SPI

THE SOCIETY OF THE PLASTICS INDUSTRY, INC.

250 PARK AVENUE • NEW YORK, NEW YORK 10017 • 212/687-2675

June 21, 1968

To: Members, SPI Food, Drug, and Cosmetic Packaging Materials Committee

Re: Results and conclusions of the PMA-SPI cooperative study on polyolefin bottles for dry drug products

Gentlemen:

By now, you have received the minutes of the last meeting of subject Committee held in New York City on April 17, 1968.

You will kindly note that in delivering a status report on the cooperative effort between SPI and the Pharmaceutical Manufacturers Association, Dr. W. B. Ackart, Union Carbide Corporation, River Road, Bound Brook, New Jersey, made reference to a somewhat lengthy report giving the experimental details, results and conclusions of the PMA-SPI cooperative study on polyolefin bottles for dry drug products. It was decided following our meeting on April 17 that it would be best to send the report under separate cover rather than attach it to the minutes of the last meeting, due principally to its unusual length.

We are therefore attaching hereto details of a report entitled "Report of the Plastic Subcommittee for Methodology for Testing Plastic Containers for Tablets, Capsules, Oral Powders, Granules, and Ophthalmic Products."

This report was prepared by Mr. W. W. Hilty of the PMA group and was published in the minutes of the October 1967, PMA meeting. Mr. Hilty has informed Dr. Ackart that the National Formulary has agreed to publish the methodology in the 1970 edition.

Dr. Ackart wishes the undersigned to point out that at our April 17th SPI Committee meeting, we discussed the use of the term "plastic" rather than "polyolefin" both in the title and the first paragraph of the report. Dr. Ackart has called this to Mr. Hilty's attention and he has promised to substitute "polyolefin" for "plastic" in the galley proofs of the N. F. publication if it can possibly be done. Hopefully, the definition of "polyolefin" which was prepared by Dr. Robert Millers' SPI Technical Information Subcommittee will also appear as a footnote. Mr. Hilty has indicated to Dr. Ackart that he will do his best to have these changes made.

If the members of the SPI Committee would like to comment on the attached document, please feel free to do so. Dr. Ackart tells us that he would welcome comments.

THE SOCIETY OF THE PLASTICS INDUSTRY, INC.

Charles L. Condit
Senior Staff Advisor

PLASTICS 100 1868-1968 KEYSTONE OF THE FUTURE

REPORT OF THE PLASTIC SUBCOMMITTEE
FOR METHODOLOGY FOR TESTING PLASTIC CONTAINERS
FOR TABLETS, CAPSULES, ORAL POWDERS, GRANULES,
AND OPHTHALMIC PRODUCTS

October 10, 1967

This committee, in collaboration with the Society of Plastic Industries, has studied various test methods as outlined and discussed in the interim report dated October 9, 1967.

From our discussions at this meeting it seems desirable that additional time be devoted to discussing and analyzing the meaningfulness of the tests studied. In almost all cases the results obtained would indicate a minimum of, or lack of response to the particular property under study.

We therefore request that the report available and the methodology attached be considered a progress report. We request that members of the Section avail themselves of the report, the methodology; and during the interim between this meeting and the meeting next Spring, make it a point to secure additional data on their own and be prepared to consider the merit of these and other tests that may be proposed.

Respectfully submitted,

REPORT OF THE PLASTIC SUBCOMMITTEE FOR METHODOLOGY FOR TESTING PLASTIC CONTAINERS FOR TABLETS, CAPSULES, ORAL POWDERS, GRANULES, AND OPHTHALMIC PRODUCTS

October 9, 1967

This subcommittee, in cooperation with the Society for Plastic Industries, has designed and conducted a collaborative study of test procedures intended to provide a means of measuring the consistency of plastic containers on a batch-to-batch basis.

Nine laboratories have participated in this study, in whole or in part, representing both the pharmaceutical and the plastic industries.

The test procedures studied represented tests for: (1) Acute Systemic Toxicity, which was designed to give an indication or measure of relative safety of the item under test; (2) Heavy Metals; (3) Non-Volatile Residue; (4) Water Vapor Permeability; (5) Light Transmission; and (6) Eye Irritation, designed to give a relative evaluation as to the advisability of using this type of container for ophthalmic preparations.

(1) In the Acute Systemic Toxicity study, the most noticeable variable was the difference among laboratories in their response (72 hour weight gain in mice) to the solvent blanks. This was examined by an analysis of variance on a computer. The laboratory x treatment mean are shown in Tables I, II, and III. For each solvent, there was evidence of a significant laboratory x plastic interaction. Because of this, any direct comparison of treatment (blank, plastic extracts) means and of laboratory means is of questionable value. In other words, the response to a particular treatment depends upon which laboratory is doing it. For this reason no treatment means or plastic means are shown in the tables. In only a few cases were there significant differences between the blank response and the plastic extract response, and these were in the direction of weight gain, which implies no significant toxicity in the plastic extracts.

PMA-SPI Collaborative Plastics Study

Acute Systemic Toxicity

Table I

5% Ethyl Alcohol in Sodium Chloride Injection, USP

Mean 72-hour weight gains in groups of mice, grams.

