Toxicological Studies on Certain Medical Grade Plastics Sterilized by Ethylene Oxide

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Ethylene oxide gas sterilization of plastics used in medical and pharmaceutical applications presents a toxicity problem which at the present time remains largely unsolved due to the increasing complexity of the plastic itself. A series of preliminary experiments was conducted to survey the problem and to present evidence of the toxic potential of plastics having a variety of chemical and physical properties. It was demonstrated that residual ethylene oxide caused extensive blood hemolysis and death to cells grown in culture.

ETHYLENE OXIDE appears to be an ideal sterilant for many heat sensitive items, but as a chemical agent it is recognized as being toxic in both its liquid and vapor phases or when prepared as a solution (1). In recent years ethylene oxide sterilization has become an important tool to biomedical personnel engaged in duties where sterilization by autoclaving is either impractical or, as in the case of many of the new plastic devices, is destructive to the product. The introduction of gas sterilization of plastics has raised questions as to the possible entrapment of ethylene oxide in a plastic, which may cause a toxic effect when placed in contact with living tissues, and the effect sorbed ethylene oxide will have on possible changes in the physical-chemical properties of the medical plastic. Certain aspects of these questions have been only partially answered in the medical literature.

Hirose *et al.* (2) and Clarke *et al.* (3) have found that plastic tubings which have been ethylene oxide sterilized can cause significant hemolysis when placed in contact with human blood. This condition was found not to exist if the materials were aerated for at least 5 days after sterilization. Similarly, Royce and Moore (4) have demonstrated that, on human volunteers, vesicular lesions occurred on the fingers, hands, and forearms when the ethylene oxide concentration in sterilized rubber gloves was greater than 2 mg./Gm. of rubber. It has been demonstrated (5) that higher concentrations of the epoxide can be taken up by polyethylene, gum rubber, and plasticized polyvinyl chloride (PVC) which reemphasizes the problem of gas entrapment in plastics. Bain and Lowenstein (6) in 1967 found that when mixed leukocyte cultures are incubated in disposable plastic tubes sterilized with ethylene oxide, the cell survival is severely affected by a toxic residue left on the plastics. The residue dissipated after 4 or 5 months' storage at room temperature, and the cell survival returned to the value obtained with ultraviolet sterilized control tubes. There have been other reports of the persistence of toxic effects after sterilization with ethylene oxide (7–12), as well as many references to the over-all procedures to be used in an effective sterilization of pharmaceutical and medical supplies (13).

This paper presents data on the ability of ethylene oxide to remain entrapped within a nonclosed system, such as a surgical tubing, gas washing bottle, plastic syringe, or plastic bottle at various temperatures above the boiling point, and also relates the hemolyzing ability of known amounts of ethylene oxide to that of freshly gas sterilized plastic pharmaceutical products. Some preliminary data on the effects of ester type plasticizers upon the sorption of ethylene oxide into PVC products are also introduced.

EXPERIMENTAL

The Effect of Plasticizer Concentration on the Uptake of Ethylene Oxide by Polyvinyl Chloride—Because literature references suggested that plasticized polymers were producing the greatest number of toxic responses following ethylene oxide sterilization, the following flexible PVC formulations were prepared for sterilization.

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Sample A—One hundred grams polyvinyl chloride,[1] 3 Gm. dibutyltin maleate heat stabilizer (M and T Chemical Co., Rahway, N. J.).

Sample B—One hundred grams PVC, 52 Gm. di-2-ethylhexyl phthalate plasticizer (Eastman Chemical Co., Bristol, Tenn), 5 Gm. epoxidized soya oil (Rohm & Haas, Philadelphia, Pa.), 2 Gm. calcium-zinc soap stabilizer (Advance Chemical Co., New Brunswick, N. J.).

Sample C—One hundred grams PVC, 20 Gm. di-2-ethylhexyl phthalate, 5 Gm. epoxidized soya oil, 2 Gm. calcium-zinc soap.

