Cellosolve phthalate

Guinea Pigs - Intraperitoneal

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
53	222	0.96	5,000	Died within 24 hours
143	191	0.82	5,000	Died within 24 hours
144	212	0.91	5,000	Died within 24 hours
153	236	1.01	5,000	Died within 24 hours
154	248	1.06	5,000	Died within 24 hours
155	230	0.98	5,000	Died in 2 days
145	191	0.66	4,000	Died within 24 hours
146	239	0.90	4,000	Died within 24 hours
147	267	0.69	3,000	Died in 6 days
148	222	0.57	3,000	None
54	263	0.45	2,000	None
149	237	0.41	2,000	None
150	282	0.48	2,000	None
25	312	0.40	1,500	None
24	189	0.20	1,200	None
151	225	0.19	1,000	None
152	322	0.28	1,000	None
41	208	0.10	500	None
23	238	0.10	500	None
42	265	0.05	250	None

Smallest Fatal Dose - 3,000 mgms. per kilo

000049

74

Sample 10  
Methyl cellosolve phthalate

Rabbits - by mouth

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
1	1.85	11.5	10,000	Died within 24 hours
2	1.42	6.1	5,000	Died within 24 hours
10	0.88	3.8	5,000	Died in 2 days
206	0.96	4.1	5,000	Died within 24 hours
207	1.05	3.6	4,000	Died in 2 days
208	1.05	3.6	4,000	Died in 2 days
209	1.28	3.3	3,000	Died in 3 days
210	1.02	2.6	3,000	Died in 4 days
219	0.99	2.6	3,000	Died in 6 days
11	1.59	3.4	2,500	Died in 14 days
220	0.99	2.1	2,500	Died in 2 days
211	1.08	1.9	2,000	Died in 6 days
212	1.19	2.1	2,000	None
222	1.36	2.4	2,000	Died in 9 days
225	0.93	2.4	2,000	Died in 3 days
223	0.93	1.2	1,500	Died in 14 days
3	1.20	1.0	1,000	None
12	1.64	1.4	1,000	None
213	1.50	1.3	1,000	None
234	0.96	0.8	1,000	None

Smallest Fatal Dose - 1,500 mgms. per kilo

000050

47

Sample 12  
Methyl cellosolve phthalate

Rabbits - intraperitoneal

Rabbit No.	weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
226	3.85	3.7	5,000	Died in 24 hours
227	3.83	3.8	5,000	Died in 24 hours
228	1.13	3.9	4,000	Died in 24 hours
230	3.88	3.0	4,000	Died in 24 hours
229	1.30	3.3	3,000	Died in 2 days
231	1.22	3.1	3,000	Died in 2 days
232	1.42	3.7	3,000	Died in 3 days
221	1.10	1.9	2,000	None
233	1.05	1.8	2,000	None
234	0.96	1.7	2,000	None
237	1.01	1.9	2,000	Died in 4 days
242	1.19	2.0	2,000	Died in 7 days
218	1.13	1.0	1,000	None
235	0.99	0.85	1,000	None
236	1.50	1.3	1,000	None
238	0.96	0.82	1,000	None
239	1.05	0.90	1,000	None
243	1.05	0.90	1,000	None
240	0.85	0.37	500	None
241	0.93	0.40	500	None

Smallest Fatal Dose - 2,000 mgms. per kilo

000051

24

Sample 12  
Methyl Cellosolve phthalate

Rats - intraperitoneal

Rat No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
88	110	0.48	5,000	Died within 24 hours
89	85	0.41	5,000	Died within 24 hours
90	104	0.42	4,000	Died within 24 hours
91	111	0.38	4,000	Died within 24 hours
105	112	0.39	4,000	Died in 2 days
92	136	0.35	3,000	Died in 3 days
93	105	0.32	3,000	None
106	101	0.31	3,000	None
107	101	0.26	3,000	None
108	111	0.29	3,000	None
94	118	0.20	2,000	Died in 7 days
95	99	0.17	2,000	Died in 5 days
109	117	0.20	2,000	None
110	103	0.18	2,000	None
111	90	0.16	2,000	None
96	104	0.09	1,000	Died in 6 days
97	108	0.09	1,000	Died in 8 days
112	117	0.10	1,000	None
113	110	0.10	1,000	None
114	116	0.10	1,000	None
200	96	0.07	300	None
201	126	0.085	800	None
202	126	0.085	800	None
203	106	0.05	600	None
204	111	0.055	600	None
205	140	0.06	500	None
206	134	0.06	500	None

Smallest Fatal Dose - 1000 mgms. per kilo

000052

Sample 12  
Methyl cellosolve phthalate

Mode - Intraperitoneal

Mouse No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
38	22.5	.200	10,000	Died within 24 hours
39	18.0	.125	8,000	Died within 24 hours
40	20.0	.105	6,000	Died within 24 hours
74	17.0	.035	6,000	Died within 24 hours
75	17.5	.090	6,000	Died within 24 hours
18	16.0	.070	5,000	Died within 24 hours
41	19.5	.065	5,000	Died within 24 hours
76	17.0	.075	5,000	Died within 24 hours
77	16.0	.070	5,000	Died within 24 hours
78	18.0	.080	5,000	Died in 4 days
19	16.5	.060	4,000	None
80	17.5	.060	4,000	None
81	17.0	.060	4,000	Died within 24 hours
82	20.0	.070	4,000	Died within 24 hours
20	19.0	.050	3,000	None
83	19.5	.050	3,000	None
84	20.0	.050	3,000	None
21	22.0	.040	2,000	None
22	24.0	.022	1,000	None

Smallest Fatal Dose - 4,000 mgms. per kilo

000053

05

Sample 12  
Methyl cellosolve phthalate

Chickens - by mouth

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
26	1.68	14.4	10,000	Died within 24 hours
27	1.50	10.3	8,000	Died within 24 hours
48	1.47	10.0	8,000	Died within 24 hours
82	1.05	7.2	8,000	Died in 4 days
49	1.28	7.7	7,000	Died in 2 days
83	1.10	6.6	7,000	Died in 2 days
50	1.36	7.0	6,000	None
84	1.36	7.0	6,000	Died within 24 hours
28	1.22	5.2	5,000	None
51	1.25	5.4	5,000	Died in 7 days
29	1.42	4.9	4,000	None
30	1.45	3.7	3,000	None
31	1.50	2.6	2,000	None
32	1.50	1.3	1,000	None

Smallest Fatal Dose - 5,000 mgms. per kilo

000054

51

Sample 12  
Methyl cellosolve phthalate

Chickens - intraperitoneal

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
43	1.59	6.8	5,000	Died within 24 hours
44	1.70	5.8	4,000	Died within 24 hours
45	1.39	3.6	3,000	Died within 24 hours
71	1.22	3.2	3,000	Died in 5 days (prostrated in 1 hour, recovered)
72	1.59	4.1	3,000	Died within 24 hours
47	1.33	2.3	2,000	None
73	1.73	3.0	2,000	None
92	1.39	2.4	2,000	None
93	1.65	2.8	2,000	None
46	1.39	1.2	1,000	None
94	1.76	1.5	1,000	None
95	1.62	1.4	1,000	None

Smallest Fatal Dose - 3,000 mgms. per kilo

000055

52

87-8211768

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Doc Title	INVESTIGATION OF TOXICITY OF CERTAIN			
	PLASTICIZERS REPORT 2. ACUTE TOXICITY TO			
	SMALL ANIMALS (CONCLUDED)			
Chemical Name (300 per name)	25	CAS No. (10)		24
ALKYL PHTHALATES		999999994		
BENZENEDICARBOXYLIC ACID DEBUTYL ESTER		84-74-2		
BENZENEDICARBOXYLIC ACID DICYCLOHEXYL ESTER		84-61-7		

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CONSULTATION AND OPINIONS IN PUBLIC HEALTH, INDUSTRIAL HYGIENE, TOXIC  
HAZARDS IN INDUSTRY, BIO-CHEMICAL PROBLEMS, BACTERIOLOGICAL PROCESSES,  
SPOILAGE, AND BACTERIOLOGICAL TRAVEL.

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PHILADELPHIA, PA.

*Investigation of  
toxicity of  
certain plasticizers*

**INVESTIGATION OF TOXICITY OF CERTAIN PLASTICIZERS**

**Report 2.**

**Acute Toxicity to Small Animals  
(Concluded)**

**February 1, 1932**

*Completed*

**RECEIVED**  
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## Scope of Report

This report covers acute toxicity tests made since our report of September 8, 1931. So far as is now known, it is a final report on acute toxicity of the materials included in our study.

## Materials

At the time our report of 9-8-31 was written, we believed Sample 9, Santicizer No. 8, to be too toxic to consider for plasticizer use. For this reason it was not administered at all to chickens or mice. Since that time we have learned that it is still considered a promising material, so that we have made enough additional tests with it to have as much acute toxicity data upon it as upon any of the samples.

The plasticizer samples for which new work is reported are:-

- Sample 9 - "Santicizer No. 8"
- 13 - Ethyl ester of o-Benzoyl Benzoic Acid
- 14 - Butyl Cellosolve Phthalate

In addition, a limited number of tests were made by feeding cellophane with waterproof coating containing Dibutyl Phthalate as plasticizer, to see if from single doses effects could be noted.

## Animals Used

The animals used for this work were similar to those used previously, and described on page 2 of the 9-8-31 report.

## Methods of dosing

Samples 9 and 14 were administered exactly as described on page 3 of the 9-8-31 report.

Sample 13, being a solid, had to be treated differently. For feeding to guinea pigs it was reduced to a thick paste with olive oil, after weighing out the required dose. This paste was placed in the animal's mouth, while it was held upside down in the operator's hand. The guinea pig, being a docile animal, swallowed the dose promptly and completely when it was fed in this way. Rabbits would not swallow the paste under any conditions. They had to be fed the sample dissolved in enough olive oil to make a perfect solution, using a stomach tube. Because of the large volumes of these doses, due to the low solubility of the sample in the oil, it was thought advisable to make control feedings of such large volumes of the oil alone. Chickens were fed weighed amounts of the sample in gelatin capsules, thrust into their crops. These samples were never regurgitated, and were ideal for chickens, but none of the other animals would swallow any size capsule at all.

Sample 13 was dissolved in olive oil and sterilized by heat for all the intraperitoneal injections. To decrease the amount of oil to be injected, these solutions were kept at 40° C, making it possible to dissolve more of the sample in a given amount of oil. Controls with

olive oil alone showed it to be perfectly harmless to the animals.

Cellophane was fed to the chickens rolled up tightly in gelatin capsules, 50 square inches to a capsule. Autopsy of chickens purposely killed showed the cellophane completely disintegrated in the intestines, so there was ample opportunity for absorption of any toxic substance from it in the bird's body. For feeding guinea pigs and rabbits, the cellophane was ground wet with water in a ball mill. Before feeding, the slurry was allowed to settle to a definite concentration and the supernatant fluid was used for grinding the next batch. Guinea pigs were fed the slurry in the same way they had been fed the liquid plasticizers, and rabbits were fed by a stomach tube. ✓

#### Observations on treated animals

The observations upon the animals were the same as those described in our 9-8-31 report, page 4.

Number of animals used

The table gives the number of animals reported upon for each material. "M" means administered by mouth, and "I.P." means by intraperitoneal injection.

Material	Guinea Pigs		Rabbits		Rats	Mice	Chickens		Total
	M.	I.P.	M.	I.P.	I.P.	I.P.	M.	I.P.	
9. Santicizer No.8	5	10	10	9	10	17	33	16	110
13. Ethyl ester of o-Benzoyl Benzoic	20	20	20	20	20	27	20	--	147
14. Butyl Cellosolve Phthalate	24	29	19	20	23	25	13	11	164
Coated Cellophane	10		10				12		32
Olive Oil Controls		2		2	4	4			14
Total for this report	59	61	61	51	57	73	78	27	467
Total 9-8-31 report	158	155	126	113	122	94	52	44	864
Total used for acute tests	433		351		179	167	201		1331

**Tabulation of Results  
(including those reported 9-8-31)**

**Smallest Dose Killing Any Animal**

(K = by mouth; I.P. = intraperitoneally)

	Guinea Pigs		Rabbits		Rats	Mice	Chickens	
	K.	I.P.	K.	I.P.	I.P.	I.P.	K.	I.P.
7. Dibutyl phthalate	2,000	1,000	3,000	2,000	1,000	3,000	over 20,000	15,000
8. Diethyl phthalate	5,000	1,000	4,000	4,000	1,000	3,000	15,000	5,000
9. "Santicizer No. 8"	1,500	250	600	250	100	500	600	800
10. "Ethox"	1,000	2,000	3,000	2,000	2,000	3,000	15,000	8,000
11. Tributyl phosphate	1,000	200	2,000	under 200	under 400			
12. Methyl cellosolve phthalate	5,000	3,000	1,500	2,000	1,000	4,000	5,000	3,000
13. Ethyl ester of o-Benzoyl Benzoic Acid	6,000	2,000	4,000	4,000	1,000	500	1,000	
14. Butyl cellosolve Phthalate	4,000	800	1,000	1,000	2,000	1,000	5,000	5,000

Coated Cellophane

No effect noted from any single dose

Guinea Pigs	10 fed	Maximum dose given = 6,000 ngs./kilo (254 sq. in./kilo)
Rabbits	10 fed	" " " 8,500 " 338 "
Chickens	12 fed	" " " 19,000 " 750 "

800000

Discussion

As in the 9-8-31 report, we have not used the term "Minimum Lethal Dose," preferring to report the smallest dose certain to kill. If other figures are desired, they can readily be obtained from the tables of doses given and results, which are part of this report.

The differences in species susceptibilities to the materials are about the same as those of the 9-8-31 report. As before, the fatal dose for intraperitoneal injection is usually smaller than for administration by mouth, probably because there is almost complete absorption of water-soluble or lipid-soluble materials from the peritoneal cavity, whereas a large portion of the latter class of materials, particularly when administered in massive doses, may pass completely through the digestive tract without being absorbed. When administering doses by mouth to most species, there is always the possibility that the animal regurgitate some of the dose. However, we are positive that this happened with none of our doses, since the animals were carefully watched with this possibility in mind.

On the basis of results of acute toxicity tests, we would arrange the plasticizers tested in the following order, the least toxic first and the most toxic last:

Fed by mouth.....	7	-	8	-	10	-	13	-	12	-	14	-	11	-	9
Injected peritoneally....	7	-	10	-	3	-	12	-	14	-	13	-	9	-	11

*Di B*  
*Di ET*

As we had anticipated, it was impossible to force enough coated cellophane down any of the animals to give acute toxic effects.

Sample 13

Ethyl ester of o-Benzoyl Benzoic Acid

Guinea Pigs - by mouth, fed as a thick paste in olive oil

Pig No.	Weight in grams	Dose in gms.	Dosage in mgms. per kilo	Result ( "None" = none seen in 14 days)
878	369	4.0	11,000	Died within 7 days
895	246	2.5	10,000	None
896	246	2.5	10,000	None
897	283	2.8	10,000	None
886	350	3.5	10,000	None
387	380	3.8	10,000	None
876	321	2.5	8,000	None
877	400	3.0	7,500	None
865	254	1.5	6,000	Died in 12 days
867	311	2.0	6,000	None
875	287	2.0	5,000	None
890	290	1.5	5,000	None
891	286	1.4	5,000	None
888	372	1.9	5,000	None
865	277	1.0	4,000	None
892	279	1.1	4,000	None
893	258	1.1	4,000	None
894	270	1.1	4,000	None
780	318	1.0	3,000	None
829	255	0.5	2,000	None

Smallest Fatal Dose - 6,000 mgms. per kilo

Sample 13  
Ethyl Ester of o-Benzoyl Benzoic Acid

Guinea Pigs - intraperitoneally; as a 10% solution in Olive Oil. See Page 31 for control tests on Olive Oil alone

Pig No.	Weight in grams	Dose in c.c. of 10% sol.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
900	334	33.0	10,000	Died within 24 hours
899	339	16.5	5,000	Died within 24 hours
920	311	7.75	2,500	Died within 24 hours
921	223	8.05	2,500	Died within 24 hours
938	293	5.9	2,000	Died within 24 hours
939	306	6.1	2,000	None
940	288	5.2	2,000	Died within 24 hours
941	307	6.1	2,000	None
922	342	5.15	1,500	None
923	310	4.65	1,500	None
898	328	3.33	1,000	None
924	332	3.3	1,000	None
925	343	3.4	1,000	None
942	318	3.2	1,000	None
943	299	3.0	1,000	None
926	320	1.6	500	None
927	386	1.9	500	None
928	360	1.8	500	None
944	370	1.9	500	None
945	312	1.5	500	None

Smallest Fatal Dose - 2,000 mgms. per kilo

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Sample 13  
Ethyl ester of o-Benzoyl Benzoic Acid

Rabbits - by Mouth; Fed as a 12.5% solution in olive oil. See page 31  
 for control tests on the oil alone.

Rabbit No.	Weight in kilos	Dose in c.c. 12.5% solution	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
332	0.99	80.	10,000	Died within 24 hours
333	1.30	104.	10,000	Died in 2 days
334	1.30	104.	10,000	Died within 24 hours
335	1.13	72.	8,000	Died in 2 days
336	1.20	77.	8,000	Died in 2 days
337	1.50	96.	8,000	Died in 2 days
338	1.28	52.	5,000	Died in 5 days
339	1.05	42.	5,000	Died in 4 days
340	1.06	43.	5,000	Died in 6 days
341	0.99	32.	4,000	None
342	1.22	39.	4,000	None
343	1.53	49.	4,000	Died in 4 days
344	1.59	38.	3,000	None
345	1.73	42.	3,000	None
346	1.13	18.	2,000	None
347	1.45	23.	2,000	None
348	1.22	20.	2,000	None
349	1.59	25.	2,000	None
350	1.13	9.	1,000	None
351	1.10	4.	500	None

Smallest Fatal Dose - 4,000 mgms. per kilo.

Sample 13  
Ethyl ester of o-Benzoyl Benzoic acid

Rabbits - intraperitoneally; injected as a 10% solution in olive oil.  
See page 31 for control tests on the oil alone.

Rabbit No.	Weight in kilos	Dose in c.c. 10% solution	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
312	1.45	53.0	4,000	Died within 24 hours
313	1.13	45.0	4,000	Died within 24 hours
314	1.60	48.0	3,000	Died within 24 hours
315	1.13	34.0	3,000	None
316	1.13	28.0	2,500	None
317	0.96	19.2	2,000	Died in 5 days
318	1.30	26.0	2,000	None
319	1.50	20.0	2,000	None
320	1.68	34.0	2,000	None
321	1.82	27.2	1,500	None
322	1.02	15.2	1,500	None
323	1.32	20.0	1,500	None
324	1.40	21.0	1,500	None
325	1.36	20.4	1,500	None
326	1.42	14.2	1,000	None
327	1.42	14.2	1,000	None
328	1.70	17.0	1,000	None
329	1.79	16.0	1,000	None
330	1.73	17.0	1,000	None
331	1.30	13.0	1,000	None

Smallest Fatal Dose - 2,000 mgms. per kilo

Sample 13  
Ethyl Ester of o-Benzoyl Benpic acid

Rats - Intraperitoneally; as 10% solution in olive oil. See Page 31 for control tests on Olive Oil alone

Rat No.	Weight in grams	Dose in c.c. 10% sol.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
133	154	15.4	10,000	Died within 24 hours
134	146	7.3	5,000	Died within 24 hours
135	102	5.1	5,000	Died within 24 hours
136	96	4.8	5,000	Died within 24 hours
137	73	2.2	3,000	Died within 24 hours
138	123	3.7	3,000	Died within 24 hours
139	111	2.2	2,000	Died within 24 hours
140	103	2.1	2,000	Died within 24 hours
141	140	2.8	2,000	Died within 24 hours
142	133	1.3	1,000	None
143	148	1.5	1,000	None
144	58	0.58	1,000	None
145	95	0.95	1,000	None
146	179	1.8	1,000	None
147	133	1.3	1,000	None
148	91	0.9	1,000	Died within 24 hours
149	134	1.1	800	None
150	128	1.0	800	None
151	117	0.7	600	None
152	104	0.6	600	None

Smallest Fatal Dose - 1000 mgms. per kilo

Sample 13  
Ethyl ester of o-Benzoyl Benzoic acid

Mice - intraperitoneally; as 10% solution in olive oil. See page 91 for control tests on olive oil alone

Mouse No.	Weight in grams	Dose in c.c. 10% solut.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
112	22	2.2	10,000	Died within 24 hours
113	25	1.25	5,000	Died within 24 hours
114	24	1.2	5,000	Died within 24 hours
115	29	0.58	2,000	Died within 24 hours
116	20	0.40	2,000	Died within 24 hours
117	26	0.52	2,000	Died in 3 days
118	29	0.29	1,000	Died within 24 hours
119	21	0.21	1,000	None
120	25	0.25	1,000	None
121	26	0.26	1,000	None
122	25	0.25	1,000	Died in 3 days
123	22	0.22	1,000	Died in 3 days
124	25	0.20	800	None
125	27	0.22	800	None
126	23	0.16	800	Died in 3 days
127	24	0.12	500	Died in 10 days
128	31	0.15	500	None
129	27	0.14	500	Died in 10 days
130	22	0.11	500	None
131	24	0.07	300	None
132	24	0.07	300	None
133	23	0.07	300	None
134	22	0.044	200	None
135	24	0.048	200	None
136	22	0.04	200	None

Smallest Fatal Dose - 500 mgms. per kilo

Sample 13  
Ethyl Ester of o-Benzoyl Benzoic Acid

Chickens - by Mouth (in capsules)

Chick No.	Weight in kilos	Dose in grams	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
147	0.96	7.5	8,000	Died in 4 days
148	0.71	3.5	5,000	Died in 2 days
149	0.85	4.0	5,000	None
150	1.28	5.0	4,000	None
151	0.59	2.0	3,500	Died in 5 days
152	0.91	2.5	3,000	Died in 11 days
153	1.25	3.0	2,500	None
154	1.39	2.5	2,000	None
155	0.91	1.5	1,500	None
156	1.19	2.0	1,500	None
157	0.88	1.0	1,000	Died in 9 days
158	1.05	1.0	1,000	None
159	0.85	1.0	1,000	None
160	0.91	0.5	500	None
161	1.19	0.5	500	None
162	1.79	1.0	500	None
163	1.25	0.4	300	None
164	1.53	0.4	300	None
165	1.50	0.3	300	None
166	1.59	0.4	300	None

Smallest Fatal Dose - 1,000 mgms. per kilo

**Sample 14**  
**Butyl Cellosolve Phthalate**

**Guinea Pigs - by mouth**

<b>Pig No.</b>	<b>Weight in grams</b>	<b>Dose in c.c.</b>	<b>Dosage in mgms. per kilo</b>	<b>Result ("None" = none seen within 14 days)</b>
868	305	2.88	10,000	Died within 24 hours
869	260	2.43	10,000	Died in 2 days
870	174	1.32	8,000	Died in 2 days
871	230	1.74	8,000	Died within 24 hours
879	281	1.43	6,000	None
880	175	1.00	6,000	Died within 24 hours
828	310	1.47	5,000	None
901	279	1.58	6,000	None
881	245	1.16	5,000	None
882	291	1.38	5,000	None
883	296	1.40	5,000	Died within 24 hours
902	313	1.48	5,000	Died within 24 hours
903	291	1.10	4,000	None
904	353	1.33	4,000	Died in 3 days
905	271	1.05	4,000	None
906	318	1.19	4,000	None
788	283	0.80	3,000	None
929	317	0.90	3,000	None
930	320	0.91	3,000	None
933	321	0.91	3,000	None
783	392	0.74	2,000	None
931	350	0.66	2,000	None
932	340	0.64	2,000	None
782	262	0.25	1,000	None

Smallest Fatal dose - 4,000 mgms. per kilo

Sample 14  
Butyl Cellosolve Phthalate

Guinea Pigs - intraperitoneally

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
872	271	1.28	5,000	Died within 24 hours
907	334	1.57	5,000	Died within 24 hours
908	334	1.26	4,000	Died within 24 hours
909	411	1.55	4,000	Died in 2 days
910	371	1.25	4,000	Died within 24 hours
873	246	0.70	3,000	None
911	371	1.05	3,000	Died in 2 days
912	325	0.92	3,000	None
913	337	0.95	3,000	Died within 24 hours
934	310	0.59	2,000	None
935	306	0.58	2,000	Died in 2 days
936	342	0.65	2,000	None
937	375	0.71	2,000	None
946	307	0.58	2,000	Died within 24 hours
952	271	0.52	2,000	None
874	281	0.27	1,000	None
947	258	0.24	1,000	Died within 24 hours
948	342	0.32	1,000	Died within 24 hours
949	307	0.29	1,000	Died within 24 hours
953	355	0.34	1,000	None
954	371	0.35	1,000	None
955	359	0.34	1,000	None
950	362	0.27	800	Died within 24 hours
951	257	0.19	800	None
956	410	0.31	800	None
957	378	0.29	800	None
958	350	0.26	800	None
959	369	0.17	500	None
960	421	0.20	500	None

Smallest Fatal Dose - 800 mgms. per kilo

Sample 14  
Butyl Cellosolve Phthalate

Rabbit - by Mouth

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
260	1.84	17.6	10,000	Died within 24 hours-
261	3.1	14.6	5,000	Died within 24 hours
262	1.33	6.3	5,000	Died within 24 hours
263	1.25	3.6	3,000	Died in 2 days
264	1.62	4.6	3,000	Died within 24 hours
265	2.64	5.0	2,000	Died within 24 hours
266	1.65	3.2	2,000	Died in 6 days
267	1.87	3.5	2,000	Died in 7 days
268	2.25	4.2	2,000	None
269	2.30	4.3	2,000	Died within 24 hours
270	2.30	2.2	1,000	None
271	2.04	1.9	1,000	Died within 24 hours
272	1.16	1.1	1,000	None
273	1.22	1.2	1,000	None
274	1.22	0.93	800	None
275	1.22	0.93	800	None
276	1.36	1.03	800	None
277	0.93	0.45	500	None
278	1.45	0.68	500	None

Smallest Fatal Dose - 1,000 mgms. per kilo

- 17 -

000020

Sample 14  
Butyl Cellosolve Phthalate

Rabbits - intraperitoneally

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
240	1.36	6.4	5,000	Died within 24 hours
241	1.30	6.1	5,000	Died within 24 hours
242	1.16	3.3	3,000	Died in 2 days
243	1.47	2.8	2,000	Died in 2 days
244	1.02	1.9	2,000	Died in 7 days
245	1.33	2.5	2,000	Died within 24 hours
246	1.25	1.2	1,000	None
247	1.02	0.95	1,000	Died in 14 days
248	1.62	1.5	1,000	None
249	1.39	1.3	1,000	None
250	1.30	1.2	1,000	None
251	1.29	1.3	1,000	None
252	1.22	0.98	800	None
253	1.30	0.98	800	None
254	1.19	0.90	800	None
255	1.30	0.98	800	None
256	1.93	1.45	800	None
257	1.50	0.85	600	None
258	1.59	0.90	600	None
259	1.65	0.93	600	None

Smallest Fatal Dose - 1,000 mgms. per kilo

Sample 14  
Butyl Cellosolve Phthalate

Rats - intraperitoneally

Rat No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
153	143	0.68	5,000	None
184	185	0.90	5,000	Died within 24 hours
155	138	0.39	3,000	Died within 24 hours
156	175	0.49	3,000	None
157	179	0.34	2,000	Died in 3 days
158	142	0.27	2,000	None
159	138	0.26	2,000	None
160	165	0.32	2,000	None
161	156	0.30	2,000	None
162	168	0.16	1,000	None
163	153	0.14	1,000	None
164	167	0.16	1,000	None
165	179	0.17	1,000	None
166	118	0.11	1,000	None
167	130	0.12	1,000	None
168	133	0.10	800	None
169	150	0.11	800	None
170	195	0.15	800	None
171	214	0.16	800	None
172	169	0.08	500	None
173	116	0.055	500	None
174	153	0.07	500	None
175	159	0.075	500	None

Smallest Fatal Dose - 2,000 mgms. per kilo

Sample 14  
Butyl Cellosolve Fthalate

Mice - intraperitoneally

Mouse No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
137	27	0.25	10,000	Died within 24 hours
138	25	0.12	5,000	Died within 24 hours
139	25	0.12	5,000	Died within 24 hours
140	25	0.10	4,000	Died within 24 hours
141	26	0.10	4,000	Died within 24 hours
142	27	0.08	3,000	Died in 6 days
143	22	0.06	3,000	Died within 24 hours
144	23	0.06	3,000	Died within 24 hours
145	21	0.04	2,000	Died within 24 hours
146	23	0.05	2,000	None
147	21	0.04	2,000	Died within 24 hours
148	27	0.05	2,000	None
149	29	0.03	1,000	None
150	23	0.02	1,000	Died in 5 days
151	25	0.025	1,000	Died in 5 days
152	24	0.025	1,000	None
153	23	0.02	1,000	None
154	25	0.025	1,000	None
155	31	0.025	800	None
156	25	0.02	800	None
157	23	0.02	800	None
158	20	0.015	800	None
159	23	0.01	500	None
160	25	0.01	500	None
161	22	0.01	500	None