(Number of surviving mice in parentheses)

Laboratories	A	B	C	D	E
Blank	1.29(10)	4.85(20)	-1.70(10)	0.45(10)	-1.00 (20)
Plastics					
Polypropylene (Continental Can)	1.35(10)	4.55(10)		0.70(10)	
High Density Polyethylene (Union Carbide)		4.85(10)	-0.90(10)	-0.45(10)	
Pigmented High Density Polyethylene (Owens-Illinois)	1.87(10)		-1.40(10)		
Ethylene Butene Copolymer (Phillips Petroleum)					-0.32 (40)

PMA-SPI Collaborative Plastics Study

Acute Systemic Toxicity

Table II

Cottonseed Oil, USP

Mean 24 hour weight gain in groups of mice, grams.
(Number of surviving mice in parentheses)

Laboratories	A	B	C	D
Blank	1.72(10)	4.78(20)	0(10)	2.10(10)
Plastics				
Polypropylene (Continental Can)	0.41(10)		0.40(10)	
High Density Polyethylene (Union Carbide)	1.26(10)			
Pigmented High Density Polyethylene (Owens-Illinois)		5.00(10)		2.20(10)
Ethylene Butene Copolymer (Phillips Petroleum)		4.55(10)	1.20(10)	1.65(10)

PMA-SPI Collaborative Plastics Study

Acute Systemic Toxicity

Table III

Polyethylene Glycol 400, USP

Mean weight gains in groups of mice, grams.

(Number of surviving mice in parentheses)

Laboratories	A	B	C	D
Blank	-1.29(10)	-2.05(13)	-1.16(6)	2.50(10)
Plastics				
Polypropylene (Continental Can)		1.10(5)	-2.625(8)	0.70(10)
High Density Polyethylene (Union Carbide)		0.24(7)		0.80(10)
Pigmented High Density Polyethylene (Owens-Illinois)	-0.76(10)			
Ethylene Butene Copolymer (Phillips Petroleum)	-0.71(10)		-1.70(10)	

On the other hand, there appeared to be no practical difference within laboratories when comparing the several plastic extracts with the solvent blanks. There appeared to be no significant difference among the several forms of plastics.

(2) Heavy Metals test results indicated no particular difficulty in any laboratory and no positive responses to the heavy metals test were noted.

(3) The test procedure for Non-Volatile Residue appeared to work satisfactorily in all laboratories, and there appeared to be no evidence of non-volatile residue in any of the plastics tested. One laboratory did have a significant amount of non-volatile residue in its blanks.

(4) Water Vapor Permeability was originally tested under a procedure that proved inadequate for the task. This procedure was revised to incorporate specific instructions as outlined by the Plastic Industry representatives. The results of this test as it is now incorporated in the proposed methodology appear to yield a significant laboratory effect. However, on a relative basis they will provide a means of evaluation.

(5) In an attempt to provide a test procedure for light transmission it became apparent quickly that this can be measured only under very complex conditions involving special equipment not generally available in the usual laboratory in the pharmaceutical industry. The reason for this is that polyolefins refract light and scatter it so, that unless all refracted and scattered light is measured no true value of transmitted light can be obtained. Therefore, it seems pertinent to delete this as an evaluation test and indicate that if a non-transmittant light substance is desirable it should contain a pigment such as titanium oxide.

(6) The Eye Irritation Test was found to be workable and to yield results that indicated plastic containers can be evaluated in this manner so far as eye irritation is concerned.

(7) An additional test for Apparent Density has been added to this list of test methods. It has been checked in one pharmaceutical firm and several plastic industry firms and is described in the Book of ASTM Standards, Part 26, June 1965, ASTM Designation: D 1622-63.

WATER VAPOR PERMEABILITY

Weight Change Rate, gm./day

Material	Monsanto	UCC Trial 1	UCC Trial 2	Lilly
A. High Density Polyethylene (UCC)	.00590	.0066	.00617	.00328
B. Polypropylene (Continental Can)	.00733	.0065	.00700	.00449
C. Ethylene-Butene Copolymer (Phillips)	.00368	.0048	.00369	.00199
D. Pigmented High Density Polyethylene (Owens-Illinois)	.00627	.0046	.00431	.00331

Permeation Factor

$$P = \frac{RW\ (100)}{.01639\ DA^2}$$

Material	Monsanto	UCC Trial 1	UCC Trial 2	Lilly
A. High Density Polyethylene (UCC)	0.776	0.851	0.803	0.320
B. Polypropylene (Continental Can)	1.093	0.961	1.044	0.510
C. Ethylene-Butene Copolymer (Phillips)	0.616	0.854	0.656	0.238
D. Pigmented High Density Polyethylene (Owens-Illinois)	0.546	0.403	0.375	0.232

