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FATE IN HUMANS OF THE PLASTICIZER, DI(2-ETHYLHEXYL)
PHTHALATE, ARISING FROM TRANSFUSION OF PLATELETS STORED
IN VINYL PLASTIC BAGS

Robert J. Rubin, Ph.D.

Professor of Environmental Medicine

Department of Environmental Medicine

Johns Hopkins School of Hygiene and Public Health

615 N. Wolfe Street

Baltimore, Maryland 21205

and

Charles A. Schiffer, M.D.

Section of Medical Oncology

National Cancer Institute

Baltimore Cancer Research Center

at

University of Maryland Hospital

22 South Green Street

Baltimore, Maryland 21201

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PLASTICIZER, DI (2-ETHYLHEXYL) PHTHALATE,
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SUMMARY

Platelet concentrates were shown to contain 18-38 mg/100 ml of a phthalate plasticizer (DEHP) which arose by migration from the vinyl plastic packs in which the platelets were prepared and stored. Transfusion of these platelets into 6 adult patients with leukemia resulted in peak blood plasma levels of DEHP ranging from 0.34 - 0.83 mg/100 ml (approximately 0.02 mg/100 ml plasma per mg DEHP administered per square meter of surface area). The blood levels fell mono-exponentially with a mean rate of 2.83 percent per minute and a half-life of 28.0 minutes. Urine was assayed by a method that would measure unchanged DEHP as well as all phthalic acid-containing metabolites. In two patients, at most 60 and 90% of the infused dose, respectively, was excreted in the urine collected for 24 hours post-transfusion. These estimates, however, could be high due to the simultaneous excretion of DEHP remaining from previous transfusions or arising from uncontrolled environmental exposures.

Di(2-ethylhexyl)phthalate (DEHP) is widely used as a plasticizer in the formulation of vinyl plastics. It serves to make the polymeric plastic pliable; in this role its concentration may reach 30-40% of the total weight of the finished product. In 1972 Jaeger and Rubin¹ reported the migration of this plasticizer from vinyl plastic bags into human blood stored for transfusion. The rate of migration was found to be 0.25mg/100ml blood/24 hours of storage at 4°C. Marcel and Noel^{2,3} have reported similar observations. In 1973 Jaeger and Rubin⁴ also reported on the migration of DEHP into platelet-rich plasma stored in vinyl plastic bags. The rate of plasticizer migration was found to be approximately 20mg/100ml plasma/24 hours of storage at room temperature. These results have recently been confirmed by Valeri, et al⁵.

DEHP has been found in a variety of tissues obtained at autopsy from humans who had received transfusions of blood stored in vinyl plastic bags¹. The plasticizer has also been identified in tissues of humans who have not received transfusions of blood nor had any direct contact of their own blood with a medical plastic device⁶. Presumably, these latter observations are due to environmental contamination⁷, although endogenous synthesis of DEHP in trace amounts in mammalian tissues has not been entirely ruled out.

Schulz and Rubin⁸ have reported that in rats intravenously administered DEHP (emulsified in a solution of bovine serum albumin) disappears from the circulation at a bi-exponential rate, the initial phase having a half-life of 4.5-9 minutes and the latter phase having a half-life of 22 minutes. The initial rapid phase was shown to be due to a rapid uptake into the liver. More recently⁹, DEHP solubilized in a non-ionic detergent (Tween 80) has been shown to disappear from rat blood at a single exponential rate having a half-life of 21 minutes. Under these conditions, accumulation in the liver does not occur. These data emphasize the influence of the physical state of DEHP on its fate in vivo.

Patients with leukemia frequently receive transfusions of platelet units that have been prepared and stored in vinyl plastic bags and thus contain high levels of migrated DEHP. This paper reports on the blood levels and kinetics of disappearance of DEHP in 6 such patients who received DEHP during transfusion of platelet-rich plasma that had been stored for 24 hours at room temperature in vinyl plastic bags. In addition, the urinary excretion of DEHP and its metabolic derivatives have been determined in two of these patients.

