Toxicology of Di-2-ethylhexyl Phthalate and Other Phthalic Acid Ester Plasticizers
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The first poly(vinyl chloride) blood bag containing di-2-ethylhexyl phthalate (DEHP) as a plasticizer was introduced in medicine in 1950. Since then, millions of liters of blood collected in plastic have been transfused. Aside from the obvious advantages of plastic over glass containers for the collection, storage and transfusion of blood, plastic containers have made blood component therapy possible; at least six different blood fractions can now be separated aseptically from whole blood and administered individually to the patient as needed.

Poly(vinyl chloride) plastics containing DEHP also have been employed in a variety of other biomedical devices, including transfusion and infusion assemblies, administration sets, artificial kidney connecting tubing, bubble-type oxygenators, catheters, enema and urinary drainage bags, etc. Plasticized poly(vinyl chloride) plastics were selected for these medical applications principally because of their unique physical properties, ease of fabrication, low cost, and apparent safety. To provide the necessary flexibility, relatively high levels of plasticizer (up to 40%) must be blended into the plastic formulation.

Recent reports have identified milligram-% quantities of DEHP in blood or plasma stored in poly(vinyl chloride) blood bags (1–3) and microgram per gram amounts in certain tissues of recipients (3, 4). These findings have raised questions of possible hazard for man exposed to such levels of plasticizer.

This paper is a review of the available toxicologic data on DEHP and other phthalate plasticizers to determine: (1) the current status of the toxicologic information on these materials, and (2) the types of studies needed to complete the safety evaluation.

Acute Toxicity

Median Lethal Dose

Because the phthalate esters as a group and DEHP in particular are viscous oils with poor water solubility, it is not possible to determine an intravenous median lethal dose (LD₅₀) for these compounds. However, several laboratory groups have determined acute toxicity by other routes of administration on a number of phthalate esters. These data are summarized in Table 1.

It is evident as noted by many investigators that the shorter chain phthalate esters are more toxic than the longer chain compounds. A wide range of LD₅₀ values has been reported for DEHP. To what extent this variation in toxicity may reflect variable contamination with free phthalic acid [intra-
peritoneal LD<sub>50</sub> = 0.55 g/kg (5)] or other impurities is not known. The DEHP evaluated in our laboratory (11) had an intraperitoneal LD<sub>50</sub> in mice of greater than 75 g/kg and was more than 99% pure as determined by gas chromatography and contained less than 0.01% free phthalic acid.

In any event, DEHP administered either orally or intraperitoneally has a low order of toxicity. The magnitude of the LD<sub>50</sub> values by these routes of administration (as high as 10% of body weight) likely indicates a nonspecific type of toxicity, possibly the result of impurities or of local effects of the oily compound within the gastrointestinal tract or peritoneal cavity. Unabsorbed DEHP can be recovered in appreciable quantities from the peritoneum for at least 3 days after intraperitoneal injection. Absorption of DEHP into the systemic circulation from such injection sites therefore is quite limited. Quantitative studies of its rate of transfer across biologic membranes have not been reported.

**Effects on Cells in Tissue Culture**

One of the most sensitive measures of biocompatibility is the tissue culture assay, first applied to the evaluation of plastics and plastic ingredients by Guess and co-workers (16). A number of phthalate esters since have been tested for toxic effects on tissue culture cells. These data are summarized in Table 2.

Bioincompatibility of the phthalates in these isolated cell systems is seen with the lower chain dialkyl esters, which also have greater water solubility than the higher chain homologs. Dillingham and Autian in this symposium (19) have reported that the dose–cytotoxicity response curves for four lower-chain dialkyl esters is limited, not unexpectedly, by solubility. Probably because of its low aqueous solubility, DEHP has not