FIGURE A

High Density Polyethylene
Union Carbide

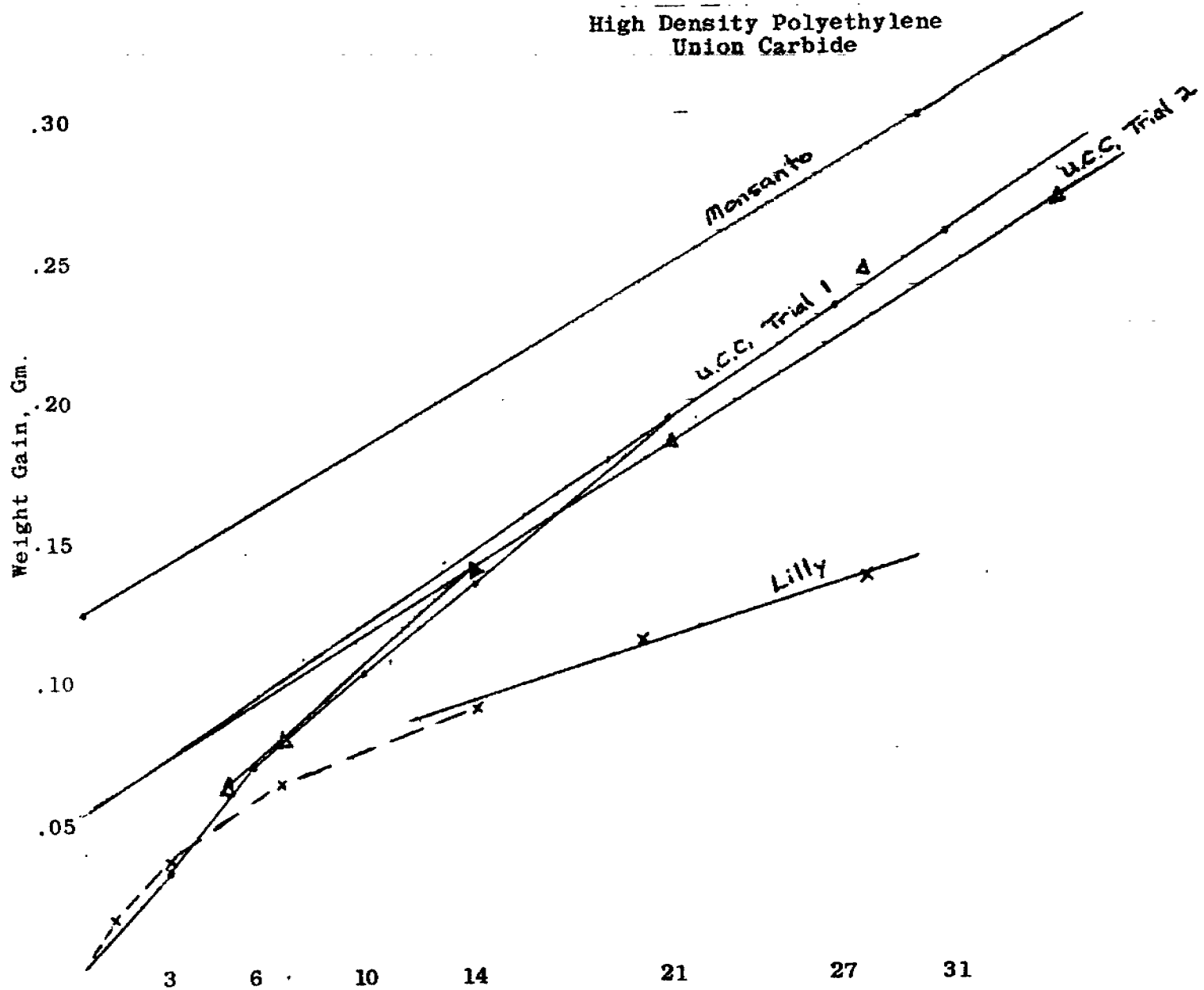


FIGURE B

Polypropylene
Continental Can

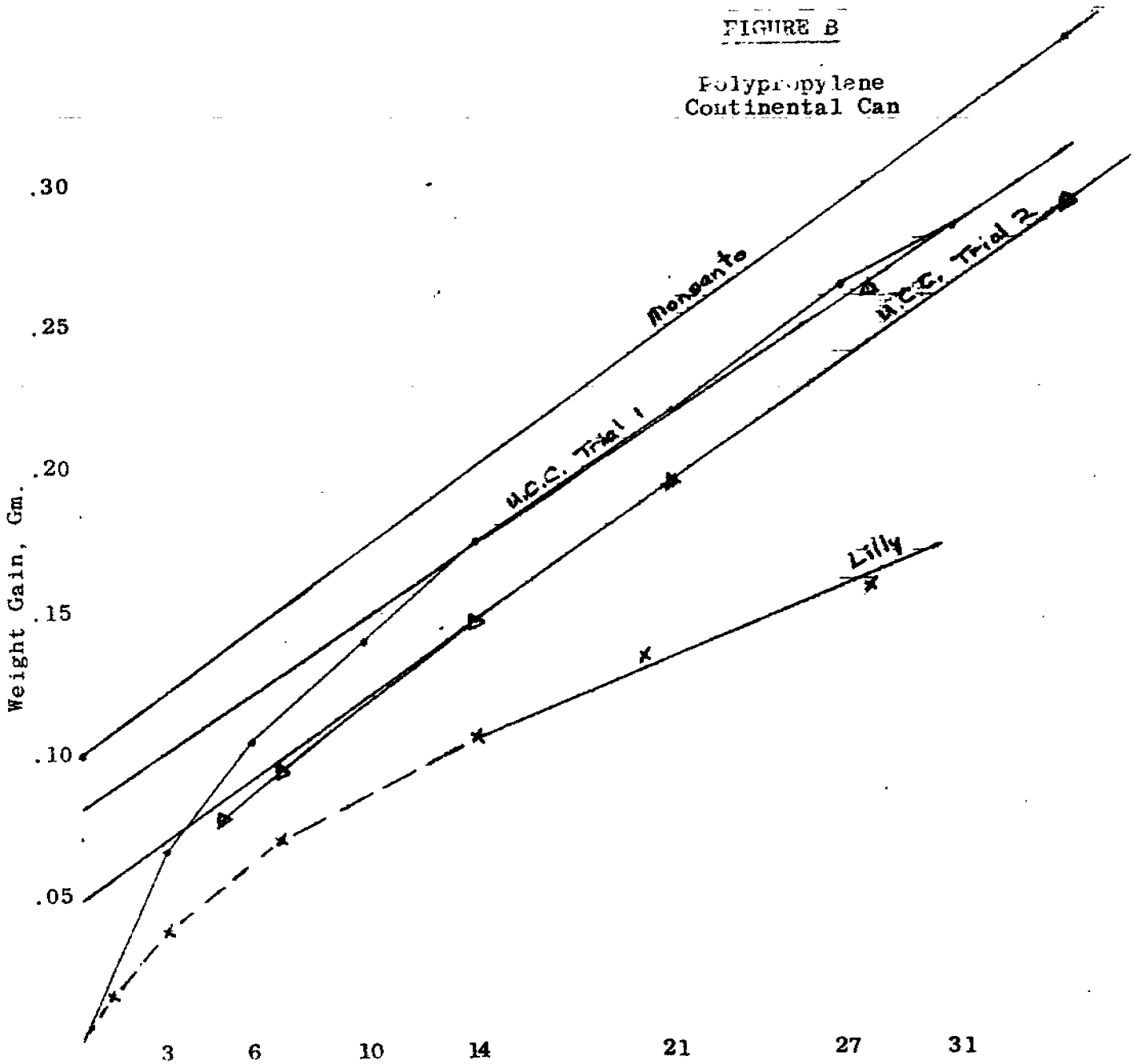


