METHYL AND ETHYL ACRYLATES #79

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METHYL AND ETHYL ACRYLATES

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SUMMARY

Description

Methyl and ethyl acrylates are colorless, volatile, liquids. Both are quite soluble in alcohol and in ether, but only slightly soluble in water. Methyl acrylate has an unpleasant acrid odor. The ethyl ester has a characteristic intense, penetrating and persistent, fruity odor (16).

Methyl and ethyl acrylates polymerize readily on standing at temperatures above + 10°C; heat, light, and peroxides speed up the process (46). Polymerization in a closed container may cause a violent explosion (48). Polymerization inhibitors, however, such as hydroquinone, monomethyl ether of hydroquinone, or monomethyl ether of t-butyl-hydroquinone, are usually used in commercial grades (11,31,36). If pure, both esters can be stored without undergoing polymerization if the temperature is maintained below + 10°C (46).

Occurrence

Methyl acrylate is used in the manufacture of paper and textile coatings, plastic films, leather-finish resins, nitron fiber, and synthetic rubber, as well as, vitamin B_1, amphoteric surfactants, and chemical intermediates (27, 46).

Ethyl acrylate occurs naturally in pineapples and raspberries (19,26). The synthetic product is used as a flavoring agent in certain foods (19,23). It is widely

employed also in the manufacture of plastics, paper and textile coatings, adhesives, water emulsion paint vehicles, leather-finish resins, and intermediates (11,27,46).

Acute Toxicity

Methyl acrylate has an intraperitoneal LD_{50} in mice of 254 mg/kg BW (31). For rats, orally, a value of 300 mg/kg BW was obtained (44); the LD_{50} was 1000 ppm by inhalation (44). An oral MLD of 180-280 mg/kg BW in the rabbit has been reported (56). The LD_{50} value, via the skin, was 1235 mg/kg BW (44).

Ethyl acrylate has an intraperitoneal LD_{50} in mice of 599 mg/kg BW (31). In rats, oral LD_{50}'s of 1020 and 2080 have been reported (36,57); the Minimum Lethal Concentration (MLC) by inhalation was 2000 ppm (36). In the rabbit, an oral MLD of 280-420 mg/kg BW was found (56); by cutaneous application, the LD_{50} value was 1790 (36).

Short-Term Studies

Repeated 7-hour exposures to methyl acrylate in air at levels of 237-578 ppm proved fatal for rats, guinea pigs, and rabbits (56). Lethargy, dyspnea, and convulsive movements were observed before death (56). Pulmonary congestion, edema, and toxic degenerative changes in heart muscle, liver, and kidney occurred also (56).

Twenty-four oral sublethal doses, (23 mg/kg BW), of methyl acrylate over a period of 33 days, caused growth retardation and small weight losses in rabbits during the period of treatment (56). Growth was normal, however, throughout the remainder of the three month study (56). No significant

changes were observed in gross and microscopic examination of tissues at the conclusion of the study (56).

Small transitory weight losses were the only systemic adverse effects noted in rabbits following methyl acrylate applications to the skin at levels as high as 34 mg/kg BW (56).

A monkey subjected to 130 seven hour exposures, over a period of 185 days of up to 31 ppm of methyl acrylate in air, suffered a weight loss of 1251 grams (56).

Rats and guinea pigs repeatedly exposed to ethyl acrylate vapors at levels of 272-540 ppm, had mortality rates as high as 50-100% (36,56). Lungs, liver, and kidneys of the victims showed damage (56). Toxic degenerative changes in the heart muscle were noted also (56). Lethargy and respiratory difficulties occurred (56). Weight gains were below normal (36,56). Rabbits repeatedly exposed to ethyl acrylate vapors at a level of 272 ppm had a mortality rate of 100%. Weight changes and other symptoms as well as the nature of tissue damage were essentially the same as noted in rats and guinea pigs above (56).

Repeated oral sublethal doses of ethyl acrylate, (25 doses of 31.5 mg/kg BW each, over 35 days), caused growth retardation or small losses of weight in rabbits during the period of administration (56). Growth was normal, however, during the two months following treatment. No significant gross or microscopic changes were noted at the

conclusion of the study.

Ethyl acrylate caused death in rabbits within eight hours when applied to the skin at a level of 49.8 g/kg BW, in divided doses (56). Generalized edema and congestion of the lungs as well as cloudy swelling and congestion of heart, liver, and kidneys were found (56). The survivors experienced small transitory weight losses; no significant gross or microscopic abnormalities were noted, however, upon sacrifice some months after treatment (56).

Ethyl acrylate caused emesis in dogs when first administered orally at a dietary equivalent of 1000 ppm (11). However, when the dose was raised gradually to that level daily, no further vomiting episodes occurred over a feeding period of two years (11). Food intake and weight gains were slightly low, but not significantly so, according to the authors (11). No other adverse effects were reported.

Ethyl acrylate vapor at the 1204 ppm level, proved fatal for a monkey during the third 7-hour exposure (56). Prior to death, convulsive movements and spasmodic respiration were noted. A second animal subjected to 28 seven-hour exposures at the 272 ppm level was slightly lethargic but survived. There were no toxic signs in single animals subjected to 130 seven-hour exposures at the 26 and 24.5 ppm levels (56).

Both ethyl and methyl acrylate are highly toxic to man

by ingestion, inhalation, and skin absorption (27,46,48). Lethargy and convulsions may occur if the vapors are inhaled in high concentrations (46). The Threshold Limit Value (TLV) of ethyl acrylate is 25 ppm (49); that of the methyl ester is 10 ppm (48,49). Sensitization of the skin and respiratory tract may result from concentrated short-term exposure to either compound (49).

Long-Term Studies

Ethyl acrylate administered to rats for two years in the drinking water at a level of 2000 ppm caused a significant depression of body weight, fluid intake, and food consumption (11). Females showed a significant decrease in weight throughout the study, but the males were affected during the first year only (11). The minimum toxic level was 6-7 ppm.

Fluid intake was depressed significantly for both sexes throughout, but food consumption was significantly lower overall in the female group only (11).

There were 17 deaths amongst the 50 animals at the 2000 ppm level but 21 of the 50 controls succumbed also (11).

No microscopic lesions or abnormalities attributable to ethyl acrylate were noted. Hematologic values, urine concentrations of protein and reducing substances, as well as organ-to-body weight ratios, were within normal limits (11).

Special Studies

No reports on carcinogenicity, teratogenicity, or mutagenicity by methyl or ethyl acrylate were found. Acrylic acid, however, caused teratogenic effects in rats (42), and this information was included in the monograph because of its possible relevance to the potentialities of methyl and ethyl acrylates in this respect. Hydrolysis in the body of either ester would yield acrylic acid.

Unusually small fetuses ($P \leq 0.01$) and skeletal malformations (elongated and fused sternebrae and frontal ribs) resulted from intraperitoneal administration of as little as 0.0023 ml/kg BW (1/10 LD_{50}) of acrylic acid to pregnant rats on days 5,10, and 15 of gestation (42). The minimum toxic level was not determined in this study.

Gross abnormalities in the form of hemangiomas were found in 9.1% of fetuses at the 0.0075 ml/kg level and in 3.7% at the 0.0045 ml/kg level (42).

Fetal mortality was 6% at the highest level (42). No fetal deaths occurred at the two lower levels but resorptions were detected at the middle level (42).

Biochemistry

Methyl acrylate applied to the skin caused significant changes in the gamma-globulins, ascorbic acid, reduced glutathione, residual amino nitrogen, and packed cell volume of the blood, as well as in body temperature in rabbits (50,51,54,56). Workers in occupational contact with methyl acrylate also showed changes in the residual

amino nitrogen of the blood and the amino nitrogen content of the urine (51).

Pure methyl acrylate applied to the skin of rabbits at a level of 950 mg/kg BW caused a significant drop in the gamma-globulin level by the 10th day which extended through the 20th day but returned to normal ten days later ($P < 0.001$) (50).

When a 1% solution of methyl acrylate was applied daily for 60 days at a level of 19 mg/kg, there was an insignificant increase in gamma-globulins through the first 40 days followed by a sharp increase by the 60th day ($P < 0.001$) with a return to normal by the 80th day (50). The alpha and beta globulins were affected also (50).

The same dosage and schedule indicated above, caused a decrease in the residual amino nitrogen of the blood ($P < 0.001$) which returned toward normal by the end of the experiment (51).

Methyl acrylate at a level of 950 mg/kg BW (in equally divided doses, with a 24-hour interval) applied to the skin of rabbits caused a decrease in the ascorbic acid content of the blood during the first 20 days ($P < 0.001$) which remained low throughout the 30-day experiment ($P < 0.002$) (54). The reduced glutathione of the blood also decreased at the same rate and to the same extent for 20 days but returned to normal by the 30th day (54).

Workers in occupational contact with methyl acrylate also

showed a low blood residual amino nitrogen level ($P < 0.001$) which was directly proportional to the length of employment ($P < 0.05$) (51). The urinary amino nitrogen was lower also than that of the controls (51).

Both methyl and ethyl acrylates orally caused a decrease in body temperature of rabbits and an increase in the hematocrit (56). Single doses of either ester as low as 180 mg/kg caused hypothermia (56). The minimum dose which caused an increase in the hematocrit was 180 mg/kg for methyl acrylate and 280 mg/kg for the ethyl ester (56).

Ethyl acrylate administered orally to dogs at dietary equivalents of 500 and 1000 ppm caused emesis (11). When the dose was increased gradually from 300 ppm, to a dietary equivalent of 1000 ppm again, over a 16-week period, no further emetic effects were reported over the remainder of the two year period. There was no mention of emesis at the lower dose levels (dietary equivalents of 10, 100, and 300 ppm).

Consumer Exposure

Ethyl acrylate is employed as a flavoring agent in a variety of foods, confections, and beverages (19,22,23). Estimated average daily intakes range from 0.08 mg for infants to 2.6 mg for children and adults (21). Maximum daily intakes vary from 0.15 mg for infants to 4.95 mg for children and adults (21). Foods in which the agent is employed at the maximum use level are frozen dairy products (15.2 ppm), baked goods (13.8 ppm), and soft candy (13.3 ppm) (22).

Ethyl acrylate occurs naturally also in pineapples and raspberries (19,23).

Both methyl and ethyl acrylate are widely used in the manufacture of a variety of plastics, paper and textile coatings, adhesives, water emulsion paint vehicles, as well as synthetic rubber. Some of these are employed in food packaging material and articles for holding food, including heating prepared food (11,27,46).

Both acrylates are included in the category of substances which may migrate to foods from materials used in food packaging or handling, which are generally regarded as safe for their intended use within the meaning of federal food additive regulations (04).

CHEMICAL INFORMATION

Methyl Acrylate

I. Nomenclature

A. Common names: Methyl acrylate. (Methyl propenoate; Methyl 2-propenoate) (01,15).

B. Chemical names: Acrylic acid, methyl ester. (Propenoic acid; methyl ester: 2-propenoic acid, methyl ester) (01,15).

C. Trade names: No information.

D. Chemical Abstracts Services Unique Registry Number: 96333 (15).

II. Empirical Formula (15)

$O_2C_4H_6$

III. Structural Formula (46)

$$CH_2{=}CH{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}O{-}CH_3$$

IV. Molecular Weight (15)

86.04

V. Specifications

A. Chemical

Typical specifications of commercial grades of methyl acrylate are as follows (38):

1.	Assay (Gas Chromatography),%	98.5 min.
2.	Acidity (as acrylic acid),%	0.009 max.
3.	Water (by Karl Fischer),%	0.15 max.
4.	Inhibitors*, ppm	10-20 MEHQ, 180-220 MEHQ
5.	Color when shipped (APHA)	10 max.

* MEHQ - monomethyl ether of hydroquinone.

B. Food Grade

Food Chemicals Codex, 2nd edition, 1972, gives no information concerning methyl acrylate (16).

VI. Description

A. General characteristics

Methyl acrylate is a colorless, volatile liquid with an unpleasant acrid odor. It is a lacrimator (46,48).

B. Physical properties

Soluble in alcohol and in ether. Slightly soluble in water (6 g/100 ml at 20°C) (46,48).

Boiling point: 80°C (56)
Melting point: -76.5°C (46)
Specific Gravity: 0.95 (48)
Refractive Index: 1.400 (56)

C. Stability

Methyl acrylate polymerizes easily on standing at temperatures above + 10°C; heat, light, and peroxides speed up the process (28). Polymerization in a closed container may cause a violent explosion (48). However, it usually contains an inhibitor to prevent self-polymerization (48). Hydroquinone and hydroquinone monomethyl ether* are used for this purpose in commercial grades (40,46). If pure, methyl acrylate can be stored without undergoing polymerization if the temperature is maintained below + 10°C (46).

Methyl acrylate is a fire and explosion hazard. It is flammable and the vapor forms explosive mixtures with air (48). The vapor is heavier than air and may flash back. Flash point is 27°F (48).

VII. Analytical Methods

A typical industrial analytical procedure for methyl acrylate is as follows (39):

I. GAS LIQUID CHROMATOGRAPHY (GLC)

This GLC method may be used for methyl or ethyl acrylate or methyl or ethyl methacrylate; it can be adapted to the analysis of other acrylic and methacrylic esters.

Apparatus

Perkin-Elmer Vapor Fractometer, Model 154-C, or equivalent instrument, with a thermistor detector and 0-5 mv recorder.

Column of stainless steel or aluminum tubing, 1/4" O.D., 20 feet long, packed with 20% UCON 50 HB -2000 on 40-60 mesh firebrick.

Microsyringe, 50 μl, Hamilton miniature #705, fitted with B-D Yale No. 27 5/8-inch needle.

Operating Conditions

Column Temperature	100 ± 5°C.
Carrier Gas	Helium
Input Pressure	20 psig
Flow Rate	60 ml/min
Detector Voltage	8.0 v
Sample Size	10 μl
Chart Speed	30 inches/hr

Procedure

1. Cleaning Injection Equipment. Draw acetone through the syringe and the needle separately with an aspirator until each is thoroughly flushed. Dry with a stream of dried compressed air. Wash the plunger with acetone and wipe dry with a clean tissue. Insert the plunger into the syringe, but do not attach the needle as yet.

2. Sampling Technique. Draw a sample from a container into the syringe by slowly withdrawing the plunger. To remove air bubbles, hold the syringe in a horizontal position, remove the plunger slowly, gently incline the syringe backwards, and reinsert the plunger when a solid column of liquid forms at the back end of the syringe. With the syringe in the same position, attach the needle, and push the plunger carefully into the barrel of the

syringe until liquid appears at the needle tip. Invert the syringe to normal position, and depress the plunger slowly until the meniscus is at 10 μl. Remove excess liquid adhering to the needle tip by pressing it rapidly against the glass sample container or the fingers. Do not use a tissue or other absorbent substance.

3. Injection of Sample. Align the syringe, force the needle through the septum, inject the sample, and withdraw the syringe. This operation should be performed in the minimum possible time, not more than two seconds. It is convenient to inject the sample at the same time that the pen of the recorder passes over a major axis line on the chart paper; elution times can then be read easily.

4. The order of elution, typical retention times, and retention times with respect to methyl methacrylate (relative retention time = 1.00) are listed below. Values for the major component are indicated in italic type.

Retention Times, minutes

Material	Methyl Acrylate		Ethyl Acrylate		Methyl Methacrylate		Ethyl Methacrylate	
	Typical	Relative	Typical	Relative	Typical	Relative	Typical	Relative
Acetone	7.90	0.34	7.90	0.34	7.90	0.34	7.90	0.34
Methanol	9.75	0.42			9.75	0.42		
Ethanol			11.55	0.50			11.55	0.50
Methyl Acrylate	*14.15*	*0.61*	14.95	0.65	14.95	0.65	14.95	0.65
Ethyl Acrylate	21.35	0.92	*21.15*	*0.92*	21.40	0.92	21.30	0.92
Methyl Methacrylate	23.10	1.00	23.00	1.00	*23.15*	*1.00*	23.00	1.00
Ethyl Methacrylate	31.70	1.4	31.70	1.4	31.70	1.4	*32.05*	*1.4*

5. Calculations. Compare sample runs to synthetic samples of about the same, known composition. Correction must first be made for variations in the size of the sample. The peak height of the major component MC is the measure of sample size. The peak height of the major component (MC) in the synthetic sample is considered constant.

a. Compute a factor to adjust the peak height of MC in the test sample to the peak height of MC in the synthetic sample:

$$\text{Factor} = \frac{\text{peak height of MC in synthetic sample}}{\text{peak height of MC in test sample}}$$

b. Draw a baseline wherever a peak appears on the chart. Measure the peak height above each baseline in centimeters or blocks. Multiply each peak height by the above factor to obtain an adjusted value. For trace components, little or no correction occurs if the sample size is constant within ± 2 percent.

c. Compare the adjusted peak height of each component to the corresponding peak in the synthetic sample. If an unexpected peak appears, calculate the concentration of the material by using the height of the nearest peak in the synthetic sample as a standard. Calculate the concentration of each minor component from the equation.

$$\text{weight \%} = \frac{\text{\% component in synthetic sample X adjusted peak height in test sample}}{\text{peak height in synthetic sample}}$$

d. To calculate the assay of MC, subtract from 100 the sum of the percent concentrations of minor components (GLC), the water content (Karl Fischer), the acidity (titration), and the inhibitor content:

$$\text{assay, \%} = 100 - [\text{\% minor components} + \text{\% water} + \text{\% acidity} + \text{\% inhibitor}]$$

Water and acidity are not normally determined by GLC methods. Operating instructions for the Perkin-Elmer Vapor Fractometer are supplied by the Perkin-Elmer Co., Instrument Division, 702 Main Street, Norwalk, Conn. 06852. Information on other instruments is available from the manufacturers of laboratory supply houses.

VIII. Occurrence and levels

A. Plants

No information.

B. Animals

No information.

C. Synthetics

Methyl acrylate is used in making vitamin B_1, amphoteric surfactants, and chemical intermediates, as well as paper and textile coatings, plastic films, leather-finish resins, nitron fiber, and synthetic rubber (27,46,51). Its resin is regarded as the hardest of the acrylate series (46).

D. Natural Inorganic Sources

None.

BIOLOGICAL DATA

Methyl Acrylate

I. Acute Toxicity

A. Mice

Lawrence et al.(31) injected male albino ICR mice, 25 ± 5 g body weight, (age not specified), in groups of ten, intraperitoneally, with methyl acrylate (monomer)[a], at several dose levels, and observed the animals seven days for mortalities. The LD_{50} dose was calculated by the Cornfield-Mantel modification of Karber's method (31). The value obtained was then analyzed with the use of mathematical models of Free and Wilson, and Hansch, to relate structure to toxicity (See article for details) (31). The results are presented in Acute Toxicity Table 1 and in Table 2 (31). Seventeen other acrylates (including ethyl acrylate) were included in the study.

Regarding comparative toxicities, the authors stated - "Acrylate monomers are more toxic than the corresponding methacrylate monomers. Lower (in molecular weight) members of the series tend to be more toxic than the higher members. The straight chain substituent is less toxic than the corresponding branched chain. Simple aliphatic substituents are less toxic than substituents that contain hydroxyl or amine functional groups" (31).

[a] The methyl acrylate preparation used was obtained from Rohm and Haas Company, Philadelphia, Pa., and contained monomethyl ether of hydroquinone (15 ppm) to inhibit polymerization.

Table 1

Acute Toxicity

SUBSTANCE	ANIMAL	SEX & NO.	ROUTE	DOSAGE mg/kg BW	MEASURE-MENT	REFERENCE
Methyl acrylate	Mice ICR albinos	Males 10/Dose	i.p.	254	LD_{50}	Lawrence (31)
Methyl acrylate	Rats	6-10/Dose	p.o.	300	LD_{50}	Smyth (44)
Methyl acrylate	Rats	6/Level	Inhalation	1000[a]	LD_{50}	Smyth (44)
Methyl acrylate	Rabbits	11 Females	p.o.	180-280	MLD	Treon (56)
Methyl acrylate	Rabbits	6-10/Dose	Cutaneous	1235	LD_{50}	Smyth (44)
Ethyl acrylate	Mice ICR albinos	Males 10/Dose	i.p.	599	LD_{50}	Lawrence (31)
Ethyl acrylate	Rats	40 Males	p.o.	1020	LD_{50}	Pozzani (36)
Ethyl acrylate	Rats	- - -	p.o.	2080	LD_{50}	Union Carbide (57)
Ethyl acrylate	Rats	30	Inhalation	2000[a]	MLC	Pozzani (36)
Ethyl acrylate	Rabbits	12 Females	p.o.	280-420	MLD	Treon (56)
Ethyl acrylate	Rabbits	30	Cutaneous	1790	LD_{50}	Pozzani (36)

[a] Parts per million.

Table 2

Intraperitoneal LD_{50} Values of Methyl and Ethyl Acrylate in Mice (31)

Compound	Acute IP Toxicity in Mice: LD_{50} (mol/10^4 gm)	95% Confidence Limits	q^{σ} (C)*	π†	MEHQ‡ (ppm)
Glacial acrylic acid	0.225	0.169- 0.295	0.1806	−0.53	200
Methyl acrylate	2.949	2.281- 3.828	0.1666	0.21	15
Ethyl acrylate	5.986	4.619- 7.760	0.1662	0.73	15
Butyl acrylate	6.653	5.475- 8.083	0.1661	1.77	5
Isobutyl acrylate	5.932	4.862- 7.231	0.1662	1.64	§
2-ethylhexyl acrylate	7.195	5.991- 8.643	0.1661	3.72	50
Glacial methacrylic acid	0.564	0.482- 0.658	0.1778	−0.01	100
Methyl methacrylate	11.217	9.378- 13.412	0.1638	0.73	10
Ethyl methacrylate	10.896	9.328- 12.734	0.1634	1.25	15
Butyl methacrylate	10.481	8.893- 12.346	0.1634	2.29	10
Isobutyl methacrylate	8.398	7.345- 9.601	0.1634	2.16	10
Isodecyl methacrylate	14.251	12.041- 16.875	0.1634	5.28	‖
Lauryl methacrylate	84.531	65.694-108.769	0.1634	6.45	‖
t-butylaminoethyl methacrylate	0.937	0.853- 1.031	0.1635	1.19	100
Dimethylaminoethyl methacrylate	0.618	0.505- 0.761	0.1635	0.30	2,000
Hydroxyethyl methacrylate	4.060	3.178- 5.195	0.1636	−0.55	1,200
1,3-butylene dimethacrylate	16.063	14.335- 18.005	0.1631	2.50	100
Trimethylolpropane trimethacrylate	8.537	8.023- 9.082	0.1634	3.53	200

* Net σ charge on the carbonyl carbon as calculated by the method of Del Re.
† Calculated as the sum of substituent constants, π selected from the literature.
‡ MEHQ = monomethyl ether of hydroquinone, used to inhibit polymerization.
§ No information given concerning the inhibitor or its concentration.
‖ Contains 100 ppm hydroquinone as the polymerization inhibitor.

B. Rats

Smyth et al. (44) determined the oral LD_{50} of methyl acrylate in rats (age, weight, sex, and strain not specified) using six to ten animals per group. The data are presented in Acute Toxicity Table 1 and Table 2A.

The same authors (44) exposed rats, six per group, (age, weight, sex, and strain not given), to methyl acrylate vapors of varying concentrations for a period of four hours and determined the inhalation LD_{50} value over a span of 14 days (See Acute Toxicity Table 1 and Table 2A).

C. Rabbits

1. Smyth et al. (44) determined in rabbits the cutaneous LD_{50} of methyl acrylate using six to ten animals per group, (age, weight, sex, and strain not specified), and an exposure period of one day. The results are given in Acute Toxicity Table 1 and Table 2A.

2. Treon et al. (56) fed female rabbits, one to four per group, (age, weight, and strain not specified), by stomach tube, single doses of methyl acrylate ranging from 120 mg/kg to 1000 mg/kg BW and observed the animals over a period of two months. The time of death was recorded for all fatalities and the Minimum Lethal Dose (MLD) determined. Survivors were sacrificed and examined for gross microscopic pathologic changes. The results are presented in Acute Toxicity Table 1 and in Tables 3 and 4.

The MLD of methyl acrylate was found to be 180-280 mg/kg BW in this study (Table 3). Death was preceded by lethargy, distension of ear veins, tremors, spasms of the diaphragm, dyspnea, and cyanosis. Death occurred after six hours or less.

Pathologic changes in the heart, liver, lungs, kidneys, brain, and spleen, as well as evidence of severe local irritation of the gastrointestinal tract

Table 2A

Oral, Cutaneous, and Inhalation Acute Toxicity Data on Methyl Acrylate (44)

Capital Letters Designate Chemicals Having the Respective Hazard in the Same Order of Magnitude as the Material Studied, and Refer to the List Following the Table

MATERIAL STUDIED	SINGLE DOSE ORAL TOXICITY TO RATS		PENETRATION OF RABBIT SKIN*		SATURATED VAPOR INHALATION BY RATS		INHALATION OF KNOWN VAPOR CONCENTRATION BY RATS				PRIMARY SKIN IRRITATION	EYE INJURY FROM FLUID	
	R.F. LD_{50}		R.F. LD_{50}		Maximum for no death		Concentration	Time	Mortality in 11 days			Grade	
Methyl acetoacetate.....	3.0	O	(>10)	Z	8 hr.	Z	—	—	—	—	U	7	N
Methyl acrylate..	0.3	A'	1.3	A	—	—	1000	4	3/6	L	U	4	U
Methylbenzyl "cellosolve"..	2.29	A	5.0	O	4 hr.	I	—	—	—	—	—	3	S
Methyl "cellosolve" acetate.	3.39	O	5.25	O	—	—	7000	4	2/6	Q	—	2	Z
Methyl diacetoacetate.....	1.7	A	(>10)	Z	8 hr.	Z	—	—	—	—	C'	5	V

The words "Cellosolve" and "Carbitol" are trade marks of Carbide and Carbon Chemicals Corporation for the solvents that are ethers of ethylene and diethylene glycol respectively. These letter codes have the same significance as in our 1944 article (1). The symbol > before a letter in the table means significantly more toxic than

A Methyl "Cellosolve" solvent
B Formaldehyde
E Lactic acid
I "Cellosolve" acetate solvent
J Triisopropanolamine
L Mesityl oxide
N Ethylene chlorhydrin
O "Cellosolve" solvent
Q Cyclohexanone
S Ethanol
T Dimethyl sulfate
U Ethyl acetoacetate
V Acetone
W Ammonium hydroxide (28% NH_3)
Z "Carbitol" solvent
A' Crotonaldehyde
B' Benzene
C' Diethanolamine

Table 3

Mortality Among Rabbits Given an Oral Dose of Methyl or Ethyl Acrylate (56)

DOSAGE	METHYL ACRYLATE		ETHYL ACRYLATE	
	Number of animals that died/number given ester	Length of survival	Number of animals that died/number given ester	Length of survival
gm./kg.		*hr.*		*hr.*
1.00	1/1	2.8	—	—
0.94	—	—	1/1	3.6
0.62	1/1	4.1	1/1	6.4
0.42	1/1	4.0	2/2	12–89
0.28	2/2	5.3–5.8	0/4	—
0.18	0/4	—	0/3	—
0.12	0/2	—	0/1	—

were observed in animals that succumbed.

Methyl acrylate lowered body temperature and increased the packed cell volume of the blood. The extent of the latter was directly proportional to the dose (See Table 4).

Hypothermia and a transient weight loss were the only symptoms observed with sublethal doses. After two months, "slight to moderately severe hepatocellular degeneration and minimal degeneration of the renal tubules" were detected in survivors at autopsy.

II. Short-Term Studies

A. Rats

Treon *et al*. (56) subjected rats (specifications not given), over periods as long as 185 days, to 7, 12, 50, or 130 seven-hour exposures, to levels of 578, 237, 95, or 31 ppm, respectively, of methyl acrylate in air. Two animals were used at each level (See Table 5).

The animals were observed during and after exposure for essential signs of intoxication and weight changes. Microscopic examinations were made of tissues from lungs, heart, liver, and kidneys. The peripheral blood was also examined for any changes in the formed elements. The results are presented below and in Table 5.

Fatalities occurred at the 578 ppm level only. Both exposed animals succumbed after seven-hour exposures. Weight losses were observed at the two higher levels of exposure but not at the lower concentrations (Table 5). Irritation of mucous membranes of eyes, nose, and mouth as well as lethargy, dyspnea, and convulsive movements were toxic signs observed in the animals

Table 4

The Effects of the Oral Administration of Methyl and Ethyl Acrylate in One Dose upon the Packed Cell Volume (Hematocrit) of the Blood and upon the Rectal Temperature of Rabbits (56)

Dose	Methyl acrylate				Ethyl acrylate			
	Percentage change in packed cell volume after administration	Hours elapsed between administration and withdrawal of blood sample	Drop in rectal temperature following administration		Percentage change in packed cell volume after administration	Hours elapsed between administration and withdrawal of blood sample	Drop in rectal temperature following administration	
			°F.	Hours elapsed between administration and observation			°F.	Hours elapsed between administration and observation
gm./kg.								
0.94					+67.0	3.6	0.2	0.7
							1.2	1.7
							2.8	2.8
							4.8	3.5
0.42	+51.8	4	1.5	0.8	+27.5	6.5	1.2	0.9
			1.8	2.0			1.4	2.5
			2.9	3.3			2.4	6.3
			3.5	3.8				
0.28	+27.4	5.3	0.5	0.7	+8.0	3.8	1.5	0.5
			2.3	1.7			2.2	1.7
			3.3	2.7			1.4	3.0
			4.4	4.7			1.4	3.0
0.28	+28.9	5.8	1.6	1.1				
			1.7	2.1				
			2.7	3.0				
			4.1	4.0				
			2.8	5.5				
0.18	+8.2	5.0	0.9	1.0	0.0	4.9	1.9	1.3
			0.9	2.1			1.9	2.6
			1.2	3.5			1.7	4.0
			1.2	4.8			1.3	4.9
0.18	−6.9	3.3	0.2	1				
			0.3	3.3				

Table 5

Mortality, Weight Changes, and Signs of Intoxication Induced in Animals by Inhaled Methyl Acrylate (56)

Concentration mg./l.	Concentration p.p.m.	Duration of Exposure	Number of Various Species Exposed	Number of Animals That Died	Average Change in Weight During Exposure	Signs of Intoxication
		hr.			*gm.*	
9.04	2,620.7	2.75	4 rabbits	4	—	Distention of ear veins, conjunctival irritation, lacrimation, sneezing, nasal irritation, salivation, coughing, labored respiration, prostration, convulsions, trembling, jerking of body, cyanosis
8.70	2,522.1	1	4 rabbits	2	−146	Distention of ear veins, conjunctival irritation, contraction of pupils of eyes, lacrimation, sneezing, rubbing and twitching of nose, nasal irritation, coughing, salivation, labored respiration, rales, prostration, convulsive kicking
1.99 ±0.07	577.8	2 × 7	4 rabbits	4	−311	Excitation, distention of ear veins, closure of eyes, lacrimation, twitching and reddening of nose, salivation, labored respiration, rales, lethargy, running-like movements of legs, opisthotonos, cyanosis
		3 × 7	2 guinea pigs	2	−99	Closure of eyes, slight lacrimation, nasal and oral irritation, salivation, labored respiration, lethargy, few convulsive movements
		7 × 7	2 rats	2	−68	Closure of eyes, conjunctival irritation, nasal irritation, salivation, labored respiration, lethargy
0.817 ±0.038	236.8	11 × 7	5 rabbits	5	−609	Closure of eyes, conjunctival irritation, lacrimation, rubbing of nose, sneezing, nasal irritation, coughing, salivation, labored respiration, lethargy
		12 × 7	2 guinea pigs	2	−228	Closure of eyes, nasal irritation, salivation, labored respiration, lethargy
		12 × 7	2 rats	0	−6	Eyes closed, nasal irritation, lethargy
0.328 ±0.003	95.1	50 × 7	4 rabbits	0	+419	Slight conjunctival irritation, slight nasal irritation
		50 × 7	2 guinea pigs	0	+110	Not remarkable
		50 × 7	2 rats	0	+120	Not remarkable
0.107 ±0.007	31.0	130 × 7	4 rabbits	0	−299	Not remarkable
		130 × 7	2 guinea pigs	0	−39	Not remarkable
		130 × 7	2 rats	0	+63	Not remarkable
		130 × 7	1 monkey	0	−1251	Not remarkable

exposed to the higher levels of methyl acrylate (See Table 5 for a more detailed description of symptoms). At the 31 and 95 ppm levels, no remarkable signs of intoxication were reported (56).

Pulmonary congestion, edema and toxic degenerative changes in heart muscle, liver and kidneys were detected in animals subjected to the higher levels of methyl acrylate (Table 5) (56). The highest level which failed to give microscopic evidence of injury was 237 ppm; the least concentration which gave rise to injury was 578 ppm (56). No changes in the formed elements of the peripheral blood were reported (56).

B. Guinea pigs

Treon et al. (56) subjected guinea pigs also to methyl acrylate vapors in the study reported above, (11), at the same levels and under essentially the same conditions as reported therein (See Table 5). Two animals, (specifications not given), were exposed at each level. The observations made and results obtained are given below and in Table 5.

Mortality was 100% at the 578 and 237 ppm exposure levels. No deaths occurred at the two lower levels (95 and 31 ppm).

Toxic symptoms were essentially the same at the two higher levels (578 and 237 ppm) where irritation of the eyes, nose, and mouth as well as lethargy and dyspnea were noted. Also, at the 578 ppm level, a few convulsive movements were reported. Toxic effects were reported as not remarkable, at 95 and 31 ppm, the two lower exposure levels (Table 5).

Microscopic changes, and results of peripheral blood studies, were the same as those reported for the rats in the study mentioned above. The highest level of methyl acrylate which did not give microscopic evidence of injury was 95 ppm; the least concentration which gave rise to injury was 237 ppm (56).

C. Rabbits

Treon et al. (56) studied the effect on rabbits of multiple oral sublethal doses (approx. 1/10 lethal dose) of methyl acrylate over a period of approximately three months.

Rabbits, (strain, age, sex, and weight not specified), over a period of 33 days were given daily oral doses of methyl acrylate consisting of 23 mg/kg BW, five times weekly, until 24 doses had been administered. Two animals were used.

Growth retardation and small weight losses during the period of administration were the only toxic symptoms noted (56). Growth was normal throughout the remainder of the observation period (56).

Both animals survived. When they were sacrificed at end of the study, no significant gross or microscopic abnormalities were noted (56). The authors stated that apparently no cumulative effect resulted from methyl acrylate in the manner administered (56).

The same authors (56) investigated the effect on rabbits, over a period of from one day to two months, of methyl acrylate when administered by way of the skin. Rabbits, (strain, sex, age, and weight not given), over a period of one or two days, received multiple applications of undiluted methyl acrylate, 1-5 ml portions, at intervals of ten minutes and contact periods of one to three hours, on the intact skin. (See Table 6 for complete description.) The material was retained in contact with the skin by means of a glass cover and a resilient bandage around the animal's body, except for two instances where the application was allowed to evaporate freely. At the end of the contact period, the skin was washed with water and a sulfonated oil, and

dried. A wide neck collar prevented the animal from licking the area. Adequate ventilation was maintained to minimize the possibility of the animals breathing vapors of the compound. One animal was used for each level of treatment. Results are presented in Table 6.

Local irritation, edema, etc. occurred in all animals, but the condition of the skin returned to normal within a week after the last application (56). Small transitory weight losses occurred (56). All animals survived. No significant gross or microscopic abnormalities were noted in one animal sacrificed two months after treatment (56).

Table 6

The Effects of the Application of Undiluted Methyl or Ethyl Acrylate Upon the Intact Skin of Rabbits (56)

AREA COVERED	AMOUNT APPLIED EACH TIME	INTERVAL BETWEEN SUCCESSIVE APPLICATION	NUMBER OF APPLICATIONS PER DAY	NUMBER OF DAYS ON WHICH APPLICATIONS WERE MADE	DURATION OF CONTACT EACH DAY	TOTAL DOSAGE		FATE	LENGTH OF SURVIVAL AFTER REMOVAL FROM SKIN
						ml./kg.	gm./kg.		
Methyl acrylate									
sq in.	*ml.*	*min.*			*hr.*				
30	5	10	16	1	2.67	34.4	32.6	Survived	8 weeks; sacrificed
30	5	10	18	1	3.0	29.9	28.4	Survived	1 day; sacrificed
4.5*	1	10	6	2	1.0	4.5	4.3	Survived	2 months; sacrificed
Ethyl acrylate									
4.5*	5	5 and 10	38†	1	6.0	75.3	69.1	Died	16 hours
4.5*	5	10	30	1	5.0	54.3	49.8	Died	7.5 hours
4.5*	5	10	24	1	4.0	44.4	40.7	Survived	53 days; sacrificed
4.5*	5	20	3	2	1.0	11.7	10.7	Survived	3 months; sacrificed
4.5*	3	20	3	2	1.0	5.9	5.4	Survived	3 months; sacrificed

* Evaporation was reduced to a minimum since the material was applied beneath a glass cover.

† The first four applications were made at 5 minute intervals; the next 34 applications, at 10 minute intervals.

The same authors (56) subjected rabbits to methyl acrylate in air over periods of up to 185 days, with exposures varying from a single one-hour period up to 130 seven-hour periods, and acrylate levels ranging from 31 to 2,621 ppm (See Table 5). Four to five rabbits (specifications not given), were used for each ester concentration and exposure schedule.

The animals were observed during and after exposure for signs of intoxication and weight changes. Microscopic examinations were made of tissues from lungs, heart, liver, and kidneys. The peripheral blood was examined for any changes in the formed elements. Blood clotting time was measured by the prothrombin method of Kato. Also, the inorganic-to-total sulfates relationship in the urine was determined. The results are given below and in Table 5.

Fatalities occurred at all levels of methyl acrylate above 95 ppm. The ratios of deaths to total number of animals used were as follows:

2,621 ppm - 4/4
2,522 ppm - 2/4
578 ppm - 4/4
237 ppm - 5/5
95 ppm - 0/4
31 ppm - 0/4

The weight changes that occurred are presented in Table 5 also. Weight losses occurred at all levels where measured, except at the 95 ppm level (56).

Irritation of mucous membranes of eyes, nose, and mouth as well as lethargy, dyspnea, and convulsive movements were observed in animals exposed to the higher levels of methyl acrylate. (See Table 5 for a more detailed description of symptoms at these levels).

Slight conjunctival irritation and nasal irritation were the only symptoms mentioned at the 95 ppm exposure. Toxic signs at the 31 ppm level were reported as not remarkable (Table 5).

Pulmonary congestion, edema, and toxic degenerative changes in heart muscle, liver, and kidneys were reported (56). The highest level of methyl acrylate which failed to give microscopic evidence of injury was 95 ppm; the least concentration which gave rise to injury was 237 ppm (56).

The authors reported that in the exposed animals, "no consistent or general trend was apparent with respect to changes in the formed elements of the peripheral blood". Blood clotting time, (by Kato's method), remained within normal limits (56). Also, no significant alteration occurred in the relationship of inorganic-to-total sulfates in the urine of rabbits (56).

D. Monkeys

Treon et al. (56) subjected one monkey over a period of 185 days, to 130 seven-hour exposures to 31 ppm of methyl acrylate in air, and observed the animal for signs of intoxication and changes in weight. Microscopic examinations of tissues from the heart, liver, and kidney were made. The peripheral blood was examined for any changes in the formed elements. The results are given below and in Table 5.

Although the animal survived, the average change in weight during exposure was determined as minus 1251 g (Table 5). Signs of intoxication were reported as not remarkable, however, and no changes in the tissues were observed (56). With respect to changes in the formed elements of the peripheral blood, no consistent or general trend was apparent (56).

E. Human Toxicity

Methyl acrylate is highly toxic by ingestion, inhalation, and skin absorption (27). Lethargy and convulsions may occur if the vapor is inhaled

in high concentrations (46). The Threshold Limit Value (TLV) is 105[a] ppm (48,49). Locally it is highly irritating to the eyes, skin, and mucous membranes (46). Steere (48) warned that prolonged contact with skin or eyes may cause serious damage. Also, the skin or respiratory tract may become sensitized after concentrated short-term exposure (49).

[a] "S" (Skin) refers to the potential contribution to the over-all exposure by the cutaneous route including mucous membranes and eye, either by airborne, or more particularly, by direct contact with the substance" (49).

BIOCHEMICAL ASPECTS

Methyl Acrylate

I. Breakdown

Methyl acrylate polymerizes easily on standing (46). Heat, light, and peroxides speed up the process (46). Usually, however, a polymerization inhibitor is added (48). Monomethyl ether of hydroquinone has been used in commercial methyl acrylate for this purpose (31).

If pure, methyl acrylate can be stored below + 10°C without undergoing polymerization (46).

II. Absorption-Distribution

Treon *et al*. (56) noted in rabbits, following *oral* administration of methyl acrylate, that the characteristic odor of the ester was noticeable in all tissues of fatally poisoned animals and that it was particularly strong in the stomach.

The same authors (56) observed that *inhaled* methyl acrylate caused death in rats, guinea pigs, and rabbits (See BIOLOGICAL DATA, Methyl Acrylate, pp. 11,14,17).

According to the Merck Index (46), in man, *inhalation* of methyl acrylate vapors may be accompanied by lethargy and convulsions if the concentration is high enough.

Suvorov (51) stated that methyl acrylate has the capacity to penetrate through the intact *skin* and cause a subsequent systemic effect.

Smyth *et al*. (44) found that in rabbits, application of methyl acrylate to the *skin* caused death (See BIOLOGICAL DATA, Methyl Acrylate, p. 9).

III. Metabolism and Excretion

No information.

IV. Effects on Enzymes and Other Biochemical Parameters

A. Rabbits

1. Treon et al. (56) reported that rabbits given a single oral dose of either methyl or ethyl acrylate suffered a decrease in body temperature and an increase in the packed cell volume of the blood (See Table 4).

Both effects were noted at doses as low as 180 mg/kg BW although not all animals showed an increase in the packed cell volume of the blood (hematocrit) at this level. The authors point out that the extent of rise in the hematocrit varied directly with the size of the dose administered (See Table 4).

2. Suvorov, in a series of investigations, studied the effect of methyl acrylate via the skin on a number of constituents of the blood and urine of rabbits and man.

In the first study, Suvorov (50) determined the effect of cutaneous methyl acrylate applications on the alpha-, beta-, and gamma-globulins, albumin, and total serum protein in the rabbit. Two experiments were performed. In the first, rabbits (specifications not given), were studied over a period of 30 days, after receiving 950 mg/kg BW total, of pure methyl acrylate, in the form of two 1 ml doses (24-hr. interval between applications), on the skin of the left flank. Thirty animals were used.

Blood samples were taken from the marginal ear vein before the experiment and 10, 20, and 30 days after application of the acrylate. Total protein, albumin, and the alpha-, beta-, and gamma-globulin content of the serum specimens were determined.

There was a significant drop in the gamma-globulin level by the 10th day ($P < 0.001$), and it was still low on the 20th day ($P < 0.001$). By the 30th day, however, the concentration approximated the initial level. The author points out that, according to other workers, changes in the content of this globulin fraction are associated with changes in the synthesis of protective (defense) proteins. The alpha-globulin level had dropped significantly also by the 10th day ($P < 0.001$), but on the 20th day an increase was noted ($P < 0.01$), and the content remained high through the 30th day ($P < 0.001$). A statistically unreliable increase in the beta-globulin fraction was noted on the 10th day. By the 20th day the level had increased ($P < 0.001$) but it dropped close to the initial level again by the 30th day. Changes in the total protein and albumin fraction of the blood serum were not as significant as the above-mentioned changes in the globulin fractions.

In the second experiment, Suvorov (50) gave rabbits (specifications not given), in a study lasting 80 days, daily applications, 4 ml each, of 1% methyl acrylate solution (19 mg/kg BW), on the skin for a period of 60 days. Thirty animals were used.

Blood samples were taken prior to application of the solution, on the 20th, 40th, and 60th days of the experiment, and 20 days after the last application. Total protein, albumin, alpha-, beta-, and gamma-globulins of the blood serum and the albumin-globulin ratio were determined. An insignificant increase in gamma-globulins through the first 40 days ($P < 0.05$), followed by a sharp increase by the 60th day ($P < 0.001$), and a return to the normal value by the 80th day, was found. The total protein content decreased significantly up to the 40th day ($P < 0.001$). A significant increase, in comparison with the 40th

day level, was noted on the 60th day. However, by the 80th day, the level had decreased sharply and was even lower than the original value ($P < 0.001$). The alpha-globulin fraction decreased, then increased and remained high through the 80th day ($P < 0.02$). The beta-globulins increased up to the 60th day but returned to their normal value by the 80th day. Significance of the parallel increase in total protein and gamma-globulins by the 60th day was discussed.

The author concluded that methyl acrylate can penetrate into the organism through the skin and cause changes in the protein metabolism and particularly in the ratio of individual protein fractions.

In the second investigation, Suvorov and Kudin (54) studied the effect of methyl acrylate via the skin on the blood ascorbic acid and glutathione content of the rabbit.

Rabbits (specifications not given) were studied over a period of 30 days following two applications of pure methyl acrylate, 1 ml each (950 mg/kg BW, total dose), to the skin over an area of 3 x 3 cm^2, with exposure periods of five hours and an interval between applications of 24 hours. Thirty rabbits were treated with the acrylate and ten held as controls.

Blood levels of ascorbic acid and glutathione were determined before treatment and 10, 20, and 30 days after the applications. The animals were observed also for evidence of local toxicity. The blood ascorbic acid of treated animals decreased in comparison with that of the controls during the first ten days ($P < 0.001$). There was no substantial change during the ensuing ten day period. By the 30th day however, although the level remained low, there was a slight increase ($P < 0.002$). Absorption of ascorbic acid by blood proteins was found to be greater also in the treated rabbits by the

10th day ($P < 0.01$), and continued to increase up to the 20th day, at least. However, by the 30th day, absorption did not differ, according to the author, from the analogous indices in the controls ($P < 0.05$).

The reduced blood glutathione of treated animals decreased also during the first ten days ($P < 0.001$). On the other hand, the oxidized ($P < 0.001$) and total ($P < 0.02$) glutathione levels increased. By the 20th day, reduced glutathione remained at substantially the same level while oxidized and total glutathione continued to decrease. Thirty days after methyl acrylate application, the author stated that reduced, oxidized, and total glutathione levels did not differ from analogous indices in the controls ($P < 0.05$).

In the investigation, Suvorov (51) studied the effect of methyl acrylate on the residual amino nitrogen of the blood serum and the amino nitrogen content of the urine. In the first experiment, lasting 30 days, chinchilla rabbits, 8 months of age (sex and weight not specified), were given two 1-ml applications of pure methyl acrylate (950 mg/kg BW, total dose), five-hour exposures with a 24-hour interval between, via the skin (fur shorn before application) in a manner precluding its evaporation. Thirty animals were treated and ten held as untreated controls.

Blood samples for chemical analysis were taken from the marginal aural vein before the experiment (prior to feeding) and 10, 20, and 30 days following the applications. The data are given in Table 7. A decrease of residual amino nitrogen was detected in the blood serum of the acrylate-treated rabbits on the 10th day when compared with the controls ($P < 0.001$) (51). By the end of the experiment, however, there was indication that the decrease was lessening (See Table 7).

Degeneration and necrotic changes in the skin, and subsequent healing, paralleled the changes in blood residual amino nitrogen (51). The author mentioned that these changes possibly reflected disturbances in protein metabolism.

Urine analysis results were not reported.

Table 7

Residual Amino Nitrogen Content of Blood Serum of Rabbits Given Two Cutaneous Applications of Methyl Acrylate (51)

Statistical index	Rabbits	Time of investigation			
		before experiment	10th day of experiment	20th day of experiment	30th day of experiment
Mean ± S.E.	Experimental	5.7 ± 0.2	3.6 ± 0.1	3.1 ± 0.2	4.9 ± 0.3
	Control	5.8 ± 0.3	5.7 ± 0.1	6.1 ± 0.4	5.9 ± 0.6
P		> 0.05	< 0.001	< 0.001	> 0.05

In the second experiment, 80 days duration, Suvorov (51) gave rabbits, (same specifications as preceding experiment), daily applications of 4-ml portions of 1% methyl acrylate solution (19 mg/kg BW), five-hour exposures, for 60 days, on the skin in the manner described in the first study. Blood samples for chemical analysis were taken before the experiment and on the 20th, 40th, and 60th days of the treatment period, and also 20 days after the last application (80th day of study). The data are given in Table 8.

Repeated applications of 1% methyl acrylate solution caused effects similar to those detected following two applications of pure methyl acrylate in the preceding experiment. A lower residual amino nitrogen concentration in the blood serum of the acrylate-poisoned rabbits in comparison with the

controls was found on the 20th day ($P < 0.001$) (51). The difference was less however, by the 60th and 80th days of the study (See Table 8). The possible metabolic significance of the observed changes was indicated by the author. Necrosis at the application site and subsequent healing were observed (51). Urine analysis results were not reported.

Table 8

Residual Amino Nitrogen Content of Blood Serum of Rabbits Given 60 Daily Cutaneous Applications of Methyl Acrylate (51)

Statistical index	Rabbits	Time of investigation				
		before experiment	20th day of experiment	40th day of experiment	60th day of experiment	80th day of experiment
Mean ± S.E.	Experimental	5.6 ± 0.1	4.1 ± 0.1	3.4 ± 0.2	4.6 ± 0.1	5.1 ± 0.2
	Control	5.7 ± 0.5	5.6 ± 0.3	5.9 ± 0.3	5.8 ± 0.5	5.9 ± 0.3
P		> 0.05	< 0.001	< 0.001	< 0.05	> 0.05

B. Man

Suvorov (51) also examined workers in occupational contact with methyl acrylate for residual amino nitrogen in the blood serum and the amino nitrogen content of the urine. Thirty-six workers in comparison with 30 "donors" took part in the first study (blood residual amino nitrogen); 46 workers and 20 donors participated in the latter (amino nitrogen content of urine). Morning urine specimens only were analyzed in order to exclude, the author stated, the influence of physical stress and various physiological and emotional factors on the specimen's composition. Specimens from women were limited to the intermenstrual period.

The blood residual amino nitrogen content of the workers was low (4.6 ± 0.1 mg%) in comparison with that of the donors (5.7 ± 0.1 mg%) ($P < 0.001$).

Moreover, workers with more than two years service had lower levels (4.0 ± 0.1 mg%) than those with less service (5.2 ± 0.04 mg%) ($P < 0.05$).

The urinary amino nitrogen content of the workers also was lower than that of the donors, according to the author. The values given are 41.7 ± 3.1 and 28.1 ± 1.3 mg per 100 ml ($P < 0.001$). (Note: the sequence of these values, which are given in the original article, seems to indicate the reverse of the author's statement. However, in the summary, the author stated that "the urine of workers engaged in the manufacture of methyl acrylate has a reduced content of amino nitrogen.")

V. Drug Interaction

No information.

VI. Consumer Exposure

Methyl acrylate is not a direct food additive (62). However, it is used in the manufacture of a variety of products and coatings, some of which come into contact with food (02,11,46,51). It is one of the substances, for instance, that may be used in preparing vinylidene chloride copolymers employed in coatings on nylon-film intended for food contact use (02). Federal regulations limit the use of these coatings that contain methyl acrylate polymer units, however, to nonalcoholic foods having a pH above 5.0 (02). Methyl acrylate is used also in making vitamin B_1, amphoteric surfactants, and chemical intermediates as well as textile coatings, paper coatings, plastic films, leather-finish resins, nitron fiber, and synthetic rubber (27,46,51). Its resin is regarded as the hardest in the acrylate series (46). It is included in the list of "substances migrating to food from paper and paperboard products used in food packaging that are generally

recognized as safe for their intended use", within the meaning of Federal food additive regulations (04).