| Phthalate ester | Animal species | Route of administration | LD<sub>50</sub>, g/kg | Reference |
|----------------|---------------|-------------------------|----------------------|-----------|
| Dimethyl       | Mouse         | IP                      | <0.97-2.86           | (5-7)     |
| Diethyl        | Mouse         | IP                      | <1.11-2.83           | (6,7)     |
| Di-<i>n</i>-propyl | Mouse  | IP                      | <1.25                 | (7)       |
| Diisopropyl    | Mouse         | IP                      | <1.25                 | (7)       |
| Di-<i>n</i>-butyl | Mouse  | IP                      | <1.39-5.76           | (5-7)     |
|                | Rat           | IM                      | >8                   | (8)       |
|                |               | PO                      | ca. 8                | (8)       |
|                |               | PO                      | 23.0                 | (9)       |
| Diisobutyl     | Mouse         | IP                      | 4.5                  | (6)       |
| Di-<i>n</i>-hexyl | Rat         | PO                      | 29.6                 | (10)      |
| Di-2-ethylhexyl | Mouse    | IP                      | 14.19->128           | (6,11,12) |
|                | Rat           | IP                      | >23.8->70            | (12-14)   |
|                |               | PO                      | 30.6->60             | (12-14)   |
|                | Rabbit        | IP                      | >31                  | (13)      |
|                |               | PO                      | 33.9                 | (14)      |
| Dialkyl (79)   | Mouse         | IP                      | >20                  | (15)      |
|                |               | PO                      | >20                  | (15)      |
|                | Rat           | IP                      | >20                  | (15)      |
|                |               | PO                      | >20                  | (15)      |
| Di-<i>n</i>-capryl | Mouse  | IP                      | 14.19                | (6)       |

a IP = intraperitoneal; IM = intramuscular; PO = by mouth.
Table 2. Effects of phthalate esters on cells in tissue culture.

| Phthalate ester | Effects on tissue cultures a | Reference |
|-----------------|-------------------------------|-----------|
|                 | Mouse fibroblast L-cells      | 10-Day chick embryo cells |
|                 | Pure ester SSS b Emulsion c   | Pure ester SSS b Emulsion c |
| Dimethyl        | + - + -                      | - - (6,7,17) |
| Diethyl         | +,- - +,- +,-                 | - - (6,7,17) |
| Di-n-propyl     | + NT NT + NT NT             | - NT NT (7) |
| Diisopropyl     | + NT NT + NT NT             | - NT NT (7) |
| Di-n-butyl      | + NT NT - ± NT -            | - NT (6,7) |
| Diisobutyl      | ± - - + - -                 | - (6,7,17) |
| Di-n-pentyl     | ± NT NT - NT NT             | - NT NT (7) |
| Di-n-hexyl      | ± NT NT - NT NT             | - NT NT (7) |
| Di-2-ethylhexyl | - NT - NT NT -             | - NT (6,18) |
| Di-n-octyl      | - - NT - NT -              | - NT (7,17) |
| Di-n-capryl     | - - - - - -                | - (6,17) |

a Symbols: +, cell death; -, no cell toxicity; ±, equivocal results; NT, not tested.
b Saturated saline solution of ester.
c 0.05 ml of 50 mg/ml emulsion of ester.

been demonstrated to be cytotoxic in any of these cell culture systems.

Human blood stored in anticoagulant-nutrient solution, however, is a tissue culture system which contains significant quantities of lipophilic substances. Extensive experience has now been accumulated on the use of banked blood stored for up to 21 days in flexible bags made of poly(vinyl chloride) with DEHP plasticizer.

Tests on plastic blood collection units prepared from 24 consecutive batches of plastic showed that in vivo red blood cell survival in man averaged 82% with a standard deviation of 6.2% for blood stored 21 to 23 days at 4°C (20). Red cell survival was greater than the National Institutes of Health minimum permissible level of 70% in 96% of such tests, although up to 11.5 mg-% of DEHP has been shown to accumulate in blood stored in plasticized poly(vinyl chloride) blood bags for 21 days (1).

Miscellaneous Effects

The phthalate esters have been evaluated also for both local and systemic “subtle” pharmacologic and toxicologic effects in a number of other biologic systems. These studies include intradermal irritancy (6), effects on the electroencephalogram, blood pressure, and respiration (6), effects on hexobarbital sleeping time (6, 21) and on zoxazolamine paralysis time (21), interaction effects of phthalates and antibody (22), effects on conditioned behavior, reticuloendothelial function, and on aggregates of microemboli in stored blood (21).