FIGURE C

Ethylene-butene Copolymer
Philips Petroleum

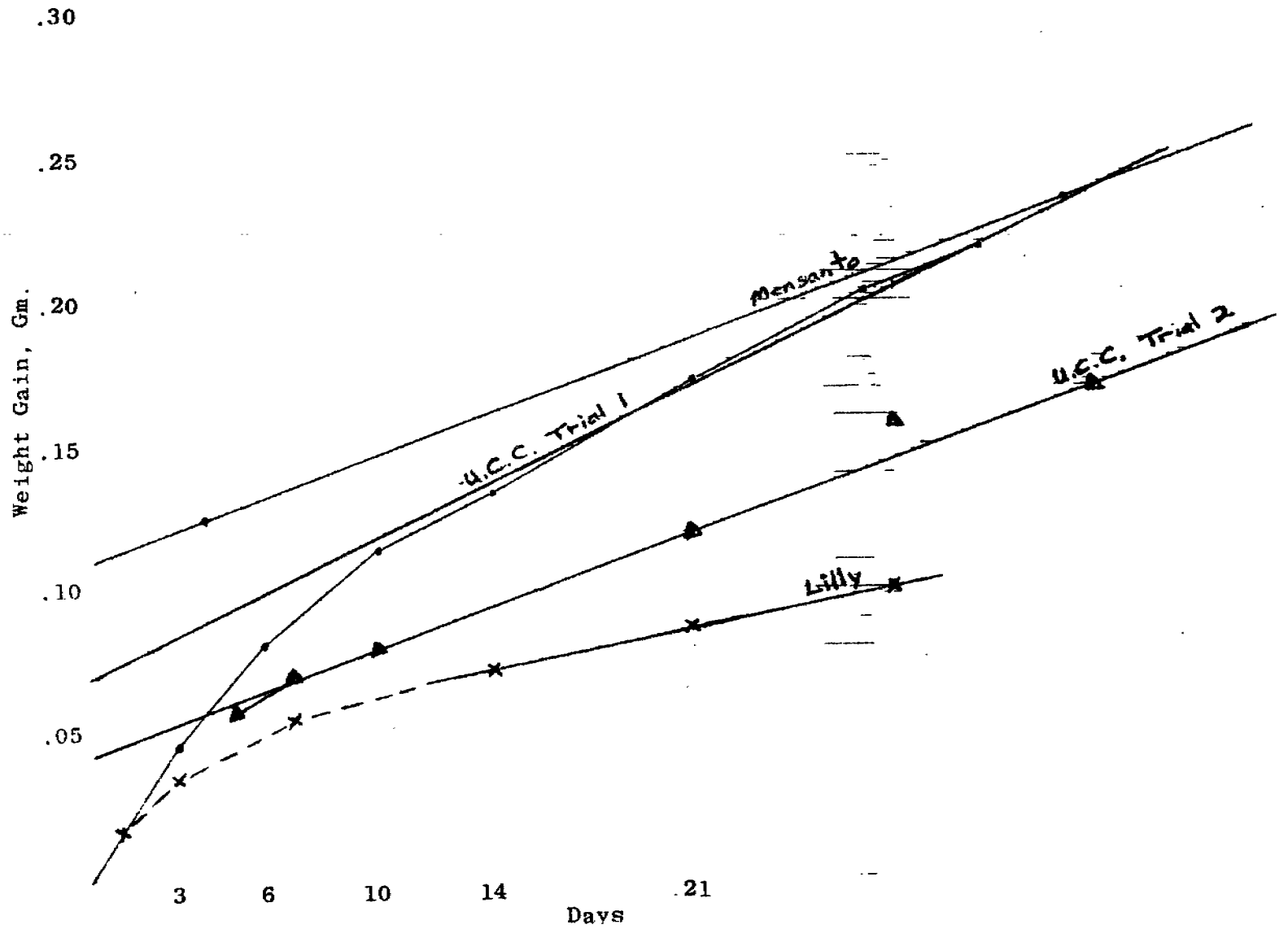
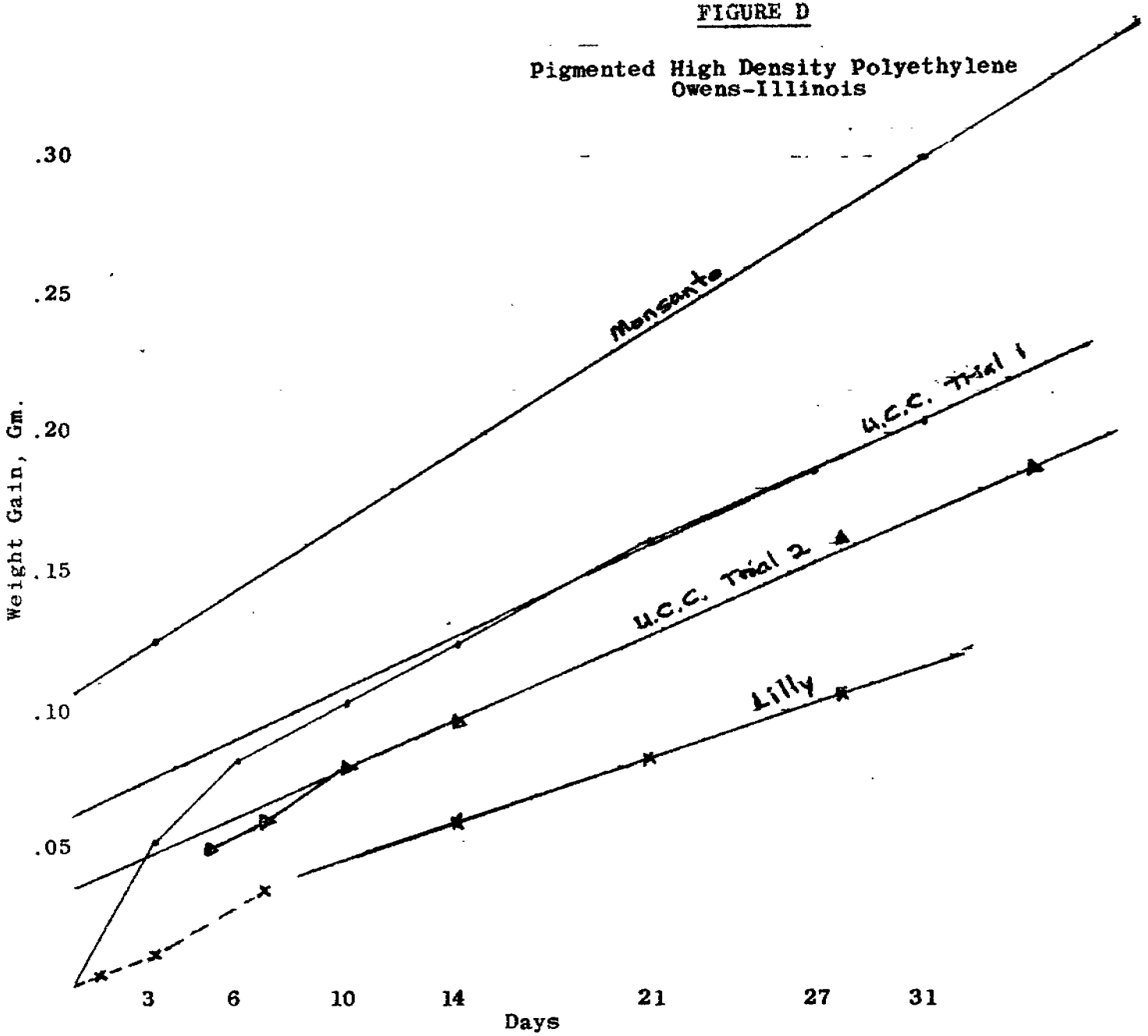