Smallest Fatal Dose - 1,000 mgms. per kilo

Sample 14  
Butyl Cellosolve Phthalate

Chickens - by Mouth

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
• 167	0.95	16.0	20,000	None
168	1.42	26.8	20,000	None
169	1.56	29.4	20,000	None
170	1.56	29.4	20,000	None
171	0.94	8.9	10,000	None
172	1.05	10.0	10,000	Died within 24 hours
173	1.62	15.3	10,000	None
174	1.96	18.5	10,000	None
175	1.13	5.3	5,000	None
176	1.56	7.4	5,000	None
177	1.06	5.0	5,000	None
178	1.42	6.7	5,000	Died in 14 days
179	1.47	2.8	2,000	None

Smallest Fatal Dose - 5,000 mgms. per kilo

Sample 14  
Butyl Cellulosate Phthalate

Chickens - intraperitoneally

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
180	1.02	9.6	10,000	Died in 7 days
181	0.99	9.3	10,000	None
182	0.85	4.0	5,000	None
183	1.33	6.3	5,000	None
184	0.76	3.6	5,000	Died in 5 days
185	1.87	3.8	5,000	None
186	1.68	4.9	3,000	None
187	1.62	4.6	3,000	None
188	1.99	5.6	3,000	None
189	1.50	4.2	3,000	None
190	1.08	2.0	2,000	None

Smallest Fatal Dose - 5,000 mgms. per kilo

Sample 9  
Santicizer No. 8

Guinea Pigs - by Mouth; Animals marked with an asterisk (\*) were previously tabulated in our report of 9-8-31

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen in 14 days)
194*	205	1.72	10,000	Died within 24 hours
195*	210	1.42	8,000	Died within 24 hours
250*	157	1.05	8,000	Died within 24 hours
196*	200	1.00	6,000	None
251*	259	1.50	6,000	Died in 8 days
203*	245	1.02	5,000	None
252*	256	1.08	5,000	Died in 2 days
197*	165	0.55	4,000	None
225*	257	0.37	4,000	Died within 24 hours
253*	202	0.68	4,000	None
198*	190	0.48	3,000	Died within 24 hours
226*	300	0.75	3,000	None
254*	308	0.78	3,000	None
255*	186	0.47	3,000	None
228*	195	0.42	2,500	None
199*	200	0.34	2,000	None
227*	160	0.27	2,000	None
256*	283	0.48	2,000	None
229*	320	0.41	1,500	Died in 9 days
741	292	0.37	1,500	None
756	282	0.35	1,500	None
200*	190	0.16	1,000	None
230*	180	0.15	1,000	None
231*	215	0.18	1,000	None
530	240	0.20	1,000	None
543	387	0.33	1,000	None
739	344	0.29	1,000	None
201*	190	0.08	500	None
202*	215	0.04	200	None

Smallest Fatal Dose - 1,500 mgms. per kilo

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Sample 9  
Santicizer No. 8

Guinea Pigs - Intraperitoneally; animals marked with an asterisk (\*) were previously tabulated, in our report of 9-8-31

Pig No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
216*	138	0.79	5,000	Died within 24 hours
217*	128	0.42	4,000	Died within 24 hours
218*	214	0.54	3,000	Died within 24 hours
219*	195	0.33	2,000	Died within 24 hours
220*	255	0.22	1,000	Died within 24 hours
257*	263	0.22	1,000	Died within 24 hours
701	280	0.24	1,000	None
258*	274	0.21	300	None
259*	244	0.16	750	Died within 24 hours
221*	210	0.09	500	Died within 24 hours
222*	226	0.09	500	None
260*	275	0.12	500	None
516	230	0.12	500	None
781	363	0.15	500	None
606	259	0.11	500	None
261*	244	0.08	400	None
262*	189	0.06	400	None
263*	240	0.06	300	None
264*	295	0.07	300	None
223*	222	0.05	250	Died within 24 hours
224*	188	0.04	250	None
714	292	0.06	250	None
832	311	0.07	250	None
769	290	0.06	250	None
795	277	0.06	250	None
265*	267	0.05	200	None
266*	271	0.05	200	None
225*	138	0.01	100	None
226*	175	0.015	100	None
267*	353	0.03	100	None
268*	283	0.03	100	None
523	300	0.03	100	None
792	300	0.03	100	None

Smallest Fatal Dose - 250 mgms. per kilo

Sample 9; Santicizer No. 8

Rabbits - by Mouth; Animals marked with an asterisk (\*) were previously tabulated in our report of 9-8-31

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
77*	1.08	4.6	5,000	None
82*	1.16	4.9	5,000	Died within 24 hours
78*	1.10	3.7	4,000	Died
83*	1.70	5.7	4,000	Died within 24 hours
79*	1.02	2.6	3,000	Died within 24 hours
84*	1.20	3.2	3,000	Died within 24 hours
85*	1.22	3.2	3,000	Died within 24 hours
133*	1.22	3.1	3,000	Died within 24 hours
80*	0.96	1.6	2,000	Died within 24 hours
86*	1.70	2.9	2,000	Died in 2 days
87*	1.79	3.0	2,000	None
134*	1.10	1.9	2,000	Died within 24 hours
135*	1.25	2.1	2,000	Died within 24 hours
81*	1.05	0.89	1,000	None
136*	1.59	1.3	1,000	None
137*	1.68	1.4	1,000	None
288	1.10	0.92	1,000	None
90*	1.25	0.84	800	Died in 11 days
91*	1.25	0.84	800	None
289	0.96	0.64	800	None
92*	1.05	0.53	600	Died in 12 days
93*	1.05	0.53	600	Died in 11 days
290	1.19	0.50	500	None
291	2.90	1.22	500	None
292	2.96	1.25	500	None
293	1.02	0.26	300	None
294	1.33	0.34	300	None
295	1.25	0.32	300	None
296	2.61	0.66	300	None
297	2.58	0.63	300	None

Smallest Fatal Dose - 600 mgms. per kilo

Sample 9; Santicizer No. 8

Rabbits - Intraperitoneally; animals marked with an asterisk (\*) were previously tabulated in our report of 9-8-31

Rabbit No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
94*	0.91	3.8	5,000	Died within 24 hours
95*	1.45	4.9	4,000	Died within 24 hours
96*	1.22	3.1	3,000	Died within 24 hours
97*	1.36	3.4	3,000	Died within 24 hours
139*	1.59	2.9	2,500	Died within 24 hours
140*	1.47	3.1	2,500	Died within 24 hours
97*	1.25	3.1	2,000	None
141*	1.62	2.6	2,000	Died within 24 hours
142*	1.10	1.9	2,000	Died within 24 hours
89*	1.16	0.97	1,000	None
98*	0.85	0.72	1,000	Died within 24 hours
143*	1.02	0.85	1,000	None
144*	1.25	1.04	1,000	None
145*	1.45	0.91	750	Died within 24 hours
146*	1.10	0.69	750	None
149*	1.10	0.47	500	None
147*	1.30	0.55	500	None
279	0.93	0.39	500	None
148*	1.13	0.24	250	None
150*	1.28	0.27	250	Died in 8 days
280	0.93	0.20	250	Died in 14 days
281	1.25	0.26	250	None
282	2.72	0.57	250	None
283	2.42	0.51	250	None
151*	1.47	0.12	100	None
152*	1.39	0.12	100	None
284	0.82	0.07	100	None
285	1.22	0.10	100	None
286	1.25	0.11	100	None
287	2.42	0.20	100	None

Smallest Fatal Dose - 250 mgms. per kilo

Sample 9  
Senticizer No. 8

Rate - intraperitoneally; Animals marked with an asterisk (\*) were previously tabulated in our report of 9-8-31

Rat No.	Weight in grams	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
37*	102	0.17	2,000	Died in one hour
50*	135	0.23	2,000	Died in 5 hours
38*	112	0.14	1,500	Died in one hour
39*	122	0.15	1,500	Died in one hour
40*	117	0.10	1,000	Died in 5 hours
41*	163	0.14	1,000	Died in one hour
42*	155	0.10	750	None
43*	115	0.07	750	Died in 5 hours
73*	81	0.05	750	Died within 24 hours
74*	98	0.06	750	Died within 24 hours
75*	110	0.06	600	None
76*	134	0.07	600	Died within 24 hours
44*	124	0.05	500	None
45*	154	0.07	500	Died in 5 hours
77*	106	0.04	500	None
78*	126	0.05	500	Died in 6 days
79*	102	0.03	400	Died in 11 days
80*	119	0.04	400	Died in 8 days
81*	140	0.04	300	Died in 12 days
82*	137	0.04	300	Died in 9 days
123	122	0.03	300	None
46*	130	0.03	250	Died in 4 days
47*	130	0.03	250	None
124	114	0.02	200	Died in 7 days
48*	134	0.01	100	Died in 7 days
49*	140	0.01	100	Died in 8 days
125	127	0.01	100	None (as 20% solution in Olive Oil)
126	122	0.01	100	None "
127	131	0.011	100	None "
128	144	0.012	100	None "
129	97	0.004	50	None (as 10% solution in Olive Oil)
130	70	0.003	50	None
131	151	0.006	50	None
132	171	0.007	50	None "

Smallest Fatal Dose - 100 Gms. per kilo

Sample 9  
Santicizer No. 8

Mice - intraperitoneally † as 10% solution in olive oil. See page 31 for results on olive oil alone, as controls.

Mouse No.	Weight in grams	Dose in c.c. 10% solut.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
95	25	1.05	5,000	Died within 24 hours
96	25	0.85	4,000	Died within 24 hours
97	29	0.70	3,000	Died within 24 hours
98	27	0.60	2,500	None
99	22	0.50	2,500	None
100	26	0.43	2,000	Died within 24 hours
101	25	0.21	1,000	Died within 24 hours
102	24	0.21	1,000	Died in 2 days
103	25	0.105	500	None
104	23	0.10	500	Died within 24 hours
105	27	0.11	500	None
106	25	0.105	500	None
107	26	0.1	500	Died in 2 days
108	25	0.05	250	None
109	26	0.05	250	None
110	23	0.08	250	None
111	25	0.05	250	None

Smallest Fatal Dose - 500 mgms. per kilo

Sample 9: Santicizer No. 8Chickens - by Mouth

Chick	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
98	1.93	16.6	10,000	Died in 24 hours
99	2.33	15.5	8,000	Died in 2 days
100	2.36	9.9	5,000	Died in 2 days
101	1.87	7.9	5,000	Died in 2 days
102	1.68	7.0	5,000	None
103	1.45	4.9	4,000	None
104	1.99	6.7	4,000	None
105	1.76	4.4	3,000	None
106	1.62	4.1	3,000	Died in 2 days
107	2.10	5.3	3,000	None
108	1.96	4.9	3,000	Died in 10 days
109	1.93	4.9	3,000	None
110	2.19	3.7	2,000	None
111	1.90	3.2	2,000	None
112	1.79	3.0	2,000	None
113	0.99	1.7	2,000	Died within 24 hours
114	1.25	2.1	2,000	None
115	0.99	1.13	1,500	None
116	1.25	1.57	1,500	None
117	1.08	0.91	1,000	None
118	0.59	0.50	1,000	None
119	1.90	1.6	1,000	Died within 24 hours
120	1.56	1.3	1,000	Died within 24 hours
121	0.89	0.75	1,000	Died in 6 days
122	1.73	1.2	800	Died within 24 hours
123	1.13	0.76	800	None
124	1.42	0.60	500	None
125	1.39	0.56	500	None
126	1.50	0.63	500	None
127	1.59	0.67	500	None
128	1.39	0.47	300	None
129	1.38	0.47	300	None
130	1.90	0.64	300	None

Smallest Fatal Dose - 800 mgms. per kilo

Sample 9  
Santicizer No. 8

Chickens - intraperitoneally

Chick No.	Weight in kilos	Dose in c.c.	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
131	1.70	2.9	2,000	Died within 24 hours
132	1.96	1.7	1,000	Died in 2 days
133	1.08	0.91	1,000	Died within 24 hours
134	1.13	0.95	1,000	None
135	1.73	1.16	800	None
136	1.76	1.18	800	None
137	0.99	0.66	800	Died within 24 hours
138	1.13	0.76	800	Died within 24 hours
139	1.65	0.69	500	None
140	1.90	0.80	500	None
141	1.28	0.54	500	None
142	1.25	0.53	500	None
143	1.96	0.50	500	None
144	0.99	0.25	300	None
145	1.36	0.34	300	None
146	1.08	0.29	300	None

Smallest Fatal Dose - 800 mgms. per kilo

Controls  
Olive Oil (sterilized)

Intraperitoneal

Species	Number	Weight in grams	Dose in c.c.	Result ("None" = none seen within 14 days)
Mouse	162	26	0.1	None
"	163	24	0.2	None
"	164	26	2.5	None
"	165	28	2.5	None
Rat	176	142	15.0	None
"	177	116	15.0	None
"	178	127	15.0	None
"	179	80	15.0	None
G. Pig	961	278	25.0	None
"	962	395	25.0	None
Rabbit	298	1130	25.0	None
"	299	1420	50.0	None

By Mouth

Rabbit	300	1160	50.0	None
"	301	1790	100.0	None

Coated Cellophane

Guinea Pigs - Fed by Mouth ( as suspension of ground material in water. 1 c.c. =  
4 square inches cellophane)

Pig No.	Weight in grams	Dose in sq. inches	Dosage in sq. inches per kilo	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
810	314	80	254	6,000	None
525	340	30	234	6,000	None
830	349	80	229	6,000	None
740	279	43	154	4,000	None
831	268	40	149	3,500	None
786	301	40	133	3,500	None
785	342	40	117	3,000	None
537	353	40	113	3,000	None
715	300	21.5	72	2,000	None
727	291	13	45	1,000	None

No fatal Dose fed

Coated Cellophane

Rabbits - fed by Mouth: (as suspension of ground material in water, administered through stomach tube. One c.c. = 4 square inches cellophane)

Rabbit No.	Weight in Kilos	Dose in sq. inches	Dosage in sq. inches per kilo	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
302	1.27	430	338	8,500	None
303	1.30	430	330	8,000	None
304	1.05	344	328	8,000	None
305	1.10	344	312	8,000	None
306	1.39	430	309	7,500	None
307	0.73	172	236	6,000	None
308	1.05	246	234	6,000	None
309	1.28	86	67	1,500	None
310	0.82	86	105	2,500	None
311	1.79	25	14	400	None

No fatal dose fed

000036

Coated Cellophane

Chickens - fed by Mouth (cellophane rolled tightly in gelatine capsules)

Chick No.	Weight in kilos	Dose in sq. inches	Dosage in sq. inches per kilo	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
191	1.33	1000	750	19,000	None
192	1.68	1000	595	15,000	None
193	1.82	1000	550	14,000	None
194	1.84	1000	545	13,000	None
195	1.87	1000	535	13,000	None
196	1.22	500	410	10,000	None
197	1.28	500	390	10,000	None
198	1.82	500	275	7,000	None
199	1.62	350	210	5,000	None
200	1.16	200	172	4,000	None
201	1.53	100	65	1,600	None
202	1.39	52.5	38	1,000	None

No fatal Dose fed

OFFICE OF TOXIC SUBSTANCES  
CODING FORM FOR GLOBAL INDEXING

REV. 7/27/82

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Doc Type	• R I • U P • H E A S D 8 D . . . S U H S F N			22
Doc Title	INVESTIGATION OF TOXICITY OF			23
CERTAIN PLASTICIZERS REPORT NO. 3. CHRONIC				
TOXICITY TO SMALL ANIMALS				
Chemical Name (300 per name)	25		CAS No. (10)	24
ALKYL PHTHALATES			99999994	
BENZENEDECARBOXYLIC ACID, DEBUTYL ESTER			84-74-2	
BENZENEDECARBOXYLIC ACID, DICYCLOHEXYL ESTER			84-61-7	

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CONSULTATION AND INVESTIGATIONS IN PUBLIC HEALTH, INDUSTRIAL HYGIENE, TOXIC  
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**INVESTIGATION OF TOXICITY OF CERTAIN PLASTICIZERS**

**Report 3.**

**Chronic Toxicity to Small Animals**

April 13, 1932

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## Scope

This report covers investigations of the chronic toxicity of Dibutyl phthalate, "Santicizer No. 8" and Moisture-Proof Cellophane when administered by mouth to small animals. A few feedings of Diethyl phthalate were made before we received orders to drop this material. Acute toxicity of all these materials was considered in our reports of September 8, 1931, and February 1, 1932.

The plasticizers studied were chosen from the group of eleven originally submitted to us for examination on the basis of their acute toxicity, and their availability for commercial use according to information from the DuPont Company. The actual specimens used were those received for the acute work.

## Animals and their Care

The animals used in this work were guinea pigs, white rats and rabbits. A total of 462 received repeated small doses and results obtained from that number are in this report. Approximately 15 per cent of the total number of animals were controls, receiving no doses at all.

The guinea pigs weighed approximately 300 grams when received and were practically all males. The rats weighed between 100 and 150 grams when received and were of mixed sexes. In almost every case each test group of rats was half males and half females. The rabbits weighed between 1000 and 1500 grams when received and were of mixed sexes. In general, the

test groups of rabbits were also of mixed sexes. The chickens weighed from 1000 to 2000 grams and were barred Plymouth Rocks, of mixed sexes. No animal was used for these chronic toxicity tests until at least two weeks' observation and weighings had shown that it was normal and apparently in good health. Several hundred animals were discarded during this two weeks' observation period because they were unfit for chronic tests.

Each group of guinea pigs consisted of 12 animals receiving identical treatments and living in the same cage. Each group of rats consisted of 12 animals receiving identical treatment and living in two separate cages, because this number would have unduly crowded one rat cage. Each group of rabbits consisted of six animals living in the same cage and receiving identical treatment. The chickens all lived together in one house and run.

The guinea pig and rabbit cages were built of wood with wire mesh at the front and back. Each cage was approximately 36 x 24 x 18 inches high and the cages were arranged in tiers of three. These animals were kept on timothy-hay bedding, which was replaced twice a week. During cleaning of the cages the walls and floors were sprayed with a cresol solution, and the cages were allowed to air 24 hours before animals were put in them again. The rats were kept in wire mesh cages approximately 12 x 16 x 12 inches high. Their bedding of fine wood shavings was placed in a galvanized iron pan inside the cage. The bedding was changed twice a week,

and approximately monthly the cages were boiled out in a disinfectant solution. All of these cages were kept in well-ventilated, light rooms and the animals were subjected to as little excitement as possible.

, The rabbits and guinea pigs were fed a diet of oats, carrots and cabbage, in addition to the hay bedding which they ate freely, and they received water. The food of the rats consisted of a mixture of ground whole wheat, whole milk powder and salt. They received abundant water through siphon bottles attached to the sides of the cage. The chickens ate wheat and dry mash, and received plenty of water.

Each animal was weighed weekly and its general condition recorded.

#### Methods of Dosing

The guinea pigs were fed their doses five or six days each week. All the materials were administered in the way described in our report of September 8th; that is, while the animal was held upside down in the hand the dose was measured into the mouth from a pipette and the animal held in this position until the dose was swallowed. The rabbits were fed the plasticizers five or six days a week in the same way as the guinea pigs were fed. The rats received their doses mixed with their diet in definite proportions, so that they were fed seven days a week. The chickens were fed only the cellophane. They received it rolled tightly into gelatine capsules, 50 square inches or 1.24 grams, to a capsule. These capsules were thrust into the birds' crops five or six times a week.

Each week the doses to be administered to the animals were calculated on the basis of the total weight of each group receiving the same dose. For example, if the 12 guinea pigs receiving 400 mgms. per kg. weighed 2 kgs., then 800 mgms. of the material would be the daily dose for that group. Since there were 12 animals, each one would receive 67 mgms of the sample. This weight was then converted to volume and the doses were measured from a pipette. In the case of the rats it was necessary to determine not only how much of the material should be added to their diet, but also at the end of the week how much of the material they had actually eaten in their diet, since almost never did they eat the exact amount of food that we had expected, so that their dosage never came out to the exact figure that we had anticipated. Since they were fed the powdery diet in McCollum feeding pans, practically none of it was lost, and the amount actually eaten could be determined fairly closely.

For administering the doses of cellophane it was necessary to grind the material. Definite quantities of cellophane were ground in a ball mill with water to give suspensions of definite concentrations. The guinea pigs were fed these suspensions directly. This made it impossible to feed large doses of cellophane to the guinea pigs, since they could not be made to swallow more than 10 cc. of fluid. We felt that passing a stomach tube on a guinea pig time after time would be too much strain on the animal, since no tube that we could obtain could be passed without temporarily stopping

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the animal's breathing. Rabbits were fed much larger doses of this ground cellophane. This was done through a stomach tube two or three times a week. Passing the stomach tube down a rabbit's esophagus does not inconvenience the animal at all, but we hesitated to do this every day because of the possibility of setting up chronic inflammations. To obtain a material suitable for feeding rats, the ground cellophane was dried at 100° and pounded in a mortar until it was quite fine. This powdered cellophane was then mixed with the diet of the rats in definite proportions, so that they received cellophane seven days a week.

#### Doses Administered

All doses were calculated and are reported in terms of milligrams administered per kilogram body weight of the animal. We consider this the most logical means for expressing experimental toxicity data. Our figures in these units can be approximately converted to the units "ccs. per milligram" by dividing our numbers by 10,000.

The doses for repeated administration were chosen on the basis of the acute toxicity results obtained with the same materials. Since no very great cumulative effect was anticipated, the doses given were a fairly large proportion of those found to have significance acutely. The largest dose administered chronically was one-fifth of the smallest fatal dose we had found in the examination of the acute toxicity of the same materials. That is to say, the largest dose of dibutyl phthalate given chronically was 400 milligrams

per kilogram. Three other doses of this material were administered, each half of the one higher; that is, 200, 100 and 50 milligrams per kilogram. Similarly, the doses of "Santicizer No. 8" given chronically were 300, 150, 75 and 37.5 milligrams per kilogram. The doses of diethyl phthalate were 1000, 500, 250 and 125 milligrams per kilogram. Since we had found no acute damage from any dose of moisture-proof cellophane which we could administer, our largest chronic dose of cellophane was chosen as great as could be given to the animal in question. For guinea pigs this was only 20 square inches per animal, or approximately 40 square inches per kilogram body weight. For rabbits it was 200 square inches per animal, or approximately 120 square inches per kilogram of body weight. For rats the largest dose was 200 square inches per kilogram of body weight. With the rats this represented approximately 20 per cent of the total diet of the animal. Moisture-proof cellophane was also given chronically to each species of animal in exactly half of the doses mentioned above.

After approximately one month of feeding, one-third of the animals in each cage were killed for examination. After two months a second third, and after three months one-half of the last third were killed and dosing was stopped. The last animals in the group were kept under observation without doses for one month longer before being killed for examination. In this way it was hoped to obtain a picture of the rate at which small doses of the test materials affected the different organs.

All the animals were killed by severing the spinal cord with a sharp, heavy instrument. They were then promptly autopsied and any abnormalities noted, and bits of the organs (usually the liver, kidney and spleen, often lung, in addition) were fixed, dehydrated, embedded in paraffine, sections stained and studied microscopically for evidence of toxic action of the doses. Over 1300 microscopic preparations from this study are part of our permanent files.

### Observations.

Detailed observations on the individual animals, so far as they appear to be significant or to show deviations from the normal, are presented in the tables from pages 22 to 62. Necessarily the entries in the tables are abbreviated because of lack of space. The meaning of the abbreviations used can be found on page 21, although most of them are obvious. Observations showing no deviation from normal have been omitted from the tabulations, except in the case of micropathology where "N" has been entered merely to show that the tissue section was studied. These detailed tables are included in the report for purposes of record only, since they are much too bulky for convenient consultation. The significant data, therefore, have been collected in shorter, more concise tables, which will be referred to below.

### Behavior and Appearance.

At no time was any abnormality noticed in the behavior or appearance of the animals which could be blamed on

the toxic action of materials fed. Animals having pneumonia or tuberculosis were observed to be emaciated, their coats rough and unkempt, their noses running (often slightly bloody) and breathing labored. However, those cases of abnormal appearance were always found to be caused by disease conditions quite distinct from any possible action of the materials fed. Therefore, no tabulation or other comment appears on behavior.

### Diseases.

Table 1, page 9, gives all cases of infectious diseases found among the experimental animals, with this qualification that only diseases which had progressed far enough to affect the micropathology of the organs are noted. It is seen that the most frequent disease was kidney infection which was found in approximately one-third of the total number of rabbits. This apparently was a contagious disease, starting from a few cases which were not detected during the preliminary observation period, and later spreading to a large proportion of the rabbits. A description of the criteria used for diagnosing this disease from the micropathology will be given later in the section on interpretation of micropathology. We believe that the infection is completely independent of the dose received because the four groups fed Santicizer No. 8 and having the most kidney infection came from a different batch of animals, being received at a later date and doses started later than the other rabbits. It is probable that the infection was originally present in this group alone, and the other cases found were transmitted from infected

Table 1.

Diseases  
Severe enough to Affect the Micropathology, found among the Experimental Animals.

Material fed	Dose in mgms. per kilo per dose	Guinea Pigs			Rats			Rabbits			% of animals diseased	Diseased conditions found								Toxic deaths				
		Animals in group	Survivors diseased	Deaths of disease	Deaths from doses	Animals in group	Survivors diseased	Deaths of disease	Deaths from doses	Animals in group		Survivors diseased	Deaths of disease	Deaths from doses	Pneumonia; G.Pig	" " Rat	Tuberculosis; G.Pig	" " Rabbit	Kidney Inf; G.Pig		" " Rat	" " Rabbit	Liver Cysts	Mastoid infection
Controls		26	1	2		24	1	2		15	1	5		16		1	1		2	1	6		1	
Dimethyl phthalate	125	12												0										
	250	12												0										
	500	12												0										
	1000	12												0										
Dibutyl phthalate	50	12	1			12		3		6			13		3	1								
	100	13		1		12				7	1	1	10		1	1				1	1			1
	200	14		2		12	1			6		1	13	2	1					1	1			
	400	13	1	1		12				6		1	7	1	1	1					1			1
Santicizer No. 8	37½	12				12		1		11	1	8	28		1		2				7			
	75	12				12	1	1		10		4	15		1		2				2			
	150	13			1	12		1		10	2	4	20		1		1				4	1		1
	300	12			1	12				9		6	18								6			1
Moisture- proof cell- ophane	500	12											0											
	1000	12		1									8	1										
	1500									10	1	3	40			1					3			
	2500					12	3	1					33		1								3	
	5000					12		3		11	2	3	44		3						5			3
Totals		199	3	7	2	144	6	11	0	101	8	35	1		4	12	4	6	2	1	35	2	4	3

Animals tabulated - 444; non-toxic deaths - 53 = 11.9%; total disease - 70 = 15.8%

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animals of this particular purchase.

The next most common disease was pneumonia among the rats. This infected approximately one-half of the number of rats used. In addition to this pneumonia, we were not satisfied with the general condition of the rat groups, and believe that the diet they received was deficient in some respect, although we had previously used it without trouble.

Diseases among the chickens have been omitted from the tabulation because of the small number of birds fed. Among the group of 18 chickens receiving repeated doses of cellophane, six were found to be sufficiently abnormal so that their micropathology could not be considered. Four of these six were infested with parasites which had produced many cysts in the livers and had killed the fowls. One had died from a protozoal intestinal infection, and another which had not died was suffering from a kidney infection. These diseases were not among the fowls receiving the larger doses, but were among a small number of fowls started later than most of the chickens and having been purchased in a separate lot. As with the rats, it appears that the diseases had come from a purchased lot of poor fowls rather than being due to the materials fed.

Considering the diseases as a whole, we do not feel that the total - approximately one-sixth of the animals used - is unduly high for experiments lasting over several months. The diseases were not distributed among the different

Table 2.

Weight Gains of Surviving Animals (Average of Group).

Material Fed	Dosage in mgms. per kilo per dose	Guinea Pigs				Rats				Rabbits		
		After 25 Doses	After 50 Doses	After 75 Doses	After 75 Doses And one Month	After 25 Doses	After 50 Doses	After 75 Doses	After 75 Doses and one Month	After 25 Doses	After 50 Doses	After 75 Doses
Controls		92	198	159	240	29	39	61	66	328	780	830
Dibutyl phthalate	50	117	234	272	352	15	21	32		216	650	985
	100	78	164	234	339	26	20	29	29	368	540	1030
	200	84	179	252	292	20	18	1	12	356	710	930
	400	72	209	256	343	13	14	38	61	398	730	805
Santicizer No. 8	37½	126	242	331	406	16	19	28	54	190	505	665
	75	109	222	340	457	29	18	25	43	310	915	1400
	150	122	217	330	394	12	15	15	19	215	485	380
	300	103	245	279	373	20	17	21	39	490	660	1015
Moisture-proof cellophane	500	135	352	340	430					15 Doses	25 Doses	35 Doses
	1000	113	261	388	401							
	1500									447	518	620
	2500					19	20	22	20			
	3000									473	530	880
5000					25	37	29	24				

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Table 3.

Micropathology  
Summary, giving number of animals with organs abnormal microscopically.

