# Toxicology of a Series of Phthalate Esters

By DAVID CALLEY, JOHN AUTIAN, and WALLACE L. GUESS

**Certain types of plastic materials require the addition of appreciable quantities of plasticizers to impart specific physical-chemical properties to the final item. Presently, many vinyl devices used with drug products may contain plasticizers of the phthalate type. For this reason, a series of phthalic acid esters were evaluated for parenteral toxicity including $LD_{50}$ values and hexobarbital narcosis. Experiments utilized in the study also included i.p. injections in mice for acute toxicity profiles, i.v. administration in rabbits for blood pressure and respiration effects, and intradermal injections into rabbits for irritation effects. A further study was conducted to note what effects repeated i.p. doses of the phthalates would have on mice over a period of time, examining the effects on organs, weight gain, and the blood. Finally, tissue culture experiments were conducted to attempt to correlate certain of the toxicity manifestations. The most salient feature of the toxicity of these compounds was the central depression on the animals and the rather low order of toxicity by the parenteral route of administration.**

CERTAIN plastic materials (*e.g.*, polyvinyl chloride) require the introduction of an appreciable quantity of plasticizers to impart specific physicochemical and mechanical properties. In medical practice, these materials are used as bags, tubings, vehicles for administration, and collection or storage devices. A high degree of safety must be established and maintained in order to prevent any adverse effect when and if an ingredient from the plastic device migrates into a parenterally administered product. Previous reports from this laboratory have indicated that certain plastic devices used medically can release one or more ingredients into tissue or solvent systems (1–3). Furthermore, these "migrated" ingredients, when in sufficient concentrations, can elicit definite pharmacological responses. One study by Guess, Autian, and Meyers (4) demonstrated that a group of citric acid esters used as plasticizers for vinyl formulations produced definite toxicological effects when administered by parenteral routes. These same compounds, however, were extremely safe even in large quantities when administered orally to animals.

Presently, one of the most commonly used groups of plasticizers is the phthalic acid esters. These and related compounds generally have low volatility (5), low water solubility (6), some degree of absorption through the skin, and toxicity when taken into the body by the inhalation of vapors or by the oral route (7–9). Little information, however, is available as to the degree of toxicity or modes of actions of the phthalic acid esters when they are introduced parenterally (10, 11). This paper reports a series of biological experiments in which 8 of the esters were evaluated as to parenteral toxicity to mice and rabbits and *in vitro* toxicity to cell cultures.

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## EXPERIMENTAL

### Materials

Dimethyl phthalate, diethyl phthalate, dibutyl phthalate, di-isobutyl phthalate, di-(methoxyethyl) phthalate (Eastman Chemical Products, Inc.), butyl benzyl phthalate (Monsanto Chemical Co.), di-(2-ethylhexyl) phthalate (Eastman Chemical Products, Inc.), and dicapryl phthalate (Harchem Division, Wallace & Tiernan, Inc.).

All of the compounds were commercial products. Thin-layer chromatography and infrared analysis demonstrated that each of the samples had a relatively high degree of purity.

### Methods

**Acute Toxicity.**—*$LD_{50}$ Determinations.*—These were conducted according to the method and tables described by Thompson and Weil (12) and Weil (13). Each phthalate was administered intraperitoneally in Swiss Webster white mice of uniform weight and age in 4 dosage levels ranging from 0.5 Gm./Kg. to 16 Gm./Kg. Dosage levels were spaced in geometric progression, increasing by a factor of 2.

*Effect of Phthalate on Hexobarbital Narcosis.*—This study was conducted to measure acute CNS stimulation or depression following sublethal intraperitoneal administration of emulsified phthalates. Groups of 10 white mice weighing 14 to 20 Gm. were administered 500-mg./Kg. doses of the phthalate esters and, after an interval of 30 min., injected i.p. with 60 mg./Kg. of sodium hexobarbital. A control group of 10 mice received an equivalent volume of 3% acacia in place of the phthalate.