FIGURE D

Pigmented High Density Polyethylene
Owens-Illinois



The methods have been re-edited and put into compendia style and are attached to this report.

In the matter of proposing limits for these tests it is the opinion of the committee that the following recommendations can be suggested.

PHYSICOCHEMICAL TESTS

a. Heavy Metals

The test procedure for heavy metals presented no problems, and is straightforward. Moreover, the study indicated there were no positive responses to the test. It is therefore recommended the limits be set at "not more than 3 ppm."

b. Non-Volatile Residue

Although the test methodology is accurate and precise, it is recommended that no limits be established for this test. The non-volatile residue will vary dependent upon the type of plastic being tested.

c. ΔpH

The pH difference between the Sample Extract and the Blank is again dependent, to a large degree, upon the type of material being tested. Even by establishing a limit for the ΔpH of 2.5, several of the plastic material tested would not meet this limit; therefore, no specific limits are recommended at this time.

d. Water Vapor Permeability

Using the revised procedure received from the plastics industry, the test appeared to be workable. As was indicated by Dr. Breunig's statistical summary, there appeared to be a significant laboratory effect; however, it is believed the test is quite reliable but unfeasible to most laboratories. Therefore, additional testing by this method would be necessary before any meaningful limits could be established.

BIOLOGICAL TESTS

a. Acute Systemic Toxicity

If any animals treated with the Sample show only slight signs of toxicity, and not more than one animal shows gross symptoms of toxicity or death, the test should be

repeated using the same number of mice. On the repeat test, all animals treated with the Sample should show no significant reaction above the Blank animals during the observation period.

b. Eye Irritation Test

If any irritability of either eye is noticed, the test should be repeated using the same number of rabbits. On the repeat test, all animals treated with the Sample should show no more significant reaction above the Blank animals during the observation period.

PLASTICS - GENERAL TESTS AND APPARATUS

The following Physicochemical and Biological Test Procedures are the result of the combined efforts of the Plastics Committee of the Quality Control Section of the Pharmaceutical Manufacturers Association and interested representatives from The Plastic Industry. The tests are general in scope but applicable for testing a wide variety of plastic containers in use today. The purpose of this study is to establish procedures for monitoring batch to batch production of plastic materials which may come into contact with pharmaceutical compounds.