Material Fed	Dosage in mgms. per kilo per dose	Guinea Pigs				Rats			Rabbits			Total, all Species					Organs affected						
		Animals studied	Free of infection	Possible toxic action	Definite toxic action	Animals studied	Free of infection	Possible toxic action	Definite toxic action	Animals studied	Free of infection	Possible toxic action	Definite toxic action	% showing possible toxic action	% showing definite toxic action	Liver	Kidney	Spleen					
Controls		25	25			24	21			15	9				64	53							
Diethyl phthalate	125	3	3												3	3							
	250	3	3	3											3	3	3		100		1	3	
	500	4	4	4											4	4	4		100		2	3	
	1000	4	4		4										4	4	4		100		4	4	
Dibutyl phthalate	50	12	11			12	9			6	6				30	26					1	1	
	100	13	12			12	12			7	8	1			32	29	1		4	4	1	2	
	200	14	12	3		12	11			6	8		1		32	28	3		11	7	3	3	
	400	13	11	3	1	12	12	1	1	6	6		1*		31	29	4	2	14	7	2	4	1
Santicizer No. 8	37½	12	12	2		11	10			11	2				34	24	2		8				
	75	12	12	2		12	11			10	6		1		34	29	2	1	7	3	1	2	
	150	13	13		1*	12	11			10	4		1		35	28		1		3	1	2	
	300	12	12		1*	12	12			9	3		1		33	27		2	7	2	1		
Moisture proof cell-o-phane	500	12	12																				
	1000	12	11																				
	1500									10	6				68	52							
	2500					12	8																
	3000									10	6												
5000					12	9																	
Totals		164	155	17	7	143	126	1	1	100	58	1	4	407	339	19	12			14	27	1	

Percentages are calculated on basis of disease-free animals

\* indicates animal died from toxic action

Table 4.

Doses Received by Animals showing Toxic Effects.  
 (No animals not in this tabulation showed any micropathology due to toxic action of the doses)

Material	Dosage in mgms. per kilo per dose	Guinea Pigs			Rats			Rabbits		
		Number	Toxic Action	Doses	Number	Toxic Action	Doses	Number	Toxic Action	Doses
Diethyl Phthalate	250	3	possible	12						
	500	4	possible	12						
	1000	4	definite	12						
Dibutyl Phthalate	50							1	definite	75
	100							1	possible	75
	100							1	died	62
	200	2	possible	25						
	200	1	possible	75						
	400	3	possible	25	1	possible	25			
400	1	definite	75	1	definite	50				
Santicizer No. 8	37½	1	possible	25						
	37½	1	possible	50						
	75	2	possible	25				1	definite	75
	150	1	died	16						
	300	1	died	57				1	definite	75
Moisture-proof cellophane	6600	1 chicken, some effect in liver, after 50 doses								

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groups in proportion to the magnitude of the doses fed, so that we do not feel that any of the doses weakened animals to such an extent that they became easy victims of infections existing in the colonies.

#### Fecundity.

The number of young produced by the animals was so low that it has not been tabulated. The reason that the guinea pigs did not produce any young was probably the fact that almost all of them were males. Although the rats were of mixed sexes - six males and six females in each group - they produced very few litters, not over 50 young being born during the entire experiment. We blamed this on the deficient diet mentioned above rather than on any effects of the doses, because fecundity did not appear to be related in any way with the material administered or the magnitude of the dose. Low fecundity was a general failing of the entire colony of rats rather than of any particular groups. The rabbits were all mixed sexes, and possibly six litters were born during the experiment. This small number of litters was probably due to the fact that the animals were young to start with, and only a few of them had reached maturity by the time the experiment was ended.

#### Weight Gain.

Table 2, page 11, gives in summary the average weight gain of each group of animals. Since some animals were killed after 25 doses, some after 50, and some after 75, while still more out of each group were kept one month beyond

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the 75th dose, it was necessary to tabulate four average weights for each group. The first weight is the average weight gain during the first 25 doses, while the second column is the average weight gain during the first 50 doses of those animals which received that many doses, etc.

None of the doses administered apparently were severe enough in their action to affect the weight gain of the animals. Among the guinea pigs it can be seen that the treated animals grew in almost every case more rapidly than did the controls. With the rabbits the treated animals in all but three cases grew as rapidly as did the controls, or more so. These three cases where treated animals did not gain weight as rapidly as did the controls were found to correspond with cages where a large proportion of the diseased rabbits lived; that is to say, apparently the low average weight gain of these cages was due to infection rather than to toxic action. The weight gains of the rats are all unsatisfactory, independent of the dose received. We must blame this on the deficient diet mentioned before. Among the rats, the controls, although they did not gain as much as rats on a normal diet should gain, yet they did gain more than any of the treated animals. We believe this is due to the fact that the taste of the dose administered, which it will be remembered was mixed with the entire diet of the rats, was sufficiently unpleasant to prevent the treated animals from eating as much as they otherwise would have done.

### Deaths from Toxic Action.

Only three animals out of the entire 444 died from toxic action. One guinea pig died after 16 doses of 150 mgms. per kilo of Santicizer No. 8, and one after 57 doses of 300 mgms. per kilo. One rabbit died after 62 doses of 100 mgms. per kilo of Dibutyl Phthalate.

These deaths are too few to be very important alone, so they are tabulated with the data on micropathology.

### Micropathology.

Table 3, page 12, indicates the number of animals showing toxic changes in the tissues and Table 4, page 13, shows the number of doses these animals had received.

In the differential diagnosis based on tissue findings, we have used the terms "nephritis" and "acute infection," differentiating these conditions from the toxic degeneration attributable to the materials fed the animals. Where nephritis or acute infection appear there was little if any evidence of change in the liver, with cloudy swelling in the kidneys, usually marked, accompanied by engorgement of the glomeruli and frequently with evidence of exudate in the capsules of the glomeruli pressing on the capillary network. The cloudy swelling was usually very marked, but there was not often evidence of cell disintegration in the tubules.

Associated with this renal condition there was often an apparent decided increase in prominence of the monocuclear splenocytes in the spleen. Where toxic action was diagnosed, in addition to cloudy swelling in the kidneys, there was frequently evidence of epithelial degeneration and cell disintegration

in the tubules without the characteristic glomeruli changes described above. This condition was usually associated with cloudy swelling and at times fatty degeneration in the liver. In most cases where the diagnosis of acute infection or nephritis was made, the animals succumbed after comparatively few doses of the material, while the majority of uninfected animals survived much longer periods, even when their tissues showed marked toxic action.

Also, control animals for the same group showed the same characteristic renal changes. These infectious cases also at times showed evidence of pulmonary involvement, and when this was marked they were diagnosed as pneumonia or tuberculosis. Where there was definite action from the materials fed, there was almost invariably evidence of action on the liver to as great an extent and even greater extent than on the kidneys, while in the infections this condition was reversed.

It is seen from the tables that usually only a small proportion of the animals receiving any one dose were affected by it, and that all three species of animals were not equally affected, the rats being least so and the guinea pigs most so.

#### Summary.

Diethyl phthalate was fed in dosages up to 100 mgms. per kilo to guinea pigs only. No fatalities due to this material occurred. Three animals fed 125 mgms. per kilo and four fed 500 mgms. per kilo showed probable toxic action

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evidenced in both liver and kidney pathology in three and kidney pathology in the fourth after 12 doses. Four animals fed 1000 mgms. per kilo all showed toxic changes in both liver and kidney after 12 doses. After these doses had been completed, we found the material was not considered available for commercial use, so no more tests were made.

Judging by these animals alone, any dosage of 250 mgms. per kilo, or over, per day (possibly equivalent to 17.5 grams for a 70 kilo man) might produce some toxic effect in a comparatively short time

Santicizer No. 8 was fed to guinea pigs, rats and rabbits, in doses up to 300 mgms. per day for as high as 75 days. With both this and Dibutyl Phthalate the rabbits showed irregular effects, occasionally being harmed by small doses, but not affected by the larger ones. As these animals are very susceptible to a variety of harmful agents, including infections, food conditions, and toxic substances, we feel that it is safe to disregard these cases where only one of a group of six was affected, although they are included in the tabulations.

With guinea pigs the lower dosages of 37-1/2 and 75 mgms. per kilo showed doubtful toxic action in two animals of each group. With the larger doses of 150 and 300 mgms. per kilo one animal in each group died, evidently from the effects of the feedings. This material affected both liver and kidneys, sometimes one being more affected, sometimes the other.

No effect whatever was produced on rats with the feeding of any of the doses mentioned. With rabbits one animal fed 75 mgms. per kilo showed toxic effect, as did one animal fed 300 mgms. per kilo. Considering the effect on the 3 groups of animals, we would say that any dosage over 75 mgms. per kilo might be productive of harm to susceptible individuals, and in some cases might lead to a fatal termination after frequent repetition. The average individual, however, will probably not be affected even after frequent feedings with as high as 300 mgms. per kilo (possibly equivalent to 21 grams for a 70 kilo man).

Dibutyl phthalate was fed in dosages up to 400 mgms. per kilo and for up to 75 doses. No effects were produced by 50 and 100 mgm. dosages, except in the case of three rabbits out of 12 fed, but as larger doses did not affect the rabbits, we think that these were accidental, or probably incidental to some other unrecognized condition. With dosages of 200 mgms. per kilo no rats were affected, but three guinea pigs showed renal lesions that may have been attributable to the material. With 400 mgms. per kilo definite toxic effects were observed in one rat and one guinea pig, and possible toxic action in one rat and three guinea pigs. In the lower doses this material affected the kidney primarily and in the high doses both kidney and liver were affected.

We were able to find no evidence of harm from the feeding of moisture-proof cellophane in doses up to 5000 mgms.

per kilo or 200 square inches per kilo repeated up to 35 times on the same animals. This would probably be equivalent to 14,000 square inches for a 70 kilo man or 350 grams, and it is not conceivable that anyone would either intentionally or accidentally ingest this much cellophane - certainly not at frequent intervals. One chicken was apparently affected to some degree after receiving 6600 mgms. per kilo for 50 consecutive feedings.

The results obtained from chronic feedings of these materials in general followed those obtained from the acute feedings. We were unable to give sufficient cellophane to any animal to produce harm in a single dose or in several doses.

Two different methods of administration to five different types of animals of dibutyl phthalate for acute toxic effect showed 5900 mgms. per kilo as the average smallest dose killing any animal. With diethyl phthalate the similar average was 4700 mgms. per kilo. Santicizer No. 8 gave an average of 600 mgms. per kilo as the smallest dose killing any animal.

Diethyl phthalate proved to have more cumulative action than did the other two, for its significant dose for chronic feeding is a smaller part of its least fatal acute dose than is the case with the others. Santicizer No. 8 did not prove to have as much cumulative action as did the others, compared to its acute toxicity.

## Conclusion.

Our work gives no evidence of possible harm from the feeding of moisture-proof cellophane in any amount that we can possibly conceive of an individual's voluntarily taking.

Dibutyl phthalate proved definitely toxic to some animals in 400 mgm. per kilo dosage, possibly toxic to some guinea pigs in 200 mgm. per kilo doses, and harmless in 100 mgm. per kilo doses. These amounts would be contained in 1250, 625, and 310 square inches of cellophane, respectively, (provided our information on the formula of coated cellophane is correct) These figures would indicate that there would probably be no harm in the feeding of well over 20,000 square inches of cellophane to a 70 kilo man at frequently repeated intervals. It would appear to us that this material is a perfectly safe plasticizer for continued use.

Santicizer No. 8, while somewhat more toxic than No. 7, could probably also be used with impunity, as there was no definite toxic action from 75 mgm. per kilo doses, although there would be a possibility of susceptible individuals being affected by massive doses of this material, since some individual animals were affected by smaller doses.

As far as work with small animals can go, we believe we have shown that Cellophane coated with material containing Dibutyl Phthalate to be free from hazard of either acute or chronic toxicity.

**Explanation of Tables , pages 22 to 62, inclusive**

Entries under "weight change" are expressed in grams.

In all tables save those on pages 60, 61, and 62 the column headed "25" corresponds to the weight change during the first month, "50" during the second month, etc.

On pages 60 and 61, the column headed "15" corresponds to the weight change during the first month and one-half, "25" during the first two and one-half months, and "35" during the first three and one-half months. On page 62 "38" is during the first two months, and "50" during the first three months.

Abbreviations used:- (capital letters indicate marked pathology)

ag	abundant glycogen
c	congested
cg	congested glomeruli
cs	cloudy swelling
csc	cloudy swelling of convoluted tubules
cl	cloudy swelling of loop tubules
cat	cloudy swelling of convoluted tubules (same as "csc")
d	desquamation
de	detritus
eg	engorged (engorged glomeruli, in kidney)
etb	early tuberculosis
exg	exgate in glomeruli
fd	fatty degeneration
gp	granular pigment
h	hemorrhagic
inf	infiltration
K	Kupfer cells prominent
loy	liver cysts
lgo	lung congested (autopsy finding)
lm	liver mottled (autopsy finding)
lp	liver pale (autopsy finding)
mg	moderate glycogen
mn	large mononuclear cells prominent
N	normal
ng	no glycogen
ns	not studied
ph	phagocytosis
pn	pneumonia
sg	scant glycogen
sp	splenocytes prominent
st	secretion in tubes
tb	tuberculosis
td	toxic degeneration
tgt	toxic degeneration (same as "td")

000025

Chronic Feeding  
Controls

Guinea Pigs - Cage 64

a. Animals killed for autopsy (None died during the experiment)

Pig No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, Page 21, for meaning of entries)				
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
670	25	+129				N	N	N	N	
671	25	+ 40				N,mg	N	N	N	
672	25	+142				N	N	N	nc	
673	25	+ 61				N,sg	N	N	ns	
674	50	+ 73	+210			N,mg	N	N	ns	
675	50	+114	+199			N,mg	N	N	ns	
676	50	+ 79	+171			N,mg	N	N	ns	
677	50	+ 87	+212			N,sg	N	N	ns	
678	75	+119	+172	+183		N	N	N	ns	
681	75	+ 74	+124	+ 20		N	N	N	ns	
679	75+	+128	+257	+245	+264	N	N	N	ns	Skin infection
680	75+	+ 43	+257	+319	+168	N	N	N	ns	" "
Average		+ 91	+200	+194	+216	Normal				

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000026

Chronic Feedings  
Controls

Guinea Pigs - Cage 55

A. Animals killed for autopsy

Pig No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
682	25	+103				N	N	N	ns
684	25	+102				N,sg	N	N	N
635	25	+ 87				cs,ng	cs	N	c,tb
885	25	- 4				N	N	ns	ns
686	50	+116	+246			N,mg	N	N	N
687	50	+141	+267			N	N	N	N
689	50	+106	+191			N	N	N	N
841	50	+134	+ 86			N	N	N	ns
690	75	+ 89	+192	+ 96		N	N	N	ns
691	75	+121	+193	+ 92		N	N	N	ns
692	75+	+122	+187	+121	+259	N	N	N	ns
693	75+	+103	+208	+186	+267	N	N	N	ns
Average		+102	+196	+124	+263	Normal			

B. Animals dying during the experiment

Pig No.	Doses Received	Weight change during experiment		Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
683	20	-52		c,cs	C,cst,csl	N	ns	infection
688	20	+38	no autopsy					

23

000027

Chronic Feeding  
Controls

Rats ( Pages 9 and 10 )

a. Animals killed for autopsy ( None died during the experiment)

Rat No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
916	25	+23				N	N	N	ns	Mastoid
754	25	+46				N	N	N	N	
742	25	+52				N	N	N	N	
716	25	+ 4				c,cs	N	N	N	
735	50	+21	+25			N	N	N	ns	
744	50	+32	+34			N	N	N	ns	
745	50	+38	+44			N	N	ns	ns	
871	50	+ 3	+ 7			N	N	N	ns	
723	75	+ 2	+10	+17		N	N	N	ns	
724	75	+50	+51	+60		N	N	N	ns	
749	75+	+35	+55	+70	+73	N	N	N	ns	
731	75+	+57	114	134	119	ns	ns	ns	ns	
Average		+30	+42	+70	+96	animals normal				

Chronic Feeding  
Controls

Rats ( Cages 21 and 22 )

A. Animals killed for autopsy

Rat No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
914	25	+53							ns	
922	25	+17							ns	
733	50	+35	+14						ns	
691	50	+21	+10						ns	
635	50	+27	+37						ns	
874	50	+10	+16						ns	
759	36	+49	+72	+36			ns	ns	ns	
755	75	+15	+22	+23					ns	
781	75+	+52	+55	+53	+45				ns	
782	75+	+51	+64	+44	+26				ns	
Average		+26	+36	+52	+35	Animals normal				

B. Animals dying during the experiment

Rat No.	Doses received	Weight change during experiment	Autopsy findings (see code page 21)	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
810	40	-25	pneumonia	K	cs1, nephritis	ns	c, pn	pneumonia
877	16	+1	mastoid	CS, K	CS, CG, ST	N	N	Mastoid

Chronic Feeding  
Controls

Rabbits - Page 46

1. Animals killed for autopsy

Rab. No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
13	25	+230				N	N	N	N
41	50	+510	+1000			N	N	N	N
2	75	+600	+1000	+660		N	ns	N	ns
61	75+	+460	+ 910	+250	+1250	ns	ns	ns	ns
Average		+450	+990	+755	+1250	Normal			

2. Animals dying during the experiment

Rab. No.	Doses Received	Weight change during experiment	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
			Liver	Kidney	Spleen	Lung	
20	45	+670	c,cs	de,eg,c	nm	c	Bacterial infection
193	20	-560	cs	de,eg,c	ns	ns	
40	25	+110	c,ng	c,csl,cst,de,eg	N	c	
47	25	+320	ns	c,eg,SSC, CSL, de	ns	c	
			Kidney infections				

000030

Chronic Feeding  
Controls

Rabbits (Cage 47)

A. Animals killed for autopsy

Rab. No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
1	25	+230				K, mg	N	N	K	Kidney infection
7	25	+350				N, mg	K	K	H	
9	50	+ 90	+940			K, mg	N	N	ns	
153	50	+590	+200			cs,ng	cs	N	ns	
64	75	+400	+910	+1130		ns	ns	ns	ls	
62	75	-150	+230	+ 680		K,sg	N	K	ns	
Average		+206	+570	+305		Normal, save for kidney infection				

B. Animals dying during the experiment

Rab. No.	Doses Received	Weight change during exper-	autopsy findings	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
46	45	+550		C S,C	de, eg	mn	c	Bacterial infection

Chronic Feeding  
Sample 8 - Diethyl Phthalate

Guinea Pigs - 1000 mgms. per kilo per dose ( cage 66)

.. animals killed for autopsy (None died during the experiment).

Fig No.	Doses received	Weight change during experiment	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
			Liver	Kidney	Spleen	Lung
706	12	+46	ii; ag	c;eg;cs1	E	ns
707	12	+38				
708	12	+44	c;mg	cs1;cs2;d;de;tgt	N	ns
709	12	+27	c;cs;ng	c;cs1;cs2;d;de;tgt	N	ns
710	12	+38				
711	12	+ 5	c;mg	c;cs1;cs2	N	ns
712	12	+27				
713	12	+39				
716	12	+29				
717	12	+27				
755	12	+41				
757	12	+24				
Average		+32.1	Slight but definite toxic action demonstrated from 12 doses			

18

000032

Tonic Feeding  
Sample 8 - Diethyl Pthalate

Guinea Pigs - 500 mms. per kilo per dose ( Cage 67 )

a. Animals killed for autopsy (None died during the experiment)

Pig No.	Doses received	Weight change during experiment	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
			Liver	Kidney	Spleen	Lung
718	12	+26				
719	12	+32				
720	12	+22	N, mg	cs1	N	ns
721	12	+53				
722	12	+32				
723	12	+25				
724	12	+27	N, mg	cs1	N	ns
725	12	+ 8				
726	12	+46	c,cs	cs1	N	ns
728	12	+38				
729	12	+40	cs,sg	N	N	ns
758	12	+43				
Average		+32.5	Questionable toxic action demonstrated from 12 doses			

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000033

Chronic Feeding  
Sample 8 - Diethyl Phthalate

Guinea Pigs - 250 mcms. per kilo per dose (Cage 68)

a. Animals killed for autopsy (None died during the experiment)

Fig No.	Doses received	Weight change during experiment	Interpretation of micro pathology of organs (See code, page 21, for meaning of entries)			
			Liver	Kidney	Spleen	Lung
730	12	+35				
731	12	+57				
732	12	+45				
733	12	+50				
734	12	+40				
735	12	+57				
736	12	+51				
737	12	+60				
738	12	+53	N,mg	csl,cst	N	ns
759	12	+68	N,mg	c,csl	N	ns
760	12	+26				
761	12	+80	c,sg	c,csl	N	ns
Average		+51.8	questionable action demonstrated from 12 doses			

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000034

Chronic Feeding  
Sample 8 - Diethyl Pthalate

Guinea Pigs - 125 mgms. per kilo per dose (Cage 69)

a. Animals killed for autopsy (None died during the experiment)

Pig No.	Doses received	Weight change during experiment	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
			Liver	Kidney	Spleen	Lung
742	12	+44	N, ag	c, cal	N	ns
743	12	+48				
744	12	+54	c, sg	c	N	ns
745	12	+32	N, mg	c	c	ns
746	12	+47				
747	12	+40				
748	12	+ 8				
749	12	+57				
750	12	+40				
751	12	+57				
752	12	+47				
753	12	-12				
Average		+38.5	No definite toxic action from 12 doses			

Chronic Feeding  
Sample 7 - Dibutyl Phthalate

Guinea Pigs - 400 mcgms. per kilo per dose ( Cage 60)

A. Animals killed for autopsy

Fig No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (see code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
501	25	+33				N, mg	c, cse, csl	N	c
502	25	+68				N, mg	csl	N	c
504	25	+71				N, mg	csl	N	N
635	25	114				N	N	N	N
506	50	+66	+151			N, mg	N	N	ns
510	50	121	+284			N, mg	N	N	ns
511	50	110	+247			N	N	N	etb
514	50	+84	+265			N, mg	N	N	ns
517	75	+33	+141	+188		ns	N	N	ns
518	75	+10	+157	+261		N, sg	csc, csl, exg	mm	ns
521	75+	+73	+220	+274	+296	N	N	N	ns
522	75+	+86	+210	+300	+389	N	N	N	ns
Average		+72	+209	+256	+343	Probable slight but definite toxic action on kidneys, apparently recovered from on stopping the doses			

B. Animals dying during the experiment

Fig No.	Doses Received	Weight change during experiment.	Autopsy findings (see code page 21)	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
509	4		lp	c, fd	c, cst, csl	ns	c, h	pneumonia

Chronic Feeding  
Sample 7 - Dibutyl Adipate

Rats - 400 mgms. per kilo per dose (Cages 1 and 2)

a. Animals killed for autopsy (None died during the experiment)

Rat No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
870	25	+20				N	N	N	ns
900	25	+11				N	N	N	ns
629	25	+31				c,cs,ng	N	N	I
869	25	+29				N	N	N	ns
633	50	+30	+36			N	N	N	ns
654	50	+27	+32			N	N	ns	ns
580	50	-29	-34			N	N	N	qn
649	50	- 2	0			fd	N	N	ns
566	75	+16	= 7	=12		N	N	N	ns
632	75	- 8	+28	+31		N	N	N	ns
657	75+	+ 8	- 7	+48	+64	N	N	N	ns
648	75+	+30	+31	+52	+59	N	N	N	ns
Average		+13	+14	+38	+61	Questionable toxic action			

Chronic Feeding  
Sample 7 - Dibutyl Pthalate

Rabbits - 400 mms. per kilo per dose ( Cage 40 )

a. Animals killed for autopsy (None died during the experiment)

Rab. No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
15	25	+ 90				N, mg	N	N	N
16	25	+460				N, c, sg	N	N	N
17	50	+310	+790			N, mg	N	N	ns
18	50	+460	+720			N, mg	N	N	ns
22	75	+790	+830	1020		N, mg	N	N	ns
19	75+	+280	+510	+590	+480	N, sg	N	N	ns
Average		+398	+730	+805	+480	No definite toxic action			

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000038

Chronic Feeding  
 Sample 7 - Dibutyl Antihalate

Guinea Pigs - 300 mgms. per kilo per dose ( Cage 61)

A. Animals killed for autopsy

Pig No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
525	25	-21				N,mg	N,osl	N	c	
532	25	+79				N,mg	N,osl	N	N	
836	25	132				N	N	N	ns	
837	25	137				N	N	N	ns	
535	50	214	+223			N	N	N	N	
551	50	+22	+132			N, mg	N	N	N	
552	50	+47	+123			N, mg	N	N	N	
592	50	+42	+156			N, sg	N	N	N	
597	75	121	+216	+260		N,mg	N	N	ns	
598	75	103	+232	+202		N, mg	N	N	ns	
600	75+	+80	+207	+302	+312	N	st	N	ns	
604	75+	+15	+132	+236	+271	N	N	N	ns	
Average		+84	+179	+252	+292	Possible slight action on Kidney				

B. Animals dying during the experiment

Pig No.	Doses received	Weight change during experiment	Autopsy findings (see code, page 21)	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
593	12	- 4	lm	c,cs,fd	C, EG, CSG, CSL	N	pn	pneumonia
603	14	-46	lgc	c,cs	c,eg,csc,osl	N	pn	pneumonia

Chronic Feeding  
Sample 7 - Dibutyl antihalate

Dose - 200 mgms. per kilo per dose ( Cages 3 and 4)

No. animals killed for autopsy ( None died during the experiment)

Rat No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
697	25	+14				N	N	N	N
593	25	+18				N	N	N	N
911	25	+57				N	N	N	ns
923	25	+69				N	N	N	ns
693	50	+11	+20			N	N	N	ns
696	50	+21	+50			N	N	N	ns
615	50	+ 6	- 1			c	N	N	pn
628	50	+ 7	+18			N	N	N	ns
695	75	+ 5	+10	-12		N	N	N	ns
700	75	+ 2	+ 5	- 1		N	N	ns	ns
694	75+	+23	+45	+33	+32	N	N	N	ns
626	75+	+ 6	- 5	-10	- 7	N	N	N	ns
Average		+20	+18	+ 1	+12	Animals normal			

Chronic Feeding  
Sample 7 - Dibutyl Phthalate

Rabbits - 200 mgms. per kilo per dose (Cage 43)

A. Animals killed for autopsy

Rab. No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
21	25	+260				ng	N	N	ns	cysts.
28	50	+400	+570			ng	N	N	ns	
29	50	+490	+940			ng	N	N	ns	
55	75	+310	+540	+770		ng	N	N	ns	
53	75+	+320	+800	1090	+920	ng	N	N	ns	
Average		+356	+710	+930	+920	No signs of toxic action				

B. Animals dying during the experiment

Rab. No.	Doses Received	Weight change during experiment	Autopsy findings	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lungs	
4	20	-150	liver cysts	cs,ng,tb	cs,csl,ds,td	nn	ns	acute infection

Chronic Feeding  
Sample 7 - Diobutyl Phthalate

Guinea Pigs - 100 mms. per kilo per dose ( Cage 62 )

A. Animals killed for autopsy

Pig No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (see code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
		609	25	+113				N	N
610	25	+ 47				N,mg	N	N	ns
613	25	+115				N,mg	N	N	N
840	25	+ 79				N,mg	N	N	ns
615	50	+ 45	+155			N,mg	N	N	N
632	50	+ 55	+196			N,mg	N	N	ns
649	50	+ 87	+210			N,sg	N	N	N
663	50	+ 82	+156			N,mg	N	N	N
664	75	+104	+143	+120		N	N	N	ns
666	75	+ 16	+ 86	+129		ns	N	N	ns
667	75+	+109	+213	+407	+367	N	N	N	ns
668	75+	+65	+157	+289	+307	N	N	N	N
Average		+ 78	+164	+234	+339	No definite toxic action seen			

B. Animals dying during the experiment

Pig No.	Doses Received	Weight change during experiment	Autopsy findings (see code, page 21)	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
662	19	-22	lm	c,FD, GP	c,tgt, st	sp	c,pn	pneumonia or early tuberculosis

Chronic Feeding  
Sample 7 - Dibutyl Phthalate

Rats - 100 mgms. per kilo per dose ( Cages 5 and 6)

a. Animals killed for autopsy ( None died during the experiment)

Rat	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs ( See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
906	25	+24				N	N	N	ns
936	25	+51				N,mg	N	N	N
937	25	+50				N	N	N	ns
943	25	+27				N	N	N	ns
663	50	-11	-22			N	N	N	ns
872	50	+46	+59			N	N	N	ns
709	50	+32	+31			N	N	N	ns
939	50	+26	+34			ns	ns	ns	c
705	75	+15	+17	+39		N	N	N	ns
624	75	+23	+18	+31		N	N	N	ns
713	75+	+ 9	+ 7	+15	+22	N	N	N	ns
693	75+	+20	+14	+32	+36	N	N	N	ns
Average		+26	+20	+29	+29	Animals normal			

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000043

Chronic Feeding  
Sample 7 - Dibutyl Phthalate

Rabbits - 100 mms. per kilo per dose (Cage 41)

A. Animals killed for autopsy

Rab. No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
27	25	+280				sg	d	N	N	liver cysts cysts cysts
74	25	+390				c,sg	N	N	ns	
32	50	+370	+460			N	N	N	ns	
34	50	+370	+400			N	N	N	ns	
37	75+	+430	+770	+1030	+1014	sg	C,st	ns	ns	
Average		+368	+540	+1030	+1014	Animal Number 37 probably shows toxic signs.				