*Rabbit Intradermal Irritation Tests.*—Phthalate emulsions in concentration of 100 mg./ml. were injected 0.2 ml. intradermally into the cleanly shaven backs of rabbits. Inflammatory response at the injection site was measured by injection of 1 ml./Kg. of 1% trypan blue into the marginal ear vein after an interval of 15 min. (14, 15).

*Acute Intravenous Toxicity.*—Effects of phthalate emulsions on rabbit blood pressure, respiration rate, electrocardiogram pattern, and electroencephalogram pattern were recorded on the Grass Polygraph. Rabbits were anesthetized with approxi-

mately 33 mg./Kg. of sodium pentobarbital. Phthalates were emulsified in buffered 3% acacia solution and administered in repeated doses of 50 mg./Kg. through the cannulated external jugular vein directly into the heart. Blood pressure changes were recorded *via* the cannulated common carotid artery with the Statham transducer. Respiratory changes were determined from the cannulated trachea connected to a low pressure transducer (model PT 5A) through the Grass recorder. The EEG was taken using occipital leads, and the EKG taken from leads inserted subdermally in the limbs and chest. Two rabbits receiving only 3% acacia in equivalent volumes served as controls for record comparison.

*Tissue Culture.*—Toxicity of the phthalates to strain L 929 mouse fibroblast cells and to chick embryo cells was tested according to a method developed in our laboratory (16). Porous pads were wet with 0.05 ml. of a 50 mg./ml. emulsion of phthalates. The pads were placed on the surface of the agar and the cultures observed over a 48-hr. period for appearance of cell mortality.

**Subacute Toxicity Studies.**—These studies were conducted using 4 phthalic acid esters: diethyl, di-(methoxyethyl), butyl benzyl, and di-(2-ethylhexyl). From 20 to 30 white mice of uniform initial weight and age were injected daily with an emulsion of each phthalate for a period of 6 weeks. Dosage levels ranged from 125 mg./Kg. to 500 mg./Kg., depending on the toxicity or tolerance level for each. Weight gains were recorded weekly. Organ-to-body weight ratios for liver, kidney, lungs, heart, spleen, and testes were calculated at the end of the study and compared with normal values of a control group receiving only 3% acacia suspension. Pathology studies were made on certain of the organs using the standard formalin fixation and hematoxylin-eosin stains. Blood studies were made before injections began and again at the termination of the project before sacrifice. These studies included white and red cell counts, white cell differential counts, hematocrit, and hemoglobin levels.

## RESULTS AND DISCUSSION

### Acute Toxicity Studies

**$LD_{50}$ Determination.**—The results of this study indicated that acute lethal i.p. toxicity of the phthalic acid esters studied was generally of a low order. $LD_{50}$ values ranged from 1.58 Gm./Kg. for dimethyl phthalate to 14.19 Gm./Kg. for di-(2-ethylhexyl) and dicapryl phthalate (Table I). Although water solubility of all the compounds was extremely low, ranging from 1.5 Gm./100 Gm. to insolubility, a correlation appeared to exist between this factor and degree of toxicity indicated by $LD_{50}$ values. The 3 esters with greatest solubility also exhibited greatest toxicity. As would be expected, an inverse relationship existed between toxicity and molecular weight of the compounds.

**Effect of Phthalates on Hexobarbital Narcosis.**—Sleeping time of the control group was 46 min. (Table II). Only 2 compounds gave indications of CNS stimulation as represented by significantly shortened sleeping time. These were di-(2-ethylhexyl) and dicapryl phthalate, each with a sleeping time of 36 min. These averages were significantly different ($p > 0.05$) from the control average. All other phthalates appeared to demonstrate CNS depression. Notable were diethyl, di-isobutyl, and butyl benzyl phthalates, with sleeping times of 88, 72, and 62 min. These 3 values were also significantly different ($p > 0.05$) from the control group.

