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ACUTE PULMONARY PATHOLOGY AND SUDDEN DEATH IN RATS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF THE PLASTICIZER, DI(2-ETHYLHEXYL) PHTHALATE, SOLUBILIZED WITH TWEEN SURFACTANTS

Abbreviated Title:

Acute Toxicity of Solubilized DEHP

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3. Portions of the results presented in this paper were presented at 13th Annual Meeting of the Society of Toxicology, March, 1974 and at the FASEB meetings, April, 1974.

ABSTRACT

Acute Pulmonary Pathology and Sudden Death in Rats Following the Intravenous Administration of the Plasticizer, Di(2-ethylhexyl) phthalate, Solubilized with Tween Surfactants. C.O. Schulz, R.J. Rubin, and G.M. Hutchins. Toxicol. and Appl. Pharmacol. 00, 000-000.

Intravenous administration of 200-300 mg/kg of di(2-ethylhexyl)phthalate (DEHP) solubilized in aqueous solutions of several Tween surfactants caused respiratory distress in rats. There was a dose-dependent lethality with death generally occurring within 90 minutes after injection. The lungs from DEHP:Tween treated animals were enlarged, generally darkened, and in some cases showed hemorrhagic congestion. At doses of 200 mg/kg and above, the ratios of wet lung weight to body weight for rats treated with DEHP:Tween were significantly elevated over those for vehicle-injected or non-injected controls. Histologic examination of lungs from DEHP:Tween 80 treated rats revealed an edematous swelling of the alveolar wall and a marked infiltration of polymorphonuclear leukocytes. These effects were evident at doses as low as 50 mg DEHP/kg. Neither the overt symptoms nor the morphologic alterations resulting from DEHP:Tween administration could be reproduced by intravenous administration of aqueous Tween solutions alone. The absence of pulmonary

abnormalities following the intravenous administration of DEHP as an aqueous emulsion given either alone or even as soon as 2 minutes after pretreatment with Tween 80, suggests that the specific *in vivo* interaction between DEHP and Tween surfactants depends on the prior formation of water-soluble micelles of DEHP.

INDEX TERMS

Di(2-ethylhexyl)phthalate

DEHP

Tween Surfactants

Acute Pulmonary Pathology

Lethality

Tween-solubilized DEHP

INTRODUCTION

Within the past several years increased attention has been focused on the phthalate esters, a class of water-insoluble organic chemicals used primarily as plasticizers in polyvinyl chloride (PVC) formulations. As a result of their versatility and economy, phthalate esters are extensively produced and widely used in myriad applications (Graham, 1973). Recently, they have been identified as wide-spread environmental contaminants (Mayer, et al, 1972; Gross and Colony, 1973; Hites, 1973; Thomas, 1973; Wildbrett, 1973; Williams, 1973). Of particular importance to humans are the recent findings that phthalate esters are leached from PVC medical devices into the biological fluids which they contain (Jaeger and Rubin, 1972; Jaeger and Rubin, 1973a; Marcel, 1973; Needham and Luzzi, 1973; Valeri, et al, 1973).

Studies have been reported of the biological effects of the most widely used phthalate, di(2-ethylhexyl)phthalate (DEHP) (Jaeger and Rubin, 1973b) as well as of its distribution, metabolism, and excretion (Schulz and Rubin, 1973). Since DEHP is a water-insoluble liquid, a problem arises in finding a suitable vehicle for parenteral administration. Previous intravenous studies were performed using sonicated emulsions of

DEHP which were opaque and contained from 10^7 to 10^9 particles/ml with diameters ranging from 1.9 to 7.5 μm (Rubin and Schulz, 1974). However, when DEHP was sonicated in aqueous solutions of the Tween surfactants (polyoxyethylene sorbitan esters of fatty acids), optically clear solutions were obtained which contained no particles detectable by phase contrast microscopy (Rubin and Schulz, 1974). This paper describes the results of a study of the pathology and lethality associated with intravenous injections of this Tween-solubilized DEHP in rats.[3]

MATERIALS AND METHODS

Materials

DEHP was provided by the Monsanto Company, St. Louis, Missouri. Tween 20 (polyoxyethylene sorbitan monolaurate), Tween 60 (polyoxyethylene sorbitan monostearate), and Tween 80 (polyoxyethylene sorbitan monooleate) as well as arachidonic acid, 99% pure, were obtained from Sigma Chemical Co., St. Louis, Missouri. The rats were adult male Wistars (175-250g) from Charles River, Inc., Boston, Massachusetts.