The above formulations were then processed in the following manner. The polymer and the additives (in the desired concentration) were placed into a 1-gal. laboratory Hobart mixer and mixed for 30 min. The films of the polymers were prepared by milling them on a Farrell-Birmingham mill with a chromium-plated, variable-speed roller. Prior to milling each film, the rollers were carefully cleaned with practical grade stearic acid. The temperature of the rollers was maintained at 175° C., and the duration of the milling was 5 min. During the final phase of the operation, the separation of the rollers was adjusted so as to furnish films having thicknesses in the range of 0.040–0.045 in. Cut samples $5^7/_8 \times 5^7/_8$ in. from the milled stock were then press polished on the Dake compression press. The molds containing the samples were then heated for 3.5 min. at 35,000 lb. of pressure and rapidly cooled to 120° before removing the test sample from the mold. All samples were finally allowed to condition at room temperature and 50% relative humidity for 48 hr. prior to their use. The 3 PVC plastics, containing 0, 15.8, and 32.7% di-2-ethylhexyl phthalate, were sterilized in a 10 × 16-in. Cryotherm experimental gas sterilizer.[2] The specific sterilization involved prehumidifying the test samples in the chamber at 50% relative humidity, 57° C., under 660 mm. of Hg vacuum for 1 hr. A mixture of 11.0% ethylene oxide, 78.3% trichlorofluoromethane[3] (CCl_3F), 10.7% dichlorodifluoromethane[4] (CCl_2F_2) was added to the chamber until a pressure of 16 p.s.i.g. was reached. The plastics used were then sterilized for 4 hr. At the end of this time interval, a vacuum of 660 mm. of Hg was placed on the system in order to remove the ethylene oxide–CCl_3F–CCl_2F_2 mixture. The samples were analyzed by a procedure previously described by Gunther (5). A plot of the concentration of ethylene oxide detected *versus* the concentration of plasticizer is presented in Fig. 1. These data were obtained on the plastics after they had been aerated at room temperature for 24 hr.

Ethylene Oxide Solubility Studies of a Series of Dialkyl Benzene Carboxylate Plasticizers—Because the experiments described in the preceding section suggested that the presence of an ester-type plasticizer in PVC permits a greater degree of ethylene oxide sorption to occur, an investigation of the solubility of this gas in a series of phthalic acid esters was undertaken. It was hoped that this information would help in the rationalization of the fact that plasticized PVC plastics produce a greater degree of toxicity following sterilization.

[1] Geon 101EP. B. F. Goodrich Co., Cleveland, Ohio.
[2] American Sterilizer Co., Erie, Pa.
[3] Freon 11. E. I. du Pont de Nemours and Co., Wilmington, Del.
[4] Freon 12. E. I. du Pont de Nemours and Co., Wilmington, Del. (commonly known as Cryoxide).

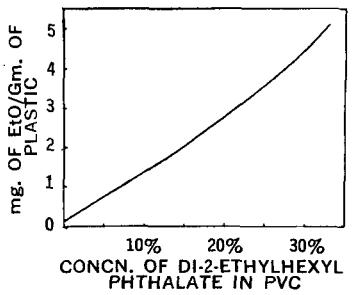

Fig. 1—A plot of di-2-ethylhexyl phthalate concentration in polyvinyl chloride versus *sorption of ethylene oxide after 24 hr. aeration.*

Description of Test System—Pure ethylene oxide vapor was distilled into a 125-ml. gas washing bottle containing an accurately weighed sample. The bottle was stoppered with a ground-glass cap which was provided with a venting tube connected to a desiccator. After approximately 5 ml. of liquid ethylene oxide had accumulated in the bottom of the container, it was removed from the ice bath and placed in a constant-temperature bath. Twenty minutes were allowed for the ethylene oxide–plasticizer mixture to reach equilibrium at the desired temperature. A glass-coated stirring bar provided agitation for the system. The bottle was then removed from the bath and titrated rapidly with a standardized HBr–glacial acetic acid reagent according to the method described by Gunther (5). The indicator was 0.1% crystal violet in glacial acetic acid. The concentration of ethylene oxide was calculated by:

$$\frac{\text{mg. of ethylene oxide}}{\text{Gm. of plasticizer}} = \frac{\text{ml. HBr} \times \text{normality} \times \text{equivalent wt.}}{\text{wt. of sample}}$$