METHODS

Six adult thrombocytopenic males with acute nonlymphocytic leukemia who were being treated at NCI Baltimore Cancer Research Center were studied. All of the patients had received prior

transfusions with packed red blood cells and platelet concentrates. The patients were in stable clinical condition and had normal renal function as assessed by urinalysis and serum blood urea nitrogen and creatinine. Two patients (#2 and #3) had mild abnormalities of hepatic function, probably secondary to viral hepatitis.

Platelet concentrates (PC) were prepared in acid-citrate-dextrose (ACD) anticoagulant by plateletpheresis technique. Each unit of PC was stored in a final volume of 20-30ml in plastic bags (Fenwal PA220 transfer pack) for 22-26 hours at room temperature (approximately 24°C) with continuous gentle agitation. Six to eight units of PC were pooled just prior to administration. An aliquot of the pooled concentrate was centrifuged at 4000xg for ten minutes in glass test tubes and the platelet-poor plasma was separated and used for the measurement of DEHP.

The platelets were infused over a twenty to thirty minute period except in one patient (#5) who received a one-hour transfusion. A 6-7ml venous blood sample was obtained from the opposite arm prior to transfusion, immediately post-transfusion and every 15 minutes thereafter for one hour. Samples were drawn using glass syringes and were transferred to glass test tubes containing 1ml of ACD anticoagulant. These were kept in ice prior to separation of plasma for measurement of DEHP. Plasma

samples were maintained in the frozen state prior to assay. In two patients, urine was collected for the 24 hours pre-transfusion and for the 24 hours post-transfusion.

DEHP was assayed in plasma by the method of Piechocki¹⁰ involving the separation of the DEHP on a Celite 545 column. DEHP was measured by gas chromatography on a 6 foot 3% SE-30 column using a flame ionization detector and hexacosane (C26) as an internal standard. All samples were corrected by subtracting a blank value that was obtained by processing an aliquot of distilled water through the entire analytical procedure. This was done for each set of samples from each patient. The individual blank values ranged from 0.03-0.15 mg/100ml with a mean of 0.07 mg/100ml. Plasma samples were further corrected for dilution of the initial blood sample with ACD solution.

DEHP and all phthalic acid-containing metabolic intermediates of DEHP in urine were hydrolyzed to phthalic acid by the method of Shaffer, et al¹¹ as previously described¹. Following methylation with diazomethane, the phthalic acid content was determined by gas chromatographic analysis of the formed dimethylphthalate on a 6ft. column of 3% SE-30 using flame ionization detection. Hexadecane (C16) was used as an internal standard for quantitation. From the phthalic acid content of the hydrolyzed urine, the equivalent amount of DEHP could be calculated. This latter amount is presented as "DEHP equivalents" and it represents the amount

of DEHP that appears in urine unchanged as well as that which is excreted in the form of phthalic acid-containing metabolites. The amount of unchanged DEHP excreted into the urine was not separately determined.

The disappearance rate of DEHP from blood and the corresponding half-life was determined for each patient following calculation of the line of best fit of the data by a linear regression analysis.

RESULTS

In Table 1 it can be seen that the preparation and storage of platelets in vinyl plastic bags resulted in levels of contamination of DEHP ranging from 18-38 mg/100ml, thus confirming earlier reports^{4,5}. The total amount of DEHP administered to patients ranged from 26-82 mg (or 14-44 mg per square meter of surface area). It can be seen from the Table that prior to the transfusion, trace amounts of DEHP could be found in the plasma of 4 of the 6 patients. At the termination of the transfusion period, plasma levels ranging from 0.34 - 0.83 mg/100 ml were observed. These plasma levels correlated reasonably well with the dose of the administered DEHP in that the patient who received the lowest dose per square meter of surface area (patient #1) had the lowest blood level while the other patients receiving 2-2.5 times as much of the plasticizer had blood levels approximately 2-2.5 times as high.