Methods

Appropriate volumes of surfactant and 0.9% (w/v) aqueous sodium chloride were sonicated for approximately 1 minute using

a Branson Sonifier^{R} Cell Disruptor (Danbury, Conn.) with the standard flat tip probe. Clear solutions of DEHP were prepared by combining appropriate volumes of DEHP and the surfactant: saline solution and then sonicating at approximately 75 watt output until the solution became transparent and no visible droplets of DEHP appeared on the surface. Tween 60 solutions of DEHP required only brief sonication to achieve clarity (approximately 1 minute). Tween 80 preparations required five to ten minutes of sonication and Tween 20 preparations did not become completely transparent even after fifteen minutes of sonication. When used, dimethylsulfoxide (DMSO) was added to the transparent solution resulting from this sonication and mixed on a vortex mixer. At room temperature DEHP:Tween preparations remained clear for 30 to 60 minutes and then gradually became opaque. Upon minimal sonication, however, these solutions became transparent again. In all cases the concentration of DEHP in the solution was 50 mg/ml. Thus, different doses of DEHP were achieved by injecting different volumes of the DEHP solution. In each experiment control rats were injected with an equal volume of the appropriate vehicle alone.

Emulsions of DEHP in 4% bovine serum albumin (BSA) were prepared by probe sonicating mixtures of appropriate volumes

of DEHP and 4% bovine serum albumin (Fraction V) in 0.9% aqueous sodium chloride. This resulted in milky white emulsions. The homogeniety and stability of these emulsions has been demonstrated by adding a small amount of ^{14}C-labelled DEHP prior to sonication and then analyzing multiple samples for homogenous dispersion of radioactivity and reproducibility as a function of storage time.

Intravenous injections were administered over a period of approximately 15 seconds via the penis vein of rats that had been lightly anesthetized with ether. Rats were sacrificed by capitation. They were thoroughly exsanguinated and the lungs excised, rinsed in ice cold 0.9% saline, blotted dry, and weighed. One lung from each animal was fixed in 10% formaldehyde for histologic examination while the remaining lung was reweighed, then air dried at 105°C for at least twenty-four hours and weighed again in order to determine the ratio of wet lung weight to dry lung weight.

Formaldehyde-preserved lungs were imbedded in paraffin. Four-micron sections were stained with hematoxylin and eosin, and examined under a microscope.

RESULTS

Symptoms of acute respiratory distress were observed in rats receiving intravenous injections of 250 to 300 mg DEHP/kg

solubilized in a vehicle cortaining 10% Tween 80 and 25% dimethylsulfoxide (DMSO). These symptoms included watery secretions at the mouth and nostrils, irregular and gasping respiration, usually occuring within one minute, cyanosis, and lethargy frequently followed by death. Rats that died, did so usually within ninety minutes after the injection. Animals given equal volumes of the vehicle alone displayed none of these symptoms and no deaths were observed. The animals that survived this initial period generally began to show improvement and by the following day they were overtly indistinguishable from non-injected or vehicle-injected control rats.

Necropsy of rats which died following intravenous injections of DEHP in the Tween 80:DMSO vehicle revealed that the lungs were grossly enlarged and darkened compared to lungs from vehicle-injected control animals (Figure 1). In addition, the lungs were often covered with dark red-brown areas indicative of hemorrhagic congestion. The extent of this congestion was more variable from animal to animal than was the enlargement or the generalized darkening. Lungs from vehicle-injected control animals could not be distinguished from those from non-injected animals.

Table 1 shows the results of a study designed to obtain a more quantitative assessment of the pulmonary effect of DEHP solubilized in Tween 80 and DMSO. The data include only those values determined for lungs from rats which survived the critical ninety minute period and were sacrificed at that time by decapitation. Lungs from rats which died prior to ninety minutes were not included because blood trapped in the pulmonary vasculature gave artificially high weights. As indicated in Table 1 the vehicle by itself had no significant effect on lung weights nor any lethality in the range studied. Overtly, vehicle-injected control rats did not demonstrate any behavior or symptoms which would distinguish them from saline-injected rats or from rats that are non-injected but handled like the injected animals. On the other hand, intravenous administration of 50 mg/ml solutions of DEHP in the vehicle produced respiratory distress symptoms at all levels shown. Normalized wet lung weights were clearly elevated at 200 mg DEHP/kg but because of the variability seen at this dose the elevation was not statistically significant. However, wet lung weights were significantly elevated at a dose of 250 mg DEHP/kg. At a dose of 300 mg DEHP/kg three of the five treated rats died and the lungs of the two surviving rats were grossly enlarged.