Treon et al. (56) reported that the characteristic odor of methyl acrylate was detected in metal storage containers after having been soaked in kerosine for some months.

Methyl acrylate is present also in industrial effluents, according to Chekhovskaya et al. (14), who found that the ester at a level of 300 mg/l sharply inhibited the growth of bacteria involved in water purification processes and biodegradation of effluents (14).

Ethyl Acrylate

CHEMICAL INFORMATION

Ethyl Acrylate

I. **Nomenclature**

A. Common names: Ethyl acrylate (Ethyl propenoate: Ethyl 2-propenoate) (01,15).

B. Chemical names: Acrylic acid, ethyl ester (Propenoic acid, ethyl ester: 2-Propenoic acid, ethyl ester) (01,15).

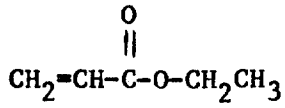
C. Trade names: No information.

D. Chemical Abstracts Services Unique Registry Number: 140885 (15).

II. **Empirical Formula** (01)

$O_2C_5H_8$

III. **Structural Formula** (23)



IV. **Molecular Weight** (15)

100.05

V. **Specifications**

A. Chemical

Typical specifications of commercial ethyl acrylate are as follows (38):

1.	Assay (Gas Chromatography), %	98.5 min.
2.	Acidity (as acrylic acid), %	0.009 max.
3.	Water (by Karl Fischer), %	0.15 max.
4.	Inhibitors*, ppm	10-20 MEHQ
		180-220 MEHQ
5.	Color when shipped (APHA)	10 max.

* MEHQ - monomethyl ether of hydroquinone.

B. Food Grade

Food Chemicals Codex (16) gives the following specifications for ethyl acrylate:

Assay. Not less than 99.5 percent of $C_5H_8O_2$.

Specific Gravity. Between 0.916 and 0.919.

Limits of Impurities

Acidity (as acrylic acid). Not more than 0.005 percent.

Antioxidants. Not more than 220 parts per million (0.022 percent) of hydroquinone and/or hydroquinone monomethyl ether.

Water. Not more than 0.05 percent.

VI. Description

A. General characteristics

Ethyl acrylate is a clear, colorless, mobile liquid with a characteristic intense, penetrating, and persistent fruity odor. It acts as a lacrimator (16,23).

B. Physical properties

It is miscible in all proportions with alcohol and with ether. One ml is soluble in about 50 ml of water (16).

Density (25°C, g/cc):	0.917	(56)
Boiling point: 99°C		(56)
Freezing point: -70°C		(56)
Refractive index: 1.404		(56)

C. Stability

The Merck Index (46) points out that ethyl acrylate polymerizes easily on standing. Heat, light, and peroxides speed up the process (46). Polymerization inhibitors, hydroquinone (36), monomethyl ether of hydroquinone

(31), and monomethyl ether of t-butylhydroquinone (11) have been used in commercial grades of ethyl acrylate.

Pure ethyl acrylate, like the methyl ester, can be stored without undergoing polymerization if the temperature is kept below + 10°C (46). However, it is flammable and always a dangerous fire and explosion hazard (27).

VII. Analytical Methods

Food Chemical Codex (16) gives the following analytical methods for ethyl acrylate:

> Assay. Determine the percent of ethyl acrylate by gas-liquid chromatography (See p. 886) using an instrument containing a thermal conductivity detector. Prepare a 2.5-meter x 6-mm column packed with 25 percent, by weight, of Carbowax 20M on 42/60 GC-22. Observe the following operating conditions during the determination: Sample, 10 µl; Injector temperature, about 300°; Column temperature, 50° to 225°, programmed at a rate of 5.6° per minute; Detector temperature, about 300°; Detector current, 200 ma; and Helium flow, about 100 ml per minute. Under the conditions described the retention time for ethyl acrylate is 9 minutes. The area of the ethyl acrylate peak is not less than 99.5 percent of the total area of all peaks.
>
> Specific gravity. Determine by any reliable method (See p.5).
>
> Acidity. Transfer 50 ml (46 grams) of the sample into a 250-ml Erlenmeyer flask, and add 50 ml of methanol previously neutralized to bromothymol blue T.S. with 0.1 N alcoholic potassium hydroxide. Add an additional 5 or 6 drops of the bromothymol blue T.S., and titrate with 0.1 N alcoholic potassium hydroxide to a bluish green end-point. Each ml of 0.1 N alcoholic potassium hydroxide is equivalent to 7.21 mg of $C_3H_4O_2$.

VIII. Occurrence and Levels

A. Plants

Ethyl acrylate occurs naturally in pineapples and raspberries (19,23,26).

B. Animals

No information.

C. Synthetics

Ethyl acrylate is a direct food flavor additive for a variety of foods (See BIOCHEMICAL ASPECTS, Ethyl Acrylate, VI). It is used also in the manufacture of plastics, paper and textile coatings, adhesives, water emulsion paint vehicles, leather-finish resins, and intermediates (11,27,46).

D. Natural inorganic sources

No information.

BIOLOGICAL DATA

Ethyl Acrylate

I. Acute Toxicity

A. Mice

Lawrence et al. (31) determined the intraperitoneal LD_{50} of ethyl acrylate (monomer) with mice of the same specifications, and using the same procedure exactly, as described previously for the intraperitoneal LD_{50} determination of methyl acrylate (See Biological Data, Methyl Acrylate p. 6).

The ethyl acrylate preparation was obtained from Rohm and Haas company, Philadelphia, Pa., and contained monomethyl ether of hydroquinone (15 ppm) to inhibit polymerization (31). Seventeen other acrylates were included in the study. The results are presented in Acute Toxicity Table 1 and in Table 2.

Regarding comparative toxicities of methyl and ethyl acrylates and the other compounds included in the study. See Biological Data, Methyl Acrylate, p. 6 .

B. Rats

1. Pozzani et al. (36) gave male albinos rats 90-120 g (strain and age not specified), ten animals per group, by stomach tube, ethyl acrylate[a] in single doses ranging from 795-1580 mg/kg BW (in "Tergitol"7 penetrant solution, 1%, vehicle) and determined the LD_{50} value from the mortality data over an observation period of 14 days. The rats were not fasted before administration of the compound.

[a] Commercial grade, inhibited with 0.25 percent hydroquinone (36).

The value obtained, 1020 mg/kg BW (950-1100), indicated that ethyl acrylate was more toxic per os than methyl methacrylate, ethyl methacrylate, and ethyl crotonate, but less toxic than methyl acrylate, according to the authors (36).

All fatalities but one occurred within the first 24 hours. Sluggishness, prostration, and narcosis preceded death (36). Gross pathological findings were: pale liver, kidneys, and spleen as well as an irritated gastrointestinal tract, and congested lungs. The authors further stated that the viscera seemed to be "macerated" by the compound (36).

The same authors (36) exposed rats, six per group, (strain, sex, age, and weight not specified) to vapors of ethyl acrylate at levels of 1000, 2000, and 4000 ppm, for four-hour periods and determined the minimum lethal concentration over a 14-day observation period. Survivors were weighed and sacrificed at end of study. The data are presented in Acute Toxicity Table 1.

All six animals at the 4000 ppm level died, five of six at the 2000 ppm level died, but none of the six at the 1000 ppm level succumbed (36). Respiratory distress was noted at the highest concentration. Marked redness of nose, ears, and feet occurred at all three levels (36). Severe congestion and hemorrhage were found in the lungs of the victims (36). The authors stated that, for rats in acute inhalation tests, ethyl acrylate was more toxic than methyl or ethyl methacrylate but less toxic than methyl acrylate (36).

2. Union Carbide Corporation (57) determined the acute oral LD_{50} in rats, (specifications not available), of ethyl acrylate, single dose procedure. The LD_{50} value obtained, 2080 mg/kg BW, indicated that this compound was approximately as toxic as a 10% solution of acrylic acid which has

an LD_{50} value of 2500 mg/kg BW, according to the authors (57).

C. Rabbits

1. Pozzani et al. (36) administered to rabbits, ten animals per group (strain, sex, age, and weight not given), via clipped skin of the trunk, ethyl acrylate (undiluted)[a] in varying amounts, (limits were at least 1580 mg/kg BW and 2520 mg/kg BW), for a period of 24 hours, and determined the LD_{50} value over a 14-day observation period. (The acrylate was retained in contact with the skin by means of a jacket of flexible "vinylite" sheeting). Survivors were sacrificed at the end of the observation period and autopsied.

The LD_{50} value obtained was 1790 mg/kg BW (1650-1950). (Acute Toxicity Table 1). The authors stated that ethyl acrylate was thus about as toxic as methyl acrylate. Pathologic findings, at autopsy, included congestion and hemorrhage of the lungs, liver mottling, congestion and occasional hemorrhage of the kidneys, and injection of intestinal blood vessels (36).

2. Treon et al. (56) fed rabbits, females, one to four per group, (age, weight, and strain not given), by stomach tube, single doses of ethyl acrylate ranging from 120 mg/kg to 940 mg/kg, and determined the minimum lethal dose in the same study reported previously for methyl acrylate (See p. 15 for observations made). The results are presented in Acute Toxicity Table 1, and in Tables 3 and 4.

The minimum lethal dose (MLD) of ethyl acrylate was found to be 280-420 mg/kg in this study (Table 3). Symptoms and pathologic changes were the same for ethyl acrylate as for the methyl ester reported above (56). Effects on body temperature and packed cell volume of the blood were the same also (56).

[a] Commercial grade, inhibited with 0.25 percent hydroquinone.

Both esters in sublethal doses produced the same symptoms and the same changes that were noted in the survivors at autopsy (56). See Table 4.

II. Short-Term Studies

A. Rats

1. Pozzani et al. (36) exposed Sherman albino rats, males and females, approximately two months old, for a maximum of 19 days, five days per week for seven hours per day, to a vapor concentration of ethyl acrylate of 540 ppm. Twenty animals (ten of each sex) were used. In addition, animals of the same specifications were exposed for a maximum of 30 days, according to the same schedule, to concentrations of 70 to 300 ppm. Thirty rats (15 of each sex) were used for each of these exposure levels. Untreated control groups were included for each acrylate dosage. (See Table 7 for other procedural details, observations made, and data obtained.)

Exposed surviving animals were anesthetized (0.1-0.2 ml of 6% "Nembutal" i.p.) and the spinal cord sectioned. Liver and kidneys were removed and weighed after withdrawal of blood until the heart action had ceased. Tissue slices were then taken from the liver, kidneys, heart, lungs, pancreas, spleen, and adrenals for histopathologic examination. The results are presented in Table 9, Fig. 1, and below. Mortality rates at the various concentrations of ethyl acrylate were as follows:

540 ppm - 65%	Control group - No deaths
300 ppm - 60%	Control group - No deaths
70 ppm - 10%	Control group - 6.7% mortality

Table 9

Results of Repeated Exposure of Rats to Ethyl Acrylate Vapors (36)

Concentration (p.p.m.) (metered)	500	0	250	0	62.5	0
Concentration (p.p.m.) (interferometer mean)	540	0	300	0	70.	0
Exposure (days)	19	20	30	30	30	30
Mean weight gain of survivors (gm.)	+9.3*	+54.6	+38.1*	+73.2	+89.7	+88.6
Mean body length of survivors (mm.)	—	—	206.2	208.2	205.7	203.0
Fatness of survivors (gm./mm.)	—	—	0.908*	1.068	1.064	1.071
Liver weight as % of body weight	—	—	4.28	4.23	4.22	4.05
Kidney weight as % of body weight	—	—	0.84*	0.78	0.84	0.80
No. of rats that died of lung infection	12*	0	11*	0	2	2
Mean no. of days to death of victims	13.8*	—	14.8*	0	13.3	11.5
No. of survivors	6*	20	12*	30	27	23
Sets of tissues examined	17	—	21	29	29	28
Sets with major pathologic changes other than "lung infection"	0	—	0	0	0	0

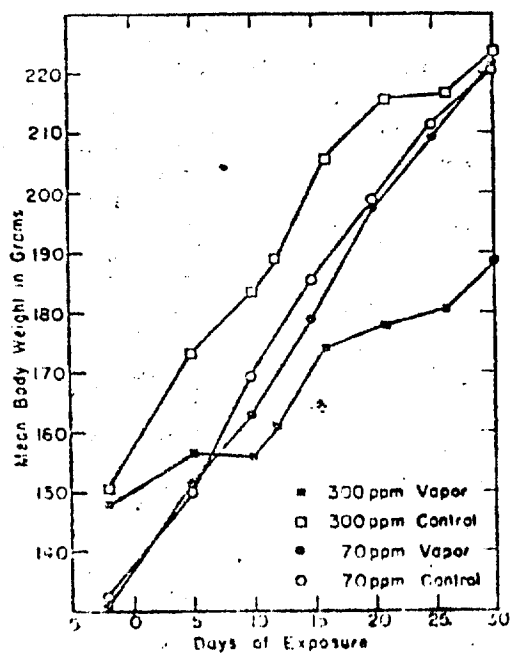


Fig. 1. Mean weights of rats exposed to ethyl acrylate vapor. (36).

Histopathologic examination of the victims revealed the following:

Lungs - pneumonic involvement.
At the 540 ppm level:
12/13 positive for above-mentioned effect.
1/13 not analyzed due to severe autolysis.
At the 300 ppm level:
10/18 positive for above-mentioned effect.
6/18 congestion of lungs.
2/18 not analyzed because of autolysis.
At the 70 ppm level:
All fatalities - No significant changes as compared with controls.
(Two control animals "died of lung infection".)

Kidneys - cloudy swelling of renal tubules.
At the 540 ppm level:
12/13 lung involvement mentioned above was "usually accompanied" by the renal tubule changes.
1/13 too far autolyzed for analysis.
At the 300 ppm level:
10/18 same as at 540 ppm.
6/18 no effect.
2/18 too far autolyzed for examination.
At the 70 ppm level:
All fatalities - Increase in kidney weight of males only. No histopathologic abnormalities. No other significant changes as compared with controls.

Liver - congestion and cloudy swelling.
At the 540 ppm level:
12/13 lung involvement mentioned above "occasionally" accompanied by the liver effect.
1/13 not analyzed; severe autolysis.
At the 300 ppm level:
15/18 same as at the 540 ppm level.
1/18 ?
2/18 not analyzed; autolysis.
At the 70 ppm level:
All fatalities - No significant changes as compared with controls.

Lower weight gains of exposed animals as compared with controls at the 540 and 300 ppm levels were considered statistically significant[a] (Table 9) (36). At the 70 ppm level, weight gain was not significantly affected.

[a] The data of exposed and control rats, given in Table 9, were compared by use of the "t" test. "If the probability associated with the "t" and specific degrees of freedom was less than 0.05, the difference was accepted as statistically significant and probably not due to random errors of measurement".

Histopathologic examination of surviving animals that had been exposed to ethyl acrylate showed the following:

Lungs - incipient pneumonic involvement.
At the 540 ppm level:
5/6 positive for above-mentioned change.
1/6 normal lungs.
At the 300 ppm level:
3/12 "only minor histologic alterations of the lung".
9/12 no change.
Liver -
At the 540 ppm level:
1/6 light cloudy swelling.
5/6 no hepatic damage.
At the 300 ppm level:
3/12 minor histologic alteration.
9/12 no change.
At the 70 ppm level:
All animals - no change.
Kidneys - no renal damage.
All levels and all animals.

2. Treon et al. (56) studied the effects of ethyl acrylate in rats, (specifications not mentioned), over periods up to 199 days during which time they were subjected to 2, 13, 28, 50, 62-130, or 87-130 seven-hour exposures, at levels of 1204, 501, 272, 75, 26, and 24.5 ppm, respectively, of the acrylate in air. Two animals were used at each level (See Table 10).

The rats were observed during and after exposure for essential signs of intoxication and weight changes. Microscopic examinations were made of tissues from lungs, heart, liver, and kidneys. The peripheral blood was examined also for any changes in the formed elements. The results are presented below and in Table 10.

Fatalities occurred at the 1204 and 501 ppm levels only. Both animals exposed at the 1204 level died. One of the two rats at the 501 ppm level succumbed. Weight losses occurred at the three higher exposure levels only. (See Table 10). Irritation of the mucous membranes of the eyes, nose, and mouth, as well as lethargy, dyspnea, and convulsive movements were noted in the animals exposed to the higher concentrations of the ester. (See Table 10

Table 10

Mortality, Weight Changes and Signs of Intoxication Induced in Animals by Inhaled Ethyl Acrylate (56)

Concentration mg./l.	Concentration p.p.m.	Duration of Exposure	Numbers of Various Species Exposed	Number of Animals That Died	Average Change in Weight During Exposure	Signs of Intoxication
		hr.			*gm.*	
4.83	1,204	7	4 rabbits	4	−159	Coughing, salivation, rales, conjunctival and nasal irritation, prostration, ataxia, convulsive movements, spasmodic respiration, diarrhea
		7	2 guinea pigs	2	−38	Hiccoughing, salivation, rales, conjunctival and nasal irritation, prostration, convulsive movements, spasmodic respiration
		2 × 7	2 rats	2	−29	Salivation, conjunctival and nasal irritation, prostration, convulsive movements, spasmodic respiration
		2.2 × 7	1 monkey	1	−627	Salivation, conjunctival and nasal irritation, prostration, convulsive movements, spasmodic respiration
2.01 ±0.034	501	4 × 7	4 rabbits	4	−308	Salivation, rales, conjunctival and nasal irritation, prostration, gasping, convulsive movements, diarrhea
		13 × 7	2 guinea pigs	2	−79	Salivation, rales, conjunctival and nasal irritation, lethargy, gasping
		13 × 7	2 rats	1	−18	Salivation, conjunctival and nasal irritation, lethargy, labored respiration
1.09 ±0.019	272	(8 to 17) × 7	4 rabbits	4	−478	Moderate conjunctival and nasal irritation, lethargy, gasping, convulsive movements
		28 × 7	2 guinea pigs	1	−72	Moderate conjunctival and nasal irritation, lethargy, moderate gasping
		28 × 7	2 rats	0	−29	Slight salivation, slight conjunctival and nasal irritation, lethargy, diarrhea
		28 × 7	1 monkey	0	−476	Slight irritation of mucous membranes, slight lethargy
0.300 ±0.002	74.8	50 × 7	4 rabbits	0	+891	None
		50 × 7	2 guinea pigs	0	+81	None
		50 × 7	2 rats	0	+27	None
0.105 ±0.001	26.2	130 × 7	2 guinea pigs	0	+322	None
		(62 to 130) × 7	2 rats	0	+25	None
		130 × 7	1 monkey	0	+336	None
0.099 ±0.007	24.5	(108 to 130) × 7	4 rabbits	0	+1058	None
		130 × 7	2 guinea pigs	0	+93	None
		(87 to 130) × 7	2 rats	0	+65	None
		130 × 7	1 monkey	0	−401	None

for a more detailed description of symptoms). No signs of intoxication were reported at the 75, 26, and 24.5 ppm levels. Pulmonary congestion, edema, and toxic degenerative changes in heart muscle, liver, and kidneys occurred at the higher levels of exposure. The highest concentration at which there was no microscopic evidence of injury was 272 ppm; the least concentration which caused injury was 501 ppm. No changes in the formed elements of the peripheral blood were reported.

The authors stated that the results of their study were in good agreement with those reported by Smyth and co-workers (13). For further comparisons, see the preceding study (II.,A,1.).

B. Guinea pigs

Treon et al. (56) subjected guinea pigs to ethyl acrylate vapors in the study reported immediately above, at the same levels and under essentially the same conditions as reported therein (See Table 10). Two animals, (specifications not given) were exposed at each level. The observations made and results obtained are given below and in Table 10.

Mortality was 100% at the 1204 and 501 ppm levels, 50% at the 272 ppm level, and 0% at the lower levels (75, 26, and 24.5 ppm). Toxic symptoms at the higher levels were the same as those reported for rabbits above, i.e., irritation of mucous membranes of eyes, nose, and mouth as well as lethargy, dyspnea, and convulsive movements. No toxic effects were observed at the lower levels (75, 26, and 24.5 ppm). Pulmonary congestion, edema, and toxic degenerative changes in heart muscle, liver, and kidneys were detected in the exposed animals at the higher levels. The highest concentration of ethyl acrylate which failed to give microscopic evidence of injury was 75 ppm; the least concentration which gave rise to injury was 272 ppm. The authors stated that "no consistent or general trend was apparent with respect to changes in the formed elements of the peripheral blood".

C. Rabbits

1. Treon et al. (56) investigated the effect of multiple oral sublethal doses of ethyl acrylate on rabbits, in parallel with those of the methyl ester, in the short-term study reported earlier in this monograph (See Biological Data, Methyl Acrylate, p. 9). Rabbits, (strain, age, sex, and weight not specified), over a period of 35 days, were given daily doses of ethyl acrylate at a level of 31.5 mg/kg BW, five times weekly, until 25 doses had been administered orally. Two animals were used in the study.

Toxic signs, weight changes, survival, gross and microscopic pathology were the same as reported for methyl acrylate (Biological Data, Methyl Acrylate, p. 9) (56). The authors stated that it would appear no cumulative effect resulted from ethyl acrylate in the manner administered (56).

The same authors (56) studied the action of ethyl acrylate on rabbits, over periods ranging from a few hours to three months, when the ester was administered via the intact skin. The animals employed, (strain, sex, age, and weight not specified), were given multiple applications of the undiluted compound, 3-5 ml portions, at intervals of 5 to 20 minutes and contact periods of one to six hours, over one to two days. (See Table 6 for number of applications, area covered, and so forth). Most of the other procedural details were the same as given in the methyl acrylate study described earlier (See Biological Data, Methyl Acrylate, p.15). One animal was used for each level of treatment. The data are given in Table 6. Two of five animals that received massive applications of ethyl acrylate died within 24 hours. The minimum lethal dose was 40.7-49.8 g/kg BW (56). Pathologic changes in the animals that succumbed were cloudy swelling and congestion of the heart, liver, and kidneys; generalized edema and congestion of the lungs; severe local

lesions (56). Small transitory losses of weight occurred (56). Local effects were the same as described in the study referred to above with the methyl ester. No significant gross or microscopic abnormalities were noted in the survivors sacrificed some months after treatment with ethyl acrylate.

The same authors (56) exposed rabbits to ethyl acrylate in the gaseous state over periods up to 199 days, with exposures varying from a single seven hour period to 130 seven-hour periods, and acrylate levels ranging from 24.5 to 1204 ppm. Four animals were used for each ester concentration and exposure schedule (See Table 10).

The rabbits were observed during and after exposure for toxic signs and weight changes. Microscopic examinations were made of tissues from lungs, heart, liver, and kidneys. The peripheral blood was examined for any changes in the formed elements. The blood clotting time also was measured by the prothrombin method of Kato. The inorganic-to-total sulfates relationship in the urine was determined. The results are given below and in Table 10.

Mortality was 100% at all levels above 75 ppm. No deaths occurred, however, at the 75 and 24.5 ppm levels. Pulmonary congestion, edema, and toxic degenerative changes in heart muscle, liver, and kidney were detected. The highest level of ethyl acrylate which did not give microscopic evidence of injury was 75 ppm; the least concentration which gave rise to injury was 272 ppm. Irritation of mucous membranes of eyes, nose, and mouth as well as lethargy, dyspnea, and convulsive movements were observed in animals exposed to the higher levels. (See Table 10 for a more detailed description of symptoms). There were no toxic signs at the 75 and 24.5 ppm exposures. Weight losses were noted at all fatality levels but not at the 75 and 24.5 concentrations.

No consistent or general trend was apparent, according to the authors, with regard to changes in the formed elements of the peripheral blood. Blood clotting time also remained within normal limits. There were no significant alterations in the relationship of inorganic-to-total sulfates in the urine of exposed rabbits. The authors stated that the results obtained were in good agreement with those reported by Smyth and his co-workers (13).

2. Pozzani et al. (36) also studied the effect of ethyl acrylate vapors on rabbits. Albino males, (strain, age, and weight not specified), were exposed for a period of 19 or 30 days, five days per week for seven hours per day, to vapor concentrations of 540, 300, or 70 ppm. Four animals were used at the 540 ppm level and 19-day exposure period. Nine rabbits were exposed to 300 ppm and 70 ppm for 30 days. Each exposure group had its own set of untreated control animals.

Fatalities were as follows:

540 ppm level	- All of the rabbits (4) died within 2 days.
Controls	- 2 of 4 died within 30 days.
300 ppm level	- All animals (9) died within 7 days.
Controls	- 3 of 8 succumbed.
70 ppm level	- 2 of 8 rabbits died within 30 days.
Controls	- 3 of 8 succumbed within 30 days.

Pathologic changes detected were catarrhal pneumonic involvement and moderate damage to the liver. At the 300 ppm level, eight of the nine fatalities showed these effects. Three of eight controls, however, died from pulmonary infections also. Results at the 70 ppm level were inconclusive (36).

D. Dogs

Borzelleca et al. (11) gave dogs, purebred beagles, both sexes (averaging six months of age at beginning of experiment), over a two-year period, monomeric ethyl acrylate dissolved in corn oil in gelatin capsules, once each

day at feeding time, in doses equivalent to dietary concentrations of 0, 10, 100, or 300 to 1000 ppm. (Initial 1000 ppm doses caused emesis which necessitated a gradual elevation from 300 to 1000 ppm during the early weeks.) The acrylate contained 10 ppm of monomethyl ether of t-butylhydroquinone as a polymerization inhibitor[a]. Four animals (four males and four females) were used for each dose level and for the control group (0 level).

The following observations and determinations were made: gross toxic effects, daily food consumption, weight change (at 13-week intervals), hematologic values (hematocrit, hemoglobin, total white and differential white cell counts), urine concentrations of reducing substances and protein, organ-to-body weight ratios at sacrifice. Also, histopathologic studies on each dog of heart, lung, liver, kidney, urinary bladder, spleen, gastroenteric, skeletal muscle, bone marrow, skin, brain, thyroid, adrenal, pancreas, pituitary, and gonad tissues. The data are presented below and in Table 11.

Emesis was noted early in the study. At the 1000 ppm level, initially all animals vomited. A dose of 500 ppm on the second day caused emesis in 50% of the dogs. However, when the amount was reduced to 300 ppm and then gradually increased over the first 16 weeks to 1000 ppm, the dose was retained throughout the remainder of the experiment. Levels of 10 and 100 ppm did not cause any emetic effects (11). Weight gains were slightly less at the 1000 ppm level than those at the 0 level (controls), and the authors stated that this effect was correlated with slightly lower food consumption (See Table 11) (11). Statistically, the effect was viewed as not significant (11). At the 100 ppm

[a] The ethyl acrylate preparation was supplied by the Rohm and Haas Company, Philadelphia, Pennsylvania.

level, weight gains of treated animals exceeded those of the controls; at the 10 ppm level, they were greater over most of the experimental period.

Hematologic findings and urine concentrations of reducing substances and protein were within normal limits in all groups (11). There were no significant adverse effects on organ weights (11). Histopathologic studies revealed no lesions attributable to ethyl acrylate (11). No deaths were reported.

Table 11

Body Weight Data on Dogs Receiving Ethyl Acrylate or Methyl Methacrylate for Two Years (11)

Material[a] (ppm)	Average body weight (kg)								
	Start	13 Wk	26 Wk	39 Wk	52 Wk	65 Wk	78 Wk	91 Wk	104 Wk
0	6.4	9.6	9.9	10.4	9.9	9.6	10.0	10.7	11.3
E-10	6.4	9.1	10.1	10.0	10.1	10.8	10.9	11.3	11.5
E-100	6.7	9.7	10.5	10.6	10.5	11.2	11.2	11.8	12.0
E-1000[b]	6.3	8.4	9.0	8.9	8.8	9.1	9.5	10.3	10.6
M-10	6.4	8.5	9.1	9.0	9.0	9.7	10.1	10.5	10.8
M-100	6.3	9.5	10.2	10.7	10.3	10.6	10.8	11.1	11.7
M-1500[b]	6.3	8.2	8.7	8.9	9.1	9.4	9.6	9.9	10.0

[a] The letter E identifies ethyl acrylate and M identifies methyl methacrylate.
[b] For dosage levels during the early weeks, see section on Methods.

E. Monkeys

Treon et al. (56) subjected four monkeys (strain, sex, and age not given), over a period of 199 days, to 2.2, 28, or 130 seven-hour exposures, at levels of 1204, 272, 26, and 24.5 ppm, respectively, of ethyl acrylate in air. One animal was used at each level (See Table 8).

The monkeys were observed during and after exposure for essential signs of intoxication and variation in weight. The peripheral blood was examined also for any changes in the formed elements. Tissues of the one fatality and

three survivors were studied microscopically for pathologic alterations. The results are presented below and in Table 10.

Ethyl acrylate at a concentration of 1204 ppm proved fatal, during the third seven-hour exposure, for the single monkey used at that level. Prior to death, salivation, conjunctival and nasal irritation, prostration, convulsive movements, and spasmodic respiration were noted. The weight loss by the time of death was 627 grams. The three animals exposed to lower levels of the acrylate survived (Table 10). The single monkey subjected to 28 seven-hour exposures at the 272 ppm level was slightly lethargic and suffered a weight loss of 476 g. Slight irritation of the mucous membranes was noted also.

There were no signs of intoxication in the animals subjected to 130 seven-hour exposures at the 26 and 24.5 ppm levels. The latter animal, however, showed a 401 g weight loss during exposure. The authors state that animals subjected to the higher concentrations of methyl and ethyl acrylates showed microscopic changes in the form of pulmonary congestion, edema, and toxic degenerative alterations in the heart muscle, liver, and kidneys. The highest concentration of ethyl acrylate which failed to give microscopic evidence of injury was 272 ppm; the least concentration which gave rise to injury was 1204 ppm. There was no consistent or general trend apparent with respect to changes in the formed elements of the peripheral blood in any of the animals.

F. Human toxicity

Ethyl acrylate is highly toxic by ingestion, inhalation, and skin absorption (27). Lethargy and convulsions may occur if the vapor is inhaled in high concentrations (46). The Threshold Limit Value (TLV) is 25 S[a] ppm (49).

[a] "S" (Skin) refers to the potential contribution to the over-all exposure by the cutaneous route including mucous membranes and eye, either by air-borne, or more particularly, by direct contact with the substance (49).

The compound is highly irritating to eyes, skin, and mucous membranes (46). Sensitization of the skin and respiratory tract may result from concentrated short-term exposure (49).

III. Long-Term Studies

Rats

Borzelleca *et al*. (11) gave rats, Wistar strain albinos, young males and females (average body weight at start - males: 63 g; females 60 g), over a period of two years, ethyl acrylate in the drinking water, according to the following schedule:

4 months - 0, 6, 60 or 2000 ppm

20 months - 0, 7, 70 or 2000 ppm

The acrylate contained 10 ppm of monomethyl ether of t-butylhydroquinone as a polymerization inhibitor[a]. Fifty animals (25 males and 25 females) were used for each level of treatment with littermate distribution within the series.

The rats were caged individually and provided with a diet of finely ground Purina Dog Chow Kibbled Meal consumed *ad libitum*. Fluid and food intake values were determined over a three-day period at the end of one and four weeks, then monthly through 6 months, and on even months thereafter; diet equivalents of ethyl acrylate consumed in the drinking water were calculated from these measurements (11). The low and medium concentrations of ester in the drinking water were chosen with the expectation that the diet equivalents would approximate 10 and 100 ppm. The highest concentration was selected after preliminary tests indicated that it would significantly depress fluid intake

[a] The ethyl acrylate was supplied by Rohm and Haas Company, Philadelphia, Pennsylvania.

(11). The authors concluded that concentrations were equivalent to 10, 100, and 3000 ppm in the diet, approximately, if allowance was made for loss of the ester by evaporation (11). (See Table 12 and article for details.)

Table 12

Fluid and Food Consumption Data and Diet Equivalents of Test Materials for Rats Receiving Ethyl Acrylate or Methyl Methacrylate in Their Drinking Water (11)

Sex	Fluid conc. (ppm)	Fluid consumption (ml/rat/day)[c]	Food consumption (g/rat/day)[c]	Diet equivalent of fluid conc. (ppm)[c]
Female	0	32.5 ± 4.9	15.6 ± 2.2	—
	E-6[a]	32.2 ± 4.5	15.5 ± 2.0	13.9 ± 1.5
	E-60[b]	30.3 ± 5.1	15.4 ± 2.1	132 ± 5
	E-2000	24.5 ± 5.3[d]	13.8 ± 1.8[d]	3530 ± 430
	M-6[a]	33.4 ± 4.6	15.6 ± 2.0	14.3 ± 1.4
	M-60[b]	32.7 ± 4.9	15.9 ± 2.0	137 ± 4
	M-2000	25.2 ± 4.8[d]	15.0 ± 2.3	3360 ± 290
Male	0	35.8 ± 4.6	19.6 ± 3.4	—
	E-6[a]	35.1 ± 5.0	20.0 ± 3.1	11.8 ± 1.3
	E-60[b]	35.4 ± 4.5	19.9 ± 3.4	120 ± 5
	E-2000	29.0 ± 4.7[d]	18.8 ± 2.7	3080 ± 220
	M-6[a]	35.8 ± 4.8	19.6 ± 2.8	12.2 ± 1.2
	M-60[b]	34.9 ± 4.2	20.4 ± 2.6	115 ± 3
	M-2000	31.3 ± 4.9[d]	19.6 ± 3.3	3210 ± 220

[a] Changed to 7 ppm at start of fifth month.
[b] Changed to 70 ppm at start of fifth month.
[c] Mean ± SD calculated from means obtained at 16 observation periods during the two-year period.
[d] Differ from control value by $P < 0.05$.

The letter E identifies ethyl acrylate, and M identifies methyl methacrylate.

The following observations and determinations were made: fluid intake, food consumption, weight changes (weekly weighings), survival, hematology (hematocrit, hemoglobin, total white and differential white cell counts), urinalysis (protein and reducing substances), organ-to-body weight ratios (heart, spleen, kidney, liver, testes) on two-year survivors, and histopathologic studies (heart, lung, liver, kidney, urinary bladder, spleen,

gastroenteric, skeletal muscle, bone marrow, skin, brain, thyroid, adrenal, pancreas, pituitary, and gonad tissues) on two-year survivors as well as on fatalities which were not autolyzed too extensively. The hematologic values and urinary concentrations of reducing substances were determined at three-month intervals for each level of acrylate used on five rats of each sex (pooled samples from the five rats were used for the urinalyses) (11). The results are presented below and in Tables 12 and 13.

Depression of body weight, fluid intake, and food consumption were the significant ($P < 0.05$) adverse effects reported (11). A significant decrease in body weight (average) at the 2000 ppm level was noted in female rats throughout the two year period; males showed this effect during the first year only. Weight losses at the lower levels (60-70 and 6-7 ppm) were not indicated as being significant, by the author. The minimum toxic level in this respect was 6-7 ppm (See Table 13). Fluid intake (mean) was depressed significantly at the 2000 ppm level for both sexes throughout the study. Decreases were very slight, however, at the two lower levels (Table 12). Food consumption (mean) was depressed significantly at the 2000 ppm level overall in the female group only (Table 12). The authors pointed out, however, that for the other rats at this level, periods of depressed food consumption tended to parallel periods of depressed growth.

The fatality rate was not affected, according to the authors, by the levels of ethyl acrylate employed. Approximately 40% of the controls died and mortality ranged from 35-50%, approximately, in the treated groups (See Table 13). Histopathological studies revealed no lesions or abnormalities, by type or incidence, at variance with those occurring naturally in rats of the strain employed and at the age used in the study (11). Hematologic values and urine concentrations of protein and reducing substances were within normal

Table 13

Body Weight and Survival Data on Rats Receiving Ethyl Acrylate or Methyl Methacrylate in Their Drinking Water for Two Years (11)

Sex	Fluid[a] conc. (ppm)	Average weight of rats (g)									Number of survivors
		Start	1 Wk	3 Wk	6 Wk	13 Wk	26 Wk	52 Wk	78 Wk	104 Wk	
Female	0	60	92	145	194	243	284	340	366	388	16
	E-6[b]	60	92	145	191	240	281	325	349	371	18
	E-60[c]	60	90	142	190	239	286	341	373	360	9
	E-2000	60	85[d]	133[d]	172[d]	221[d]	259[d]	307	315[d]	331[d]	15
	M-6[b]	60	96	151	197	250	295	365	403	382	18
	M-60[c]	60	95	149	199	250	292	350	382	400	18
	M-2000	60	83[d]	144	186	235	271	323	343	344	15
Male	0	63	100	186	283	403	483	562	561	563	13
	E-6[b]	63	101	186	287	411	498	558	594	527	12
	E-60[c]	63	102	187	283	396	485	564	590	532	15
	E-2000	63	93[d]	167[d]	260[d]	376[d]	451[d]	528[d]	543	524	18
	M-6[b]	63	99	179	283	393	470	548	554	516	18
	M-60[c]	63	102	187	285	406	468	550	571	533	15
	M-2000	63	87[d]	168[d]	271	397	474	550	558	518	13

[a] The letter E identifies ethyl acrylate, and M identifies methyl methacrylate.
[b] The concentration was raised to 7 ppm at the start of the fifth month.
[c] The concentration was raised to 70 ppm at the start of the fifth month.
[d] Differs significantly from control value ($P = < 0.05$).

limits (11). The authors indicated that organ-to-body weight ratios of the treated animals did not differ significantly from those of the controls (11).

IV. Special Studies

No reports on carcinogenicity, teratogenicity, or mutagenicity by methyl or ethyl acrylate were found in the literature review for this monograph. Acrylic acid, however, has been found to cause teratogenic effects in rats (42), and this information was included in the monograph because of its possible relevance to the potentialities of methyl and ethyl acrylates in this respect. Either ester on hydrolysis in the body would release acrylic acid. (Since acrylic acid was not included in the literature search for this monograph, other studies on its teratogenic effects may have been reported.)

Singh *et al*. (42) determined in rats, adult Sprague-Dawley virgin females 175-225 g BW), up to the 20th day of gestation (one day before expected parturition), the effect of acrylic acid on the fetus when, on days 5, 10, and 15 of gestation, 0.0075, 0.0045, or 0.0023 ml/kg BW was injected intraperitoneally. The dose levels were 1/3, 1/5, and 1/10 of the acute intraperitoneal LD_{50} (42). Five pregnant rats were used for each group (See article for breeding details). An untreated group of rats was maintained as controls. Distilled water, normal saline, and cottonseed oil controls were included also for comparative purposes although no diluent or vehicle was used for acrylic acid or any of the test compounds (Table 14).

The animals were sacrificed (ether inhalation) on the 20th day of gestation (one day before expected parturition) and the uterine horns and ovaries were exposed for counting and recording the numbers of corpora lutea, resorption sites, dead, and viable fetuses. The fetuses were then weighed dry and examined for gross abnormalities. Approximately 50% were prepared as transparent

Table 14

Embryonic-Fetal Toxicity of a Group of Methacrylate Esters on Rat Fetuses (42)

Treatment Groups	Volume Injected (ml/kg)	No. of CL*	Resorptions	Dead Fetuses	Live Fetuses	Mean Weight of Fetuses†
Untreated controls	...	60	0	0	59 (100%)	4.83 ± 0.01
Distilled water	0.8222	53	3 (7.7%)	0	36 (92.3%)	3.82 ± 0.12§
Normal saline	0.8222	60	4 (7.4%)	0	50 (92.6%)	4.15 ± 0.11§
Cottonseed oil	0.8222	53	0	0	50 (100%)	3.85 ± 0.08§
Methyl methacrylate	0.4427	51	3 (5.9%)	0	48 (94.1%)	4.17 ± 0.05§
	0.2656	54	3 (5.7%)	0	50 (94.3%)	4.22 ± 0.06§
	0.1328	53	2 (4.4%)	0	43 (95.6%)	4.22 ± 0.12§
Ethyl methacrylate	0.4076	58	7 (12.1%)	0	51 (87.9%)	3.80 ± 0.08§
	0.2446	53	6 (12.5%)	0	42 (87.5%)	3.30 ± 0.37§
	0.1223	53	5 (9.4%)	0	48 (90.6%)	4.26 ± 0.36
n-Butyl methacrylate	0.7680	54	18 (34.6%)	0	34 (65.4%)	3.86 ± 0.20§
	0.4608	58	3 (5.2%)	0	55 (94.8%)	3.95 ± 0.12§
	0.2304	55	1 (2.0%)	0	48 (98.0%)	3.98 ± 0.12§
Isobutyl methacrylate	0.4666	55	9 (16.4%)	0	46 (83.6%)	4.08 ± 0.16§
	0.2799	52	2 (4.0%)	0	48 (96.0%)	4.01 ± 0.06§
	0.1400	51	4 (8.2%)	0	45 (91.8%)	3.89 ± 0.08§
Isodecyl methacrylate	0.8222	54	19 (44.2%)	0	24 (55.8%)‡	3.42 ± 0.20§
	0.4933	57	14 (25.0%)	2 (3.6%)	40 (71.4%)	3.13 ± 0.21§
	0.2467	60	3 (5.1%)	0	56 (94.9%)	4.09 ± 0.08§
Acrylic acid	0.0075	52	3 (6.0%)	3 (6%)	44 (88.0%)	3.80 ± 0.34§
	0.0045	56	2 (3.6%)	0	54 (96.4%)	3.96 ± 0.11§
	0.0023	57	0	0	57 (100%)	4.09 ± 0.08§

Note: Five pregnant female rats were injected in each group on days 5, 10, and 15 of pregnancy.
* Number of corpora lutea.
† Numbers represent the average values (gm) ± the standard error of the mean for each group.
‡ One rat aborted all ten fetuses.
§ Significantly different from untreated controls ($P \leq 0.01$).

specimens for evaluation of skeletal malformations.

The following observations and determinations were made: embryonic-fetal toxicity (resorptions and stillbirths), gross fetal malformations (external), skeletal malformations, and fetal size (weight). The data are given in Tables 14 and 15.

The fetal mortality rate was 6% (3/47) at the highest level of acrylic acid. No fetal deaths occurred at the two lower levels; resorptions were detected, however, at the middle level (See Tables 14 and 15). In the untreated control group, no dead fetuses or resorption sites were found. Gross and skeletal teratogenic effects were found in fetuses from rats given intraperitoneal injections of acrylic acid. Gross abnormalities in the form of hemangiomas[a] were found at the highest level in 9.1% of the fetuses and in 3.7% at the middle level. The authors point out that both of these values are greater than the 95% confidence interval of the pooled volume control (Table 15). At the lowest level of acrylic acid, there were no gross defects and none were found in the untreated control group. Skeletal malformations, and unusually small fetuses also, were found at all levels of acrylic acid administered (Table 14, 15, and 16). The authors pointed out that weight differences between fetuses from treated and control rats were significant statistically at the 99% level ($P \leq 0.01$).

[a] Congenital anomalies that resemble neoplasms (47).

Table 15

Gross and Skeletal Malformations Produced by Methacrylate Esters (42)

Treatment Groups	Volume Injected (ml/kg)	Resorptions*	Gross Abnormalities†	Skeletal Abnormalities‡
Untreated controls	...	0	0	0
Distilled water	0.8222	3 (7.7%)	0	1 (5.0%)
Normal saline	0.8222	4 (7.4%)	1 (2.0%)	2 (7.7%)
Cottonseed oil	0.8222	0	1 (2.0%)	4 (15.4%)
Methyl methacrylate	0.4427	3 (5.9%)	8 (16.7%)§	0
	0.2656	3 (5.7%)	4 (8.0%)§	0
	0.1328	2 (4.4%)	1 (2.3%)	0
Ethyl methacrylate	0.4076	7 (12.1%)§	8 (15.7%)§	3 (11.1%)
	0.2446	6 (12.5%)§	5 (11.9%)§	2 (7.7%)
	0.1223	5 (9.4%)§	3 (6.3%)§	1 (5.0%)
n Butyl methacrylate	0.7680	18 (34.6%)§	4 (11.8%)§	2 (10.5%)
	0.4608	3 (5.2%)	2 (3.6%)§	2 (6.7%)
	0.2304	1 (2.0%)	2 (4.2%)§	2 (7.7%)
Isobutyl methacrylate	0.4666	9 (16.4%)§	5 (10.9%)§	2 (8.0%)
	0.2799	2 (4.0%)	3 (6.3%)§	2 (7.7%)
	0.1400	4 (8.2%)	3 (6.7%)§	0
Isodecyl methacrylate	0.8222	19 (44.2%)§	1 (4.2%)§	2 (13.3%)
	0.4933	14 (25.0%)§	1 (2.5%)	1 (4.3%)
	0.2467	3 (5.1%)	1 (1.8%)	0
Acrylic acid	0.0075	3 (6.0%)	4 (9.1%)§	4 (16.7%)§
	0.0045	2 (3.6%)	2 (3.7%)§	3 (10.7%)
	0.0023	0	0	3 (9.7%)

* Percentage resorptions are based on total number of resorptions, dead and live fetuses.
† Percentage gross abnormalities are based on total number of fetuses.
‡ Percentage skeletal abnormalities are based on total number of stained fetuses (~50% of total fetuses).
§ Values greater than the 95% confidence interval of the pooled volume control.

Table 16

Skeletal Malformations Attributed to Acrylic Acid (42)

Volume Injected (ml/kg)	Abnormality	Incidence	
0.0075	Elongated and fused sternebrae or frontal ribs or both	4/24[a]	16/7%[b]
0.0045	Elongated and fused sternebrae and frontal ribs	3/28[a]	10.7%
0.0023	Elongated and fused sternebrae and frontal ribs	3/31[a]	9.7%
Untreated controls	none	0	0

[a]Number of stained fetuses.

[b]"Value greater than the 95% confidence interval of the pooled volume control".

BIOCHEMICAL ASPECTS

Ethyl Acrylate

I. Breakdown

Ethyl acrylate, like the methyl ester, polymerizes easily on standing and heat, light, and peroxides speed up the process (46). If pure, however, both esters can be stored without incurring this change if the temperature is kept below + 10°C (46). Polymerization inhibitors, such as hydroquinone (36), monomethyl ether hydroquinone (31), and monomethyl ether of t-butylhydroquinone (11), have been used in commercial grades of ethyl acrylate. Food Chemicals Codex (16) indicates that the first two compounds (antioxidants) may be used in concentrations up to 220 ppm (0.022%). (See Chemical Information, Ethyl Acrylate, p.30).

II. Absorption-Distribution

Treon et al. (56) reported that, in rabbits, after oral administration of ethyl acrylate, the characteristic odor of the compound was noticeable in all tissues of fatally poisoned animals, and that it was especially strong in the stomach (56).

The same authors (56) indicated that two rabbits given 30 to 38 skin applications of 5 ml each in a single day, died in a matter of hours after the last application and showed changes in the heart, liver, kidneys, and lungs (56).

Union Carbide Corporation reports (57) indicated that acute toxicity data on skin penetration by ethyl acrylate in rabbits suggested that harmful amounts may penetrate after massive or prolonged skin contact (57).

The Merck Index (46) states that, in man, inhalation of ethyl acrylate

vapors in high concentrations may result in lethargy and convulsions.

III. Metabolism and Excretion

No information.

IV. Effects on Enzymes and Other Biochemical Parameters

Treon et al. (56) noted that rabbits given a single oral dose of ethyl acrylate experienced a decrease in body temperature accompanied by an increase in the hematocrit (packed cell volume of the blood). (See Biological Data, Ethyl Acrylate, p.35 and Table 4). Body temperature decreases occurred with doses as low as 180 mg/kg (minimum quantity reported). The authors indicated, however, that the decrements were greater in the fatally poisoned animals than in survivors (Table 3 and 4). Increases in the packed cell volume of the blood were found at doses as low as 280 mg/kg but not at the 180 mg/kg level. The authors pointed out that the extent of increase varied directly with the amount of acrylate administered (See Table 4).

Borzelleca et al. (11) fed four dogs gelatin capsules containing ethyl acrylate at a dietary equivalent of 1000 ppm and noted that all of the animals experienced emetic effects on the first day. On the second day, a dietary equivalent of 500 ppm caused emesis in two of the animals. When the dose was reduced to a dietary equivalent of 300 ppm, all of the animals retained the material. The dose was then increased gradually, without emetic episodes, over a period of 16 weeks to a dietary equivalent of 1000 ppm again for the remainder of a two-year period. No further emesis was reported. There was no mention of emetic effects at the lower dose levels (11). (See Biological Data, Ethyl Acrylate, p.44).

V. Drug Interaction

No information.

VI. Consumer Exposure

Ethyl acrylate is a direct food additive employed as a flavoring agent in fruit, liquor, and rum flavors (05,19,22,23). Federal regulations permit its use, along with certain other synthetic flavoring substances and adjuvants, "in the minimum quantity required to produce their intended effect, and otherwise in accordance with all the principles of good manufacturing practice" (05). Some of the foods and confections in which ethyl acrylate is used are: baked goods, gelatin puddings, ice cream and ices, candy, and chewing gum; it is used also in beverage (19,22,23).

Estimated average daily intakes from all food categories, according to the Comprehensive GRAS Survey - NAS/NRC 1972, range from 0.08 mg for infants (0-5 months of age) to 2.6 mg for children and adults (2-65+ yrs. of age) (Table 17) (21). Maximum estimated daily intakes vary from 0.15 mg for infants to 4.95 mg for children and adults (Table 17) (21).

Foods in which ethyl acrylate is employed at the maximum use level, as reported in the survey mentioned above, are frozen dairy products (15.2 ppm), baked goods (13.8 ppm), and soft candy (13.3 ppm) (Table 18) (22).

The total 1970 poundage reported to FEMA and NAS was 153 pounds (20).

Ethyl acrylate is a natural constituent of pineapples and raspberries (19, 23,26). Between 13.6 and 17.8 million cases[a] of pineapple, and 13,704-15,354 thousand cases[b] of pineapple juice, were canned in the United States during the period 1960-1970 (59).

[a] In terms of 24 No. 2 1/2 cans.
[b] Equivalent cases of 24 No. 2 cans.