Animals dying during the experiment

Rab. No.	Doses Rec'd.	Weight change during experiment	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
			Liver	Kidney	Spleen	Lung	
30	6		c,cs,ng	CSC, CSL	ph	na	acute infection
35	62	+1060	na	c,cs,td,st,d	N	na	toxic action

000044

Chronic Feeding  
Sample 7 - Dibutyl Sebacate

Guinea Pig - 50 mgms. per kilo per dose (Cage 63)

n. Animals killed for autopsy (None died during the experiment)

Pig No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (see code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	lung
669	25	+107				N,mg	N	N	ns
694	25	+121				N,mg	N	N	c,etc
695	25	+ 80				N,mg	N	N	ns
696	25	+121				N,mg	N	N	N
697	50	+116	+200			N,mg	N	N	N
698	50	+112	+133			N,mg	N	N	N
699	50	+116	+279			N	N	N	ns
700	50	+145	+346			N	N	N	ns
702	75	+129	+262	+341		N	N	ns	ns
703	75	+118	+226	+233		N	N	N	ns
704	75+	+116	+196	+255	+312	N	N	N	ns
705	75+	+122	+223	+260	+391	N	N	N	ns
Average		+117	+234	+272	+352	Animals normal, not affected by the doses			

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000045

Chronic Feeding  
Sample 7 - Dibutyl Phthalate

Rats - 48 mgms. per kilo per day (Cages 7 and 8)

2. Animals killed for autopsy

Rat No.	Doses Received	Weight change during number of doses indicated			Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	Liver	Kidney	Spleen	Lung
917	25	+10			N	N	N	N
919	25	+27			N	N	N	N
727	50	+51	+77		N	N	N	N
873	50	+7	+9		N	N	N	N
867	50	+13	+24		N	N	N	N
707	50	+6	+13		N	N	N	N
912	50	+29	+22		N	N	N	N
938	50	+3	-12		N	N	N	N
733	75	+36	+34	+32	N	N	N	N
Average		+15	+21	+32	Normal, no signs of toxic action			

3. Animals dying during the experiment

Rat No.	Doses	Weight change during experiment	Autopsy findings	Micropathology of organs not studied because of autopsy findings
725	56	+4	pneumonia	
719	56	+27	pneumonia	
721	56	+1	pneumonia	

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000046

Chronic Feeding  
Sample 7 - Dibutyl Phthalate

Rabbits - 50 mgms. per kilo per dose (Cage 42)

A. Animals killed for autopsy (None died during the experiment)

Rab. No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
38	25	+280				sg	N	N	N
39	25	+370				c,sg	N	N	N
60	50	+ 60	+490			N	N	N	ns
54	50	+120	+610			mg	N	N	ns
63	75	+180	+690	+1000		mg	N	N	ns
78	75+	+290	+800	+ 970	+1200	cs,fd	c,st	N	ns
Average		+216	+650	+985	+1200	Possible toxic action on number 78			

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000047

Chronic Feeding  
 Sample 9 - Senticizer No. 8

Guinea Pigs - 300 mms. per kilo per dose (Cage 70)

A. Animals killed for autopsy

Fig. No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
767	25	+109				N,sg	N	N	N
768	25	+120				N,sg	N	N	N
770	25	+ 67				N,mg	N	N	N
771	25	+146				N,mg	N	N	N
772	50	+ 60	+156			N,sg	N	N	ns
773	50	+107	+347			N,mg	N	N	ns
774	50	+148	+297			N,mg	N	N	ns
775	50	+125	+295			N	N	N	ns
779	75	+105	+179	+266		N,mg	N	N	ns
776	75+	+ 70	+205	+234	+320	N	N	N	ns
777	75+	+ 77	+237	+337	+427	N	N	N	ns
Average		+103	+245	+279	+373				

B. Animals dying during the experiment

Fig No.	Doses Rec'd	Weight change during experiment	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
			Liver	Kidney	Spleen	Lung	
778	57	+50	cs,fd	c,cst	mn	c	pneumonia, or toxic effect.

No definite signs of toxic action, save possibly one death, not certainly due to toxic effect

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000048

Chronic Feeding  
Sample 9 - Santicizer No. 8

Rats - 300 mgms. per kilo per dose (Cages 11 and 12)

a. Animals killed for autopsy (None died during the experiment)

Rat No.	Doses Received	weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 31, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
876	25	+21				N	N	N	ns
813	25	+40				N	N	N	ns
915	25	+40				N	N	N	ns
880	25	+ 5				N	N	N	ns
743	50	+10	+ 5			N	N	N	ns
752	50	+29	+27			N	N	N	ns
730	50	+11	+ 3			N	N	N	ns
747	50	+14	+12			N	N	N	ns
765	75	+17	+23	+11		N	N	N	ns
732	75	+36	+30	+31		N	N	N	ns
761	75+	+26	+38	+53	+61	N	N	N	ns
722	75+	-14	- 4	- 9	- 3	N	N	N	ns
Average		+20	+17	+21	+39	Animals Normal			

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000049

Chronic Feeding  
Sample 9 - Santicizer No. 8

Rabbits - 300 mgms. per kilo per dose (Cage 44)

A. Animals killed for autopsy

Rab. No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
59	50	+310	+400			ng	N	N	ns
69	75	+690	1030	+1290		ng	N	N	ns
71	75+	+480	+570	+ 740	+320	ng,cs,fd	N	N	ns
Average		+490	+666	+1015	+320	Toxic effect on animal number 71			

B. Animals dying during the experiment

Rab. No.	Doses Received	Weight change during experiment	Autopsy findings (see code page 21 for abbreviations)	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
98	42	+1020		cs,td	exg,CSC	nn	ns	nephritis
10	25	+ 290	liver cysts	c,ng	csc,csl	nn	ns	nephritis
12	30	+ 280	liver cysts	C	c,eg,csc	c	N	nephritis
66	22	- 200	liver cysts	C,cs	CSC,CSL	nn	N	nephritis
58	3		liver cysts	c,cs,	csc	ns	c,h	acute infection
72	2		liver cysts	na	ns	ns	ns	acute infection

000050

Chronic Feeding  
Sample 9 - Senticizer No. 8

Guinea pigs - 150 mms. per kilo per dose (Cage 71)

A. Animals killed for autopsy

Pig No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
816	25	+130				ng,mg	cs1	N	h
817	25	+121				ng,mg		N	h
818	25	+119				ng,mg		N	h
884	25	+194				ng,mg		N	ns
820	50	+146	+243			ng,mg		N	ns
821	50	+103	+214			ng,mg		N	ns
822	50	+137	+243			ng,mg		N	ns
824	50	+101	+136			ng,mg		N	ns
763	75	+128	+194	+289		ng,mg		N	ns
764	75	+ 95	+196	+342		ng,mg		N	ns
765	75+	+ 61	+183	+218	+335	N		N	ns
766	75+	+123	+280	+364	+454	N		N	ns
Average		+122	+217	+303	+394				

B. Animals dying during the experiment

Pig No.	Doses received	Weight change during experiment	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
			Liver	Kidney	Spleen	Lung	
819	16	-50	ng,cs,fd	de,cs	ns	c	Possibly toxic effect

No definite signs of toxic action, save one death possibly due to toxic effect

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000051

Chronic Feeding  
Sample 9 - Santicizer No. 8

Rats - 150 mms. per kilo per dose - (Cages 23 and 24)

A. Killed for autopsy

Rat No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
931	25	+53				N	N	N	ns
930	25	+40				N	N	N	ns
927	25	+ 7				N	N	N	ns
926	25	+15				N	N	N	ns
881	50	+13	+41			N	N	N	ns
883	50	-11	+ 9			N	N	N	ns
879	50	0	+23			N	ns	N	ns
886	50	- 2	+ 2			N	N	N	ns
762	75	- 9	- 4	+ 3		N	N	N	ns
763	75	+ 7	+10	0		N	N	N	ns
764	75+	+ 9	+24	+43	+19	N	N	N	ns
Average		+12	+15	+15	+19	Animals normal			

B. Animals dying during the experiment

Rat No.	Doses Received	Weight change during experiment	Autopsy findings (see code page 21)	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
759	75+	-43	pn, lcy	CS, FD	cst	N	ns	Pneumonia and cysts

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000052

Chronic Feeding  
Sample 9 - Senticizer No. 8

Rabbits - 150 mgms. per kilo per dose (Cage 48)

A. Animals killed for autopsy

Rab. No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		25	50	75	75+ obe.	Liver	Kidney	Spleen	Lung	
5	25	+130				c,cs,cyst	N	N	ns	
52	25	+230				sg	N	N	ns	
23	50	+370	+600			sg	N	N	ns	
24	50	+110	+480			ng	N	N	ns	
85	75	+110	+470	+170		ng	cas	ns	tb	tuberculosis
88	75+	- 90	+400	+590	+1020	ng	N	N	ns	
Average		+215	+485	+380	+1020	No toxic action				

B. Animals dying during the experiment

Rab. No.	Doses received	Weight change during experiment	Autopsy findings (see code page 21 for abbreviations)	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
87	8	+370		C,cs	c,CS,td	nn	ns	nephritis
89	10	+170	liver cysts	cs,C	eg,cs,cal	ns	c	nephritis
91	45	+510		c,cs,ng	c,cs,de	ns	na	nephritis
93	46	+940		c	cs,cal,de	N	na	nephritis

000053

Chronic Feeding  
Sample 9 - Sulfadiazine No. 8

Guinea pigs - 75 mgms. per kilo per dose ( Cage 72)

a. Animals killed for autopsy ( None died during the experiment)

Pig No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
302	25	+117				N	cs1	N	ns
303	25	+110				N	N	N	h
304	25	+ 94				N,mg	cs1	N	N
805	25	+ 92				N	N	N	ns
807	50	+148	+226			N	N	N	ns
808	50	+150	+232			N,mg	N	N	ns
809	50	+ 89	+139			N,mg	N	N	ns
811	50	+ 33	+130			N,mg	N	N	ns
812	75	+ 81	+197	+272		N,mg	N	N	ns
813	75	+134	+266	+389		N,mg	N	N	ns
814	75+	+150	+283	+388	+512	N	N	N	ns
815	75+	+109	+193	+312	+402	N	N	N	ns
Average		+109	+222	+340	+457	No definite signs of toxic action			

Chronic Feeding  
Sample 9 - Santicizer No. 8

Rats - 75 mgms. per kilo per dose ( Cages 25 and 26)

A. Animals killed for autopsy ( None died during the experiment)

Rat No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
894	25	+10				N	N	N	ns	
896	25	+ 8				N	N	N	ns	
898	25	- 7				N	N	N	ns	
929	25	+70				N	N	N	ns	
767	50	+30	+42			N	N	N	ns	
768	50	+44	+50			N	N	N	ns	
770	50	+ 2	-13			N	N	N	ns	
796	50	+37	+10			N	N	N	ns	
799	75	+25	-41	-44		ns	c, eg, de, cst	c	ns	Pneumonia
800	75	+83	+41	+48		N	N	N	ns	
766	75+	+ 5	+ 5	+34	+51	N	N	N	ns	
771	75+	+35	+46	+31	+35	N	N	N	ns	
Average		+29	+18	+25	+43	Animals Normal				

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000055

Chronic Feeding  
Sample 9 - Santicizer No. 8

Rabbits - 75 mms. per kilo per dose (Cage 45)

A. Animals killed for autopsy

Rab. No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
51	25	+380				sg	N	N	ns
3	25	+ 30				sg	N	N	ns
67	50	+710	+1340			sg	N	N	ns
76	50	+200	+370			sg	N	N	ns
79	75	+ 200	+1040	+1820		N	N	N	ns
75	75+	+340	+910	+ 980	+1180	ng,cs,fd	N	N	ns
Average		+310	+915	+1400	+1180	Toxic effect on animal number 75			

B. Animals dying during the experiment

Rab. No.	Doses received	Weight change during experiment	Autopsy findings (see code page 21 for abbreviations)	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
81	4			C,cs,ng	c,CSC,CST	mn	c,ex	Tuberculosis
83	10	+ 60		c,cs	c,csa,csi	mn	c,h	acute infection
84	4		tuberculosis	na	c,osc,cal,fd	mn	c	Tuberculosis
14	8	-400	liver cycts	C,CS	est,de,fd	N	ns	acute infection

000056

Chronic Feeding  
Sample 9 - Sulficizer No. 8

Guinea Pigs - 37.5 mgms. per kilo per dose (Cage 73)

... Animals killed for autopsy (None died during the experiment)

Pig No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
787	25	+121				N, mg	N	N	N	
789	25	+133				N, mg	cs1	N	N	
790	25	+109				N, mg	N	N	N	
791	25	+183				N	N	N	ns	
793	50	+122	+244			N, mg	N	N	ns	
794	50	+111	+226			N	csc, cs1	N	ns	
796	50	+123	+290			N, mg	N	N	ns	
797	50	+111	+238			N, mg	N	N	ns	
798	75	+101	+257	+415		N, mg	N	N	ns	
799	75	+109	+216	+334		N, mg	c	N	ns	
800	75+	+206	+281	+378	+420	ns	ns	ns	ns	
801	75+	+108	+183	+199	+392	N	N	N	ns	
Average		+126	+242	+331	+406	No definite signs of toxic action				

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000057

Chronic Feeding  
Sample 9 - Santicizer No. 8

Rats - 38 mgms. per kilo per dose (Cages 27 and 28)

A. Animals killed for autopsy

Rat No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
936	25	+19				N,lg	N	N	ns
895	25	+20				N	N	N	ns
899	25	+ 6				N	N	N	ns
897	50	+16	+20			N	N	N	ns
892	50	+18	+17			N	N	N	ns
893	50	+ 7	+ 4			N	N	ns	ns
779	75	+21	+ 5	+ 7		N	N	N	ns
807	75	+13	+37	+29		N	N	N	ns
778	75+	+20	+14	+10	+28	N	N	N	ns
776	75+	+16	+35	+44	+80	N	N	N	ns
Average		+16	+19	+28	+54	Animals normal			

B. Animals dying during the experiment

Rat No.	Doses received	Weight change during experiment	Autopsy findings (see code page 21)	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
941	12	+25	killed by accident	-	tissues not studied			
000774	50	-29	pn	N	cst,cs1,cg	N	pn	pneumonia

Chronic Feeding  
Sample 9 - Senticizer No. 8

Rabbits - 37.5 mgms. per kilo per dose (Cage 49)

A. Animals killed for autopsy

Rab. No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		25	50	73	75+ obs.	Liver	Kidney	Spleen	Lung	
94	25	-170				ng	csc,osl,de	nn	ns	acute infection
36	75	+260	+350	+490		ng	N	N	ns	
26	75	+480	+660	+840		ng	I	N	ns	
Average		+190	+505	+665		No toxic action				

B. Animals dying during the experiment

Rab. No.	Doses Received	Weight change during experiment	Autopsy findings (see code, page 21 for abbreviations)	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
99	40	+660	tb	c,cs,fd	cs,de,exg	nn	C,tb	tuberculosis
42	16	+450		cs	csc,osl	N	ns	acute infection
31	17	+140	liver cysts	c,cs,ng	csc,osl,exg	N	ns	acute infection
33	44	+430	pneumonia	c,ogats	CSC,osl,de	nn	pn	acute infection
95	32	+370	liver cysts	c,ng	csc	N	c	acute infection
96	37	+660	tb	c,cs,fd	c,cat,de	nn	c,tb	tuberculosis
100	37	+600	liver cysts	c,cs,ng	csc	nn	ns	acute infection
97	14	0	liver cysts	c,cs	N	nn	N	acute infection

000059

**Chronic Feeding**  
**Moisture-Proof Cellophane**

**Guinea Pigs - 20 sq. inches (500 milligrams) per kilo per dose (Cage 74)**

**A. Animals killed for autopsy (None died during the experiment)**

Pig No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
839	25	+138				N	N	N	ns
842	25	+154				N	N	N	ns
843	25	+162				N	N	N	N
844	25	+103				N	N	N	ns
853	50	+145	+274			N	N	N	ns
854	50	+ 78	+298			N	N	N	ns
855	50	+143	+433			N	N	N	ns
856	50	+110	+361			N	N	N	ns
850	75	+ 91	+323	+355		na	na	na	ns
851	75	+145	+280	+354		N	N	N	ns
845	75+	+134	+295	+371	+498	N	N	N	ns
852	75+	+114	+277	+282	+362	N	N	N	ns
Average		+135	+352	+340	+430	No toxic action seen			

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000060

**Chronic Feeding  
Moisture-Pure Cellophane**

**Guinea Pigs - 40 sq. inches (1000 milligrams) per kilo per dose (Cage 75)**

**A. Animals killed for autopsy**

Fig No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
857	25	+ 89				ng	N	N	N
858	25	+ 93				ng	N	N	N
859	25	+131				ng	N	N	ns
860	25	+123				ng	N	N	ns
861	50	+117	+198			N	N	N	ns
862	50	+108	+246			N	N	N	ns
863	50	+150	+328			N	N	N	ns
864	75	+ 63	+286	+410		N	N	N	ns
846	75	+120	+329	+470		N	N	N	ns
847	75+	+133	+157	+298	+352	N	N	N	ns
838	75+	+117	+285	+376	+450	N	N	N	ns
Average		+113	+261	+388	+401	No toxic action seen			

**B. Animals dying during the experiment**

Fig No.	Doses Received	Weight change during experiment	Autopsy findings	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
848	40	+ 66	pneumonia	c, f&	ost, cal, de, d	sm	c, pn, de	pneumonia

Chronic Feeding  
Moisture-proof Cellophane

Rats - 100 square inches (2500 mcms.) per kilo per dose (Cages 13 and 14)

A. Animals killed for autopsy

Rat No.	Doses Received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				
		25	50	75	75+ obs.	Liver	Kidney	Spleen	Lung	
797	25	+29				N	N	N	c	lastoid
791	25	+53				N	N	N	ns	lastoid
890	25	+13				N	N	N	N	
823	25	+19				N	N	N	ns	lastoid
825	25	+13				c	N	N	ns	
790	50	+23	+44			N	N	N	ns	lastoid
812	50	+25	+67			N	N	N	ns	
827	50	+16	- 8			N,mg	N	N	N	
852	75	+33	- 3	+33		N	N	N	ns	
817	75+	-24	-32	-28	-29	N	N	N	ns	
820	75+	+32	+51	+62	+69	N	N	N	ns	
Average		+19	+20	+22	+20					

B. Animals dying during the experiment

Rat No.	Doses Received	Weight change during experiment	Autopsy findings	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
				Liver	Kidney	Spleen	Lung	
821	35	-32	pneumonia	cs,k	CST	ns	c,pn	pneumonia

No evidence of toxic action

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000062

Chronic Feeding  
Moisture-proof Cellulose

Rats - 200 square inches (5000 mms.) per kilo per dose (Cages 15 and 16)

2. Animals killed for autopsy

Rat No.	Doses received	Weight change during number of doses indicated				Interpretation of micropathology of organs (See code, page 21, for meaning of entries)			
		5	50	75	75+ obs.	Liver	Kidney	Spleen	Lung
824	25	+40				N	N	N	ns
838	25	+11				N	N	N	ns
841	25	+ 6				N	N	N	ns
842	25	+25				N	N	N	ns
831	50	+25	+46			N	N	N	ns
853	75	+ 8	+17	+20		N	N	N	ns
839	75	+40	+51	+40		N	N	N	ns
851	75+	+27	+22	+18	+17	N	N	N	ns
855	75+	+47	+36	+38	+31	N	N	N	ns
Average		+25	+37	+29	+24				

3. Animals dying during the experiment

Rat No.	Doses received	Weight change during experiment	Autopsy findings	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable Cause of Death
				Liver	Kidney	Spleen	Lung	
822	40	+21	pneumonia	c,cs,fd	csc,csl,exg	Ln	pn	pneumonia
846	50	+14	pneumonia	c,cs	c,cst	N	pn	pneumonia
862	50	-36	pneumonia	c,cs	cst,csl	N	pn	pneumonia

No evidence of toxic action

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000063

Chronic Feeding  
Moisture-Proof Cellophane

Rabbits - 120 sq. inches (3000 milligrams) per kilo per dose (Cage 50)

A. Animals killed for autopsy

Rab. No.	Doses Received	Weight change during number of doses indicated			Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		15	25	35	Liver	Kidney	Spleen	Lung	
189	15	+540			ng	N	N	ns	nephritis  semi-paralysis
181	15	+600			ng,fd,c	c,csc,st	N	ns	
50	25	+370	+450		ng	N	N	ns	
86	25	+430	+230		ng	N	N	ns	
80	35	+490	+810	+1010	ng	N	N	ns	
44	35	+410	+630	+750	ng	N	N	ns	
Average		+473	+530	+880	No toxic action				

B. Animals dying during the experiment

Rab. No.	Doses Rec'd	Weight change during experiment	Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Probable cause of death
			Liver	Kidney	Spleen	Lung	
183	12	- 50	ng,cs	csc	N	ns	nephritis
45	6	-170	c,ca,ng,K	C,ag,csc,csi,de	ns	C	nephritis
49	6	+260	cs	exg,csc,cst	ns	cell- ophane	drowned and nephritis
56	4	+ 80	ns	ns	ns	na	drowned
184	7	+170	c,cs,ng	c,ag,cst	N	ns	nephritis

000064

Chronic Feeding  
Moisture-Proof Cellophane

Rabbits - 67 sq. inches (1500 milligrams) per kilo per dose (Cage 51)

A. Animals killed for autopsy

Rab. No.	Doses Received	Weight change during number of doses indicated			Interpretation of micropathology of organs (See code, page 21, for meaning of entries)				Other information
		15	25	35	Liver	Kidney	Spleen	Lung	
197	15	+600			N	N	N	ns	tuberculosis
188	15	+610			ng	N	N	ns	
48	25	+640	+740		ng	N	N	tb	
73	25	+540	+640		ng	N	N	ns	
8	35	+ 80	+110	+280	ng	N	N	ns	
65	35	+390	+560	+810	N	N	N	ns	
90	35	+270	+540	+770	ng	N	N	ns	
Average		+447	+518	+620	No toxic action seen				

B. Animals dying during the experiment

Rab. No.	Doses Received	Weight change during experiment	Interpretation of micropathology of organs (see code, page 21, for meaning of entries)				Probable cause of death
			Liver	Kidney	Spleen	Lung	
25	5	- 40	c,ng	c, eg, csc, csl	N	c	nephritis
6	14	+440	c,ng	c, eg, csc, csl, de	N	ns	nephritis
188	5	-110	c, cs, ng	c, eg, cs	eg	c	nephritis

000065

Chronic Feeding  
Coated Cellophane

Chickens - various repeated doses

A. Animals killed for autopsy

Chick No.	Doses rec'd.	Amount of each dose		Weight change during number of doses indicated			Interpretation of Micropathology or Organs (see code, page 21, for meaning of entries)			
		sq.in.	mgms./kg.	4	38	50	Liver	Kidney	Spleen	Lung
52	50	500	5200		+130	+240	N	N	N	ns
53	50	500	6600		+310	+240	c,cs	N	N	ns
54	50	250	2800		-140	-110	N	N	N	ns
55	50	250	2800		+60	+200	N	N	N	ns
56	50	250	2600		+540	+790	N	N	N	ns
57	50	150	1700		+630	+720	N	N	N	ns
58	50	150	1900		0	+560	N	N	N	ns
49	50	150	2500		+140	0	N	N	N	ns
63	38	100	1650		0		cs	cst	N	ns
61	36	100	2300		+170		N	N	N	ns
65	38	50	850		+570		N	N	N	ns
97	4	500	7500	0			ns	ns	ns	ns
96	4	500	8700	0			ns	ns	ns	ns
Average					+210	+377	6600 mgms./kg. for 50 doses gave questionabl effect. All rest probably normal.			

micropath. probably not due to cellophane

B. Animals dying during the experiment

Chick No.	Doses rec'd.	Amount of each dose		Weight change during experiment	Autopsy (see code, page 21, for meaning of abbreviations)	Micropathology of organs (see code, page 21, for meaning)		
		sq.in.	mgms./kg.			Liver	Kidney	Spleen
51	10	500	8000	-590	Liver cysts	c,cs	N	N
59	46	150	1800	+170	Liver cysts, Lung infection	CS	cst,c, d	N
62	36	100	2200	+100	Diarrhea, emaciated	N	N	N
64	10	50	1400	-720	Liver cysts	c,cs	N	N
66	11	50	1200	-220	Liver cysts	c,cs	N	N

Probably none of the birds dying did so from the effects of cellophane, since all had some abnormality or infection.

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INVESTIGATION OF TOXICITY OF CERTAIN PLASTICIZERS

Report No. 4

Chronic Toxicity to Large Animals

May 13, 1932

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## Scope

This report covers investigations of the chronic toxicity of Dibutyl phthalate, "Santicizer No. 8", and Moisture-Proof Cellophane (with dibutyl phthalate as plasticizer) when administered by mouth to monkeys and dogs.

## Animals and Their Care

The original group of monkeys consisted of 14 *Macacus rhesus* specimens of mixed sexes, weighing two to three kilos each. When received they were placed singly in small wooden cages for observation. Each one was tested for tuberculosis by intramuscular injection of tuberculin followed by observation of rectal temperature for 48 hours. As a result of these tests two animals were killed to protect the others, and two other monkeys were noted as suspicious. At the conclusion of the feedings it was found that the monkeys noted as suspicious really had tubercular lesions in the lungs. Following this quarantine period the animals were marked for identification with dyes, and were placed in two large cages approximately 3 x 6 x 6 feet, built of wire with metal floors. The floors of the cages were kept covered with plenty of clean sawdust, which was renewed daily.

The monkey diet was the same as that used in the Philadelphia Zoological Garden. The morning meal was a portion of cake baked from cornmeal, meat, carrots, Crisco, molasses, baking powder, and a complex mixture of salts designed to overcome any mineral deficiency in the rest of the

diet. The noon and evening meals were different each day to give the animals variety, but in general they included boiled eggs, lettuce, oranges, and apples. Water was given occasionally.

The original group of dogs included 12 specimens of indeterminate breeds and mixed sexes. These animals were not "de-barked." Upon receipt they weighed from 5 to 10 kilos each. They were kept in pairs in wire cages with concrete floor and a wooden shelf, upon which the dogs spent most of their time. The cages and shelves were cleaned daily by scraping, followed by flushing with a hose. The animals were fed meat, bone, and vegetable scraps, and received all the water they wanted.

#### Methods of Dosing

The two plasticizers were fed exactly as received. Their doses were calculated in terms of mgms. per kilo of body weight of the animal, but were actually measured in cubic centimeters. The cellophane was fed as a suspension in water, one c.c of which contained four square inches of cellophane. Our Report No. 3 of April 13, 1932, page four, described the preparation of this suspension.

The monkeys were fed their doses by a stomach tube two or three times a week; not oftener because of fear that we would set up chronic irritations in the throat from the passage of the tube, which was somewhat large for the esophagus. During the passing of the tube the monkey was stretched on its back on a table, and its hind legs were

secured by a hobble, which passed over two nails in the table. Its front legs were held by an assistant. A wooden gag, somewhat like a large spool, was inserted between the jaws and a stomach tube well lubricated with paraffine oil was gently passed down into the stomach. The required dose was then forced down the tube by gentle air pressure. When the dose was large it was warmed to body temperature before feeding to avoid the shock of a large volume of cold fluid.

The dogs received cellophane in the same way that the monkeys received all their doses; that is, by stomach tube, except that since the dogs had larger throats and their mucous membranes were less tender, we felt it safe to make feedings five times a week. The dogs swallowed small doses perfectly when the material was placed in the back of their throats, so that for the plasticizers stomach tubes were not used.

#### Doses Administered

The program of doses given is not completely balanced because we were requested to stop this work sooner than we had expected.

Since little effect had been obtained by feeding these plasticizers to small animals (see Report No. 3), the largest dose previously fed was taken as the starting point for dosing large animals; that is to say, the smallest dose of plasticizer fed the large animals was 400 mgms.

Abbreviations used in Table 1, page 4.

Con.	=	Control animal
DBP		Dibutyl phthalate
S-8		Santicizer No. 8
Cel.		Moisture-proof Cellophane, using Dibutyl phthalate as plasticizer
N		Normal
TB		Tuberculosis
lcs		light cloudy swelling
ost		light cloudy swelling of tubules
c		congestion
p		parasitic cysts
pn		pneumonia

Table 1.

## Summary of Doses Fed and Results

(See page 4b for meaning of abbreviations used)

Species	Animal Number	Material Fed	Weeks Fed	Single Doses Fed	Dose in mgms. Per kilo	Weight in kilos at first dose	Change in weight	Millions change in Red blood cells	Change in Hemo- globin, in %	Urine; abnormal specimens found			Autopsy notes	Micropathology			
										Albumin	Casts	Sugar		Liver	Kidney	Spleen	Lung
Monkeys	12	Con.	14			2.64	+ .03	-1.4	+ 8	0	0	0		N	N	N	
	13	Con.	14			2.84	+ .06	-1.5	+14	0	1	1		N	N	N	
	4	DBP	13	29	1200	2.78	+ .06	-3.0	-15	0	0	1		N	N	N	
	1	DBP	9	19	800	2.42	+ .06	-1.2	+10	0	0	0	dose in lung	N	N	N	
	14	DBP	10	21	400	2.98	+ .15	-1.2	+13	0	0	0		N	N	N	
	7	S-8	13	28	1200	2.67	+ .00	-0.8	-10	1	0	0	TB in lung	loc	cat	c	TB
	6	S-8	8	17	800	3.27	+ .11	-1.7	-13	1	1	0	carbuncle, spleen cysts	N	N	v	
	11	S-8	10	24	400	2.42	+ .08	0.0	+ 8	0	0	0		N	N	N	
	8	Cel.	13	28	4200	2.16	+ .45	+0.5	0	0	0	1		N	N	N	
	9	Cel.	6	15	3800	2.59	+ .02	-0.3	+ 8	0	0	0		N	N	N	
10	Cel.	10	24	3700	2.56	+ .26	+0.2	+13	1	0	0	TB in lung	loc	cat	c	TB	
3	Cel.	7	15	3200	3.15	+ .00	+0.8	0	2	0	0	dose in lung, spleen cysts	c	N	v		
Dogs	739	Con.	12			6.3	+0.7			0	0	0		N	N	N	
	698	DBP	11	40	1200	5.1	+1.2			0	0	0		N	N	N	
	703	DBP	11	31	1200	5.4	+1.1			0	0	0		N	N	N	
	736	DBP	8	27	800	7.0	-0.4			0	0	0	TB in lung	N	N	N	
	691	DBP	11	39	400	7.7	+1.6			0	0	0		N	N	N	
	726	S-8	11	43	600	6.7	+2.3			0	0	0		N	N	N	
	683	S-8	8	28	500	6.4	+0.4			0	0	0		N	N	N	
	685	S-8	11	40	400	4.3	+0.3			0	0	0		N	N	N	
	615	Cel.	5	20	1500	6.4	+0.5			0	0	0		N	N	N	
	701	Cel.	5	17	1200	7.2	+0.4			0	0	0		N	N	N	
684	Cel.	5	19	1000	9.4	0.0			0	0	0		N	N	N		

per kilo of body weight. We found that the dogs would not tolerate doses of Santicizer No. 8 greater than about 600 mgms. per kilo. Invariably larger doses caused the dog to vomit, so that there was no point in administering such doses. An attempt was made to accustom the animal to the effect of this material by gradually increasing the dose from a small starting point, but it was found that this did not avoid the vomiting when doses of approximately 600 mgms. had been reached.