GENERAL TESTS, PROCESSES AND APPARATUS FOR PLASTICS

PLASTICS - CONTAINERS FOR INJECTIONS

Plastics can be of natural origin or produced synthetically and are generally divided into two broad categories in accordance with their response to applied heat, thus are classified as being either thermoplastic or thermosetting. A thermoplastic material is usually rigid at normal temperatures but will soften upon the application of heat and deform under pressure while a thermosetting material will undergo physical and chemical changes under influences of heat, pressure or catalysts to become substantially infusible and insoluble. Very few plastics are single chemical entities but rather consist of a mixture of homologous compounds having a wide range of molecular weights. Moreover, plastics frequently contain other substances such as residues from the polymerization process, plasticizers, stabilizers, antioxidants, pigments and lubricants.

DEFINITIONS

For the purpose of these tests for plastics, the following definitions apply: A Container is the device which holds the drug and which is or may be in direct contact with the drug. The Sample is the plastic specimen under test or the extract prepared from such a specimen. A Blank consists of the same quantities of the same extracting medium used for the extraction of the specimen under test treated in the same manner as the extracting medium containing the specimen under test. A Negative Control is a plastic specimen which gives no reaction under the conditions of the test.

PLASTICS - PHYSICOCHEMICAL TEST PROCEDURES

The following tests are designed to determine such physical and chemical properties of plastics and their extracts as are specified in individual monographs. Moreover, these test procedures are designed to test the suitability of a plastic for use as a container, or accessory thereto. Since the tests are based on the extraction of a plastic, it is essential that the designated amount of the plastic be used. The specified surface area must be for extraction at the designated temperature.

EXTRACTING MEDIA

Special distilled water is to be used. Water equivalent to Sterile Water for Injection (U.S.P. XVII, page 754) is suitable.

SPECIAL APPARATUS

Oven - Use a forced-circulation model, which will maintain an operating temperature of 70° ± 1°C.

PREPARATION OF APPARATUS

Clean all glassware thoroughly with chromic acid cleaning solution and, if necessary, with hot nitric acid followed by prolonged rinsing with distilled water.

EXTRACTION CONTAINERS

Use only containers such as ampuls or screw-cap culture test tubes, of borosilicate glass. If used, culture test tubes are closed with screw caps having suitable rubber liners. The exposed surface of the rubber liner is protected with an inert solid disk 0.05 mm. to 0.075 mm. in thickness. A suitable disk can be fabricated from a polytetrafluorethylene resin.

PROCEDURE

Preparation of Sample - Select from a homogeneous plastic sample the amount listed in the Table I for each 10 ml. of extracting medium and subdivide the sample as indicated. Remove particulate matter, such as lint and free particles, by treating each subdivided sample as follows: Place the cuts in a clean 250 ml., glass-stoppered graduated cylinder of borosilicate glass, and add about 160 ml. of water for injection. Agitate for about 30 seconds and drain off the water. Repeat this step and dry those pieces prepared for the extraction in a oven not exceeding 50°. Note - Do not clean the plastic with a dry or wet cloth or by rinsing or washing with an organic solvent, a surfactant, etc.

TABLE I - SURFACE AREA OF PLASTIC TO BE USED

FORM OF PLASTIC	THICK-NESS*	AMOUNT OF PLASTIC PER 10 ml. EXTRACTING MEDIUM	SUBDIVIDED INTO
Film or sheet	a	60 $cm.^2$ total surface area	Strips of about 10 cm. x 0.3 cm.
	b	30 $cm.^2$ total surface area	
Tubing	a†	Length = $\frac{60\ cm.^2}{\text{I.D. circumference + O.D. circumference}}$	Sections of about 10 cm. x 0.3 cm.
	b†	Length = $\frac{30\ cm.^2}{\text{I.D. circumference + O.D. circumference}}$	
Monofilament	a	Length = $\frac{60\ cm.^2}{\text{circumference}}$	Sections of about 10 cm.
	b	Length = $\frac{30\ cm.^2}{\text{circumference}}$	
Slabs, tubing, and molded items	c	2.0 Cm. (based on material of specific gravity 1.0)	Pieces of about 0.3 cm. in over-all diameter.

*a = 0.5 mm. (0.020 in.); b = 0.5 mm. to 1 mm. (0.020 in. to 0.040 in.); c = 1 mm. (0.040 in.).
†Wall thickness of tubing.

tested in separate extraction flasks and add to each flask 100 ml. of special distilled water. Also prepare one 100 ml. Blank of the medium. Extract by heating in an oven at 70° for 24 hours. Allow a[illegible] the liquid in the container to reach the extraction temperature. [Note - The extraction conditions should not in any instance cause physical changes such as fusion or melting of the plastic pieces in any way beyond a slight adherence of one portion to another. Always add the cleaned pieces individually to the extraction medium.] Cool all containers to about room temperature but not below 22°, shake vigorously, and decant each extract, using aseptic precautions, into a dry sterile vessel. Store the extracts at a temperature between 22° and 30°, and test within the following 24 hours.

pH Change - Standardize a pH meter with a standard buffer solution (page 912). Rinse the electrodes several times with special distilled water. Prepare a portion of the Blank solution for pH determination by the addition of 0.30 ml. of saturated potassium chloride solution to 100 ml. of the autoclaved Blank solution. Rinse the electrodes with this solution, then take pH measurements on successive portions of the solution until a reproducible result is obtained. Rinse the electrodes several times with special distilled water. Prepare a portion of the Sample Extract solution for pH determination by the addition of 0.30 ml. of saturated potassium chloride solution to 100 ml. of the autoclaved Sample Extract. Rinse the electrodes with this solution, then take pH readings on successive portions of the solution until a reproducible result is obtained. The pH change (ΔpH) is the difference between the pH of the Blank solution and the Sample Extract solution.