The validity of these results implying specific pharmacologic or toxicologic actions of DEHP however needs to be given careful scrutiny. In most of these studies large doses much in excess of the solubility limit of DEHP were used to demonstrate effects, necessitating administration of DEHP in a nonphysiologic dosage form as an oil emulsion. In no case were non-DEHP emulsions included as controls to establish the nonspecific effects of the emulsions themselves. Further, responses to only a single or at most two dose levels were determined so that a complete dose-response curve over a broad dosage range was not established.
Except for the micro-aggregate studies of Jaeger (21), DEHP has not been demonstrated to produce any effects in any of these systems at dosages or concentrations that could be completely solubilized and which therefore necessarily would represent true pharmacologic actions. The micro-aggregate system studied by Jaeger is complex, and though he found an apparent increase in micro-aggregate formation in blood stored in poly(vinyl chloride) plastic, his experiments did not exclude other possible causes for this effect. In the absence of conclusive data obtained from more critical experimentation demonstrating specific pharmacologic responses to DEHP, it is premature to conclude that such effects of DEHP have been well established.

**Chronic Toxicity**

Only a few of the phthalate esters have been evaluated in long term toxicity studies and these have been done by the oral route of administration only. Ninety-day oral studies of dialkyl 79 phthalate, a widely used plasticizer in the United Kingdom, have been conducted by Gaunt et al. (15). A "no-effect" dietary level was determined to be 0.125%, equivalent to a daily dose of approximately 60 mg/kg (see Table 3). Dibutyl phthalate, along with some non-phthalate plasticizers, was studied by Smith (8) in a 1 yr rat study. A dietary level of 1.25% killed five of ten animals during the first week, but the survivors at this level and all animals at the next lower level of 0.25%

| Phthalate ester | Animal species | Number of animals in study | Study duration, weeks | No-effect level | Toxic signs | Reference |
|-----------------|----------------|----------------------------|-----------------------|----------------|------------|-----------|
| Dialkyl 79      | Rat            | 30                         | 120                   | 13             | 0.125      | Growth retardation, increased kidney and liver weight | (15) |
| Di-n-butyl      | Rat            | 10                         | 40                    | 52             | 0.25       | Death at 1.25%                                      | (8) |
| Di-2-ethylhexyl | Rat            | 5                          | 20                    | 13             | 0.375      | Growth retardation, testicular degeneration, tubular atrophy | (14) |
| Rat             | b              | 272                        | 104                   | 0.13-0.4       | 60-200     | Growth retardation, increased kidney and liver weight | (23) |
| Guinea pig      | 86             | 172                        | to 104                | 0.1            | 40-80      | Same                                                | (13) |
| Dog             | 4              | 5                          | 52                    | 0.13           | 60         | Increased liver weight in females only               | (23) |
| Dog             | 0              | 2                          | 14                    | -              | 59         | Fatty vacuolization and congestion in subcapsular area of liver and moderate kidney congestion | (23) |
| Dog             | 0              | 2                          | 14                    | -              | 100        | Weight loss and cholecystitis reported in one dog    | (13) |

*a At higher than no-effect level.

b Number not given but "appropriate controls" were included in the study.
(approximately 110–350 mg/kg/day) showed no signs of clinical or tissue toxicity (Table 3).

DEHP has been the subject of more investigations of long term toxicity than any other phthalate ester. Oral studies lasting from 90 days to 2 years in the rat, 1 year in the guinea pig, and up to 1 year in the dog have been conducted at several different laboratories (13, 14, 23). These investigations have established a “no-effect” oral dose of about 60 mg/kg/day. Higher dosages were associated with growth retardation and increased liver and kidney weights in rats. No microscopic abnormalities attributable to DEHP were noted. The dog studies to date have not challenged a large enough group of animals to characterize adequately the clinical and tissue toxicity of DEHP at toxic doses in this species. The study conducted by Carpenter et al. (23) showed that a dose of 0.06 ml/kg administered five times a week for 1 yr produced no apparent clinical toxicity or gross or microscopic pathology of the major organs, except for one animal that did show slight tubular degeneration of the kidney. This latter change had a questionable relationship to DEHP administration.

Carpenter et al. (23) also studied the effects of DEHP on reproductive performance of both male and female rats and on survival and histopathology of first-generation offspring. More specific teratologic studies of DEHP and other phthalates have been conducted by McLaughlin et al. (24) Guess et al. (22), Bower and co-workers (25), and by Singh, Lawrence, and Autian (26). These studies will be the subject of the next presentation in this symposium (19).