One hundred c.c. of fluid was all that we felt could safely be fed the animals. This volume, then, was the limiting dose for cellophane and represented 400 square inches of the material, or approximately 10 grams. Accordingly, the cellophane doses administered to the large animals were less when expressed as mgms. per kilo than the cellophane doses which had been given to the small animals, because of the fear that larger volumes would choke the monkeys or the dogs. Table 1, page 4, gives the magnitude of dose and number of times repeated for every animal considered in this report.

### Observations

Behavior, appearance, and weight, determined weekly, were recorded for every animal. Every two weeks a blood count, including red and white cells, hemoglobin and differential white count, was made on each monkey, and every two weeks each monkey's urine was analyzed for sugar, albumin,

specific gravity and casts. A similar urianalysis was made for each dog just before the animal was killed. No blood counts were made for the dogs, partly due to lack of facilities where the dogs were kept, and partly to the fact that since nothing abnormal had been found in the monkey bloods, it was felt to be a waste of time to examine the dog bloods.

### Behavior and Appearance

Nothing abnormal whatever was observed in the behavior and appearance of any of the large animals. They were all active and alert at all times.

### Diseases

The only infectious diseases noted among the monkeys were the two cases of tuberculosis mentioned above. Since these two animals had given suspicious tuberculin-test temperature charts, it is certain that their infection occurred before we received them. Two other monkeys had parasitic cysts in the livers, but their infestation was not sufficiently severe to have affected the pathology of the organs.

Only one case of disease of any kind was found among the dogs. This was a mild pneumonia in a female which had given birth to a litter just before our feeding experiment started. The probability is that this pneumonia was caused by the littering.

## Weight Gain

Table 1, page 4, shows the weight changes of the animals during treatment. Only two monkeys made what we consider satisfactory weight gains during the period. We are somewhat at a loss to explain this, since there was no infection in the group and the animals had adequate exercise and food. Monkey No. 13, a control, lost weight during the feeding period, and twice its urine showed albumin and once sugar. It seems probable that this animal was not normal, so that it will be disregarded and the other control used as a criterion for judging the treated animals.

Of the treated monkeys four gained less weight than did the control. Of these four, two had accidentally received small amounts of their doses in the lungs, and one had an infection of tuberculosis. These three, then, need not be explained by any effect of the materials fed. The fourth animal had received cellophane. This is the only deviation from normal weight gains among the treated monkeys which cannot be explained by disease or accident. Two other monkeys receiving doses of cellophane made excellent weight gains.

In general, the dogs gained weight satisfactorily. Six dogs gained less than did the control dog. One of these cannot be considered due to toxic effect, because it was suffering from pneumonia. Three of the remaining had received cellophane, one had received Santicizer No. 8, and one dibutyl phthalate, neither of the last two receiving

the largest doses fed of the test materials. Apparently the cellophane did have an effect on the weight gain of the dogs. It is possible that this effect was due only to the bulk of the dose fed, which decreased the appetite of the animal for its normal diet. Since the dogs were fed four or five times a week, such a decrease in appetite would have more effect on their weight gain than it would on the monkeys, which were fed only two or three times a week.

#### Blood Examination

Table 1, page 4, shows the changes in red cell count and hemoglobin per cent experienced by the monkeys during treatment. Practically all the animals lost red cells, which is possibly due to the fact that they received no direct sunlight in their cages. At the same time the average gain in hemoglobin for the group was 2 per cent.

Monkey No. 4, receiving dibutyl phthalate at 1200 mgmc. per kilo, lost more red cells and hemoglobin than did the control. Monkey No. 7, receiving Santicizer No. 8, at 1200 mgms. per kilo, lost more hemoglobin; Monkey No. 6, receiving Santicizer No. 8 at 800 mgms. per kilo, lost more red cells and hemoglobin than did the control, and the three monkeys receiving cellophane (omitting the one animal infected with tuberculosis) lost more hemoglobin. The only gains in red cells among the whole monkey group were by three animals receiving cellophane.

It is possible that the largest dose of both dibutyl phthalate and Santicizer No. 8 had some effect in

decreasing both hemoglobin and red cells, and that the cellophane doses had some effect in decreasing hemoglobin, although there is just as much evidence that they stimulated red cell formation at the same time.

The total white cell counts and differential white cell counts were perfectly normal and are not reported in detail.

### Urine Examination

Only two of the treated monkeys showed high sugar (over 0.2 per cent to Benedict's Solution). One of these had received dibutyl phthalate at 1200 mgms. per kilo, and the other had received the largest dose of cellophane fed. Each of the two showed high sugar in only one out of seven tests, and neither one was over 0.5 per cent. The phenylhydrazine test proved the sugar to be dextrose in both cases.

Six treated monkeys showed albumin in the urine from time to time, although not consistently. The two monkeys receiving the highest doses of dibutyl phthalate had albumin in half of the samples examined, and the two receiving the highest dose of Santicizer No. 8 each had albumin in a sixth of the samples examined. In addition, two monkeys receiving cellophane showed albumin occasionally. In only one instance were casts found in the urine. These were hyaline casts and were associated with albumin in the monkey receiving Santicizer No. 8 at 800 mgms. per kilo.

Only one dog showed anything unusual in the urine. The dog receiving Santicizer No. 8 at 400 mgms. per kilo showed a trace of albumin.

None of the materials caused the consistent excretion of abnormal urine. However, both the plasticizers produced albumin in some cases with the two larger doses, - dibutyl phthalate producing it more frequently than Santicizer No. 8. Cellophane also produced albumin, but less frequently than did either plasticizers.

#### Deaths

No animals died during the experiment from any cause whatever.

#### Micropathology

Liver, kidney and spleen sections were studied microscopically and the preparations are part of our permanent files. No abnormal cell structure was found in any of these organs, except with animals known to be infected with parasites, some of which were encysted in liver or spleen; that is to say, there is no evidence that any of the doses fed produced structural changes in liver, kidney or spleen of any test animal.

## Summary

Since no evidence of microscopic structural change was found in the organs examined, it has been taken for granted that none of the minor symptoms noted (blood findings, transient urine abnormalities and weight gain) were due to the doses fed. It is much more likely that these minor symptoms were due to extraneous causes rather than to toxic action, although they have been noted faithfully in this report in case others desire to draw their own conclusions from them.

Santicizer No. 8 was fed to monkeys three times weekly in doses of 400, 800 and 1200 mgms. per kilo for 10, 8 and 13 weeks, respectively. In no instance was there any evidence of harmful effect attributable to this material in this time, either in weight, blood or renal changes or tissue degeneration. The failure to gain weight, loss in hemoglobin and occasional presence of albuminuria in one case were traceable to tuberculosis and the blood and urine changes in another to a staphylococcus infection.

Dogs fed Santicizer No. 8 at 400, 500 and 600 mgms. per kilo on five days a week for 8 to 11 weeks showed no harmful effects attributable to this material, unless it be the presence of transient albuminuria in the dog receiving the smallest dose. No tissue changes were noted except in the animal fed 500 mgms. per kilo, which developed pneumonia after labor.

These tests show no evidence of any harm from the administration of Santicizer No. 8 up to 1200 mgms. per kilo to monkeys for 39 doses extending over a 13-week period, and up to 600 mgms. per kilo to dogs for 55 doses extending over an 11-week period.

Dibutyl phthalate was fed to monkeys three times weekly in doses of 400, 800 and 1200 mgms. per kilo for periods of 10, 9 and 13 weeks, respectively. No tissue pathology attributable to this material was noted, but the second animal lost slightly in weight and developed irregular albuminuria; and the third showed considerable decrease in red blood corpuscles and in hemoglobin, with albuminuria and slight glycosuria.

Dogs received the same doses five days weekly for 11, 8 and 11 weeks, respectively, and showed no evidence of harmful effects.

Neither type of animal was harmed by the largest dose previously given to smaller animals, but monkeys were apparently somewhat affected by the larger doses, - much larger doses than would ever accidentally be taken by man.

Moisture-proof Cellophane (with dibutyl phthalate as plasticizer) given to monkeys in up to 4200 mgms. per kilo showed no harmful effects in up to 39 doses over a 13-week period except in one case (3800 mgm. per kilo) where a slight reduction in red blood cells and hemoglobin was found. Two animals made fair weight gains, better than the control, and two very slight gains, but the animal receiving the largest doses showed the best gain of any animal in the group. Two showed

transient albuminuria, one having tuberculosis and one accidentally receiving some of the material in the lung.

Dogs received 1000, 1300 and 1500 mgms. per kilo five times weekly for five weeks, and showed no harmful effects other than poor weight gains possibly attributable to lost appetite from receiving such large amounts of inert material daily.

No evidence of harm was found from feeding cellophane to monkeys or dogs in massive doses for 39 doses in monkeys and 25 doses in dogs. The doses fed correspond to doses of 3000 to 12,000 square inches for a man, repeated 15 to 24 times.

#### Conclusion

The results obtained with the larger animals, monkeys and dogs, with these materials agree as far as they were carried out with the results obtained with the small animals, and indicate that there is no likelihood of harm being produced in man from any amounts of dibutyl phthalate or Santicizer No. 8 likely to be ingested accidentally or of moisture-proof cellophane containing the plasticizers likely to be eaten purposely.

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Doc Type	• RI • UP • HEADS • 8D • SU HS FN			22
Doc Title	TOXICITY OF DIBUTYL PHTHALATE TO LABORATORY ANIMALS			23
Chemical Name (300 per name)			CAS No. (10)	24
ALKYL PHTHALATES			999999994	
BENZENEDICARBOXYLIC ACID DIBUTYL ESTER			84-74-2	
BENZENEDICARBOXYLIC ACID DICYCLOHEXYL ESTER			84-61-7	

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The Toxicity of Dibutyl Phthalate to Laboratory Animals

87-8211712

... toxicity of dibutyl phthalate (DBP) has been determined...  
... administration by means of the stomach tube...  
... laboratory...  
... that dibutyl phthalate was...  
... experiments on the toxicity of...  
... dibutyl phthalate in which rabbits given daily...  
... without the occurrence of... or... of... following...

The rabbits were then treated daily for eight days with...  
... of dibutyl phthalate... At the end of this period...  
... had lost... and was on the point of...  
... significant pathological changes were found...  
... in the kidney... and...  
... The second animal after eight days had lost...  
... and was suffering from anorexia. Histopathological changes...  
... in kidney and slight protein leakage in kidney...  
... and slight atrophy and...  
... of the liver.

... administration of DBP by Dr. ...  
... established on LD50 of 11 cc per kg. compared with a corresponding value  
... of 6.4 cc. per kg. for dibutyl phthalate (DBP). The corresponding LD50  
... in rats were DBP 17 cc. per kg., DBP 6.3 cc. per kg.

Neither of these compounds produce significant...  
... phthalate appears to be more toxic than dibutyl phthalate when taken  
... internally, whereas dibutyl phthalate is significantly toxic if applied in  
... a daily course of generous doses applied directly to the skin. Dibutyl  
... phthalate is more toxic under these conditions.

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Dibutyl phthalate  
FDA

87-8211712

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Toxicity tests by the British indicate that DDF is less toxic than DFP when given subcutaneously to rabbits, doses of 9 cc. per kg. being tolerated. Doses of 6 cc. per kg. administered orally were fatal to rabbits. Repeated (12) skin applications of 1 cc. per kg. a day produced no ill effects. Large doses of DDF produce, in rabbits, significant pathological changes including tubular degeneration and necrosis in the kidneys and occasional liver damage. They conclude that both DDF and DFP are of low toxicity.

It was emphasized by Dr. Braise (FDA) that a compound such as DDF may be quite toxic when directly applied to the skin but may exert no harmful effect if it is used in impregnated clothing in direct contact with the skin even though the concentration of the compound in the fabric is quite high. Dr. Braise is waiting samples of fabric impregnated with DDF to be submitted for toxicity tests. These samples are to be supplied by the Army. He is quite hopeful that such samples will prove to be safe for use.

References: CC-8-1 Letter to Dr. C. C. Stark from Dr. R. O. Calvery, October 4, 1944.  
CC-8-10 Toxicity of Insect Repellents and Larvicides. Bi-monthly progress Rep. of Dr. Calvery, R. O. et al. FDA, OMSD contract W-2218, June 1, 1944.  
CC-148 Toxicity of DDT Dissolved in Dimethyl Phthalate and Dibutyl Phthalate. Cameron, G.R. and F. Burgess. Ministry of Production, LDF (44) 104, July 18, 1944.

8-4-46 Richard A. Grunbee, Tech. Aide, OMSD

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REPORT UPON THE TOXICITY OF PLASTICIZERS

Submitted to Dr. G. H. Gehrman, Medical Director,

E. I. DuPont de Nemours and Co., Wilmington, Del.

Report submitted by

Dr. C. M. McCay, Cornell Univ., Ithaca, N. Y.

Oct. 1, 1931

- 1. Dibutyl phthalate
- 2. Diethyl phthalate
- 3. Santizer
- 4. Ethox

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Subject. The toxicity of Plasticizers Nos. 1, 2, 3 and 4.

Object. The preliminary experiments recorded in this report were undertaken to determine the relative toxicity of the series of plasticizers. At the same time we have attempted to determine the nature of the injuries to the organs and bodies of various animal species after the administration of these compounds in various ways.

Animals and Technique; This report covers preliminary studies upon the following animal species: chickens, rats, mice, goats, cats, dogs and guinea pigs. All animals tested have an aversion to consuming these compounds in their feed. Therefore when given orally they must be administered in capsules or forcibly. No histological work is incorporated in this report since we felt such work had better be reserved for a later date after the completion of relatively crude testing. Quite widely different techniques were used with the various species. This was done in order to give the most information that can be obtained in a brief period concerning the toxicology and pharmacology of the compounds in question.

Summary of Results. All species tested seem quite sensitive to the compounds in question. In a future report we plan to give a series of figures for the m. l. d. for various species. Such data in this case will probably have questionable value since we are dealing with compounds that lower the body temperature. The studies with guinea pigs show clearly that the animal will live if kept warm after the administration of a given compound while

it will die if kept at a normal room temperature. The nature of these compounds, therefore, makes the values for the m. l. d. relatively crude.

Toxicity. Of the four compounds tested No. 3 is more toxic than the others. All species support this conclusion. The toxicity of the other three seems quite the same. These compounds seem toxic whether administered by mouth or subcutaneous injection. The autopsy findings and the symptoms before death seem quite the same for the various species and independent of the mode of administration of the compound. All compounds seem to effect their greatest injury in the mucosa of the intestinal tract. This injury seems to result from both feeding and injecting the various compounds subcutaneously. In many cases the lungs and liver are also affected. These compounds seem to inhibit the activity of certain strains of bacteria since the fecal material in the intestines of chickens seemed free from putrefaction. No. 3 seems to be especially injurious to the intestinal tract. This is best illustrated in the section dealing with the goat experiments. These compounds all seem to increase the flow of bile very markedly. It is possible that this is the channel of excretion although the cysts that form on the intestines after the administration of the various compounds indicate there may be some passage thru the intestinal wall possibly through the lymph.

Experiments with rats.

The rats used in these experiments were healthy adults of known genetic history. They weighed about 250 grams each.

Technique. Since the number of animals available for these preliminary tests was limited, we started by injecting a small dose of each of the compounds subcutaneously at intervals of two days. We usually started with a small injection of .01 cc. As no ill effects were observed after these injections of such small amounts of compounds Nos. 1-4, the dose injected was gradually increased until 1 cc. of each compound was given each rat at intervals of two days. Records were kept of the total amounts injected.

Tolerance of compounds injected.

Soln. No.	Rat No.	Span of Time	Total Amt. Injected.	Remarks
1	1	3	14 cc.	Alive--appetite maintained
1	2	19	10 cc.	Killed for post mortem
1	4	43	11 cc.	Alive--appetite maintained
2	6	43	11 cc.	Alive--appetite maintained
2	7	43	15 cc.	Alive--appetite maintained
2	8	19	10 cc.	Killed for post mortem.
3	17	3	2 cc.	Died
3	16	14	6 cc.	Died
3	14	31	4.5 cc.	Died
3	15	19	5 cc.	Post mortem
3	9	1	1 cc.	Died
3	10	5	3 cc.	Died

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4	13	19	10 cc.	Killed for post mortem.
4	11	43	14 cc.	Alive--appetite maintained.
4	12	43	11 cc.	Alive--appetite maintained.

Symptoms Observed After the Injection of Sub-Lethal Doses.

Soln. No.	Amt. to produce Symptoms.	Symptoms Observed.
1	0.5-1 cc.	Slight dizziness with recovery after a few hours. Slight temperature drop of 1° F. after injection in one case.
2	1 cc.	Dizziness and temp. drop of same magnitude as No. 1.
3	0.5 cc.	Marked drop in body temperature. In one case the body temperature dropped from a normal of 101° F. to 93° F. in the course of 2.5 hours after the injection. In another case the body temp. dropped to 78° F. before the animal died. In both cases the animals were in coma and unconscious. Some symptoms of blindness have been observed.
4	1 cc.	Dizziness with recovery after several hours. Temperature drops of as much as 12° F. with ultimate recovery have been observed.

Post Mortem Observations.

All four solutions injected subcutaneously produced cysts or nodules upon the mucosa of the small intestine. No. 1 may be more slowly absorbed than the others as shown by an earlier formation of subcutaneous nodules or cysts. No. 3 produces an excessive flow of bile into the small intestine as observed in the form of reddish-yellow fluid in the upper sections of the small intestine. All solutions produce an enlarged and somewhat

congested spleen. The lungs were congested in the case of Soln. 3. No changes were observed in the livers of rats except in one case where gross inspection showed a mottled liver. Bladder distension was observed in two cases after the injection of No. 3. Gross examination showed no kidney injury. After the injection of No. 3 an inflamed mesentery was found in one case and an opaque eye in another.

#### ORAL ADMINISTRATION OF THE COMPOUNDS To Rats

Technique. Sixteen mature rats were divided into four groups. One member of each group received 0.5 cc., another 1 cc., a third 1.5 cc. and a fourth 2 cc. by mouth daily, of the various compounds. They were fed a standard stock ration known to be satisfactory for growth, maintenance and reproduction. This experiment has been in progress for two weeks with no evidence of toxicity thus far. This experiment will be continued with an improved technique at a slightly later date. At present the rats are fed at intervals each day with an eye dropper. A small stomach tube will be used in the next series.

#### Experiments with Mice

Sixteen small mice in a preliminary trial were injected with varying amounts of the various compounds, 0.05-0.3 cc. No conclusive data resulted from this experiment since these young mice seemed very sensitive and died too quickly for symptoms to be observed. Some spotted livers were observed. Fascia at the site of injection were rendered opaque in some cases. No correlation with the material injected has been made thus far.

With a limited number of adult mice the order of toxicity was found as follows:-

No. 3. Most toxic killing with two injections subcutaneously with doses of 0.2 cc. and a time interval of 2 days.

No. 1 was next in toxicity killing with 3 doses of 0.2 cc.

No. 2 was next killing with 6 doses of 0.2 cc. in the course of ten days.

No. 4 is still alive after 8 injections of 0.2 cc. each in the course of 14 days.

Pathological Findings.

No. 2 showed a stained liver and considerable bile in the intestine.

No. 3 showed a few large nodules on the intestines.

Experiments with Goats.

Two goats weighing about 15 kg. each were available.

No. 1 was injected as follows with compound No. 3., subcutaneously:

Date	vol. injected	Observations.
9/18	5 cc.	No effects
9/19	5 cc.	" "
9/20	5 cc.	" "
9/21	10 cc.	" "
9/22	15 cc.	" "
9/23	20 cc.	Died 18 hrs. later

From the time of this final injection the body temperature

fell at the rate of about one degree per hour until a minimum of 94° F. was observed. Bloating started after about 9 hrs. and continued regularly until the rumen was much distended with gas at the time of death. Retching movements were also observed. The muscles of the neck seemed to tend to pull the head well back. Pulse irregular shortly after the injection with a tendency toward normal in later hours.

Autopsy Findings.

Rumen distended with gas but no marked evidences of putrefaction.

The mucosa of all the stomach linings showed marked injury and a tendency to peel readily. The small intestine showed inflammation and multiple cysts were found on the mucosa. The mesenteric lymph glands were enlarged and darker than normal. Lungs were congested. Liver, adrenals and ovaries were normal. There was no excess bile. The eyelids were slightly inflamed.

The second goat was injected as follows with compound No. 1.

Date	volume injected subcutaneously.
9/18	5 cc.
9/19	5 cc.
9/20	5 cc.
9/21	10 cc.
9/22	15 cc.
9/23	20 cc.
9/24	25 cc.
9/25	30 cc.
9/26	35 cc.
9/28	40 cc.
9/29	50 cc.

This goat was alive and normal at the time of the last injection. No injurious symptoms observed one day after the last injection.

Experiments with Chickens.

The chickens used in these experiments were growing cockerels weighing approximately 1.4 kg. each. They were fed a standard poultry ration.

The compounds were administered by mouth in capsules since animals refuse food mixed with the compounds.

Two preliminary experiments have been completed. In the first one a preliminary dose of 1.5 cc. of the four compounds per kg. body wt. <sup>was</sup> ~~were~~ fed the first day. This was increased by 1.5 cc. per kg. every other day. The survival periods and the total compound consumed afford measures of the relative toxicity. The results of this experiment are summarized in the following table:

Compound No.	Survival Period (days)	Total Oil Given (cc.)	Maximum Single Dose in cc. per kg.
1	Normal at end 10 days	44	5.6
2	7	30	4.3
3	3	7	2.9
3	6	23	2.7
4	8	36	4.7

In a second experiment a daily dosage of 1.8 cc. per kg. was administered until death ensued. The results of this experiment are summarized in the following table:-

Compound No.	Survival Period	Total Compound Fed in cc.
1	11	28.6
2	19	49.5
3	3	10.4
4	Alive after 22 days and fed	57.2 but badly emaciated

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### Symptoms

The symptoms leading to death were: listlessness, pale comb, watery eyes, slight diarrhoea, emaciation, unsteady gait, lowering of temperature, inability to rise or stand, coma, death. In test 1 all of these symptoms, except coma and death were exhibited by a preceding dose from which the animal recovered. In general the initial symptoms were noted within an hour after dosage becoming progressively worse and followed by death or apparent recovery in 48 hours. In test 2 the symptoms developed more gradually over a longer period prior to death. There were no periods of apparent recovery. Emaciation was a marked feature.

### Post-mortem Findings.

The following were common to all animals which died: narcotic areas on gizzard lining, stomach lining corroded, intestinal lining inflamed, gall bladder distended and full, spleen small and pale. The heart and lungs were normal. In some instances the kidneys were inflamed and in one case the liver appeared congested. The pathological findings were much more marked in the case of Oil # 3. The bird which survived the 10-day period in the first test with oil # 1 with no apparent deleterious effects showed a slight distension of the gall bladder and a thickening of the small intestine.

Experiments with Dogs, Cats and Guinea Pigs.

Subcutaneous injections.

Subcutaneous injections of # 1 in guinea pigs, in doses varying from 1 cc. to 4 cc. per kg. body weight, produced no apparent physiological or anatomical symptoms. Very slow absorption of the chemical from the subcutaneous tissues is strikingly indicated since these same doses, when given intraperitoneally were followed by rather characteristic physiological symptoms.

Subcutaneous injections of 2 cc. of # 2 per kg. body weight in guinea pigs produces no apparent physiological or anatomical abnormalities.

Subcutaneous injections of 2 cc. of # 3 per kg. body weight in guinea pigs resulted in coma, subnormal body temperatures and death. Autopsy: Ashy-gray discoloration of the lungs; distended gall bladder and appreciable quantities of bile in the small intestine.

Subcutaneous injections of 0.36 cc. of # 4 per kg. body weight in a dog was without any noticeable effect on the animal. Injections of 2 cc. per kg. body weight in guinea pigs were also without physiological effect. No post-mortems were conducted since the animals were not killed.

Intraperitoneal injections.

In guinea pigs, 1 cc. of # 1 resulted in the following mild symptoms: sluggishness, rough hair, and subnormal temperature. These persisted for 24 to 72 hours. This was taken to indicate that the chemical is not readily absorbed from the peritoneum. If

the animals are not kept warm by artificial means, their body temperature may fall considerably. It seems quite apparent that the heat regulating mechanism is affected adversely.

Intraperitoneal injections of # 1 in cats in doses of 3 cc. per kg. body weight, produce nausea, retching movements, vomiting, loss of appetite, lethargy, and ultimately coma and death. Body temperature falls considerably before death. This symptom, however, is not so prominent in the early stages of the poisoning as in the case of the guinea pigs. Postmortem: Unusually large quantities of fluid in the peritoneal cavity but the liquid does not have the odor of the injected chemical. Bile secretion is stimulated by the chemical. This is indicated by a distension of the gall bladder and unusual amounts of bile in the small intestine. There was no other pathological physiology or anatomy.

Intraperitoneal injections of # 2 were given to guinea pigs in doses varying from 2 to 8 cc. per kg. body weight. Two cc. appeared to be about the minimal toxic dose. The following symptoms resulted: lethargy, narrowed palpebral apperatures, subnormal body temperatures (these could be prevented by the application of heat). This indicates a disturbance of the heat regulating mechanism. With larger doses, there resulted a hyperproes coma, and death. No apparent pathological anatomy was found at the postmortem; neither were there any signs of infection.

The sensivity of guinea pigs to the toxicity of # 2 is not noticeably increased by previous injections of this same chemical.

Intraperitoneal injections of # 2 in cats to the extent of 1 to 3 cc. per kg. body weight are followed by lethargy, loss of appetite, nausea, retching movements, vomiting and increased irritability; with larger doses coma results, the body temperature falls

and death ultimately supervenes, usually following a period of slowly diminishing respiratory exchange.

Dogs can withstand rather large doses of # 2 when injected subcutaneously without physiological or anatomical symptoms. Intraperitoneal injections, up to 2 cc. per kg. body weight, are non-lethal. With these larger injections, however, the animals may become slightly lethargic, refuse food, and become very irritable, but show no appreciable alterations in respiratory exchange or body temperature. Pulse rates are not reliable in dogs, unless the animals are trained, because of psychic disturbances which may arise from handling.

Intraperitoneal injections of # 3 in dogs, in doses of 0.36 cc. to 1 cc. per kg. body weight, were made. The smallest dose produced sluggishness, but no other physiological symptoms. Within 2 minutes after 1 cc. per kg. body weight was injected, one dog lay comatose and died 25 minutes later from a gradual failure of respiration. Body temperature remained normal until death. No other symptoms of physiological importance arose. Autopsy: Mottled appearance of lungs with ashy pink discoloration. There were no other pathological findings. The survival in this animal was, of course, too short for the development of marked changes.

Intraperitoneal injection of 0.66 cc. of # 3 per kg. body weight in a kitten resulted in vomiting, coma, and death. Coma developed within 10 minutes. The animal slowly expired. Autopsy: there were no pathological findings.

Intraperitoneal injections of # 3 in guinea pigs in doses of 2 cc. per kg. body weight resulted in lethargy, coma, subnormal body temperature (this was preventable by the application of artificial heat), and death. Death occurred after about 2 hours.

Autopsy: Ashy-pink discoloration of the lungs.

In doses of 0.5 cc. per kg. body weight in guinea pigs this compound produced only moderately severe symptoms similar to those following larger doses. These animals recovered completely.

The duration of the symptoms following the injection of # 3 is comparatively short when compared with those for Nos. 1, 2, and 4. It would seem that absorption of # 3 is more rapid.

Intraperitoneal injections of # 4 in guinea pigs in doses of 2 cc. per kg. body weight, is about the minimal toxic dose for some animals, others show no toxic symptoms with this dose. The symptoms are : sluggishness and subnormal body temperatures which exist from 12 to 48 hours. Recovery occurred in all cases. With doses of 12 cc. per kg. body weight (6 cc. per animal) more severe symptoms developed, including prostration, coma, and death within 6 hours. Autopsy: the urinary and gall bladders were distended with their respective excretions. An unusual amount of bile was present in the small intestine. From its odor, the chemical was easily detected in the fluid of the peritoneal cavity.