Table 17

Possible Daily Intakes of FEMA Questionnaire Substances Not in NAS Appendix A (Group III), Per Food Category and Total Dietary, Based on Food Consumption by Total Sample (21)

Substance Name Survey No.	Food Category No.	Name	No. of Firms	Age	Possible Daily Intake, mg. Average	High A	High B
Ethyl Acrylate	01	Baked Goods(R)	10	0-5 mo.	0.039941	0.052863	0.046779
				6-11 mo.	0.298383	0.608513	0.349466
FEMA 24180				12-23 mo.	0.640231	1.054913	0.749838
				2-65+yr.	1.611738	2.394112	1.887666
	07	Frozen Dairy(R)	6	0-5 mo.	0.010596	0.043442	0.015203
				6-11 mo.	0.100?59	0.279727	0.144431
				12-23 mo.	0.152578	0.358136	0.218927
				2-65+yr.	0.271251	0.653756	0.389203
	10	Meat Prods(R)	*	0-5 mo.	0.000110	0.000290	0.000110
				6-11 mo.	0.002070	0.005580	0.002070
				12-23 mo.	0.003020	0.005190	0.003020
				2-65+yr.	0.007840	0.013010	0.007840
	16	Soft Candy(R)	8	0-5 mo.	0.002028	0.020277	0.002660
				6-11 mo.	0.022304	0.068941	0.029262
				12-23 mo.	0.035484	0.094286	0.046554
				2-65+yr.	0.058802	0.178434	0.077146
	20	Gelatin Pud(R)	7	0-5 mo.	0.013530	0.018266	0.021689
				6-11 mo.	0.086594	0.262487	0.138807
				12-23 mo.	0.093359	0.22730	0.149651
				2-65+yr.	0.138009	0.355170	0.221223

Table 17 (cont'd)

Substance Name Survey No.	Food Category No.	Name	No. of Firms	Age	Possible Daily Intake, mg. Average	High A	High B
	23	Bev Type I(R)	10	0-5 mo.	0.011388	0.017082	0.020994
				6-11 mo.	0.107714	0.368694	0.198566
				12-23 mo.	0.257184	0.771078	0.474110
				2-65+yr.	0.493490	1.317713	0.909731
	24	Bev Type II(R)	*	0-5 mo.	0.000000	0.000000	0.000000
				6-11 mo.	* * * *	0.000038	* * * *
				12-23 mo.	* * * *	0.000075	* * * *
				2-65+yr.	0.012188	0.035400	0.032500
	31	Chewing Gum(R)	*	0-5 mo.	* * * *	* * * *	* * * *
				6-11 mo.	0.000007	0.000007	0.000011
				12-23 mo.	0.000007	0.000021	0.000011
				2-65+yr.	0.000014	0.000026	0.000022
	99	All Categories	15	0-5 mo.	0.077593	0.152220	0.107435
				6-11 mo.	0.617731	1.593987	0.862613
				12-23 mo.	1.181864	2.511008	1.642110
				2-65+yr.	2.593331	4.947624	3.525332

Table 18

Usage Levels Reported for FEMA Questionnaire Substances Not in NAS Appendix A (Group III) - Regular Foods Only (22)

Substance Name (Survey No.)	Food Category No.	Food Category Name	No. Firms Reporting	Usual Use Wtd Mean, ppm	Maximum Use Wtd, Mean, ppm
Ethyl Acrylate FEMA 24180	01	Baked Goods(R)	10	11.747362	13.758497
	07	Frozen Dairy(R)	6	10.595727	15.203252
	10	Meat Prods(R)	*	00.100000	00.100000
	16	Soft Candy(R)	8	10.138322	13.301087
	20	Gelatin Pud(R)	7	6.765134	10.844271
	23	Bev Type I(R)	10	4.745095	8.747414
	24	Bev Type II(R)	*	0.375000	1.000000
	31	Chewing Gum(R)	*	0.070000	0.110000

The commercial pack of frozen raspberries (1960-1971) varied from a high of 38,773,000 pounds in 1963 to a low of 26,044,000 pounds in 1968; in 1971, 28,102,000 pounds were put up (59).

The amount of ethyl acrylate produced in the United States during 1969 was 199,720,000 pounds; 68,115,000 pounds were sold. In 1970, 207,269,000 pounds were produced and 68,202,000 pounds sold (60).

Ethyl acrylate is very widely used in the United States in the manufacture of a variety of products and coatings such as plastics, paper and textile coatings, adhesives, water emulsion paint vehicles, and synthetic rubber. Some of these are employed in food packaging materials and articles for holding food, including heating of prepared food (02,06,11,27,46,51). It is included in the category of substances which may migrate to foods, from materials used in food packaging or handling, that are generally regarded as safe for their intended use within the meaning of Federal food additive regulations (04).

APPENDIX

Polymerization Inhibitors Used in Methyl and Ethyl Acrylates

I. Hydroquinone (p-Hydroquinone) (40,46)

A. Synonyms (40,46)

p-Dihydroxybenzene; 1,4-Benzenediol; Quinol; Hydroquinol; Tecquinol.

B. Empirical Formula (46)

$C_6H_6O_2$

C. Structural Formula (46)

D. Molecular Weight (46)

110.11

E. Description

Hydroquinone is a colorless solid which crystallizes in the form of hexagonal prisms (40).

It is freely soluble in alcohol and in ether, slightly soluble in benzene, and soluble in 14 parts of water (46).

Physical constants are (40):

mp: 170.5°C
bp: 286.2°C
d : 1.358 at 20°/4°C
flash p: 329°F (C.C.)

F. Uses

Photography reducer and developer; analytical reagent for phosphates; antioxidant (46).

G. Toxicity

a. Experimental animals

Species	Route	LD_{50} mg/kg	Reference
Mice	i.p.	160-170	Sax (40)
Rats	p.o.	320	Merck Index(46)
Rats	p.o.	370-390	Sax (40)
Rats	i.p.	170-175	Sax (40)
Rabbits	i.p.	125-150	Sax (40)

b. Human toxicity

1. Acute toxicity

Ingestion of 5 g has caused death (40,46).

Ingestion of 1 g has produced tinnitis, nausea, vomiting, headache, dizziness, sense of suffocation, increased respiration rate, pallor, muscular twitchings, dyspnea, cyanosis, convulsions, delirium, and collapse (40,46).

As an acute toxic hazard, hydroquinone has been rated as follows (40):

Acute local: Irritant 2: Allergen 1

Acute systemic: Ingestion 2; Inhalation 2

("1 SLIGHT: Causes readily reversible changes which disappear after end of exposure"; 2 MODERATE: May involve both irreversible and reversible changes not severe enough to cause death or permanent injury") (40).

2. Chronic toxicity

Staining and opacification of the cornea occur in personnel exposed for prolonged periods to concentrations of vapor not high enough to produce systemic effects (46).

Keratitis and discoloration of the conjunctiva have resulted among persons exposed to concentrations of 10-30 mg of the vapor or dust per cubic meter of air. "It is considered to be more toxic than phenol". The Threshold Limit

Value - TLV:ACGIH (recommended) is 2 mg per cubic meter of air (40).

As a chronic toxic hazard, hydroquinone has been rated as follows:

Chronic local: Allergen 2

Chronic systemic: Ingestion 2: Inhalation 2.

("2 MODERATE: May involve both irreversible and reversible changes not severe enough to cause death or permanent injury") (40).

One authority states: "Under conditions of ordinary use, hydroquinone does not present a serious hazard. Relatively safe in very low concentrations. Has been proposed as a food antioxidant" (46).

II. Hydroquinone Monomethyl Ether (40)

A. Synonyms

4-Methoxy phenol; Hydroxy anisole (40).

B. Empirical Formula (40)

$C_7H_8O_2$

C. Structural Formula

D. Molecular Weight (40)

124.13

E. Description (40)

Hydroquinone monomethyl ether is a white, waxy solid.

Physical constants are (40):

mp: 52.5°C
bp: 246°C
d : 1.55 at 20°/20°C

It can react with oxidizing materials (40). Regarded as a slight fire hazard when exposed to heat or flame (40). As an explosion hazard, details are unknown (40).

F. Toxicity

As a toxic hazard, hydroquinone monomethyl ether has been rated as follows (40):

Acute local: U

Acute systemic: Ingestion 2; Inhalation 2

Chronic local: U

Chronic systemic: U

("U UNKNOWN: No information on humans considered valid by the authors; 2 MODERATE: May involve both irreversible and reversible changes not severe enough to cause death or permanent injury") (40).

III. Monomethyl Ether of t-Butylhydroquinone

No information.

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(h) Substances migrating to food from paper and paperboard products used in food packaging that are generally regarded as safe for their intended purpose, within the meaning of Section 409 of the Act
21 Code of Federal Regulations 121.101:32-33

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COPIES OF ARTICLES CITED IN TEXT

American Chemical Society. 1972

Chemical Abstracts. Chemical Substances
Index P-Z, 2-Propenoic acid (79-10-7)

Chemical Abstracts Service, Ohio State
University, Columbus
76 (Jan.-June):1972, p. 2963CS and
following

(b) Specifications:

Nylon resins	Specific gravity	Melting point	Solubility in boiling 4.2N HCl	Maximum extractable fraction in selected solvents (expressed as percent by weight of resin).			
				Water	95 percent ethyl alcohol	Ethyl acetate	Benzene
* * *	* * *	* * *	* * *	*Percent* * * *	*Percent* * * *	*Percent* * * *	*Percent* * * *
(1) Nylon 11 resins * * *	* * *	* * *	* * *	0.30	0.35	0.25	0.30
(2) Nylon 11 resins * * *	* * *	* * *	* * *	0.35	1.60	0.35	0.40
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington, D.C. 20201, written objections thereto, preferably in quintuplicate. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof.

Effective date. This order shall become effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348 (c)(1))

Dated: August 28, 1967.

J. K. KIRK,
Associate Commissioner for Compliance.

[F.R. Doc. 67-10326; Filed, Sept. 1, 1967; 8:47 a.m.]

PART 121—FOOD ADDITIVES

Subpart F—Food Additives Resulting From Contact With Containers or Equipment and Food Additives Otherwise Affecting Food

VINYLIDENE CHORIDE COPOLYMER COATINGS FOR NYLON FILM

The Commissioner of Food and Drugs, having evaluated the data submitted in petitions (FAP 7B2063, 7B2064) filed by Morton Chemical Co., Div. of Morton International, Inc., 110 North Wacker Drive, Chicago, Ill. 60606, and other relevant material, has concluded that the food additive regulations should be amended to provide for the use of methacrylic acid and methyl acrylate as components of vinylidene chloride copolymers used in coatings on nylon film intended for food-contact use. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348 (c)(1)) and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (21 CFR 2.120), § 121.2599(b) is revised to read as follows:

§ 121.2599 Vinylidene chloride copolymer coatings for nylon film.

* * * * *

(b) The coatings are prepared from vinylidene chloride copolymers produced by copolymerizing vinylidene chloride with one or more of the monomers acrylonitrile, ethyl acrylate, methacrylic acid, and methyl acrylate. The finished copolymers contain at least 50 weight percent of polymer units derived from vinlyidene chloride. Finished vinylidene chloride copolymer coatings containing polymer units derived from methyl acrylate and/or methacrylic acid are limited to use in contact with nonalcholic foods having a pH above 5.0.

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington, D.C. 20201, written objections thereto, preferably in quintuplicate. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof.

Effective date. This order shall become effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1))

Dated: August 28, 1967.

J. K. KIRK,
Associate Commissioner for Compliance.

[F.R. Doc. 67-10327; Filed, Sept. 1, 1967; 8:47 a.m.]

SUBCHAPTER C—DRUGS

PART 141a—PENICILLIN AND PENICILLIN-CONTAINING DRUGS; TESTS AND METHODS OF ASSAY

PART 141c—CHLORTETRACYCLINE (OR TETRACYCLINE) AND CHLORTETRACYCLINE- (OR TETRACYCLINE-) CONTAINING DRUGS; TESTS AND METHODS OF ASSAY

PART 146c—CERTIFICATION OF CHLORTETRACYCLINE (OR TETRACYCLINE) AND CHLORTETRACYCLINE- (OR TETRACYCLINE-) CONTAINING DRUGS

PART 148j—NOVOBIOCIN

PART 148o—PAROMOMYCIN

PART 148r—TYROTHRICIN

PART 148x—LINCOMYCIN

Miscellaneous Amendments

Under the authority vested in the Secretary of Health, Education, and Welfare by the Federal Food, Drug, and Cosmetic Act (sec. 507, 59 Stat. 463, as amended; 21 U.S.C. 357) and delegated by him to the Commissioner of Food and Drugs (21 CFR 2.120), the antibiotic drug regulations are amended as follows to effect minor technical and editorial changes:

1. In § 141a.113 *Clemizole penicillin G*, paragraph (c) is amended by changing "use 1.0 milligram" to read "use 1.0 milliliter".

2. In § 141c.266 *Demethylchlortetracycline hydrochloride tablets*, paragraph (a) is amended in the second sentence by changing "of demethylchlortetracycline that" to read "of demethylchlortetracycline hydrochloride that".

3. In § 146c.263 *Demethylchlortetracycline-nystatin for oral suspension*, paragraph (a) is amended by deleting the word "hydrochloride" the first place that it occurs.

4. In § 148j.2 *Calcium novobiocin*, paragraph (b)(4) is amended by changing "solution" to read "suspension".

5. In § 148o.3 *Paromomycin sulfate syrup*, paragraph (b)(1) is amended by changing "point" to read "concentration".

6. In § 148r.4 *Tyrothricin cream*, paragraph (b) is amended by changing "25 milliliters of 80 percent ethyl alcohol" in two places to read "20 milliliters of 80 percent ethyl alcohol", and by changing "10-milliliter volumetric flask" to read "100-milliliter volumetric flask".

7. In § 148x.1 *Lincomycin hydrochloride monohydrate*, paragraph (b)(1)(vii) is amended by changing "incubated for 10 to 18 hours" to read "incubated for 16 to 18 hours".

The changes effected by this order are merely of a minor technical or editorial nature; therefore, notice and public procedure and delayed effective date are not prerequisites to this promulgation.

21 CODE of Fed. Reg. 21:101, 32, 33

tiated by the Commissioner will likewise be published. Following such publication, the proceedings shall be the same as prescribed by section 409 of the act and the regulations in this part for the promulgation of a regulation.

(b) "Reasonable grounds" shall include an explanation showing wherein the person has a substantial interest in such regulation and an assertion of facts (supported by data if available) showing that new information exists with respect to the food additive or that new uses have been developed or old uses abandoned, that new data are available as to toxicity of the chemical, or that experience with the existing regulation or exemption may justify its amendment or repeal. New data should be furnished in the form specified in § 121.51 for submitting petitions.

§ 121.75 Exemption for investigational use and procedure for obtaining authorization to market edible products from experimental animals.

A food additive or food containing a food additive intended for investigational use by qualified experts shall be exempt from the requirements of section 409 of the act under the following conditions:

(a) If intended for investigational use in vitro or in laboratory research animals, it bears a label which states prominently, in addition to the other information required by the act, the warning:

Caution. Contains a new food additive for investigational use only in laboratory research animals or for tests in vitro. Not for use in humans.

(b) If intended for use in animals other than laboratory research animals and if the edible products of the animals are to be marketed as food, permission for the marketing of the edible products as food has been requested by the sponsor, and authorization has been granted by the Food and Drug Administration in accordance with § 135.3 of this chapter or by the Department of Agriculture in accordance with § 309.20 of Title 9 (9 CFR 309.20), and it bears a label which states prominently, in addition to the other information required by the act, the warning:

Caution. Contains a new food additive for use only in investigational animals. Not for use in humans.

Edible products of investigational animals are not to be used for food unless authorization has been granted by the U.S. Food and Drug Administration or by the U.S. Department of Agriculture.

[36 F.R. 18377, Sept. 14, 1971]

Subpart B—Exemption of Certain Food Additives From the Requirement of Tolerances

§ 121.101 Substances that are generally recognized as safe.

(a) It is impracticable to list all substances that are generally recognized as safe for their intended use. However, by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, sugar, vinegar, baking powder, and monosodium glutamate as safe for their intended use. The lists in paragraph (d) of this section include additional substances that, when used for the purposes indicated, in accordance with good manufacturing practice, are regarded by the Commissioner as generally recognized as safe for such uses.

(b) For the purposes of this section, good manufacturing practice shall be defined to include the following restrictions:

(1) The quantity of a substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritional, or other technical effect in food; and

(2) The quantity of a substance that becomes a component of food as a result of its use in the manufacturing, processing, or packaging of food, and which is not intended to accomplish any physical or other technical effect in the food itself, shall be reduced to the extent reasonably possible.

(3) The substance is of appropriate food grade and is prepared and handled as a food ingredient. Upon request the Commissioner will offer an opinion, based on specifications and intended use, as to whether or not a particular grade or lot of the substance is of suitable purity for use in food and would generally be regarded as safe for the purpose intended, by experts qualified to evaluate its safety.

(c) The inclusion of substances in the list of nutrients does not constitute a finding on the part of the Department that the substance is useful as a supplement to the diet for humans.

(f) *Trace minerals added to animal feeds.*[1] These substances added to animal feeds as nutritional dietary supplements are generally recognized as safe when added at levels consistent with good feeding practice.

Element	*Source compounds*
Cobalt	Cobalt acetate.
	Cobalt carbonate.
	Cobalt chloride.
	Cobalt oxide.
	Cobalt sulfate.
Copper	Copper carbonate.
	Copper chloride.
	Copper gluconate.
	Copper hydroxide.
	Copper orthophosphate.
	Copper oxide.
	Copper pyrophosphate.
	Copper sulfate.
Iodine	Calcium iodate.
	Calcium iodobehenate.
	Cuprous iodide.
	3,5-Diiodosalicylic acid.
	Ethylenediamine dihydroiodide.
	Potassium iodate.
	Potassium iodide.
	Sodium iodate.
	Sodium iodide.
	Thymol iodide.
Iron	Iron ammonium citrate.
	Iron carbonate.
	Iron chloride.
	Iron gluconate.
	Iron oxide.
	Iron phosphate.
	Iron pyrophosphate.
	Iron sulfate.
	Reduced iron.
Manganese	Manganese acetate.
	Manganese carbonate.
	Manganese citrate (soluble).
	Manganese chloride.
	Manganese gluconate.
	Manganese orthophosphate.
	Manganese phosphate (dibasic).
	Manganese sulfate.
	Manganous oxide.
Zinc	Zinc acetate.
	Zinc carbonate.
	Zinc chloride.
	Zinc oxide.
	Zinc sulfate.

(g) Synthetic flavoring substances and adjuvants that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

[1] All substances listed may be in anhydrous or hydrated form.

Acetaldehyde (ethanal).
Acetoin (acetyl methylcarbinol).
Aconitic acid (equisetic acid, citridic acid, achilleic acid).
Anethole (parapropenyl anisole).
Benzaldehyde (benzoic aldehyde).
N-Butyric acid (butanoic acid).
d- or *l*-Carvone (carvol).
Cinnamaldehyde (cinnamic aldehyde).
Citral (2,6-dimethyloctadien-2,6-*al*-8, geranial, neral).
Decanal (*N*-decylaldhehyde, capraldehyde, capric aldehyde, caprinaldehyde, aldehyde C-10).
Diacetyl (2,3-butandeione).
Ethyl acetate.
Ethyl butyrate.
3-Methyl-3-phenyl glycidic acid ethyl ester (ethyl-methyl-phenyl-glycidate, so-called strawberry aldehyde, C-16 aldehyde).
Ethyl vanillin.
Eugenol.
Geraniol (3,7-dimethyl-2,6 and 3,6-octadien-1-*ol*).
Geranyl acetate (geraniol acetate).
Glycerol (glyceryl) tributyrate (tributyrin, butyrin).
Limonene (*d*-, *l*-, *and dl*-).
Linalool (linalol, 3,7-dimethyl-1,6-octadien-3-*ol*).
Linalyl acetate (bergamol).
1-Malic acid.
Methyl anthranilate (methyl-2-aminobenzoate).
Piperonal (3,4-methylenedioxy-benzaldehyde, heliotropin).
Vanillin.

(h) Substances migrating to food from paper and paperboard products used in food packaging that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Acetic acid.
Alum (double sulfate of aluminum and ammonium potassium, or sodium).
Aluminum hydroxide.
Aluminum oleate.
Aluminum palmitate.
Ammonium chloride.
Ammonium hydroxide.
Calcium chloride.
Calcium hydroxide (lime).
Calcium sulfate.
Casein.
Cellulose acetate.
Clay (kaolin).
Copper sulfate.
Cornstarch.
Corn sugar (sirup).
Dextrin.
Diatomaceous earth filler.
Ethyl cellulose.
Ethyl vanillin.
Ferric sulfate.
Ferrous sulfate.
Formic acid or sodium salt.

Glycerin.
Guar gum.
Invert sugar.
Iron, reduced.
Locust bean gum (carob bean gum).
Magnesium carbonate.
Magnesium chloride.
Magnesium hydroxide.
Magnesium sulfate.
Methyl and ethyl acrylate.
Mono- and diglycerides from glycerolysis of edible fats and oils.
Oleic acid.
Oxides of iron.
Potassium sorbate.
Propionic acid.
Propylene glycol.
Silicon dioxides.
Pulps from wood, straw, bagasse, or other natural sources.
Soap (sodium oleate, sodium palmitate).
Sodium aluminate.
Sodium carbonate.
Sodium chloride.
Sodium hexametaphosphate.
Sodium hydrosulfite.
Sodium hydroxide.
Sodium phosphoaluminate.
Sodium silicate.
Sodium sorbate.
Sodium sulfate.
Sodium thiosulfate (additive in salt).
Sodium tripolyphosphate.
Sorbitol.
Soy protein, isolated.
Sulfamic acid.
Sulfuric acid.
Starch, acid modified.
Starch, precelatinized.
Starch, unmodified.
Sucrose.
Talc.
Urea.
Vanillin.
Zinc hydrosulfite.
Zinc sulfate.

(i) Substances migrating to food from cotton and cotton fabrics used in dry food packaging that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Acacia (gum arabic).
Acetic acid.
Beef tallow.
Calcium chloride.
Carboxymethylcellulose.
Coconut oil, refined.
Corn dextrin.
Cornstarch.
Fish oil (hydrogenated).
Gelatin.
Guar gum.
Hydrogen peroxide.
Japan wax.
Lard.
Lard oil.
Lecithin (vegetable).
Locust bean gum (carob bean gum).
Oleic acid.
Peanut oil.
Potato starch.
Sodium acetate.
Sodium bicarbonate.
Sodium carbonate.
Sodium chloride.
Sodium hydroxide.
Sodium sulfate.
Sodium silicate.
Sodium tripolyphosphate.
Sorbose.
Soybean oil (hydrogenated).
Stearic acid.
Talc.
Tall oil.
Tallow (hydrogenated).
Tallow flakes.
Tapioca starch.
Tartaric acid.
Tetrasodium pyrophosphate.
Urea.
Wheat starch.
Zinc chloride.

(Secs. 201(s), 409, 701(a), 52 Stat. 1055, 72 Stat. 1784, 1785 et seq., as amended; 21 U.S.C. 321(s), 348, 371(a)) [30 F.R. 15845, Dec. 23, 1965, as amended at 33 F.R. 5519, Apr. 11, 1968; 34 F.R. 17064, Oct. 21, 1969; 35 F.R. 1049, Jan. 27, 1970]

§ 121.102 Adjuvants for pesticide chemicals.

Adjuvants, identified and used in accordance with 40 CFR 180.1001 (c) and (d), which are added to pesticide use dilutions by a grower or applicator prior to application to the raw agricultural commodity, are exempt from the requirement of tolerances under section 409 of the act.

(Sec. 409, 72 Stat. 1785; 21 U.S.C. 348)

Subpart C—Food Additives Permitted in Feed and Drinking Water of Animals or for the Treatment of Food-Producing Animals

AUTHORITY: The provisions of this Subpart C issued under sec. 409, 72 Stat. 1785; 21 U.S.C. 348, unless otherwise noted.

§ 121.200 Definitions and interpretations applicable to Subpart C.

(a) Regulations prescribing conditions under which additives may be safely used in animal feed, animal feed supplements, concentrates, or premixes or in animals intended for food use shall not be construed to relieve such additives from the provisions of sections 505 and

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§ 121.1164 Synthetic flavoring substances and adjuvants.

Synthetic flavoring substances and adjuvants may be safely used in food in accordance with the following conditions.

(a) They are used in the minimum quantity required to produce their intended effect, and otherwise in accordance with all the principles of good manufacturing practice.

(b) They consist of one or more of the following, used alone or in combination with flavoring substances and adjuvants generally recognized as safe in food, prior-sanctioned for such use, or regulated by an appropriate section in Subpart D of the food additive regulations.

Acetal; acetaldehyde diethyl acetal.
Acetaldehyde phenethyl propyl acetal.
Acetanisole; 4'-methoxyacetophenone.
Acetophenone; methyl phenyl ketone.

Adipic acid; 1,4-butanedicarboxylic acid.
Allyl anthranilate.
Allyl butyrate.
Allyl cinnamate.
Allyl cyclohexaneacetate.
Allyl cyclohexanebutyrate.
Allyl cyclohexanehexanoate.
Allyl cyclohexanepropionate.
Allyl cyclohexanovalerate.
Allyl disulfide.
Allyl 2-ethylbutyrate.
Allyl hexanoate; allyl caproate.
Allyl α-ionone; 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1,6-heptadiene-3-one.
Allyl isothiocyanate; mustard oil.
Allyl isovalerate.
Allyl mercaptan; 2-propene-1-thiol.
Allyl nonanoate.
Allyl octanoate.
Allyl phenoxyacetate.
Allyl phenylacetate.
Allyl propionate.
Allyl sorbate; allyl 2,4-hexadienoate.
Allyl sulfide.
Allyl tiglate; allyl *trans*-2-methyl-2-butenoate.
Allyl 10-undecenoate.
Ammonium isovalerate.
Ammonium sulfide.
Amyl alcohol; pentyl alcohol.
Amyl butyrate.
α-Amylcinnamaldehyde.
α-Amylcinnamaldehyde dimethyl acetal.
α-Amylcinnamyl acetate.
α-Amylcinnamyl alcohol.
α-Amylcinnamyl formate.
α-Amylcinnamyl isovalerate.
Amyl formate.
Amyl heptanoate.
Amyl hexanoate.
Amyl octanoate.
Anisole; methoxybenzene.
Anisyl acetate.
Anisyl alcohol; *p*-methoxybenzyl alcohol.
Anisyl butyrate.
Anisyl formate.
Anisyl phenylacetate.
Anisyl propionate.
Beechwood creosote.
Benzaldehyde dimethyl acetal.
Benzaldehyde glyceryl acetal; 2-phenyl-*m*-dioxan-5-ol.
Benzaldehyde propylene glycol acetal; 4-methyl-2-phenyl-*m*-dioxolane.
Benzenethiol; thiophenol.
Benzoin; 2-hydroxy-2-phenylacetophenone.
Benzophenone; diphenylketone.
Benzyl acetate.
Benzyl acetoacetate.
Benzyl alcohol.
Benzyl benzoate.
Benzyl butyl ether.
Benzyl butyrate.
Benzyl cinnamate.
Benzyl 2,3-dimethylcrotonate; benzyl methyl tiglate.
Benzyl disulfide; dibenzyl disulfide.
Benzyl ethyl ether.
Benzyl formate.
3-Benzyl-4-heptanone; benzyl dipropyl ketone.
Benzyl isobutyrate.
Benzyl isovalerate.
Benzyl mercaptan; α-toluenethiol.
Benzyl methoxyethyl acetal; acetaldehyde benzyl β-methoxyethyl acetal.
Benzyl phenylacetate.
Benzyl propionate.
Benzyl salicylate.
Birch tar oil.
Borneol; *d*-camphanol.
Bornyl acetate.
Bornyl formate.
Bornyl isovalerate.
Bornyl valerate.
2-Butanone; methyl ethyl ketone.
Butter acids.
Butter esters.
Butter starter distillate.
Butyl acetate.
Butyl acetoacetate.
Butyl alcohol; 1-butanol.
Butyl anthranilate.
Butyl butyrate.
Butyl butyrylactate; lactic acid, butyl ester, butyrate.
α-Butylcinnamaldehyde.
Butyl cinnamate.
Butyl 2-decenoate.
Butyl ethyl malonate.
Butyl formate.
Butyl heptanoate.
Butyl hexanoate.
Butyl *p*-hydroxybenzoate.
Butyl isobutyrate.
Butyl isovalerate.
Butyl lactate.
Butyl laurate.
Butyl levulinate.
Butyl phenylacetate.
Butyl propionate.
Butyl stearate.
Butyl sulfide.
Butyl 10-undecenoate.
Butyl valerate.
Butyraldehyde.
Cadinene.
Camphene; 2,2-dimethyl-3-methylenenorbornane.
d-Camphor.
Carvacrol; 2-*p*-cymenol.
Carveol; *p*-mentha-6,8-dien-2-ol.
Carvacryl ethyl ether; 2-ethoxy-*p*-cymene
4-Carvomenthenol; 1-*p*-menthen-4-ol; 4-terpinenol.
Carvyl acetate.
Carvyl propionate.
β-Caryophyllene.
Caryophyllene alcohol.
Caryophyllene alcohol acetate.
Cedarwood oil alcohols.
Cedarwood oil terpenes.
1,4-Cineole.
Cinnamaldehyde ethylene glycol acetal.
Cinnamic acid.
Cinnamyl acetate.
Cinnamyl alcohol; 3-phenyl-2-propen-1-ol.
Cinnamyl anthranilate.
Cinnamyl benzoate.

Cinnamyl butyrate.
Cinnamyl cinnamate.
Cinnamyl formate.
Cinnamyl isobutyrate.
Cinnamyl isovalerate.
Cinnamyl phenylacetate.
Cinnamyl propionate.
Citral diethyl acetal; 3,7-dimethyl-2,6-octadienal diethyl acetal.
Citral dimethyl acetal; 3,7-dimethyl-2,6-octadienal dimethyl acetal.
Citral propylene glycol acetal.
Citronellal; 3,7-dimethyl-6-octenal; rhodinal.
Citronellol; 3,7-dimethyl-6-octen-1-ol; *d*-citronellol.
Citronelloxyacetaldehyde.
Citronellyl acetate.
Citronellyl butyrate.
Citronellyl formate.
Citronellyl isobutyrate.
Citronellyl phenylacetate.
Citronellyl propionate.
Citronellyl valerate.
p-Cresol.
Cuminaldehyde; cuminal; *p*-isopropyl benzaldehyde.
Cyclohexaneacetic acid.
Cyclohexanethyl acetate.
Cyclohexyl acetate.
Cyclohexyl anthranilate.
Cyclohexyl butyrate.
Cyclohexyl cinnamate.
Cyclohexyl formate.
Cyclohexyl isovalerate.
Cyclohexyl propionate.
p-Cymene.
γ-Decalactone; 4-hydroxy-decanoic acid, γ-lactone.
δ-Decalactone; 5-hydroxy-decanoic acid, δ-lactone.
Decanal dimethyl acetal.
1-Decanol; decylic alcohol.
2-Decenal.
3-Decen-2-one; heptylidene acetone.
Decyl acetate.
Decyl butyrate.
Decyl propionate.
Dibenzyl ether.
4,4-Dibutyl-γ-butyrolactone; 4,4-dibutyl-4-hydroxy-butyric acid, γ-lactone.
Dibutyl sebacate.
Diethyl malate.
Diethyl malonate; ethyl malonate.
Diethyl sebacate.
Diethyl succinate.
Diethyl tartrate.
2,5-Diethyltetrahydrofuran.
Dihydrocarveol; 6-*p*-menthen-2-ol; 6-methyl-3-isopropenylcyclohexanol.
Dihydrocarvone.
Dihydrocarvyl acetate.
m-Dimethoxybenzene.
p-Dimethoxybenzene; dimethyl hydroquinone.
2,4-Dimethylacetophenone.
α,α-Dimethylbenzyl isobutyrate; phenyldimethylcarbinyl isobutyrate.
2,6-Dimethyl-5-heptenal.
2,6-Dimethyl octanal; isodecylaldehyde.
3,7-Dimethyl-1-octanol; tetrahydrogeraniol.
α,α-Dimethylphenethyl acetate; benzylpropyl acetate; benzyldimethylcarbinyl acetate.
α,α-Dimethylphenethyl alcohol; dimethylbenzyl carbinol.
α,α-Dimethylphenethyl butyrate; benzyldimethylcarbinyl butyrate.
α,α-Dimethylphenethyl formate; benzyldimethylcarbinyl formate.
Dimethyl sulfone.
1,3-Diphenyl-2-propanone; dibenzyl ketone.
delta-Dodecalactone; 5-hydroxydodecanoic acid, delta-lactone.
γ-Dodecalactone; 4-hydroxydodecanoic acid, γ-lactone.
2-Dodecenal.
Estragole.
p-Ethoxybenzaldehyde.
Ethyl acetoacetate.
Ethyl 2-acetyl-3-phenylpropionate; ethylbenzyl acetoacetate.
Ethyl aconitate, mixed esters.
Ethyl acrylate.
Ethyl *p*-anisate.
Ethyl anthranilate.
Ethyl benzoate.
Ethyl benzoylacetate.
α-Ethylbenzyl butyrate; α-phenylpropyl butyrate.
Ethyl brassylate; tridecanedioic acid cyclic ethylene glycol diester; cyclo 1,13-ethylenedioxytridecan-1,13-dione.
2-Ethylbutyl acetate.
2-Ethylbutyraldehyde.
2-Ethylbutyric acid.
Ethyl cinnamate.
Ethyl crotonate; *trans*-2-butenoic acid ethyl ester.
Ethyl cyclohexanepropionate.
Ethyl decanoate.
Ethyl formate.
2-Ethylfuran.
Ethyl 2-furanpropionate.
4-Ethylguaiacol; 4-ethyl-2-methoxyphenol.
Ethyl heptanoate.
2-Ethyl-2-hepteual; 2-ethyl-3-butylacrolein.
Ethyl hexanoate.
Ethyl isobutyrate.
Ethyl isovalerate.
Ethyl lactate.
Ethyl laurate.
Ethyl levulinate.
Ethyl maltol; 2-ethyl-3-hydroxy-4H-pyran-4-one.
Ethyl 2-methylbutyrate.
Ethyl myristate.
Ethyl nitrite.
Ethyl nonanoate.
Ethyl 2-nonynoate; ethyl octyne carbonate.
Ethyl octanoate.
Ethyl oleate.
Ethyl phenylacetate.
Ethyl 4-phenylbutyrate.
Ethyl 3-phenylglycidate.
Ethyl 3-phenylpropionate; ethyl hydrocinnamate.
Ethyl propionate.
Ethyl pyruvate.
Ethyl salicylate.

Ethyl sorbate; ethyl 2,4-hexadienoate.
Ethyl tiglate; ethyl *trans*-2-methyl-2-butenoate.
Ethyl undecanoate.
Ethyl 10-undecenoate.
Ethyl valerate.
Eucalyptol; 1,8-epoxy-*p*-menthane; cineole.
Eugenyl acetate.
Eugenyl benzoate.
Eugenyl formate.
Eugenyl methyl ether; 4-allylveratrole; methyl eugenol.
Farnesol; 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol.
d-Fenchone; *d*-1,3,3,-trimethyl-2-norbornanone.
Fenchyl alcohol; 1,3,3- trimethyl-2-norbornanol.
Formic acid.
(2-Furyl)-2-propanone; furyl acetone.
1-Furyl-2-propanone; furyl acetone.
Fusel oil, refined (mixed amyl alcohols).
Geranyl acetoacetate; *trans*-3,7-dimethyl-2,6-octadien-1-yl acetoacetate.
Geranyl benzoate.
Geranyl butyrate.
Geranyl formate.
Geranyl hexanoate.
Geranyl isobutyrate.
Geranyl isovalerate.
Geranyl phenylacetate.
Geranyl propionate.
Glucose pentaacetate.
Glyceryl monooleate.
Guaiacol; *o*-methoxyphenol.
Guaiacyl acetate; *o*-methoxyphenyl acetate.
Guaiacyl phenylacetate.
Guaiene; 1,4-dimethyl-7-isopropenyl-Δ9, 10-octahydroazulene.
Guaiol acetate; 1,4-dimethyl-7-(α-hydroxyisopropyl)-Δ9,10-octahydroazulene acetate.
γ-Heptalactone; 4-hydroxyheptanoic acid, γ-lactone.
Heptanal; enanthaldehyde.
Heptanal dimethyl acetal.
Heptanal 1,2-glyceryl acetal.
2,3-Heptanedione; acetyl valeryl.
3-Heptanol.
2-Heptanone; methyl amyl ketone.
3-Heptanone; ethyl butyl ketone.
4-Heptanone; dipropyl ketone.
Heptyl acetate.
Heptyl alcohol; enanthic alcohol.
Heptyl butyrate.
Heptyl cinnamate.
Heptyl formate.
Heptyl isobutyrate.
Heptyl octanoate.
1-Hexadecanol; cetyl alcohol.
ω-3-Hexadecenolactone; 16-hydroxy-6-hexadecenoic acid, ω-lactone; ambrettolide.
γ-Hexalactone; 4-hydroxyhexanoic acid, γ-lactone; tonkalide.
Hexanal; caproic aldehyde.
2,3-Hexanedione; acetyl butyryl.
Hexanoic acid; caproic acid.
2-Hexenal.
2-Hexen-1-ol.
3-Hexen-1-ol; leaf alcohol.
2-Hexen-1-yl acetate.
3-Hexenyl isovalerate.
3-Hexenyl 2-methylbutyrate.
Hexyl acetate.
2-Hexyl-4-acetoxytetrahydrofuran.
Hexyl alcohol.
Hexyl butyrate.
α-Hexylcinnamaldehyde.
Hexyl formate.
Hexyl hexanoate.
2-Hexylidene cyclopentanone.
Hexyl isovalerate.
Hexyl 2-methylbutyrate.
Hexyl octanoate.
Hexyl propionate.
Hydroxycitronellal; 3,7-dimethyl-7-hydroxyoctanal.
Hydroxycitronellal diethyl acetal.
Hydroxycitronellal dimethyl acetal.
Hydroxycitronellol; 3,7-dimethyl-1,7-octanediol.
N-(4-Hydroxy-3-methoxybenzyl)-nonanamide; pelargonyl vanillylamide.
5-Hydroxy-4-octanone; butyroin.
4-(*p*-Hydroxyphenyl-2-butanone; *p*-hydroxybenzyl acetone.
Indole.
α-Ionone; 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one.
β-Ionone; 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one.
α-Irone; 4-(2,5,6,6-tetramethyl-2-cyclohexene-1-yl)-3-buten-2-one; 6-methylionone.
Isoamyl acetate.
Isoamyl acetoacetate.
Isoamyl alcohol; isopentyl alcohol; 3-methyl-1-butanol.
Isoamyl benzoate.
Isoamyl butyrate.
Isoamyl cinnamate.
Isoamyl formate.
Isoamyl 2-furanbutyrate; α-isoamyl furfurylpropionate.
Isoamyl 2-furanpropionate; α-isoamyl furfurylacetate.
Isoamyl hexanoate.
Isoamyl isobutyrate.
Isoamyl isovalerate.
Isoamyl laurate.
Isoamyl nonanoate.
Isoamyl octanoate.
Isoamyl phenylacetate.
Isoamyl propionate.
Isoamyl pyruvate.
Isoamyl salicylate.
Isoborneol.
Isobornyl acetate.
Isobornyl formate.
Isobornyl isovalerate.
Isobornyl propionate.
Isobutyl acetate.
Isobutyl acetoacetate.
Isobutyl alcohol.
Isobutyl angelate; isobutyl *cis*-2-methyl-2-butenoate.
Isobutyl anthranilate.
Isobutyl benzoate.
Isobutyl butyrate.
Isobutyl cinnamate.
Isobutyl formate.

Isobutyl 2-furanpropionate.
Isobutyl heptanoate.
Isobutyl hexanoate.
Isobutyl isobutyrate.
α-Isobutylphenethyl alcohol; isobutyl benzyl carbinol; 4-methyl-1-phenyl-2-pentanol.
Isobutyl phenylacetate.
Isobutyl propionate.
Isobutyl salicylate.
Isobutyraldehyde.
Isobutyric acid.
Isoeugenol; 2-methoxy-4-propenylphenol.
Isoeugenyl acetate.
Isoeugenyl benzyl ether; benzyl isoeugenol.
Isoeugenyl ethyl ether; 2-ethoxy-5-propenylanisole; ethyl isoeugenol.
Isoeugenyl formate.
Isoeugenyl methyl ether; 4-propenylveratrole; methyl isoeugenol.
Isoeugenyl phenylacetate.
Isojasmone; mixture of 2-hexylidenecyclopentanone and 2-hexyl-2-cyclopenten-1-one.
α-Isomethylionone; 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one; methyl γ-ionone.
Isopropyl acetate.
p-Isopropylacetophenone.
Isopropyl alcohol; isopropanol.
Isopropyl benzoate.
p-Isopropylbenzyl alcohol; cuminic alcohol; p-cymen-7-ol.
Isopropyl butyrate.
Isopropyl cinnamate.
Isopropyl formate.
Isopropyl hexanoate.
Isopropyl isobutyrate.
Isopropyl isovalerate.
p-Isopropylphenylacetaldehyde; p-cymen-7-carboxaldehyde.
Isopropyl phenylacetate.
3-(p-Isopropylphenyl)-propionaldehyde; p-isopropylhydrocinnamaldehyde; cuminyl acetaldehyde.
Isopropyl propionate.
Isopulegol; p-menth-8-en-3-ol.
Isopulegone; p-menth-8-en-3-one.
Isopulegyl acetate.
Isoquinoline.
Isovaleric acid.
Lauric aldehyde; dodecanal.
Lauryl acetate.
Lauryl alcohol; 1-dodecanol.
Lepidine; 4-methylquinoline.
Levulinic acid.
Linalool oxide; *cis*- and *trans*-2-vinyl-2-methyl-5-(1′-hydroxy-1′-methylethyl) tetrahydrofuran.
Linalyl anthranilate; 3,7-dimethyl-1,6-octadien-3-yl anthranilate.
Linalyl benzoate.
Linalyl butyrate.
Linalyl cinnamate.
Linalyl formate.
Linalyl hexanoate.
Linalyl isobutyrate.
Linalyl isovalerate.
Linalyl octanoate.
Linalyl propionate.
Maltol; 3-hydroxy-2-methyl-4H-pyran-4-one.
Menthadienol; p-mentha-1,8(10)-dien-9-ol.
p-Mentha-1,8-dien-7-ol; perillyl alcohol.
Menthadienyl acetate; p-mentha-1,8(10)-dien-9-yl acetate.
p-Menth-3-en-1-ol.
Menthol; 2-isopropyl-5-methylcyclohexanol.
Menthone; p-menthan-3-one.
Menthyl acetate; p-menth-3-yl acetate.
Menthyl isovalerate; p-menth-3-yl isovalerate.
o-Methoxybenzaldehyde.
p-Methoxybenzaldehyde; p-anisaldehyde.
o-Methoxycinnamaldehyde.
2-Methoxy-4-methylphenol; 4-methylguaiacol; 2-methoxy-p-cresol.
4-(p-Methoxyphenyl)-2-butanone; anisyl acetone.
1-(4-Methoxyphenyl)-4-methyl-1-penten-3-one; methoxystyryl isopropyl ketone.
1-(p-Methoxyphenyl)-1-penten-3-one; α-methylanisylidene acetone; ethone.
1-(p-Methoxyphenyl)-2-propanone; anisyl methyl ketone; anisic ketone.
2-Methoxy-4-vinylphenol; p-vinylguaiacol.
Methyl acetate.
4′-Methylacetophenone; p-methylacetophenone; methyl p-tolyl ketone.
2-Methylallyl butyrate; 2-methyl-2-propen-1-yl butyrate.
Methyl anisate.
o-Methylanisole; o-cresyl methyl ether.
p-Methylanisole; p-cresyl methyl ether; p-methoxytoluene.
Methyl benzoate.
Methylbenzyl acetate, mixed o-,m-,p-.
α-Methylbenzyl acetate; styralyl acetate.
α-Methylbenzyl alcohol; styralyl alcohol.
α-Methylbenzyl butyrate; styralyl butyrate.
α-Methylbenzyl isobutyrate; styralyl isobutyrate.
α-Methylbenzyl formate; styralyl formate.
α-Methylbenzyl propionate; styralyl propionate.
2-Methylbutyl isovalerate.
Methyl p-*tert*-butylphenylacetate.
2-Methylbutyraldehyde; methyl ethyl acetaldehyde.
3-Methylbutyraldehyde; isovaleraldehyde.
Methyl butyrate.
2-Methylbutyric acid.
α-Methylcinnamaldehyde.
p-Methylcinnamaldehyde.
Methyl cinnamate.
2-Methyl-1,3-cyclohexadiene.
Methylcyclopentenolone; 3-methylcyclopentane-1,2-dione.
Methyl disulfide; dimethyl disulfide.
Methyl ester of rosin, partially hydrogenated (as defined in § 121.1059); methyl dihydroabietate.
Methyl heptanoate.
2-Methylheptanoic acid.
6-Methyl-5-hepten-2-one.
Methyl hexanoate.
Methyl 2-hexenoate.
Methyl p-hydroxybenzoate; methylparaben.
Methyl α-ionone; 5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-4-penten-3-one.

Methyl β-ionone; 5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-4-penten-3-one.
Methyl δ-ionone; 5-(2,6,6-trimethyl-3-cyclohexen-1-yl)-4-penten-3-one.
Methyl isobutyrate.
2-Methyl-3-(p-isopropylphenyl)-propionaldehyde; α-methyl-p-isopropylhydrocinnamaldehyde; cyclamen aldehyde.
Methyl isovalerate.
Methyl laurate.
Methyl mercaptan; methanethiol.
Methyl o-methoxybenzoate.
Methyl N-methylanthranilate; dimethyl anthranilate.
Methyl 2-methylbutyrate.
Methyl 2-methylthiopropionate.
Methyl 4-methylvalerate.
Methyl myristate.
Methyl β-naphthyl ketone; 2′-acetonaphthone.
Methyl nonanoate.
Methyl 2-nonenoate.
Methyl 2-nonynoate; methyloctyne carbonate.
2-Methyloctanal; methyl hexyl acetaldehyde.
Methyl octanoate.
Methyl 2-octynoate; methyl heptine carbonate.
4-Methyl-2,3-pentanedione; acetyl isobutyryl.
4-Methyl-2-pentanone; methyl isobutyl ketone.
β-Methylphenethyl alcohol; hydratropyl alcohol.
Methyl phenylacetate.
3-Methyl-4-phenyl-3-butene-2-one.
2-Methyl-4-phenyl-2-butyl acetate; dimethylphenylethyl carbinyl acetate.
2-Methyl-4-phenyl-2-butyl isobutyrate; dimethylphenyl-ethylcarbinyl isobutyrate.
3-Methyl-2-phenylbutyraldehyde; α-isopropyl phenylacetaldehyde.
Methyl 4-phenylbutyrate.
4-Methyl-1-phenyl-2-pentanone; benzyl isobutyl ketone.
Methyl 3-phenylpropionate; methyl hydrocinnamate.
Methyl propionate.
3-Methyl-5-propyl-2-cyclohexen-1-one.
Methyl sulfide.
3-Methylthiopropionaldehyde; methional.
2-Methyl-3-tolylpropionaldehyde, mixed o-, m-, p-.
2-Methylundecanal; methyl nonyl acetaldehyde.
Methyl 9-undecenoate.
Methyl 2-undecynoate; methyl decyne carbonate.
Methyl valerate.
2-Methylvaleric acid.
Myrcene; 7-methyl-3-methylene-1,6-octadiene.
Myristaldehyde; tetradecanal.
d-Neomenthol; 2-isobutyl-5-methylcyclohexanol.
Nerol; *cis*-3,7-dimethyl-2,6-octadien-1-ol.
Nerolidol; 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol.
Neryl acetate.
Neryl butyrate.
Neryl formate.
Neryl isobutyrate.
Neryl isovalerate.
Neryl propionate.
2,6-Nonadien-1-ol.
γ-Nonalactone; 4-hydroxynonanoic acid, γ-lactone; aldehyde C-18.
Nonanal; pelargonic aldehyde.
1,3-Nonanediol acetate, mixed esters.
Nonanoic acid; pelargonic acid.
2-Nonanone; methylheptyl ketone.
3-Nonanon-1-yl acetate; 1-hydroxy-3-nonanone acetate.
Nonyl acetate.
Nonyl alcohol; 1-nonanol.
Nonyl octanoate.
Nonyl isovalerate.
Nootkatone; 5,6-dimethyl-8-isopropenyl-bicyclo[4,4,0]-dec-1-en-3-one.
Ocimene; *trans*-β-ocimene; 3,7-dimethyl-1,3,6-octatriene.
γ-Octalactone; 4-hydroxyoctanoic acid, γ-lactone.
Octanal; caprylaldehyde.
Octanal dimethyl acetal.
Octanoic acid; caprylic acid.
1-Octanol; octyl alcohol.
2-Octanol.
3-Octanol.
2-Octanone; methyl hexyl ketone.
3-Octanone; ethyl amyl ketone.
3-Octanon-1-ol.
1-Octen-3-ol; amyl vinyl carbinol.
1-Octen-3-yl acetate.
Octyl acetate.
3-Octyl acetate.
Octyl butyrate.
Octyl formate.
Octyl heptanoate.
Octyl isobutyrate.
Octyl isovalerate.
Octyl octanoate.
Octyl phenylacetate.
Octyl propionate.
ω-Pentadecalactone; 15-hydroxypentadecanoic acid, ω-lactone; pentadecanolide; angelica lactone.
2,3-Pentanedione; acetyl propionyl.
2-Pentanone; methyl propyl ketone.
4-Pentenoic acid.
1-Penten-3-ol.
Perillaldehyde; 4-isopropenyl-1-cyclohexene-1-carboxaldehyde; p-mentha-1,8-dien-7-al.
Perillyl acetate; p-mentha-1,8-dien-7-yl acetate.
α-Phellandrene; p-mentha-1,5-diene.
Phenethyl acetate.
Phenethyl alcohol; β-phenylethyl alcohol.
Phenethyl anthranilate.
Phenethyl benzoate.
Phenethyl butyrate.
Phenethyl cinnamate.
Phenethyl formate.
Phenethyl isobutyrate.
Phenethyl isovalerate.
Phenethyl 2-methylbutyrate.
Phenethyl phenylacetate.
Phenethyl propionate.
Phenethyl salicylate.