The results of an experiment in which DEHP was solubilized only in 13.3% Tween 80 are shown in Table II. Rats injected with 50 mg/ml solutions of DEHP in this vehicle showed the same symptoms of respiratory distress as animals treated with the DMSO-containing vehicle and the same enlargement and darkening of the lungs as seen in Figure 1 were apparent upon necropsy. Again, at the levels studied, the vehicle by itself had no effect. On the other hand, the data indicate a DEHP dose-related increase in wet lung weight and lethality with a statistically significant elevation in the former at a dose of 200 mg DEHP/kg. The ratio of wet to dry lung weight was calculated for each animal in Table II. At no dose level was this ratio significantly higher for DEHP-treated rats than for vehicle-injected animals. Thus, the increase in wet lung weights shown in Table II is not solely the result of an accumulation of plasma water in the lung tissue, ruling out edema fluid as the sole cause of the increased total weight.

The intravenous administration of aqueous emulsions of DEHP in acacia or BSA solutions had not previously revealed any respiratory distress syndrome. Doses of DEHP as high as 500 mg/kg administered as 40 to 100 mg/ml emulsions in either 4% bovine serum albumin or 3% acacia solution, which elicited

no symptoms of respiratory distress, were found to result in no gross alteration in lung appearance or weight nor was any lethality observed.

Experiments were undertaken to evaluate the role of a non-specific effect of the intravenous administration of any water-insoluble liquid solubilized in a surfactant solution. Corn oil was sonicated in a Tween 80, DMSO, saline (10:25:65) vehicle to give an optically clear solution of 50 mg corn oil/ml. An intravenous dose of 300 mg corn oil/kg (6.0 ml/kg) of this solution caused no overt indications of respiratory distress. Moreover, ninety minutes after injection the lungs appeared grossly normal and the mean normalized wet lung weight for the corn oil treated rats was 4.22×10^{-3} vs. 4.96×10^{-3} for vehicle-treated rats. Although there was a tendency toward lower lung weights, the decrease was not statistically significant.

Di(2-ethylhexyl)sebacate (DEHS) was solubilized in a 13.3% Tween 80:saline vehicle. DEHS is physically and chemically quite similar to DEHP and it is used for many of the same industrial applications. Intravenous doses of 300 mg DEHS/kg caused no overt symptoms of respiratory distress among the treated animals nor were the lungs grossly abnormal. Moreover, the mean ratio of wet lung to body weight for these animals was

4.89 x 10^{-3} which was not significantly different from control values.

In another series of experiments, 3 groups of rats were pretreated with intravenous injections of the 13.3% Tween 80 in saline vehicle. At 24 hours, 90 minutes, or 2 minutes after this pretreatment one-half of each group of rats were given intravenous injections of 250 mg DEHP/kg as a 40 mg/ml emulsion in 3% BSA solution. As a control, the remaining rats were given 3% BSA solution at the same time intervals. Even when the DEHP emulsion was administered within two minutes of the Tween vehicle injection, there were no symptoms of respiratory distress, the lungs were not grossly abnormal, and the normalized wet lung weights were not significantly elevated. There was no lethality associated with any of these treatments. The results of these experiments indicate that the observed effects of DEHP:Tween 80 injections are dependent on the actual physical dissolution of DEHP in the Tween vehicle.

The acute lung effects were found not to be specific to Tween 80. When DEHP was solubilized in saline solutions of two other Tween surfactants and injected intravenously, similar respiratory effects were observed. As shown in Table III a dose of 200 mg DEHP/kg in the Tween 60 vehicle caused a significant increase in wet lung weight. An equal dose of

DEHP in the Tween 20 vehicle resulted in grossly enlarged lungs although the large variability in lung weights precluded a statistically significant level. These experiments also indicated a trend toward greater lethality with the Tween 60 and Tween 20 vehicles as compared to Tween 80.

Figure 2 shows, on the left side, a histological section from the lungs of a rat injected with vehicle and, on the right, from a rat injected with DEHP in Tween 80. The lesion is characterized by an extensive edematous thickening of the inter-alveolar septa and a marked engorgement of the pulmonary vasculature with polymorphonuclear leukocytes. Although not shown here, examination of many sections from several animals indicate that the leukocytes, following margination on the capillary endothelium, migrate across the septal tissue into the air spaces themselves. In focal areas the migration of leukocytes is accompanied by a transudation of cellular and proteinaceous material from the bloodstream accounting for the hemorrhagic appearance and elevated dry lung weights observed in the DEHP-treated animals. No evidence of platelet aggregrates, fibrin thrombi, or particulate infiltration were observed in any of the specimens.

Studies relating the severity of the morphologic alterations to the dose (Table IV) and the time course of its development (Table V) were carried out. All samples were coded so that the pathologist grading the specimens was unaware of their treatment history. In Table IV it can be seen that the lungs from vehicle-injected rats were histologically indistinguishable from those from non-injected rats. Both showed some evidence of slight polymorphonuclear leukocytic invasion. It should be noted that even though a 50 mg DEHP/kg dose caused no overt symptoms of respiratory distress and no grossly visible lung enlargement, it did cause a very definite pathological response.