Intraperitoneal injections of 2 cc. of # 4 into dogs per kg. body weight resulted in no apparent pathological symptoms; 4 cc. per kg. body weight also were without effect in producing symptoms.

Per Os, or by Stomach Tube.

Animals will not voluntarily take any one of the chemicals by mouth with food or in any other way. The taste, or possibly the irritation to the mucous membranes of the mouth, is disliked very much. When the chemicals are given by force, the animals struggle and fight ferociously.

Ten cc. or 0.6 cc. per kg. body weight of # 1 given by mouth (dogs), by force, resulted in no immediate or delayed symptoms.

In another animal, 21 cc., or 3 cc. per kg., given by tube resulted, within 5 minutes, in coma, marking time effect with feet, gasping respiratory movements, tremors and twitchings of the muscles, respiratory spasms alternating with periods of hyperpnoea, and finally respiratory failure and death. Postmortem showed a mottled appearance of the lungs, frothy mucus in the stomach and general irritation of the gastric mucosa.

Five-tenths cc. of # 4 per kg. body weight when given by tube to a dog produced salivation. No other disturbances were noticeable. With doses of 3 cc. per kg. body weight, one animal was dead in three minutes. This was probably not due to its direct action on the stomach or to its absorption from the gastrointestinal tract, but to disturbances of respiration, apparently some of the chemical entered the trachea.

#### Intravenous Injections.

Intravenous injections of approximately 0.2 cc. of # 4 per kg. body weight in a dog resulted in almost immediate stimulation of salivation, and slight stimulation of respiration. Increase in respiratory exchange was apparently not the result of a rise in body temperature since this did not occur. The symptoms soon passed off. The dog was not killed for autopsy.

Intravenous injections of # 1 in doses of 0.3 cc. per kg. body weight were followed within 7 minutes, by panting, salivation, and nervousness. The animal refused to eat. Recovery was complete.

When 0.7 cc. of # 2 per kg. body weight was injected intravenously into a dog, symptoms appeared almost immediately. One minute after the injection the animal was prostrate, fighting and gasping for breath. The pulse rate was increased; the temperature

remained at the normal level; recovery was complete in 30 minutes. No. 2 is apparently not suitable for intravenous injection. The symptoms would probably indicate that the disturbances were due to physical rather than chemical effects of the drug. They suggested the formation of emboli by the injected drug.

Intravenous Injection in Dogs under Barbital-sodium Anaesthesia.

This series of experiments was conducted for purpose of studying the effects of these compounds in the blood stream on the following: (1) blood pressure, (2) respiration, rate and depth, (3) the heart, and (4) bile secretion. Blood pressure was measured from the carotoid artery and recorded graphically on smoked kymograph paper. Respiration was recorded by a pneumograph placed around the thorax. Bile secretion was measured by cannulating the common bile duct, after tying off the cystic duct, and recording, by electrical methods, the number of drops of bile secreted. The following effects were produced by the respective compounds:

# 1. a. Fall of blood pressure.

b. With or without acceleration of the heart rate.

c. Increase in rate of respiration, with usually an increase in depth.

d. Stimulation of bile secretion.

# 2.

a. Marked fall of blood pressure.

b. With or without acceleration of the heart.

c. Decrease in rate and depth of respiration.

d. Bile flow is not appreciably affected in this animal but is indicated in other experiments.

# 3.

- a. Fall in blood pressure.
- b. Increased rate and depth of respiration.
- c. Heart not appreciably affected.
- d. No record of bile flow. Other experiments have shown that bile secretion is increased by it.

# 4.

- a. Fall in blood pressure.
- b. variable rate and variable depth of respiration,
- c. Acceleration of the heart.
- d. Marked acceleration of bile secretion.

Tentative conclusions.

Each of the four chemicals produce disturbances of the heat regulating mechanism.

Since bile secretion is stimulated by each of the drugs would indicate that this is the likely channel for their excretion.

Chemical # 3 is decidedly the most toxic; fewer experiments were made with it since it invariably proved fatal. Chemical # 2 is the next most toxic and then follow # 1 and # 4, respectively.

The relative absorbability of these chemicals needs further investigation. The prolonged duration of the symptoms was taken to indicate a probable slow absorption or delayed excretion of the chemicals.

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ASSISTANT TECHNICAL DIRECTOR  
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SOME EFFECTS OF TWO PHTHALIC ACID ESTERS ON THE LIFE  
CYCLE OF THE MIDGE (Chironomus plumosus)



A Thesis

Presented to

The Faculty of the Graduate School  
University of Missouri - Columbia



In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science

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HASKELL LABORATORY

by

Jonathan M. Streufert

December 1977

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John R. Jones

Thesis Supervisor

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## INTRODUCTION

Phthalic acid esters (PAEs) are organic chemicals that have caused environmental concern because of the variety of their application and annual production rates (Great Lakes Water Quality Board 1975). PAEs are the esters of the ortho form of benzenedicarboxylic acid. About 95% of the PAEs produced are used as plasticizers, primarily of polyvinylchloride (U.S. Tariff Commission 1974). As such, they lend flexibility and extensibility to the original resin, which may contain up to 60 parts per hundred of these PAEs in the final formulation (Nematollahi et al., 1967). Two PAE plasticizers of considerable interest are di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DBP). DEHP has been used as an orchard acaricide, while DBP has been used as an insect repellent (Farm Chemicals 1977) and in pesticide formulations to retard volatilization (Brooks and School 1964). Total phthalate anhydride ester production in the United States for 1972 was  $519.8 \times 10^6$  kg of which DEHP accounted for  $197.5 \times 10^6$  kg and DBP amounted to  $13.2 \times 10^6$  kg (U.S. Tariff Commission 1974). Projected annual PAE production for 1981 is  $705 \times 10^6$  kg (Chemical and Engineering News 1976).

Phthalate esters have been reported by several sources to occur in many segments of the environment. PAE residues have been detected in fish, water, and sediments (Table 1).

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Table 1. Locations and types of PAE contaminated samples.

Location	Sample Type	DBP (ng/g)	DEHP (ng/g)	PAE (ng/g)	Source
Mississippi River, mouth	Water	-	600	-	Corcoran 1973
Gulf of Mexico	Water	-	Detectable	-	Ibid.
Great Lakes Area	Waste Treatment Effluent	-	-	1-1200	Gt. Lks. Water Qual. Bd. 1975
	Sewage	-	884,000	-	Ibid.
	Fishes	-	-	0-1300	Ibid.
Black Bay, L. Superior Ontario	Water	-	300	-	Mayer et al. 1972
	Sediment	100	200	-	Ibid.
	Walleye	-	800	-	Ibid.
Missouri River, McBaino, Missouri	Water	0.09	4.9	-	Ibid.
Mississippi and Arkansas Indust. and Agric. area)	Channel Catfish	-	3200	-	Ibid.
Lake Huron, Michigan	Water	-	5.0	-	Ibid.
Iowa Fish Hatchery	Channel Catfish	200	400	-	Ibid.
	Dragonfly, Naiad	200	200	-	Ibid.
	Tadpoles	500	300	-	Ibid.
---	Commercial Fish Food	-	2000-7000	-	Ibid.
Charles River, Massachusetts	Water	-	-	1.9	Hites 1973
Tama River, Japan	Water	3.14	4.4	-	Morita et al. 1974
	Sediment	350	-	-	Ibid.

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Streams and effluents in the Great Lakes Region had PAE concentrations ranging from 1 to 1200 µg/l, while sewage sludge and fishes contained up to 884,000 and 1300 ng/g (dry wt), respectively (Great Lakes Water Quality Board 1975). Mayer et al. (1972) found DEHP concentrations of 300 µg/l in water, 3200 ng/g in fishes, 200 ng/g in invertebrates, and 200 ng/g in sediment. DBP concentrations as high as 100 ng/g were found in sediment and up to 500 ng/g in tadpoles.

Sources of PAEs are most likely municipal and industrial effluents (Hites 1973; Lake Michigan Toxic Substances Committee 1974). Monitoring surveys by several agencies in the Great Lakes states showed that effluents of industrial and municipal waste treatment facilities contained PAEs in concentrations ranging from less than 1 to 1200 µg/l and tributaries to Lake Michigan contained 1 µg/l or less (Great Lakes Water Quality Board 1975). The fate of PAEs discharged into these tributaries is not well defined, but analyses of settleable solids showed residues ranging from 1 to 75 µg/g (dry wt), which suggests that PAEs may be adsorbed to particulate matter in streams and ultimately deposited in bottom sediments.

Data on toxicity of PAEs to aquatic organisms are limited, but there is some indication that acute toxicity of DEHP and DBP is low. The 96-h LC50s of DBP to two invertebrates and four fishes ranged from 0.73 mg/l for the bluegill (Lepomis macrochirus) to > 10.0 mg/l for the crayfish (Orconectes nais). The 96-h LC50 for DEHP was above 10.0

mg/l for all organisms tested (Mayer and Sanders 1973). Less information is available concerning chronic effects of DEHP and DBP on aquatic organisms. Mayer and Sanders (1973) reported that dietary exposure of zebrafish (Brachydanio rerio) to DEHP could reduce fry survival. These authors also noted that a DEHP concentration of 3 µg/l caused a 60% decrease in the production of Daphnia magna.

Direct effects of DEHP on aquatic organisms are not the only interaction to be considered. An aquatic contaminant accumulated by organisms near the base of the food chain may indirectly affect organisms higher in the food chain. Metcalf et al. (1973) found that invertebrates accumulated DEHP several thousand times that of the water concentration to which they were exposed. Mayer and Sanders (1973) also observed rapid initial uptake of DEHP and DBP by aquatic invertebrates, but noted that PAE residues were lost rapidly when the organisms were transferred to uncontaminated water.

The lack of aquatic toxicity data on PAEs, their possible reproductive impairment of certain aquatic species, and their accumulation by other species indicates a need for further examination of their effects on aquatic organisms. One organism important to the diets of many fishes is the aquatic larva of the midge, Chironomus plumosus. The objectives of this study were to determine the acute toxicities of DEHP and DBP to midge larvae; to determine the effects of these chemicals on midge emergence and reproduction; to determine the potential of the larvae to accumulate these PAEs; and to develop a method for using hydrosol in the

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chronic exposure of benthic macroinvertebrates to aquatic contaminants. Because PAEs have been found at relatively high concentrations in hydrosol, it was desirable to examine the effect of sand and hydrosol substrates on the interactions between the PAEs and midges.

## MATERIALS AND METHODS

### Test organism and water quality

Larvae and egg cases of the midge Chironomus plumosus were obtained from cultures maintained at the CNFRL, Columbia, Mo. Rearing techniques described by Biever (1965) were used to maintain a continuously reproducing population of midges. Water used for cultures and all toxicity tests was from a deep well. The water had a pH of 7.5, a hardness of 272 mg/l as CaCO<sub>3</sub> and alkalinity of 237 mg/l. Other chemical characteristics of this water have been summarized by Mayer et al. (1975). Dissolved oxygen (DO) concentrations of inflow water were measured weekly and averaged 8.5 mg/l; DO levels in chronic test chambers were maintained above 2.0 mg/l. All toxicity tests were conducted at 22 ± 1 C in a photoperiod controlled for 16 h light and 8 h dark.

### Chemicals tested

The following phthalate compounds and metabolites were tested: DEHP, DBP, mono-2-ethylhexyl phthalate (MEHP), phthalic acid (PA), and 2-ethylhexanol (2-EH). DEHP, 2-EH, and PA were provided by Monsanto Chemical Co., St. Louis, Missouri. DBP was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. The <sup>14</sup>C-ring-labelled DEHP (specific activity 10.52 mCi/mM = 55 dpm/ng) used in residue dynamic studies was purchased from Pathfinder Laboratories Inc.,

St. Louis, Missouri. DDT (1,1,1-Trichloro-2,2-bis(p-chlorophenyl)-ethane) was from City Chemical Corp., New York, N.Y., and it was used as a base line chemical for both acute and chronic tests. Purities of all chemicals were over 90%.

Ethanol and acetone were used as solvents to increase the solubility of the chemicals. Solvent concentrations never exceeded 1.8 ml/l in acute tests, a level which did not affect larval survival, and 0.12 ml/l in chronic exposures. Solvent limits suggested by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) are 0.5 ml/l for acute and 0.1 ml/l for flow-through tests. It was necessary to exceed these recommendations somewhat due to the insolubility of the PAEs tested.

#### Hydrosoil

Hydrosoil used as larval substrate in the chronic tests and in the <sup>14</sup>C-DEHP uptake tests was taken from Little Dixie Reservoir, Callaway County, Mo. This is a typical Midwestern hardwater reservoir similar in water chemistry to those described by Jones (1977). The hydrosoil was obtained from a depth of 1.0 to 1.5 m with an Ekman dredge and only the top three cm of each sample were used. The sediment was oven-dried at 37 C and ground to a powder.

Hydrosoil used in initial range-finding tests was taken from 0.1 ha ponds at the CNFRL and prepared in the manner described.

Eight soil samples from Little Dixie Reservoir and

three from the CNFRD ponds were analyzed for organic matter, pH, Ca, and other chemical parameters relevant to the interactions between midge larvae, the PAEs, and the substrate. The results of these analyses are presented in Table 2. Soil chemistry tests were conducted by the School of Agronomy in the College of Agriculture, University of Missouri - Columbia, according to methods described by Brown et al. (1977). The chemical parameters of the Little Dixie hydro-soil indicate a close similarity to and derivation from the surface soils of surrounding farms in the reservoir's watershed (Brown, personal communication). The cation exchange capacity (CEC) of this soil is intermediate between the CECs of kaolinite and montmorillonite clays (Weber and Coble 1968).

#### Acute toxicity tests

Acute toxicity tests were conducted according to procedures recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). In static tests 10 late-third and early-fourth instar larvae were exposed to concentrations of phthalate compounds for 48 h in 250 ml of solution in glass jars. The dead and affected larvae were recorded at 24 and 48 h. Defining larval mortality was difficult and death was recorded when larvae became turgid, turned pale, or no longer responded to touch. Other investigators have used similar criteria (Augenfeld 1967; Karnak and Collins 1974). Affected larvae were defined as those unable to make coordinated swimming motions when touched

Table 2. Chemical characteristics of hydrosols used in chronic and uptake studies.

Soil Source		kg P <sub>2</sub> O <sub>5</sub> /ha		OM*	pH <sub>w</sub>	me H <sup>+</sup> /100g	me Ca /100g	me Mg /100g	me P /100g	me Na /100g
	n	P-I	P-II	(%)						
Little Dixie Reservoir	2	18.6** (4.14)	178 (21)	1.6 (0.14)	7.3 (0.04)	0.2 (0.14)	11.3 (0.74)	1.9 (0.14)	0.54 (0.03)	0.25 (0.01)
CHFRL Ponds	4	63.9 (1.65)	183 (8)	3.0 (0.10)	7.0 (0.05)	1.5 (0.30)	15.1 (0.25)	2.6 (0.05)	0.44 (0.02)	0.22 (0.01)

\*Organic matter (dry weight basis).

\*\* $\bar{x} \pm SE$ .

(Sanders, personal communication). Mortality data were plotted on log-arithmetic probability paper and the 48-h EC50s, LC50s, and 95% confidence limits were calculated by the Hitchfield and Wilcoxon (1949) method. EC50s are those concentrations which immobilize or affect 50% of the organisms, while LC50s are those concentrations lethal to 50% of the organisms.

Second instar (96 h old) larvae were also exposed in static tests with DEHP and DBP to determine if toxicities of the chemicals changed with age of the larvae. These tests were conducted as described above, except that exposure was in 10 ml of solution in the cells of commercial jelly trays.

Acute tests were also performed with the base line chemical DDT.

#### Chronic tests

The methods for chronically exposing midge larvae to phthalate compounds followed the procedures recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Chronic tests were conducted by exposing 100 first instar larvae to the chemicals in flow-through systems modified after Mount and Brungs (1967) and Chandler et al. (1974). Exposure chambers measured 10 x 20 x 20 cm with screened drain pipes adjusted so that each chamber contained 2 l of solution. The containers were made from glass cemented together with Dow-Corning 78i building sealant. Chemical stock solutions in stock bottles drained approximately 1 ml of solution into mixing chambers. The

dilution apparatus delivered an additional 9 ml of well water to each mixing chamber. The resulting 1:10 diluted solutions were delivered to the chambers every 7.5 min, which resulted in a turnover rate of approximately one volume per day.

Solvent concentrations in control chambers equalled the highest solvent concentration used in test chambers. DO levels were monitored weekly during the tests to determine proper feeding rates. The larvae were fed 0.12 g of Hartz Mountain Dog Kisses<sup>®</sup> every four days for the first 12 days and 0.15 g every four days thereafter up through day 24. Substrate for the larvae was either 200 g (dry wt) of hydrosoil from Little Dixie Reservoir or 200 g of sand.

#### Effects of DEHP and DBP on midge emergence

Effects of the PAEs on the emergence of adult midges were determined by exposing 100 first instar larvae to selected concentrations of DEHP and DBP. Pupation and emergence started about day 15 and continued for 20 to 25 days. Tests were terminated when no exuviae were present in any of the eight exposure chambers for two consecutive days following the onset of emergence. The exuviae were removed and recorded daily. The effects of PAEs on midge emergence were determined by conducting analyses of variance on the arcsin transformation for proportions ( $\text{angle} = \arcsin \sqrt{\text{proportion}}$ ) followed by a least significant difference test (Snedecor and Cochran 1974). Significance in these analyses and throughout this study were taken at the  $P = 0.05$  level.

As in the acute studies, base line tests were performed using DDT.

#### Effects of DEHP on midge reproduction

Effects of DEHP on the reproductive aspects of the midge life cycle were examined to determine if the chemical affected either the hatchability of eggs produced by PAE-exposed adults or the average number of eggs in each egg case. Screened covers, consisting of two 7 x 22 cm pieces of glass connected by a 8 x 22 cm piece of netting, were placed over exposure chambers at day 16. They allowed for DO determinations and food introduction at appropriate intervals. Egg cases were laid by emerging adults that had been exposed to DEHP solutions. The egg cases were removed daily and placed in separate exposure containers. Within six days all viable eggs had hatched. It was not determined whether the failure of eggs to hatch was due to non-mating or to a chemical effect. Some virgin female midges raised alone laid normal appearing egg cases, a fact also noted by Hilsenhoff (1965). Only those egg cases which hatched were used for calculating hatchabilities and average numbers of eggs per egg case. After six days the unhatched eggs were counted.

Larvae hatching from the tests were further exposed to DEHP. The experimental design was previously described and only the emergence effects were investigated.

#### Determination of chronic concentrations

The chemical concentration delivered to each chamber in

chronic tests was calculated from the amounts of stock solution and water delivered within three day time intervals, from day 0 to day 3, then for day 2 to day 5, and so on until the last day of the test. The calculations were performed using the formula:

$$\text{Concentration} = \frac{(\text{Liters of stock delivered}) (\text{Stock Conc.})}{(\text{Liters stock delivered}) + (T) (K_{\text{cell}})}$$

where T is the number of times the diluter delivered water to the mixing chamber and  $K_{\text{cell}}$  is the average number of milliliters delivered to a particular mixing chamber. The resulting concentrations for a chamber were averaged to obtain calculated toxicant and solvent concentrations. Toxicant concentrations were measured once during the DEHP and DDT chronic tests, as recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

In the DEHP tests triplicate 40 ml aliquots from the highest PAE concentration chamber were extracted according to the method of Johnson (1977). Triplicate 10 ml aliquots of methylene chloride were used to extract the DEHP from the 40 ml. The methylene chloride was evaporated to 3 to 5 ml, 5 ml of isooctane added, and this was then evaporated to about 4 ml. The isooctane was brought up to 5 or 10 ml by the addition of petroleum ether. DDT measurements were taken from one high, one medium, and one low concentration test chamber and extracted in the manner previously described.

Gas chromatograph analyses were performed using a Packard 803 oven. Column length was 180 cm with an internal diameter of 2 mm. It was packed with Chromasorb W-HP 80 to

100 mesh Corning glass beads coated with 1% OV-7. Column temperature was 260 C at the inlet, 280 C at the outlet, and 235 C at the detector. The detector was a  $^{63}\text{Ni}$  electron capture cell. The flow rate of the  $\text{N}_2$  carrier gas was 30 ml/min. DEHP's retention time was about 27 min at a chart speed of 8 mm/min, and its detection limit was 100 ng/l (Whitener, personal communication). Concentrations were determined by comparison against known standards.

By correcting for extraction efficiency the concentrations in the 40 ml aliquots could be derived. Dividing the average measured concentration by the calculated concentration for the preceding three day period, correction factors (CFs) were obtained (Table 3). The CF for a particular test was then used to adjust downward the calculated values.

Extraction efficiencies were determined using radio-labelled DEHP and DDT. Spiked samples were extracted according to the aforementioned method but using toluene instead of isooctane. Radiometric analyses determined an extraction efficiency of 0.72 for DEHP and 0.94 for DDT.

#### Extraction of DEHP from hydrosol

DEHP extractions were performed according to the method of Hesselberg and Johnson (1972) on triplicate 20 g samples of wet hydrosol which had been decanted for 30 minutes. Eighty grams of  $\text{Na}_2\text{SO}_4$  were thoroughly mixed with each sample and packed into an extraction column. Four 25 ml aliquots of diethyl ether were used to extract the DEHP from the hydrosol. After evaporation of the diethyl

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THE EFFECTS OF PHTHALIC ACID ESTERS ON MICROBIAL  
PROCESSES AND THE BIOGEOCHEMICAL CYCLING OF  
NUTRIENTS IN FRESHWATER HYDROSOIL

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Presented to  
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## Chapter I

### INTRODUCTION

During the past several decades, the production and distribution of synthetic organic chemicals has expanded rapidly (U.S. Tariff Commission, 1974). These chemicals are used as pesticides, herbicides, and in the manufacture of substances used in the medical, automotive, and construction industries (Graham, 1973). Subsequently, it is not surprising that the occurrence of contaminants have been found in the aquatic environment (Katz, et al., 1970). Several methods have been developed to determine the acute and chronic effects of these contaminants on aquatic organisms (Committee on Aquatic Bioassays, 1971; Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975). In conjunction with these tests, biodegradation studies are routinely conducted to determine the persistency of environmental contaminants (Swisher, 1970; Alexander, 1965).

Toxicity tests and biodegradation studies do not provide important information, such as: do contaminants that persist in sediments for long periods of time affect the microbial communities associated with those sediments; do persistent chemicals affect the biogeochemical cycling of nutrients occurring in hydrosols? These questions should

be routinely asked in the research and development of new compounds. It is imperative that man determine what ecological changes chemicals may produce, which changes are permanent or temporary, and decide which changes are acceptable (Kaufman and Plimmer, 1972).

The important role of microorganisms in cyclic processes (nitrogen cycle, carbon cycle, sulfur cycle, etc.), essential to continued functioning of ecosystems, demands careful study of the effects of new compounds to avoid permanent disruption of these processes (Kaufman and Plimmer, 1972). Newman and Downing (1958) discuss some of the effects of phenoxyacetic herbicides on nitrification, ammonification, and carbon dioxide evolution. Some of the effects of these herbicides on the above processes were only temporary. Domsch (1970) suggests that the ecological significance of temporary changes in the composition of microbial populations can only be judged when the roles of groups of microorganisms within the ecosystem are properly identified and quantified.

Therefore, the purpose of this study was to determine what ecological effects di-2-ethylhexyl phthalate (DEHP) and its degradation products, 2-ethylhexanol (2-EH) and phthalic acid (PA) have on the resident microbial processes and the biogeochemical cycling of nutrients associated with freshwater hydrosol. The kinds and numbers of physiological groups of microorganisms were monitored to determine whether changes occurred due to the presence DEHP. A secondary

objective was to develop a method to follow population  
shifts of specific physiological groups of microorganisms in  
an aquatic microcosm.

## Chapter II

### LITERATURE REVIEW

The term phthalate ester is restricted to the ortho form of benzenedicarboxylic acid prepared by the reaction of phthalic acid with a specific alcohol to form the desired ester (Autian, 1973). They are colorless liquids, have high boiling points, low volatility, and are lipophilic (Graham, 1973). Phthalic acid esters (PAE's) are used in clothing, medical, home furnishings, automotive, and construction industries (U.S. Tariff Commission, 1974). Their chief use is as plasticizers with vinyl chloride polymers which can contain up to 60% phthalate plasticizer (Graham, 1973).

It is estimated that the total production of 20 different phthalates produced in 1973 was  $450 \times 10^6$  kg (Mathur, 1974). Of these, di-2-ethylhexyl phthalate (DEHP) accounted for 39% of the market and di-n-butyl phthalate comprised about 2% (Johnson, et al., 1977). It is estimated that the demand for plasticizers, mainly phthalate esters, will grow from  $7.9 \times 10^8$  kg in 1976 to  $1.1 \times 10^9$  kg in 1981 (Chemical and Eng. News, 1976).

Due to the large quantities of PAE's produced each year, and the many ways in which they are used, presence in the environment is not unexpected. Mayer, Stalling, and

Johnson (1972) reported the occurrence of PAE's in fish, aquatic invertebrates, and water samples from North America (Table 1). Corcoran (1973) found a concentration of DEHP in the Mississippi River near the Gulf of Mexico of about 600  $\mu\text{g}/\text{l}$  and Hites (1973) found concentrations of PAE's in the Charles River, Massachusetts, ranging from 0.89  $\mu\text{g}/\text{l}$  to 1.9  $\mu\text{g}/\text{l}$ . Giam, et al. (1978) found water concentrations of DEHP in the Gulf of Mexico ranging from 6  $\mu\text{g}/\text{l}$  to 316  $\mu\text{g}/\text{l}$ . Ogner and Shnitzer (1970) have proposed a probable mechanism for the transport of phthalate esters in the environment as a water soluble complex of fulvic acid, a component of humic substances in soil.

With the presence of phthalates in the environment confirmed, questions arose concerning their effects on aquatic organisms. Mayer and Sanders (1973) reported that acute toxicities of PAE's were low to aquatic organisms. Mayer, et al. (1973) reported that scud (Grammarus pseudolimnaeus) accumulated di-n-butyl phthalate (DBP) and DEHP at 6,700 and 13,000 times the water concentration (0.1  $\mu\text{g}/\text{l}$ ) within 14 days. This study indicated that the compounds may be detrimental to aquatic organisms at low chronic concentrations. Mayer and Sanders (1973) reported that DEHP decreased production of daphnids (Daphnia magna) 60% at 3  $\mu\text{g}/\text{l}$ , 70% at 10  $\mu\text{g}/\text{l}$ , and 83% at 30  $\mu\text{g}/\text{l}$ . Mayer, et al. (1972) showed that DEHP was readily accumulated by fathead minnows (Pimephales promelas) exposed continuously to water

Table 1

Phthalate Ester Residues Found in Selected Samples from North America (after Mayer, et al., 1972)

Source	Sample type	DBP (ng/g)	DEHP (ng/g)
Mississippi and Arkansas (Industrial and Agricultural area)	Channel catfish	trace	3200
Fairport National Fish Hatchery, Iowa	Channell catfish	200	400
	Dragonfly niads	200	200
	Tadpoles	500	300
Black Bay, Lake Superior, Ontario	Walleye	-	800
	Water	-	300
	Sediment	100	200
Hammond Bay, Lake Huron, Michigan	Water	.040	-
Lake Huron, Michigan	Water	-	5.0
Missouri River, McBaine, Missouri	Water	.09	4.9

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concentrations of 2.5 µg/l phthalate. Metcalf, et al. (1973) studied the uptake of DEHP in organisms within a model ecosystem and Johnson, et al. (1977) studied the fate of DEHP in a modular food chain.

The biodegradability of PAE's have been studied by several investigators. Saeger and Tucker (1973) demonstrated that all PAE's tested underwent complete aerobic degradation in activated sludge and river water. Johnson and Lulves (1975) found marked differences between the conditions and the rates of degradation of DBP and DEHP in hydrosol shake-flask studies. Within 24 h, under aerobic conditions, 46% of the DBP was degraded to mono-n-butyl phthalate and nearly 98% of the radioactivity disappeared after 5 days. DEHP was completely resistant to microbial attack under anaerobic conditions.

Questions remain regarding the effects of PAE's on microbial processes and whether or not their presence affects the cycling of nutrients in hydrosol communities. Past studies, such as Tu (1970) and Walker, Seesman, and Colwell (1975) have investigated the effects of organophosphate insecticides and crude oil on soils and estuarine sediments, respectively. They also found oil to be toxic to proteolytic, chitinolytic, and cellulolytic microorganisms of estuarine sediments.

This study was conducted using laboratory hydrosol microcosms to expose the sediments to test compounds. The

use of microcosms as a tool in ecological research has been discussed and demonstrated (Draggan, 1976; Taub, 1976). Hargrave (1975) has shown that physical stratification and metabolic processes within a sediment-water interface rapidly stabilize to steady-state levels after disturbance.

This study is an attempt at developing a method of monitoring the effects of PAE's on microbial processes in hydrosol microcosms and whether or not their presence affects the cycling of nutrients in hydrosol communities.