Phenethyl senecioate; phenethyl 3,3-dimethylacrylate.
Phenethyl tiglate.
Phenoxyacetic acid.
2-Phenoxyethyl isobutyrate.
Phenylacetaldehyde; α-toluic aldehyde.
Phenylacetaldehyde 2,3-butylene glycol acetal.
Phenylacetaldehyde dimethyl acetal.
Phenylacetaldehyde glyceryl acetal.
Phenylacetic acid; α-toluic acid.
4-Phenyl-2-butanol; phenylethyl methyl carbinol.
4-Phenyl-3-buten-2-ol; methyl styryl carbinol.
4-Phenyl-3-buten-2-one.
4-Phenyl-2-butyl acetate; phenylethyl methyl carbinyl acetate.
1-Phenyl-3-methyl-3-pentanol; phenylethyl methyl ethyl carbinol.
1-Phenyl-1-propanol; phenylethyl carbinol.
3-Phenyl-1-propanol; hydrocinnamyl alcohol.
2-Phenylpropionaldehyde; hydratropaldehyde.
3-Phenylpropionaldehyde; hydrocinnamaldehyde.
2-Phenylpropionaldehyde dimethyl acetal; hydratropic aldehyde dimethyl acetal.
3-Phenylpropionic acid; hydrocinnamic acid.
3-Phenylpropyl acetate.
2-Phenylpropyl butyrate.
3-Phenylpropyl cinnamate.
3-Phenylpropyl formate.
3-Phenylpropyl hexanoate.
2-Phenylpropyl isobutyrate.
3-Phenylpropyl isobutyrate.
3-Phenylpropyl isovalerate.
3-Phenylpropyl propionate.
2-(3-Phenylpropyl)-tetrahydrofuran.
α-Pinene; 2-pinene.
β-Pinene; 2(10)-pinene.
Pine tar oil.
Piperidine.
Piperine.
d-Piperitone; *p*-menth-1-en-3-one.
Piperonyl acetate; heliotropyl acetate.
Piperonyl isobutyrate.
Polylimonene.
Polysorbate 20; polyoxyethylene (20) sorbitan monolaurate.
Polysorbate 60; polyoxyethylene (20) sorbitan monostearate.
Polysorbate 80; polyoxyethylene (20) sorbitan monooleate.
Potassium acetate.
Propenylguaethol; 6-ethoxy-*m*-anol.
Propionaldehyde.
Propyl acetate.
Propyl alcohol; 1-propanol.
p-Propyl anisole; dihydroanethole.
Propyl benzoate.
Propyl butyrate.
Propyl cinnamate.
Propyl disulfide.
Propyl formate.
Propyl 2-furanacrylate.
Propyl heptanoate.
Propyl hexanoate.
Propyl *p*-hydroxybenzoate; propylparaben.
3-Propylidenephthalide.
Propyl isobutyrate.
Propyl isovalerate.
Propyl mercaptan.
α-Propylphenethyl alcohol.
Propyl phenylacetate.
Propyl propionate.
Pulegone; *p*-menth-4(8)-en-3-one.
Pyridine.
Pyroligneous acid extract.
Pyruvaldehyde.
Pyruvic acid.
Rhodinol; 3,7-dimethyl-7-octen-1-ol; *l*-citronellol.
Rhodinyl acetate.
Rhodinyl butyrate.
Rhodinyl formate.
Rhodinyl isobutyrate.
Rhodinyl isovalerate.
Rhodinyl phenylacetate.
Rhodinyl propionate.
Rum ether; ethyl oxyhydrate.
Salicylaldehyde.
Santalol, α and β.
Santalyl acetate.
Santalyl phenylacetate.
Skatole.
Sorbitan monostearate.
Styrene.
Sucrose octaacetate.
α-Terpinene.
γ-Terpinene.
α-Terpineol; *p*-menth-1-en-8-ol.
β-Terpineol.
Terpinolene; *p*-menth-1,4(8)-diene.
Terpinyl acetate.
Terpinyl anthranilate.
Terpinyl butyrate.
Terpinyl cinnamate.
Terpinyl formate.
Terpinyl isobutyrate.
Terpinyl isovalerate.
Terpinyl propionate.
Tetrahydrofurfuryl acetate.
Tetrahydrofurfuryl alcohol.
Tetrahydrofurfuryl butyrate.
Tetrahydrofurfuryl propionate.
Tetrahydro-pseudo-ionone; 6,10-dimethyl-9-undecen-2-one.
Tetrahydrolinalool; 3,7-dimethyloctan-3-ol.
Tetramethyl ethylcyclohexenone; mixture of 5-ethyl-2,3,4,5-tetramethyl-2-cyclohexen-1-one and 5-ethyl-3,4,5,6-tetramethyl-2-cyclohexen-1-one.
2-Thienyl mercaptan; 2-thienylthiol.
Thymol.
Tolualdehyde glyceryl acetal, mixed *o*, *m*, *p*.
Tolualdehydes, mixed *o*, *m*, *p*.
p-Tolylacetaldehyde.
o-Tolyl acetate; *o*-cresyl acetate.
p-Tolyl acetate; *p*-cresyl acetate.
4-(*p*-Tolyl)-2-butanone; *p*-methylbenzylacetone.
p-Tolyl isobutyrate.
p-Tolyl laurate.
p-Tolyl phenylacetate.
2-(*p*-Tolyl)-propionaldehyde; *p*-methylhydratropic aldehyde.
Tributyl acetylcitrate.
2-Tridecenal.
2,3-Undecadione; acetyl nonyryl.

γ-Undecalactone; 4-hydroxyundecanoic acid γ-lactone; peach aldehyde; aldehyde C-14.
Undecenal.
2-Undecanone; methyl nonyl ketone.
9-Undecenal; undecenoic aldehyde.
10-Undecenal.
Undecen-1-ol; undecylenic alcohol.
10-Undecen-1-yl acetate.
Undecyl alcohol.
Valeraldehyde; pentanal.
Valeric acid; pentanoic acid.
Vanillin acetate; acetyl vanillin.
Veratraldehyde.
Zingerone; 4-(4-hydroxy-3-methoxyphenyl)-2-butanone.

(c) δ-Decalactone and δ-dodecalactone when used separately or in combination in oleomargarine are used at levels not to exceed 10 parts per million and 20 parts per million, respectively, in accordance with § 45.1 of this chapter.

(d) BHA (butylated hydroxyanisole) may be used as an antioxidant in flavoring substances whereby the additive does not exceed 0.5 percent of the essential (volatile) oil content of the flavoring substance.

[30 F.R. 15345, Dec. 23, 1965, as amended at 31 F.R. 7563, May 26, 1966; 33 F.R. 5773, Apr. 11, 1967; 32 F.R. 7947, June 2, 1967; 33 F.R. 10569, July 25, 1968; 34 F.R. 7374, May 7, 1969]

§ 121.1165 Rhizopus oryzae.

Carbohydrase from *Rhizopus oryzae* may be safely used in the production of dextrose from starch in accordance with the following prescribed conditions:

(a) *Rhizopus oryzae* is classified as follows: Class, *Phycomycetes;* order, *Mucorales;* family, *Mucoraceae;* genus, *Rhizopus;* species, *Rhizopus oryzae.*

(b) The strain of *Rhizopus oryzae* is nonpathogenic and nontoxic.

(c) The carbohydrase is produced under controlled conditions to maintain nonpathogenicity and nontoxicity, including the absence of aflatoxin.

(d) The carbohydrase is produced by a process which completely removes the organism *Rhizopus oryzae* from the carbohydrase product.

(e) The carbohydrase is maintained under refrigeration from production to use and is labeled to include the necessity of refrigerated storage.

§ 121.1166 Petrolatum.

Petrolatum may be safely used in food, subject to the provisions of this section.

(a) Petrolatum complies with the specifications set forth in the U.S. Pharmacopeia XVII for white petrolatum or in The National Formulary XII for petrolatum.

(b) Petrolatum meets the following ultraviolet absorbance limits when subjected to the analytical procedure described in § 121.1156(b):

Ultraviolet absorbance per centimeter path length:

Millimicrons:	*Maximum*
280-289	0.25
290-299	.20
300-359	.14
360-400	.04

(c) Petrolatum is used or intended for use as follows:

Use	*Limitation (inclusive of all petroleum hydrocarbons that may be used in combination with petrolatum)*
In bakery products; as release agent and lubricant.	With white mineral oil, not to exceed 0.15 percent of bakery product.
In confectionery; as release agent and as sealing and polishing agent.	Not to exceed 0.2 percent of confectionery.
In dehydrated fruits and vegetables; as release agent.	Not to exceed 0.02 percent of dehydrated fruits and vegetables.
In egg white solids; as release agent.	Not to exceed 0.1 percent of egg white solids.
On raw fruits and vegetables; as protective coating.	In an amount not to exceed good manufacturing practice.
In beet sugar and yeast; as defoaming agent.	As prescribed in § 121.1099.

(d) Petrolatum may contain any antioxidant permitted in food by regulations issued in accordance with section 409 of the act, in an amount not greater than that required to produce its intended effect.

[30 F.R. 15345, Dec. 23, 1965, as amended at 33 F.R. 15415, Oct. 17, 1968]

§ 121.1170 Bacterial catalase.

Bacterial catalase derived from *Micrococcus lysodeikticus* by a pure culture fermentation process may be safely used in destroying and removing hydrogen peroxide used in the manufacture of cheese, in accordance with the following conditions.

21 Code Fed. Reg. 121: 2525, pg. 250

(ii) The film, when exposed to 50 percent ethyl alcohol at 120° F. for 24 hours, yields chloroform-soluble extractives not to exceed 0.5 milligram per square inch of film surface exposed to the solvent.

(2) *Conditions of use.* The film is used for packaging, transporting, or holding alcoholic beverages that do not exceed 50 percent alcohol by volume.

(g) Uncoated polyethylene terephthalate film consisting of a base sheet prepared from substances identified in paragraph (d)(4)(i) of this section and conforming with the specifications prescribed in subparagraph (1) of this paragraph is used as provided in subparagraph (2) of this paragraph:

(1) *Specifications.* (i) The film, when exposed to distilled water at 250° F. for 2 hours, yields chloroform-soluble extractives not to exceed 0.02 milligram per square inch of film surface exposed to the solvent; and

(ii) The film, when exposed to *n*-heptane at 150° F. for 2 hours, yields chloroform-soluble extractives not to exceed 0.02 milligram per square inch of film surface exposed to the solvent.

(2) *Conditions of use.* The film is used to contain foods during oven baking or oven cooking at temperatures above 250° F.

[30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 15570, Dec. 10, 1966]

§ 121.2525 Acrylate ester copolymer coating.

Acrylate ester copolymer coating may safely be used as a food-contact surface of articles intended for packaging and holding food, including heating of prepared food, subject to the provisions of this section:

(a) The acrylate ester copolymer is a fully polymerized copolymer of ethyl acrylate, methyl methacrylate, and methacrylic acid applied in emulsion form to molded virgin fiber and heat-cured to an insoluble resin.

(b) Optional substances used in the preparation of the polymer and in the preparation and application of the emulsion may include substances named in this paragraph, in an amount not to exceed that required to accomplish the desired technical effect and subject to any limitation prescribed: *Provided, however,* That any substance named in this paragraph and covered by a specific regulation in Subpart F of this chapter must meet any specifications in such regulation.

List of substances	Limitations
Aluminum stearate	
Ammonium lauryl sulfate	
Borax	Not to exceed the amount required as a preservative in emulsion defoamer.
Disodium hydrogen phosphate	
Formaldehyde	Do.
Glyceryl monostearate	
Methyl cellulose	
Mineral oil	
Paraffin wax	
Potassium hydroxide	
Potassium persulfate	
Tallow	
Tetrasodium pyrophosphate	
Titanium dioxide	

(c) The coating in the form in which it contacts food meets the following tests:

(1) An appropriate sample when exposed to distilled water at 212° F. for 30 minutes shall yield total chloroform-soluble extractables not to exceed 0.5 milligram per square inch.

(2) An appropriate sample when exposed to *n*-heptane at 120° F. for 30 minutes shall yield total chloroform-soluble extractables not to exceed 0.5 milligram per square inch.

[30 F.R. 15845, Dec. 23, 1965, as amended at 31 F.R. 15570, Dec. 10, 1966; 32 F.R. 4050, Mar. 15, 1967]

§ 121.2526 Components of paper and paperboard in contact with aqueous and fatty foods.

Substances identified in this section may be safely used as components of the uncoated or coated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding aqueous and fatty foods, subject to the provisions of this section. Components of paper and paperboard in contact with dry food of the type identified under type VIII of table 1 in paragraph (c) of this section are subject to the provisions of § 121.2571.

(a) Substances identified in subparagraphs (1) through (5) of this paragraph may be used as components of the food-contact surface of paper and paperboard. Paper and paperboard products shall be exempted from compliance with the extractives limitations prescribed in paragraph (c) of this section: *Provided,*

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Studies on the Chronic Oral Toxicity of Monomeric Ethyl Acrylate and Methyl Methacrylate[1]

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Esters of acrylic acid and methacrylic acid are now widely used as monomers in the preparation of polymeric materials of considerable technological importance. Many of these polymers in the form of plastics, paper coatings, cloth coatings, etc., may be expected to come in contact with foods. Minor amounts of the unpolymerized monomers may be present in the final polymers. There is, therefore, the possibility that these monomers can be transferred to food at the parts per million (ppm) level. For this reason, it was deemed advisable to further develop our toxicologic information concerning the two most important esters, ethyl acrylate and methyl methacrylate.

Toxicologic studies on monomeric ethyl acrylate and methyl methacrylate appear to have been limited to observations on the effects of acute and subacute exposures.

Pozzani *et al.* (1949) obtained an acute oral LD_{50} for ethyl acrylate in rats of 1.02 g/kg, and a percutaneous LD_{50} in rabbits of 1.95 g/kg. Prostration and narcosis preceded death in rats, death occurring within 24 hours. In inhalation studies over 4-hour periods, 1000 ppm killed no rats of 6, 2000 ppm killed 5 of 6, and 4000 ppm killed 6 of 6. Repeated exposures (30 days) to 300 ppm produced lung damage and secondary effects on liver and kidneys. A concentration of 50 ppm was suggested as the maximum for repeated human inhalation.

For methyl methacrylate, acute oral LD_{50}'s of 8.4 g/kg (Deichmann, 1941) and 10.0 ml/kg (Spealman *et al.*, 1945) have been reported for rats. Oral LD_{50}'s of 0.3 and 5.0 ml/kg, respectively, have been estimated for guinea pigs and dogs (Spealman *et al.*, 1945). As with ethyl acrylate, prostration and narcosis preceded death. In inhalation studies, Deichmann (1941) found that rats, rabbits, and guinea pigs survived 8-hour exposures to 14.3 mg/l, but all three species succumbed to 19 mg/l. In unpublished observations made in our laboratory, the LD_{50} for 2-hour exposures in rats was estimated to lie between 45 and 50 mg/l. In albino mice subjected to 3-hour exposures, Spealman *et al.* (1945) estimated the LD_{50} at 55 mg/l, and Karpov (1954) found the minimum lethal concentration to be 13.1 mg/l for 2-hour exposures. Karpov from observations on workers exposed to methyl methacrylate vapors suggested a tolerance of 0.05 mg/l for industrial establishments, a value considered to be on the high side by Raines (1957).

The experiments reported below were performed to study the tolerance of animals to chronic ingestion of ethyl acrylate and methyl methacrylate.

[1] This study was supported by a grant from the Rohm and Haas Company, Philadelphia, Pennsylvania, who also furnished the ethyl acrylate and methyl methacrylate used.

METHODS

The undiluted stocks of ethyl acrylate and methyl methacrylate were inhibited from polymerization with 10 ppm of monomethyl ether of *t*-butylhydroquinone.

Rats

Both test materials were too volatile to add to the diet and were dissolved in the drinking water instead. Tests with 6-ounce bottles equipped with ordinary drinking tubes showed that substantial losses of these materials developed in the water near the end of the tube. By replacing the tube with a Bakelite cap with a 2.7-mm hole drilled in it (no. 36 drill), thus reducing the surface for volatilization and minimizing the distance between the rat's access to water and the body of the reservoir, losses were markedly reduced.

Tests for losses were conducted in the Rohm & Haas Laboratories[2] in the following manner. Six-ounce bottles were filled with water containing 1000 ppm ethyl acrylate or methyl methacrylate, capped, and inverted. The action of a rat drinking was stimulated by gently tapping a finger on the hole in the cap thus displacing a small quantity of fluid from the bottle. Tapping was repeated periodically on each sample during each day but was suspended for 1 hour prior to sampling. Samples were drawn from just inside the cap at the end of 24, 48, and 72 hours. At least 3 replicates were taken in all cases. The samples were analyzed polarographically for monomer content; the results are recorded in the accompanying tabulation.

Ethyl acrylate		Methyl methacrylate	
Time (hours)	Ppm	Time (hours)	Ppm
24	936 ± 34	24	852 ± 68
48	965 ± 38	48	843 ± 31
72	950 ± 67	72	894 ± 23

It is apparent that there was essentially no loss of ethyl acrylate and a maximum loss of 15% of the methyl methacrylate.

In the animal studies, stock solutions of the monomers were prepared in tightly stoppered carboys once a week and the drinking bottles were filled twice a week, water remaining in the bottles at refilling being discarded. Rats quickly learn to drink from these bottles. Dripping does not occur unless the cage or bottle is jarred; evaporation loss averages 5 ml in 72 hours at 72°C.

Young albino rats (Wistar strain) were divided into 7 groups of 25 males and 25 females each. One group was placed on each of the following concentrations of test materials: 0 (control), 6, 60, and 2000 ppm of ethyl acrylate; 6, 60, and 2000 ppm of methyl methacrylate; low and medium levels were raised to 7 and 70 ppm at the start of the fifth month. Littermate distribution was achieved within each of the two test series and rats numbered 1—12 in the controls were littermates of the corresponding numbers in the ethyl acrylate groups; and numbers 13—25, of the corresponding numbers in the methyl methacrylate groups.

The rats were individually caged and weighed once a week. The diet consisted of finely ground Purina Dog Chow Kibbled Meal consumed ad libitum. Fluid con-

[2] Analyses kindly performed by Mr. Vincent DeMarco.

sumption values were determined over a 3-day period at the end of 1 and 4 weeks, monthly through 6 months, and on even months thereafter; food consumptions were measured over 3-day periods at the same time intervals. From the fluid and food consumption measurements, diet equivalents of the monomers consumed in the drinking water were calculated. The low and medium concentrations in the water were selected with the expectation that the diet equivalents would approximate 10 and 100 ppm. The high concentration was selected following preliminary tests that indicated that this level would significantly depress fluid consumption.

Hematologic values (hematocrit, hemoglobin, total white and differential white cell counts) were obtained on 5 rats of each sex on each treatment level at 3-month intervals. Semiquantitative tests for urinary concentrations of reducing substances (Morris-Anthrone method through 18 months, succeeded by Uristix method) and protein (Pro-Teen method initially, succeeded by Shevky and Stafford method) were performed on urines pooled from 5 rats of each sex on each treatment level at 3-month intervals.

At sacrifice of 2-year survivors, organ to body weight ratio measurements were made for heart, spleen, kidney, liver, and testes. Tissues preserved from 2-year survivors and animals dying during the study in which excessive autolysis had not set in were: heart, lung, liver, kidney, urinary bladder, spleen, gastroenteric, skeletal muscle, bone marrow, skin, brain, thyroid, adrenal, pancreas, pituitary, and gonad. Histopathologic studies were done on all tissues execept those from rats on the low concentrations of test materials.

Dogs

The materials were dissolved in corn oil for oral dosing in gelatin capsules. Preliminary tests, made at the Rohm & Haas Laboratories, on 1000 ppm solutions of the monomers in corn oil indicated that the amount disappearing at room temperature over a 5-day period was no more than 5%. Solutions given to the dogs were prepared once a week and kept at 4°C prior to administration.

Purebred beagle dogs were used. Prior to placing them on experiment at an average age of about 6 months, the dogs were immunized against distemper, hepatitis, and leptospirosis; treatment for intestinal parasites was administered periodically.

During the conditioning period and for the first 6 weeks of administration of the test materials, the diet consisted of Purina Dog Chow Kibbled Meal moistened with an equal weight of water. Owing to the appearance of areas of eczema in some of the dogs (also frequently seen in caged dogs in earlier studies in our laboratory), it was decided to switch to a high-fat diet at the start of the seventh week. High-fat diets in the prevention and treatment of skin lesions in the dog have been reported on by Hansen and Wiese (1951). The diet chosen consisted of 87% Foundation Diet (Hill Packing Company), 12% corn oil, and 1% cod liver oil, mixed fresh daily and moistened with an equal weight of water prior to feeding. The lesions healed rapidly and there was no recurrence. Food consumptions were measured daily and the average daily intakes for each dog for each week were calculated. The test materials were administered once a day at feeding time, the daily dose for any one week being adjusted to be equivalent to a given number of parts per million in the diet consumed during the preceding week. The concentrations of test materials in corn oil

were adjusted so that the total volume given per day was equivalent to 2 ml of solution per 500 g of food (prior to addition of water) consumed.

Twenty-eight dogs were divided into 7 groups of 2 males and 2 females each. One group was initially placed on each of the following dietary equivalents of the test materials: 0 (control), 10, 100, and 1000 ppm of ethyl acrylate; 10, 100, and 1000 ppm of methyl methacrylate. The dogs receiving 10 and 100 ppm equivalents of ethyl acrylate retained their doses without apparent difficulty. However, on the first day the 4 receiving 1000 ppm all vomited. On the second day the dose was reduced to the equivalent of 500 ppm and 2 of the 4 vomited. Dosing of these 4 was discontinued for the remainder of the first week. With the start of the second week, dosing was reinstituted at an equivalent of 300 ppm, which was retained. Thereafter, the level was raised uneventfully to 400 ppm at 5 weeks, to 500 ppm at 7 weeks, to 600 ppm at 9 weeks, to 700 ppm at 12 weeks, to 800 ppm at 14 weeks, and to 1000 ppm at 16 weeks, this level being maintained through the rest of the study. Dogs receiving methyl methacrylate retained it without apparent difficulty and those on 1000 ppm were raised to 1200 ppm at 5 weeks, to 1400 ppm at 7 weeks and to 1500 ppm at 9 weeks, and for the rest of the study.

Hematologic studies (same as for rats above) were made prior to placing the dogs on the test materials, at 2, 4, and 13 weeks and at 3-month intervals thereafter. Urine concentrations of reducing substances and protein (same methods as for rats above) were determined at the same periods as the hematologic studies.

At sacrifice, organ to body weight ratios were determined and the same tissues preserved as for rats, above. Histopathologic studies were made on all tissues from each dog.

RESULTS

Rats

Body weight data at representative periods are summarized in Table 1, as are also data on numbers of 2-year survivors. Survival differences show no trends indicating adverse effects from the treatments. Body weights of female rats receiving 2000 ppm of ethyl acrylate in their drinking water were significantly depressed or bordered on this throughout the study, and this concentration also significantly depressed body weights of males through the first year. Similar depression among rats receiving 2000 ppm of methyl methacrylate did not continue beyond the first few weeks of the study.

Fluid consumption data obtained at 16 observation periods during the study are summarized in Table 2. The overall data indicate significant depression in fluid consumption by rats receiving 2000 ppm of the test materials, although the degree of this tended to regress toward the end of the study.

Food consumption data obtained at 16 observation periods are also summarized in Table 2. The overall data indicate significant depression only in female rats receiving 2000 ppm of ethyl acrylate in the drinking water. However, at individual observation periods, depressed food consumptions tended to parallel periods of depressed growth reported for the other rats receiving 2000 ppm of the test materials (see Table 1).

Diet equivalents of the test materials as calculated from the fluid and food con-

TABLE 1

BODY WEIGHT AND SURVIVAL DATA ON RATS RECEIVING ETHYL ACRYLATE OR METHYL METHACRYLATE IN THEIR DRINKING WATER FOR TWO YEARS

Sex	Fluid[a] conc. (ppm)	Average weight of rats (g)										Number of survivors
		Start	1 Wk	3 Wk	6 Wk	13 Wk	26 Wk	52 Wk	78 Wk	104 Wk		
Female	0	60	92	145	194	243	284	340	366	388		16
	E-6[b]	60	92	145	191	240	281	325	349	371		18
	E-60[c]	60	90	142	190	239	286	341	373	360		9
	E-2000	60	85[d]	133[d]	172[d]	221[d]	259[d]	307	315[d]	331[d]		15
	M-6[b]	60	96	151	197	250	295	365	403	382		18
	M-60[c]	60	95	149	199	250	292	350	382	400		18
	M-2000	60	83[d]	144	186	235	271	323	343	344		15
Male	0	63	100	186	283	403	483	562	561	563		13
	E-6[b]	63	101	186	287	411	498	558	594	527		12
	E-60[c]	63	102	187	283	396	485	564	590	532		15
	E-2000	63	93[d]	167[d]	260[d]	376[d]	451[d]	528[d]	543	524		18
	M-6[b]	63	99	179	283	393	470	548	554	516		18
	M-60[c]	63	102	187	285	406	468	550	571	533		15
	M-2000	63	87[d]	168[d]	271	397	474	550	558	518		13

[a] The letter E identifies ethyl acrylate, and M identifies methyl methacrylate.
[b] The concentration was raised to 7 ppm at the start of the fifth month.
[c] The concentration was raised to 70 ppm at the start of the fifth month.
[d] Differs significantly from control value ($P = < 0.05$).

sumption data obtained at the 16 observation periods are also summarized in Table 2. In these calculations, corrections were not made for evaporation losses of the test materials from the drinking water, the orders of magnitude of which are given under Methods above. Allowing for such losses, it would appear that the concentrations of test materials in the drinking water were equivalent to approximately 10, 100, and 3000 ppm in the diet.

TABLE 2

FLUID AND FOOD CONSUMPTION DATA AND DIET EQUIVALENTS OF TEST MATERIALS FOR RATS RECEIVING ETHYL ACRYLATE OR METHYL METHACRYLATE IN THEIR DRINKING WATER

Sex	Fluid conc. (ppm)	Fluid consumption (ml/rat/day)[c]	Food consumption (g/rat/day)[c]	Diet equivalent of fluid conc. (ppm)[c]
Female	0	32.5 ± 4.9	15.6 ± 2.2	—
	E-6[a]	32.2 ± 4.5	15.5 ± 2.0	13.9 ± 1.5
	E-60[b]	30.3 ± 5.1	15.4 ± 2.1	132 ± 5
	E-2000	24.5 ± 5.3[d]	13.8 ± 1.8[d]	3530 ± 430
	M-6[a]	33.4 ± 4.6	15.6 ± 2.0	14.3 ± 1.4
	M-60[b]	32.7 ± 4.9	15.9 ± 2.0	137 ± 4
	M-2000	25.2 ± 4.8[d]	15.0 ± 2.3	3360 ± 290
Male	0	35.8 ± 4.6	19.6 ± 3.4	—
	E-6[a]	35.1 ± 5.0	20.0 ± 3.1	11.8 ± 1.3
	E-60[b]	35.4 ± 4.5	19.9 ± 3.4	120 ± 5
	E-2000	29.0 ± 4.7[d]	18.8 ± 2.7	3080 ± 220
	M-6[a]	35.8 ± 4.8	19.6 ± 2.8	12.2 ± 1.2
	M-60[b]	34.9 ± 4.2	20.4 ± 2.6	115 ± 3
	M-2000	31.3 ± 4.9[d]	19.6 ± 3.3	3210 ± 220

[a] Changed to 7 ppm at start of fifth month.
[b] Changed to 70 ppm at start of fifth month.
[c] Mean ± SD calculated from means obtained at 16 observation periods during the two-year period.
[d] Differ from control value by $P < 0.05$.

Hematologic values varied within normal ranges in all groups of rats throughout the study, and urine concentrations of protein and reducing substances showed no trends that appeared relatable to treatment.

Organ to body weight ratios obtained at sacrifice of 2-year survivors differed from the controls only in significantly increased kidney ratios in female rats receiving 2000 ppm of methyl methacrylate (controls 0.0082 ± 0.0019; treated 0.0094 ± 0.0011).

Histopathologic findings showed no abnormalities or lesions, in kind or incidence, not explicable on the basis of naturally occurring ones in this strain of rat at this age.

Dogs

Except for initial emetic effects produced by the high level dosing with ethyl acrylate (for details see section on Methods above), no gross toxic effects were observed from the administration of either test material.

Body weight data at representative intervals are summarized in Table 3. Slightly lesser weight gains were achieved by the dogs receiving the high level doses of either

TABLE 3

BODY WEIGHT DATA ON DOGS RECEIVING ETHYL ACRYLATE OR METHYL METHACRYLATE FOR TWO YEARS

Material[a] (ppm)	Average body weight (kg)								
	Start	13 Wk	26 Wk	39 Wk	52 Wk	65 Wk	78 Wk	91 Wk	104 Wk
0	6.4	9.6	9.9	10.4	9.9	9.6	10.0	10.7	11.3
E-10	6.4	9.1	10.1	10.0	10.1	10.8	10.9	11.3	11.5
E-100	6.7	9.7	10.5	10.6	10.5	11.2	11.2	11.8	12.0
E-1000[b]	6.3	8.4	9.0	8.9	8.8	9.1	9.5	10.3	10.6
M-10	6.4	8.5	9.1	9.0	9.0	9.7	10.1	10.5	10.8
M-100	6.3	9.5	10.2	10.7	10.3	10.6	10.8	11.1	11.7
M-1500[b]	6.3	8.2	8.7	8.9	9.1	9.4	9.6	9.9	10.0

[a] The letter E identifies ethyl acrylate and M identifies methyl methacrylate.

[b] For dosage levels during the early weeks, see section on Methods.

material, this being correlated with slightly lesser food consumptions in the dogs receiving ethyl acrylate, but not in those receiving methyl methacrylate.

Hematologic findings and urine concentrations of reducing substances and protein were within normal ranges in all animals. Organ to body weight ratios differed statistically significantly from those of control dogs only for spleens of dogs receiving 100-ppm equivalents of methyl methacrylate (0.0057 ± 0.0014 vs 0.0032 ± 0.0011, respectively). The biologic significance of this, if any, is not apparent.

Histopathologic findings revealed no lesions attributable to the test materials.

SUMMARY

Ethyl acrylate or methyl methacrylate have been added to the drinking water of rats in concentrations of 0, 6–7, 60–70, and 2000 ppm for a period of two years. On the basis of fluid and food consumption observations, these concentrations of the test materials were estimated to be equivalent to about 10, 100, and 3000 ppm in the food. Mortality was unaffected. Body weights of female rats receiving 2000 ppm of ethyl acrylate in the water were significantly depressed or bordered on this throughout the study, and this concentration also significantly depressed body weights of males through the first year. Similar depression among rats receiving 2000 ppm of methyl methacrylate did not last beyond the first few weeks. Depressed food consumptions tended to parallel the periods of depressed growth. For both materials, fluid consumptions were significantly lower in rats receiving 2000 ppm. Hematologic values and urine concentrations of protein and reducing substances varied within normal limits for all groups of rats. Organ to body weight ratios were comparable for all groups except for significantly elevated kidney ratios in female rats receiving 2000 ppm of methyl methacrylate. Histopathologic findings revealed no lesions attributable to either test material.

Ethyl acrylate, dissolved in corn oil, was administered to beagle dogs in gelatin capsules for a period of two years in amounts equivalent to the following concentrations in the diet: 0, 10, 100, and 300–1000 ppm. Doses equivalent to 1000 ppm, attempted at the outset, caused emesis. After the dose was lowered to 300 ppm and gradually raised to 1000 ppm over the first 16 weeks, no further difficulty was encountered. Methyl methacrylate was similarly administered to beagle dogs in amounts equivalent to 0, 10, 100, and 1000–1500 ppm in the diet (emesis did not occur and the 1000 ppm level was raised stepwise to 1500 by the ninth week). Dogs receiving the high level of either material achieved slightly lesser weight gains than the controls, but the difference was not statistically significant. Hematologic values and urine concentrations of protein and reducing substances were comparable in all groups. Organ to body weight ratio measurements for test animals differed from controls only in statistically significantly lower spleen ratios for dogs receiving 100 ppm of methyl methacrylate; the biologic significance of this, if any, is not apparent. Histopathologic findings revealed no lesions attributable to either test material.

ACKNOWLEDGMENTS

The technical assistance of Miss A. M. Lea and Mrs. K. R. McCoig is gratefully acknowledged.

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THE ASSAY OF ACUTE VAPOR TOXICITY, AND THE GRADING AND INTERPRETATION OF RESULTS ON 96 CHEMICAL COMPOUNDS*

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THE toxicity evaluation of a large number of chemical compounds each year to indicate the hazards of production and utilization has been accomplished by means of the range finding test (1, 2, 3). This series of individual tests provides toxicity data on ingestion, skin penetration, vapor inhalation, skin irritation and eye injury. The presentation of these data to the medical and production departments has served to inform them of the relative hazards attendant upon the manufacture of these compounds. As soon as large-scale production is anticipated the toxicity studies may be extended as necessary to delineate further the scope of possible safe uses.

The results obtained on inhalation of known vapor concentrations of 96 compounds at the range finding level are presented in table 1. Preliminary to testing known concentrations, all compounds are screened by exposing 6 rats to substantially saturated vapor generated at room temperature. The hazard of vapor inhalation is considered negligible if all of the rats survive or if only one dies after an 8-hour exposure. If 2 or more rats die as a result of an 8-hour exposure, or if shorter exposures cause the death of a portion or all of the animals exposed to saturated vapors, then exposures to controlled concentrations are made. It should be emphasized that exposures to saturated vapors measure hazard rather than quantitative toxicity, because the amount inhaled is governed by the vapor pressure at room temperature.

Exposures to known concentrations are made to evaluate the relative toxicity of a compound independently of its vapor pressure. The exposure time is held constant at 4 hours in order to eliminate one variable, and the concentration is varied in a geometric progression increasing by a factor of 2 until the concentration which kills 2, 3, or 4 of 6 rats within a 14-day observation period is obtained. On occasion, when one concentration kills none of the 6 rats exposed and all are killed when it is doubled, the toxicity value must be determined by interpolation. Our method offers a less cumbersome basis for comparing different compounds than would be available if neither of the variables was fixed.

The equipment utilized for vapor exposures is not explosion-proof and concentrations are therefore kept below the lower explosive limit, which for many compounds is on the order of 10,000 p.p.m. or 1 per cent by volume.

Occasionally, small quantities of compounds are submitted for evaluation of vapor toxicity. If the sample available is insufficient to permit the use of dynamic exposures, static exposures are made in a 60-liter glass cube housed in a well ventilated hood. Calculations show that deprivation of oxygen may have been the causative factor in the death of the rats exposed to the compounds listed in grades 1 and 2. If such was the case, the data err on the side of caution, as the deaths may not have been caused by the toxic action of these compounds. Whatever the case, it has been shown that these vapors have a low order of toxicity for rats.

PROCEDURE

Male or female albino rats of the Sherman strain from our own colony are used in this test. Six rats, usually within the 100 to 150 gm. weight range, constitute an exposure group. While the exposure is in progress an observer watches for the first appearance of any abnormal symptoms and records the time that has elapsed until their occurrence. The check list which serves as a guide includes: continuous blinking, lacrimation, nasal irritation, roughening of the coat, excessive salivation, accelerated respiration, gasping, tremors, convulsions, loss of coordination, prostration, and narcosis.

Autopsies are performed on all of the rats to ascertain that they did not die of extraneous infection. Gross pathologic change is recorded and portions of lung, liver, spleen, adrenal gland,

* Presented at the Annual Meeting of the American Industrial Hygiene Association, Detroit, Michigan, April 5–8, 1949.

TABLE I

1. 256,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6*
   Ethyl fluoride

2. 128,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6*
   1-chloro-1,1-difluoroethane
   1,1-difluoroethylene (vinylidene fluoride)

3. 64,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
   1,1-difluoroethane

4. 32,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
   1,1-dichloroethylene (vinylidene chloride)

5. 16,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
   Acetic acid
   Benzene
   1,1-dichloroethane (ethylidene dichloride)
   1,1-dimethoxyethane (dimethyl acetal)
   Dioxolane
   Ethylene glycol monoisopropyl ether (isopropyl "Cellosolve")
   Isopropanol
   2-methyl-1,3-dioxolane
   Silicon tetrafluoride
   Vinyl ethyl ether

6. 8,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
   Acetonitrile
   Cyclohexanone
   2,6-dimethyl-1,4-dioxane
   2,6-dimethyl tetrahydro-1,4-pyrone
   Ethylene glycol diethyl ether (diethyl "Cellosolve")
   Ethylene glycol methyl ether acetate (methyl "Cellosolve" acetate)
   Ethyl orthoformate
   Silicon tetrachloride
   Toluene
   Trichloroethylene

7. 4,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
   n-butylamine
   Carbon tetrachloride
   Chlorotrifluoroethylene
   1,1-diethoxyethane (acetal)
   Ethylene glycol monoethyl ether ("Cellosolve")
   Ethylene oxide
   2-ethyl hexene-1
   2-ethylhexyl-6-chloride
   Propylene fluorohydrin
   Tetrachloroethylene
   p-thioxane (oxathiane)
   Vinyl acetate

TABLE I—Continued

8. 2,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
   Ammonia
   Amyl trichlorosilane
   Amyl mercaptan
   Benzyl alcohol
   1-chloro propene-2 (allyl chloride)
   2-chloro-1,1,3-triethoxy propane
   1,2-dichloropropane (propylene dichloride)
   Diisobutyl ketone
   Ethylbutyl ketone
   Ethylene glycol ethylether acetate ("Cellosolve" acetate)
   Ethylene glycol monomethyl ether (methyl "Cellosolve")
   Ethylene glycol mono-n-propyl ether (propyl "Cellosolve")
   Hexanedione-2,5 (acetonyl acetone)
   4-methylpentanol-2 (methyl amyl alcohol)
   Methyl ethyl ketone
   1,1,2-trichloroethane
   Vinyl trichlorosilane

9. 1,000 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
   Acetic anhydride
   Chloroacetyl chloride
   1,1-dichloro-2,2-difluoro ethylene
   1,2-dichloroethane (ethylene dichloride)
   Diethoxychlorosilane
   Mesityl oxide
   Methyl acrylate
   Pentanedione-2,4
   1,1,2,2-tetrachloroethane
   Tetraethyl orthosilicate
   Trichlorosilane

10. 500 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
    Acrylonitrile
    1,2-dichloropropanol-3
    2,5-dimethyl furane
    Ethyl trichlorosilane
    Propylene imine

11. 250 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
    Allyl acetate
    Allyl alcohol
    Aniline
    2-chloroethyl vinyl ether
    2,2′-dichloroethyl ether
    Diethyl sulfate
    Epichlorhydrin
    3-ethoxy propionaldehyde
    Formaldehyde
    3-pentene-2-one (ethylidene acetone, methy vinyl ketone)
    1,3,3-triethoxy propene-1

TABLE I—*Continued*

12. 125 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
    1-chloropropane-2,3-diol
    2-ethylhexyl amine
    2-methyl acrolein (methacrylaldehyde)

13. 62 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
    Di-2-chloroethyl formal
    Ethylene imine

14. 32 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
    Dimethyl sulfate
    Ethylene chlorhydrin

15. 16 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
    Cyanomethyl acetate
    1,2-dichloroethyl acetate
    2,3-dichloro propionaldehyde
    Tetrachloro tetrafluoro propane (mixed isomers)

16. 8 p.p.m. for 4 hrs. kills 2/6, 3/6, or 4/6
    Acrolein
    1-propene-2-chloro-1,3-diol-diacetate (chloroallylidene diacetate)
    1-propene-1,3-diol diacetate (allylidene diacetate)
    1-propene-1,3-diol diacetate

* Static exposures.

kidney, and testis are taken for histopathological interpretation from 2 of the rats which died during the 14 day observation period and also from 2 that survived. The survivors are weighed and killed by transecting the spinal cord in the cervical region. An attempt is made to take the tissues for histopathological study from rats exposed to the concentration which killed either 2, 3, or 4 of the 6 exposed.

Apparatus

The vapor concentration is prepared by delivering the fluid sample, from a 10 ml. hypodermic syringe activated by a "Monodrum", through a 26-gauge, 6-inch hypodermic needle into an evaporator through which metered air is forced. The rats are exposed to the vapor-air mixture which flows through a 9-liter desiccator fitted with inlet and outlet ports built in a standard taper stopper.

The "Monodrum"[1] is a single-drum kymograph which has been adapted for this use by removal of the drum and substitution of a screw drive to push the plunger of a syringe mounted in a vertical position with the delivery tip upright. The drive is powered by a synchronous motor which is linked by a gear train so that 22 different speed ratios, each 1.6 times faster than the preceding one, may be obtained. A fluid output ranging from less than 0.0008 ml. to above 3.6 ml. per minute may be furnished with a 10 ml. syringe. With this range of delivery and not over 20 liters per minute of dilution air, this apparatus will adequately cover the range of concentrations employed in this test. The rate of flow of dilution air is measured by a Fischer-Porter "Rotameter" covering a range of 0.5 to 30 liters per minute.

[1] Manufactured by Gorrell and Gorrell, Haworth, New Jersey.

TABLE 2

Vapor Concentration Activity Grades

4 Hour inhalation of listed concentration kills 2, 3, or 4 of 6 rats within 14 days

Degree of Hazard	Grade	Concentration	Range
		p.p.m.	*p.p.m.*
Slight	1	256,000	(Over 179,000)
	2	128,000	(89,000 to 179,000)
	3	64,000	(45,000 to 88,000)
	4	32,000	(22,000 to 44,000)
	5	16,000	(11,000 to 21,000)
Moderate	6	8,000	(5,600 to 10,900)
	7	4,000	(2,800 to 5,500)
	8	2,000	(1,400 to 2,700)
	9	1,000	(700 to 1,390)
Definite	10	500	(350 to 690)
	11	250	(175 to 340)
	12	125	(88 to 174)
	13	62	(43 to 87)
Serious	14	32	(22 to 42)
	15	16	(11 to 21)
	16	8	(6 to 10)
	17	4	(3 to 5)
	18	2	(1.4 to 2.9)
	19	1	(Under 1.4)

The evaporator consists of a Pyrex tube 1 inch in diameter and 13 inches long in which 33 parallel ridges gradually varying from 3 to 9 mm. in width have been blown. The ridges are in a plane perpendicular to the long axis of the tube, and they serve to increase the surface area and slow down the passage of liquid. The outside of this tube is encircled with uninsulated 28-gauge nichrome wire

which is connected through a suitable rheostat to the current supply. This unit is incased in a 1½ inch Pyrex tube which serves as an insulating jacket. Rubber stoppers are used to seal and align the jacket tube. Fluid is delivered into the top of the column and flows downward over the inner corrugated wall. The principal advantage of this evaporator is that current passing through the resistance wire may be regulated to cause evaporation of the product without charring. The vapors are carried to the exposure chamber by the counter current of dilution air entering the bottom of the evaporator.

The vapors enter the desiccator through the central tube, which extends to within an inch of the bottom, and flow out through the side arm connected to the standard taper joint in the top.

No analytical check is made on the concentration of the prepared vapor. The concentration is based upon empirical calculation. Careful observation of flowmeter reading, Monodrum delivery, and vaporizer performance is relied upon to eliminate errors. Experience indicates that the calculated concentrations are slightly higher than would actually be found if it were practical to determine them analytically on the exposure air. In our opinion there is definite advantage in using an all-glass vessel for the exposure chamber, as it is impervious to vapor and easily cleaned. Furthermore, the mixing problem is eliminated by virtue of its small volume and the fact that the vapors enter beneath the desiccator grid upon which the rats are supported.

Discussion

The information obtained from this test permits a compound to be assigned to a definite position in a 19 grade series. Reference may then be made to other more familiar compounds that have been graded so that plant personnel may gain a better conception of the relative hazard of the material in question. In reporting on compounds to the manufacturer we have chosen to speak of grades 1 through 5 as indicating a slight hazard, grades 6 through 9 a moderate hazard, grades 10 through 13 a definite hazard, and grades 14 through 19 a serious hazard (table 2).

We are fully aware of the fact that the inclusion of a given chemical in one of the established grades may be misleading because no evaluation of the chronic effect has been considered. However, for the particular purpose in mind—namely, evaluating the hazard to life or health from single vapor exposure—we feel that it is a sound method upon which to base our judgment of relative toxicity.

Summary

The method used for grading the activity of a chemical on the basis of the response of rats to a 4-hour vapor exposure in a 14-day observation period is described. A list of 96 compounds that have been assigned to the various grades is given, together with a justification of the use of this method at the range finding level of toxicity study.

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Biochemical Oxidation of Methyl Acrylate

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Source: Vodosnabzhenie, Kanalizatsiya, Gidrotekhnicheskiya Sooruzheniya No. 5, pp. 14-16, 1967.

Methyl acrylate is rather widely used in industry; namely, in the production of Nitron fiber, acrylic emulsions and plexiglas.

Methyl acrylate (methyl ester of acrylic acid), $CH_2 = CHCOOCH_3$, is a transparent liquid with a very unpleasant odor, and having a narcotic and general toxic effect. Thus, for white mice, the minimum lethal concentration of methyl acrylate during a two-hour exposure, is equal to 9.3 mg/l.

According to N. V. Lazarev, the maximum permissible concentration of methyl acrylate in the air is 0.02 mg/l.

In view of the fact that methyl acrylate is present in industrial effluents, and since its effect on saprophytic microorganisms, which take part in self-purification processes of water reservoirs and in the biochemical purification (decontamination) of effluents under artificial conditions has not been studied, investigations in this direction were carried out and the results obtained are reported in this article.

In order to determine the ability of methyl acrylate to undergo biochemical oxidation, tests were carried out to observe the course of oxygen consumption (demand) by a methyl acrylate solution. Complete BOD (biochemical oxygen demand) terminates after 20 days, and 1 mg methyl acrylate consumes 1 mg oxygen. The calculated and practical (actual) COD (chemical oxygen demand) of 1 mg methyl acrylate is equal to 1.4 mg oxygen. Thus, complete BOD amounts to 71.4% of the calculated COD.

With the aid of a method developed at the All-Union Scientific Research Institute for Water Supply, Sewer Systems, Hydrotechnical structures and Hydrogeological Engineering, we have studied the effect of an aqueous solution of chemically pure methyl acrylate, in concentrations ranging from 1 to 300 mg/l, on saprophytic microorganisms, which carry on the process of biochemical purification of effluents.

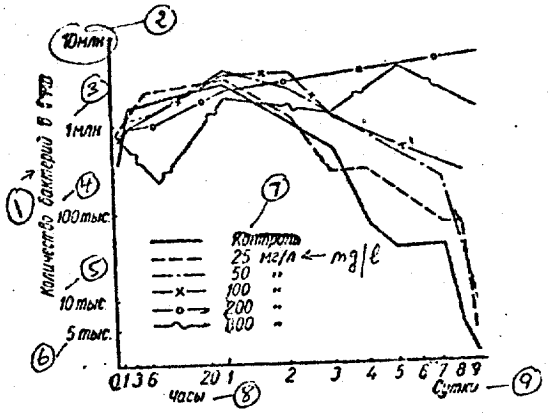
A household effluent, diluted with dechlorinated water in such a way that its permanganate oxidizability was equal to 30 mg/l, served as a control and was added in the same amount to all samples as a source of contamination.

During the first 24 hours of the test, inoculation of the bacteria was done after 1, 3, 6, and 20 hours, and then every 24 hours. The duration of the test depended on the nature of the growth and dying of bacteria. The number of saprophytic bacteria was determined by counting the colonies on meat-peptone agar after 2 days of incubation at 20°C. A hydrobiological analysis of the liquid samples was carried out daily during the duration of the test.

In order to check the dynamics of the process of mineralization of the substance, the following determinations were regularly performed: pH, bichromate oxidizability, ammonium nitrogen, nitrite and nitrate nitrogen, and methyl acrylate content.

The effect of methyl acrylate on saprophytic microorganisms is illustrated in Figure 1. Our studies have shown that in concentrations up to 100 mg/l methyl acrylate is well assimilated by bacteria. A concentration of 200 mg/l methyl acrylate extends the lag phase by 24 hours; however, the maximum growth of bacteria at this concentration after the lag phase is greater than the maximum growth of bacteria in the control vessel. In a concentration of 300 mg/l, methyl acrylate sharply inhibits the growth of bacteria.

FIGURE 1



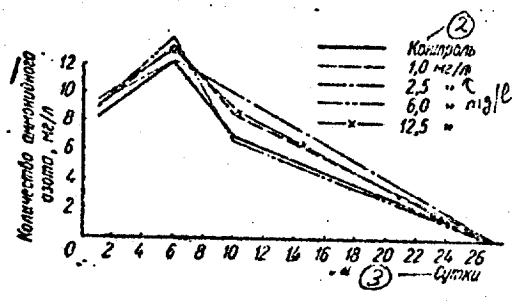
1--Number of bacteria in 5^3ml ? (illegible)
2--10 million
3-- 1 million
4--100,000
5-- 10,000
6-- 5,000
7--Control
8--Hours
9--Days

Growth of bacteria in methyl acrylate

Microscopic examination of the samples showed that flagellates and infusioria appear after 24 hours in methyl acrylate solutions at concentrations up to 100 mg/1, as well as in the control vessel. The development (growth) of infusoria is arrested for 6 days at a methyl acrylate concentration of 100 mg/1.

The values of bichromate oxidizability dropped sharply in methyl acrylate solutions, and nitrification processes took place. It should be noted that methyl acrylate, at a concentration of 12.5 mg/1, does not affect the ammonification process, while this process is slightly inhibited at a concentration of 25 mg/1 (Figs. 2 and 3).

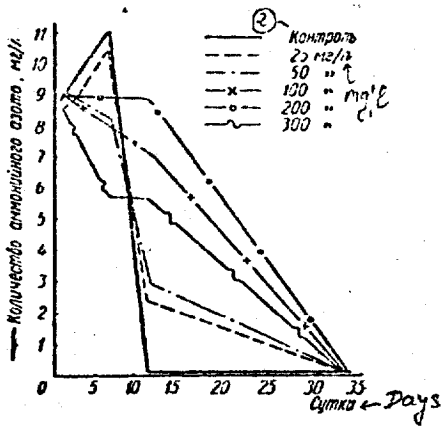
FIGURE 2



1--Amount of ammonium nitrogen, mg/1 3--Days
2--Control

Variation in the content of ammonium nitrogen in methyl acrylate solutions.

FIGURE 3

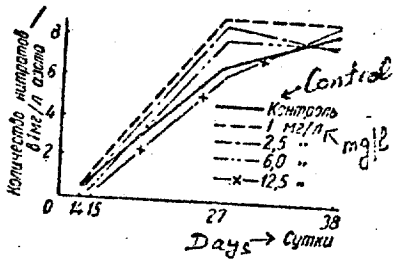


1--Amount of ammonium nitrogen, mg/1
2--Control

Variation in the amount of ammonium nitrogen in methyl acrylate solutions.

Methyl acrylate, in a concentration of 12.5 mg/1, does not affect the nitrification process, whereas this process is slightly inhibited at a concentration of 25 mg/1 methyl acrylate. On the 11th day, 1.9 mg/1 of nitrate nitrogen was found in the control, while none was found at a concentration of 25 mg/1 (Fig. 4)

FIGURE 4



1--Amount of nitrates, in 1mg/1 nitrogen

Nitrification process (II phase) in methyl acrylate solutions.

At the end of the test, after 30 days, the amount of nitrate nitrogen in the control vessel and in methyl acrylate solutions with initial concentrations of 25, 50, and 100 mg/1 is almost equal.

Methyl acrylate, in a concentration of 300 mg/1, inhibits the nitrification process.

Vodosnabzh. Kanaliz., Gidrotekh. Sooruzheniya 5:14-16, 1967

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БИОХИМИЧЕСКОЕ ОКИСЛЕНИЕ МЕТИЛАКРИЛАТА

Метилакрилат довольно широко применяется в промышленности: в производстве волокна нитрон, акриловых эмульсий, органического стекла.

Метилакрилат (метиловый эфир акриловой кислоты) $CH_2 = CHCOOCH_3$ представляет собой прозрачную жидкость с очень неприятным запахом, обладающую наркотическим и общетоксическим действиями. Так, для белых мышей минимальная смертельная концентрация метилакрилата при 2-часовом воздействии составляет 9,3 *мг/л*.

По Н. В. Лазареву предельно допустимая концентрация метилакрилата в воздухе 0,02 *мг/л*.

Ввиду того, что метилакрилат присутствует в промышленных сточных водах и влияние его на сапрофитные микроорганизмы,

участвующие в процессах самоочищения водоемов и биохимической очистки стоков в искусственно созданных условиях, не изучено, были проведены исследования в этом направлении, и результаты их представлены в настоящем сообщении.

Для того, чтобы установить способность метилакрилата биохимически окисляться, были проведены опыты по наблюдению за ходом потребления кислорода раствором метилакрилата. Полное БПК заканчивается на 20-е сутки, 1 *мг* метилакрилата потребляет 1 *мг* кислорода. Расчетное и практическое ХПК 1 *мг* метилакрилата равно 1,4 *мг* кислорода. Таким образом, полное БПК составляет 71,4% расчетного ХПК.

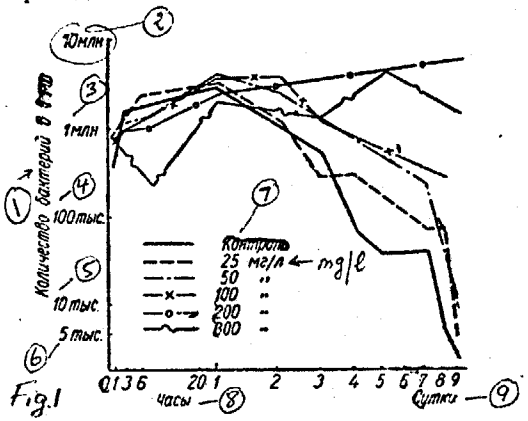

Рис. 1. Развитие бактерий в растворах метилакрилата.