HEAVY METALS (page 876)

Pipette 20 ml. aliquots of the Blank and Sample Extract solutions into separate Nessler tubes. Also pipette 2 ml. (1 ppm), 6 ml. (3 ppm), and 10 ml. (5 ppm) of Standard Lead Solution into separate Nessler tubes. Add 2 ml. of dilute acetic acid to all five tubes. Dilute each tube to 25 ml. with special distilled water. Add 10 ml. of freshly prepared hydrogen sulfide T.S. to each tube, mix (preferably with a stirring rod having a loop at the lower end), allow to stand for 5 minutes and view downward over a white surface. Determine the amount of heavy metals (as lead) in the Blank and Sample Extract solutions by comparing the amount of darkening in each solution against that in the standard solutions. If the Blank or Sample Extract solution is darker than the highest standard, repeat the procedure using larger quantities of Standard Lead Solution. The Heavy Metals content is the difference between the Blank and Sample Extract values.

NONVOLATILE RESIDUE

Pipette 50 ml. aliquots of the Blank and Sample Extract solutions into separate, previously tared crucibles that have been dried to

constant weight under the same conditions to be employed in the determination. Evaporate on a steam bath, using a current of air, to dryness. Dry the crucibles in a 105° oven for 1 hour. Cool and weigh. [Note - If an oily residue is obtained, inspect the crucible repeatedly during the evaporation and drying period since such oils may creep along the walls of the crucible.] The difference obtained from the Sample Extract and the Blank is the nonvolatile residue.

APPARENT DENSITY

Scope - This method covers a procedure for determining the apparent density of plastic materials. It is intended for rigid cellular plastics.

Apparatus - (1) analytical balance, (2) Micrometer Dial Gauge or calipers.

Test Specimen - (1) The specimen shall be of a shape whose volume can be calculated, (2) The specimen shall be free from surface skin whenever possible, (3) The specimens should be conditioned and tested in a room or enclosed space maintained at 25° ±1° and 50° ± 2 percent relative humidity.

PROCEDURE

Weigh a minimum of five test specimens and record the weight. Measure the five specimens with a sliding caliper gauge or with steel scale or tape and calculate the volume. Measure all dimensions to a precision of ± 1 percent. Calculate the density to three significant figures by using the following formula:

$$D = \frac{Ws}{V} \times 0.0618$$

Where

D = density of specimen in grams per cubic centimeter
Ws = weight of specimen in grams
V = volume of specimen in cubic inches

WATER VAPOR PERMEABILITY

Definition - Water vapor permeability is a property of polyolefins describing their ability to transmit water vapor from one side of a sheet or film to the other side. Water vapor permeability may be defined as the quantity of water vapor passing through a specified thickness of plastics per unit of time, per unit of area, under controlled conditions of temperature and relative humidity.

APPARATUS

Constant temperature and relative humidity cabinet 100°F. ± 2°, 92% ± 2% relative himidity,, calcium chloride (anhydrous)-4 or 8 mesh, polyethylene/aluminum laminate, heat sealing iron, calipers and rule capable of measuring to the nearest hundredth inch, and an analytical balance.

Procedure:

Weigh the empty plastic container without the cap. Determine the density of the plastic container. Determine the total surface area of the test container by using the calipers to measure the diameters of the body (D) and neck (d) and the height (h) of the container from the shoulder to the bottom. The surface area can be calculated as follows:

$$A = 2\pi\frac{D}{2} \times h + \left(2 \times 2\pi\frac{D}{4}^2\right) - \pi\left(\frac{d}{2}\right)^2$$

[Note: This formula is only correct when the area between the shoulder and neck minus the top opening is equivalent to the area of the bottom. Containers shaped differently must be calculated accordingly. Moreover, no calculation is made for the area of the neck.]

Place the anhydrous calcium chloride into the plastic container to be tested and fill to the shoulder neck opening. Seal the containe with polyethylene/aluminum laminate, cap with the conventional closure and weigh. Place the container into the controlled atmosph cabinet. Weigh the container after 1 day, 3 days, 7 days, 14 days, and 28 days. [Note - After removal of the container from the cabinet, allow only enough time for the container to cool to ambien temperature before weighing. Replace bottle immediately after weig ing. At each weighing interval, shake container in order to expose the fresh surface of calcium chloride to vapor inside container.] Plot the total weight gained against time interval (days) on linear graph paper. Determine the weigh gain per day by calculating the slope of the line at equilibrium, i.e. where the curve becomes line toward the end of the test. The last 3 points should be on a strai line. Further testing until equilibrium is reached may be necessar The water vapor permeability is calculated from the following formu

$$W.V.P. = \left(\frac{Gms.}{day}\right)\left(\frac{Wt.\ of\ Container\ (Grams)}{Density}\right)\left(\frac{6100}{A^2}\right)$$

BIOLOGICAL TESTS - PLASTIC CONTAINERS

The following biological test procedures are designed to test the suitability of a plastic for use as a container, or accessory thereto. The object of each procedure provided is to test the reaction of living animal and of normal animals to the presence of portions of the plastic or of injections of extracts of it. These tests are designed for application to plastics in the condition in which they are used. If a plastic is to be exposed to any cleansing or sterilization process prior to its end-use, then the tests are to be conducted on a Sample prepared from a specimen pre-conditioned by the same processing. Care must be exercised in the preparation of the extracts to prevent contamination with microorganisms and other foreign matter.