Control Value for System—Before investigating the solubility of ethylene oxide in dimethyl, dipentyl, and dinonyl phthalate, it was necessary to determine if any ethylene oxide would remain in the test system in the absence of a sample. Five determinations were made at 45.5, 57.0, and 71.5° C. for the ethylene oxide which would remain in the gas washing bottle under the test conditions described above. Table I summarizes the data and presents the mean values obtained as well as the probable deviation of the mean (14).

Prior to testing the aforementioned plasticizers,

TABLE I—EFFECT OF TEMPERATURE ON RESIDUAL ETHYLENE OXIDE IN GLASS BOTTLES

Experiment	Wt., mg. 45.5° C.	57.0° C.	71.5° C.
1	459	399	382
2	473	456	391
3	481	452	371
4	482	413	399
5	466	399	439
Mean values	472	424	396
S. E.	3.0	8.5	7.8

TABLE II—SOLUBILITY OF ETHYLENE OXIDE IN A SERIES OF PHTHALATE ESTERS AT 45.5° C.

Dimethyl Phthalate		Dipentyl Phthalate		Dinonyl Phthalate	
mg./-Gm.	Gm./-mole	mg./-Gm.	Gm./-mole	mg./-Gm.	Gm./-mole
Mean Values					
224.1	43.5	170.0	52.1	118.7	49.6
5.1	1.0	3.9	1.2	5.9	2.5

it was imperative to know that these esters would not interfere with the titration method of analysis. An evaluation of the amount of ethylene oxide which could be detected in the presence and absence of the esters showed that these plasticizers did not contribute to the titers.

Ethylene Oxide Solubility in Phthalates—Various amounts of dimethyl, dipentyl, and dinonyl phthalate were accurately weighed into the gas washing bottle previously described; they were then saturated with ethylene oxide prior to being placed in the constant temperature bath at 45.5° C. In this study only this one temperature (45.5° C.) was evaluated. Five replicate experiments were performed for each of the esters. The data obtained from the titration were corrected for the value of ethylene oxide obtained from the standard values in Table I. The corrected data appear in Table II, and are expressed both as Gm. of ethylene oxide per Gm. of ester and as Gm. per mole of ester. Probable deviations of the mean values were also calculated.

Application of Raoult's Law of Partial Pressure—Once an evaluation of the number of moles of ethylene oxide present in the phthalate plasticizers had been made, it was possible to evaluate the vapor pressure of ethylene oxide over this solution. Raoult's law states:

$$P = P_0 X_0$$

where P is the vapor pressure of the solvent or volatile solute over its solution, X_0 is the mole fraction, and P_0 is the vapor pressure of the pure solvent or the volatile solute at the same temperature as the solution. The mole fraction was obtained by dividing the number of moles of ethylene oxide in the plasticizer at 45.5° C. by the number of moles of plasticizer and ethylene oxide. Before carrying out this operation it was necessary to evaluate the vapor pressure of pure ethylene oxide with the Moor-Kanep formula which states that (15):

$$\text{EtO log vapor pressure [mm. Hg]} = 6.839 - \frac{1410}{T°\text{K.}}$$

DISCUSSION

For the sake of clarity and continuity, the discussion that follows will coincide with the sequence of topics covered under *Experimental*.