С помощью методики, разработанной во ВНИИ ВОДГЕО, изучено влияние водного раствор химически чистого препарата метилакрилата в концентрациях от 1 до 300 *мг/л* на сапрофитные микроорганизмы, ведущие процесс биохимической очистки сточных вод.

Бытовая жидкость, разведенная дехлорированной водой так, чтобы ее перманганатная окисляемость составляла 30 *мг/л*, служила контролем и в одинаковом количестве вносилась во все пробы в качестве источника заражения.

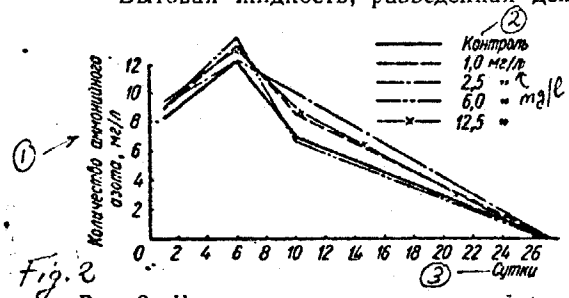

Рис. 2. Изменение содержания аммонийного азота в растворах метилакрилата.

В первые сутки опыта посев бактерий производился через 1, 3, 6, 20 *ч*, а затем каждые сутки. Продолжительность опыта зависела от характера роста и отмирания бактерий. Количество сапрофитных бактерий определялось счетом колоний на мясо-пептонном агаре через 2 суток инкубации при 20° С. Ежедневно на протяжении опыта проводился гидробиологический анализ проб жидкости.

Для наблюдения за динамикой процесса минерализации вещества регулярно определялись pH, окисляемость бихроматная, азот аммонийный, азот нитритов и нитратов, содержание метилакрилата.

Влияние метилакрилата на сапрофитные микроорганизмы представлено на рис. 1. Исследования показали, что в концентрациях до 100 *мг/л* метилакрилат хорошо усваивается бактериями. Концентрация 200 *мг/л* метилакрилата удлиняет лагфазу на 1 сутки, однако максимальное развитие бактерий при этой концентрации после лагфазы выше максимального развития бактерий в контрольном сосуде. Метилакрилат в концентрации 300 *мг/л* резко тормозит развитие бактерий.

Микроскопированием проб установлено, что в растворах метил-

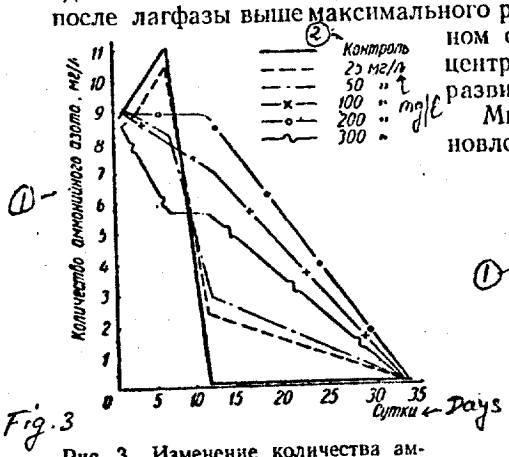

Рис. 3. Изменение количества аммонийного азота в растворах метилакрилата.

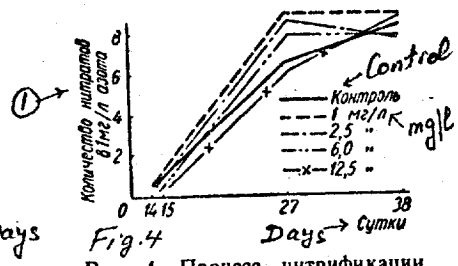

Рис. 4. Процесс нитрификации (II фаза) в растворах метилакрилата.

акрилата при концентрациях до 100 *мг/л* так же, как и в контрольном сосуде, через сутки появляются жгутиковые и инфузории. Развитие инфузорий задерживается на 6 суток при концентрации метилакрилата 100 *мг/л*.

Величины бихроматной окисляемости в растворах метилакрилата интенсивно снижались, происходили процессы нитрификации. Следует отметить, что при концентрации 12,5 *мг/л* метилакрилат не влияет на процесс аммонификации, при концентрации 25 *мг/л* он несколько тормозит этот процесс (рис. 2, 3).

Метилакрилат в концентрации 12,5 *мг/л* не влияет на процесс нитрификации, в то время как при 25 *мг/л* метилакрилата наблюдается некоторое торможение этого процесса. На 11-е сутки азота нитратов было в контроле 1,9 *мг/л*, а при концентрации 25 *мг/л* он отсутствовал (рис. 4).

К концу опыта, на 30-е сутки, в контрольном сосуде и в растворах с начальными концентрациями метилакрилата 25, 50, 100 *мг/л* количество азота нитратов почти выравнивается. Метилакрилат в концентрации 300 *мг/л* тормозит процесс нитрификации.

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J. Amer. Chem. Soc. 67: 1646–1650, (1945)

[Contribution from the William G. Kerckhoff Laboratories of the Biological Sciences of the California Institute of Technology]

Chemical Studies of Pineapple (*Ananas sativus* Lindl). I. The Volatile Flavor and Odor Constituents of Pineapple[1]

By A. J. Haagen-Smit, Justus G. Kirchner, Arthur N. Prater[2] and Clara L. Deasy[3]

Our chemical knowledge of the flavors of the fleshy fruits is largely based on comparison with the smell of synthetically-obtained esters. Few investigations have been carried out with the object of isolating and identifying the substances actually present in the fruit and responsible for their flavor. The production of these substances is an expression of the metabolism going on during ripening and their accurate knowledge is of both scientific and technical value. A direct contribution is also made by substitution of the analytical chemical determination of these compounds for the subjective scale of grading used at present in the fruit industry. In this way, the effects of climatological factors, changes in agricultural methods, and the results of breeding experiments may be investigated.

The following investigations on pineapple (*Ananas sativus* Lindl) were carried out to determine the chemical nature of the volatile constituents and, if possible, to establish a correlation between flavor and these substances. It has been observed that the taste of pineapple varies with the season in which it is grown. Although there is a great variation in individual fruits, in general the fruit grown during the summer season has a much sweeter and more fruity flavor than that of the winter crop, and it was, there-

(1) This investigation was supported by a grant from the Pineapple Research Institute of Hawaii.

(2) Present address: C. B. Gentry Co., Los Angeles, California.

(3) Present address: Temple University of Medicine, Philadelphia, Penn.

fore, decided to investigate both types of fruits separately.

The fresh fruit was subjected to vacuum distillation at 20 mm. and the volatile material condensed in a number of traps kept at different temperatures ranging from 25° to the temperature of liquid air. The small amount of oil thus obtained is typical for the pineapple flavor. From fractionation data on these oils, an immediate difference was apparent between the summer and winter fruit. The more aromatic summer fruit has a much greater volatile oil content than the winter fruit: 190 mg. per kilogram of trimmed summer fruit (1940) and 15.6 mg. per kilogram of winter fruit (1939–40). This difference is most pronounced in the low boiling fractions, mainly in the ethyl acetate and ethyl alcohol content. The acetaldehyde content, although small, was twice as great in the summer fruit, amounting to 1.35 mg. per kilogram of trimmed fruit. A similar observation was made on the fractions boiling above 100° at 745 mm. Since a slightly different method of processing was adopted in working up the 1940 winter fruit, thereby increasing the yield of the higher boiling fractions through extraction, a fresh quantity of summer fruit was distilled in 1942. The 1940 winter fruit was shown to contain 10.75 mg. of oil per kilogram of material with a boiling point above 100° at 745 mm. as compared to 17.64 mg. per kilogram for the 1942 summer fruit. These fractions were then individually analyzed for their constituents, and, if necessary, hydrolyzed with potassium hydroxide in ethylene glycol.[4] In all cases the identities of the original compounds or their hydrolysis products were established by the preparation of appropriate derivatives, *i. e.*, dinitrobenzoates for the alcohols, *p*-phenylphenacyl esters for the acids and 2,4-dinitrophenylhydrazones for the carbonyl compounds.

Since it was desirable to know the composition of the individual esters, chemical treatment was postponed until a thorough fractionation had segregated some of the oil components in narrow fractions.

The quantitative separation of the lower fatty acids was accomplished by utilizing the solvent partition method of Crowell.[5] By this method, using saturated $CaCl_2$–KCl solution and kerosene, acetic acid remains in the aqueous solution while butyric and higher molecular weight acids go into the kerosene phase. Propionic acid, if present, would be distributed between the two phases.

In both winter and summer fruit, small amounts of sulfur-containing fractions were obtained. The negative reaction for nitrogen excludes the presence of an isocyanate.[6]

In order to give an over-all picture of the various components identified in the winter and summer fruit, Table I gives the approximate amounts in terms of mg. per kilogram of trimmed fruit.

(4) Redemann and Lucas, *Ind. Eng. Chem., Anal. Ed.*, **9**, 521 (1937).

(5) Crowell, THIS JOURNAL, **40**, 453 (1918).

(6) Hoagen-Smit, *et al.*, THIS JOURNAL, **67**, 1651 (1945).

TABLE I

SUMMARY OF COMPONENTS OF THE VOLATILE OIL OF PINEAPPLE

Winter fruit (1939–1940)	Mg./kg. fruit
Ethyl acetate	2.91
Acetaldehyde	0.61
Methyl isocaproate	1.4
Methyl isovalerate	0.6
Methyl *n*-valerate	.49
Methyl caprylate	.75
Methyl ester of five carbon hydroxy acid	Trace
Sulfur-containing compounds	1.07
Summer fruit (1940)	
Ethyl acetate	119.6
Ethyl alcohol	60.5
Acetaldehyde	1.35
Ethyl isovalerate	0.39
Methyl *n*-propyl ketone	Trace
Ethyl acrylate	0.77
Ethyl *n*-caproate	.77
Summer fruit (1942) boiling above 100° at 745 mm.	
Acetic acid	0.49
Ethyl ester of a C_5 unsaturated acid	1.08
Methyl ester of a C_5 unsaturated acid	0.68
Methyl ester of a C_5 keto acid	Trace
Sulfur-containing compound	0.88

Experimental

Fresh pineapple of the smooth Cayenne variety was supplied by the Pineapple Research Institute of Hawaii. The trimmed summer fruit (1940), amounting to 1161 kg. (total weight of whole fruit with peel and core, 1611 kg.) was cut into small pieces and was distilled from a twelve-liter Pyrex balloon flask. The distillation was carried out at 20 mm. of mercury with the still contents being kept below 40° at all times. The vapors passed through a spiral glass condenser into a flask at room temperature, through three flasks immersed in an ice–salt bath, through three receivers at −80° and finally into a trap cooled with liquid air. The condensates in the receivers held at room temperature and at the temperature of the ice–salt mixture were returned to the still and topped to remove the volatile material. By this redistillation procedure the desired volatile material was collected in the third ice-cooled receiver and the subsequent ones at lower temperature. The bulk of the desired distillation products was contained in the first receiver cooled to −80°. The liquid air trap contained only solid carbon dioxide and no liquid condensate. The oil–aqueous condensates from the traps were combined, the aqueous phase saturated with ammonium sulfate to salt out as much of the oil phase as possible and the two phases separated. The oil phase was dried over anhydrous sodium sulfate.

The trimmed winter fruit (1939–40, 995 kg. from 1450 kg. whole fruit) was treated in an analogous manner except that after redistillation of the material from the room temperature receiver, the water remaining in the still and the condensate in the ice–salt trap was extracted with ether yielding 6.4 g. of higher boiling material.

In order to obtain more working material for the smaller fractions of the volatile constituents, an additional 740 kg. of trimmed summer fruit (1942, 1089 kg. whole fruit) was distilled. In this case, the peeled fruit was run through a Waring Blendor. The juice was squeezed out in a press and the solid residue again run through the Blendor with a quantity of water. After a final pressing, the fibers which remained were discarded.

TABLE II

FRACTIONATION OF 8.6 G. OF VOLATILE OIL FROM 995 KG. OF TRIMMED WINTER FRUIT (1940)

Fraction no.	Distilln. Temp., °C.	Press., mm.	Fraction wt., g.	Sapn. no.
1	40 - 58	745	0.02	...
2	58 - 64.5	745	1.40	93.2
3	64.5- 70	745	1.20	127.5
4	70 - 87	745	0.30	121.5
5	87 -109.5	745	.90	115.5
6	109.5-115	745	.60	133
7	64 - 71	100	.60	159
8	71 - 87	100	.45	158
9	87 - 99	100	1.40	163
10	99 -115	100	0.49	158
11	115 at 100.0 mm. to 123 at 52.0 mm.		0.75	175
Residue			0.00	

TABLE III

FRACTIONATION OF THE ETHER EXTRACT OF THE AQUEOUS DISTILLATE OF WINTER FRUIT (1939-1940)

Fraction no.	Distilln. temp., °C.	Press., mm.	Fraction wt., g.
12	40-60	746	0.05
13	33-41	11	.12
14	57-67	11	.07
15	67-69	11	.46
16	69-71	11	.45
17	71-79	11	.22
18	69-78	6	.31
19	78-86	6	.32
20	71-77	2	.46
21	residue		3.5

TABLE IV

CHARACTERIZATION OF ALCOHOL PART OF VOLATILE OIL AS 3,5-DINITROBENZOATE

Fraction	M. p., °C.	Mixed m. p., °C.	% Analyses Carbon	Hydrogen	Nitrogen
2, 3, 4	85 - 85.5	No depr. with Et deriv.	44.80	3.28	
6	104 -104.5	No depr. with Me deriv.	42.38	2.66	12.48
9	106.5-107	No depr. with Me deriv.	42.32	2.66	12.50
10	107 -108	No depr. with Me deriv.	42.39	2.64	12.36
11	107.5-108	No depr. with Me deriv.	42.85	2.81	12.51
Ethanol	92	.....	calcd. 44.99	3.36	11.67
Methanol	108	.....	calcd. 42.49	2.67	12.39

Winter Fruit (1939-1940)

The aqueous-oil, two-phase condensate was saturated with ammonium sulfate and separated. The aqueous phase (215 g.) gave a positive test for aldehydes. A crystalline 2,4-dinitrophenylhydrazone was obtained identical with that of acetaldehyde, as shown by analyses and mixed melting point with the synthetic derivative.

Anal. of 2,4-dinitrophenylhydrazone[7]: Calcd. for $C_9H_8N_4O_4$: N, 24.95. Found: N, 24.75.

The total amount of acetaldehyde present in the aqueous phase is 0.585 g., as determined by triplicate quantitative determinations.

The volatile oil phase from the cold traps amounted to 8.6 g. of anhydrous oil. This oil was fractionated at atmospheric pressure through a 20-cm. Vigreux column until the lower boiling material had been removed. The remainder was then fractionated under reduced pressure. Table II gives the distillation data.

(7) Analyses in this paper were carried out by G. Oppenheimer and G. Swinehart.

The 6.4 g. of higher boiling material obtained by ether extraction gave the fractions listed in Table III.

The lowest fraction, 40-58°, contained acetaldehyde, which was absent in higher fractions, as shown by tests with fuchsin sulfite reagent.

With the exception of Fraction 2 which contained ethyl esters, the higher boiling material consisted of methyl esters (Table IV).

Fractions 9, 10 and 11 contain small amounts of a sulfur compound and analyzed for 0.11, 0.69 and 0.75% sulfur, respectively. In addition, the higher boiling fractions contained sulfur in larger amounts, Fraction 13 contained 4.67% and fractions 15 through 20 contained 16.86, 17.89, 13.36, 10.95, 2.60 and 1.10%, respectively.

The acids characterized from these fractions are listed in Table V with their derivatives. Fraction 6 was chiefly methyl isovalerate. Fraction 9 was almost pure methyl isocaprylate, while fraction 10 consisted mainly of methyl *n*-valerate with a trace of a methyl ester of a hydroxyvaleric acid.

Summer Fruit (1940)

The distillation products from 1161 kg. of trimmed summer fruit (1940) consisted of an oil-aqueous mixture, which, after saturating with ammonium sulfate and separating, yielded 320.5 g. of aqueous phase and 220.3 g. of anhydrous volatile oil phase.

Acetaldehyde was identified and determined in the aqueous phase as described for the winter fruit and amounted to 1.134 g.

Most of the volatile oil phase (197.7 g. out of 220.3 g. total) was slowly fractionated at atmospheric pressure through a 24-inch Widmer column until the lowest boiling material had been removed. The residue then was distilled at atmospheric pressure through a 20-cm. air-jacketed Vigreux column, and finally the column was substituted by a 5-cm. column and stillhead and the distillation continued at reduced pressure.

A five to nine milligram sample of each fraction was hydrolyzed by heating in a glass-stoppered 3-ml. test-tube with an excess of tenth normal potassium hydroxide in ethylene glycol. After hydrolysis, the excess base was back titrated with one-hundredth normal hydrochloric acid. From the data, saponification numbers were cal-

TABLE V

p-PHENYLPHENACYL DERIVATIVES OF ACID PART OF HYDROLYZED FRACTIONS

Fraction	M. p., °C.	Mixed m. p., °C.	% Analyses Carbon	Hydrogen
2, 3, 4	108 -109	108 -109	75.56	5.73
Acetic acid	108 -109		calcd. 75.57	5.56
6	71.5- 72	71 - 72	77.60	6.78
Isovaleric acid	74.0- 74.8		calcd. 77.00	6.80
9	69.5- 70	69.5- 70	77.34	7.12
Isocaproic acid	70 - 70.5		calcd. 77.20	7.45
10 (1st deriv.)	67.5- 68	68 - 69	77.45	6.98
10 (2nd deriv.)	98 - 99		73.96	6.43
n-Valeric acid	69		calcd. 76.97	6.82
Hydroxyvaleric acid			calcd. 73.03	6.47
11	63 - 64	63 - 65	78.16	6.88
n-Caprylic acid	67		calcd. 78.07	7.75

culated, expressed as the apparent molecular weight if the entire fraction were an ester. Table VI contains the distillation data and the saponification numbers of the fractions obtained.

TABLE VI

FRACTIONATING OF 197.7 G. OF VOLATILE OIL CORRESPONDING TO 1042 KG. OF SUMMER FRUIT (1940)

Fraction no.	Distilln. temp., °C.	Press., mm.	Fraction wt., g.	Supn. no.
S-1	up to 69	745	4.3	162
S-2	69 - 70.0	745	116.8	118
S-3	70.0- 70.4	745	41.7	123
S-4	70.4- 75.5	745	7.3	175
S-5	75.5- 76	745	6.6	439
S-6	76 - 77.5	745	1.9	958
S-7	77.5- 78.0	745	0.4	1213
S-8	78.0- 78.7	745	.9	790
S-9	78.7- 78.8	745	.8	1289
S-10	78.8- 79.0	745	.8	889
S-11	79.0- 81.0	745	.4	1790
S-12	81.0- 88.0	745	trace	698
S-13	88.0- 89.8	745	0.4	1232
S-14	89.8- 91.0	745	.4	1001
S-15	91.0- 94.0	745	.5	311
S-16	36 - 98.5	100	.3	289
S-17	98.5-101.5	100	.8	234
S-18	101.5-105	100	.8	263
S-19	105 -109	100	.5	245
S-20	109 at 100 mm. to 124.5 at 61 mm.		.5	302
S-21	124.5 at 61 mm. to 125 at 20 mm.		.4	323

Identification of Fraction S-1.—This fraction was found to contain 9% of acetaldehyde, thus accounting for 0.388 g. In addition, there was 0.044 g. of acetaldehyde in the unfractionated oil, making a total of 1.566 g. in the summer fruit. Fraction S-1 also contained ethyl acetate, which was identified as before. From the saponification value, the quantity of ethyl acetate can be calculated to be approximately 55% of the fraction, which with the 9% aldehyde leaves 36% to be accounted for by the ethyl alcohol which was found to be present.

Anal. of 2,4-dinitrophenylhydrazone: Calcd. for $C_8H_8N_4O_4$: N, 24.95: Found: N, 24.72.

Anal. of 3,5-dinitrobenzoate: Calcd. for $C_9H_8N_2O_6$: C, 44.99; H, 3.36. Found: C, 44.76; H, 3.22.

Anal. of *p*-phenylphenacyl ester: Calcd. for $C_{16}H_{14}O_3$: C, 75.57; H, 5.56. Found: C, 75.71; H, 5.61.

Identification of Fractions S-2, S-3 and S-4.—These fractions consist of a mixture of mainly ethyl alcohol and ethyl acetate. From the saponification values the ethyl acetate content can be calculated to be approximately 75, 71.5 and 50%, respectively.

Identification of Fraction S-5.—This fraction gave upon hydrolysis ethyl alcohol and an acid portion yielding a low melting mixture of *p*-phenylphenacyl esters. Accordingly, the acid portion was therefore separated by partitioning between a saturated $CaCl_2$–KCl solution and kerosene.[5]

By identifying the acids after the separation, it was found that the esters originally present were ethyl acetate and ethyl isovalerate. The following quantitative results were obtained in this separation: 2.000 g. of fraction 5 was saponified with excess potassium hydroxide, the mixture acidified to *p*H 3 with sulfuric acid and distilled until all the organic acid had distilled over. This distillate was carefully neutralized with standard base and the solution dried to constant weight. It was found that 0.00274 equivalent of base was used and 0.3068 g. of potassium salts was obtained. From this, it can be calculated that the 2.000 g. of fraction 5 consists of: 0.118 g. or 5.9% ethyl isovalerate, 0.162 g. or 8.1% ethyl acetate and 1.720 g. or 86% ethyl alcohol (by difference).

The dried potassium salts of the mixed acids were moistened with water, and to the solution was added an amount of standard hydrochloric acid equivalent to the amount of organic acids present. The solution was then partitioned between kerosene and saturated $CaCl_2$–KCl. The phases were carefully separated, and the kerosene treated with silver sulfate to remove halogen ions and then distilled. The kerosene-organic acid distillate was titrated with standard base, and it was found to require 0.000776 equivalent. This amount multiplied by the factor 1.12 (determined by Crowell[5] to be a correction factor for losses in the partitioning procedure) yields 0.000869 equivalent or 0.113 g. calculated as ethyl isovalerate. This checks well with the previous figure of 0.118 g. calculated from the potassium salt mixture. The neutral aqueous phase obtained by titrating the kerosene-organic acid distillate, was made acid with a drop of sulfuric acid and refluxed with ethyl alcohol and *p*-phenylphenacyl bromide. A derivative was formed which was identified as the isovaleric acid compound.

Anal. of *p*-phenylphenacyl ester of isovaleric acid, m. p. 73.5–74.5°: Calcd. for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.84; H, 6.62.

The saturated $CaCl_2$–KCl solution was diluted with alcohol and, after filtering off the salts, the *p*-phenylphenacyl ester was formed as before. Acetic acid was identified in this material.

Fractions S-6 through S-11 proved to be practically all ethyl alcohol. In addition, they contained traces of esters which were not identified because of the small amounts.

Identification of Methyl *n*-Propyl Ketone.—Since fraction S-13 gave a positive ketone test, it was shaken with an aqueous hydrochloric acid solution of 2,4-dinitrophenylhydrazine. A small amount of crystalline material was obtained which was extracted with ether. After removal of the ether, this material was recrystallized from aqueous ethanol and yielded orange-yellow needles that melted over a range of 126–134°. This was dissolved in purified carbon tetrachloride and passed through a 50-cm. column of magnesium oxide.[8] Two bands developed: a fairly narrow upper band and a diffuse lower band. The colored bands were eluted with ethyl alcohol. Only very small amounts of the purified products were obtained. The derivative from the upper band melted 70–100°, while that from the lower band melted at 141–142°. The latter gave no depression with the 2,4-dinitrophenylhydrazone of methyl *n*-propyl ketone (m. p. 139.5–140.5°).

Thus, while not enough material was obtained for micro-analysis, the data accumulated indicate the presence of methyl *n*-propyl ketone Fractions 12, 14 and 15 contain the substance in such small amounts that no appreciable amounts of derivative could be obtained from this.

Fractions S-16, S-17 and S-18 contained esters of ethyl alcohol as determined by the formation of the characteristic 3,5-dinitrobenzoates.

The acid part of fraction S-17 gave a *p*-phenylphenacyl derivative, melting at 109–110°, which turned orange when melted. This analyzed as the derivative of acrylic acid.

Anal. of *p*-phenylphenacyl ester: Calcd. for $C_{17}H_{14}O_3$: C, 76.69; H, 5.26. Found: C, 76.13; H, 5.58.

The acid part of fraction S-18 gave a *p*-phenylphenacyl derivative melting at 69.5–70°. A mixed melting point with the derivative (m. p. 70.8–71°) of *n*-caproic acid gave 67.5–69.5°.

Anal. of *p*-phenylphenacyl ester: Calcd. for $C_{20}H_{22}O_3$: C, 77.39; H, 7.14. Found: C, 76.96; H, 7.26.

Fractions S-16 through S-21 contained sulfur, ranging from 0.09% in fraction S-16 to a maximum of 3.30% in fraction S-20. Fraction S-21 contained 2.17% of sulfur.

(8) Strain, THIS JOURNAL, 57, 758 (1935).

Summer Fruit (1942)

Since the ether extract of the water distillate from the winter fruit yielded a considerable quantity of high boiling material, this technique was applied to a fresh quantity of summer fruit (1942). Fractionating of the total volatile oil from this fruit yielded the data in Table VII.

TABLE VII

FRACTIONATING OF 215.3 G. OF VOLATILE OIL FROM 740 KG. OF TRIMMED SUMMER FRUIT (1942)

Fraction no.	Distilln. temp., °C.	Press., mm.	Fraction, wt., g.
F-1	35-45	745	14.4 (mainly residual ether)
F-2	45-55	745	5.1
F-3	68-76	745	181.3
F-4	48-55	123	3.44
F-5	45-50	11	1.37
F-6	50-60	11	1.31
F-7	60-70	11	1.25
F-8	70-80	2	0.82
F-9	80-83	at 2	
		at 1	.50
F-10	Residue	..	5.2

Since fractions F-4 and F-5 were decidedly acidic, they were washed with dilute potassium hydroxide solution. The neutral residues were combined and fractionated as shown in Table VIII. Likewise, the acidic fractions were combined and fractionated, as shown in Table IX.

TABLE VIII

FRACTIONATION OF NEUTRAL PORTION OF FRACTIONS F-4 AND F-5 OF THE SUMMER FRUIT (1942)

Fraction no.	Distilln., temp., °C.	Press., mm.	Fraction, wt., g.
N-1	76 - 81.5	745	0.097
N-2	81.5- 87.5	745	.089
N-3	87.5- 90	745	.085
N-4	90 - 95	745	.067
N-5	95 -100	745	.161
N-6	76 - 82	100	.108
N-7	90 -100	100	.128

TABLE IX

FRACTIONATION OF ACIDIC MATERIAL FROM FRACTIONS F-4 AND F-5 OF THE SUMMER FRUIT (1942)

Fraction no.	Distilln. temp., °C.	Press., mm.	Fraction wt., g.
A-1	87-98	745	0.026
A-2	53-55	120	3.261
A-3	54-64	100	0.366
A-4	65-44	100	
		to 10	0.115

Identification of Fractions A-2 and A-3.—The presence of mainly acetic acid in both fractions was shown by preparing the *p*-phenylphenacyl derivatives.

Anal. of *p*-phenylphenacyl ester, m. p. 109.5-110: Calcd. for $C_{16}H_{14}O_3$: C, 75.57; H, 5.56. Found: C, 76.12; H, 5.38.

Identification of Fraction F-8.—The alcohol part of this ester fraction was identified as ethyl alcohol (3,5-dinitrobenzoate, m. p. 90.8-91, no depression with synthetic ethyl derivative).

The *p*-phenylphenacyl ester of the acid part melted at 122-123.2° and turned yellow after melting. Analysis indicated that it was an acid with five carbon atoms and one double bond. Eleven structures for the acid are possible. The *p*-phenylphenacyl esters of two of the possible acids, tiglic and angelic, did not melt at the same temperature as the naturally occurring acid, and did not turn yellow after melting.

Identification of Fraction F-9.—The alcohol part of the esters did not contain ethyl alcohol, but was identified as methyl alcohol by conversion to the 3,5-dinitrobenzoate, m. p. 98.5-102°. A mixed melting point with synthetic ethyl 3,5-dinitrobenzoate gave a melting point of 70-90°, and with synthetic methyl 3,5-dinitrobenzoate (m. p. 108°) a melting point of 104-107°.

The acid part was chiefly the same C_5 unsaturated acid as occurred in the fraction immediately previous, fraction F-8. It also contained a small amount of an acid, whose *p*-phenylphenacyl derivative melted at 109.5-110.5°. This analyzed as the derivative of a C_5 keto acid.

Anal. of *p*-phenylphenacyl derivative, m. p. 109.5-110.5°: Calcd. for $C_{19}H_{18}O_4$: C, 74.07; H, 6.17. Found: C, 74.92; H, 6.15.

Summary

The chemical composition of the volatile oil from fresh pineapple has been investigated, and a comparison made between the oils from the winter and summer crops.

The volatile oil content of summer fruit (1940) is 190 mg.; that of winter fruit (1939-40) is 15.6 mg. per kg. of trimmed fruit. This larger yield for the summer fruit is chiefly caused by the larger percentage of low boiling material, consisting of ethyl acetate and ethyl alcohol, respectively, 119.6 mg. and 60.5 mg. per kg. of trimmed fruit. The difference in amounts of the higher boiling material was not so pronounced. The winter fruit contains 10.75 mg. of oil boiling above 100° at 745 mm. per kg. of trimmed fruit, compared to 17.64 mg. for the summer fruit.

In addition to ethyl alcohol and ethyl acetate, the following compounds were found in the summer fruit: acetaldehyde, ethyl isovalerate, methyl *n*-propyl ketone, ethyl *n*-caproate, ethyl acrylate (probably), acetic acid, an ethyl ester of a C_5 unsaturated acid, some methyl ester of the same acid, and an ethyl ester of a C_5 keto acid.

The winter fruit contained, besides ethyl acetate, the following: acetaldehyde, methyl isocaproate, methyl isovalerate, methyl *n*-valerate, a methyl ester of a C_5 hydroxy acid and methyl caprylate.

Both the winter and summer fruit contained a sulfur-containing compound which was present in the higher boiling fractions.

Except for the ethyl acetate, all the remaining esters investigated in the winter oil were methyl esters. In the summer fruit, only one methyl ester was found.

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Use of Mathematical Models in the Study of Structure-Toxicity Relationships of Dental Compounds: I. Esters of Acrylic and Methacrylic Acids

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The toxicity of a series of esters of acrylic and methacrylic acids was determined in groups of mice, and the resultant LD_{50} values were analyzed with the use of the mathematical models of Free and Wilson and of Hansch to relate structure of the compounds to toxicity. Both models proved valuable in relating structures to toxicity parameters, but the more useful method for predicting toxicity was the Hansch model.

Synthetic polymeric materials are receiving greater attention in dentistry for a number of uses, including restorative materials, liners, protective films, adhesives, oral prosthetic devices, tissue substitutes, and implantable plastic teeth. With the increasing use of man-made materials in dentistry and medicine, questions of toxicity are being asked about the final polymerized material and the monomers and other agents that may become part of the final plastic or elastomeric item.

One of the most successful synthetic polymeric dental materials belongs to the family of acrylic resins, of which poly(methyl methacrylate) is the most widely used. The monomer is the methyl ester of methacrylic acid (methyl methacrylate). Other esters of acrylic or methacrylic acid have been or may be used to produce polymers with a variety of physical, chemical, and mechanical properties. We became interested in the toxicogenic properties of a series of acrylic monomers, with the hope of being able to correlate the acute toxic response of the monomer with its structure and one or more physicochemical properties. From this basic approach, it was thought that one or more mathematical models could be used to predict or approximate the specific toxic effect when new compounds in the series or related compounds were to be considered. The Free-Wilson[1] and the Hansch[2] mathematical models were used in this study. The toxicogenic parameter used in the models is the acute LD_{50} value of the monomers.

THE FREE-WILSON MODEL.—Free and Wilson[1] developed a quantitative structure-activity equation based on the assumption that for an analogous series of compounds, the observed activity of a given compound can be expressed as a sum of mutually independent contributions from the various segments of the molecule. Thus, the activity of each compound in a series can be represented in the form of a linear equation. Assuming the compound has N segments, the relationship can be depicted as

$$\text{biologic activity} = \text{overall average} + \text{contribution of segment 1} + \text{contribution of segment 2} + \cdots\cdots + \text{contribution of segment } N. \quad (1)$$

The different segments are assumed to be those portions of the general structure that change between any two compounds in the series. A number of different substituents may be found at each segment; for this reason each compound in the series will have a different linear equation for its activity. The activity contributions of the substituents thus can be treated as the unknowns in a system of independent, linear equations that are set up for the series of compounds under consideration. These equations can be solved by use of standard regression analysis techniques.[3]

From such an analysis, one usually can determine how well the linear model fits the data and the calculated relative activity contributions of the substituents at a given segment. If the model fits the data, the substituent contributions can be used to predict activities of untested compounds.

THE HANSCH MODEL.—Hansch and Fujita[2] have developed a free energy-related quantitative structure-activity model, which in its most common form, is expressed as

$$-\log(\text{biologic response}) = a\,\pi^2 + b\,\pi + c\,\sigma + d. \quad (2)$$

Here, π is the log of the octanol-water partition coefficient and σ is the Hammett[4] or Taft[5] substituent constant for the compound (drug, toxicant, etc) considered. When values are specified for the biologic response (eg, LD_{50}, TIC_4, in molar units), π and σ for each compound in an analogous series, the corresponding equations can be solved for the coefficients a, b, c, and d. The parameter π is considered to be a measure of the transport (eg, across membranes) and hydrophobic binding properties of the drug or toxicant.[2] Accordingly, a high level of correlation of activity with π may be indicative of the importance of this property with regard to activity, and the Hammett or Taft σ value is related to the ability of a substituent to affect the electron distribution within the molecule.

These mathematical models presuppose a common mode of biologic action for all members of the series. Initial justification for this assumption is based on toxicity studies of lower members of the methacrylate series by Deichmann[6] and Spealman et al,[7] in which they reported respiratory failure as the cause of death in several species of experimental animals when the compounds were administered by various routes. Similarly, studies on methyl and ethyl acrylate found that the acutely toxic effects caused respiratory failure in experimental animals.[8,9]

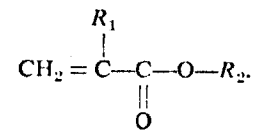
Materials and Methods

LD_{50} DETERMINATIONS.—All acrylic and methacrylic monomers were obtained from a commercial source* as liquids. Each of the monomers contained from 5 to 2,000 ppm of the polymerization inhibitor, monomethyl ether of hydroquinone, or 100 ppm hydroquinone. The monomers were used as such with no further purification. Male albino ICR mice† that weighed 25 ± 5 gm each were used as the test animals. Eighteen compounds were used in this study.

Several dose levels of each compound were injected intraperitoneally into groups of ten mice and the mice were observed for seven days for mortalities. Injections were made with a clinical syringe when the dose to be administered was greater than 1 ml. With a dose of 0.1 to 1.0 ml a tuberculin syringe was used, and for doses of less than 0.1 ml a Hamilton microliter syringe was used. For some highly toxic monomers, accurate measurement of the dose made it necessary to dilute the compound with normal saline (when water soluble) or cottonseed oil (when not water soluble) before injection. All mice were given laboratory chow‡ and water ad libitum.

From the seven day mortalities for each compound, the LD_{50} dose was calculated by the Cornfield-Mantel modification of Karber's method,[10] with the use of a computer.§

FREE-WILSON ANALYSES.—The series of compounds reported in this paper can be represented as



When R_1 = H, a series of acrylic esters results, and when $R_1 = CH_3$, a series of methacrylic esters is formed. To formulate the linear equations, log $(1.0/LD_{50})$ was used as the biologic activity. For convenience in calculation, the original experimental LD_{50} values (determined as ml/kg) were converted to mol/10^6 gm.

HANSCH ANALYSES.—The general form of Hansch's equation used in these studies for

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* Rohm and Haas, Philadelphia, Pa.
† Harlan Industries, Cumberland, Ind.
‡ Ralston-Purina Co.
§ IBM model 1620.

structure-toxicity activity can be expressed as

$$\log (1.0/\mathrm{LD}_{50}) = a\,\pi^2 + b\,\pi + cQ^{\sigma}(\mathrm{C}) + d. \quad (3)$$

As with the Free-Wilson model, the LD_{50} values were expressed as mol/10^6 gm. Sigma electron distributions were calculated for members of each series and the net σ charge on the carbonyl carbon [represented as $Q^{\sigma}(\mathrm{C})$] included in the regression analyses, and values for the parameter π were calculated as the sums of substituent constants obtained from the literature.[11] Sigma electron distributions were obtained by the method of Del Re[12] with the use of parameters determined to reproduce dipole moments.[12,13] No attempt was made to obtain suitable Hammett or Taft σ values.

CRITERIA OF ANALYSES.—As measures of the "goodness of fit" of the data to the Free-Wilson or the Hansch models, the square of the multiple correlation coefficient (R^2), the overall F ratio, and the explained variance for the regression analyses may be calculated.[3] The multiple correlation coefficient gives an indication of the degree of correspondence between the experimentally observed and the calculated activities ($R^2 = 1.0$ indicates perfect correlation). The overall F ratio provides, by tabular interpretation, a measure of the significance of the calculated coefficients (substituent contributions in this instance) considered altogether. Explained variance is the fraction of the variance of the activity data that can be accounted for by the model. A value of 1.0 is perfect; a negative value is possible if, on a per-degree-of-freedom basis, the simple average value of the observed activities is better than the activity calculated with the theoretical linear equation. The explained variance is often the most useful statistic. In addition, the student's t test for individual coefficient significance also was carried out for the Hansch analyses. This allows one to assess the individual significance of the coefficients of the π, π^2, and $Q^{\sigma}(\mathrm{C})$ terms when they are included together in an analysis.

The Del Re molecular orbital calculations and the Free-Wilson and Hansch analyses were carried out on a computer* with the use of programs developed in our laboratories.

Results

The acute toxicity data are presented in Table 1 as the LD_{50} value and 95% confidence limits in units of mol/10^6 gm. The calculated parameters [$Q^{\sigma}(\mathrm{C})$ and π] for the Hansch model and the polymerization inhibitor and its concentration also are included in this table. Acrylate monomers are more toxic than the corresponding methacrylate monomers. The lower (molecular weight) members of the series tend to be more toxic than the higher members, the straight chain substituent is less toxic than the corresponding branched chain (compare butyl and isobutyl), and simple aliphatic substituents ($C_n H_{2n+1}$) are less toxic than substituents that contain hydroxyl or amine functional groups.

The toxicities of the 18 acrylates and methacrylates were subjected to a regression analysis with the use of the model of Free and Wilson.[1] This analysis yields substituent toxicity contributions that may be used to rank the substituents that appear at a given segment according to their toxic contributions. Table 2 includes substituent contributions when all of the compounds were used in the analysis, when all of the compounds except butyl methacrylate were used, and when all of the compounds were used except butyl methacrylate and molecules in which R_2 contained an amine or hydroxyl group. (The three categories were made to facilitate further discussion.) The series of molecules considered in this report is not, however, sufficiently extensive to allow a statistical characterization of the results of the Free-Wilson analyses. Several of the substituents at R_2 occur in only a single molecule, thereby ruling out a statistical treatment.

Twenty-eight analyses with the Hansch model[2] were carried out by varying the

* IBM model 1620.

TABLE 1
EXPERIMENTAL LD_{50} VALUES, CALCULATED $Q^{\sigma}(\mathrm{C})$ AND π VALUES, AND POLYMERIZATION INHIBITOR CONCENTRATION

Compound	Acute IP Toxicity in Mice: LD_{50} (mol/10^6 gm)	95% Confidence Limits	$Q^{\sigma}(\mathrm{C})$*	π†	MEHQ‡ (ppm)
Glacial acrylic acid	0.225	0.169- 0.295	0.1806	−0.53	200
Methyl acrylate	2.949	2.281- 3.828	0.1666	0.21	15
Ethyl acrylate	5.986	4.619- 7.760	0.1662	0.73	15
Butyl acrylate	6.653	5.475- 8.083	0.1661	1.77	5
Isobutyl acrylate	5.932	4.862- 7.231	0.1662	1.64	§
2-ethylhexyl acrylate	7.195	5.991- 8.643	0.1661	3.72	50
Glacial methacrylic acid	0.564	0.482- 0.658	0.1778	−0.01	100
Methyl methacrylate	11.217	9.378- 13.412	0.1638	0.73	10
Ethyl methacrylate	10.896	9.328- 12.734	0.1634	1.25	15
Butyl methacrylate	10.481	8.893- 12.346	0.1634	2.29	10
Isobutyl methacrylate	8.398	7.345- 9.601	0.1634	2.16	10
Isodecyl methacrylate	14.251	12.041- 16.875	0.1634	5.28	‖
Lauryl methacrylate	84.531	65.694-108.769	0.1634	6.45	‖
t-butylaminoethyl methacrylate	0.937	0.853- 1.031	0.1635	1.19	100
Dimethylaminoethyl methacrylate	0.618	0.505- 0.761	0.1635	0.30	2,000
Hydroxyethyl methacrylate	4.060	3.178- 5.195	0.1636	−0.55	1,200
1,3-butylene dimethacrylate	16.063	14.335- 18.005	0.1631	2.50	100
Trimethylolpropane trimethacrylate	8.537	8.023- 9.082	0.1634	3.53	200

* Net σ charge on the carbonyl carbon as calculated by the method of Del Re.
† Calculated as the sum of substituent constants, π selected from the literature.
‡ MEHQ = monomethyl ether of hydroquinone, used to inhibit polymerization.
§ No information given concerning the inhibitor or its concentration.
‖ Contains 100 ppm hydroquinone as the polymerization inhibitor.

TABLE 2
STRUCTURE-ACTIVITY ANALYSES FOR ACRYLATE-METHACRYLATE SERIES USING FREE-WILSON MODEL

Segment	Substituent	Substituent Contributions, −log (LD 50) Units, Based on: a*	b†	c‡
R_1	H	0.2120	0.2249	0.2001
	CH_3	−0.1060	−0.1229	−0.1493
R_2	H	1.0846	1.0671	1.2100
	$CH_2CH_2N(CH_3)_2$	1.0051	1.0026	...
	$CH_2CH_2NHC(CH_3)_3$	0.8241	0.8216	...
	CH_2CH_2OH	0.1881	0.1856	...
	CH_3	−0.1229	−0.1404	−0.0032
	$CH_2C(CH_2{=}C(CH_3)\text{-}COOCH_2)_2C_2H_5$	−0.1349	−0.1374	−0.0062
	$CH_2CH(CH_3)_2$	−0.2114	−0.2289	−0.0853
	C_2H_5	−0.2714	−0.2895	−0.1453
	C_4H_9	−0.2844	−0.3773	−0.2352
	$(CH_2)_7CH(CH_3)_2$	−0.3539	−0.3564	−0.2128
	$CH_2CH(C_2H_5)CH_2CH_2CH_3$	−0.3789	−0.4125	−0.2677
	$CH_2CH_2CH(CH_3)OOCC(CH_3){=}CH_2$	−0.4139	−0.4164	−0.2728
	$(CH_2)_{11}CH_3$	−1.1239	−1.1264	−0.9828
Average log $1/\mathrm{LD}_{50}$, μ		−0.6901	−0.6706	−0.7879

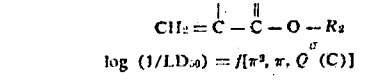
$$CH_2{=}C(R_1)-C(=O)-O-R_2$$

$$-\log(\mathrm{LD}_{50}) = \mu + \sum \text{substituent contributions}$$

* Calculations based on all of the 18 compounds listed in Table 1.
† Calculations based on all of the compounds listed in Table 1 except butyl methacrylate.
‡ Calculations based on all of the compounds in Table 1 except butyl methacrylate and molecules in which R_2 contains an amine or hydroxyl group.

TABLE 3
STRUCTURE-ACTIVITY ANALYSES FOR ACRYLATE-METHACRYLATE SERIES USING HANSCH MODEL

$f[\pi^2, \pi, Q^\sigma(C)]$	R^2	Significance Level (%)	Explained Variance
$-0.240\ \pi - 0.255$	0.55	99.9	0.52
$-0.035\ \pi^2 - 0.449$	0.41	99.5	0.37
$85.041\ Q^\sigma(C) - 14.805$	0.47	99.5	0.43
$0.020\ \pi^2 - 0.348\ \pi - 0.196$	0.57	99.5	0.51
$-0.177\ \pi + 55.174\ Q^\sigma(C) - 9.526$	0.71	99.9	0.67
$-0.027\ \pi^2 + 68.588\ Q^\sigma(C) - 11.889$	0.69	99.9	0.65
$-0.004\ \pi^2 - 0.152\ \pi + 56.785\ Q^\sigma(C) - 9.809$	0.71	99.9	0.65



$\log(1/LD_{50}) = f[\pi^2, \pi, Q^\sigma(C)]$

π = log octanol-water partition coefficient calculated by use of substituent constants taken from the literature. $Q^\sigma(C)$ = sigma charge on carbonyl carbon.

combinations of parameters [π^2, π, $Q^\sigma(C)$] which were included, and the makeup of the series investigated (all compounds, all acrylates, all methacrylates, etc). The statistical results for these analyses are presented in Tables 3 through 7.

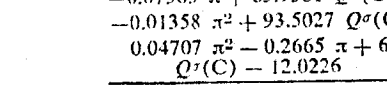
To test the predictive utility of the Free-Wilson and the Hansch models, a number of analyses that omitted either butyl acrylate or butyl methacrylate were carried out for variously constituted series of toxicants. This allows one to calculate the activity of the deleted compound for comparison with the observed value. These findings are presented in Table 8.

Discussion

FREE-WILSON ANALYSIS.—Inspection of the substituent rankings in Table 2 reveals that of the two substituents at segment R_1 (hydrogen and methyl), hydrogen is calculated to contribute more strongly to toxicity. As one might have surmised on inspection of the LD_{50} values in Table 1, the acrylates are predicted to be more toxic than the corresponding methacrylates. At segment R_2, hydrogen (corresponding to the acid) and short chains that contain amine or hydroxyl group appear to enhance toxicity, whereas simple alkyls and substituents with additional ester linkages decrease toxicity.

TABLE 4
STRUCTURE-ACTIVITY ANALYSES FOR ACRYLATE SERIES USING HANSCH MODEL

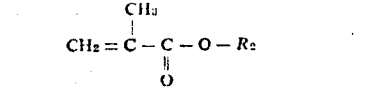
$f[\pi^2, \pi, Q^\sigma(C)]$	R^2	Significance Level (%)	Explained Variance
$-0.27942\ \pi - 0.15768$	0.51	85.0	0.38
$-0.04563\ \pi^2 - 0.35275$	0.17	55.0	−0.04
$97.2579\ Q^\sigma(C) - 16.9097$	0.96	99.9	0.95
$0.16778\ \pi^2 - 0.82482\ \pi - 0.04607$	0.87	95.0	0.79
$-0.07363\ \pi + 85.9381\ Q^\sigma(C) - 14.9083$	0.98	99.5	0.97
$-0.01358\ \pi^2 + 93.5027\ Q^\sigma(C) - 16.2300$	0.97	99.0	0.95
$0.04707\ \pi^2 - 0.2665\ \pi + 69.3084\ Q^\sigma(C) - 12.0226$	0.99	97.5	0.98



$\log(1/LD_{50}) = f[\pi^2, \pi, Q^\sigma(C)]$

TABLE 5
STRUCTURE-ACTIVITY ANALYSES FOR METHACRYLATE SERIES USING HANSCH MODEL

$f[\pi^2, \pi, Q^\sigma(C)]$	R^2	Significance Level (%)	Explained Variance
$-0.226\ \pi - 0.308$	0.55	99.0	0.50
$-0.034\ \pi^2 - 0.494$	0.47	97.5	0.42
$79.605\ Q^\sigma(C) - 13.887$	0.26	90.0	0.19
$0.006\ \pi^2 - 0.259\ \pi - 0.287$	0.55	95.0	0.45
$-0.196\ \pi + 46.698\ Q^\sigma(C) - 8.059$	0.63	97.5	0.55
$-0.030\ \pi^2 + 59.477\ Q^\sigma(C) - 10.321$	0.61	97.5	0.52
$-0.007\ \pi^2 - 0.153\ \pi + 49.110\ Q^\sigma(C) - 8.486$	0.63	95.0	0.49



$\log(1/LD_{50}) = f[\pi^2, \pi, Q^\sigma(C)]$

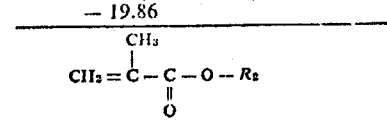
In this latter grouping, there is a rough inverse relationship between the size of the substituent and its calculated toxicity contribution.

HANSCH ANALYSES.—As can be seen in Table 1, the parent acids of both series (acrylic acid and methacrylic acid) were the most toxic or active members in each series. This suggested that perhaps hydrolysis or a more general nucleophilic attack at the carbonyl carbon of the esters might occur, which in turn could contribute to the total toxic manifestation of one or more of the esters. The tendency of an ester to undergo nucleophilic attack can be related to the electronic charge on the carbonyl carbon atom.[14] This charge may be considered to be the sum of π electrons and σ electron components. Because the π electron system in the vicinity of the ester linkage does not vary from one molecule of the series to another (ignoring hyperconjugation, etc), any variation in the net charge will arise from changes in the σ electron distributions.

When the toxicities of the 18 molecules (acrylates and methacrylates) were subjected to regression analyses with the Hansch structure-activity model, as much as 52%

TABLE 6
STRUCTURE-ACTIVITY ANALYSES FOR REDUCED METHACRYLATE SERIES USING HANSCH MODEL

$f[\pi^2, \pi, Q^\sigma(C)]$	R^2	Significance Level (%)	Explained Variance
$-0.1998\ \pi - 0.4634$	0.56	97.5	0.50
$-0.0263\ \pi^2 - 0.7073$	0.45	95.0	0.38
$97.82\ Q^\sigma(C) - 17.14$	0.71	99.5	0.66
$0.0220\ \pi^2 - 0.3436\ \pi - 0.3218$	0.59	90.0	0.45
$-0.1185\ \pi + 72.37\ Q^\sigma(C) - 12.62$	0.86	99.5	0.81
$-0.0182\ \pi^2 + 81.73\ Q^\sigma(C) - 14.28$	0.90	99.9	0.87
$-0.0596\ \pi^2 + 0.3171\ \pi + 113.13\ Q^\sigma(C) - 19.86$	0.95	99.5	0.92



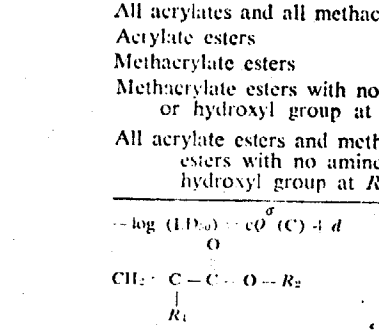
$\log(1/LD_{50}) = f[\pi^2, \pi, Q^\sigma(C)]$

Note: Molecules in which R_2 contains an amine or hydroxyl group are omitted from this series.