The time course data in Table V indicate that the lesion is fully developed within 15 minutes after the injection and persists for at least two days thereafter even though the treated animals are overtly asymptomatic within one day after the injection.

Recently, Silver and co-workers (1974) have described respiratory distress and sudden death in rabbits given arachidonic acid intravenously. Aspirin pretreatment prevented these effects, leading the authors to conclude that they were

mediated by the release of a prostaglandin. Table VI summarizes the results of an experiment designed to determine whether aspirin might protect rats against the lethal effect of the intravenous administration of Tween-solubilized DEHP and thus indicate a possible role of prostaglandins. Arachidonic acid was used as a positive control in this experiment. The data show that like rabbits, rats are also sensitive to arachidonic acid with 9 out of 9 animals dying within a few minutes following pretreatment with either saline or sodium acetate. Examination of the lungs from two of these rats indicated that they were grossly enlarged and hemorrhagic. On the other hand, the uniform dark red discoloration characteristic of the response to solubilized DEHP was not observed in these lungs. Among the seven rats pretreated wtih aspirin, six survived the treatment with arachidonic acid. Although all survivors displayed signs of rapid respiration for a brief period after injection, they appeared completely normal five minutes after the injection. Four hours after arachidonic acid two of these rats were sacrificed and their lungs were not abnormal either in gross appearance or in wet weight. On the other hand, the data indicate that aspirin pretreatment had no protective effect against intravenous injections of 300 mg DEHP/kg.

All 6 of these animals displayed the previously described symptoms of respiratory distress and died between 10 and 57 minutes after injection. At autopsy their lungs were enlarged, engorged with blood, and hemorrhagic in spite of pretreatment doses of aspirin in 4 of the animals that were twice that required to protect against the direct effects of arachidonic acid.

DISCUSSION

The results presented in this paper indicate that in rats the intravenous injection of Tween solubilized DEHP causes an acute pulmonary reaction which, if sufficiently severe, leads to respiratory distress and even death. The lesion is characterized by very rapid development, the leukocytic engorgement being seen as early as 15 minutes; in some cases death has been seen within two minutes of injection.

The events that lead to the pathological response are not clear at this time. Thus, it is not known whether the solubilized DEHP produces a direct, injurious effect on the pulmonary epithelium or on the endothelial cells of the vasculature resulting in a inflammatory response. Alternatively, the Tween-solubilized DEHP could produce some sort of injury to the polymorphonuclear leukocytes causing them to adhere to (or marginate on) the endothelial cells in the lung. Following

this adhesion, possible degranulation and fragmentation of the leukocytes could give rise to the release of intracellular components that would compromise the integrity of the alveolar membrane. Other types of chemical mediators could also be released that contribute to the lethal response. Such mechanisms have been proposed for endotoxin and other types of shock which result in the same light microscopic picture of lung pathology as shown here (Balis, et al, 1974).

The possibility can be raised that the water-insoluble DEHP separates from the plasma following its injection as a solubilized preparation and is trapped as insoluble droplets in the lung microvasculature. This can be ruled out, however, by the observation in our laboratory that the in vitro addition of Tween-solubilized DEHP to rat plasma in the ratio to be expected from in vivo injection does not result in the formation of any particles visible by phase contrast microscopy.

The acute lung effect, then, is the result of some specific interaction of DEHP and the Tween surfactant. In fact, the DEHP must be physically solubilized in the Tween solution in order for the effect to be observed as shown by the experiments in which a Tween solution and a DEHP emulsion were administered sequentially within 2 minutes to the same rat without effect.

The specific interaction between DEHP and Tween surfactants could result from the potentiation of a toxic effect inherent in either component of the mixture. This could come about either by an increased tissue accumulation or increased tissue sensitivity. No significant difference in lung content of DEHP has been found following emulsified or Tween-solubilized DEHP (Schulz and Rubin, 1973; Rubin and Schulz, 1974). However, the possibility remains that the Tween might make some cellular or subcellular site of toxic action more accessible to the small amounts of DEHP present in the lung. With regard to an increased sensitivity, it should be pointed out that intravenous doses of non-solubilized DEHP as high as 500 mg/kg do not cause respiratory difficulties or gross lung abnormalities. Thus, the combined effect of DEHP and Tween does not appear to be an extension of any known toxicologic response to DEHP alone.