## Chapter III

### METHODS AND MATERIALS

#### HYDROSOIL SAMPLING

Hydrosoil samples used in this study were taken from Little Dixie Lake, an 83 ha impoundment located in an agricultural watershed in Callaway County, 16 km east of Columbia, MO. Chemical characteristics within the reservoir are similar to other surface waters in central Missouri (Jones, 1977). Alkalinity is entirely bicarbonate with values averaging 50 mg/l (Heman, Campbell, and Redman, 1969). Hydrosoil samples were collected from the littoral zone (approximately 1.5 m) by using an Eckman dredge. Only the upper 3 cm of sediment was used as this is where the highest microbial activity occurs (Hayes and Anthony, 1959). Chemical characteristics of the hydrosoil were analyzed by the College of Agriculture, Department of Agronomy, University of Missouri-Columbia (Table 2).

The hydrosoil in Little Dixie Lake was chosen because the lake is situated in an agricultural watershed typical of those receiving runoff from pesticide treated crops. This choice was supported by the fact that the chemical characteristics of the sediment (Table 2) was

Table 2

Chemical Characteristics of Little Dixie Lake Hydrosol Used in  
all Microcosm and Shake-flask Experiments

kg P <sub>2</sub> O <sub>5</sub> /ha		OM*	pH <sub>w</sub>	me H <sup>+</sup>	me Ca	me Mg	me K	me Na
P-I	P-II	(%)		/100g	/100g	/100g	/100g	/100g
18.6	178	1.6	7.3	0.2	11.3	1.9	0.54	0.15

\*Organic matter (dry weight basis).

identical to the chemical characteristics of the average Callaway County farm soil (Brown, personal communication). This indicates that the chemical make-up of the soil changes very little upon entering the lake.

All laboratory analyses were conducted in the Microbiology Laboratory of the Columbia National Fishery Research Laboratory, Columbia, MO. This laboratory is run by the Fish and Wildlife Service, U.S. Department of the Interior, for the purpose of conducting toxicological research of agricultural and industrial chemicals on aquatic organisms.

Phthalate standards used in this study were provided by Monsanto Chemical Co. of St. Louis, MO.

#### WATER CHEMISTRY

Alkalinity values for pond water from the Columbia National Fishery Research Laboratory averaged 4.0 mg/l  $\text{OH}^-$  and 187.0 mg/l as  $\text{CaCO}_3$ . Hardness averaged 200.0 mg/l  $\text{CaCO}_3$  and specific conductance 500  $\mu\text{mhos cm}^{-1}$ .

After the pond water was autoclaved the alkalinity values averaged 6.6 mg/l as  $\text{OH}^-$  and 120 mg/l as  $\text{CaCO}_3$ . Total hardness decreased to 127.0 mg/l  $\text{CaCO}_3$  and specific conductance decreased to 360  $\mu\text{mhos cm}^{-1}$ .

#### MICROCOSMS

To test the effects of phthalates on the physiological activity of microorganisms in hydrosol, microcosms were

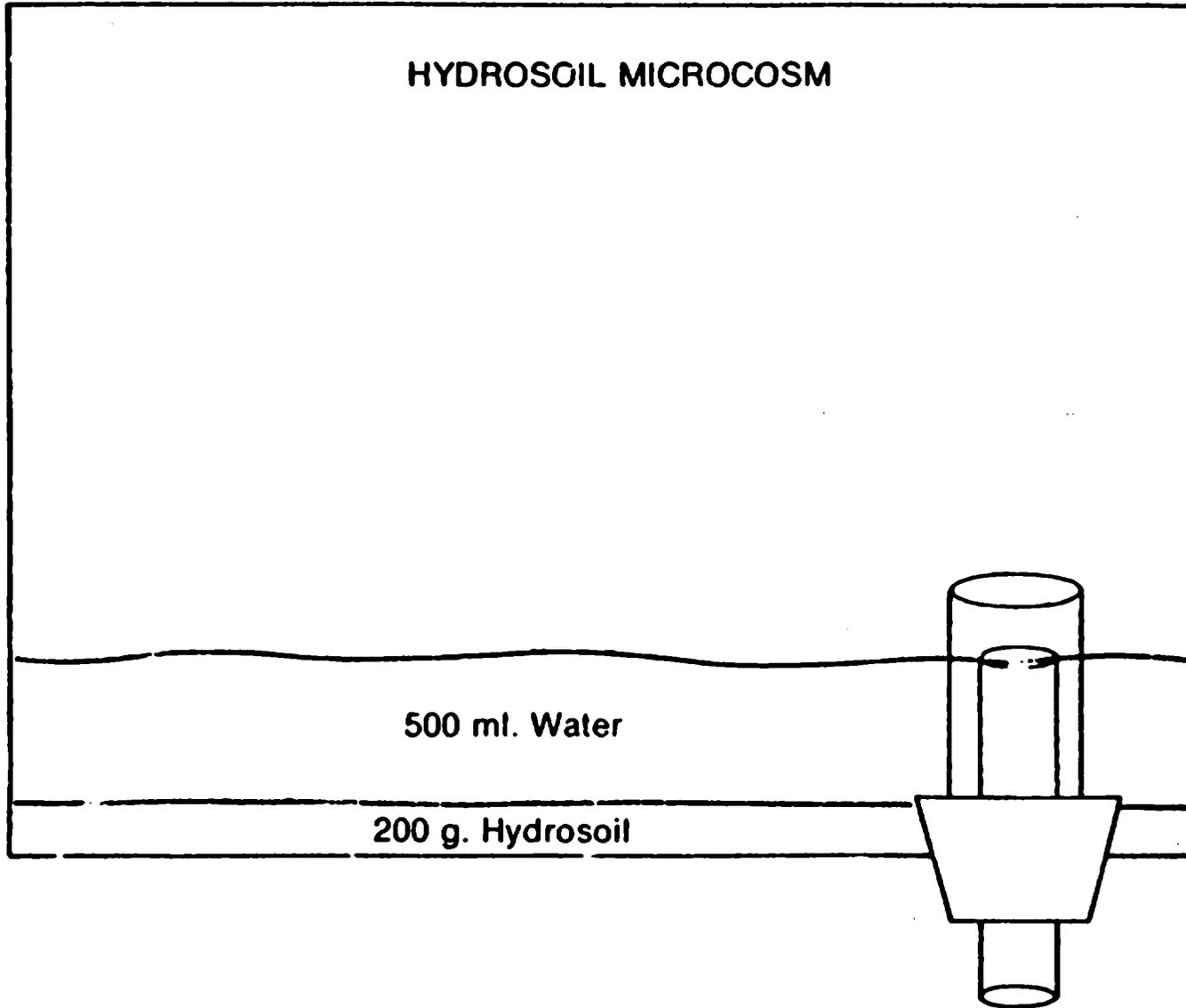
constructed using 7.1 l glass aquaria (Figure 1). Each aquarium was equipped with a drain pipe. The drain pipes were fitted with glass sleeves notched on the bottom to allow for drainage. The sleeve extended above the surface of the water and prevented water added to the aquarium from moving along the surface and out the drain without adequate mixing. The drain pipe was adjusted to maintain a constant volume of 500 ml. To each microcosm, 200 g of hydrosol and 500 ml of sterilized pond water were added 3 days before the beginning of an experiment. All microcosms were maintained under constant light and 22 C. Each experiment consisted of 3 treated aquaria, an acetone solvent control, and a water control. During the 17-day experiments, phthalate compounds were added daily in 500 ml of sterilized pond water for 14 days. On the last consecutive 3 days 500 ml of phthalate-free water was added to the microcosms to determine the effect of phthalate removal on physiological processes in the sediments.

Microcosms were sampled on days 0, 1, 7, 14 and 17 of an experiment. Sampling involved using a spatula to collect at random a composite 5 g sample from within each microcosm. This method sampled the entire depth of the sediment layer, approximately 2 cm.

The effects of phthalates on microbial physiological processes were estimated from physiological profiles of the hydrosol. Differential media was used in conjunction with

Figure 1. A diagram of a hydrosol microcosm used in all flow-through studies.

HYDROSOIL MICROCOSM



500 ml. Water

200 g. Hydrosoil

the Most-Probable-Number technique of Cochran (1950) to determine the kinds of physiological groups present and the number of organisms in each group. The processes monitored are given in Table 3.

After collection, the composite 5 g samples from each microcosm were diluted in 45 ml of sterilized pond water to prevent osmotic shock. A seven-fold serial dilution was made of each sample. One-tenth ml aliquots of each dilution from  $10^{-3}$  to  $10^{-7}$  were placed in triplicate test tubes. Each sample dilution was inoculated onto all 6 media (Table 3) in the same manner. After 96 h incubation at 20 C, the tubes were examined. Reagents specific for each medium were used to determine the number of organisms performing a given function (Table 3). Only those tubes yielding a positive reaction after the addition of the reagent were considered positive, even though microbial growth may have been seen at greater dilutions.

Using the three tube Most-Probable-Number technique, it is necessary to determine the highest dilution at which all three triplicate tubes are positive. One then determines the number of positive tubes in each of the following two dilutions. From this a significant number is derived. Significant numbers are three digit numbers corresponding to the number of positive tubes in three sequential dilutions. Once a significant number is obtained the most probable number of organisms can be determined by referring to a MPN

Table 3

**List of Media and Reagents Used in Profiling  
Physiological Groups of  
Hydrosoil Microflora**

Process Monitored	Medium	Reagent
Protein digestion	Frazier Gelatin Agar	Frazier Gelatin Developer (Pelczar and Chan, 1972)
Ammonification	1% Peptone	Nessler's Reagent (Lord, 1962)
Starch activity	Starch Agar	Gram's Iodine (Lord, 1962)
Hydrogen sulfide	Iron-Peptone Agar	None
Denitrification	Indole Nitrite Broth	Acetic Acid 2-naphthylamine (Pelczar and Chan, 1972)
Total heterotroph	Casein-Peptone- Starch Agar (Jones, 1970)	None

table (Finstain, 1972). The exponent is derived from the highest dilution containing three positive tubes. Each number from an MPN table has 95% confidence limits associated with it.

#### NUTRIENT CYCLING

Shake-flask studies were conducted to determine whether phthalate esters affected the ability of hydrosol microflora to carry out essential geochemical processes. Three specific processes were investigated: ammonification, nitrification, and sulfur oxidation. Studies were done in 125 ml Erlenmeyer flasks containing 10 g of hydrosol and 40 ml of sterilized pond water. One mg/l concentrations of DEHP, 2-ethylhexanol, and phthalic acid were tested in triplicate with an acetone solvent control and a water control. Tests were run for 7 days on a rotary shaker with samples taken on days 1 and 7.

Ammonification was monitored by following the conversion of 100 mg of Bacto Peptone to ammonia by using an Orion specific ion ammonia electrode (Orion Research Inc.) and a Corning pH meter (Model #12). Samples were removed from the shaker and allowed to settle for approximately 30 minutes. The overlying water was decanted into 50 ml centrifuge tubes and centrifuged for 20 min. at 10,000 revolutions per minute. The overlying water was poured into 100 ml beakers and analyzed following the procedure outlined in the electrode instruction manual (Orion Research Inc.).

Sulfur oxidation was monitored by following the oxidation of sulfite to sulfate. One mg of sulfite was added to each flask as  $\text{Na}_2\text{SO}_3$ . At the time of sampling, each flask was extracted with 0.2 g  $\text{CaCl}_2$  and allowed to settle. Five ml of the supernatant was filtered through a 0.45  $\mu\text{m}$  Millipore filter and analyzed by using the method of Tabatabai (1974).

Nitrification was monitored by following the conversion of nitrite to nitrate. One mg of nitrite was added to each flask as  $\text{KNO}_2$ . At the time of sampling, each flask was extracted with 0.2 g  $\text{CaSO}_4$ . Each sample was shaken vigorously on a rotary shaker for 10 min. and allowed to settle. A 20 ml aliquot of the supernatant was filtered through a 0.45  $\mu\text{m}$  Millipore filter and analyzed using the phenoldisulfonic acid method of Bremner (1965).

A cadmium reduction column was another method used in an attempt to monitor nitrification (Strickland and Parsons, 1965). Sample preparation was similar to the first method except that the 20 ml aliquot was diluted to 100 ml with distilled water and passed through the column.

#### PURE CULTURE GROWTH CURVES

The growth rate of a pure culture hydrosol isolate was followed to determine whether phthalate compounds inhibited growth. The isolate was obtained from a  $10^{-2}$  hydrosol dilution inoculated onto a Petri dish containing

nutrient agar. Cultures were grown in 50 ml Delong flasks containing a 50% nutrient broth solution. The inoculum was introduced in 0.1 ml of nutrient broth solution. DEHP, 2-ethylhexanol, and phthalate acid were tested at 1 mg/l in triplicate along with an acetone solvent control, a non-manipulated control, and a positive control containing 0.1%  $\text{HgCl}_2$ . All flasks were incubated on a rotary shaker at 22 C and growth was measured every 4 h for 24 h as optical density at 560 nm using a Bausch and Lomb Spectronic 20.

#### DISC SENSITIVITY

Disc sensitivity tests were performed to determine whether phthalates inhibited microorganisms from carrying out certain physiological processes. Discs, approximately 0.5 cm in size, were made from Whatman No. 1 filter paper. The discs were soaked in 1 mg/ml or 1 mg/l solutions of DEHP, 2-ethylhexanol, and phthalic acid, dried, and placed on Petri plates inoculated with 0.1 ml of a  $10^{-3}$  dilution of 1.0 g (wet wt.) hydrosol. Each plate contained 3 discs of the low concentration, an acetone solvent control, and a control disc soaked in 0.1%  $\text{HgCl}_2$ .

A slightly modified Defined medium, as described by Lord (1962), was used in the tests. The glucose recommended in the medium was used and was also substituted with the following nutrients: gelatin, starch, peptone, urea, sulfite (added as  $\text{Na}_2\text{SO}_4$ ), and nitrite (added as  $\text{KNO}_2$ ).

Each medium was run in triplicate. After 48 h the zones of inhibition, around each disc, if any, were measured with a ruler.

#### INCORPORATION OF PHTHALATES INTO THE MEDIUM

In order to delineate whether the ability of phthalates to move through the medium affected the results of the disc sensitivity tests, the compounds tested were incorporated into the medium before pouring the plates. Each medium and compound was run in triplicate. Acetone and 0.1%  $\text{HgCl}_2$  served as controls. After 48 h the plates were examined for microbial growth.

## Chapter IV

### RESULTS AND DISCUSSION

#### NUTRIENT CYCLING

This portion of the study was devoted to following the effects of phthalates on the ability of hydrosol to cycle nutrients. This approach differs from the microcosm studies because nutrient concentrations were followed instead of the associated microflora. Agricultural studies routinely investigate the effects of a pesticide on nutrient cycling in soils (Brown, 1954; Bartha, Lanzilotta, and Pramer, 1967; Tu, 1970). Such studies attempt to determine whether interruption of a step in a cycle may lead to excessively high or low levels of essential nutrients. In aquatic ecosystems, Hasler and Einsele (1948) have shown that high levels of hydrogen sulphide may stimulate the release of phosphate from a ferrous phosphate complex. The phosphates generated may stimulate the growth of algae and cause a reduction in aesthetic or commercial use of that body of water. Also, high levels of ammonia and hydrogen sulphide have been shown to interfere with fish respiration (Trussel, 1972; Smith, et al., 1976).

In order to monitor a given nutrient cycle, a one-step conversion process was followed for each cycle. Due to

low levels of ammonia, sulfate, and nitrate in the hydrosol used in this study, it was necessary to add high levels of intermediary nutrients such as nitrite, sulfite, and peptone. Shake-flask studies were then conducted to determine the effects of 1 mg/l concentrations of DEHP, 2-EH, and PA on sulfur oxidation, ammonification, and nitrification.

The effects of these three compounds on sulfur oxidation are summarized in Table 4. An average of 165  $\mu$ /l of sulfates were produced during the 7-day study in each of the three treated samples and the acetone solvent control. There were no significant differences (one-way ANOVA;  $p \geq 0.05$ ) between the phthalate treated samples and the controls. A 250 mg/l concentration of sodium azide effectively inhibited sulfur oxidation.

The results of the ammonification study are summarized in Table 5. In one day approximately 28 mg/l of ammonium was produced from 100 mg of peptone. By day 7 an average of 83 mg/l of ammonium was detected in all treated samples and the solvent controls. No significant differences (one-way ANOVA;  $p \geq 0.05$ ) were found. Sodium azide effectively inhibited all ammonification (Table 5).

An attempt was made to follow the effects of phthalates on nitrification by following the conversion of nitrite to nitrate. Nitrite was found to be an unsuitable intermediary product to be used because of its spontaneous conversion to nitrate in the presence of oxygen.

Table 4

The Effects of 1 mg/l Concentrations of DEHP, 2-EH, and PA on Sulfur Oxidation in Hydrosoil Shake-flask Studies (values reported are  $\mu\text{g/l}$  of sulfate).

Compound	Days Exposure	
	1	7
DEHP	420 (2.04) *	600 (1.81)
2-EH	460 (2.45)	640 (1.92)
PA	500 (1.43)	660 (1.71)
Acetone Control	540 (2.30)	680 (1.20)
Sodium Azide	0.0	0.0

\*Each value is the mean of three samples with the standard error in parentheses.

Table 5

The Effects of 1 mg/l Concentrations of DEHP, 2-EH,  
and PA on Ammonification in Hydrosoil Shake-  
flask Studies (values reported  
are mg/l of  $\text{NH}_4^+$ )

Compound	Days Exposure	
	1	7
DEHP	28 (0.93)*	84 (1.09)
2-EH	28 (0.85)	82 (0.97)
PA	30 (0.79)	85 (0.84)
Acetone Control	27 (0.89)	82 (1.10)
Sodium Azide	0.0	0.0

\*Each value is the mean of three samples  
with the standard error in parentheses.

In addition, peptone was investigated as an alternative nitrogen source. However, peptone was found to be unsuitable as a nitrate nitrogen source since no detectable levels of nitrates were found after 7 days.

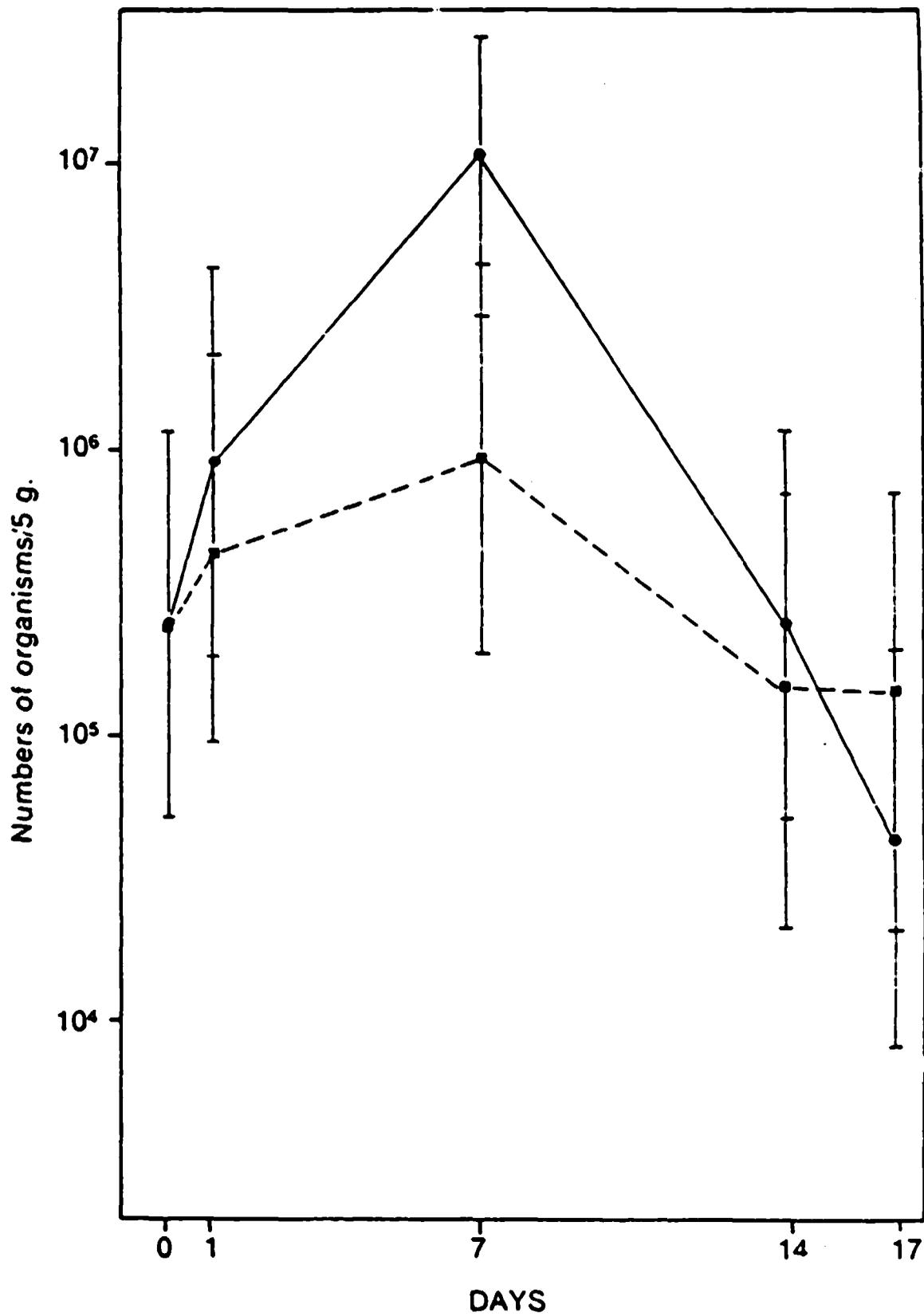
#### MICROCCSMS

One mg/l concentrations of DEHP and 2-EH and a 100 mg/l concentration of 2-EH were tested in the microcosms. The results of the Most-Probable-Number (MPN) test (Cochran, 1950) performed for each experiment are given in Appendix 1. The numbers reported are the most probable number of organisms performing a given function in the microcosm at the time of sampling. Within each microcosm, the results of the MPN's conducted on each sampling day were compared to the preceding days. Comparisons were also made between each treated microcosm and the acetone and water controls.

Although there were wide fluctuations in microbial numbers, none could be attributed to the presence of the compounds. The data in Appendix 1 shows that there were instances in which it appeared that a steady increase or decrease in numbers was occurring, but the large 95% confidence limits associated with the three tube MPN technique made it difficult to obtain statistically significant changes.

Figure 2 is an example of population shifts of ammonifying microorganisms occurring in a microcosm treated

Figure 2. A comparison of population shifts of ammonifying microorganisms occurring in a microcosm treated with 1 mg/l DEHP (broken line) and a control microcosm (solid line) over a 17-day period. The 95% confidence limits are derived from a 3-tube Most-Probable-Number table (Appendix 2).



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with 1 mg/l DEHP and a control microcosm over a 17-day period. From this figure it can be seen that, due to the large confidence limits, almost a 100-fold shift is required before a change can be considered significant. Studies such as Walker, et al. (1975), that report 10-fold changes in microbial numbers as significant, must describe the method of enumeration and report associated confidence limits. Without 95% confidence limits, it is possible to mistake large shifts in numbers as significant, when both extremes may actually fall within the confidence limits of the counting technique used.

The confidence limits of the MPN technique can be narrowed by increasing the number of tubes used per dilution to five or more (Alexander, 1965). Rowe, Todd, and Waide (1977) describe the use of a microtechnique incorporating eight replicates per dilution. As one increases the number of replicates (tubes) used per dilution, the use of microtechnique would greatly reduce the time and expense involved in running an MPN analysis.

Test compounds were added daily to the overlying water, so that exposure of the sediments to those compounds was brought about by natural processes; such as coprecipitation with suspended solids or through some physical-chemical attraction of the molecule for sediment particles (Pionke and Chesters, 1973). In both instances the compound will come in contact with the surface of the sediments

first. Depending on the ability of the compound to exert an effect on microbial populations, the hydrosol surface will be the area where this effect is first noted. The method of sampling the microcosms for MPN analysis mixed a sample of the entire sediment layer. This may have masked any effects on microbial populations at the surface of the sediments. Future studies should consider sampling at several sediment depths to determine whether microbes at the sediment-water interface are more susceptible to contaminants introduced via the water.

To determine if the method of sampling the microcosms was capable of detecting perturbations to hydrosol microflora, a positive chemical control was needed that had antimicrobial properties. A 0.1 mg/l solution of sodium hypochlorite, which was added daily to the microcosms for 7 days, failed to cause any significant reduction in numbers.

Johnson and Lulves (1975) reported that a 250 mg/l solution of sodium azide effectively inhibited microbial activity in hydrosol shake-flask studies. To test the possibility of using sodium azide as a control, 250 mg/l was added to the microcosms daily for 7 days. No reduction in microbial activity was noted. The inhibition of microbial activity using sodium azide was noted by Johnson and Lulves. This inhibition may have been attributable to the shaking of the sediment-water mixture. Active shaking would have ensured maximum exposure of the microorganisms to the

chemical, thereby increasing the chances of the chemical exerting an effect. It may also be possible that the sodium azide was actually inhibiting the microflora in the microcosms, but by removing the sediment sample and placing it in sterilized pond water (used in making the MPN dilution series), activity may have resumed. The method of sampling may have also removed the microbes from exposure to the inhibitory substance. Except for those compounds capable of exerting long-lasting or permanent non-lethal effects on a microorganism, 96 h incubation in media containing no inhibitor may mask any temporary inhibition present in the microcosm.

Some consideration was given to the idea of placing each compound tested in the microcosms in the dilution water used in the MPN analysis. A problem arises here in that this technique exposes the microorganisms to the test compound twice; once in the microcosm, and once in the dilution water. Using this technique, it would be impossible to tell where inhibition was occurring. Inhibition would be more likely to occur in the dilution water, because as a serial dilution is made, the number of sediment particles capable of adsorbing and reducing the availability of the compound would decrease as would the number of microorganisms exposed to a given concentration of inhibitor.

If the test compound had killed a significant portion of the microflora present, the MPN analysis would show a

significant decrease. It is the author's belief that by conducting an MPN analysis without the test compound added to the media or the dilution water, the only effect that could be measured would be a reduction in numbers of microflora.

The results of a profile incorporating sodium azide in the dilution water and one in which the dilution water was free of sodium azide are compared in Table 6. One hydrosoil shake-flask, treated with 250 mg/l sodium azide, was profiled twice as described previously. The results of both treated profiles were compared to a control profile. The treated profile containing sodium azide in the dilution water showed significantly ( $p \geq 0.05$ ) lower populations of starch, protein, and ammonifying organisms than the profile without sodium azide in the dilution water. This indicates that those compounds that have an inhibitory effect on microflora in a hydrosoil system may not produce such an effect in an MPN analysis.

#### PURE CULTURE GROWTH CURVES

Bacterial pure culture growth curves were conducted with an unidentified gram-negative rod to determine if 1 mg/l concentrations of DEHP, 2-EH, and PA could produce an effect on the growth rate of a pure culture isolated from hydrosoil. The results of these tests are summarized in Table 7. The data show that at approximately 12 h the

Table 6

The Effects of Adding Sodium Azide to the  
Dilution Water on the MPN Analysis of a  
Sodium Azide Treated Shake-flask

Activity Monitored	NaN <sub>3</sub> in Dilution Water	NaN <sub>3</sub> Not in Dilution Water
Starch	0	2.5 X 10 <sup>4</sup> *
Protein digestion	0	9.5 X 10 <sup>3</sup>
Denitrification	.9 X 10 <sup>3</sup>	7.5 X 10 <sup>3</sup>
Ammonification	0	9.5 X 10 <sup>3</sup>
Total Heterotroph Numbers	2.5 X 10 <sup>3</sup>	9.5 X 10 <sup>4</sup>

\*Numbers of organisms per gram of hydrosol.

Table 7

The Effects of 1 mg/l Concentrations of DEHP, 2-EH, and PA on a  
Hydrosoil Bacterial Pure Culture Growth Rate (values  
are reported as optical density at 560 nm)

Compound	Time (hours)							
	12		16		20		24	
DEHP	0.001	(0.0008)*	0.07	(0.016)	0.33	(0.022)	0.40	(0.016)
2-EH	0.001	(0.0008)	0.05	(0.021)	0.35	(0.017)	0.39	(0.010)
PA	0.0	--	0.07	(0.011)	0.31	(0.021)	0.41	(0.012)
Acetone Control	0.0	--	0.09	(0.019)	0.33	(0.016)	0.41	(0.021)
Sodium Azide	0.0	--	0.0	--	0.0	--	0.0	--

\*Each value represents the mean of three samples with the standard error in parentheses.

optical density of the treated and solvent control flasks began to increase and continued increasing up to the termination of the study at 24 h. There were no significant differences (one-way ANOVA;  $p \geq 0.05$ ) between the growth rates of the phthalate treated samples and the solvent controls. Sodium azide effectively inhibited all microbial growth during the 24 h test.

#### DISC SENSITIVITY

Filter paper discs, soaked in 1 mg/l and 1000 mg/l solutions of DEHP, 2-EH, and PA were placed on the surface of agar plates to determine whether these compounds inhibited the growth of specific physiological groups of microorganisms. A slightly modified defined medium was used (Lord, 1962). Different medias were made by substituting the glucose called for in the medium with gelatin, starch, peptone, urea, sulfite, and nitrite. Of the above nutrients, sulfite, nitrite, and urea were not capable of supporting growth of hydrosol microflora. Growth was greatest on those plates containing peptone and glucose. The basic medium, without any substrates added, did not support growth. Neither the high (1000 mg/l) or the low (1 mg/l) concentrations of the compounds tested inhibited growth. The control discs, containing 0.1% mercuric chloride, consistently produced zones of inhibition 3-4 mm around the disc.