Ethylene Oxide Solubility Studies in Phthalate Esters—One of the most interesting observations made in the solubility experiments of ethylene oxide in phthalic acid esters was that this gaseous vapor has the ability to remain in a nonclosed system in high equilibrium concentrations at relatively high temperatures. Since ethylene oxide boils at 10.7° C., it was not anticipated that any significant amount of ethylene oxide would remain in the gas washing bottle system (previously described) at 71.5° C. However, at 7 times the boiling temperature, there was still almost 0.5 Gm. of residual sterilant (see Table I). If one considers the porous nature of certain plastic devices sterilized for medical use, it immediately becomes obvious that a thorough degassing technique must be employed following sterilization. It is not known at this time whether the ethylene oxide is sorbed in much the same manner as some tin compounds onto the surface of a glass system. In light of this observation, a possible explanation of the toxicity found in certain tubing systems following ethylene oxide sterilization might be better rationalized in terms of sorption processes into the polymeric matrices. One immediate example of where this residual ethylene oxide phenomenon could present a hazard would be in the sterilization of such devices as syringes. If the barrel and plunger of the syringe were engaged too soon after sterilization, the residual gas might produce a toxicity from the oxidation of the epoxide to a glycol, which in turn could be further oxidized to glyoxal, glyoxalic acid, glycolic acid, or even oxalic acid. All of these compounds could potentially produce a toxic response. As for the effect of the gas or its oxidation products upon the syringe contents, it is difficult to begin to even enumerate the many novel toxicities that could be produced.

In addition to the possibility of absorption of the ethylene oxide gas, it has been found that this relatively polar ether does have significant ability to solvate such nonpolar hydrocarbon esters as dinonyl phthalate. Considering that PVC tubing will contain approximately 35% of dioctyl phthalate, and that dinonyl phthalate has a solubility of 119.7 mg./Gm., as well as the fact that as the length of the hydrocarbon ester moiety decreases the EtO solubility increases (see Table II), it thus becomes possible that a flexible tubing weighing 1 Gm. might contain as much as 40 mg. of ethylene oxide. It has been shown that this is a sufficient amount to cause significant hemolysis in human blood. At the present time it is not known whether the plasticizer in the polymer will attain the same solubility limits for ethylene oxide as it does in the pure state. This information will be investigated in future studies.

The application of Raoult's law and the Moor-Kanep formula proves to be rewarding intuitively and practically. It was learned that the partial vapor pressure of ethylene oxide in the presence of dialkyl phthalates decreases as a linear function of the length of the dialkyl chain. This suggests

TABLE III—PARTIAL PRESSURES AND MOLE FRACTIONS OF ETHYLENE OXIDE IN A SERIES OF PHTHALATE ESTERS AT 45.5° C.

Plasticizer	Moles of EtO	Moles of Phthalate	Moles of EtO and Ester	Mole Fraction	Vapor Pressure, mm. Hg
Dimethyl phthalate	27.66	121.39	149.05	0.18558	490.3
Dipentyl phthalate	28.58	162.58	191.16	0.14951	395.0
Dinonyl phthalate	31 72	256.52	288.24	0.11005	290.75

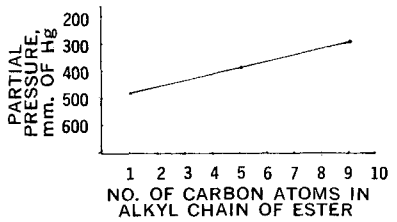

Fig. 2—Plot of the partial pressure of ethylene oxide in phthalic acid esters at 45.5°C. versus *length of alkyl moiety of the ester.*

that the lower esters would degas at a faster rate than their higher molecular weight congeners. (See Table III and Fig. 2.) However, as can be seen from Table II, it appears that this is not the only major consideration. The solubility of the ester in the epoxide also rises with the decrease of the number of carbon atoms in the hydrocarbon moiety. Thus one might rationalize that the higher alkyl esters of the phthalate type would be the preferred plasticizers for ethylene oxide sterilized plastics. If the solubility of the ester in the epoxide is to be the predominant consideration, then it can be said that for every four carbons added to the alkyl chain there will be a 25% decrease in the ethylene oxide solubility.

Table III presents the mole fractions, vapor pressure, and partial pressures just described. Figure 2 illustrates the linear function of the partial pressure of ethylene oxide to the length of the alkyl ester moiety.