TABLE I.—ESTIMATED $LD_{50}$ VALUES FOR A SERIES OF PHTHALATE ESTERS

| Phthalate Ester | i.p. $LD_{50}$ in Mice, Gm./Kg. | 95% Confidence Limits | Mol. Wt. | Solubility in Water, Gm./100 Gm. |
|---|---|---|---|---|
| Dimethyl phthalate | 1.58 | 0.98 to 1.99 | 194 | 0.45 |
| Diethyl phthalate | 2.83 | 2.42 to 3.29 | 222 | 0.1 |
| Dibutyl phthalate | 4.00 | 2.94 to 5.45 | 278 | Insol. |
| Di-isobutyl phthalate | 4.50 | 3.36 to 6.02 | 278 | 0.01 |
| Di-(methoxyethyl) phthalate | 2.51 | 1.82 to 3.45 | 282 | 0.85 |
| Butyl benzyl phthalate | 3.16 | 2.51 to 3.98 | 312 | Insol. |
| Di-(2-ethylhexyl) phthalate | 14.19 | 12.62 to 15.76 | 390 | 0.01 |
| Dicapryl phthalate | 14.19 | 11.21 to 15.87 | 390 | 0.03 |

TABLE II.—HEXOBARBITAL SLEEPING TIME IN MICE

| | Mice in Group, No. | Sleeping Time ± S.E., min. | CNS Effect: Depression | CNS Effect: Stimulation |
|---|---|---|---|---|
| Control group (0.5 ml. 3% acacia) | 10 | 46 ± 1.66 | | ... |
| Dimethyl phthalate | 10 | 52 ± 1.15 | + | ... |
| Diethyl phthalate | 10 | 88 ± 2.94[a] | + | ... |
| Dibutyl phthalate | 10 | 55 ± 2.18 | + | ... |
| Di-isobutyl phthalate | 10 | 72 ± 2.83[a] | + | ... |
| Di-(methoxyethyl) phthalate | 10 | 50 ± 1.71 | + | ... |
| Butyl benzyl phthalate | 10 | 62 ± 2.45[b] | + | ... |
| Di-(2-ethylhexyl) phthalate | 10 | 36 ± 1.14[b] | ... | + |
| Dicapryl phthalate | 10 | 36 ± 1.14[b] | ... | + |

[a] Indicates significant difference from control value ($p > 0.01$). [b] Indicates significant difference from control value ($p > 0.05$).

No satisfactory explanation for the evidence of CNS stimulation found for di-(2-ethylhexyl) and dicapryl phthalate could be elicited from these experiments, particularly in view of the fact that both of these showed a tendency to lower blood pressure at higher dose levels. The only supporting evidence of stimulation was seen in the EEG patterns after i.v. administration of dicapryl phthalate (see below). The order of the potentiation of narcosis followed the water solubility of the compounds. In view of the extremely lipid nature of the CNS, this order of narcosis potentiation was surprising. However, these compounds were administered as emulsions by the i.p. route, and from this route, the degree of absorption and consequently evidence of narcosis potentiation could have been due to a concentration at the site of action effect.

**Rabbit Intradermal Irritation Tests.**—Three phthalates—dimethyl, diethyl, and di-(2-ethylhexyl)—caused rapid and intense concentration of extravasated trypan blue at the injected sites (Table III), indicating marked inflammatory response.

TABLE III.—IRRITATIVE RESPONSE IN RABBITS TO INTRADERMAL INJECTIONS OF PHTHALATE ESTERS

| Phthalate Ester | Degree of Extravasation[a] 10 min. | 15 min. | 26 min. |
|---|---|---|---|
| Dimethyl phthalate | ++ | +++ | +++ |
| Diethyl phthalate | +++ | +++ | +++ |
| Dibutyl phthalate | + | + | ++ |
| Di-isobutyl phthalate | − | − | ++ |
| Di-(methoxyethyl) phthalate | ++ | ++ | ++ |
| Butyl benzyl phthalate | − | + | ++ |
| Di-(2-ethylhexyl) phthalate | +++ | +++ | +++ |
| Dicapryl phthalate | − | + | + |
| 0.85% NaCl (negative control) | − | − | − |
| 20.00% EtOH (positive control) | +++ | +++ | +++ |

[a] Inflammatory response indicated by degree of dye extravasation; −, no color, negative reaction; +, mild; ++, moderate; +++, marked.