By varying only one substance in the basic medium, some important variables were eliminated. The use of completely different medias to estimate the sensitivity of microorganisms to phthalate soaked discs may cause differences due to variations in the mobility of the compounds in the various medias. By using only one basic medium, this variable was eliminated. Another important factor to consider is that many differential medias allow more than one physiological group of organisms to grow. By using a medium containing only one substrate capable of supporting microbial growth, a zone of inhibition is proof that those organisms utilizing that substrate were inhibited by the compound contained on the disc.

#### INCORPORATION OF TEST COMPOUNDS INTO MEDIA

To further determine whether the ability of a compound to move through the medium was a factor affecting the results of the discs sensitivity test, the test compounds were incorporated into the medium. The only concentration tested was 1 mg/l, because the 1000 mg/l concentration was insoluble and formed a film on the surface of the medium.

DEHP, 2-EH, and PA, at 1 mg/l, did not inhibit the growth of any physiological group tested. Those plates containing 0.1% mercuric chloride effectively inhibited all growth.

The concentration of phthalates used in this study were chosen so that they exceeded the solubility of the compound in water. No special attempts were made to solubilize them in any hydrosoil system. This allowed concentrations to equilibrate according to the natural limits of the system. Phthalates added to a hydrosoil shake-flask may have achieved greater concentrations by adsorbing to sediment particles through mixing than would similar concentrations in a microcosm. In using concentrations below solubility limits, one would need to be concerned with those processes which tend to lower the actual concentration tested; such as adsorption to containers, sediment particles, and glass. Had effects been noted, a concentration-effect relationship would have been established.

## Chapter V

### SUMMARY

Using the methods developed in this study DEHP, 2-EH, and PA did not interfere with nutrient cycling or microbial processes in hydrosol. Pure culture and disc sensitivity tests showed that the toxicity to soil microorganisms of these compounds was low. This study used a three-tube MPN technique to monitor microbial populations in a hydrosol microcosm. A discussion of some of the problems associated with using an MPN technique to monitor microbial inhibition is presented.

While the MPN technique may not be perfectly suited for monitoring microbial toxicity, it can be used in conjunction with degradation studies to determine the kinds and numbers of organisms present in a sample and to monitor changes during the study. For example, the River Die-Away test (Weaver and Coughlin, 1964) incorporates the use of untreated river water to determine the biodegradability of contaminants. Little is known of the biological content of the river water used in these studies. Microbial populations present in the sample may be transitory, resulting from the movement of a contaminant down the river, therefore, the kinds and numbers of microbes present may vary from study to study (Hynes, 1971). By conducting a

physiological profile of the water used in such a study one can quantify the microorganisms present and can monitor them concomitant with degradation.

Future research in this area should investigate the possibilities of using microcosms to monitor the effects of xenobiotics on geochemical cycles. Microcosms have been shown to be valid ecological tools in studying the effects of pollutants on aquatic ecosystems (Taub, 1976). By adding a stable nutrient such as sulfur, peptone, or some other nitrogenous organic compound, it may be possible to follow an entire cycle within a microcosm. A stable mud-water interface, allowing for the formation of oxidized and reduced zones in the hydrosol, will allow the investigator to follow both oxidizing and reducing segments of a cycle (Hargrave, 1975). The microcosm approach would be ideally suited for the use of specific ion electrodes, making it possible to monitor nutrient levels within the microcosm.

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APPENDIX 1

## APPENDIX 1

Physiological profiles of three 17-day experiments exposing hydrosol microcosms to 1 mg/l concentrations of 2-EH and DEHP and 100 mg/l concentrations of 2-EH. Each experiment consisted of three treated microcosms, an acetone solvent control, and a water control. Values given are the number of organisms per 5 g of hydrosol ( $\times 10^5$ ).

Experiment 1  
1 mg/l 2-EH  
Sept. 25, 1976 to Oct. 11, 1976

Activity Monitored	Days				
	0	1	7	14	17
<u>Microcosm A (Treated 1 mg/l 2-EH)</u>					
Amyolytic	25	140	140	9.5	25
Proteolytic	0.45	0.45	0.25	0.95	0.25
Denitrification	45	110	2.0	15	2.5
Ammonification	25	2.5	0.25	2.5	0.95
Total Heterotrophs	0.15	45	2.5	2.5	2.5
Hydrogen Sulfide Production	0.2	0.075	0.009	0.025	0.002
<u>Microcosm B (Treated 1 mg/l 2-EH)</u>					
Amyolytic	140	0.95	140	30	0.45
Proteolytic	0.95	0.45	0.095	4.5	0.25
Denitrification	45	9.5	1.5	45	4.5
Ammonification	9.5	2.5	0.45	2.5	0.25
Total Heterotrophs	4.5	110	0.95	25	25
Hydrogen Sulfide Production	0.15	0.045	0.095	0.045	0.045
<u>Microcosm C (Treated 1 mg/l 2-EH)</u>					
Amyolytic	140	0.45	140	140	7.5
Proteolytic	2.5	0.45	0.25	0.25	0.45
Denitrification	45	9.5	4.5	20	4.5
Ammonification	9.5	9.5	2.5	0.95	2.5
Total Heterotrophs	4.5	25	4.5	45	11.5
Hydrogen Sulfide Production	0.095	0.025	0.045	0.25	0.025
<u>Microcosm D (Solvent Control)</u>					
Amyolytic	140	4.5	140	25	0.45
Proteolytic	2.5	0.25	0.25	0.45	0.25
Denitrification	25	15	0.45	25	0.95
Ammonification	4.5	4.5	1.5	0.45	0.45
Total Heterotrophs	4.5	140	4.5	4.5	2.5
Hydrogen Sulfide Production	0.025	0.095	0.045	0.025	0.045
<u>Microcosm E (Water Control)</u>					
Amyolytic	140	9.5	140	9.5	0.45
Proteolytic	9.5	0.25	2.5	0.25	2.5
Denitrification	25	45	2.5	4.5	2.5
Ammonification	45	4.5	0.45	2.5	0.45
Total Heterotrophs	4.5	45	2.5	4.5	4.5
Hydrogen Sulfide Production	0.009	0.02	0.025	0.095	0.045

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Experiment 2  
1 mg/l DEHP  
Oct. 25, 1976 to Nov. 12, 1976

Activity Monitored	Days				
	0	1	7	14	17
<b>Microcosm A (Treated 1 mg/l DEHP)</b>					
Amyolytic	0.45	0.95	2.5	2.5	4.5
Proteolytic	0.45	0.25	0.45	1.15	0.45
Denitrification	9.5	4.5	20	25	1.5
Ammonification	9.5	4.5	9.5	0.95	9.5
Total Heterotrophs	4.5	2.0	4.5	2.5	4.5
Hydrogen Sulfide Production	0.0	0.025	0.025	0.0	0.015
<b>Microcosm B (Treated 1 mg/l DEHP)</b>					
Amyolytic	0.45	2.5	1.5	0.95	0.45
Proteolytic	0.45	0.25	0.75	0.25	0.45
Denitrification	0.45	4.5	15	2.5	0.95
Ammonification	7.5	9.5	9.5	4.5	4.5
Total Heterotrophs	2.5	2.5	4.5	2.5	7.5
Hydrogen Sulfide Production	0.045	0.009	0.009	0.0	0.045
<b>Microcosm C (Treated 1 mg/l DEHP)</b>					
Amyolytic	1.5	2.5	2.5	0.45	0.25
Proteolytic	1.5	0.25	2.5	0.45	0.45
Denitrification	0.95	2.5	110	2.5	4.5
Ammonification	2.5	4.5	9.5	1.5	1.5
Total Heterotrophs	2.5	2.5	4.5	0.45	2.5
Hydrogen Sulfide Production	0.0	0.015	0.009	0.0	0.006
<b>Microcosm D (Solvent Control)</b>					
Amyolytic	2.5	0.95	1.5	0.45	1.5
Proteolytic	0.75	0.095	0.75	0.45	0.15
Denitrification	2.5	0.95	15	0.95	0.95
Ammonification	4.5	9.5	7.5	2.5	2.5
Total Heterotrophs	2.5	4.5	7.5	2.5	4.5
Hydrogen Sulfide Production	0.004	0.25	0.009	0.0	0.075
<b>Microcosm E (Water Control)</b>					
Amyolytic	0.95	4.5	7.5	0.45	0.95
Proteolytic	0.45	0.75	15	0.95	0.45
Denitrification	2.5	9.5	25	1.5	2.0
Ammonification	2.5	9.5	110	2.5	0.45
Total Heterotrophs	2.5	9.5	2.5	0.25	2.5
Hydrogen Sulfide Production	0.0	0.115	0.011	0.0	0.009

Experiment 3  
100 mg/l 2-EH  
Nov. 20, 1976 to Dec. 6, 1976

Activity Monitored	Days				
	0	1	7	14	17
<u>Microcosm A (Treated 100 mg/l 2-EH)</u>					
Amyolytic	2.5	0.45	0.45	2.5	2.5
Proteolytic	0.045	0.45	1.5	0.45	1.5
Denitrification	0.95	0.45	9.5	0.95	0.45
Ammonification	4.5	2.5	0.45	0.45	2.5
Total Heterotrophs	0.95	0.95	0.45	0.95	0.95
Hydrogen Sulfide Production	0.009	0.045	0.009	0.009	0.045
<u>Microcosm B (Treated 100 mg/l 2-EH)</u>					
Amyolytic	0.45	0.45	0.45	0.25	0.45
Proteolytic	0.25	0.095	0.045	0.25	0.095
Denitrification	0.45	0.75	25	2.5	0.75
Ammonification	0.30	2.5	2.5	2.5	4.5
Total Heterotrophs	0.75	0.25	0.75	0.75	0.75
Hydrogen Sulfide Production	0.045	0.009	0.025	0.0	0.009
<u>Microcosm C (Treated 100 mg/l 2-EH)</u>					
Amyolytic	0.95	0.45	0.75	0.45	0.75
Proteolytic	0.075	0.25	0.075	0.25	0.25
Denitrification	1.5	0.45	45	4.5	1.5
Ammonification	0.95	2.5	2.5	2.5	0.95
Total Heterotrophs	0.45	0.45	0.25	0.25	0.45
Hydrogen Sulfide Production	0.0	0.0	0.015	0.009	0.015
<u>Microcosm D (Solvent Control)</u>					
Amyolytic	0.25	0.25	0.25	0.45	0.25
Proteolytic	0.045	0.45	0.095	0.25	0.45
Denitrification	0.75	0.20	45	4.5	4.5
Ammonification	0.45	0.95	0.45	0.75	0.45
Total Heterotrophs	0.75	0.45	0.45	0.25	0.25
Hydrogen Sulfide Production	0.0	0.009	0.009	0.009	0.0
<u>Microcosm E (Water Control)</u>					
Amyolytic	0.75	0.45	0.75	0.95	0.25
Proteolytic	0.25	0.45	0.095	0.095	0.25
Denitrification	1.5	2.5	45	2.5	4.5
Ammonification	0.95	0.95	0.95	0.75	0.75
Total Heterotrophs	2.5	0.45	1.15	2.5	4.5
Hydrogen Sulfide Production	0.004	0.0	0.004	0.009	0.004

**APPENDIX 2**

APPENDIX 2

A Table of Most-Probable-Number of Organisms With  
Three Tubes Per Dilution (after Finstein, 1972)

Signi- ficant Number	Probable Number	95% Confidence Limits	Signi- ficant Number	Probable Number	95% Confidence Limits	Signi- ficant Number	Probable Number	95% Confidence Limits
000	0.0		201	1.4	0.30 - 6.6	302	6.5	1.4 - 30
001	0.3	0.064 - 1.1	202	2.0	0.45 - 9.4	310	4.5	0.96 - 21
010	0.3	0.084 - 1.4	210	1.5	0.21 - 7.0	311	7.5	1.6 - 35
011	0.6	0.13 - 2.8	211	2.0	0.43 - 9.4	312	11.5	2.5 - 54
020	0.6	0.13 - 2.8	212	3.0	0.64 - 14	313	16.0	3.4 - 75
100	0.4	0.085 - 1.9	220	2.0	0.43 - 9.4	320	9.5	2.0 - 44
101	0.7	0.15 - 3.3	221	3.0	0.64 - 14	321	15.0	3.2 - 70
102	1.1	0.24 - 5.2	222	3.5	0.75 - 16	322	20.0	4.3 - 94
110	0.7	0.15 - 3.3	223	4.0	0.85 - 19	323	30.0	6.4 - 140
111	1.1	0.24 - 5.2	230	3.0	0.64 - 14	330	25.0	5.3 - 120
120	1.1	0.24 - 5.2	231	3.5	0.75 - 16	331	45.0	7.6 - 200
121	1.5	0.32 - 7.0	232	4.0	0.86 - 19	332	110.0	24 - 520
130	1.6	0.34 - 7.5	300	2.5	0.53 - 12	333	140.0	30 - 660
200	0.9	0.19 - 4.2						

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Doc Title	ACUTE TOXICITY OF PHANEX "D" AND SANTICIZER NO. 10 TO GUINEA PIGS PREPARE BY HYGIENE LAB			23
Chemical Name (300 per name)		25	CAS No. (10)	
ALKYL PHTHALATES			999999994	
BENZENE DICARBOXYLIC ACID DIBUTYL ESTER			84-74-2	
BENZENE DICARBOXYLIC ACID DICYCLOHEXYL ESTER			84-61-7	

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ACUTE TOXICITY OF PHANEX "D" AND SANTICIZER NO. 10  
TO GUINEA PIGS

April 7, 1933.

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*Phanex D = Dicyclohexyl phthalate*

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This report covers the acute toxicity to guinea pigs of Phanex "D" and Santicizer No. 10, when administered by mouth, and by intraperitoneal injection.

Since the materials were solids, they had to be handled in Olive Oil, a material having no toxicity to the animals. The doses fed by mouth were ground in a mortar with just enough oil to give a stiff paste.

The doses injected were dissolved in oil to make a solution, 1 c.c. of which = 0.25 grams plasticizer. The stock of this solution remained clear at 40°C., but formed abundant crystals when cooled to room temperature.

Other methods used were exactly the same as those used previously in similar work, reported to the Cellophane Company September 8, 1931 and February 1, 1932.

The largest dosages administered were as large as can be handled conveniently. That is, it is not practical to feed or inject guinea pigs with more than 20,000 mgms. per kilo of their body weight (2% of their body weight). Obviously materials having no toxic action when administered in such huge dosages can be spoken of as non-toxic.

Our results are tabulated on the next page, along with results obtained by the same method with other plasticizers, for comparison.

Smallest Fatal Dose, when administered to Guinea Pigs  
(expressed in Mgs. plasticizer per kilo  
body weight of animal)

	<u>By Mouth</u>	<u>Intraperitoneally</u>
Phanex "D"	20,000	15,000
Santicizer No. 10	20,000	1,000
Dibutyl phthalate	2,000	1,000
Ethyl ester o-benzoyl benzoic acid	6,000	2,000
Methyl cellosolve phthalate	5,000	3,000
Diethyl phthalate	5,000	1,000
Butyl cellosolve phthalate	4,000	800
Santicizer No. 3	1,500	250
Ethox	1,000	2,000
Tributyl phosphate	1,000	200

It can be seen from these results that Phanex "D" has a low acute toxicity to guinea pigs when administered both by mouth and intraperitoneally, while Santicizer No. 10 has a low toxicity by mouth, and a higher one intraperitoneally. The detailed results on the pages following indicate that these two plasticizers do not have quite the same acute effects by mouth, since 3/7 of the animals receiving 20,000 mgms. per kilo of Santicizer No. 10 died, while only 1/6 of those receiving the same dose of Phanex "D" died; but in either case the toxicity by mouth was quite low.

Phanex "D" (di-cyclo-hexyl phthalate)

Guinea Pigs - by Mouth

Fig No.	Weight in grams	Dose in grams	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
64	300	6.0	20,000	Died in 7 days
65	290	5.8	20,000	None
66	275	5.5	20,000	None
88	300	6.0	20,000	None
89	310	6.2	20,000	None
90	290	5.8	20,000	None
29	314	3.14	10,000	None
30	308	3.08	10,000	None
51	250	2.50	10,000	None
52	252	2.52	10,000	None
63	310	3.10	10,000	None
28	296	1.48	5,000	None
49	306	1.53	5,000	None
50	260	1.30	5,000	None
91	300	1.50	5,000	None
92	340	1.70	5,000	None
93	360	1.80	5,000	None
17	255	0.64	2,500	None
18	240	0.60	2,500	None
16	396	0.40	1,000	None

Smallest Fatal Dose - 20,000 mgms. per kilo

Phanex "D" (di-cyclo-hexyl phthalate)

Guinea Pigs - Intraperitoneally (as solution in Olive Oil)

Pig No.	Weight in grams	Dose in grams	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
55	335	6.7	20,000	None
56	310	6.2	20,000	None
57	360	7.2	20,000	Died in 6 days
58	330	6.6	20,000	Died in 2 days
11	295	5.9	20,000	None
67	410	6.1	15,000	None
69	370	5.5	15,000	None
68	345	5.2	15,000	Died in 2 days
10	328	3.28	10,000	None
12	345	3.45	10,000	None
31	270	2.70	10,000	None
32	312	3.12	10,000	None
43	285	2.85	10,000	None
3	260	1.30	5,000	None
33	386	1.93	5,000	None
34	345	1.72	5,000	None
44	305	1.57	5,000	None
2	353	0.88	2,500	None
1	405	0.40	1,000	None

Smallest Fatal Dose - 15,000 mgms. per kilo

Santicizer No. 10

Guinea Pig - by Mouth

Pig No.	Weight in grams	Dose in grams	Dosage in mgms. per kilo	Result ("None" = none seen within 14 days)
27	285	5.7	20,000	None
61	286	5.7	20,000	Died in 4 days
62	288	5.8	20,000	None
82	310	6.2	20,000	None
83	300	6.0	20,000	Died in 4 days
86	260	5.2	20,000	None
87	280	5.6	20,000	Died in 5 days
25	300	3.00	10,000	None
26	326	3.26	10,000	None
48	208	2.9	10,000	None
59	300	3.0	10,000	None
15	244	1.22	5,000	None
46	328	1.64	5,000	None
47	248	1.24	5,000	None
84	290	1.95	5,000	None
85	285	1.42	5,000	None
14	298	0.74	2,500	None
45	349	0.87	2,500	None
13	295	0.30	1,000	None

Smallest Fatal Dose - 20,000 mgme. per kilo

Santicizer No. 10

Guinea Pigs - Intraperitoneally (as solution in Olive Oil)

Pig	Weight in grams	Dose . in grams	Dosage in mgms. per kilo	Result ("None" - none seen within 14 days)
9	268	0.80	3000	Died in 12 hours
5	268	0.67	2500	Died in 12 hours
8	375	0.75	2000	Died in 24 hours
23	295	0.59	2000	Died in 24 hours
24	260	0.52	2000	Died in 24 hours
35	330	0.50	1500	None
36	260	0.39	1500	Died in 24 hours
4	320	0.32	1000	None
7	320	0.32	1000	None
21	346	0.35	1000	None
22	346	0.35	1000	Died in 2 days
37	315	0.23	750	None
38	356	0.27	750	None
78	325	0.24	750	None
79	300	0.23	750	None
13	340	0.68	500	None
20	316	0.63	500	None
80	380	0.19	500	None
81	420	0.21	500	None
53	395	0.10	250	None
54	335	0.08	250	None
72	390	0.098	250	None
73	375	0.094	250	None
74	355	0.036	100	None
75	395	0.040	100	None
76	385	0.039	100	None
77	320	0.032	100	None

Smallest Fatal Dose - 1000 mgms. per kilo

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Doc Title	NTP MUTAGENICITY TESTING STATUS OF PHTHALATES AND RELATED COMPOUNDS			23
Chemical Name (300 per name)	25		CAS No. (10)	24
ALKYL PHTHALATES			999999794	
BENZENEDICARBOXYLIC ACID DIBUTYL ESTER			84-74-2	
BENZENEDICARBOXYLIC ACID DICYCLOHEXYL ESTER			84-61-7	

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TABLE 3. NTP MUTAGENICITY TESTING STATUS  
OF PHTHALATES AND RELATED COMPOUNDS

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CHEMICAL	STATUS	RESULTS
1. Butyl benzyl phthalate (CAS No. 85-68-7)	-Tested in <u>Salmonella typhimurium</u> by 2 laboratories -Scheduled for <u>in vitro</u> cytogenetic testing	Negative at both labs
2. Di (2-ethylhexyl) adipate (CAS No. 103-23-1)	-Tested in <u>Salmonella typhimurium</u> by 2 laboratories -Scheduled for <u>in vitro</u> cytogenetic testing -Selected for <u>Drosophila</u> testing	Negative at both labs
3. Di (2-ethylhexyl) phthalate (CAS NO. 117-81-7)	-Tested in <u>Salmonella typhimurium</u> by 3 laboratories -Scheduled for <u>in vitro</u> cytogenetic testing -Selected for <u>Drosophila</u> testing	Negative at all 3 labs
4. Diethyl phthalate (CAS NO. 84-66-2)	-Tested in <u>Salmonella typhimurium</u> by one laboratory -Scheduled for <u>in vitro</u> cytogenetic testing	Negative
5. Phthalic anhydride (CAS No. 85-44-9)	-Tested in <u>Salmonella typhimurium</u> by one laboratory -Scheduled for <u>in vitro</u> cytogenetic testing	Negative
6. Terephthalic acid (CAS No. 100-21-0)	-Tested in <u>Salmonella typhimurium</u> by one laboratory	Negative
7. Tetrachlorophthalic anhydride (CAS No. 117-08-8)	-Tested in <u>Salmonella typhimurium</u> by one laboratory	Negative

Selected for Testing in Salmonella typhimurium

- |                                                    |                                                           |
|----------------------------------------------------|-----------------------------------------------------------|
| 1. Butyl-cyclohexyl phthalate<br>(CAS No. 84-64-0) | 10. Di-n-hexyl phthalate<br>(CAS No. 84-75-3)             |
| 2. Diallyl phthalate<br>(CAS No. 131-17-9)         | 11. Di-octyl phthalate<br>(CAS No. 117-84-0)              |
| 3. Di-butyl-phthalate<br>(CAS No. 84-74-2)         | 12. Di-tridecyl phthalate<br>(CAS No. 119-06-2)           |
| 4. Di-cyclohexyl phthalate<br>(CAS No. 84-61-7)    | 13. Di-undecyl phthalate<br>(CAS No. 3648-20-2)           |
| 5. Di-isobutyl phthalate<br>(CAS No. 84-69-5)      | 14. 2-Ethylhexanol<br>(CAS No. 104-76-7)                  |
| 6. Di-isodecyl phthalate<br>(CAS No. 26761-40-0)   | 15. Mono(2-ethylhexyl) adipate                            |
| 7. Di-isononyl phthalate<br>(CAS NO. 28553-12-0)   | 16. Mono(2-ethylhexyl) phthalate<br>(CAS. No. 4376-20-9). |
| 8. Dimethyl phthalate<br>(CAS No. 131-11-3)        | 17. Phthalamide<br>(CAS No. 88-96-0)                      |
| 9. Dimethyl terephthalate<br>(CAS No. 120-61-6)    |                                                           |

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TABLE 4. NTP TOXICOLOGY AND CARCINOGENICITY TESTING STATUS OF PHTHALATES AND RELATED COMPOUNDS

<u>CHEMICAL</u>	<u>CARCINOGENICITY TESTING</u>	<u>CHRONIC/SUBCHRONIC EFFECTS</u>
1) Butyl benzyl phthalate (CAS No. 85-68-7)	-Not adequately tested in F344 male rats -Not clearly carcinogenic for F344 female rats (increased incidence of leukemias of hematopoietic system may be related to test chemical) -Not carcinogenic for B6C3F1 mice of either sex	-Internal hemorrhaging in multiple organs -Prothrombin time 20-30 x normal -Platelet counts about 1/2 normal in high dose male rats -Chronic study in male rats terminated (28th week) due to low survival
2) Diallyl phthalate (Cas No. 131-17-9)	-Chronic test in progress	-Cellular alterations in liver, with gradations from minimal to severe, at all dose levels in rats (subchronic study)
3) Di-(2-ethylhexyl) adipate (CAS No. 103-23-1)	-Not carcinogenic for F344 rats -Carcinogenic for female and possibly male B6C3F1 mice (increases in hepatocellular adenomas and carcinomas)	
4) Di-(2-ethylhexyl) phthalate (CAS No. 117-81-7)	-Hepatocellular carcinomas in B6C3F1 mice and F344 rats of both sexes	-Tubular degeneration of testis -Clear cell cytoplasmic change of hepatocytes -Hypertrophy of certain anterior pituitary cells in male rats (chronic study)
5) Diethyl phthalate (CAS No. 84-66-2)	-Prechronic test in progress	
6) Dimethyl terephthalate (CAS No. 120-61-6)	-Not carcinogenic for F344 rats or B6C3F1 mice of either sex; MTD probably not reached	
7) Phthalamide (CAS No. 88-96-0)	-Not carcinogenic for F344 rats or B6C3F1 mice of either sex	
8) Phthalic anhydride (CAS No. 85-44-9)	-Not carcinogenic for F344 rats or B6C3F1 mice of either sex	

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	25	CAS No. (10)	999999994	

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STUDIES CONDUCTED OR INITIATED  
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## ONGOING HEALTH AND SAFETY STUDIES CONDUCTED OR INITIATED BY DU PONT

### CYCLOHEXANONE

- Cataractogenesis Study - To evaluate the potential of cyclohexanone to induce cataracts in rats and guinea pigs. Study includes ophthalmologic examinations, urinalysis, hematological examinations and gross pathology. The study was completed 11/92 and final report expected 1/83. Study performed at WIL Laboratories in Cincinnati, OH, under sponsorship of the Industrial Health Foundation.

### NITROBENZENE

- Ninety-Day Inhalation Study - To evaluate the subchronic toxicity of inhaled nitrobenzene in Sprague-Dawley and Fischer-344 rats and B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice. Exposure phase complete; histopathological evaluation in progress. Study performed at CIIT, Research Triangle Park, NC.

### VINYLLIDENE FLUORIDE

- Subchronic Inhalation Study - Inhalation range-finding study in rats to establish exposure levels for a lifetime study. Study started 12/82 and a final report is expected 3/83.
- Lifetime Chronic Inhalation Study - To evaluate the chronic toxicity and oncogenicity of inhaled vinylidene fluoride in rats. These studies being performed by Prof. C. Maltoni, Institute di Oncologia, Bologna, Italy.

### PHTHALATES

- Mutagenicity Studies

Ames Assay - DEHP, DEHA, MEHP and 2-EH  
Mouse Micronucleus Assay - DEHP, DEHA, MEHP and 2-EH  
In Vitro Transformation (BALB 3T3 Cell Assay) -  
DEHP, DEHA, MEHP and 2-EH  
Unscheduled DNA Synthesis Assay - DEHP and DEHA  
Mouse Lymphoma Assay - DEHP and DEHA

All the above studies have been completed and submitted to the Agency (Sponsored by CMA).

## ONGOING HEALTH AND SAFETY STUDIES CONDUCTED OR INITIATED BY DU PONT

### PHTHALATES (Cont'd.)

- Rat Liver Peroxisomes - For purpose, see attached outline. Study conducted at MRI, Kansas City, under sponsorship of CMA. Final report expected shortly.
- Pharmacokinetics and Metabolism - To evaluate the pharmacokinetics and metabolism of DEHP. Study started late 1982 and should be completed with a final report 9/83. Study being conducted at A. D. Little under sponsorship of CMA.

### para-NITROANILINE

- Subacute Inhalation Study - To evaluate the effects of p-nitroaniline on male rats after repeated inhalation exposure. Methemoglobin levels were measured as well as other hematological parameters, urinalysis, clinical chemistry measurements, gross and histopathological evaluations. Exposure phase completed; histopathology in progress; final report expected 6/83. Study is being conducted at Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours & Co.

### ANILINE AND NITROBENZENE DERIVATIVES

- Structure-Activity Relationships: Methemoglobinemia - To evaluate and compare the correlations between toxicity, methemoglobin-forming ability and chemical structure of the following compounds:
  - N-Ethylaniline
  - N-Ethyl-m-toluidine
  - N,N-Diethyl-m-toluidine
  - m-Phenylenediamine
  - p-Phenylenediamine
  - 2,5-Dichloronitrobenzene
  - 3,4-Dichloronitrobenzene

Final report expected 6/83. Study is being conducted at Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours & Co.

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1-12-83

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**Study Outline  
Testing Phase  
(7 Compounds)\***

**I. General**

Number of rats:	5 per sex per group
Duration of treatment	3 weeks
Number of dose levels:	3 and control
Total number of animals	40

**II. Parameters to measure  
weekly body weights and food consumption  
weekly feed analysis**

**III. At sacrifice**

**A. Organ weights:**

liver  
testes  
kidney

**B. Tissues to be saved:**

liver  
kidney  
testes  
gross lesions

**C. Clinical chemistry:**

triglyceride  
total cholesterol

**D. Hepatic enzymes - all animals**

CAT  
catalase  
third enzyme - to be designated

**E. Livers of all rats will be stained and prepared for electron  
microscopy. Sections will be evaluated for two rats/sex for  
the high dose and one per sex for controls unless indicated  
by results of high dose or results of liver enzyme assays.**

**F. Light microscopy on all tissues in B for high dose and  
control only unless otherwise indicated.**

\* DBP, DUP, DIDP, DINP, 610P, 711P, BPP

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