With regard to the possibility of an enhanced toxicity to the Tween surfactant, it should be pointed out that no lethal or respiratory effects have been observed in rats in this laboratory with intravenous doses of Tween 80 up to twice as large as the biggest dose reported in this paper. Krantz, et al (1948) have reported a comparable lack of toxicologic effect of large doses of Tween 20 in rats. These observations would

argue against an extension of a known toxicity of the Tweens in rats. In dogs, Krantz, et al (1948) and Marks and Kolmen (1971) have reported that the intravenous injection of Tween 20 results in "histamine shock" which is characterized by a generalized circulatory and respiratory failure and which is protected against by pre-treatment with anti-histamines. However, the absence of platelet proliferation or aggregation which are seen in Tween 20-treated dogs (Krantz, et al, 1948 and Marks and Kolmen, 1971), and the observation in our laboratory that an anti-histamine (diphenhydramine at a dose of 10 mg/kg i.p.) failed to prevent the acute toxic effects of Tween-solubilized DEHP argue against a role of histamine in the reported effects of intravenous DEHP and Tween surfactants.

Thus, the available evidence suggests that the lung pathology observed here is not due to an extension of any known, inherent toxicity of the individual components. The exact mechanism for the toxicity of solubilized micelles of DEHP remains to be determined.

Moorhatch and Chiou (1974) have shown that dilute aqueous solutions of Tween surfactants are effective in leaching DEHP plasticizer from polyvinylchloride (PVC) bags. Since

Tween surfactants are used to solubilize fat-soluble vitamins for multivitamin infusion therapy, it is possible that human patients could receive small doses of Tween-solubilized DEHP intravenously if the multivitamin preparation is administered from a PVC bag. Thus, it would seem to be important to determine the biological effects of relatively slow, long-term infusion of low levels of DEHP and Tween surfactants, since in the studies reported here relatively rapid injections were used.

Another area which requires further investigation is the possible biological effect of chronic oral injestion of phthalate ester plasticizers either alone or in combination with Tween surfactants. Since Tween surfactants are approved for food applications (Food Additives Amendment) and the phthalates are environmentally distributed, it is important to determine whether these two classes can interact to produce a biological effect after oral ingestion. Moreover, it is important to learn whether natural surfactants such as bile salts in the gut have the capability to form solubilized micelles of DEHP which might then enter the bloodstream and give rise to subacute alterations in the lungs or other tissues.

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REFERENCES

1. Balis, J.V., Gerber, L.I., Rappaport, E.S. and Neville, W.E. (1974) "Mechanisms of Blood-Vascular Reactions of the Primate Lung to Acute Endotoxemia". Exp. & Mol. Path. 21:123-137.

2. Byers, J.M., Soin, J.S., Fisher, R.S. and Hutchins, G.M. (1974) "Acute Alveolitis in Intravenous Narcotics Abuse". Arch. Path. In Press.

3. Dale, H.H. and Laidlaw, P.P. (1919) "Histamine Shock". J. Physiol. London, 52:355.

4. Food Additives Ammendment to the Food, Drug, and Cosmetics Act, Subparts E-121.1008 and E-121.1009, Federal Register (May 7, 1969) 34 FR 7373.

5. Graham, P.R. (1973) "Phthalate Ester Plasticizers: Why and How They are Used". Environ. Health Perspectives 3:3.

6. Gross, Frederick C. and Colony, Joe A. (1973) "The Ubiquitous Nature and Objectionable Characteristics of Phthalate Esters in Aerospace Technology". Environ. Health Perspectives 3:37.

7. Hites, Ronald A. (1973) "Phthalates in the Charles and Merrimack Rivers". Environ. Health Perspectives 3:17.

8. Jaeger, Rudolph, J. and Rubin, Robert J. (1972) "Migration of a Phthalate Ester Plasticizer from Polyvinyl Chloride Blood Bags into Stored Human Blood and its Localization in Human Tissues". New England Journal of Medicine 287:1114.

9. Jaeger, Rudolph, J. and Rubin, Robert J. (1973a) "Extraction, Localization, and Metabolism of Di(2-ethylhexyl)phthalate from PVC Medical Devices". Environ. Health Perspectives 3:95.

10. Jaeger, Rudolph J. and Rubin, Robert J. (1973b) "Some Pharmacologic and Toxicologic Effects of Di(2-ethylhexyl)phthalate (DEHP) and Other Plasticizers". Environ. Health Perspectives 3:53.

11. Krantz, J.C., Jr., Carr, C.J., Bird, J.G., and Cook, S. (1948) "Sugar Alcohols XXVI. Pharmacodynamic Studies of Polyoxyalkylene Derivatives of Hexitol Anhydride Partial Fatty Acid Esters". J. Pharmacol. and Exptl. Therap. 93:188.

12. Marcel, Yves, L. (1973) "Determination of Di(2-ethylhexyl) phthalate Levels in Human Blood Plasma and Cryoprecipitates". Environ. Health Perspectives 3:119.

13. Marks, L.S. and Kolmen, S.N. (1971) "Tween 20 Shock in Dogs and Related Fibrinogen Changes". Am. J. of Physiol. 220.

14. Mayer, Foster, L., Jr., Stalling, David L., and Johnson, James L. (1972) "Phthalate Esters as Environmental Contaminants". Nature 238:411.