Others exhibited mild to moderate inflammatory response as indicated by low color intensities with greater lapses in time before appearance of the dye at the injection site. Dicapryl phthalate was the least irritating according to this test, showing only mild tissue response during the observation period. It should be noted that with the exception of di-(2-ethylhexyl) phthalate, the activity of these compounds as irritants was related to molecular weight.

**Acute Intravenous Toxicity.**—*Blood Pressure.*—With the exception of diethyl phthalate, none of the intravenously administered phthalates had dramatic or significant effect on the anesthetized rabbit blood pressure until a minimum total dose level of 350 mg./Kg. had been given. At this point, 4 of the phthalate compounds [diethyl, dimethyl, di-(2-ethylhexyl), and dicapryl] showed some depression of the blood pressure, indicating a vascular response to toxicity. In no case did the electrocardiograms or vector analysis of these results indicate direct cardiac toxicity. Therefore, the available evidence suggests an indirect cardiovascular toxicity at the higher i.v. dose levels. In the case of diethyl phthalate, each dose of 50 mg./Kg. administered i.v. caused a transient (about 3 min. duration) fall in blood pressure of about 20 mm. Hg, or approximately a 22% decrease. The blood pressure then gradually returned to the predose level, and no subsequent change ensued until administration of the next dose. A total dose of 650 mg./Kg. was given i.v. without death or other significant change in the animal, indicating a low order of toxicity. Administration of 5 doses of 3.0 ml. each of the vehicle (3% acacia) in control rabbits elicited no changes in blood pressure.

*Respiration.*—All of the phthalates administered i.v. caused an increase in the respiratory rate of the anesthetized rabbit. Since all were administered in a buffered (pH 7.0) emulsion over a period of about 2-3 min., the stimulation of respiration could not be attributed to a pH effect on the chemoreceptors. In 3 cases (dimethyl, diethyl, and diisobutyl phthalate), there were significant increases in respiratory rate after the administration of a total dose of 100 mg./Kg. The per cent increase for these 3 were 66.6, 71.2, and 114.3, respectively. The rate gradually returned toward normal over a period of about 5 min. It is of interest to note that these 3 compounds exhibited the highest order of toxicity in the $LD_{50}$ study.

*Electroencephalograms.*—The electroencephalogram tracings obtained from occipital leads showed varying patterns after the administration of different phthalates intravenously. Figure 1 depicts several of the patterns seen both pre- and post-phthalate administration. The CNS depression pattern, obtained after administration of di-(methoxyethyl) phthalate, shows decreased frequency. The stimulation pattern, obtained after administration of dicapryl phthalate shows increased frequency, which appears to be a confirmation of the CNS stimulation exhibited in the hexobarbital narcosis study. It is also recognized that other influences such as anoxia, etc., may complicate EEG tracings, but no indication of these contingencies were noted in our experiments.

**Tissue Culture Toxicity.**—None of the phthalate emulsions demonstrated toxicity to chick embryo cells in the amounts used (0.05 ml. of a 50 mg./ml. emulsion). Three of the phthalates did show toxicity to mouse fibroblast cells (L-cells) in these same amounts. Microscopic examination of the dead cell zones revealed that the cells were intact and indicated absence of corrosive activity. Other results have shown that mouse cells are more sensitive to liquid toxicants than chick cells. Table IV shows the results of each phthalate on each system used. It should be noted that only the

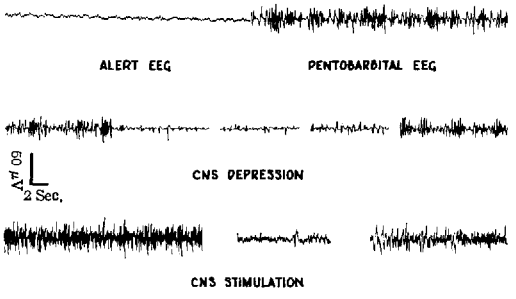


Fig. 1.—Occipital EEG patterns on rabbit. Key: ordinate and abscissa of insert (L) enlarged 2× for legibility of axis captions.