EXTRACTING MEDIA - The following are used as extracting media:

SODIUM CHLORIDE INJECTION (see page 595).
1 in 20 SOLUTION of ALCOHOL in Sodium Chloride Injection.
POLYETHYLENE GLYCOL 400 (see page 484).
COTTONSEED OIL (see page 151).

APPARATUS

Oven - Use an oven, preferably a forced-draft model, which will maintain operating temperatures of 50° or 70° within ± 1°C.

Extraction Containers - Use only containers, such as ampuls or screw-cap culture test tubes, of borosilicate glass. If used, culture test tubes are closed with screw caps having suitable rubber liners. The exposed surface of the rubber liner is completely protected with an inert solid disk 0.05 mm. to 0.075 mm. in thickness. A suitable disk can be fabricated from a polytetrafluorethylene resin.

PREPARATION OF APPARATUS

Clean all glassware thoroughly with chromic acid cleansing mixture, or if necessary with hot nitric acid, followed by prolonged rinsing with purified water. Clean cutting devises by an appropriate method (e.g., successive cleaning with acetone and methylene chloride) prior to use in subdividing a specimen. Clean all other equipment by thoroughly scrubbing with a suitable detergent and prolonged rinsing with purified water.

Render containers and devises used for extraction, and in transfer and administration of test material, sterile and dry by a suitable process. [Note - If ethylene oxide is used as the sterilizing agent, allow adequate time for complete de-gassing.]

TEST ANIMALS - For the Acute Systemic Toxidity Test select healthy, not previously used albino mice each weighing between 17 and 23 Gm.

For each test group, use only mice of the same source. Offer food and water, commonly used for laboratory animals and known as to composition, ad libitum.

For the Eye Irritation Test, select healthy, thin-skinned albino rabbits with no visable eye irritation and not previously used for any test.

PROCEDURE

Preparation of Sample - Obtain 6 Gm. of a representative sample from a homogeneous plastic sample. Cut into 3 mm. x 3 mm. pieces. Sieve and save all the pieces that pass through a No. 6 sieve (3.36 mm.) but are retained on a No. 7 sieve (2.83 mm.). Place 4.0 Gm. of the Sample pieces in a glass-stoppered 100 ml. graduated cylinder of borosilicate glass and clean by shaking for about 30 seconds with 70 ml. of water for injection. Repeat, discarding the wash water each time. Allow the portions to drain dry, and, in addition further dry those intended for extraction in Cottonseed Oil in an oven below 50° for 1 hour. Prepare in duplicate. [Note - Do not clean with a dry or wet cloth or with an organic solvent, surfactant, etc.]

Add 20 ml. portions of the appropriate Extracting Media to separate extraction containers and place a properly prepared Sample of the plastic to be tested in each of the two extraction containers. Close each container with a suitable cap or pressure sensitive tape and disperse the pieces of plastic by swirling in the extraction medium. (Repeat these directions for each extracting medium required for testing.) Prepare one 20.0 ml. Blank for each extracting medium for parallel injections and comparisons. Extract by heating in an oven at 70° for 24 hours. The extraction should not result in gross physical changes in the samples, such as fusion of the two portions in any way beyond a slight adherence of one portion to another. Cool to about room temperature, but not below 25°, shake vigorously and decant each extract, using aseptic precautions, into a dry sterile vessel. Seal and store the extracts at a temperature of 25°, and do not use for tests after 24 hours.

ACUTE SYSTEMIC TOXICITY TEST

The acute systemic toxicity test is designed to determine the biological response of mice to plastics by the single dose injection of specific extracts prepared from a plastic. Of importance is the contact of the extracting medium with the available surface area of the plastic and the time and temperature during extraction, the proper cooling, agitation and decanting process, as well as the aseptic handling and storage of the extracts following extraction. [Note - Each extract must be agitated vigorously prior to withdrawal of each injection dose to insure even distribution of the extracted matter.]

Procedure:

For this test, the mice are individually identified in a suitable manner (color, coding, ear-nicking, etc.). Each mouse in the Sample and Blank groups is weighed and the initial weights recorded.

Inject into groups of 10 mice each the extracts of the Sample and Blank as outlined in the table below:

INJECTION PROCEDURE

EXTRACT OR BLANK	DOSE per Kg.	ROUTE	INJECTION RATE (ml./sec.)
1 in 20 Solution of Alcohol in Sodium Chloride Injection	50 ml.	I.V.	0.1
Cottonseed Oil	50 ml.	I.P.	...
Polyethylene Glycol 400	10 Gm.	I.P.	...

Observe the animals immediately after injection, and at 4, 24, 48, and 72 hour intervals.

EYE IRRITATION TEST

The eye irritation test is designed to test for eye irritating properties possibly present in plastic containers used for ophthalmic products by using the eye of albino rabbits.

Procedure:

The extracting media to be tested for the eye irritation test is the extracts from (1) Sodium Chloride Injection and (2) Cottonseed Oil.

Form a cup using the lower lid of the left eye of a suitably restrained albino rabbit. Instill approximately 0.2 ml. of the Blank, being careful to bring the solution in contact with as much eye surface as possible. Maintain this contact for at least 30 seconds before allowing the lid to position normally. Repeat this procedure with the right eye, instilling the Sample extract. Repeat the above procedure using three rabbits. Examine the eyes at 24, 48, and 72 hour intervals for possible irritation.