A Dose Response Study of Ethylene Oxide Induced Hemolysis—Whole human blood, 27 days old, type O, Rh positive, was centrifuged and the plasma removed. The packed cells were washed with 0.85% saline and then recentrifuged and decanted. The procedure was repeated 8 times until the fragile cells and plasma had been thoroughly removed. A final 10% suspension of red blood cells was then prepared. This suspension was then added in varying volumes to a gas washing bottle which was known to contain the amount of ethylene oxide desired for the test. At 30, 60, and 120 min. after the red blood cell suspension was exposed to the ethylene oxide, the sample and the supernatant solutions were examined with the aid of a Spectronic 20 (Bausch & Lomb) spectrophotometer. The wavelength suggested for hemoglobin determinations is 540 mμ. The amount of hemoglobin as indicated by the percent absorption from the spectrophotometer was plotted against the time the suspension was exposed to the ethylene oxide. Figure 3 is a plot of the typical dose–hemolysis responses obtained for different concentrations of ethylene oxide at three time intervals of exposure.

An Evaluation of the Hemolysis Liability of Pharmaceutical Plastics Sterilized with Ethylene Oxide—Fourteen different plastic devices which may or may not be used to store blood were sterilized by a standard ethylene oxide sterilization technique which is presently used in many hospitals. The procedure involved exposing the devices to 7 p.s.i. (600 mg./L.) of ethylene oxide (12%) and CCl_3F–CCl_2F_2 (88%) for 4 hr. at 50% relative humidity and 57° C., followed by degassing the plastics 15 min. at 26 mm. of mercury vacuum at 57° C. The actual devices studied are given in Table IV, along with the percent absorption obtained on a Spectronic 20 from the development of hemolysis in a 10% red blood cell suspension. The devices were filled with the suspension immediately after sterilization and evaluated at varying time intervals. The controls were the same devices given the same treatment except that no ethylene oxide was allowed in the sterilization chamber. No attempt was made to control the surface-to-volume ratio.

Cell Culture Studies on the Toxicity of Ethylene Oxide Sterilized Polymers—Eighteen plastics (10-mil films) listed in Table V were sterilized by the procedure described in the previous section. The sterilized plastics were tested by a cell culture method which has been previously described (16, 17).

Table V presents the results obtained when the freshly sterilized plastics were placed on cell culture plates using the agar overlay techniques and observed 24 hr. later. It should be pointed out that all plastics listed in Table V were previously evaluated by the same cell culture technique and all were found to be nontoxic to the two cell systems utilized prior to sterilization.

TABLE IV—HEMOLYSIS LIABILITY OF SELECTED PHARMACEUTICAL PLASTICS STERILIZED WITH ETHYLENE OXIDE

Name of Device	Polymer Type	Time Blood Exposed to Plastic, min.	% Absorption at 540 mμ
Venopak disposable venoclysis set (Abbott lot No. 758-3518)	Flexible PVC	60 120	68 87
Blood administration set with plastic filter (Abbott, lot No. 740-3515)	Flexible PVC	60	22
Monoject 535S-C disposable syringe (Roehr Products Co.)	Polypropylene	40 140	18 18
Jelco disposable syringe (Jelco, lot No. 3165 JMB 130)	Polypropylene	45 130	2 8
Tomac disposable syringe (Amer. Hosp. Supply No. 4775 C2L106)	Polystyrene	85 195	15 18
Hemovial disposable blood collection unit (E. H. Wilburn Corp.)	Polyethylene	95 190	18 24
ACD—whole blood container (Pharmachem Corp.)	Polyethylene high density	75 135	33 33
Rilsan bottle (Wheaton Plastics)	Polyamide	80 150	3 14
Teflon—FEP bottle (E. I. du Pont)	Fluoroethylene Propylene	30 143	0 30
Acrylic bottle (Wheaton Plastics)	Methacrylate	100 170	22 26
Bellow's bottle (Wheaton Plastics)	Polyethylene	50 180	4 15
Natural Droptainer (Alcon)	Polyethylene	115 170	16 20
Bottle 4 oz. (Alcon)	Polyethylene	100 175	10 16
Bottle 2 oz. (Wheaton Plastics)	Blend of polypropylene and linear polyethylene	100	8