Discussion

DEHP has been evaluated more extensively for toxicity than any other phthalate plasticizer in general use. Although not relevant to the subject of this paper, it should be noted also that there are no non-phthalate plasticizers that have been studied any more carefully. Yet, admittedly, our present understanding of the pharmacology and toxicology of DEHP is incomplete. We know little about its toxicity following repeated exposure by parenteral routes of administration which must be considered for most biomedical uses of plastics. Moreover, cognizance of the special problems of solubilizing this material to provide a dosage form for administration that mimics the way in which man is exposed is necessary to determine its clinically relevant pharmacologic and toxicologic actions.

DEHP has been identified in tissues of humans exposed to biomedical plastics (4), but the relationship between biomedical exposure versus environmental or other modes of exposure to plasticizers and DEHP tissue levels has not been studied systematically. Further, the ultimate fate of tissue bound DEHP as well as the kinetics of its distribution between tissue and vascular compartments is unknown.

The presence of DEHP in the human body has prompted speculation of possible deleterious effects. While it is clear that DEHP in human tissue cannot be considered beneficial, it should be noted and emphasized in this symposium that there is no clear evidence that an adverse cause-and-effect relationship does in fact exist. As a representative of industry that manufactures blood bags and other biomedical devices made from plasticized plastics, we recognize our responsibility to take the initiative in completing the safety evaluation of DEHP. The four principal manufacturers of blood bags (Abbott, Baxter, Cutter, and McGaw Laboratories) are collaborating in this effort and have contracted the Stanford Research Institute to carry out animal studies designed to determine (1) the extent of oral absorption of DEHP as an intact diester, (2) tissue distribution of DEHP following intravenous administration, and (3) half-life of DEHP in blood and tissues and time to zero residue. Obviously, if significant oral absorption of DEHP occurs, its parenteral toxicology can be considered to be basically similar to its oral toxicology about which we have extensive data. On the other hand, if
this should not be the case, long-term parenteral toxicity studies need to be undertaken. Preliminary findings from the collaborative study indicate that there is significant absorption of DEHP from the gastrointestinal tract.

The determination of decay rates of DEHP levels in blood and tissues will place the significance of the finding of DEHP in human tissues in better perspective. In this connection, medical departments of the industry group in collaboration with the laboratories of Dr. Robert Rubin, Johns Hopkins University, and Dr. Padmanabhan Nair, Sinai Hospital, Baltimore, are conducting a study in man to establish the relationship between exposure to plasticized plastics and tissue levels of DEHP.

Finally, I want to caution this symposium that we not lose sight of the risk-to-benefit ratio in our efforts to study and define the risk associated with biomedical exposure to plasticizers. Some forms of life-saving therapy would not be possible presently without DEHP-plasticized plastics.

Summary

- DEHP and other phthalate esters have been tested for acute toxicity primarily in mice and rats and tissue culture cells. The lowerchain dialkyl esters are more toxic in these systems than their longer chain homologs.
- DEHP and other plasticizers have been assessed for “subtle” toxicity in a number of other biologic systems. The methodologic limitations of these evaluations are pointed out.
- Chronic toxicity data are available on DEHP which have established an oral “no-effect” dose of about 60 mg/kg/day for a period of one year. Chronic toxicity studies of DEHP administered by a parenteral route are not available.
- The manufacturers of plasticized blood bags are supporting a study presently being conducted at Stanford Research Institute to determine oral absorption, tissue distribution, and tissue half-life of DEHP. In addition a study of human tissue is in progress to define the relationship between exposure to biomedical plastics and DEHP tissue levels.
- Concern over risk of exposure to DEHP plasticized plastics needs to be kept in proper perspective, inasmuch as many biomedical devices incorporating such plastic provide therapeutic benefit often life-saving.