15. Moorhatch, P., and Chiou, W.L. (1974) "Interaction between Drugs and Plastic Intravenous Fluid Bags, Part II:Leaching of Chemicals from Bags Containing Various Solvent Media". Amer. J. Hosp. Pharm. 31:149.

16. Needham, T.E., Jr. and Luzzi, L.A. (1973) "Particulate Matter in Polyvinyl Chloride Intravenous Bags". New England J. of Medicine 289:1256.

17. Rubin, R.J. and Schulz, C.O. (1974) "Influence of the Physical State of the Plasticizer, Di(2-ethylhexyl) phthalate (DEHP) on its Biological Disposition and Action". Paper presented at 13th Annual Meeting of the Society of Toxicology, Wash., D.C. March, 1974.

18. Schulz, C.O. and Rubin, R.J. (1973) "Distribution, Metabolism, and Excretion of Di(2-ethylhexyl)phthalate in the Rat". Environ. Health Perspectives 3:123.

19. Silver, M.J., Hoch, W., Kocsis, J.J., Ingerman, C.M., and Smith, J.B. (1974) "Arachidonic Acid Causes Sudden Death in Rabbits". Science 183:1085.

20. Thomas, Gordon, H. "Quantitative Determination and Confirmation of Identity of Trace Amounts of Dialkyl Phthalates in Environmental Samples". Environ. Health Perspectives 3:23.

21. Valeri, C.R., Contreras, T.J. Feingold, H., Sheibley, R.H. and Jaeger, R.J. (1973) "Accumulation of Di(2-ethylhexyl) phthalate (DEHP) in Whole Blood, Platelet Concentrates and Platelet Poor Plasma". Environ. Health Perspectives 3:103.

22. Wildbrett, G. (1973) "Diffusion of Phthalic Acid Esters from PVC Milk Tubing". Environ. Health Perspectives 3 49.

23. Williams, D.T. (1973) "Gas Chromatographic Determination of Low Levels of Di(2-ethylhexyl)Phthalate in Soy Oil". J. of the AOAC 56:181.

Figure 1 - COMPARISON OF LUNGS FROM RATS INJECTED WITH DEHP IN THE TWEEN 80:DMSO:SALINE VEHICLE OR WITH THE VEHICLE ALONE.

The lungs on the left are from a rat which was sacrificed and exsanguinated 60 minutes after an intravenous injection of 250 mg DEHP/kg as a 50 mg/ml solution in 10% Tween 80:25% DMSO:saline. The lungs in the center are from a rat which was sacrificed and exsanguinated 60 minutes after an intravenous injection of 6 ml/kg of the vehicle alone. The lungs on the right are from a rat which died 15 minutes after an intravenous injection of 300 mg DEHP/kg as a 50 mg/ml solution in 10% Tween 80:25% DMSO:saline.

Figure 2 - COMPARISON OF MICROSCOPIC SECTIONS OF LUNGS FROM RATS INJECTED WITH DEHP SOLUBILIZED IN TWEEN 80: SALINE OR WITH THE VEHICLE ALONE (X160).

The section on the left is from the lung of a rat which was sacrificed and exsanguinated 90 minutes after an intravenous injection of 5 ml/kg of 13.3% Tween 80 in saline. The section on the right is from the lung of a rat which was sacrificed and exsanguinated 90 minutes after an intravenous injection of 250 mg DEHP/kg as a 50 mg/ml solution in 13.3% Tween 80:saline. The right hand section shows the edamatous thickening of the interalveolar septa and the engorgement of the alveolar wall with polymorphonuclear leukocytes, which are characteristic of the acute alveolar inflammation resulting from intravenous administration of Tween-solubilized DEHP.

TABLE I

Effect of Intravenous Injections of DEHP in a Tween 80:DMSO Vehicle on Lethality and Lung Weight in Rats

DEHP Dose[A] (mg/kg)	Lethality Among DEHP Treated Rats[B] (No. Dead/No. Treated)	Ratio of Wet Lung Weight to Body Weight ($\times 10^3$)[C]	
		Vehicle-Injected Controls[D]	DEHP Treated Rats
200	0/6	4.94 ± 0.20 (6)	5.92 ± 0.69 (6)
250	0/5	4.75 ± 0.17 (5)	5.87 ± 0.16 (5)*
300	3/5	4.97 ± 0.16 (10)	7.46 [6.81-8.12]

[A] DEHP was administered as a 50 mg/ml solution in 10:25:65 Tween 80:DMSO:Saline. Each dose was achieved by administering a different volume of this solution. Controls received a corresponding volume of vehicle alone.

[B] There was no lethality among the vehicle-injected rats.