TABLE V—CELL CULTURE TOXICITY RESULTS FOR 18 PHARMACEUTICAL PLASTICS STERILIZED WITH ETHYLENE OXIDE[a]

Plastic (10-mil Film)	"L" Cell Culture	Chick Cell Culture
Cellulose acetate	Cell death	Cell death
Polycarbonate	No effect evident	No effect evident
Polyethylene	Cell death	No effect evident
Teflon 7	No effect evident	No effect evident
PVC with 35% dioctyl phthalate	Cell death	Not tested
Nylon 6,6	No effect evident	No effect evident
Polyphenylene oxide	Cell death	Cell death
Polypropylene	No effect evident	No effect evident
PVC with 15% dioctyl phthalate	Cell death	Cell death
Rigid PVC	No effect evident	No effect evident
Cellulose triacetate	Cell death	Cell death
Rigid polyurethane	Cell death	No effect evident
Flexible polyurethane	Not tested	No effect evident
Cellulose acetate–butyrate	Cell death	No effect evident
Polymonochlorotrifluoroethylene	No effect evident	No effect evident
Polybutylene	No effect evident	No effect evident
Acrylonitrile–butadiene styrene	Cell death	No effect evident
Polyvinyl acetate	Cell death	Cell death

[a] Plastics were placed on the culture plates within 15 min. after the sterilization procedure was completed. Toxicity observations were made with the unaided eye, after 24 hr. exposure to the culture plates.

Toxicity Studies of Ethylene Oxide—The data submitted in Fig. 3 indicate that ethylene oxide is a rapid acting hemolytic agent. Independent of the dose involved, this gas will perform the major portion of its hemolyzing effect in the first 30 min. of exposure to blood. After this peak time is reached, the effect becomes asymptotically parallel with respect to the time axis. The importance of the plot in Fig. 3 is not as a direct read-out of the degree of percentage of hemolysis, but rather as a standard curve for the effect of a particular dose of ethylene oxide on human erythrocytes as compared to the same effect achieved with a sterilized plastic. For instance, in Table IV, it can be seen that Abbott's venoclysis set produced a hemolysis absorption read-out of 68% in 1 hr. According to the standard curve, it would take a concentration greater than 78.6 mg. of ethylene oxide per ml. of human blood cells (10% suspension) to produce this same hemolysis. The eye can detect this amount of hemolysis since it is so extensive. It is the opinion of the authors that the cause for the severe hemolysis liability of the Abbott devices is

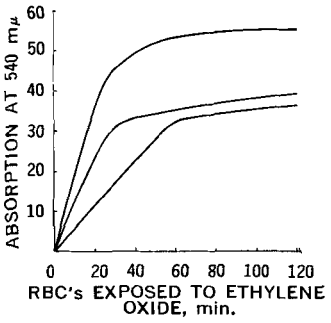

Fig. 3—Plot of ethylene oxide concentration in human blood versus *hemoglobin absorption at 540 mμ. Key: top, 78.6 mg. EtO/ml. RBC; middle, 47.2 mg. EtO/ml. RBC; bottom, 23.6 mg. EtO/ml. RBC.*

the result of the higher solubility of ethylene oxide in a plasticized PVC polymer as compared to other nonplasticized or crystalline polymers. This device also demonstrated the same phenomenon as was reported for the previously described gas washing bottle. This former device is a hollow tubular system in which residual gas pockets can develop as the ethylene oxide is desorbed from the polymer. Perhaps future studies of the effects of vacuum, temperature, and time upon the degassing of these plastics might reveal that no hemolysis liability does exist for these devices.

The different absorption readings obtained for the three syringes sterilized with Pennoxide (see Table IV) is not a reflection of the plastic used to make the barrel of the syringe, but rather the composition of the hub on the plunger. Once blood is drawn into the sterilized syringe it can be visually recorded that the hubs of the Monoject and Tomac syringes release bubbles of a gas. In the undisturbed syringe filled with the blood, hemolysis was extensive in the area next to the hub, but no noticeable difference was recorded in the rest of the barrel. The Jelco syringe did not produce this same effect and concomitantly less hemoglobin absorption was recorded.