TABLE 7
CORRELATION OF TOXICITY WITH $Q^{\sigma}(C)$ FOR SERIES WITH AND WITHOUT ACIDS INCLUDED

Description of Series	No. of Compounds	R^2	Significance Level (%)	Explained Variance
All acrylates	6	0.96	99.9	0.95
All methacrylates	12	0.26	90.0	0.19
All acrylates and all methacrylates	18	0.47	99.5	0.43
Acrylate esters	5	0.99	99.9	0.99
Methacrylate esters	11	0.08	55.0	−0.02
Methacrylate esters with no amine or hydroxyl group at R_2	8	0.02	25.0	−0.15
All acrylate esters and methacrylate esters with no amine or hydroxyl group at R_2	13	0.42	97.5	0.37

$$-\log(LD_{50}) = cQ^{\sigma}(C) + d$$



of the variance in the toxicities could be explained by use of the single parameter π (significant at the 99.9% level, see Table 3). This implies that the transport or hydrophobic properties of these molecules, or both, might be important in manifestation of toxicity, because about half of the variations in toxicity can be accounted for by this parameter. If only the charge on the carbonyl carbon [$Q^{\sigma}(C)$] is considered, up to 43% of the toxicity variations (significant at the 99.5% level) can be explained. These results indicate that the σ charge also is important, and in turn suggests the possibility that ester hydrolysis or other nucleophilic attack [$Q^{\sigma}(C)$ increased with toxicity] should be considered as playing a role in the toxicity mechanism. Alternatively, such a dependence on $Q^{\sigma}(C)$ may imply the formation of an electrostatic complex that involves the carbonyl group. Taken together, π and $Q^{\sigma}(C)$ can explain as much as 67% of the variation and are significant at the 99.9% level. Other combinations of parameters tested and results obtained for this series also are summarized in Table 3.

TABLE 8
ACTIVITY PREDICTIONS BASED ON THE STRUCTURE-ACTIVITY MODELS

Model	Test Molecule (withheld from analysis)	Series	Predicted LD_{50} (mol./10^6 gm)	Observed LD_{50} (mol./10^6 gm)
Free-Wilson	Butyl methacrylate	All acrylates and all methacrylates	14.82	10.48
	Butyl methacrylate	All acrylates and reduced* methacrylates	14.87	10.48
Hansch†	Butyl acrylate	All acrylates	5.62	6.65
	Butyl methacrylate	All methacrylates	7.30	10.48
	Butyl methacrylate	Reduced* methacrylates	11.97	10.48
	Butyl methacrylate	All acrylates and all methacrylates	8.10	10.48
	Butyl methacrylate	All acrylates and reduced* methacrylates	11.70	10.48

* Does not include compounds that contain an amine or hydroxyl group.

† Predicted LD_{50} values obtained by use of the relationship $-\log(LD_{50}) = b\pi + cQ^{\sigma}(C) + d$.

Because this series of compounds can be separated conveniently into two classes, acrylates and methacrylates, these subseries were analyzed individually. The acrylate series is composed of 6 molecules, and the methacrylate series contains 12.

The combinations of parameters considered and results obtained for the acrylate series are presented in Table 4. Although the explained variances calculated for π and π^2 separately are only 0.38 and −0.04, respectively, the combination of the two parameters increased the explained variance to 0.79. In this combination, π is significant at the 95% level and π^2 at the 99% level (based on t tests). Neither of these parameters was significant at the 95% level (based on t test) when considered alone. Such a parabolic dependence of activity on π has been considered to be indicative of the toxic compound being localized in a lipophilic phase, or swept out of the biologic test system at a rate sufficient to affect its concentration at the active site.[15] Table 4 indicates clearly that toxicity variation in this subseries can be correlated highly with $Q^{\sigma}(C)$ (explained variance = 0.95, $R^2 = 0.96$, and significant at the 99.9% level). When taken alone, it seems apparent that nucleophilic attack or electrostatic binding is implicated as an important contributing factor to the toxicity of this subseries of compounds. When π and $Q^{\sigma}(C)$ are considered together, the explained variance is increased to 0.97, and the overall significance level is 99.5%. On the basis of t tests, $Q^{\sigma}(C)$ is significant at the 99.9% level, and π is significant in the 80 to 90% range.

Combinations of parameters considered and results obtained for the methacrylate series are shown in Table 5. One may note from the information contained in the table that the high levels of correlation found for the acrylates do not carry over to the methacrylates. In the methacrylate series, the best correlation is obtained with π, which is significant at the 99% level and is able to explain as much as 50% of the variance. In this subseries, $Q^{\sigma}(C)$ leads to an explained variance of only 19% and is significant at only the 90% level. When π and $Q^{\sigma}(C)$ are considered together, 55% of the variance can be explained and the overall significance level has increased to 97.5%. On the basis of t tests, only π is significant at the 95% level. Results of the analyses for these methacrylates demonstrate that $Q^{\sigma}(C)$ (and thus perhaps nucleophilic attack or electrostatic binding) does not appear to be able to account for the observed variations in toxicity. That only about half of the variance can be explained by any of the combinations of parameters tested (Table 5) strongly suggests that other variables not included in this study must be important in the manifestation of toxicity.

To investigate this possibility further, the three methacrylates in which R_2 contains an amine or hydroxyl group were omitted and the regression analyses were repeated. The results are presented in Table 6. The extent of correlation with π or π^2, or both, remained about the same as before, but the correlation with $Q^{\sigma}(C)$ increased appreciably. With combinations of π, π^2, and $Q^{\sigma}(C)$, from 81 to 92% of the variance can be explained for this reduced series, whereas only 49 to 55% (Table 5) was possible for the complete methacrylate series. In view of this result, one might speculate that the single mode of action criterion of the Hansch model is not satisfied if the series contains molecules with a functional group in position R_2, other than another ester linkage.

The parameter $Q^{\sigma}(C)$ was introduced in this study because it should be a measure of the ability of the ester linkage to undergo nucleophilic attack. These results show that a high level of statistically significant correlation can be achieved with the use of this parameter, and that the variation of $Q^{\sigma}(C)$ with toxicity is in the proper direction for consistency with this interpretation. Correlation with $Q^{\sigma}(C)$ has been discussed to this point in terms of nucleophilic attack in general and ester hydrolysis in particular. The regression analysis results that have been presented are, however, for series that contain the parent acids. Strictly speaking, it is inappropriate to consider these results indicative of the importance of ester hydrolysis. To address this question directly, the regression analyses using $Q^{\sigma}(C)$ were repeated for series from which the acids had been omitted. The results are presented in Table 7, with those for series that include the acids. For the acrylate esters, the explained variance increases to 0.99, and for the methacrylate esters a negative explained variance results (average toxicity value is a better approximation, on a per-degree-of-freedom basis, than the calculated values).

When compounds that contain an amine or hydroxyl group in R_2 are omitted from the methacrylate ester series, the explained variance remains negative and the significance level decreases still further. Thus, although the results for the acrylate subseries would be consistent with a mechanism that involves ester hydrolysis, those for the methacrylate subseries would not.

ACTIVITY PREDICTIONS.—One of the primary objectives of this quantitative structure-activity study was to attempt to develop a basis for the prediction of activities of untested compounds. Both the Free-Wilson and the Hansch models lend themselves to this application. To assess the predictive capabilities of the mathematical formulas developed for the toxicity of acrylates or methacrylates or both, regression analyses were carried out with a representative or typical member of the series omitted. The resulting equations then were used to predict the activity of that compound. Butyl methacrylate was omitted from the series that contained methacrylates, and butyl acrylate was omitted from the acrylate series. For tests of the Hansch model, the equation

$$-\log (LD_{50}) = b + c\, Q^{\sigma}(C) + d \quad (4)$$

was used in each instance. The results are presented in Table 8.

The Free-Wilson model does not lead to good quantitative predictions. This probably reflects the fact that no substituent at R_2 occurs more than twice in the series studied. Because only the esters of acrylic and methacrylic acids were investigated in this study, no substituents can occur at R_2 more than twice, and the prospect of using the Free-Wilson model for accurate quantitative predictions becomes poor. If more than two variations are allowed at R_1, however, this problem can probably be overcome. The substituent rankings were essentially the same for each of the three variously constituted series analyzed by the Free-Wilson method (Table 2). This indicates that the model should be suitable for qualitative predictions or relative toxicities.

It is possible to achieve considerably more accurate predictions with the Hansch model if the compounds that contain an amine or an hydroxyl group in R_2 are omitted. For this reduced methacrylate series considered alone or in combination with the acrylates, good predictions for the activity of butyl methacrylate are obtained. A reasonably good prediction for butyl acrylate is found for the acrylates considered alone.

Taken all together, the results presented here indicate that the toxicity variations in acrylates and methacrylates can, under proper restrictions, be treated successfully by linear quantitative structure-activity models. The Free-Wilson model is not recommended for quantitative predictions of toxicities of untested molecules, but it does appear to provide a reliable toxicity contribution ranking of the substituents at a given segment. The Hansch free energy-related model can lead to a high level of correlation if only alkyl (or polyester) substituents are present in the series.

To achieve high levels of correlation using the Hansch model, it was necessary to exclude from the series those methacrylates that contained an amine or hydroxyl group in the substituent at R_2. This exclusion is rationalized on the basis that these functional groups might offer alternate biologic (metabolic or toxicologic) pathways for these molecules, and thus violate the single mode of action assumption of the mathematical model. The high levels of correlation obtained in the absence of amine and hydroxyl compounds tend to suggest that transport (correlation with π or π^2 or both) and the electron density at the carbonyl carbon [correlation with $Q^{\sigma}(C)$] are important factors.

Using the relationship

$$-\log (LD_{50}) = -0.0179\pi^2 + 88.21\, Q^{\sigma}(C) - 15.34 \quad (5)$$

(which was derived by using the series of all acrylates and reduced methacrylates, and resulted in R^2, 0.94: significance level, 99.5%; explained variance, 0.93), it is anticipated that one can predict reasonable LD_{50} values for as yet untested acrylates and methacrylates, if the R_2 substituent does not contain functional groups other than ester linkages.

Conclusions

The LD_{50} values for a series of esters of acrylic and methacrylic acids were determined in mice, and the resultant data were analyzed with the use of the general mathematical models of Free-Wilson and of Hansch. The parent acids for the acrylic and methacrylic monomers and for those short-chain esters that contain amine or hydroxyl groups were the more toxic compounds of the series; simple alkyls and substituents with additional ester linkages decreased toxicity. A high level of correlation of structure to toxicity was obtained with the Hansch model. With this series of compounds, the Free-Wilson model appears to be satisfactory for ranking the substituents in order of their toxicity, but does not lend itself to accurate quantitative prediction of toxicogenic activity.

Use of the Hansch model showed that as much as 52% of the variations in toxicities could be explained by use of the single parameter π (partition coefficient), which implies that transport or hydrophobic properties of these compounds or both are important in determining their toxicogenic activity. Inclusion of $Q^{\sigma}(C)$ with π increased the explained variance to 67%. When Hansch analyses were made of the acrylic and methacrylic series separately, a high correlation of structure-toxicity activity was found for the acrylic series, but not for the methacrylic series. The data suggested, however, that the R_2 substituents that contain amine or hydroxyl groups may be susceptible to alternative metabolic or toxicologic pathways. When these compounds were omitted, the explained variance increased to 92%, with a 99.5% level of significance. This data also permitted a reasonably good prediction for the toxicity of butyl methacrylate (predicted, 0.01197 mol/kg; observed, 0.01048 mol/kg). The best correlation between predicted and observed toxicity was obtained with the Hansch model with all acrylates and methacrylates except those that contained amine or hydroxyl functional groups (predicted, 0.01170 mol/kg; observed, 0.01048 mol/kg).

This work supports the idea that use of appropriate mathematical models may help show toxic potentials of new dental compounds of a homologous or related series, and may help guide chemists in the synthesis of those compounds with the least toxic liability. Further studies are being conducted in these laboratories that are relevant to dental toxicology.

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SUBACUTE VAPOR TOXICITY AND RANGE-FINDING DATA FOR ETHYL ACRYLATE*

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INTRODUCTION

ETHYL acrylate has attained considerable commercial importance in the production of resins. A few papers concerning the toxicity of the related methacrylic esters have appeared (1, 2, 3) and one paper on methyl acrylate has been published (4). No toxicological data on ethyl acrylate has come to our attention, and it therefore seemed advisable that an investigation of the ester be performed to determine the degree of toxic hazard accompanying its industrial applications.

Monomeric ethyl acrylate, $CH_2{=}HCOOC_2H_5$, has a molecular weight of 100, a specific gravity of 0.919 (25°/25°C.), a boiling point of 99.3–99.7°C., and a vapor pressure of 40 mm. of mercury at 20°C. One milligram per liter of air is equivalent to 244 parts per million (p.p.m.). The sample used in this study was the usual commercial grade, such as may be purchased on the open market, inhibited with 0.25 per cent hydroquinone.

SINGLE ORAL DOSES

Ethyl acrylate, prepared as a 10 per cent dispersion in 1.0 per cent "Tergitol" 7 penetrant solution, was administered by stomach tube to 40 male albino rats weighing from 90 to 120 gm. The rats were maintained on the "Rockland" complete rat diet and were dosed without prior starving. The deaths which occurred during a 14-day observation period in the four groups of 10 rats that received dosages of 0.795, 1.00, 1.26, and 1.58 gm. per kilogram numbered 0, 4, 10, and 10 respectively. The LD_{50} calculated by the method of Thompson (5) was found to be 1.02 (0.95 to 1.10) gm. per kilogram. The victims exhibited such symptoms as sluggishness, prostration and narcosis before dying. All deaths but one occurred within 24 hours after dosing. Gross pathological examination revealed pale liver, kidneys and spleen, congested lungs, and an irritated gastrointestinal tract. The peritoneal wall and the viscera appeared to be macerated by the action of the compound.

SKIN PENETRATION TESTS

Undiluted ethyl acrylate was applied to the clipped trunks of rabbits and was kept in contact with the skin for 24 hours by means of a jacket of flexible "Vinylite" sheeting. Deaths in groups of 10 rabbits resulting from dosages of 1.58, 2.00, and 2.52 gm. per kilogram were 1, 5, and 10 respectively. The survivors were sacrificed 14 days after the initiation of treatment. This procedure was a modification of the technique used by Draize (6). The LD_{50} by this route was found to be 1.95 (1.79 to 2.12) ml. per kilogram. Erythema, edema, and necrosis of the skin were produced. Autopsy findings included congestion and hemorrhage of the lungs, mottling of the liver, congestion and occasional hemorrhage of the kidneys, and injection of intestinal blood vessels.

SKIN AND EYE IRRITATION TESTS

A single application of 0.01 ml. of the undiluted ester was made on different areas of the clipped abdomen of each of 5 albino rabbits. After the

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treated areas had dried, the animals were caged for 24 hours and then critically examined for skin reactions at the sites of application. Two of the 5 showed minor injection of the skin capillaries and 3 were nonreactive. Ethyl acrylate is no more irritating in this test than ethyl crotonate or the insect repellent dimethyl phthalate.

Gradually decreasing amounts of the undiluted ester were applied directly to the corneas of rabbits as described by Carpenter and Smyth (7). It was found that 0.5 ml. produced severe necrosis of the corneas in 24 hours, and that 0.1 ml. produced a moderate necrosis of the corneas of 5 rabbit eyes in the same period of time. These observations indicate that the ester is as irritating to the corneas of rabbits as ethanol and about one-twentieth as irritating as ethyl crotonate (7).

Single Vapor Inhalation

A flowing stream of the appropriate air–ethyl acrylate mixture was introduced under slight positive pressure into a 9-liter exposure chamber which held the test group of 6 rats. Survivors were weighed and sacrificed 14 days after being exposed to either saturated vapor or given concentrations.

Groups of 6 rats were exposed to substantially saturated vapor produced by forcing air at the rate of 2.5 liters per minute through a fritted glass disc immersed in 50 ml. of the ester. This concentration, approximating 50,000 p.p.m., induced gasping in 3 minutes, prostration in 10 minutes, and was lethal in 15 minutes. A similar exposure of 5 minutes' duration resulted in the survival of the 6 rats exposed. Deaths were attributed to pulmonary damage, as severe congestion and hemorrhage were found in the lungs of all victims at autopsy.

The production of controlled concentrations of the vapor was accomplished by delivering the liquid ester into an evaporator at a constant rate by means of a glass syringe driven by a synchronous kymograph motor. The dilution air requisite to produce the calculated concentration was measured by means of a Fischer-Porter "Rotameter" before it was passed through the evaporator.

Concentrations of ethyl acrylate vapors of 4000, 2000, and 1000 p.p.m. killed 6 of 6, 5 of 6, and none of 6 rats respectively in 4-hour exposure periods. Respiratory distress was noticed at the highest concentration, and marked redness of the nose, ears, and feet occurred at all three levels of exposure. The gross pathological findings in the victims were similar to those noted in the rats that inhaled saturated vapors.

Subacute Vapor Studies

Prior to selecting groups of animals to be exposed to ethyl acrylate vapors, a somewhat larger group than was needed was exposed for 5 days, 7 hours per day, to room air under conditions closely simulating those of the planned test. The animals were exposed in their home cages, but food and water were witheld during the exposures. The airflow through the chambers and the heat input to the evaporators was regulated to duplicate exactly the proposed test conditions. Any animals that lost weight during this "cold" run were excluded from the population from which the final groups were to be selected by random distribution. This procedure served a dual purpose, as subnormal animals were eliminated and the remainder were conditioned to the new regimen.

Ten Sherman strain albino rats of each sex and 4 male albino rabbits were exposed for a maximum of 19 days to a vapor concentration of ethyl acrylate of 540 p.p.m. In addition 15 rats of each sex and 9 male rabbits were exposed for a maximum of 30 days to 300 and to 70 p.p.m. The animals were randomly distributed between control and treated groups and exposed 5 days per week for 7 hours per day. The exposures to various concentrations were run at different times so that each exposure group required its own set of control animals. The control animals were confined in similar chambers through which air free from vapors was drawn. The rats used in these exposures were approximately 2 months of age.

Immediately before sacrifice the rats were weighed and then anesthetized with intraperitoneal injections of 0.1 to 0.2 ml. of 6 per cent "Nembutal" to permit measurement of body length. Next the spinal cord was sectioned and the animal drained of blood until heart action ceased, after which the liver and the kidneys were excised and promptly weighed to the nearest centigram. Tissue slices for histopathological examination were taken from the liver, kidney, heart, lung, pancreas, spleen, and adrenal. A similar set of tissues was taken from the surviving rabbits, which were dispatched by a blow on the head. Data concerning the body length and organ weights of the latter species were not collected.

The concentrations of ethyl acrylate vapors which were introduced into the 550 liter exposure chambers at the rate of about 250 liters per minute

under slight negative pressure were produced by means of a displacement-type proportioning pump described by Irish and Adams (8).

The liquid was vaporized by the use of an electrically-heated vertical "Pyrex" tube 2.5 x 34 cm. The middle 25 cm. of its length was corrugated by 33 parallel ridges perpendicular to the long axis to increase surface area. The ridges were gradually increased in width from 0.3 cm. at the bottom of the tube to 0.9 cm. at the top. Bare 28 gauge nichrome wire was wound around the outside of the tube between the successive ridges. An air-insulating jacket was made from "Pyrex" tubing, 3.8 x 29 cm., and was held in place around the wired portion of the inner tube by means of rubber stoppers. A small hole was blown 2 cm. from each end of the jacket to provide for passage of the wires, which were attached to a rheostat.

The ester was introduced at the top of the vaporizing tube, and allowed to flow downward over the inner heated walls. The resultant vapors were carried off by an air-stream entering the tube at the bottom. It was found that this evaporator prevented overheating or charring of the liquid, produced a relatively constant evaporation rate, facilitated cleaning, and made possible observation of the evaporation process.

Vapor concentrations were checked 4 times daily with a 50 cm. Zeiss portable interferometer. The instrument was calibrated by empirical calculation involving the refractivities of the atoms in the ethyl acrylate molecule as described by Stamm and Whalen (9). One interferometer scale division was found to represent 21.8 p.p.m. of ethyl acrylate vapor.

Results

At the three exposure levels the mean concentrations of the ethyl acrylate vapors as determined by interferometer readings were 537.6 p.p.m., 294.1 p.p.m., and 71.2 p.p.m. with standard errors of the means of 12.1, 9.1, and 3.7 respectively. In round numbers the concentrations during exposure were 540, 300, and 70 p.p.m.

TABLE 1

RESULTS OF REPEATED EXPOSURE OF RATS TO ETHYL ACRYLATE VAPORS

CONCENTRATION (P.P.M.) (METERED)	500	0	250	0	62.5	0
Concentration (p.p.m.) (interferometer mean)	540	0	300	0	70.	0
Exposure (days)	19	20	30	30	30	30
Mean weight gain of survivors (gm.)	+9.3*	+54.6	+38.1*	+73.2	+89.7	+88.6
Mean body length of survivors (mm.)	—	—	206.2	208.2	205.7	205.0
Fatness of survivors (gm./mm.)	—	—	0.908*	1.068	1.064	1.071
Liver weight as % of body weight	—	—	4.28	4.28	4.22	4.05
Kidney weight as % of body weight	—	—	0.84*	0.78	0.84	0.80
No. of rats that died of lung infection	12*	0	11*	0	2	2
Mean no. of days to death of victims	13.8*	—	14.8*	0	18.3	11.5
No. of survivors	6*	20	12*	30	27	28
Sets of tissues examined	17	—	21	29	29	28
Sets with major pathologic changes other than "lung infection"	0	—	0	0	0	0

The data for the control group for each concentration appear in the column to the right of the data for the group exposed to that concentration of ethyl acrylate. Omissions in the table are the result of the discontinuance of the 500 p.p.m. exposure after 19 days.

* The deviation from the control is statistically significant.

The rats exposed to concentrations of 540 and 300 p.p.m. of ethyl acrylate succumbed to its toxic effects (table 1). Because only 6 of the 19 rats exposed to 540 p.p.m. survived 19 days of exposures, it was considered unnecessary to complete 30 exposures.

Upon histopathological examination, the lungs of 12 of the 13 victims of the 540 p.p.m. concentration revealed pneumonic involvement. These changes were usually accompanied by cloudy swelling of the renal tubules and occasionally by congestion and cloudy swelling of the liver. The cause of death of the thirteenth victim could not be ascertained because of severe autolysis of tissues before autopsy. None of the control rats died before the twentieth exposure day. The lungs of 5 of the

6 survivors showed incipient pneumonic involvement but no renal or hepatic damage. The other survivor had normal lungs and light cloudy swelling of the liver.

Eighteen of 30 rats died during the 30-day exposure to 300 p.p.m. ethyl acrylate. Ten of these victims exhibited the same type of pneumonic involvement and damage of kidney and liver as was seen in the victims of the 540 p.p.m. exposure. Five other victims revealed congestion of the lungs, cloudy swelling and congestion of the liver and excessive pigmentation of the spleen. Gross pathological examination of another victim revealed hemorrhage and congestion of the lung. The tissues of 2 rats could not be used because of autolysis. Nine of the 12 survivors showed no tissue changes whatsoever. The remaining 3 rats exhibited only minor histological alteration of the lung or the liver. None of the 30 control rats died during the same period of exposure.

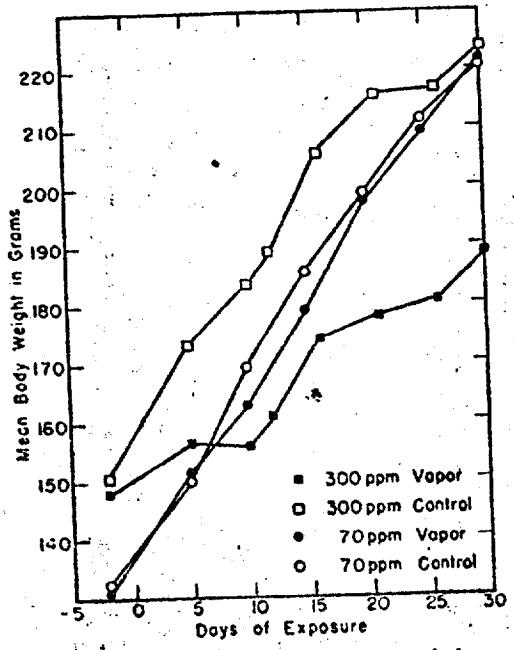

FIG. 1. Mean weights of rats exposed to ethyl acrylate vapor.

The means of the criteria of effect, listed in table 1, of the exposed and the control rats were compared by the use of the "t" test (10). If the probability associated with the "t" and the specific degrees of freedom was less than 0.05, the difference was accepted as statistically significant and probably not due to random errors of measurement.

The 12 surviving rats of the group exposed to 300 p.p.m. gained less weight, had a lower fatness index, and greater kidney weight than their controls (table 1). The probability that the difference of the mean weights of the exposed and the control rats was due to chance was 0.65 two days before exposure, 0.009 on the fifth exposure day and 0.002 on the tenth exposure day. As indicated by figure 1, the deviation between the mean weights of these exposed and control rats increased with successive exposures.

The only significant difference between the controls and the rats exposed to 70 p.p.m. was an increase in the kidney weight of male rats only. Because the females did not show this increase, and histopathological examination revealed no abnormalities, and as in all the other criteria of effect the exposed rats were not different from the controls, a concentration of 70 p.p.m. ethyl acrylate is considered safe for rats in 30-day exposures.

The 4 rabbits exposed to 540 p.p.m. died within 2 days, while 2 of the 4 in the control group succumbed within 30 days. At 300 p.p.m. the 9 exposed rabbits died within 7 days. Eight of these animals had catarrhal pneumonic involvement, usually accompanied by moderate damage of the liver. Three of the 8 controls for this concentration also died of pulmonary infection. At 70 p.p.m. 2 of 8 rabbits died within 30 days, compared with 3 of 8 controls.

The mortality caused by pulmonary infection among the rabbits exposed to 70 p.p.m. ethyl acrylate and the control group as well leads us to view these results with extreme caution. Therefore, we do not think that these data based upon the response of a relatively small group of rabbits justify the conclusion that this level is altogether without effect. This belief is substantiated, at least in part, by the brief survival time of rabbits at the concentrations of 300 and 540 p.p.m.

DISCUSSION

The oral LD_{50} of 1.02 gm. per kilogram (1.11 ml. per kilogram) of ethyl acrylate calculated for rats is considerably lower than the LD_{50} of 14.8 ml. per kilogram of ethyl methacrylate and 8.41 ml. per kilogram of methyl methacrylate reported by Deichmann (1). Thus, ethyl acrylate by the oral route is about 8 times as toxic as methyl methacrylate and 13 times as toxic as ethyl methacrylate. Further comparison shows that ethyl acrylate is one-third as toxic as methyl acrylate (4) and 3 times as toxic as ethyl crotonate (11).

The LD_{50} of 1.95 (1.79 to 2.12) ml. per kilogram of ethyl acrylate for rabbits in the skin penetration test is approximately equal to that of methyl acrylate (4). These values cannot be directly compared with the results obtained by Deichmann (1) and Spealman (2), for the methacrylic esters. These authors applied up to 40 ml. per kilogram of the compounds to the shaved belly or back of rabbits and allowed the materials to evaporate freely with no fatal results, whereas in our test the ethyl acrylate was prevented from evaporating by the impervious jacket of "Vinylite" sheeting.

Ethyl acrylate is more toxic than either methyl or ethyl methacrylate, and less toxic than methyl acrylate, for rats in acute inhalation tests. Deichmann observed that rats survived 8-hour exposures of 12.4 mg. per liter (2660 p.p.m.) ethyl methacrylate and 14.3 mg. per liter (3496 p.p.m.) methyl methacrylate (1). Carpenter and Smyth (4) reported earlier that a concentration of 1000 p.p.m. (3.48 mg. per liter) of methyl acrylate killed 3 of 6 rats in 4 hours, and all of 6 rats in 8 hours. It was found in this study that 5 of 6 rats died in a 4-hour exposure to 2000 p.p.m. (8.2 mg. per liter) of ethyl acrylate, and all of 6 rats died in an exposure to 4000 p.p.m. (16.4 mg. per liter) of the ester in the same period.

The toxic action of ethyl acrylate in rats repeatedly exposed to it is primarily that of damage of the lung accompanied by injury of the liver and the kidneys. However, we are aware of the fact that damage of liver and kidney may be found in cases of endemic infection of the lungs. The number of cases of pulmonary infection found in the control rats was negligible as compared with the high incidence in the exposed rats. Furthermore, pronounced congestion and hemorrhage of the lung were found in all victims of the acute inhalation exposures. Therefore, it is probable that the irritating action of the ethyl acrylate vapors increased the susceptibility of the animals to pneumonia. Although some victims exhibited pulmonary damage with no histological changes of the liver and the kidney, no victim showed hepatic or renal damage without the accompanying pulmonary injury.

Histopathological examination of the tissues of the rabbits that died in the repeated vapor exposures revealed the same type of pulmonary injury as was seen in the rats. On the basis of mortality, however, rabbits are more susceptible to ethyl acrylate vapors than rats.

Concentrations of ethyl acrylate as low as 8 p.p.m. may be detected readily by odor. It is tentatively concluded from the results of these experiments that the hygienic standard for repeated human exposure to ethyl acrylate vapors should not be above 50 p.p.m. when toxicity is the criterion. We believe that if a hygienic standard is selected on the basis of objectionable odor a figure below this level would be chosen.

Summary

1. The 14-day oral LD_{50} of ethyl acrylate for rats is 1.02 (0.95 to 1.10) gm. per kilogram. It is more toxic in this respect than methyl and ethyl methacrylate, and ethyl crotonate, and less toxic than methyl acrylate.

2. The LD_{50} of ethyl acrylate for the skin penetration test on rabbits is 1.95 (1.79 to 2.12) ml. per kilogram. It is thus about as toxic as methyl acrylate.

3. Minor capillary injection was produced on the bellies of 2 of 5 rabbits by 0.01 ml. of the undiluted ester. The irritating properties of this compound are comparable to those of ethyl crotonate and dimethyl phthalate.

4. Severe necrosis of the corneas of 5 rabbit eyes was produced by 0.5 ml. of the ester whereas only moderate necrosis was produced by 0.1 ml. Therefore, ethyl acrylate is as irritating to the corneas of rabbits as ethanol and only about one-twentieth as irritating as ethyl crotonate.

5. Concentrations of ethyl acrylate vapors of 4000, 2000, and 1000 p.p.m. killed all of 6, 5 of 6, and none of 6 rats respectively in 4 hours. Saturated vapors which represented a concentration of approximately 50,000 p.p.m. killed all of 6 in 15 minutes and none of 6 rats in 5 minutes. It is more toxic than methyl or ethyl methacrylate and less toxic than methyl acrylate.

6. Rabbits and rats were affected by repeated 30-day exposures to a concentration of ethyl acrylate of 300 p.p.m. The lungs were damaged, and secondary effects were noted in liver and kidney. Rats were not significantly affected by a 30-day exposure to 70 p.p.m. ethyl acrylate. The results obtained on rabbits at this concentration are inconclusive.

7. On the basis of these findings it is tentatively suggested that the hygienic standard for repeated human inhalation of ethyl acrylate vapors should not be above 50 p.p.m.

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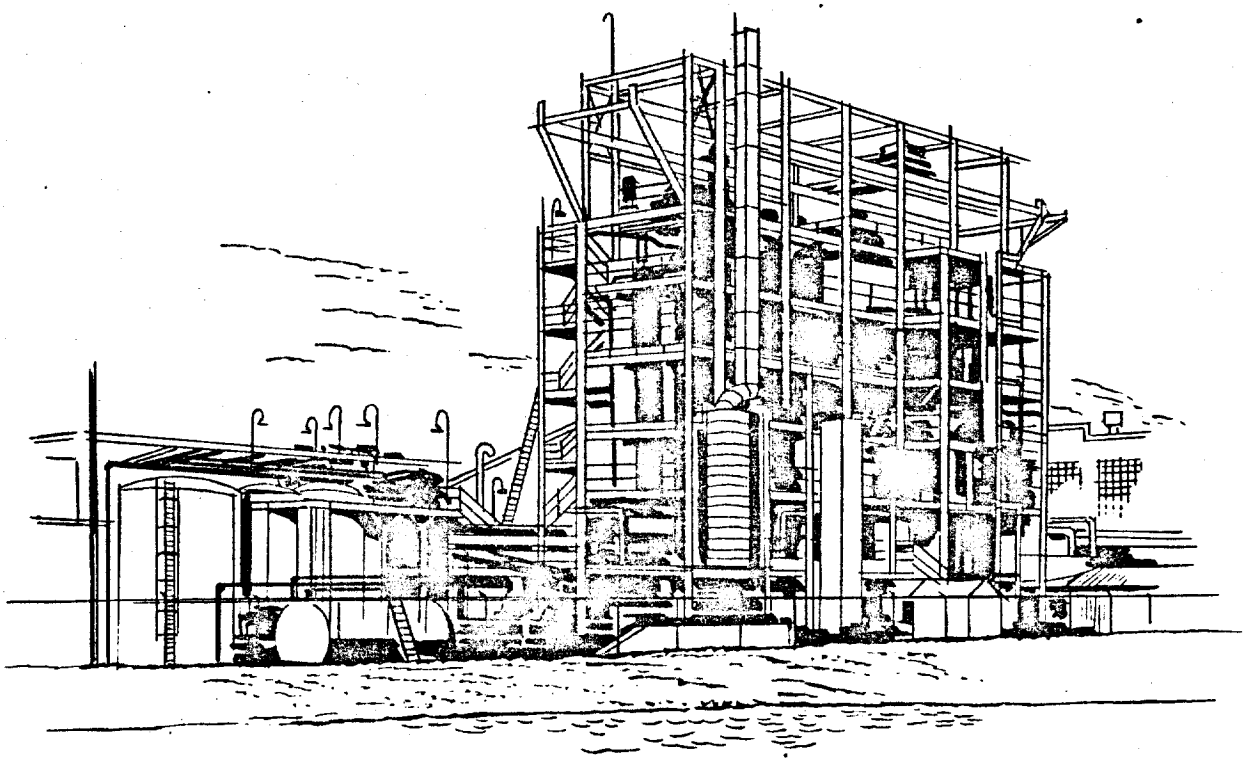

ACRYLIC AND METHACRYLIC MONOMERS

TYPICAL PROPERTIES
AND SPECIFICATIONS

APRIL 1, 1973

ROHM AND HAAS
PHILADELPHIA, PA. 19105

TYPICAL PROPERTIES AND SPECIFICATIONS OF ACRYLIC AND METHACRYLIC MONOMERS

This bulletin presents the finished product specifications that are used by Rohm and Haas Company in the manufacture and quality control of the acrylic and methacrylic monomers. The values that appear here are those in effect at the time of publication; since changes may occur between editions of this bulletin, customers should ascertain the specifications in effect when they purchase these products. The typical properties and analyses accompanying the specifications were determined by our laboratories. The methods used for these measurements are described in detail in our bulletin entitled "Analytical Methods for the Acrylic Monomers" (CM-18).

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These suggestions and data are based on information we believe to be reliable. They are offered in good faith but without guarantee, as conditions and methods of use of our products are beyond our control. We recommend that the prospective user determine the suitability of our materials and suggestions before adopting them on a commercial scale.

Suggestions for uses of our products or the inclusion of descriptive material from patents and the citation of specific patents in this publication should not be understood as recommending the use of our products in violation of any patent or as permission or license to use any patents of the Rohm and Haas Company.

I. SPECIFICATIONS AND TYPICAL PROPERTIES

A. ACRYLATE ESTERS

	METHYL ACRYLATE		ETHYL ACRYLATE		BUTYL ACRYLATE	
	Specifications	Typical Properties	Specifications	Typical Properties	Specifications	Typical Properties
1. Assay (Gas Chromatography), %	98.5 min.	98.8	98.5 min.	99.0	99.0 min.	99.2
2. Acidity (as acrylic acid), %	0.009 max.	0.001	0.009 max.	0.001	0.009 max.	0.005
3. Water (by Karl Fischer), %	0.15 max.	0.03	0.15 max.	0.03	0.10 max.	0.05
4. Inhibitors*, ppm	10-20 MEHQ	15 MEHQ	10-20 MEHQ	15 MEHQ	5-20 MEHQ	12 MEHQ
	180-220 MEHQ	200 MEHQ	180-220 MEHQ	200 MEHQ	45-60 MEHQ	50 MEHQ
5. Color when shipped (APHA)	10 max.	5	10 max.	5	10 max.	5
6. Refractive Index, n_D^{25}		1.4003		1.4034		1.4160
7. Specific Gravity (25°/15.6°C.)**		0.950		0.917		0.894
8. Flash Point, °F.						
Tag. closed cup		27		48		105
Tag. open cup		50		50		120

*MEHQ - monomethyl ether of hydroquinone
HQ - hydroquinone

**The specific gravity value refers to the density of the monomer at 25°C. compared with the density of water at 15.6°C. It is actually measured with a hydrometer that has been calibrated at 15.6°C.

I. SPECIFICATIONS AND TYPICAL PROPERTIES

A. ACRYLATE ESTERS (Continued)

	ISOBUTYL ACRYLATE		2-ETHYLHEXYL ACRYLATE	
	Specifications	Typical Properties	Specifications	Typical Properties
1. Assay (Gas Chromatography), %	98.5 min.	98.7	99.5 min.	99.5
2. Acidity (as acrylic acid), %	0.01 max.	0.001	0.02 max.	0.005
3. Water (by Karl Fischer), %	0.10 max.	0.03	0.05 max.	0.03
4. Inhibitors, ppm	80-120 MEHQ	100 MEHQ	50-70 MEHQ	50 MEHQ
	10-20 MEHQ	15 MEHQ	90-120 MEHQ	100 MEHQ
			90-120 HQ	100 HQ
5. Color when shipped (APHA)	10 max.	5	10 max.	5
6. Refractive Index, n_D^{25}		1.4115		1.4332
7. Specific Gravity (25°/15.6°C.)		0.883		0.880
8. Flash Point, °F.		107		
Tag. open cup				187
Cleveland open cup				195

I. SPECIFICATIONS AND TYPICAL PROPERTIES

B. METHACRYLATE ESTERS

	METHYL METHACRYLATE		ETHYL METHACRYLATE		BUTYL METHACRYLATE	
	Specifications	Typical Properties	Specifications	Typical Properties	Specifications	Typical Properties
1. Assay (Gas Chromatography), %	99.8 min.	99.9	98.0 min.	98.8	98.5 min.	99.1
2. Acidity (as methacrylic acid), %	0.005 max.	0.003	0.02 max.	nil	0.02 max.	0.002
3. Water (by Karl Fischer), %	0.05 max.	0.03	0.15 max.	0.03	0.10 max.	0.02
4. Inhibitors, ppm	9-12 MEHQ	10 MEHQ	12-18 MEHQ	15 MEHQ	9-15 MEHQ	12 MEHQ
	45-55 MEHQ	50 MEHQ	90-120 HQ	100 HQ	90-120 HQ	100 HQ
	90-110 MEHQ	100 MEHQ				
	22-28 HQ	25 HQ				
	54-66 HQ	60 HQ				
	30-40 BHT*	35 BHT*				
5. Color when shipped (APHA)	10 max.	5	10 max.	5	30 max.	5
6. Refractive Index, n_D^{25}		1.4120		1.4116		1.4220
7. Specific Gravity (25°/15.6°C.)		0.939		0.909		0.889
8. Flash Point, °F.						
Tag. closed cup		50		80		
Tag. open cup		55		70		106
Cleveland open cup				95		150

** BHT – butylated hydroxytoluene*

I. SPECIFICATIONS AND TYPICAL PROPERTIES

B. METHACRYLATE ESTERS (Continued)

	ISOBUTYL METHACRYLATE		ISODECYL METHACRYLATE		TRIDECYL METHACRYLATE	
	Specifications	Typical Properties	Specifications	Typical Properties	Specifications	Typical Properties
1. Assay (Gas Chromatography), %	98.5 min.	98.7	97.5 min. (Sap. No.)	97.5	97.5 min.	98.5
2. Acidity (as methacrylic acid), %	0.02 max.	0.002	0.02 max.	0.004	0.01 max.	0.004
3. Water (by Karl Fischer), %	0.10 max.	0.05	0.25 max.	0.25	0.20 max.	0.06
4. Inhibitors, ppm	8-15 MHEQ	10 MEHQ	90-120 HQ	100 HQ	80-150 HQ	100 HQ
5. Color when shipped (APHA)	30 max.	5	200 max.	60	100 max.	100
6. Refractive Index, n_D^{25}		1.4170		1.4410		1.4455
7. Specific Gravity (25°/15.6°C.)		0.883		0.878		0.871
8. Flash Point, °F.						
Tag. open cup		120				
Cleveland open cup				250		280

I. SPECIFICATIONS AND TYPICAL PROPERTIES

B. METHACRYLATE ESTERS (Continued)

	LAURYL METHACRYLATE		STEARYL METHACRYLATE	
	Specifications	Typical Properties	Specifications	Typical Properties
1. Assay (Gas Chromatography) Main Constituents, %	C_{12} Methacrylate		C_{16} Methacrylate	
	62.0 min.	66	25.0 min.	30.0
	C_{14} Methacrylate		C_{18} Methacrylate	
	22.0 min.	25	57.0 min.	60.0
2. Acidity (as methacrylic acid), %	0.02 max.	0.005	0.02 max.	0.01
3. Water (by Karl Fischer), %	0.20 max.	0.09	0.20 max.	0.09
4. Inhibitor, ppm	90-120 HQ	100 HQ	90-120 HQ	100 HQ
5. Color when shipped (APHA)	100 max.	50	200 max.	100
6. Refractive Index, n_D^{25}		1.444		1.4502
7. Freezing Point, °F.		-8		58
8. Specific Gravity (25°/15.6°C.)		0.868		0.864
9. Flash Point, °F. Cleveland open cup		270		>300

I. SPECIFICATIONS AND TYPICAL PROPERTIES

B. METHACRYLATE ESTERS (Continued)

	1,3-BUTYLENE DIMETHACRYLATE (MONOMER X-970)		TRIMETHYLOLPROPANE TRIMETHACRYLATE (MONOMER X-980)	
	Specifications	Typical Properties	Specifications	Typical Properties
1. Assay (Gas Chromatography), %	90.0 min.	90.0	Triester 89.0 min. Diester 7.0 max.	95.0
2. Methacrylic Acid, %	0.14 max.	0.10	0.10 max.	nil
3. Water (by Karl Fischer), %	0.40 max.	0.25	—	<0.2
4. Inhibitors, ppm	90-110 MEHQ	100 MEHQ	80-100 HQ	90 HQ
5. Color when shipped (APHA)	100 max.	75	200 max.	150
6. Refractive Index, n_D^{25}		1.4502		1.471

I. SPECIFICATIONS AND TYPICAL PROPERTIES

C. FUNCTIONAL GROUP METHACRYLATE ESTERS

	2-HYDROXYETHYL METHACRYLATE (94%)	HYDROXYPROPYL METHACRYLATE (94%)
	Typical Properties	Typical Properties
1. Hydroxyalkyl Methacrylate, %	86	87
2. Higher Methacrylates (mainly hydroxyalkoxyalkyl), %	6	5
3. Methacrylic Acid, %	3.5	4.5
4. Dimethacrylate, %	1.5	0.7
5. Inhibitor, ppm	1200 MEHQ	1200 MEHQ
6. Color when shipped (APHA)	150	100
7. Refractive Index, n_D^{25}	1.4498	1.4447
8. Specific Gravity, (25°/15.6°C)	1.069	1.026
9. Freezing Point, °F.	<-40	<-40
10. Flash Point, °F. Tag. Open Cup	231	220

	DIMETHYLAMINOETHYL METHACRYLATE		t-BUTYLAMINOETHYL METHACRYLATE	
	Specifications	Typical Properties	Specifications	Typical Properties
1. Assay (Gas Chromatography), %	97.5 min.	98.0	97.5 min.	98.0
2. Water (by Karl Fischer), %	0.2 max.	0.1	0.25 max.	0.1
3. Inhibitors, ppm	1750-2250 MEHQ	2000 MEHQ	850-1350 MEHQ	1000 MEHQ
4. Color when shipped (APHA)	50 max.	25	50 max.	25
5. Refractive Index, n_D^{25}	1.4372-1.4382	1.4376	1.4395-1.4405	1.4400

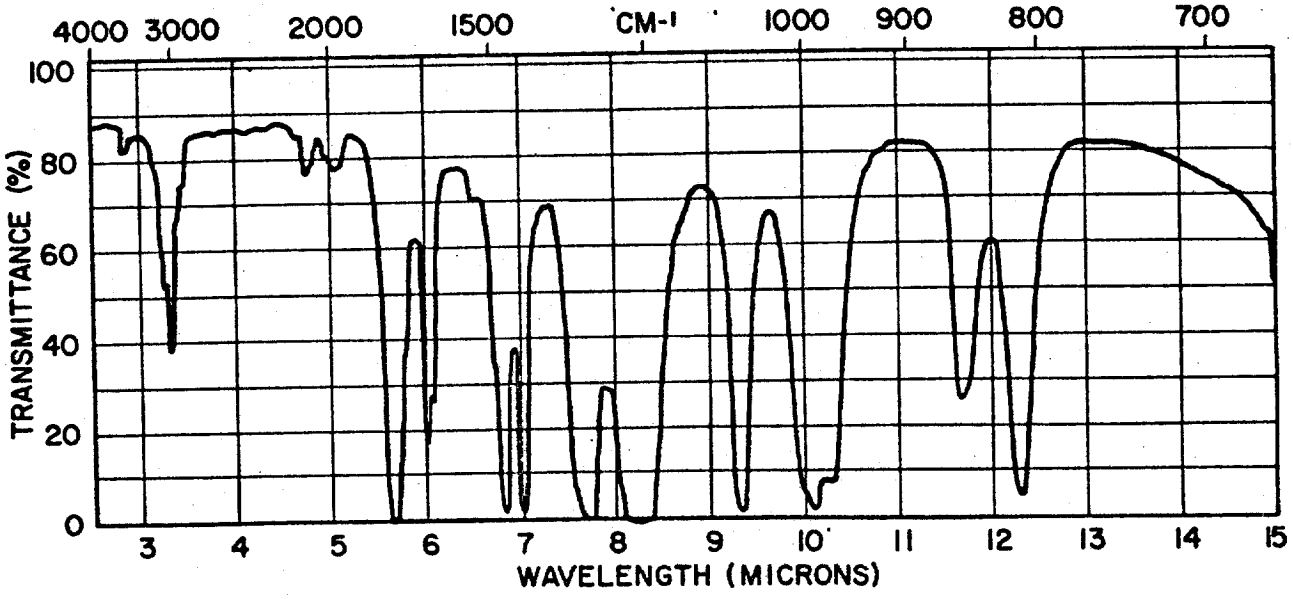
I. SPECIFICATIONS AND TYPICAL PROPERTIES

D. METHACRYLIC AND ACRYLIC ACIDS

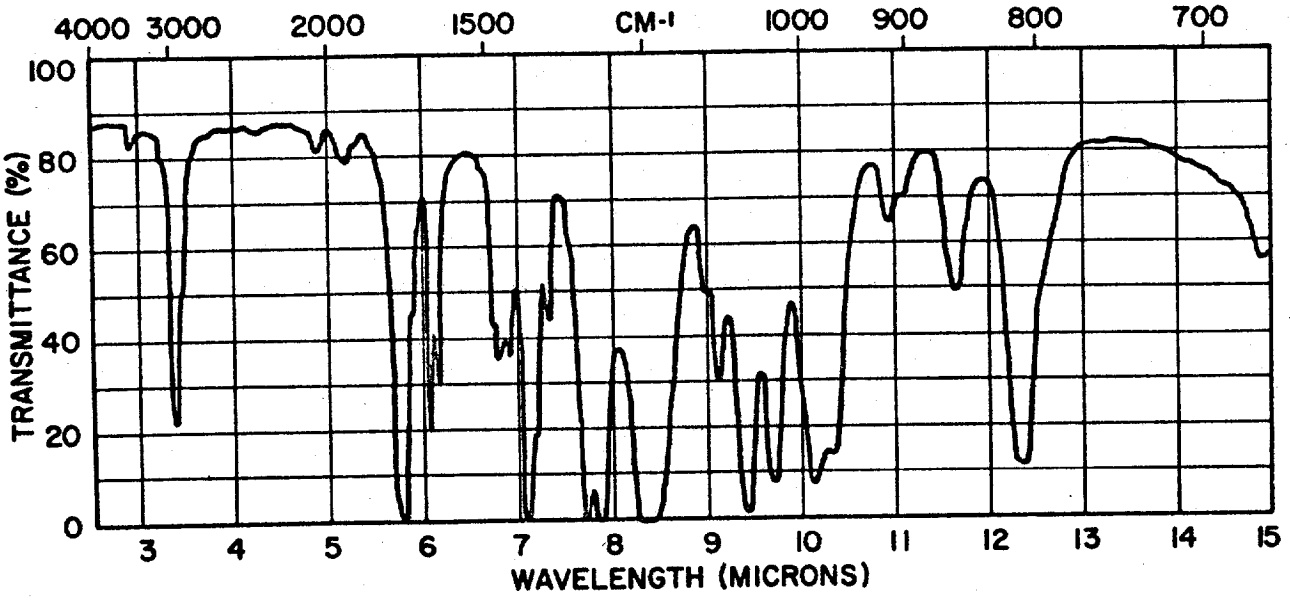
	GLACIAL METHACRYLIC ACID		GLACIAL ACRYLIC ACID	
	Specifications	Typical Properties	Specifications	Typical Properties
1. Assay (Titration), %	98.5 min.	99.4	98.0 min.	98.8
2. Water (by Karl Fischer), %	0.30 max.	0.14	0.20 max.	0.12
3. Inhibitors, ppm	90-110 MEHQ	100 MEHQ		
	225-275 MEHQ	250 MEHQ	180-220 MEHQ	200 MEHQ
4. Color when shipped (APHA)	25 max.	5	25 max.	5
5. Refractive Index, n_D^{25}		1.4288		1.4185
6. Freezing Point, °C.		14		13
°F.		57		55
7. Specific Gravity (25°/15.6°C.)		1.015		1.045
8. Flash Point, °F. Cleveland open cup		170		155
9. Viscosity, kinematic (centistokes at 25°C.)		1.30		1.10

II. MONOMER SPECTRA

INFRARED SPECTRA



METHYL ACRYLATE



ETHYL ACRYLATE

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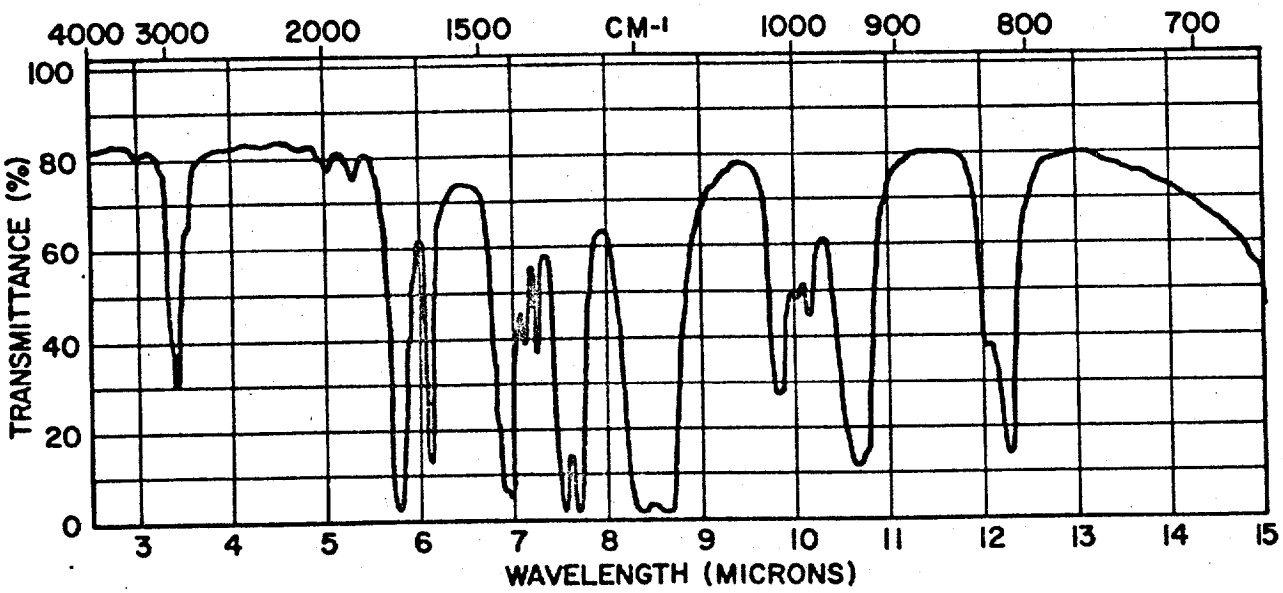
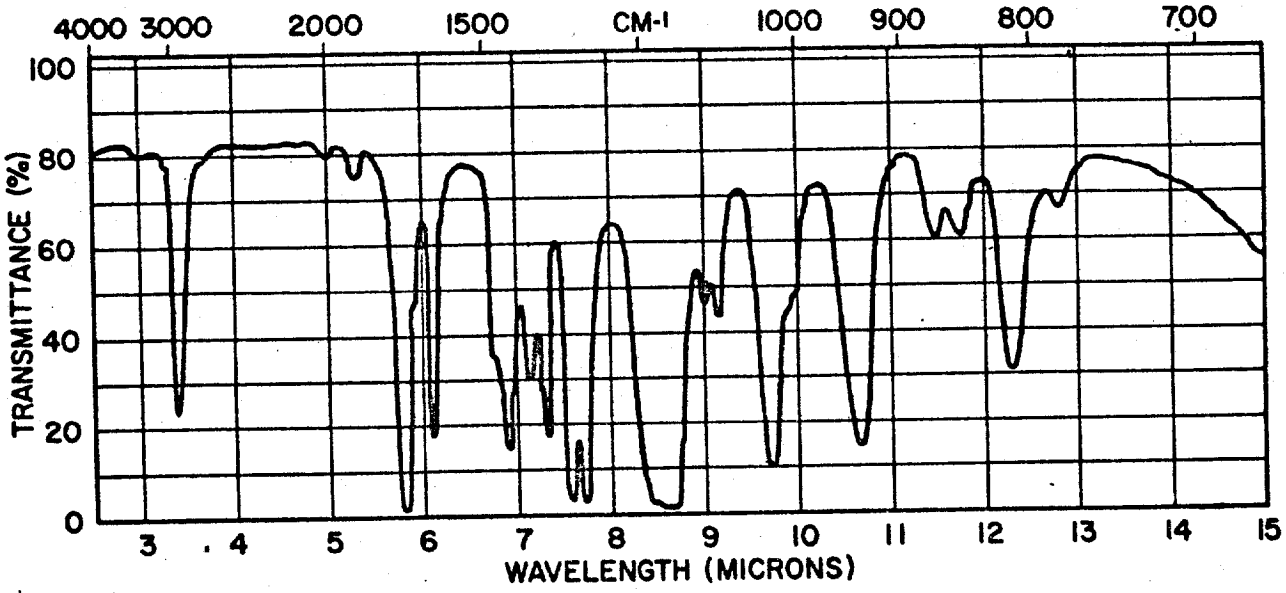
II. MONOMER SPECTRA (Continued)

INFRARED SPECTRA



METHYL METHACRYLATE



ETHYL METHACRYLATE

For ultraviolet spectra of methyl acrylate, butyl acrylate, methyl methacrylate, ethyl methacrylate, and butyl methacrylate, see H. E. Ungnade and I. Ortega, J. Am. Chem. Soc. 73, 1564 (1951).