In Figure 1 are shown the data for the disappearance of DEHP from the blood for each individual patient over a period of 60 minutes following transfusion. It can be seen that the blood levels fell in a mono-exponential fashion over this time period. In Table 2 are shown the rate constants and half-lives determined from the calculated line of best fit for each patient. The mean disappearance rate was 2.83 ± 0.45 percent per minute. This corresponded to a mean half-life of 28.0 ± 4.3 minutes.

In two of the patients, urine was collected prior to the platelet transfusion period and for 24 hours after the transfusion. The urine was assayed by a procedure (described in Methods) that measured DEHP as well as all phthalic acid-containing metabolites of DEHP including phthalic acid itself. The data, shown in Table 3, are presented as equivalent amounts of DEHP recovered in the urine. It can be seen that prior to the transfusion small amounts of DEHP equivalents (9.0 and 13.8 mgs) were excreted by the two patients. These amounts could possibly have arisen from the urinary excretion of residual amounts of DEHP received during prior transfusions. However, it should be pointed out that there have been reports of phthalic acid-containing molecules in the urine of a patient prior to open heart surgery and blood transfusions¹ and in the urine of normal controls¹². The sources of these compounds are not known but they could arise from environmental exposures. In any event, the 3-6 fold

increase in the amount of DEHP equivalents excreted during the 24 hour period after transfusion indicates excretion of a major portion of the DEHP associated with the platelet transfusions administered during this study. If the level of DEHP excreted during the pre-transfusion period is ignored, then the amounts excreted during the 24 hours post-transfusion can represent no more than 60-90% of the administered DEHP. If it is assumed that there is a continuous environmentally related level of excretion of phthalic acid-containing molecules, comparable to that seen during the pre-transfusion period, then the net difference between the pre-and post-transfusion periods would represent the amount arising from the exogenously administered DEHP. Thus, a correspondingly smaller (but still significant) fraction of the administered dose would be accounted for by excretion in the urine during the first 24 hours.

DISCUSSION

These results confirm earlier reports of the contamination of blood^{1,2,3} or blood products^{4,5} with a plasticizer, DEHP, following storage in vinyl plastic bags. They, furthermore, establish for the first time in humans the plasma levels that are attained following transfusions of DEHP-contaminated products. These levels average approximately 0.02 mg/100 ml plasma for each mg of DEHP administered per square meter of surface area.

The rate of disappearance of DEHP from plasma was mono-

exponential and relatively rapid (rate constant = $.0283 \text{ min}^{-1}$). Previous experiments in rats have shown that a suspension of DEHP, emulsified in serum albumin, disappeared from plasma bi-exponentially⁸ while a detergent-solubilized preparation disappeared mono-exponentially with a rate constant quite comparable to that shown here in humans⁹. Thus, based on kinetic behavior these results suggest that the DEHP that is administered to humans in vinyl plastic stored-blood products exists in a solubilized state rather than as discrete, suspended oil droplets. The exact nature of the physical state of DEHP following migration from a plastic surface into blood plasma is not currently known although Needham and Luzzi¹³ have recently observed droplets of oil in aqueous solutions stored in vinyl bags. Such information has important implications for the ultimate disposition of DEHP in the human body since previous studies in the rat have shown different patterns of tissue distribution for emulsified and detergent-solubilized DEHP^{8,9}.

It is of interest to note that the two patients with evidence of mild abnormalities of hepatic function (#2 and #3) did not have particularly unusual half-lives for DEHP disappearance. Although patient #2 did have the longest half-life (11.8 minutes) in Table 2, patient #3 with a half-life of 21.5 minutes was below the group mean and ranked only 3rd in the series of six. Since previous experiments in the rat¹⁴ have indicated that the liver is

the major site of metabolism of DEHP, these data suggest that the liver abnormalities in these two patients were not sufficiently severe to alter markedly their rate of DEHP disappearance from blood.