It is difficult to pin down the differences in absorption readings obtained for the various polyethylene devices. It might be said that the narrow openings of the Pharmachem products might have permitted a greater residual ethylene oxide content and build-up as desorption occurred in the device. But the bottles with the wider openings allow for a lower final equilibrium concentration after the samples were removed from the sterilizer. The variation in the densities of the individual devices might also be reflected in the different absorption values because the higher density polyethylenes would be more impermeable to sorption and diffusion of the gas. It is felt that this same type of phenomenon was responsible for the delayed hemolyzing ability of the fluoroethylene propylene produced by du Pont. This product has a high degree of crystallinity and would resist the uptake of gas. However, some gas diffusion would occur into the polymer during the 4 hr. of sterilization under pressure. Once the pressure was released and temperature was dropped, the degassing would thus occur slowly over the 1.5-hr. period. The first 30 min. failed to show hemolysis because desorption had not progressed enough to produce a toxic dose.

The cell culture toxicity evaluation of the gas sterilized plastics gave somewhat erratic results, as noted in Table V. Certain explanations may be noted, however, to at least partially account for this variation. First, the mouse fibroblasts ("L" cells) are somewhat more sensitive to toxicants than are chick embryo cells in culture, and the toxic results shown in Table V reflect this difference in sensitivity. It should be noted that the more plasticized formulations caused cell death, again reflecting a higher degree of ethylene oxide solubility in the plasticizer and a more rapid release of the gas from this material. The cell deaths noted with the nonplasticized plastics are related to the complex desorption relationships resulting from different degrees of crystallinity, branching, and molecular weights of the polymers.

In conclusion, it appears that the blood saline suspension technique is a sensitive and effective

method for evaluating hemolysis liabilities of sterilized plastics. From the results of these tests, the polypropylene devices would have to be considered the best candidates for making a medical product which would undergo ethylene oxide sterilization. However, techniques must be evaluated to prove the effectiveness of longer degassing times, higher temperatures, and the effects of vacuum. It would appear from these results that further testing is necessary to establish the actual percentage of hemolysis that occurs for a particular concentration of ethylene oxide. In any event, it is sufficient to say that hemolysis liabilities do exist from the sterilization of medical plastics of many different types.

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Keyphrases

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Solubility in phthalic esters
Glass—entrapment
Toxicity of ethylene oxide
Tissue culture
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Surface Films of Soybean Lecithin II

Interactions Between Lecithin and Lipid Substances in Mixed Monomolecular Films

By G. TOROSIAN* and A. P. LEMBERGER

Mixed films of lecithin with the fatty acids (stearic, elaidic, and oleic) as well as with the glycerol esters of mono-, di-, and triglyceride have been investigated. Evaluation of these systems has been made utilizing two properties of surface films, their mean molecular areas and their collapse points. It would appear from this investigation that in mixed film systems, lecithin–stearic acid and lecithin–monoglyceride are miscible, but without a significant degree of interaction and as such, form ideal two-dimensional liquids. Lecithin–oleic and lecithin–elaidic acids have been shown to be miscible in mixed film systems but with a significant degree of interaction so that they may be considered to be nonideal two-dimensional liquids. The data for the lecithin–di- and triglyceride systems did not conclusively show whether these systems were ideal or nonideal two-dimensional liquids in mixed surface films.

THE IMPORTANCE of phospholipids in biological membranes has been discussed by Bangham (1). Among the phospholipids, lecithin is considered particularly important since it aggregates into sheet-like micelles which are capable of further accommodating lipids, phospholipids, and sterols (2, 3). The mixed film system of lecithin–cholesterol, first investigated by Dervichian (4), showed the ability of cholesterol to condense lecithin to a smaller cross-sectional area than it exhibits alone. Van Deenen (5) indicated the molecular proportions of cholesterol to phosphotides of animal red cell membranes

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