TABLE IV.—TOXICITY OF PHTHALATE ESTERS TO CULTURED CELLS

| Phthalate Ester | Chick Embryo Cells, 50 mg./ml.[a] | Mouse Fibroblasts, 50 mg./ml.[a] |
|---|---|---|
| Dimethyl phthalate | − | + |
| Diethyl phthalate | − | + |
| Dibutyl phthalate | − | − |
| Di-isobutyl phthalate | − | − |
| Di-(methoxyethyl) phthalate | − | + |
| Butyl benzyl phthalate | − | − |
| Di-(2-ethylhexyl) phthalate | − | − |
| Dicapryl phthalate | − | − |

[a] −, no cell toxicity; +, cell death.

compounds most soluble in water demonstrated toxicity to the cultured cells. This is not too surprising since the cell system is almost entirely aqueous. Two of the 3 exhibiting cell toxicity were also most irritating in the rabbit intradermal tests.

### Subacute Toxicity Studies (Mice)

**Body Weight Gains.**—After 6 weeks of mouse intraperitoneal injections of 4 phthalates—diethyl, di-(methoxyethyl), butyl benzyl, and di-(2-ethylhexyl)—body weight in all groups, including controls, was approximately equal. The control group, however, reached maximum weight by the end of the third week, while in comparison, all phthalate-injected mice exhibited some degree of weight gain retardation, notably those receiving di-(2-ethylhexyl) phthalate, and, to a lesser extent, butyl benzyl phthalate. The diethyl phthalate group showed weekly weight gains most similar to those of the control group, and the di-(methoxyethyl) group exhibited an intermediate pattern. These differences in weight gain patterns may indicate a decreased food intake due to peritoneal cavity irritation caused by the repeated injections. It was observed on autopsy of each group of mice that some degree of peritonitis had been induced by the chemicals. The extent of peritonitis in the group of mice receiving the di-(2-ethylhexyl) phthalate was extreme. In nearly all of these cases, there was extreme adhesion formation, liver abscess, and adhesions to the diaphragm, and even testicle abscesses. These mice writhed and stretched after an injection, further supporting the observation of irritation of the entire peritoneal cavity.

**Organ Weight–Body Weight Ratios.**—Examination of organ-body weight ratios (Table V) indicated that liver weights in the di-(2-ethylhexyl) group were significantly greater and testis weights in the di-(2-ethylhexyl) and in the di-methoxyethyl) group were significantly less than the control group (in both groups $p > 0.01$). In regard to liver weights, however, it is doubtful that a genuine difference existed. Gross and pathological examination of these organs, particularly in the di-(2-ethylhexyl) group, indicated that organ weights were likely distorted by the effects of peritonitis. Adhesions, lesions, and other anomalies tended to prevent precise excision and cleaning of the organ before weighing. In addition, abscesses in the tissue changed the normal weight ratio. Testis weights indicated atrophy of this organ in the 2 above groups.

**Hematology.**—The hematological pattern of the several groups of mice receiving daily injections of the phthalate emulsions over a 6-week period indicated no significant deviations from the control group receiving only 3% acacia injections, with the possible exception of di-(2-ethylhexyl) phthalate. In this case, there was a slight decrease from original values in the hematocrit and hemoglobin and a slight increase in the red blood cell count. In all cases including controls there was a slight increase in white cell count, but this is to be expected after 6 weeks of daily injections.

**Pathology.**—In all of the test mice used in this study, there was some degree of peritonitis. Organs showing evidence of gross abnormalities were submitted for histopathological evaluation. These evaluations confirmed the original observations indicating acute peritonitis. Some organs from the phthalate injected groups were far more severely damaged than organs from the control group. In the case of di-(2-ethylhexyl) phthalate, nearly all organs showed presence of cloudy sedimentation accompanied by adhesions of the diaphragm, liver, and intestines and by abscess formation in the livers of a few. Liver and spleen of mice injected daily with di-(methoxyethyl) phthalate were found to have acute peritonitis and peri-portal hepatitis in the liver and extramedullary hematopoiesis in both the liver and spleen. The same pattern was also observed in the mice receiving the butyl benzyl phthalate, and in 1 testis there was an abscess of unknown etiology. In view of the irritation to rabbit tissue caused by intradermal injection, these results were to be anticipated. In these cases, a fairly strong irritant, comparable to the irritating action of 20% solution of ethanol, was injected daily and this was apparently enough to cause adhesions, peritonitis, and even some abscess