The urinary data indicate that a major portion of the administered DEHP is excreted within 24 hours although determination of the exact quantitative portion is complicated by the pre-transfusion excretion of significant amounts of DEHP-related material. Experiments with rats in which ¹⁴C-radiolabelled DEHP was given intravenously have indicated rather extensive biliary and fecal excretion of radioactivity. If the same mechanisms exist in humans, then determination of DEHP and/or its metabolites in feces might allow for an even greater quantitative recovery of the administered DEHP dose.

As indicated above, the assay procedure used for the urine samples does not distinguish between unchanged DEHP and phthalic acid-containing metabolites. Previous experiments with rats,^{8,15} however, have shown only trace quantities of unchanged DEHP in the urine with the major substances being water-soluble, phthalic acid-containing metabolites whose structures have been determined.¹⁵ Presumably these water-soluble metabolites comprise the DEHP equivalents found in the urine of the human patients in this study, but this has not been experimentally determined.

Autian¹⁶ has recently reviewed the toxicology of the phthalate plasticizers in animals and cell culture. However, the extensive use of vinyl-stored blood and blood products during the past decade has failed to reveal any definitive adverse effects of such storage in humans. In addition, the results presented here indicate a rapid clearance of DEHP from blood and extensive urinary excretion within 24 hours. However, these facts should not diminish the concern for any possible adverse biological effects of acute or chronic exposure to this compound, particularly when one considers the wide array of debilitated states for which blood or blood products are often transfused. For example, Jacobson, et al¹⁷ have recently reported altered hepatic morphology and function in rhesus monkeys chronically transfused for a period of one year with platelets prepared and stored in vinyl plastic bags. As long as these types of biologics are to be stored in vinyl bags and amounts of a foreign compound that are reported here are thus inadvertently administered to humans, the toxicologic potential of such exposure must be exhaustively pursued. An even better approach would be the development of alternate plastic formulations that do not result in appreciable migration of any chemical constituents.

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TABLE 1.

DEHP CONCENTRATION OF STORED PLATELETS AND PLASMA LEVELS

IN HUMANS FOLLOWING PLATELET TRANSFUSIONS

PATIENT #	TRANSFUSION VOL (ml)	DEHP Conc. of Platelet Conc. mg/100 ml	DEHP RECEIVED		PLASMA LEVEL OF DEHP (mg/100 ml)	
			Total mg	mg/m ² *	pre-trans- fusion	immediately post-trans- fusion
1	142	18.6	26.4	14.3	0.01	0.34
2	285	22.1	62.9	31.8	0.05	0.74
3	194	32.1	62.2	32.7	ND†	0.77
4	220	30.8	67.8	36.6	0.03	0.81
5	216	39.2	82.4	44.5	ND†	0.69
6	214	(lost)	--	--	0.03	0.83

*mg/square meter of surface area.

†None Detected, i.e. not different from assay blank.

TABLE 2.

DISAPPEARANCE RATES AND HALF-LIVES FOR DEHP

Patient #	Rate constant $\text{min}^{-1} \times 10^2$	$t_{1/2}$ min
1	3.32	20.9
2	1.66	41.8
3	3.22	21.5
4	2.12	32.7
5	4.75	14.6
6	<u>1.91</u>	<u>36.4</u>
Mean \pm S.E.		
	2.83 \pm 0.45	28.0 \pm 4.3

TABLE 3.

URINARY EXCRETION OF DEHP

<u>Patient #</u>	<u>Total DEHP received (mg)</u>	24 hr DEHP excretion (mg DEHP equivalents)		<u>max. % excreted</u>
		<u>pre-trans- fusion</u>	<u>post-trans- fusion</u>	
3	62.2	9.0 ^a	55.9 ^b	90
4	67.8	13.8	40.0	60

^a a 12 hour collection immediately prior to transfusion: 1250 ml urine.

^b not a complete 24 hour collection but does contain all the urine excreted during the first 8 hours and most of the urine excreted during the subsequent 16 hours: 1650 ml urine.

Figure 1. Time-course for DEHP disappearance from blood plasma following platelet transfusions in humans. Each figure represents the data from a single patient and is identified with the respective patient number.