[C] Values are reported as the mean ± the standard error with the number of animals in parenthesis. Values are reported only for animals surviving for 90 minutes after injection. The bracketed values for the 300 mg/kg dose represent the range for the two animals surviving this dose.

[D] The ratio of lung weight to body weight for each vehicle-injected control group was not significantly different from a mean value of $4.69 \pm 0.16 \times 10^{-3}$ for 10 non-injected controls.

* Significantly different from vehicle injected controls ($p<0.005$)

TABLE II

Effect of Intravenous Injections of DEHP in a Tween 80 Vehicle on Lethality and Lung Weight in Rats

DEHP Dose[A] (mg/kg)	Lethality Among DEHP Treated Rats[B] (No. Dead/No. Treated)	Ratio of Wet Lung Weight to Body Weight ($\times 10^{-3}$)[C]	
		Vehicle Injected Controls[D]	DEHP Treated Rats
200	0/10	5.02 ± 0.15 (10)	5.85 ± 0.17 (10)*
250	4/17	4.78 ± 0.14 (10)	6.18 ± 0.36 (8)*
300	10/15	4.75 ± 0.12 (13)	7.17 ± 0.62 (5)*

[A] DEHP was administered as a 50 mg/ml solution in 13.3% Tween 80 in saline. Each dose was achieved by administering a different volume of this solution. Controls received a corresponding volume of vehicle alone.

[B] There was no lethality among the vehicle-injected rats.

[C] Values are reported as the mean ± the standard error with the number of animals in parenthesis. Values are reported only for animals surviving for 90 minutes after injection.

[D] The ratio of wet lung weight to body weight for each vehicle-injected control group was not significantly different from a mean value of $4.69 \pm 0.16 \times 10^{-3}$ for 10 non-injected controls.

* Significantly different from vehicle injected controls ($p<0.005$).

TABLE III

Effect of Intravenous DEHP Solubilized in Other Tween Solutions on Lethality and Lung Weight in Rats

Tween Surfactant	DEHP Dose[A] (mg/kg)	Lethality Among DEHP Treated Animals[B] (No. Dead/No. Treated)	Wet Lung to Body Weight Ratios ($\times 10^3$)[C] Vehicle Injected Controls	DEHP Treated Rats
Tween 60	200	1/11	4.69 ± 0.17 (9)	5.53 ± 0.32 (10)*
	250	5/5	5.06 ± 0.14 (5)	———
Tween 20	200	3/10	5.24 ± 0.29 (6)	6.48 ± 0.82 (7)

[A] DEHP was administered as a 50 mg/ml solution in a 13.3% solution of the indicated Tween surfactant. Each dose was achieved by administering a different volume of these solutions. Vehicle injected controls received a corresponding volume of vehicle alone.

[B] There was no lethality among the vehicle-injected control rats.

[C] Values are reported as the mean ± the standard error with the number of animals in parenthesis. Values are reported only for those animals which survived for 90 minutes after injection.

* Significantly different from vehicle injected conrols ($p<0.05$).

TABLE IV

Relationship Between Dose and Severity of the Lesion

Treatment[A]	No. of Animals	Graded Severity of the Lesion[B]
None	2	1,1
4 ml/kg Vehicle[C]	2	1,1
5 ml/kg Vehicle[C]	3	1,1,1
50 mg DEHP/kg[D]	2	2½, 3
100 mg DEHP/kg[D]	2	1½, 2
150 mg DEHP/kg[D]	1	3
200 mg DEHP/kg[D]	1	3½
250 mg DEHP/kg[D]	1	3½

[A] Animals were sacrificed 90 minutes after injection.

[B] The grading is based on the density of polymorphonuclear leukocytes as well as the degree of alveolar wall thickening and is presented on a scale of 1 for minimal alterations to 4 for extensive changes. A value of 1/2 is assigned to intermediate evaluations. Each grade is the mean observation of 3-4 sections taken from each lobe of the lung.

[C] Vehicle is 13.3% Tween 80 in saline.

[D] DEHP is administered as the appropriate volume of a 50 mg/ml solution in 13.3% Tween 80:saline.

TABLE V

Relationship Between Time from Injection to Sacrifice and Severity of Lesion

Time from Injection[A] to sacrifice	No. of Animals	Graded Severity[B] of lesion
15 minutes	1	3
30 minutes	1	2
90 minutes	1	3½
29 hours	2	2, 2½
50 hours	2	2,2

[A] All animals were injected with 200 mg DEHP/kg as a 50 mg/ml solution in 13.3% Tween 80:saline.

[B] The grading is based on the density of polymorphonuclear leukocytes as well as the degree of alveolar wall thickening. The scale is as indicated in Table IV.