REFERENCES

1. Marcel, Y. L., and Noel, S. P. 1970. Contamination of blood stored in plastic packs. Lancet 1:35.
2. Marcel, Y. L., and Noel, S. P. 1970. Plasticizers in lipid extracts of human blood. Chem. Phys. Lipids 4:418.
3. Jaeger, R. J., and Rubin, R. J. 1970. Contamination of blood stored in plastic packs. Lancet 2:151.
4. Jaeger, R. J., and Rubin, R. J. 1970. Plasticizers from plastic devices: extraction, metabolism, and accumulation by biological systems. Science 170:460.
5. Hodge, H. C., Goldstein, M. R. and Wrightington, M. 1942. Acute toxicity for mice of phthalic acid and certain derivatives. Proc. Soc. Exp. Biol. Med. 49:471.
6. Calley, D., Autian, J., and Guess, W. L. 1966. Toxicology of a series of phthalate esters. J. Pharm. Sci. 55: 158.
7. Nematollani, J., Guess, W. L., and Autian, J. 1967. Plasticizers in medical application. I. Analysis and toxicity evaluation of dialkyl benzenedicarboxylates. J. Pharm. Sci. 56:1446.
8. Smith, C. C. 1953. Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate, and methoxyethyl oleate. Arch. Ind. Hyg. 7:310.
9. Radeva, M., and Dinoeva, S. 1966. Toxicity of dibutyl phthalate by oral application in albino rats (in Bulg.). Khig. Zdравеopazvane 9:510.
10. Smyth, H. F., Jr., et al. 1954. Range-finding toxicity data: List V. Arch. Ind. Hyg. 10:61.
11. Gesler, R. M., and Kartinos, N. J. 1970. Contamination of blood stored in plastic packs. Lancet 1:1227.
12. Hodge, H. C. 1943. Acute toxicity for rats and mice of 2-ethyl hexanol and 2-ethyl hexyl phthalate. Proc. Soc. Exp. Biol. Med. 53:20.
13. Harris, R. S., et al. 1956. Chronic oral toxicity of 2-ethylhexyl phthalate in rats and dogs. Arch. Ind. Health 13:259.
14. Shaffer, C. B., Carpenter, C. P., and Smyth, H. F., Jr. 1945. Acute and subacute toxicity of di (2-ethylhexyl) phthalate with note upon its metabolism. J. Ind. Hyg. Toxicol. 27:130.
15. Gaunt, I. F., et al. 1968. Acute (rat and mouse) and short-term (rat) toxicity studies on dialkyl 79 phthalate (a mixture of phthalate esters of alcohols having 7-9 carbon atoms). Food Cosmet. Toxicol. 6:609.

16. Guess, W. L., et al. 1965. Agar diffusion method for toxicity screening of plastics on cultured cell monolayers. J. Pharm. Sci. 54:1545.

17. Guess, W. L., and Haberman, S. 1968. Toxicity profiles of vinyl and polyolefinic plastics and their additives. J. Biomed. Mater. Res. 2:313.

18. Baxter Laboratories. Unpublished data.

19. Dillingham, E. O., and Autian, J. 1972. Teratogenicity, mutagenicity, and cellular toxicity of phthalate esters. Environ. Health Perspec. 3:81.

20. Walter, C. W., Button, L. N., and Gibson, J. G. 1964. Stability and quality of plastic transfusion equipment. Internat. Soc. Blood Trans. 9th Congr. Mexico, 1962. Proc. 11-6.

21. Jaeger, R. J. 1971. Studies on the extraction, accumulation, and metabolism of phthalate ester plasticizers from polyvinyl chloride medical devices. Ph.D. Dissertation, Johns Hopkins University.

22. Guess, W. L., et al. 1967. Characterization of subtle toxicity of certain plastic components used in manufacture of the polyvinyls. Amer. J. Hosp. Pharm. 24:495.

23. Carpenter, C. P., Weil, C. S., and Smyth, H. F. 1953. Chronic oral toxicity of di (2-ethylhexyl) phthalate for rats, guinea pigs and dogs. Arch. Ind. Hyg. 8:219.

24. McLaughlin, J., et al. 1963. The injection of chemicals into the yolk sac of fertile eggs prior to incubation as a toxicity test. Toxicol. Appl. Pharmacol. 5:760.

25. Bower, R. K., Haberman, S., and Minton, P. D. 1970. Teratogenic effects in the chick embryo caused by esters of phthalic acid. J. Pharmacol. Exp. Ther. 171:314.

26. Singh, A. R., Lawrence, W. H., and Autian, J. 1972. Teratogenicity of phthalate esters in rats. J. Pharm. Sci. 61:51.