III. ADDITIONAL PHYSICAL PROPERTIES

TABLE I
VAPOR PRESSURE – TEMPERATURE MEASUREMENTS

Temperature (°C.)	Vapor Pressure, in mm.				
	Methyl Acrylate	Ethyl Acrylate	Butyl Acrylate	2-Ethylhexyl Acrylate	Methyl Methacrylate
-20°C.	6.4	2.3	-	-	2.0
-10	12.9	4.6	-	-	4.3
0	23.4	8.7	1.1	-	8.5
10	40.5	16.5	2.1	-	16.0
20	68.2	29.5	4.0	-	29.0
30	109	50.5	7.3	-	50.0
40	170	82.0	12.8	-	81.0
50	255	126	21.3	1.0	125
60	372	190	34.0	1.9	189
70	530	280	53.5	3.3	279
80	-	400	81.5	5.6	397
90	-	560	120	9.2	547
100	-	-	173	14.8	-
110	-	-	246	23.0	-
120	-	-	342	34.7	-
130	-	-	464	51.0	-
140	-	-	617	75.5	-
150	-	-	-	108	-
160	-	-	-	150	-
170	-	-	-	210	-
180	-	-	-	280	-
190	-	-	-	375	-
200	-	-	-	492	-
210	-	-	-	640	-

TABLE II
THERMODYNAMIC PROPERTIES

	Methyl Acrylate	Ethyl Acrylate	Butyl Acrylate	2-Ethylhexyl Acrylate	Methyl Methacrylate	Ethyl Methacrylate
Latent heat of vaporization kcal./mole	7.9±0.4	8.3±0.4	9.1±0.5	10.3±0.5	8.6±0.4	9.5±0.5
Heat of sublimation kcal./mole	-	-	-	-	14.5	-
Heat of polymerization kcal./mole	18.8	18.6	18.5	14.5	13.8	13.8
Specific Heat cal./g./°C.	0.48	0.47	0.46	0.46	0.45	0.45

III. ADDITIONAL PHYSICAL PROPERTIES (Continued)

TABLE III

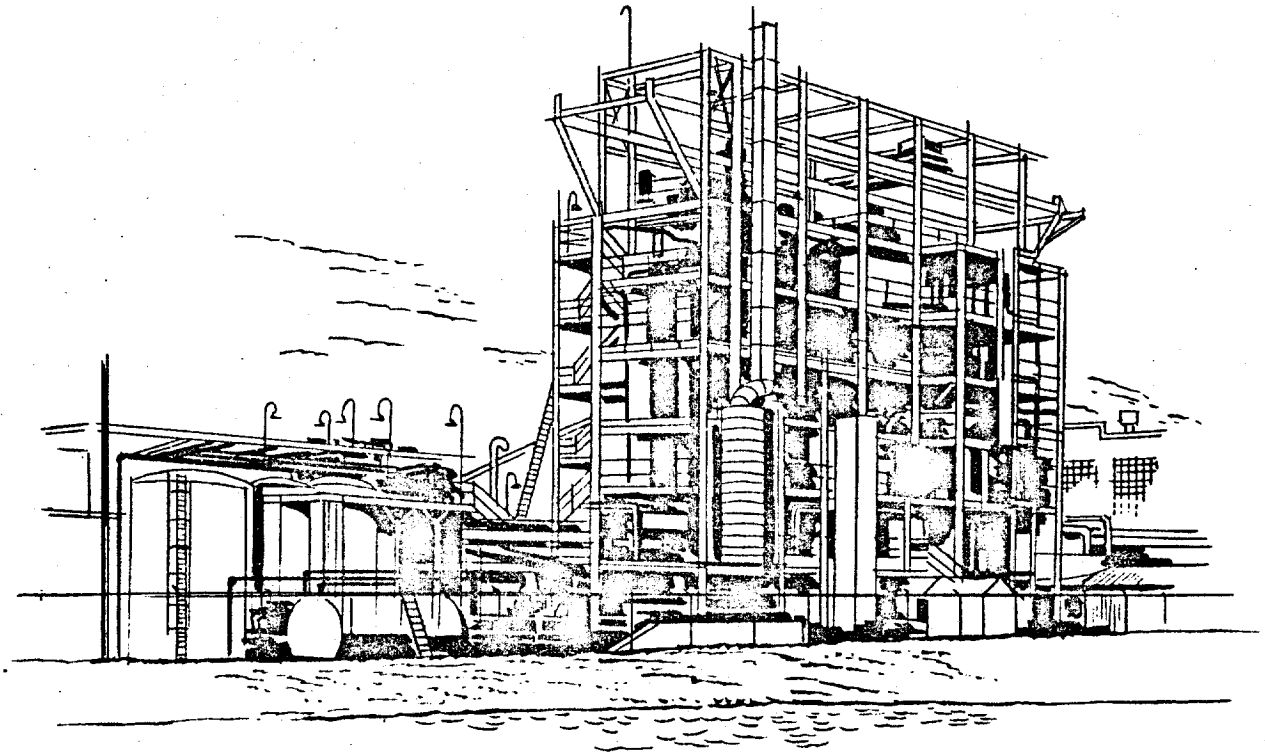
MUTUAL SOLUBILITY OF MONOMER AND WATER AT VARIOUS TEMPERATURES

Temperature (°C.)	Solubility (g./100g.)					
	Methyl Acrylate		Methyl Methacrylate		Ethyl Acrylate	
	Monomer in Water	Water in Monomer	Monomer in Water	Water in Monomer	Monomer in Water	Water in Monomer
0	-	1.57	1.85	0.85	-	-
10	-	1.94	1.72	0.99	-	-
20	-	2.32	1.59	1.15	-	-
25	5.2	-	-	-	1.51	1.51
30	5.2	2.72	1.50	1.34	-	-
40	5.2	3.18	1.43	1.56	-	-
50	5.2	3.70	1.43	1.80	-	-
60	5.2	4.39	1.49	2.07	-	-
70	5.2	5.2	1.60	2.38	-	-
80	-	-	1.80	2.74	-	-

TABLE IV

SOLVENT – MONOMER AZEOTROPES

Solvent	Methyl Acrylate	Ethyl Acrylate	Butyl Acrylate	Methyl Methacrylate
Water				
Boiling Point, °C./760 mm.	71	81.1	94.5	83
Water content, %	7.2	15	40	14
Boiling Point, °C./200 mm.	-	-	47.8/100	49
Water content, %	-	-	41	11.6
Methanol				
Boiling Point, °C./760 mm.	62.5	64.5	-	64.2
Methanol content, %	54	84.4	-	84.5
Boiling Point, °C./200 mm.	-	-	-	34.5
Methanol content, %	-	-	-	82
Ethanol				
Boiling Point, °C./760 mm.	73.5	77.5	-	-
Ethanol content, %	42.4	72.7	-	-
Boiling Point, °C./100 mm.	-	32	-	-
Ethanol content, %	-	54.4	-	-
n-Propanol				
Boiling Point, °C./760 mm.	79	-	-	-
n-Propanol content, %	5.4	-	-	-
2-Propanol				
Boiling Point, °C./760 mm.	76	-	-	-
2-Propanol content, %	46.5	-	-	-



ANALYTICAL METHODS FOR THE ACRYLIC MONOMERS

ROHM AND HAAS

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ANALYTICAL METHODS FOR THE ACRYLIC MONOMERS

A. ASSAY OF MONOMER CONTENT

I. GAS LIQUID CHROMATOGRAPHY (GLC)

This GLC method may be used for methyl or ethyl acrylate or methyl or ethyl methacrylate; it can be adapted to the analysis of other acrylic and methacrylic esters.

Apparatus

Perkin-Elmer Vapor Fractometer, Model 154-C, or equivalent instrument, with a thermistor detector and 0-5 mv recorder.

Column of stainless steel or aluminum tubing, 1/4" O.D., 20 feet long, packed with 20% UCON 50 HB -2000 on 40-60 mesh firebrick.

Microsyringe, 50 μl, Hamilton miniature #705, fitted with B-D Yale No. 27 5/8-inch needle.

Operating Conditions

Column Temperature	100 ± 5°C.
Carrier Gas	Helium
Input Pressure	20 psig
Flow Rate	60 ml/min
Detector Voltage	8.0 v
Sample Size	10 μl
Chart Speed	30 inches/hr

Procedure

1. Cleaning Injection Equipment. Draw acetone through the syringe and the needle separately with an aspirator until each is thoroughly flushed. Dry with a stream of dried compressed air. Wash the plunger with acetone and wipe dry with a clean tissue. Insert the plunger into the syringe, but do not attach the needle as yet.

2. Sampling Technique. Draw a sample from a container into the syringe by slowly withdrawing the plunger. To remove air bubbles, hold the syringe in a horizontal position, remove the plunger slowly, gently incline the syringe backwards, and reinsert the plunger when a solid column of liquid forms at the back end of the syringe. With the syringe in the same position, attach the needle, and push the plunger carefully into the barrel of the

syringe until liquid appears at the needle tip. Invert the syringe to normal position, and depress the plunger slowly until the meniscus is at 10 μl. Remove excess liquid adhering to the needle tip by pressing it rapidly against the glass sample container or the fingers. Do not use a tissue or other absorbent substance.

3. Injection of Sample. Align the syringe, force the needle through the septum, inject the sample, and withdraw the syringe. This operation should be performed in the minimum possible time, not more than two seconds. It is convenient to inject the sample at the same time that the pen of the recorder passes over a major axis line on the chart paper; elution times can then be read easily.

4. The order of elution, typical retention times, and retention times with respect to methyl methacrylate (relative retention time = 1.00) are listed below. Values for the major component are indicated in italic type.

Retention Times, minutes

Material	Methyl Acrylate		Ethyl Acrylate		Methyl Methacrylate		Ethyl Methacrylate	
	Typical	Relative	Typical	Relative	Typical	Relative	Typical	Relative
Acetone	7.90	0.34	7.90	0.34	7.90	0.34	7.90	0.34
Methanol	9.75	0.42			9.75	0.42		
Ethanol			11.55	0.50			11.55	0.50
Methyl Acrylate	*14.15*	*0.61*	14.95	0.65	14.95	0.65	14.95	0.65
Ethyl Acrylate	21.35	0.92	*21.15*	*0.92*	21.40	0.92	21.30	0.92
Methyl Methacrylate	23.10	1.00	23.00	1.00	*23.15*	*1.00*	23.00	1.00
Ethyl Methacrylate	31.70	1.4	31.70	1.4	31.70	1.4	*32.05*	*1.4*

5. Calculations. Compare sample runs to synthetic samples of about the same, known composition. Correction must first be made for variations in the size of the sample. The peak height of the major component MC is the measure of sample size. The peak height of the major component (MC) in the synthetic sample is considered constant.

a. Compute a factor to adjust the peak height of MC in the test sample to the peak height of MC in the synthetic sample:

$$\text{Factor} = \frac{\text{peak height of MC in synthetic sample}}{\text{peak height of MC in test sample}}$$

b. Draw a baseline wherever a peak appears on the chart. Measure the peak height above each baseline in centimeters or blocks. Multiply each peak height by the above factor to obtain an adjusted value. For trace components, little or no correction occurs if the sample size is constant within ± 2 percent.

c. Compare the adjusted peak height of each component to the corresponding peak in the synthetic sample. If an unexpected peak appears, calculate the concentration of the material by using the height of the nearest peak in the synthetic sample as a standard. Calculate the concentration of each minor component from the equation.

$$\text{weight } \% = \frac{\text{\% component in synthetic sample X adjusted peak height in test sample}}{\text{peak height in synthetic sample}}$$

d. To calculate the assay of MC, subtract from 100 the sum of the percent concentrations of minor components (GLC), the water content (Karl Fischer), the acidity (titration), and the inhibitor content:

assay, % = 100 - [% minor components + % water + % acidity + % inhibitor]

Water and acidity are not normally determined by GLC methods. Operating instructions for the Perkin-Elmer Vapor Fractometer are supplied by the Perkin-Elmer Co., Instrument Division, 702 Main Street, Norwalk, Conn. 06852. Information on other instruments is available from the manufacturers of laboratory supply houses.

2. PYRIDINE SULFATE DIBROMIDE (PSDB) METHOD

SCOPE AND METHOD

A solution of pyridine sulfate dibromide ($C_5H_5N.H_2SO_4.Br_2$) in glacial acetic acid is the bromine-addition reagent with mercuric acetate as the catalyst. The excess reagent is measured by adding potassium iodide and titrating the liberated iodine with sodium thiosulfate.

This method has general applicability to the determination of the purity of monomeric acrylic and methacrylic acids and esters by addition of bromine to the double bond. It is especially advantageous for the analysis of the higher members of the series which are insoluble in aqueous acetic acid and therefore give erratic results by methods employing an aqueous bromination agent. Moreover, the PSDB reagent brominates both acrylates and methacrylates rapidly, completely, and impartially; only a small excess of reagent is required and no special conditions are needed for either group of esters. The method is capable of very accurate results, but serious errors may occur if execution is not in strict accordance with the directions and explanatory notes given below.

REAGENTS

Acetic Acid, Glacial, Reagent Grade: must pass the following dichromate test.

Add 10 ml. of the glacial acid to a solution of 0.020 g. of potassium dichromate in 10 ml. of concentrated sulfuric acid cooled to 15-20°C., cooling again if necessary, and let stand at room temperature. No green color should develop in 16 hours.

To minimize the possibility of getting painful blisters on the hands, it is convenient to dispense glacial acetic acid from a device like the Ace Glass Co. Automatic Buret No. 5770 with an adapter to fit it to a 2.5 liter acid bottle. Connections to apparatus used for handling acetic acid or solutions in acetic acid should be polyethylene tubing.

Mercuric Acetate, 2.5% solution (W/V) in glacial acetic acid

Because of the reported drop in catalytic activity on standing, this solution should be prepared fresh and kept no more than a week to avoid any uncertainty about its effectiveness.

Pyridine Sulfate Dibromide, 0.1*N* solution in acetic acid.

Pour about 40 ml. of glacial acetic acid into each of three 250-ml. Erlenmeyer flasks. To one flask add 16.0 ± 0.2 g. of reagent pyridine with cooling and to another add 20.0 ± 2.0 g. of concentrated sulfuric acid, also with cooling. Mix these solutions and cool again. To the third flask add 16.0 ± 0.2 g. of bromine, pour this solution into the above mixture, and dilute to about 2000 ml. with glacial acetic acid to obtain a solution approximately 0.1*N* in available bromine.

This reagent must be prepared with great care in a well-ventilated fume hood. Each ingredient as well as the reagent itself must be regarded as hazardous. The solution is acceptably stable if it is protected from actinic light by storage in a colored glass container. The most suitable apparatus for storing and dispensing it is a gravity-filled 50-ml. buret and 2000-ml. reservoir, both of amber glass, Catalog No. 5753, Ace Glass Co., Vineland, N. J. Stopcocks should be lubricated with Sisco 3000 Stopcock Grease supplied by the Swedish Iron and Steel Co., 910 Westfield Ave., Elizabeth, N. J.

Potassium Iodide, 30% (W/V) aqueous solution

Sodium Thiosulfate, 0.1*N* solution: prepare and standardize by any acceptable method, preferably an iodometric procedure against potassium dichromate.

Starch Indicator, 1% aqueous solution

PROCEDURE

Important: This analysis must not be conducted in bright light but should be done under conditions of diffused light; see the comments below. Other provisions of the comments should also be observed.

Since the weight of sample ultimately taken for analysis should be between 0.15 and 0.50 g., depending on the molecular weight, it is best to prepare a stock solution containing a convenient amount of monomer dissolved in acetic acid. An aliquot of the stock solution is then used for the determination. The quantity of sample monomer put into the stock solution may be measured volumetrically, but it is preferable to weigh it in the interests of greater accuracy. Table I furnishes a guide for determining the sample size used in the preparation of the stock solution.

TABLE I

Monomer	Sample Vol., ml.	Monomer	Sample Vol., ml.
Acrylic Acid	1.5	Isobutyl Methacrylate	3.0
Methacrylic Acid	1.5	Isodecyl Methacrylate	5.0
Methyl Acrylate	1.5	Lauryl Methacrylate	5.0
Ethyl Acrylate	2.0	Tridecyl Methacrylate	5.0
Butyl Acrylate	2.0	Stearyl Methacrylate	5.0
Isobutyl Acrylate	2.0	1,3-Butylene Dimethacrylate	2.0
2-Ethylhexyl Acrylate	3.0	Trimethylolpropane Trimethacrylate	2.0
Methyl Methacrylate	2.0	2-Hydroxyethyl Methacrylate	2.0
Ethyl Methacrylate	2.0	Hydroxypropyl Methacrylate	3.0
Butyl Methacrylate	3.0	Dimethylaminoethyl Methacrylate	3.0
		t-Butylaminoethyl Methacrylate	4.0

To a 100-ml. volumetric flask add about 20 ml. of glacial acetic acid. Wipe dry the stopper area, stopper the flask, and weigh it on an analytical balance. Without delay, carefully pipet the designated volume of sample into the flask. Restopper the flask and reweigh. Fill to the 100-ml. mark with glacial acetic acid and mix well to give the stock solution of monomer.

Before starting the analysis, wrap each of the iodine flasks in a 18 x 18 inch square of heavy aluminum foil and prepare a 6 x 6 inch square of foil to be used for wrapping the stopper and neck after the introduction of the reaction mixture.

Into a 500-ml. foil-wrapped iodine flask, measure accurately 50 ml. of PSDB reagent. *Immediately* pipet in a 10-ml. aliquot of the sample stock solution. From a graduate cylinder *quickly* add 25 ml. of mercuric acetate solution in such a way that the ground-glass portion of the neck is thoroughly wetted. *Immediately* stopper the flask, wrap the stopper and neck with foil, mix by swirling and set aside for exactly 30 minutes.

Remove the foil covering the neck and stopper. *Quickly and immediately* fill the gutter of the flask with 30% potassium iodide solution and admit to the flask by loosening and partially removing the stopper from the flask. Restopper tightly and swirl for about 30 seconds. Add about 200 ml. of water to the flask, carefully washing down the stopper in so doing. After removing the foil, titrate with 0.1*N* sodium thiosulfate, adding 1-2 ml. of starch indicator near the end point. Call this titer A (ml. of *N* normal thiosulfate). Run a blank simultaneously according to the above procedure, but substitute 10 ml. of glacial acetic acid for the aliquot of stock solution. Call this titer B.

Calculation

$$\% \text{ Monomer (by weight)} = \frac{(B-A) \times N \times M \times 100}{\text{Wt. of sample in 10 ml. aliquot}}$$

The values of M, the milliequivalent weight of the monomer, are:

glacial acrylic acid	0.0360	isobutyl methacrylate	0.0711
glacial methacrylic acid	0.0430	isodecyl methacrylate	0.113
methyl acrylate	0.0430	lauryl methacrylate	0.131
ethyl acrylate	0.0500	tridecyl methacrylate	0.134
n-butyl acrylate	0.0641	stearyl methacrylate	0.166
isobutyl acrylate	0.0641	1,3-butylene dimethacrylate	0.0566
2-ethylhexyl acrylate	0.0921	trimethylolpropane trimethacrylate	0.0564
methyl methacrylate	0.0500	2-hydroxyethyl methacrylate	0.0656
ethyl methacrylate	0.0570	hydroxypropyl methacrylate	0.0721
n-butyl methacrylate	0.0711	dimethylaminoethyl methacrylate	0.0786
		t-butylaminoethyl methacrylate	0.0926

Comments. In the absence of mercuric acetate, the reaction between monomer and PSDB reagent is comparatively slow even in bright sunlight. When catalyst is present, however, the addition of bromine to the olefinic double bond of the monomer is very rapid even in the dark. Furthermore, the catalyst promotes substitution of hydrogen by bromine. This side reaction tends to give low results because it usually affects the blank more than the sample. Strong light, whether sunlight or artificial, must therefore be excluded from the reaction flasks during the entire course of the bromination until admittance of potassium iodide. Wrapping the flasks in aluminum foil is a convenient way to exclude light; the foil is removed during the final titration so that the end point may be observed.

Operations should be carried out as rapidly as possible to insure accurate results. When preparing the sample stock solution, for example, the high vapor pressure of acetic acid requires that the flask be unstoppered as briefly as possible during the addition of the sample to avoid an erroneous sample weight. Once the PSDB reagent has been added,

subsequent steps up to and including the capping with foil should be performed quickly and without delay. It is also necessary to measure and use immediately each individual portion of PSDB solution as needed. Do not measure beforehand all portions required in a series of determinations and then add the aliquots of stock solution. Finally, after the bromination is finished and the foil cap has been removed, potassium iodide must be added and mixed at once.

The effects of temperature must also be kept in mind. Substitution of hydrogen by bromine is accelerated by increase in temperature. In the dark, substitution is negligible at 15°C., slight at 25°, but becomes significant at 35°C. Since the PSDB reagent and the monomer stock solution consist mainly of acetic acid, which has a high coefficient of expansion, care should be taken to guard against the effect of temperature changes. It is best to run these analyses at about 20° in a constant temperature room illuminated entirely by artificial light of moderate intensity. The analyst should work several feet from the nearest source of light.

It is advisable to run this analysis in duplicate and to report results based on two sample aliquots and two blanks.

3. SAPONIFICATION NUMBER

SCOPE AND METHOD

The saponification number is obtained by reacting the sample with a measured excess of 0.5*N* alcoholic potassium hydroxide and then determining the amount of potassium hydroxide consumed by titrating the excess with standard aqueous acid. The saponification number is defined as the milligrams of potassium hydroxide required for the saponification and neutralization of one gram of sample.

REAGENTS

Alcoholic Potassium Hydroxide, 0.5*N*.

Dissolve 18 g. of silver nitrate in 12 ml. of deionized water. Pour the solution into 6,000 ml. of 2B alcohol and mix well. Dissolve 18 g. of reagent potassium hydroxide in 60 ml. of warm 2B alcohol and cool the solution. Slowly add the alcoholic alkali to the solution of silver nitrate with constant stirring. Allow the precipitate to settle and decant the clear solution through coarse filter paper. Add 170 g. of reagent potassium hydroxide to the filtrate, mix well, and let stand overnight. Filter through cotton before using the solution.

Phenolphthalein Indicator, 1% solution in alcohol.

Aqueous Hydrochloric Acid, 0.5*N*

Neutralized Alcohol

Add 1 ml. of phenolphthalein indicator solution to 1 liter of 2B alcohol and add 0.1*N* sodium hydroxide dropwise to a slightly pink color.

PROCEDURE

Blank Determination

Blank determinations should be made in triplicate at the same time and in the same manner as sample determinations. The flasks intended for use with blanks should be so marked

and are then interspersed among the sample flasks. If the total amount of the three blanks exceeds 0.45 ml., one value may be discarded and the other two averaged if they do not differ by more than 0.15 ml. If the total amount is 0.45 ml. or less, average the three values. If either of these two requirements is not met, the entire set of determinations for which these titrations represent the blanks should be discarded.

Sample Determination

All samples are analyzed in duplicate. Table II indicates the approximate weight of sample taken and lists the formula weights and saponification numbers of Rohm and Haas acrylates and methacrylates.

TABLE II

Ester	MW	SN	Sample Weight, g.
Methyl Acrylate	86.1	652	1.0
Ethyl Acrylate	100.1	560	1.2
n-Butyl Acrylate	128.2	438	1.4
Isobutyl Acrylate	128.2	438	1.4
2-Ethylhexyl Acrylate	184.3	305	1.7
Methyl Methacrylate	100.1	560	1.2
Ethyl Methacrylate	114.1	491	1.4
n-Butyl Methacrylate	142.2	395	1.7
Isobutyl Methacrylate	142.2	395	1.7
Isodecyl Methacrylate	226	248	2.0
Lauryl Methacrylate	262	214	2.5
Tridecyl Methacrylate	268	209	2.5
Stearyl Methacrylate	332	169	3.5
1,3-Butylene Dimethacrylate	226.2	496	1.4
Trimethylolpropane Trimethacrylate	338.4	497	2.0
2-Hydroxyethyl Methacrylate	130.1	430	1.4
Hydroxypropyl Methacrylate	144.2	389	1.7
Dimethylaminoethyl Methacrylate	157.2	357	1.7
t-Butylaminoethyl Methacrylate	185.3	303	1.7

The net titration for the sample should not be less than 5 ml. nor greater than 25 ml. For samples with a saponification number less than 500, weighing to 0.5 mg. is adequate; for samples with higher saponification number, weigh to 0.1 mg.

By means of an automatic pipet, transfer 50 ml. of 0.5*N* alcoholic potassium hydroxide into each flask making sure that the same volume is delivered in each case. Weigh by difference the required amount of sample into the proper flask, attach each flask to a reflux condenser, sealing the joint with a few drops of alcohol, and reflux the solutions gently for about 1.5 hours. At the end of the reflux period wash down each condenser with about 25 ml. of neutralized alcohol, remove the flask from the condenser, and rinse the tip and joint of each condenser into the corresponding flask using a stream of neutralized alcohol from a wash bottle. While the reaction mixture is still hot, add a few drops of phenolphthalein indicator and promptly titrate with standard 0.5*N* hydrochloric acid to the disappearance of the pink color. Read the buret to the nearest 0.05 ml.

Calculation:

If the sample requires A ml. and the average blank is B ml. of *N* normal hydrochloric acid,

$$\text{Saponification Number} = \frac{(B-A) \times N \times 56.1}{\text{g. sample}}$$

$$\text{Assay as } \% = \frac{(B-A) \times N \times M \times 100}{\text{g. sample}}$$

where M is milliequivalent weight:

$$M = \frac{\text{formula weight}}{1000 \times \text{number of ester groups}}$$

alternatively,

$$\text{Assay in \%} = \frac{\text{Sap. No.} \times \text{formula weight} \times 100}{56100 \times \text{number of ester groups}}$$

4. CAUSTIC TITRATION OF GLACIAL METHACRYLIC ACID

Using a weighing pipet, screw cap dropping bottle, or similar device, weigh about 2.0 g. of glacial methacrylic acid within 0.1 mg. into an Erlenmeyer flask containing 100 ml. of water and 5 drops of phenolphthalein indicator solution (1% in 50% aqueous 2B alcohol). Titrate immediately with 0.5*N* sodium hydroxide to a light pink end point.

Calculation:

$$\text{\% Methacrylic Acid} = \frac{\text{ml. NaOH} \times N \times 8.61}{\text{wt. of sample}}$$

5. ASSAY OF TOTAL ACID AND DIMER IN GLACIAL ACRYLIC ACID

a. DETERMINATION OF ACRYLOYLOXYPROPIONIC ACID

Glacial acrylic acid dimerizes spontaneously on standing to form acryloyloxypropionic acid. The extent of dimerization depends on the temperature and on the duration of storage. As the dimer content rises, the freezing point is depressed and the assay of acrylic acid is decreased. The dimer content is determined by alkaline saponification of the neutralized acid. This is carried out first and is followed by a determination of total acidity; the acrylic acid content is then obtained by difference.

REAGENTS

4.0*N* Sodium Hydroxide
0.5*N* Sodium Hydroxide
1.0*N* Potassium Hydroxide in Methanol
0.5*N* Hydrochloric Acid
1% Phenolphthalein in 50% aqueous ethanol

APPARATUS

350-ml. Citrate bottles, heat resistant.

Canvas bags (bleached and preshrunk, 8 oz.) with double drawstring, 11" long, 5.5" in diameter, Style #299, supplied by Webb Manufacturing Company, 4th and Cambria Streets, Philadelphia, Pa. 19121.

PROCEDURE

The specific gravity of the sample is determined by any suitable method. Pipet a 15-ml. sample into a heat-resistant pressure bottle and add 5 drops of the phenolphthalein

solution. Slowly add 4.0*N* sodium hydroxide solution until the solution is almost neutralized; about 50 ml. of base is required. Cool repeatedly under tap water during the neutralization. Complete the neutralization with 0.5*N* sodium hydroxide to the appearance of a pale pink end point. Do not be confused by a faint pink color possibly caused by inhibitor.

Carefully pipet 25.0 ml. of 1.0*N* methanolic potassium hydroxide into the sample bottle and into each of two similar bottles containing a volume of distilled water equal to the volume in the sample bottle. Add 5 drops of phenolphthalein solution to the latter bottles which are used for the blank determination.

The stoppered bottles are placed in the canvas bags and heated in a steam bath at 98 ± 2°C. for 30 minutes. The bottles are removed from the bath and allowed to cool to room temperature. Uncap to release pressure, remove from the bags, and wash down the inside walls with distilled water. Titrate with 0.5N hydrochloric acid to the disappearance of the pink color.

Calculation:

$$\frac{\text{meq dimer}}{\text{g. sample}} = C = \frac{(B-A) \times N}{15 \times \text{sp. gr.}}$$

where A = ml. of acid required for sample

B = ml. of acid required for blank

% dimer = C x 14.41

b. GLACIAL ACRYLIC ACID

From a weighing pipet or similar vessel, weigh a sample of 0.65-0.70 g. within 0.1 mg into an Erlenmeyer flask containing 50 ml. of water and 5 drops of phenolphthalein solution. Titrate at once with 0.5*N* sodium hydroxide solution to a light pink end point (titer A).

Calculation:

$$\frac{\text{meq total acidity}}{\text{g. sample}} = D = \frac{A \times N}{\text{wt. sample}}$$

% acrylic acid = (D-C) x 7.206

where C is meq/g. of dimer previously determined

6. DETERMINATION OF NEUTRALIZATION EQUIVALENT

The neutralization equivalent (NE) of aminoalkyl esters is determined by titration with standard acid.

REAGENTS

Alcoholic Hydrochloric Acid, 0.5*N* in ethanol: add 305 ml. of concentrated hydrochloric acid to 7 l. of 95% 2B ethanol.

Bromphenol Blue Solution, 0.1% in 1:1 (V/V) aqueous 95% 2B ethanol.

PROCEDURE

Weigh 1.5 g. within 0.1 mg. into a 250-ml. Erlenmeyer flask containing about 50 ml. of 95% 2B ethanol neutralized to Bromphenol Blue and swirl to dissolve. Add several drops of Bromphenol Blue solution and titrate to a yellow end point with alcoholic hydrochloric acid.

Calculation:

$$NE = \frac{\text{g. sample} \times 1000}{\text{ml.} \times N}$$

$$\text{Assay in \%} = \frac{\text{g. sample} \times 100{,}000}{\text{ml.} \times N \times M}$$

where M is the formula weight of the monomer and N is the exact normality of the alcoholic hydrochloric solution.

B. SPECIFIC GRAVITY

The sample is placed in a large hydrometer jar. The hydrometer of appropriate range, calibrated at 60°F. against water, is inserted in the jar and the temperature is adjusted to 25.0°C. The specific gravity is read at this temperature and is *not* corrected to 60°F.

C. REFRACTIVE INDEX

The refractive index is measured with a Valentine Precision Refractometer, Model 470, or an Abbe refractometer, usually at 25°C., using a sodium vapor lamp (A. H. Thomas Co., Catalog No. 6322-J). The lower end of the scale is checked with freshly distilled water, n_D^{20} 1.333, and the upper end with freshly distilled bromonaphthalene, n_D^{20} 1.658. Intermediate points may be calibrated with ethylene glycol, n_D^{20} 1.4314, p-cymene, n_D^{20} 1.4900, bromobenzene, 1.5598, and iodobenzene, 1.6200.

D. DISTILLATION RANGE

This determination is made according to ASTM D-1078. Stearyl methacrylate has a high melting point and boiling range; in distilling this ester, the upper part of the flask should be insulated with asbestos and warm water should be circulated through the condenser.

E. COLOR

This determination is done according to ASTM D-1209.

F. MELTING POINT AND FREEZING POINT

1. METHACRYLIC ACID

Fill a melting point capillary to a depth of about 3/4-inch with the liquid acid and chill the tube in ice for at least 5 minutes to freeze the sample. Meanwhile place a 600-ml. beaker containing a stirring bar on a magnetic stirrer and fill the beaker within an inch of the top with water *below* 10°C. Attach the chilled capillary to the chilled thermometer with a rubber band fixing the sample alongside the thermometer bulb. Suspend the thermometer in the bath with the immersion mark on the stem about level with the surface of the water. Run the magnetic stirrer at such a speed that effective stirring is produced without giving a vortex large enough to lower the water level below the immersion mark. The stirrer provides sufficient heat to warm the bath slowly. Continue stirring until the sample sinters, then melts completely. The temperature at which fusion is complete is recorded as the melting point.

2. ACRYLIC ACID

Suspend a test tube half filled with a sample of acrylic acid in a larger test tube held in an ice bath. Stir with an A.S.T.M. 90°C. thermometer covering the range 0-30°C. at 77 mm. immersion. The freezing point is the temperature at which the thermometer reading remains constant for at least two minutes while crystals are formed.

G. ACIDITY OF ESTERS (as corresponding acid)

REAGENT

Sodium Hydroxide, 0.01*N*

Bromthymol Blue Indicator

Grind 4 g. of bromthymol blue with 12.8 ml. of 0.5*N* sodium hydroxide and dilute to 10 liters with distilled water.

PROCEDURE

Pipet a 10-ml. sample of ester into an Erlenmeyer flask and add about 10 ml. of water. Titrate with 0.01*N* sodium hydroxide using 3 drops of bromthymol blue indicator to a blue end point. Swirl vigorously during the titration. Express the results as ml. of 0.01*N* alkali per 10 ml. of sample. For deeply colored samples, use a 2-ml. sample and multiply the result by 5.

Calculation

If B ml. of *N* normal alkali is used

$$\% \text{ Acidity} = \frac{B \times N \times M \times 100}{(\text{ml. sample}) \times \text{specific gravity}}$$

M = 0.072 for acrylates

M = 0.086 for methacrylates

Acidity is calculated and reported in acrylates as acrylic acid, in methacrylates as methacrylic acid.

H. DETERMINATION OF WATER BY THE KARL FISCHER METHOD

SCOPE AND METHOD

The Karl Fischer reagent, a solution of iodine, sulfur dioxide, and pyridine in methanol, is the basis of a simple, rapid, accurate, and almost universally applicable method for the determination of any concentration of water in most organic and inorganic compounds. There are certain organic substances which interfere by reaction with a component of the reagent to form water, thereby giving erronously high results. In such cases, preliminary precautions can usually eliminate the interfering reactions by simple and rapid means. However, no remedial techniques are available for a few compounds such as ascorbic acid, quinone, peroxyacids, and diacyl peroxides.

Some minor difficulties are also encountered in using the Karl Fischer reagent. The reagent is a very powerful dessicant. When exposed to moist air, it deteriorates rapidly. It is best dispensed from an all-glass automatic buret assembly filled by gravity or air pressure and fitted with drying tubes to remove moisture from incoming air. The reagent extracts moisture from other dessicants such as calcium chloride, alumina, and Drierite. Drying tubes containing these materials halt the absorption of atmospheric moisture only temporarily. A glass stopcock should therefore be installed in the line between each drying tube and the reservoir; it is kept closed except when the buret is in use. Drying tubes are preferably filled with a mixture of asbestos fibers and phosphorous pentoxide or with granular alumina. These precautions essentially eliminate the rapid but avoidable deterioration caused by entrance of air into the equipment. This loss is comparatively much greater than deterioration caused by side reactions in the reagent itself.

Karl Fischer reagent is more stable if it is not too concentrated. For ordinary use, a strength equivalent to about 2.5 of mg. of water per ml. of reagent is recommended.

Leakage around the stopcocks is largely overcome by using Sisco 300 Stopcock Grease, supplied by Swedish Iron and Steel Co., Elizabeth, N. J. Securing the stopcock with a small Universal Stopcock Adapter obtained from Emil Greiner Co., Catalog #23006, is also helpful.

Solubility considerations cause some compounds to give a false end point with Karl Fischer reagent. This may usually be prevented by dilution with several volumes of dry methanol or other suitable inert solvent before titration.

TITRATION METHODS

DIRECT TITRATION with Karl Fischer reagent is preferred to back-titration of excess reagent with a standard solution of water in methanol.

VISUAL END POINT techniques permit rapid titration with simple apparatus. They require as a minimum only an automatic buret and reservoir to hold and dispense the reagent and an Erlenmeyer titration flask for the sample. They are not easily applicable to colored samples.

ELECTROMETRIC END POINT procedures have more general applicability and greater accuracy for micro quantities. The apparatus is necessarily more complex and the manipulations more cumbersome and time-consuming than those using a visual end point.

APPARATUS

VISUAL END POINT. The following list fulfills the minimum requirements:

All-Glass 2000-ml. Reservoir and Automatic 50-ml. Buret Assembly. Any of the following is satisfactory.

Schilling Type	Ace Glass Co. #3325, Style C Reservoir
Machlett Type	Burrell Corp. #8-525
Automatic Buret	Mathieson Scientific Co. H-6380

125-ml. Erlenmeyer flasks

Manual or magnetic stirring may be used. The tip of the buret is fitted through a #4 rubber stopper; the flask is loosely stoppered yet can be shaken manually if desired during the titration. The reservoir-buret assembly should be fitted with drying tubes.

ELECTROMETRIC END POINT

Electrometric End Point Indicator. An inexpensive simple "home-made" assembly using a microammeter as indicator can be constructed as described by Mitchell and Smith, Aquametry, New York: Interscience Publishers, 1948, p. 94-95. The preferred assembly utilizes a McKinney-Hall electronic circuit having a "magic-eye" to indicate the end point and polarized electrodes; see McKinney and Hall, Annal. Chem. 15, 460 (1943), or Mitchell and Smith, Aquametry, p. 91. Magnetic stirring permits direct titration in a closed system to minimize the effect of atmospheric moisture.

Magnetic Stirrer: A. H. Thomas Co. #9235-S.

Magnetic Stirring Bar, glass covered, 7/8 x 9/32 inch, iron: A. H. Thomas Co. #9235-Ts.

Reservoir-Automatic Buret Assembly, with iron support: Burrell Corp. #8-525.

Stopcock Adapter, Universal, small size - Emil Greiner Co. #23006.

Titration Cell, Karl Fischer type: A. H. Thomas Co. #9682L-70. Individual components may be purchased for replacement.

Cell Cover, Plastic #9682L-75: Has two 5/8-inch holes for the electrodes and two 1/4-inch holes, one for the buret tip and the other for insertion of pipets.

Titration Vessel, 250 ml. capacity, #9682L-72 or preferably
Preserving Jar, 5 oz., #6276, both with #63 thread for attachment to the cell cover.

2 Platinum Electrodes, Beckman, #4937-P or preferably #4925X-40 which is sturdier.

Holder, #3235 B.

Drying Tubes as noted above.

REAGENTS

Sodium Tartrate Dihydrate, $Na_2C_4H_4O_6 \cdot 2H_2O$: Contains 15.6% water. This is used as a standard to determine the water equivalence of Karl Fischer reagent. Its water content can be checked by measuring the loss in weight of a sample dried overnight in an oven at 130°C.

Methanol, Anhydrous: water content should be below 0.05%.

Pyridine, Anhydrous: redistill if necessary to reduce the water content below 0.1%. See Mitchell and Smith, Aquametry, p. 67.

Benzene, Anhydrous.

Sulfur Dioxide, Anhydrous: the liquid is obtained by inverting the cylinder and drawing off the desired volume into a graduated cylinder.

Glacial Acetic Acid

Karl Fischer Reagent: the following directions cover the preparation of about 3.5 liters. WORK IN A HOOD.

To 1345 ml. of pyridine in a 5-liter flask, add 424 g. of iodine crystals in small portions with vigorous swirling. After complete solution of the iodine, add 1800 ml. of anhydrous methanol and mix thoroughly. Stopper the flask loosely and cool in a bath containing a slurry of chopped ice. Draw 225 ml. of liquid sulfur dioxide from the cylinder and add it rapidly but carefully to the cold solution of iodine. As an alternative, 320 g. of gaseous sulfur dioxide may be bubbled slowly into the flask tared on a torsion balance. Stopper loosely, preferably with a drying tube attached to the stopper, and allow the reagent to warm to room temperature. Stopper tightly and let stand a day or two before use.

The quantities given in the directions are not critical. A moderate excess of sulfur dioxide is necessary. The methanol content may be varied to adjust the water factor of the reagent to the desired range.

PROCEDURE

Standardization Against Water

This procedure is preferred when a visual end point is used. Accurately weigh by difference 3 drops of distilled water into a dry 125-ml. Erlenmeyer flask. Titrate at once with Karl Fischer reagent, swirling during the entire operation and adding the reagent dropwise as the end point is approached. At the end point, the color changes from chromate yellow to the reddish brown of iodine. The end point should persist for 10 seconds.

Standardization Against Sodium Tartrate Dihydrate

This is preferred for the electrometric procedure. Having adjusted the apparatus properly, weigh accurately about 0.5 g. of sodium tartrate dihydrate. Transfer the sample to a dry titration vessel containing a stirring bar and attach the vessel to the cell cover. With a pipet add 25 ml. of anhydrous methanol through the proper orifice in the cell cover, then stopper the orifice. Start the magnetic stirrer and add reagent until the tartrate is dissolved and an end point which persists at least 30 seconds is reached; call this titer A (ml. of reagent). In a similar manner run a blank on 25 ml. of methanol and call this titer B.

Calculation:

$$F \text{ (g. of water/ml. of reagent)} = \frac{\text{wt. of standard} \times 0.1565}{(A-B)}$$

Sample Titration

All unnecessary exposure to moist air of the reagent and of hygroscopic samples should be avoided, preferably by titration in a closed system. Water in acrylic monomers may be titrated directly on the sample or immediately after weighing a sample into 25 ml. of a suitable pre-titrated solvent such as anhydrous methanol, pyridine, or acetic acid. When a pre-titrated solvent is used, the first titer is disregarded and only the volume of titrant added to the sample solution is recorded.

Calculation

$$\% \text{ Water} = \frac{\text{ml. of reagent} \times F \times 100}{\text{sample weight}}$$

The sample weight may be obtained by actual weighing or by multiplying the volume of sample in ml. by the specific gravity measured with a hydrometer.

The size of sample depends on the expected water content and may vary from 50 ml. for a monomer containing less than 0.1% water to 0.2 g. for a substance containing 50% water. Materials with high water contents are best handled by taking aliquots of a stock solution prepared by diluting a measured amount of sample to a known volume with anhydrous solvent. Methanol, pyridine, glacial acetic acid, benzene, and others constitute suitable inert solvents. The best solvent for the material should be selected.

I. DETERMINATION OF INHIBITORS

1. HYDROQUINONE (HQ) BY THE IODINE METHOD

SCOPE AND METHOD

Excess iodine is added to a solution of the hydroquinone-containing material. Unconsumed iodine is measured by titration with standard thiosulfate solution. The oxidation with iodine must be carried out in a solution buffered at pH 6.0-6.8 because the reaction is accompanied by liberation of acid and may be completely reversed at pH 2.5. The method is suitable for the determination of hydroquinone in organic materials that do not react with iodine under the conditions of test. It is particularly applicable to the determination of small amounts of hydroquinone (0.0025-0.35%; 25-3500 ppm) inhibitor in acrylic and methacrylic acids and their esters up to and including butyl.

REAGENTS

Sodium Acetate-Acetic Acid Buffer

Dissolve 164 g. of anhydrous sodium acetate or 272 g. of the trihydrate in distilled or deionized water, add 5.0 ml. of acetic acid, and dilute to 1 liter. The pH of this solution is about 6.4.

Sulfuric Acid, approximately 2*N*

Iodine, 0.1*N* solution, containing 2.5% potassium iodide.

Iodine, 0.2*N* solution, prepared from 0.1*N* solution

Sodium Thiosulfate, 0.1*N* solution

Sodium Thiosulfate, 0.2*N* solution

Sodium Carbonate, 25% solution

Potassium Iodide, 10% solution

Methanol, anhydrous

Starch Indicator, 1% aqueous solution

Bromthymol Blue Indicator, 0.04% solution (see Section G)

PROCEDURE

Three variations of the procedure are given and the choice is governed by the identity and HQ content of the test monomer.

Rohm and Haas acrylic monomers need not be included in blank determinations because except for hydroquinone they contain negligible amounts of substances capable of reaction with iodine under these reaction conditions. For contaminated monomers or for materials obtained from unknown sources, a comparable amount of hydroquinone-free monomer, prepared by the directions given below, should be added to the blank solution.

HYDROQUINONE-FREE MONOMERS

In a separatory funnel, shake 1 liter of water-insoluble ester with several 200-ml. portions of a solution containing 15% of sodium hydroxide and 10% of sodium chloride. Continue the extraction until the caustic (lower) layer is practically colorless. Shake with 200-ml. portions of water until the water washes are neutral when tested with phenolphthalein. Dry over anhydrous sodium sulfate until clear and filter into a brown screw cap bottle. The uninhibited ester is stored in a refrigerator unless it is used immediately.

Do not attempt to remove the inhibitor from functionally substituted esters by this procedure. Acrylic and methacrylic acids are purified by distillation at reduced pressure using a column having 12-15 theoretical plates. Because of the possibility of dangerously violent polymerization, no more than relatively small amounts (not above 1 lb.) of these acids should be distilled at one time, holding the pot temperature below 60°C. The distillates should be condensed and stored under such conditions as to prevent their solidification and hence should be kept at 15-20°C. (60-68°F.). The preferred practice, however, is to use distilled acids promptly, thus avoiding any hazard during storage. In many cases, glacial acetic acid may be substituted for acrylic or methacrylic for use as a photometric standard.

a. Procedure for Esters Containing 0.012 (120 ppm) or Less Hydroquinone

This procedure gives excellent results when used with the methyl, ethyl, butyl, and isobutyl acrylates and methacrylates containing 0.012% (120 ppm) or less of HQ, preferably in the range 0.005-0.012% (50-120 ppm). Its application to higher esters is less satisfactory. The method is suitable for essentially colorless monomers with a sample size selected to contain not more than 6 mg. of HQ. When larger quantity of HQ are present, the yellow color of quinone produced by the oxidation obscures the end point. For monomers containing 0.006% (60 ppm) or less of HQ, use a 100-ml. sample, and for those containing 0.006-0.012% (60-120 ppm), a 50-ml. sample is taken.

Transfer the sample into a 500-ml. Erlenmeyer flask. Add 20 ml. of buffer, 125 ml. of methanol, and shake to mix. For the higher esters more than 125 ml. of methanol may be used to ensure homogeneity. Add 10 ml. of 0.02*N* iodine with swirling and let stand 1-5

minutes. Titrate with 0.02*N* thiosulfate using no starch; call this titer A. Run a blank on the reagents and call this titer B.

Calculation

$$\% \text{ HQ} = \frac{(B-A) \times N \times 5.50}{\text{ml. sample} \times \text{spec. grav.}}$$

Because starch cannot be used as an indicator, there is usually some difficulty in attaining the stoichiometric end point. The difficulty arises from the yellow color of quinone in the sample titration, whereas the blank end point is colorless. Since the intensity of the final color is proportional to the original concentration of HQ, color standards, such as an APHA color standard (ASTM D-1209), can be selected to correspond to various amounts of HQ. For example, a sample of methyl methacrylate containing 0.006% (60 ppm) of HQ is titrated to a color matching that of an APHA standard of 35. This refinement aids greatly in obtaining the maximum precision of this method.

b. Procedure for Esters Containing 0.01-0.35% (100-3500 ppm) of Hydroquinone

Transfer a 50-ml. sample into a 500-ml. Erlenmeyer flask, add 20 ml. of buffer and 50 ml. of water and mix by swirling. Add the volume of 0.1*N* iodine given below, thus providing at least 5 ml. in excess:

Estimated HQ Content		
%	ppm	Ml. 0.1N Iodine
0.01-0.10	100-1000	15
0.11-0.20	1100-2000	25
0.21-0.35	2100-3500	40

Let stand 1-5 minutes with intermittent vigorous shaking. Titrate with 0.1*N* thiosulfate solution using starch indicator and shaking vigorously; call this Titer A. Run a blank on the reagents using the same volume of iodine solution and call this Titer B. The calculation is the same as above.

c. Procedure for Acrylic and Methacrylic Acids Containing Hydroquinone

For acids containing less than 0.2% (2000 ppm) of HQ, take a 100 ml. sample; for higher HQ contents, use a proportionally smaller sample. Transfer the sample into a 1000-ml. Erlenmeyer flask. Add 5-10 drops of bromthymol blue indicator and titrate *carefully* with 25% sodium carbonate solution to a faint blue color. Carefully add 2*N* sulfuric acid until the solution becomes yellow, then add more sulfuric acid to give a pH between 6.0 and 6.8, preferably 6.2. For glacial acrylic and methacrylic acid, about 150 ml. of excess 2*N* sulfuric is required. The acid must be added VERY CAREFULLY. Swirl vigorously to liberate as much carbon dioxide as possible. Add 10 ml. of 10% potassium iodide solution and sufficient 0.1*N* iodine solution to provide an excess of at least 5 ml. Swirl to mix and let stand 1-5 minutes. Titrate with 0.1*N* thiosulfate using starch indicator to an end point persisting at least 30 seconds; call this titer A.