TABLE V.—EFFECT OF PHTHALATE ESTERS ON MOUSE TISSUE WEIGHTS

| Group | Dosage, mg./Kg. | Organ Wt.–Body Wt. Ratio × $10^3$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | Liver | Heart | Lungs | Kidney | Spleen | Testes |
| Control | | 65.00 ± 1.83 | 4.72 ± 0.15 | 7.50 ± 0.36 | 13.29 ± 0.87 | 6.97 ± 0.85 | 6.94 ± 0.53 |
| Diethyl phthalate | 125 | 65.55 ± 3.45 | 4.51 ± 0.17 | 8.71 ± 3.78 | 13.27 ± 0.83 | 7.02 ± 0.80 | 7.07 ± 0.41 |
| Di-(methoxyethyl) phthalate | 250 | 65.12 ± 3.84 | 4.26 ± 0.25 | 7.56 ± 0.56 | 11.75 ± 0.75 | 8.04 ± 0.73 | 5.65 ± 0.34[a] |
| Di-(2-ethylhexyl) phthalate | 250 | 76.02 ± 2.80[a] | 4.30 ± 0.21 | 7.97 ± 0.39 | 13.16 ± 0.36 | 7.54 ± 0.73 | 5.48 ± 0.19[a] |
| Butyl benzyl phthalate | 500 | 65.86 ± 2.73 | 4.53 ± 0.11 | 8.25 ± 0.40 | 13.20 ± 0.45 | 7.62 ± 0.54 | 6.17 ± 0.28 |

[a] Significantly different from control group averages ($p > 0.05$).

formation. The control animals in this study showed no gross or microscopic pathology.

## SUMMARY

A group of phthalic acid esters were studied for both acute and subacute toxicity in animals. The acute toxicity experiments included the evaluation of $LD_{50}$, hexobarbital sleeping time effects, rabbit intradermal tests, acute intravenous toxicity studies, and tissue culture effects. Subacute toxicity dealt with effect on body weight gain, organ–body weight ratios, effect on tissue of various organs, and effect on the hematopoietic system. Results of the study indicated that the group of phthalate esters reported in this paper had a low degree of toxicity when administered parenterally and that their degree of toxicity was parallel to their water solubility (greater solubility, greater activity) and to their molecular weight (lower molecular weight, greater activity). This low order of toxicity to parenteral administration appears to indicate that their use in applications implicated in this study are probably warranted and safe.

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# Hydrolytic Behavior of Some Alkyl-Substituted Succinanils

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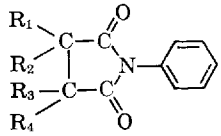
**The mechanisms and the relative rates of alkaline hydrolysis of succinanil and methyl-substituted succinanils have been investigated partly to help to elucidate the mechanism of carboxyl facilitated formation and hydrolysis of amides observed in these laboratories and also because a number of important hypnotics possess similar structures. As may be expected, the observed rates for the several imides roughly parallel those found for the corresponding anhydrides, succinanil being more reactive than the methyl-substituted compounds. The relative rates were in the order: unsubstituted > monomethyl > mesodimethyl > 2.2 dimethyl > racemic dimethyl > trimethyl > tetramethyl; the first member of the series reacting 83 times faster than the last. The tetramethyl anil was found to be sufficiently stable to co-exist as the major species in equilibrium with its cleaved product at pH of 8.**

ALTHOUGH a number of cyclic imides, including glutethimide, methsuximide, phensuximide, etc., are widely used as drugs, relatively little has appeared in the literature concerning the rate of hydrolysis of such imides. Results of studies on the effect of structure on some aspects of this hydrolytic reaction are presented at this time. In particular, the investigation has been concerned with the influence of alkyl substitution on the rate of hydrolysis of *N*-phenyl succinimides (succinanils) (I). These reactions are of interest



I

$R_1$, $R_2$, $R_3$, $R_4$, = H or alkyl

not only because of the close relationship of these compounds to pharmaceuticals but also because

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