TABLE VI

Comparison of the Protective Effect of Aspirin on Sudden Death Induced by Arachidonic Acid and Tween-Solubilized DEHP

Intraperitoneal Pretreatment	Intravenous Administration 2 hours later	Lethality (No. Dead/No. Treated)
Saline	20 mg/kg Arachidonic Acid[A]	4/4
0.6M Sodium Acetate	20 mg/kg Arachidonic Acid[A]	5/5
50 mg/kg Aspirin[B]	20 mg/kg Arachidonic Acid[A]	1/7
50 mg/kg Aspirin[B]	300 mg/kg DEHP[C]	2/2
100 mg/kg Aspirin[B]	300 mg/kg DEHP[C]	4/4

[A] 10 mg/ml in 0.1M Sodium Carbonate Solution.

[B] 25 mg/ml in 0.6M Sodium Acetate Solution.

[C] 50 mg/ml in 13.3% Tween 80:Saline Solution.

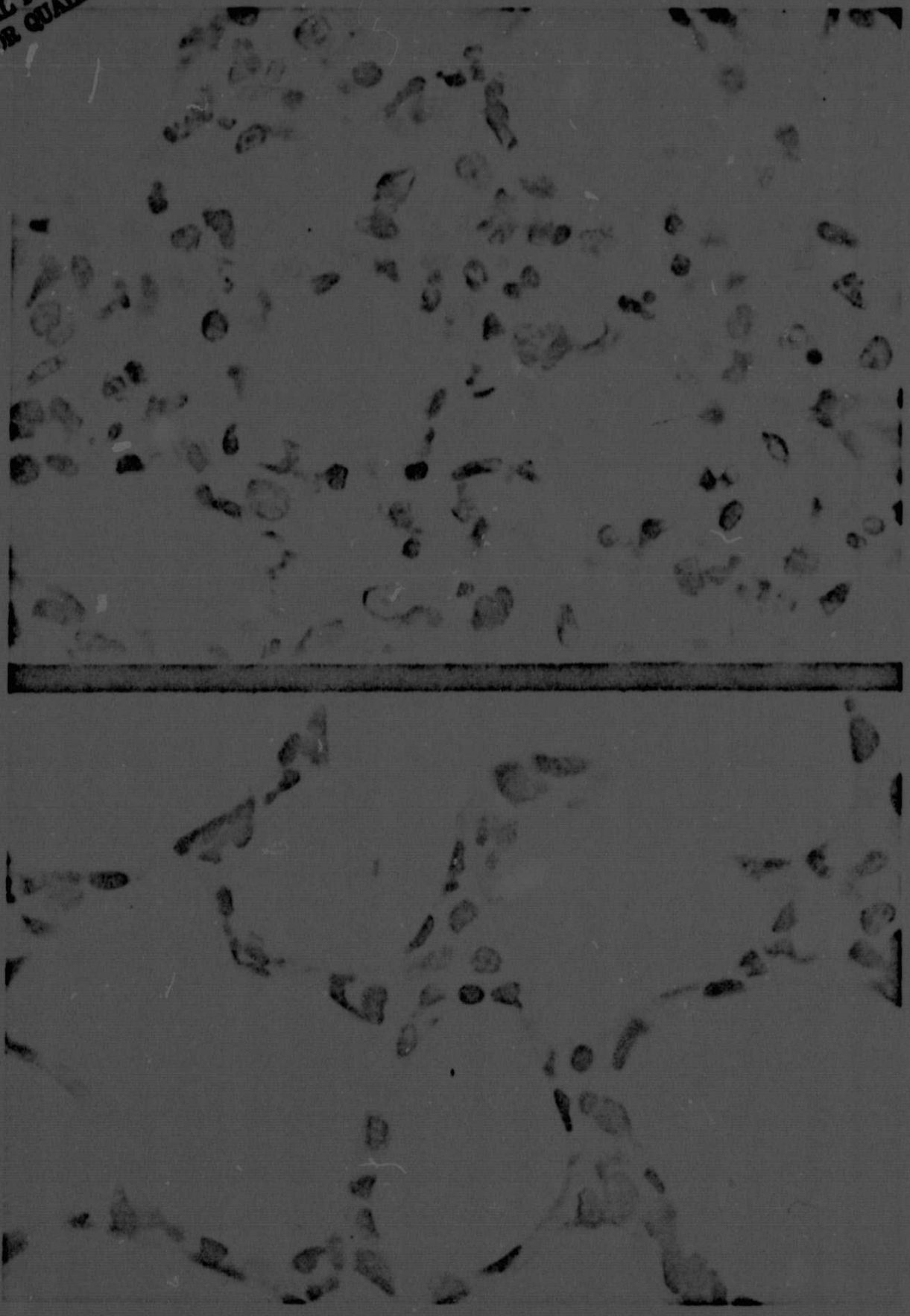

FIG. 2

FIG. 1