For the blank determination, add 5-10 drops of bromthymol blue indicator to 200 ml. of water and 20 ml. of buffer. Add the same volume of 0.1*N* iodine used above and titrate with 0.1*N* thiosulfate solution using starch indicator; call this titer B. Calculate as above.

2. HYDROQUINONE IN METHYL METHACRYLATE. ULTRAVIOLET ABSORPTION METHOD

SCOPE AND METHOD

The HQ content of methyl methacrylate can be determined accurately and rapidly by measuring the absorbance of a 10% solution of monomer in isopropyl alcohol at 293 mμ against a solution of pure monomer as reference. The procedure has been worked out specifically for methyl methacrylate containing 0.002-0.022% (20-220 ppm) of HQ. For other concentrations, the procedure may require modification. It is important that the sample and standard monomers should be free of background ultraviolet absorbers such as other inhibitors and poly(methyl methacrylate). By establishing specific standards the method could probably be adapted to other monomers inhibited only with HQ.

REAGENTS

Isopropyl Alcohol, A.C.S. Reagent

Uninhibited Methyl Methacrylate (See Section I-1).

This standard must be free of polymer. If the presence of polymer is suspected, the monomer should be checked by the procedure of section J. Grades of Rohm and Haas methyl methacrylate containing 25 or 60 ppm HQ are available as starting materials for the preparation of uninhibited monomer.

Methanol, Anhydrous

Hydroquinone, Eastman Organic Chemical No. 356 or similar grade

APPARATUS

Beckman Model DU Spectrophotometer with 10-mm. silica cells

PROCEDURE

The material to be analyzed should be clear and at room temperature before proceeding. Refrigerated monomers can be warmed safely by placing the container in the draft in front of an electric fan; do not warm refrigerated materials in hot baths or on heaters or expose them to direct sunlight. Determine the specific gravity of the sample or use the value 0.939 at 25/15.6°C. for methyl methacrylate. Pipet a 10-ml. sample into a 100-ml. volumetric flask, dilute to volume with isopropyl alcohol, and mix thoroughly. Prepare a similar reference solution of uninhibited monomer. Measure the absorbance A_s of the sample solution against the standard solution in the reference cell.

To obtain the standard absorbance A_o of HQ in pure methyl methacrylate, a solution containing 100 μg./ml. is first prepared. This can be done, for example, by dissolving 0.1000 g. of HQ in uninhibited monomer and diluting to 100 ml., then diluting a 10-ml. aliquot to 100 ml. Pipet 10.0 ml. of the standard 100 μg. solution into each of 3 100-ml. volumetric flasks and dilute to volume with isopropyl alcohol. After thorough mixing, measure the absorbance as above against a 10%(V/V) solution of pure methyl methacrylate in isopropyl alcohol.

Calculation

$$A_o = \frac{\text{average of absorbance readings}}{100}$$

$$\text{ppm HQ in sample (by weight)} = \frac{A_s}{A_o \times \text{spec. grav. of methyl methacrylate}}$$

$$\text{\% HQ (by weight)} = \frac{A_s}{A_o \times \text{spec. grav.} \times 10{,}000}$$

The value of A_o on a Beckman Model DU Spectrophotometer was found to be 0.00280. Assuming a specific gravity of 0.939 for monomer

$$\text{\% HQ (by weight)} = \frac{A_s}{26.3}$$

Notes 1. The following settings of the instrument were used:

Slit width	0.075
Zero suppression	1
Sensitivity	1
Phototube load resistor	1

2. High results are obtained if poly(methyl methacrylate), phenolic compounds such as the monomethyl ether of hydroquinone (MEHQ), or phenol are present in the test sample. Low results are obtained if these are present in the purified uninhibited monomer.

3. Purified monomer is the most practical reference because any interference caused by the absorbance of monomer in the solutions is cancelled out by using it as a reference.

3. HYDROQUINONE. CAUSTIC COLOR METHOD

SCOPE AND METHOD

HQ is simultaneously extracted from the monomer with aqueous alkali and oxidized by atmospheric oxygen. The intensity of the resulting amber color in the acidified extract is measured at 420 mμ; the absorbance is linear with HQ content up to 0.3% (3000 ppm). The method is suitable for the rapid and accurate determination of 0-0.25% (0-2500 ppm) of HQ in acrylate and methacrylate esters essentially insoluble in aqueous alkali and 0-0.12% (0-1200 ppm) of HQ in acrylic and methacrylic acids. Appropriate selection of the sample size of esters permits the analysis of all current standard HQ dosages; for acids, however, 1 ml. samples are always used. In all cases, the sample is diluted to a total volume of 100 ml. before analysis. A single standard curve obtained with methyl methacrylate is applicable to all the monomeric esters. Trace amounts of HQ (5-10 ppm) can be measured with a precision of ±0.1 ppm. MEHQ in amounts up to 0.1% (1000 ppm) does not interfere.

The color reading may be unduly increased if the aqueous extract contains haze. If a hazy extract is obtained, it can be clarified by extraction with octanol. This operation is necessary in the analysis of lauryl and stearyl methacrylates and may occasionally be required with other esters.

APPARATUS

Spectrophotometer - Beckman Model DU or equivalent instrument with 1-cm. matched stoppered cells; for trace amounts, 5- or 10-cm. cells afford higher precision.

Separatory Funnels, 250-ml. Squibb type with Teflon plug, A. H. Thomas Co. #5630-W2.

REAGENTS

Sodium Hydroxide, 10% (W/V) aqueous solution.

Acetic Acid, 6*N* aqueous solution: dilute 360 ml. of glacial acid to 1000 ml. with distilled water.

Octanol, technical grade, such as E. I. du Pont de Nemours & Co. Lorol 20 or equivalent material.

Methyl Methacrylate, uninhibited: for preparation, see p. 15.

PROCEDURE FOR ESTERS

The indicated amount of sample (see table below), uninhibited methyl methacrylate to give a total monomer volume of 100 ml., and 20 ml. of 10% caustic solution are added by pipet to a 250-ml. separatory funnel. The stoppered funnel is shaken vigorously for exactly 60 seconds, 10 ml. of 6*N* acetic acid is added at once from an automatic pipet, and the funnel is again shaken immediately for 15 seconds. After the two phases have separated completely, the clear amber lower layer is drawn off and its absorbance is measured at 420 mμ against a reagent blank prepared by mixing 20 ml. of caustic solution and 10 ml. of 6*N* acetic acid. The μg. of HQ equivalent to the observed absorbance is read from the standard curve.

For lauryl and stearyl methacrylate and for other esters giving a hazy extract, the extract is drawn off into a second separatory funnel and shaken vigorously with 100 ml. of octanol for 1 minute. The clarified lower layer is then removed and its color measured as above.

If 5 or 10-cm. cells are used, the μg. value of the standard curve should be multiplied by 5 or 10 respectively.

SAMPLE SIZE

Estimated HQ Content %	Estimated HQ Content ppm	Monomer sample, ml.
0 -0.0025	0- 25	100
0.002-0.005	20- 50	50
0.004-0.01	40- 100	25
0.009-0.025	90- 250	10
0.023-0.05	230- 500	5
0.045-0.125	450-1250	2
0.11 -0.25	1100-2500	1

If a choice of sample size is possible, the larger size should be used to increase sensitivity and accuracy.

PROCEDURE FOR ACRYLIC AND METHACRYLIC ACIDS

A 1-ml. sample of the glacial acids is pipetted into a 250-ml. separatory funnel and diluted with 100 ml. of uninhibited methyl methacrylate. After swirling gently to mix the monomers, add 25 ml. of sodium hydroxide and shake vigorously for exactly 60 seconds. Immediately add 10 ml. of 6*N* acetic acid, and shake vigorously for 15 seconds. After the two phases have been separated completely, the clear amber lower layer is drawn off and its absorbance is measured at 420 mμ against a reagent blank prepared by mixing 25 ml. of 10% sodium hydroxide and 10 ml. of 6*N* acetic acid. The μg. of HQ equivalent to the observed absorbance is read from the standard curve.

Standard Curve

A solution is prepared by dissolving 0.5000 g. of HQ in about 250 ml. of uninhibited methyl methacrylate contained in a 500-ml. volumetric flask, diluted with monomer to the mark, and mixed. A stock solution containing 100 μg. of HQ per ml. is then made by diluting 10 ml. of the above to 100 ml. with uninhibited monomer. Standard samples containing 100, 500, 1000, 2000, and 3000 μg. of HQ are then obtained by diluting respectively 1, 5, 10, 20, and 30-ml. aliquots of the stock solution to 100 ml. with uninhibited monomer in 100-ml. volumetric flasks. Each standard sample is transferred into a 250-ml. separatory funnel and the flasks are rinsed into the funnel with 1 or 2 ml. of monomer. The samples are then treated as above. The absorbances are plotted against μg of HQ to give a straight line.

Calculation

$$\text{HQ (ppm)} = \frac{\mu g \text{ of HQ (from standard curve)}}{\text{ml. of sample x spec. grav.}}$$

HQ (% by weight) = ppm/10,000

4. HYDROQUINONE (TRACE AMOUNTS). SPECTROPHOTOMETRIC METHOD

SCOPE AND METHOD

In the presence of n-butylamine, HQ is oxidized by air to a blue compound whose color intensity is measured at 345 mμ. The method offers a rapid determination of HQ in amounts up to 80 ppm in the presence of 1000 ppm MEHQ with a precision of about ± 5% relative in 5-cm. cells. The sensitivity is increased proportionally in 10-cm. cells. Acids are dissolved in water, esters in isopropyl alcohol.

REAGENTS

Hydroquinone, Eastman #356

Monomethyl Ether of Hydroquinone, Eastman #350

99% Isopropyl Alcohol, reagent

n-Butylamine, redistilled within one week of use and stored in a brown bottle.

APPARATUS

Beckman Model DU Spectrophotometer or equivalent with 5-cm. (or 10-cm) cells.

PROCEDURE

Into a 100 ml. volumetric flask, pipet 1.0 ml. of the acrylic or methacrylic acid sample into 50 ml. of water or 5.0 ml. of ester into 50 ml. of isopropyl alcohol. Add 20 ml. of n-butylamine and swirl to mix. Dilute to volume with water, mix, and pour into a 200-ml. beaker. Expose to air 15-20 minutes with frequent stirring. Measure the absorbance at 345 mμ against a reagent blank containing no monomer. A calibration curve is prepared by the same procedure using pure monomer to which known amounts of HQ are added. Read the amount of HQ from the calibration curve.

MEHQ Content %	MEHQ Content ppm	Dilution ml.	Dilution Factor D
0.01	100	0	1
0.01-0.02	100-200	10	2
0.02-0.05	200-500	40	5
0.05-0.1	500-100	90	10
0.1 -0.25	1000-2500	240	25

Pipet a 5-ml. aliquot into a 125-ml. separatory funnel. Add 1.0 ml. of 0.025*N* sodium hydroxide solution and shake *exactly 30 seconds* (Notes 1,2). *Immediately* add 1.0 ml. of 3.5*N* hydrochloric acid and shake vigorously *10 seconds*. Add 1.0 ml. of 2% sodium nitrite solution, shake 15 seconds, and allow the layers to separate. Drain the yellow (lower) layer into a 150 x 16 mm. test tube containing 1 g. of sodium chloride crystals and shake until the liquid is clear. Decant into the absorption cell and measure the absorbance at 420 mμ against a similarly prepared reagent blank using 5 ml. of chloroform with no monomer. Determine the MEHQ content in μg from the calibration curve.

Calculation

$$\text{ppm MEHQ} = \frac{\mu g \text{ MEHQ} \times D}{\text{spec. grav.}}$$

where D is dilution factor in above table and monomer sample size is 1 ml.

Notes: 1. All specified times must be measured accurately using a timer or second hand of a clock or watch.

2. Stoppers should be removed carefully because of the high vapor pressure of chloroform.

CALIBRATION CURVES (Figure I)

Prepare a stock solution containing 1000 ppm of MEHQ from 0.5000 g. of MEHQ diluted to volume with uninhibited methyl methacrylate in a 500-ml. volumetric flask. Although serial dilutions can be made containing 5-100 μg/ml., it is simpler to dilute a 10-ml. aliquot to 100 ml. with pure monomer to give a solution containing 100 μg of MEHQ per ml. Using 10-ml. aliquots, run three determinations by the sample procedure. In plotting absorbance against μg of MEHQ, draw a straight line from the absorbance value at 100 μg to the origin. Absorbance lines to be used in correcting for the presence of HQ can be obtained from samples of methyl methacrylate containing 1000 or 2000 ppm HQ with no MEHQ present. The resulting absorbance is plotted on the vertical axis at zero ppm of MEHQ and this point is connected by a straight line to the point representing the absorbance of 100 ppm of MEHQ alone. This line gives the absorbance of acrylates or methacrylates containing 1000 or 2000 ppm HQ in addition to varying amounts of MEHQ between 0 and 100 ppm. For intermediate amounts of HQ, similar lines are drawn by spacing them proportionately between the MEHQ line and the HQ-MEHQ lines; all lines should intersect at 100 ppm MEHQ. In choosing which HQ line should be used as a basis for calculation of MEHQ content, any predilution factor D must be considered.

Figure I
CALIBRATION CURVE
MEHQ IN ACRYLIC ESTERS

Beckman Model DU Spectrophotometer, 1-cm. cells at 420 mμ against reagent blank

A) MEHQ Only
B) MEHQ + 500 μg HQ
C) MEHQ + 1000 μg HQ

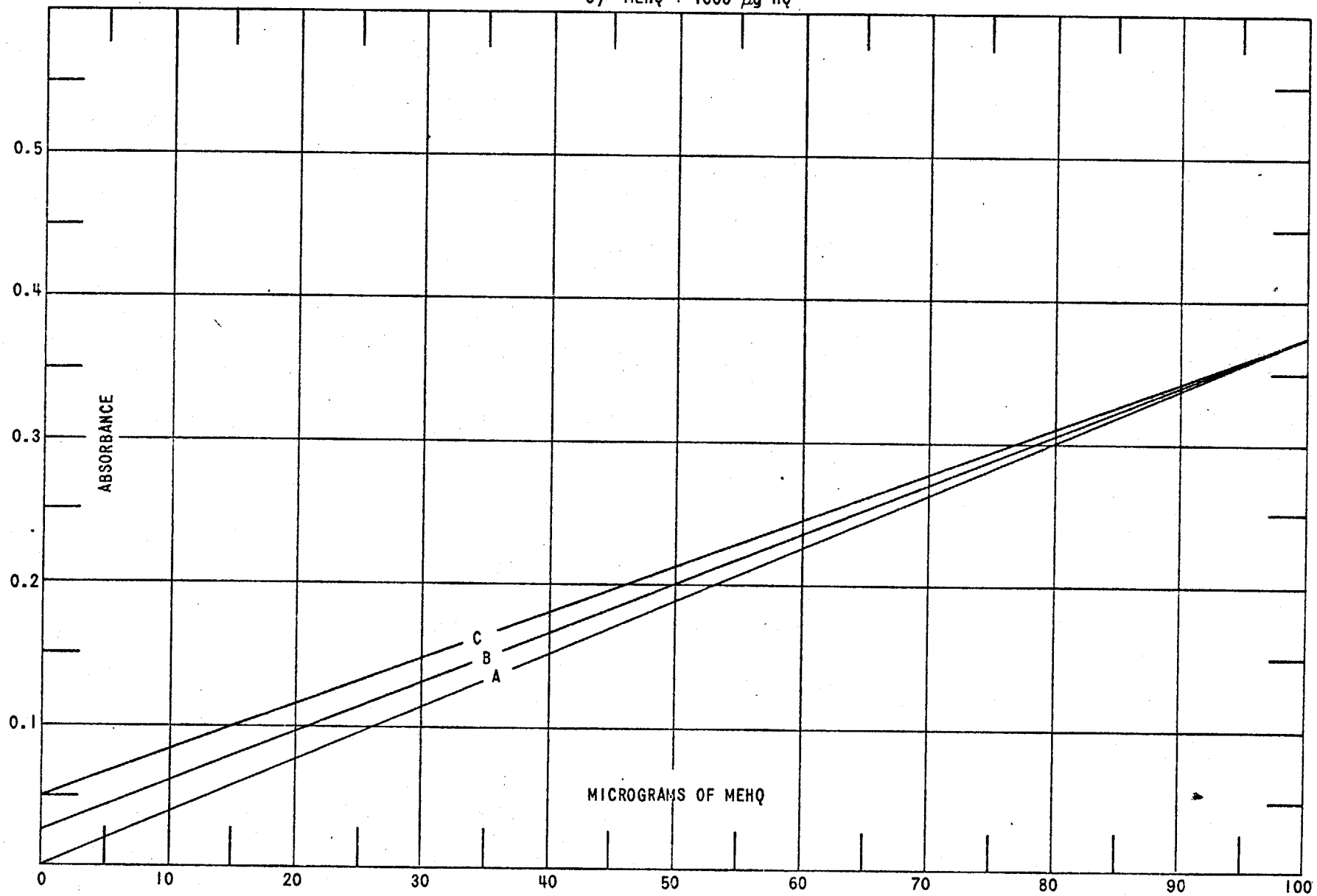
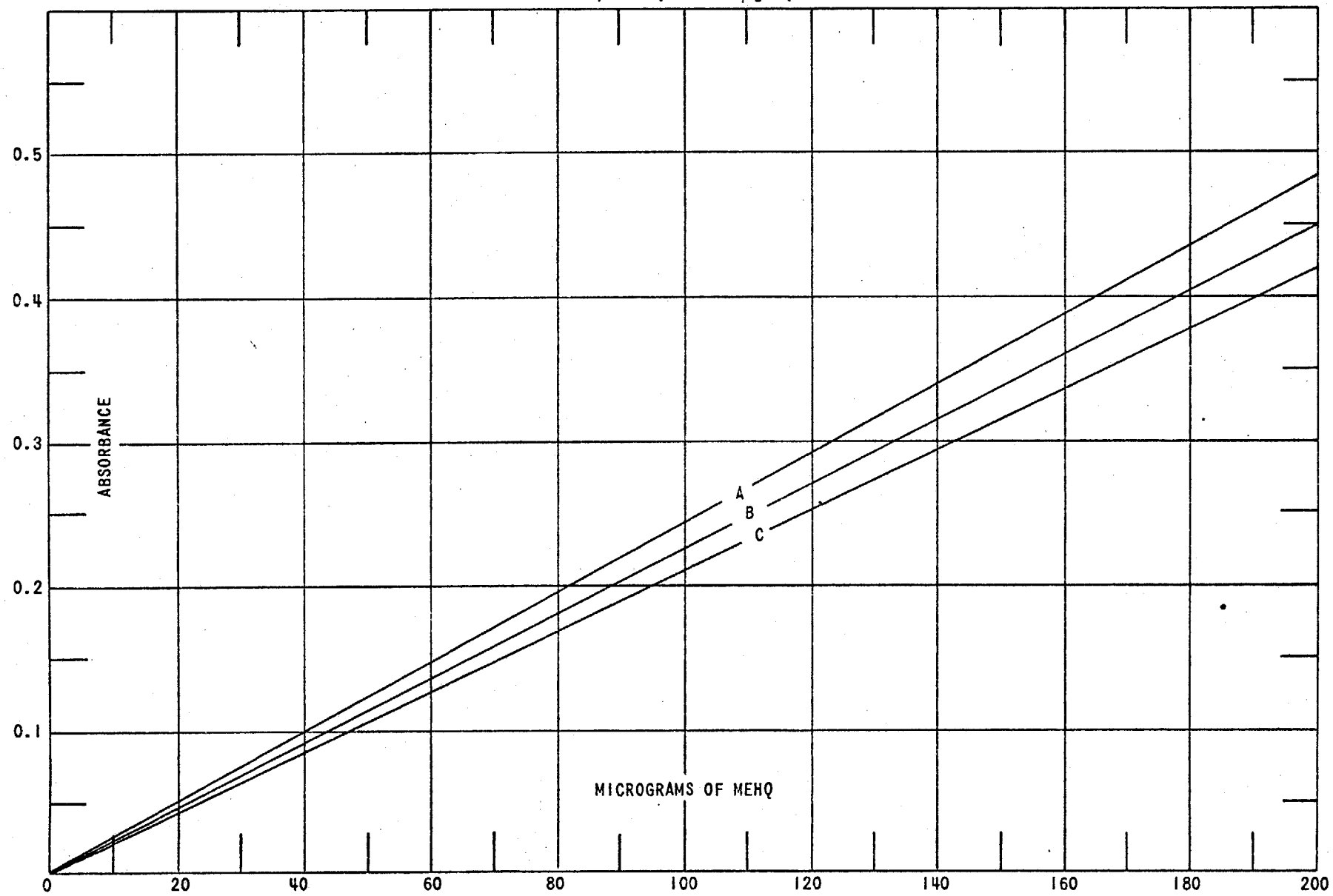


Figure 2
CALIBRATION CURVE
MEHQ IN ACRYLIC AND METHACRYLIC ACIDS

Beckman Model DU Spectrophotometer, 1-cm. cells at 420 mμ against reagent blank

A) MEHQ Only
B) MEHQ + 1000 μg HQ
C) MEHQ + 2000 μg HQ



b. ACRYLIC AND METHACRYLIC ACIDS

The following table lists the factors used in the sampling and analysis of acids.

Condition	MEHQ Content ppm	Dilution Scheme	Reagents, ml. NaOH	HCl	$NaNO_2$	Calculation Factors V	L, cm.
1	2000-5000	1:50:10:50:10	15	10	2	0.04	1
2	750-2250	2:50:10:50:10	15	10	2	0.08	1
3	200- 900	5:50:10:50:10	15	10	2	0.20	1
4	80- 360	5:100:10:50:40	60	40	8	0.10	5
5	10- 90	5:50:10:50:40	60	40	8	0.20	10
6	1- 20	10:50:10:50:40	60	40	8	0.40	10

The significance of the table will appear from the following example which describes the analysis of acids containing 200 ppm of MEHQ according to condition 4. Pipet 5 ml. of monomer into a 100-ml. volumetric flask and dilute to volume with acetic acid. After mixing thoroughly, pipet a 10-ml. aliquot into a 50-ml. volumetric flask and dilute to the mark with chloroform. Pipet 40-ml. of the well-mixed solution into a 250-ml. separatory funnel. Add 60 ml. of 2.5*N* sodium hydroxide solution and shake vigorously *exactly 30 seconds*. *Immediately* add 40 ml. of 3.5*N* hydrochloric acid and shake 10 seconds. Add 8 ml. of 2% sodium nitrite solution and shake 15 seconds. Let stand until the phases have separated sharply. Draw the lower layer into a 150 x 16 mm. test tube containing 5 g. of sodium chloride crystals and shake until clear. Decant into a 5-cm. absorption cell and measure the absorbance at 420 mμ against a reagent prepared as above starting from 10 ml. of acetic acid. Determine the amount of MEHQ from the calibration curve.

Calculation

$$\text{ppm MEHQ (By weight)} = \frac{\mu g \text{ of MEHQ}}{V \times L \times \text{spec. grav.}}$$

where V is a concentration factor and L is the cell length in cm; see the above table for values of V and L.

For conditions 1-3, use a 125-ml. separatory funnel and 1 g. of sodium chloride; for conditions 5-6, follow the directions of the example.

CALIBRATION CURVES (Figure 2)

Prepare two standard solutions of MEHQ, one without HQ, the other with 1000 ppm of HQ following the procedure above but using glacial acetic acid rather than methyl methacrylate as the diluent. If the single-point technique is adopted, run at 200 μg. of MEHQ and draw lines from the absorbance values obtained for the various HQ levels to the origin. Intermediate lines can be estimated for correcting the effect of various HQ levels on the absorbance of MEHQ. It should be noted that the presence of HQ actually depresses the absorbance of MEHQ to a small extent. This effect increases with the amount of HQ.

6. BUTYLATED HYDROXYTOLUENE (BHT) IN METHYL METHACRYLATE

SCOPE AND METHOD

BHT in methyl methacrylate is oxidized by nitric acid to a blue color, the intensity of which is measured at 385 mμ. The method is sensitive to 0.0005% (5 ppm) in 5-cm. cells;

higher sensitivity can be obtained in 10-cm. cells. This method gives reliable results for BHT only when other inhibitors are absent.

REAGENTS

BHT, Eastman Organic Chemicals #P5917

Nitric Acid, concentrated reagent

Sulfuric Acid, concentrated reagent

Acetic Acid, glacial reagent

Methyl Methacrylate, uninhibited (See section I-1)

APPARATUS

Beckman Model DU Spectrophotometer or equivalent

PROCEDURE

Pipet 10.0 ml. of BHT-inhibited monomer into a 50-ml. volumetric flask. Add 1 ml. of nitric acid, mix thoroughly, add 3 ml. of concentrated sulfuric acid, mix, and allow to react 30 seconds. Dilute to volume with acetic acid. Prepare a blank similarly from 10 ml. of uninhibited monomer. Using 5-cm. cells, obtain the absorbance at 385 mμ with the blank in the reference cell. Read the concentration of BHT in μg/10 ml. sample from the calibration curve. Empty the cells promptly and rinse well with methanol.

Calculation

$$\text{ppm BHT (by weight)} = \frac{\mu g \text{ BHT}}{10 \times \text{spec. grav.}}$$

CALIBRATION CURVE

Prepare a stock solution by diluting 0.2500 g. of BHT with uninhibited methyl methacrylate in a 500-ml. volumetric flask with thorough mixing. Pipet 1, 2, 5, 10, and 20 ml. aliquots of the stock solution into 100-ml. volumetric flasks and dilute to volume with uninhibited monomer; these solutions contain 5, 10, 25, 50, and 100 μg of BHT per milliliter respectively. Take 10-ml. aliquots of the serial solutions and treat as above, measuring the absorbance against a similarly treated blank of uninhibited methyl methacrylate. Plot net absorbance against μg of BHT per 10 ml. of monomer.

J. DETERMINATION OF TRACES OF POLYMER IN MONOMER

The presence of polymer in acrylic monomers can be tested by adding a sample to a selected solvent. The rapid appearance of haziness or turbidity usually signifies polymer. The choice of solvent varies with the monomer. It is convenient to mix the solution in a stoppered volumetric flask. The table lists the sample and solvent volumes to be used and the time during which the solution is allowed to stand.

Solutions of acrylates should be kept out of strong sunlight or artificial light.

TEST CONDITIONS FOR POLYMER IN ACRYLIC MONOMER

Monomer	Volume ml.	Solvent	Volume ml.	Time, min.
Methyl Acrylate	2	50% ($\frac{V}{V}$) H_2O-AcOH	98	5
Ethyl Acrylate	2	50% ($\frac{V}{V}$) H_2O-AcOH	98	5
Butyl Acrylate	2	Methanol	98	5
2-Ethylhexyl Acrylate	3	Methanol	10	5
Methyl Methacrylate	2	Methanol	98	5
Ethyl Methacrylate	2	Methanol	98	5
Butyl Methacrylate	2	Methanol	98	5
Lauryl Methacrylate	2	Methanol	98	5
Stearyl Methacrylate	2	Methanol	98	5[a]
1,3-Butylene Dimethacrylate	2	Methanol	98	5
2-Dimethylaminoethyl Methacrylate	2	98:2 ($\frac{V}{V}$) Ultrasene-Isopropyl Alcohol	98	15
2-t-Butylaminoethyl Methacrylate	2	98:2 ($\frac{V}{V}$) Ultrasene-Isopropyl Alcohol	98	15
Acrylic Acid[b]	2	Ultrasene	98	15
Methacrylic Acid[c]	10	25% Sodium Chloride	10	15

a - Test at 25°C. If gel-like particles are formed, warm 20 minutes at 35°. The solution should become clear.

b - Glacial grade only. The presence of more than traces of water, up to 0.5%, gives a dull-white turbidity. Polymer in glacial acrylic acid is bright white.

c - Shake the sample with the brine 30 seconds and let stand 15 minutes. Polymer is indicated by a precipitate at the interface or by turbidity throughout the lower layer.

ANALYTICAL METHODS FOR THE ACRYLIC MONOMERS

METHYL ACRYLATE
ETHYL ACRYLATE
BUTYL ACRYLATE
ISOBUTYL ACRYLATE
2-ETHYLHEXYL ACRYLATE
GLACIAL ACRYLIC ACID
METHYL METHACRYLATE
ETHYL METHACRYLATE
BUTYL METHACRYLATE
ISOBUTYL METHACRYLATE
ISODECYL METHACRYLATE
LAURYL METHACRYLATE
TRIDECYL METHACRYLATE
STEARYL METHACRYLATE
2-HYDROXYETHYL METHACRYLATE
HYDROXYPROPYL METHACRYLATE
DIMETHYLAMINOETHYL METHACRYLATE
t-BUTYLAMINOETHYL METHACRYLATE
1,3-BUTYLENE DIMETHACRYLATE
TRIMETHYLOLPROPANE TRIMETHACRYLATE
GLACIAL METHACRYLIC ACID

This bulletin presents analytical methods used by the Rohm and Haas Company in the hope that they will be useful to those working with acrylic monomers. Specifications for these monomers are listed in the bulletin entitled "Typical Properties and Specifications" (CM-16). We would be pleased to supply samples of the monomers for evaluation and a list of literature on acrylic monomers available from Rohm amd Haas Company.

ROHM AND HAAS

INDEPENDENCE MALL WEST • PHILADELPHIA, PA. 19105

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Embryonic-Fetal Toxicity and Teratogenic Effects of a Group of Methacrylate Esters in Rats

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Monomers of five methacrylate esters and acrylic acid were administered at three dose levels to female rats on days 5, 10, and 15 of gestation. The incidences of resorptions, dead fetuses, gross abnormalities, and skeletal malformations are presented for each of these groups. Mean fetal weights also were determined for each group, and those of the monomer-treated groups were significantly smaller ($P < 0.01$) than those of the untreated controls.

Methacrylate esters are the esters of methyl acrylic acid, $CH_2{:}C(CH_3)COOH$. They are transformed into resins by light, heat, oxygen, and oxygen-yielding compounds. Methacrylic resins and plastics are widely used in dentistry, in the manufacture of contact lenses, and in other industrial applications.[1] The lower members of the series have a penetrating, disagreeable odor that is easily recognized in all tissues of treated animals.[2]

Before the introduction of acrylic polymers in dentistry in 1937, materials such as porcelain, vulcanite, nitrocellulose, phenol-formaldehyde, and vinyl plastics were used for denture bases. Each of these substances, however, has certain properties that make it undesirable in some respects for this purpose. The acrylic resins were so well received by the dental profession that by 1946 it was reported that 98% of all denture bases were constructed of methyl methacrylate polymers or copolymers.[3]

Strain[4] reported that methyl methacrylate polymerized with a chemical accelerator, the so-called cold-curing acrylic resins, may contain sufficient monomeric methyl methacrylate in the finished denture to produce an allergic reaction in patients who are sensitive to the monomer. This sensitization, however, tends to diminish as the residual monomer is leached out of the plastic. On the other hand, completely polymerized materials, in which no monomer is present, do not produce true sensitization in the patient, and the sore mouth observed in these instances seems to be the result of a nonspecific pressure effect.

Toxicity studies of monomeric methyl, ethyl, and *n*-butyl methacrylates were conducted in various laboratory animals by Deichmann[2] and by Spealman et al.[5] Methyl methacrylate vapors were reported to be less acutely toxic than ethyl acetate and more toxic than acetone, and the most characteristic histologic change found in animals from prolonged exposure to methyl methacrylate vapors was degenerative changes in the liver.[5] Both groups[2,5] reported that the cause of death from acutely toxic doses, by various routes of administration, was respiratory depression.

Borzelleca et al[6] conducted tests for chronic oral toxicity of methyl methacrylate in rats and dogs, but failed to find histologic lesions or increased mortality that could be attributed to the monomer. Laskin, Robinson, and Weinmann[7] were able to produce nonmetastatic fibrosarcomas in mice from the subcutaneous implantation of polymerized methyl methacrylate. Oppenheimer et al[8] reported malignant tumors from subcutaneous implantation of polymerized methyl methacrylate in rodents, but not from painting of the monomer on the skin of the animals.

Little information is available about the embryopathic and teratogenic effects of these methacrylate esters when administered parenterally to pregnant females. To ascertain the effect such monomers might have upon the fetus, a teratogenic study was initiated in rats with acrylic acid and five methacrylate esters.

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Materials and Methods

The compounds included in this study were methyl, ethyl, *n*-butyl, isobutyl, and isodecyl methacrylate monomers and acrylic acid.* Test animals were 175 to 225 gm, adult, virgin female rats of the Sprague-Dawley strain.† Male rats of the same strain and age were used as the "stud pool."

Acute toxicity of the methacrylates was determined by administration of graded doses of these specific samples by intraperitoneal injection in rats and observation of the rats for seven days for mortality. Acute LD_{50} values and 95% confidence limits were calculated by Cornfield and Mantel's[9] modification of Karber's method or by the method of Weil.[10]

Female rats were selected for experimentation only after observation of at least two complete four- or five-day estrus cycles. Occurrence of estrus was determined by daily vaginal smears obtained by introduction of 0.2 ml fresh, clean tap water into the vagina with a smooth, clean, sterile medicine dropper; a part of the liquid was withdrawn and transferred to a clean slide. The slide was examined microscopically while fresh, and the stage of estrus (proestrus, estrus, or diestrus) was determined according to cell types found in the vaginal smear.

Five female rats were housed with one male in a large cage at room temperature (22 to 27 C): laboratory chow‡ and fresh tap water were provided ad libitum. The onset of gestation was established by the presence of sperm in the vaginal smear and was designated as day 0, with the following day as day 1 of the gestation period. At this time, the female rats were moved to individual cages where they were kept undisturbed except for specified injections. There were 22 groups, each of which was composed of five female rats.

All treatments were administered by intraperitoneal injection on days 5, 10, and 15 of gestation. Each test substance was administered at three dose levels, a tenth, a fifth, and a third of its acute LD_{50} in each injection. An untreated group of rats was maintained as controls. For comparative purposes, cottonseed oil, distilled water, and normal saline were administered at a dose of 0.8222 ml/kg, which corresponds to the largest volume of methacrylate injected (isodecyl methacrylate), although no diluent was used with the injected methacrylates because all of these compounds are liquids at room temperature.

On the 20th day of gestation, one day before expected parturition, the rats were killed by ether inhalation. The uterine horns and ovaries were exposed surgically so the numbers of corpora lutea, resorption sites, and viable and dead fetuses could be counted and recorded. Fetuses were removed, blotted dry, and weighed§ to the nearest tenth of a milligram. All fetuses, both viable and nonviable, were examined for gross abnormalities. A randomly selected number of fetuses (usually 50% of the total) were prepared as transparent specimens to permit visualization of their skeletal system for evaluation of skeletal malformations; whenever possible, fetuses that showed gross evidence of malformations were excluded. In a few instances in which the incidence of resorption was very high, all fetuses were included. Skeletal visualization was accomplished by the procedure of Staples and Schnell.[11]

The following parameters of adverse effects were investigated: embryonic-fetal toxicity, as evidenced by resorptions and stillbirths; gross (external) malformations of fetuses; skeletal malformations; and fetal size (weight). In all of these evaluations, both viable and nonviable fetuses were considered.

Results

The number of corpora lutea observed ranged from 51 to 60 per group, with a mean of 55. Of the 1,209 corpora lutea observed in this study, 1,154 (95.5%) were accounted for as live fetuses, dead fetuses, resorption sites, or aborted fetuses.

The acute intraperitoneal LD_{50} values and 95% confidence limits for the meth-

* Rohm and Haas, Philadelphia, Pa.
† Sprague-Dawley, Inc., Madison, Wis.
‡ Purina, Ralston-Purina Co., St. Louis, Mo.
§ Mettler H6T balance, Mettler Inst. Corp., Highstown, NJ.

TABLE 1
ACUTE TOXICITY OF A GROUP OF METHACRYLATE ESTERS IN RATS

Compound	LD_{50} (ml/kg)	95% Confidence Limits (ml/kg)
Methyl methacrylate	1.3280	1.0843-1.6265
Ethyl methacrylate	1.2228	1.0212-1.4644
n-Butyl methacrylate	2.3039	1.8811-2.8217
Isobutyl methacrylate	1.3999	1.1077-1.7693
Isodecyl methacrylate	2.4666	1.1765-5.1712
Acrylic acid*	0.0225	0.0178-0.0248

* Ten percent solution in saline was used for toxicity test. LD_{50} values are based on pure compound.

acrylate esters and acrylic acid are presented in Table 1. Acrylic acid was the most acutely toxic, and the methacrylate esters were orders of magnitude lower in toxicity. A detailed analysis of acute toxicity vs chemical structure of these compounds has been presented by Lawrence et al.[12]

Compound-dependent and dose-related effects on embryonic and fetal growth and development were generally observed with these methacrylates and acrylic acid. The results of developmental changes are presented in Tables 2 and 3 for the untreated controls and the experimental groups. Table 2 presents data for the number of corpora lutea, resorption sites, dead fetuses, live fetuses, and fetal weights according to treatment groups, and Table 3 is a comparison of the number and percentage of resorptions and gross and skeletal abnormalities. No resorptions, dead fetuses, or gross or skeletal abnormalities were observed in the untreated control group.

RESORPTIONS.—In addition to the untreated controls, no resorption sites were found in the groups treated with cottonseed oil or the low dose of acrylic acid. All other treatment groups exhibited one or more resorptions. The greatest incidences were seen with the high dose of butyl methacrylate (34.6%) and the two highest doses of isodecyl methacrylate (44.2% and 25.0%, respectively); the smallest incidences occurred in the groups treated with the low dose of butyl methacrylate (2.0%), middle dose of acrylic acid (3.6%), middle dose of isobutyl methacrylate (4.0%), and low dose of methyl methacrylate (4.4%).

FETAL DEATHS.—Fetal deaths were observed with much less frequency and in smaller numbers than the other criteria considered. Although most of the experimental groups showed some resorption sites, dead fetuses were found only in groups treated with the compounds isodecyl methacrylate and acrylic acid. The high dose of acrylic acid produced three dead fetuses (6% of total) and two dead fetuses (3.6%) were found in the group that received the middle dose of isodecyl methacrylate. The absence of dead fetuses in the group given the high dose of isodecyl methacrylate may be the result of its potent, early embryotoxic effect (44.2% resorption sites), or the fact that one of the five rats in this group aborted all developing fetuses (ten fetuses) two days before scheduled death, or both.

TERATOGENICITY.—*Gross abnormalities*—A significant number of malformations was observed throughout the treatment groups, and tended to show a dose-related incidence when considered as percentage of fetuses with malformations. No gross abnormalities were observed in the untreated controls or those injected with distilled water or low dose of acrylic acid, but one malformed fetus was seen in each group treated with saline, cottonseed oil, methyl methacrylate (low dose) and isodecyl methacrylate (all three doses). This represented from 1.8 to 4.2% of the fetuses in the various groups. The greatest number of gross malformations was eight per group, which occurred in the groups given high doses of methyl and ethyl methacrylates; this represented 16.7 and 15.7% of the total, respectively. A complete tabulation of the number and percentage of fetuses in which gross abnormalities were noted is presented in Table 3.

The most common gross abnormality observed was hemangiomas on various parts of the fetus; twisted hind legs occurred less frequently. One fetus in the cottonseed oil group lacked a tail; one in the group given the high dose of ethyl methacrylate had no tail and another was poorly developed and very small, with a compact head and neck.

Skeletal abnormalities.—The number of skeletal abnormalities observed for each group and its percentage of visualized fetuses is presented in Table 3. The abnormality most often seen was elongation, and frequently fusion, of the sternebrae or frontal ribs (bilateral or unilateral), or both; less frequently, one or two ribs were missing.

The incidence of skeletal abnormalities

TABLE 2
EMBRYONIC-FETAL TOXICITY OF A GROUP OF METHACRYLATE ESTERS ON RAT FETUSES

Treatment Groups	Volume Injected (ml/kg)	No. of CL*	Resorptions	Dead Fetuses	Live Fetuses	Mean Weight of Fetuses
Untreated controls		60	0	0	59 (100%)	4.83 ± 0.01
Distilled water	0.8222	53	3 (7.7%)	0	36 (92.3%)	3.82 ± 0.12§
Normal saline	0.8222	60	4 (7.4%)	0	50 (92.6%)	4.15 ± 0.11§
Cottonseed oil	0.8222	53	0	0	50 (100%)	3.85 ± 0.08§
Methyl methacrylate	0.4427	51	3 (5.9%)	0	48 (94.1%)	4.17 ± 0.05§
	0.2656	54	3 (5.7%)	0	50 (94.3%)	4.22 ± 0.06§
	0.1328	53	2 (4.4%)	0	43 (95.6%)	4.22 ± 0.12§
Ethyl methacrylate	0.4076	58	7 (12.1%)	0	51 (87.9%)	3.80 ± 0.08§
	0.2446	53	6 (12.5%)	0	42 (87.5%)	3.30 ± 0.37§
	0.1223	53	5 (9.4%)	0	48 (90.6%)	4.26 ± 0.36
n-Butyl methacrylate	0.7680	54	18 (34.6%)	0	34 (65.4%)	3.86 ± 0.20
	0.4608	58	3 (5.2%)	0	55 (94.8%)	3.95 ± 0.12§
	0.2304	55	1 (2.0%)	0	48 (98.0%)	3.98 ± 0.12§
Isobutyl methacrylate	0.4666	55	9 (16.4%)	0	46 (83.6%)	4.08 ± 0.16§
	0.2799	52	2 (4.0%)	0	48 (96.0%)	4.01 ± 0.06§
	0.1400	51	4 (8.2%)	0	45 (91.8%)	3.89 ± 0.08§
Isodecyl methacrylate	0.8222	54	19 (44.2%)	0	24 (55.8%)‡	3.42 ± 0.20§
	0.4933	57	14 (25.0%)	2 (3.6%)	40 (71.4%)	3.15 ± 0.21§
	0.2467	60	3 (5.1%)	0	56 (94.9%)	4.09 ± 0.08§
Acrylic acid	0.0075	52	3 (6.0%)	3 (6%)	44 (88.0%)	3.80 ± 0.34§
	0.0045	56	2 (3.6%)	0	54 (96.4%)	3.96 ± 0.11§
	0.0023	57	0	0	57 (100%)	4.09 ± 0.08§

Note: Five pregnant female rats were injected in each group on days 5, 10, and 15 of pregnancy.
* Number of corpora lutea.
† Numbers represent the average values (gm) ± the standard error of the mean for each group.
‡ One rat aborted all ten fetuses.
§ Significantly different from untreated controls ($P \leq 0.01$).

TABLE 3
GROSS AND SKELETAL MALFORMATIONS PRODUCED BY METHACRYLATE ESTERS

Treatment Groups	Volume Injected (ml/kg)	Resorptions*	Gross Abnormalities†	Skeletal Abnormalities‡
Untreated controls	...	0	0	0
Distilled water	0.8222	3 (7.7%)	0	1 (5.0%)
Normal saline	0.8222	4 (7.4%)	1 (2.0%)	2 (7.7%)
Cottonseed oil	0.8222	0	1 (2.0%)	4 (15.4%)
Methyl methacrylate	0.4427	3 (5.9%)	8 (16.7%)§	0
	0.2656	3 (5.7%)	4 (8.0%)§	0
	0.1328	2 (4.4%)	1 (2.3%)	0
Ethyl methacrylate	0.4076	7 (12.1%)§	8 (15.7%)§	3 (11.1%)
	0.2446	6 (12.5%)§	5 (11.9%)§	2 (7.7%)
	0.1223	5 (9.4%)§	3 (6.3%)§	1 (5.0%)
n Butyl methacrylate	0.7680	18 (34.6%)§	4 (11.8%)§	2 (10.5%)
	0.4608	3 (5.2%)	2 (3.6%)§	2 (6.7%)
	0.2304	1 (2.0%)	2 (4.2%)§	2 (7.7%)
Isobutyl methacrylate	0.4666	9 (16.4%)§	5 (10.9%)§	2 (8.0%)
	0.2799	2 (4.0%)	3 (6.3%)§	2 (7.7%)
	0.1400	4 (8.2%)	3 (6.7%)§	0
Isodecyl methacrylate	0.8222	19 (44.2%)§	1 (4.2%)§	2 (13.3%)
	0.4933	14 (25.0%)§	1 (2.5%)	1 (4.3%)
	0.2467	3 (5.1%)	1 (1.8%)	0
Acrylic acid	0.0075	3 (6.0%)	4 (9.1%)§	4 (16.7%)§
	0.0045	2 (3.6%)	2 (3.7%)§	3 (10.7%)
	0.0023	0	0	3 (9.7%)

* Percentage resorptions are based on total number of resorptions, dead and live fetuses.
† Percentage gross abnormalities are based on total number of fetuses.
‡ Percentage skeletal abnormalities are based on total number of stained fetuses (~50% of total fetuses).
§ Values greater than the 95% confidence interval of the pooled volume control.

ranged from 0% for the untreated controls, all three dose levels of methyl methacrylate, and the low dose of isobutyl and isodecyl methacrylates, to 16.7% for the high dose of acrylic acid.

FETAL SIZE.—Fetuses from all treated groups of rats were smaller by weight than the untreated controls. These differences were significant at the 99% level ($P < 0.01$) for all groups except the one that received a low dose of ethyl methacrylate. The control fetuses averaged 4.83 gm each, and those from the methacrylates and acrylic acid-treated rats averaged 3.13 to 4.26 gm, respectively (Table 2).

Discussion

Administration of acrylic acid or a methacrylate ester to pregnant rats on days 5, 10, and 15 of gestation generally produced a dose-related increase in resorptions and gross and skeletal abnormalities, and a reduction in fetal weight at birth. The types of abnormalities found with these compounds were quite similar to those reported by Singh, Lawrence, and Autian[13] for a group of phthalate esters. However, the incidences of abnormalities were greater in the phthalate-treated rats.

As shown in Tables 2 and 3, the untreated rats (controls) produced no resorption sites, dead fetuses, gross or skeletal abnormalities, and produced 59 live fetuses from 60 corpora lutea. Thus, if this is used as the base line, all forms of treatment in this study produced an effect in one or more of the parameters studied. A more conservative comparison could be made between the effects produced by acrylic acid and the methacrylates and the results of a "volume control" to compensate for any effects resulting from the physical or mechanical trauma of injection and handling. Because no diluent was used when these compounds were injected and the injected volume ranged from 0.0023 to 0.8222 ml/kg, it was decided to use distilled water, normal saline, and cottonseed oil in a quantity equivalent to the largest dose of the test compound (0.8222 ml/kg).

Because the results obtained from these three "controls" were not uniform and there

was no justification for selecting one of these over the others, the data were combined to calculate a 95% confidence interval[11] for the "pooled volume control" in regard to resorptions, and gross and skeletal abnormalities. The confidence intervals thus obtained were as follows: resorptions, 1.3 to 7.5%; gross abnormalities, 0 to 3.3%; and skeletal malformations, 1.0 to 14.8%. On this basis, it may be seen that all three doses of ethyl methacrylate, the high doses of *n*-butyl and isobutyl methacrylate, and the two highest doses of isodecyl methacrylate significantly increased resorption. The incidence of gross abnormalities was significantly increased in all treatment groups except those given the low doses of methyl methacrylate and acrylic acid, and the two lowest doses of *n*-butyl methacrylate. However, only one group (high dose of acrylic acid) showed an incidence of skeletal malformations that was greater than the confidence interval. These are indicated in the data in Table 3.

Isodecyl methacrylate was an interesting member of the series because of the strong effect this compound had on the developing embryo and fetus. A large number of fertilized ovums were detected only as resorption sites in the rats given the two higher doses, which indicates an early embryotoxic effect. One rat in the group given the high dose aborted (ten fetuses) two days before it was to be killed, thus possibly obscuring dead fetuses; two dead fetuses were found in the group given the middle dose. In spite of isodecyl's embryonic-fetal toxicity, only one gross abnormality (hemangioma) was found in each of the three dose levels. After the skeleton was visualized and stained, two of the 15 visualized fetuses of the group given the high dose had one less rib: one of the 23 in the group given the middle dose had elongated and fused sternebrae and frontal ribs; those in the group given the low dose did not show skeletal malformations.

Another compound, ethyl methacrylate, produced significant embryopathic and teratogenic effects. In the rats given the high dose, one fetus had hemangiomas on the hind leg, one on the foreleg, and three on the shoulders, one had twisted hind legs, one did not have a tail, and another was small in size with compact head and neck. Skeletal examinations of this group showed that 3 of 27 fetuses had elongated and fused ribs (unilateral). In the group given the middle dose, five fetuses had hemangiomas of the neck, and 2 of the 26 stained fetuses had elongated and fused posterior ribs. With the low dose, two fetuses had hemangiomas of the shoulders, one had twisted hind legs, and 1 of the 25 visualized fetuses had a few elongated posterior ribs.

Methyl methacrylate produced a number of gross abnormalities (hemangiomas) in offspring of treated rats, but there were no skeletal malformations observed with any dose used in this study. *n*-Butyl methacrylate produced gross and skeletal deformities. The group given the high dose contained three fetuses with twisted hind legs, and the group given the low dose had one fetus with a compact head and neck and without eyes (anophthalmia). All three dose groups contained one or two fetuses with elongated and fused ribs, and one of the stained fetuses in the group given the high dose was missing one rib. Similar gross and skeletal abnormalities were observed in groups treated with the other methacrylate esters.

Acrylic acid demonstrated considerable activity in fetuses of treated rats. In the group given the high dose, 3 dead fetuses were found, 4 had hemangiomas, and 4 of the 24 stained fetuses had skeletal malformations (elongated and fused sternebrae or frontal ribs or both). In the group given the middle dose, 2 fetuses had hemangiomas of the neck and 3 of the 28 stained fetuses had elongated and fused sternebrae and frontal ribs. In the group given the low dose, 3 of 31 stained fetuses had elongated and fused sternebrae and frontal ribs, but there were no gross abnormalities.

Conclusions

All of the methacrylate esters included in this study had a deleterious effect on the developing embryo and fetus. At one or more of the doses used, each compound produced some or all of the following effects: resorptions, gross and skeletal malformations, fetal death, and decreased fetal size. Untreated rats were used as controls to determine "normal" fetal size and as a base line of normal values. In this group there were no resorptions, gross or skeletal abnormalities, or fetal deaths. A secondary

comparison was made with the use of a 95% confidence interval obtained by pooling data from volume controls of distilled water, normal saline, and cotton-seed oil. When compared with these controls, most of the groups that showed gross abnormalities and half of those that showed resorption were significantly greater than the pooled volume control, but only one group had an incidence of skeletal malformations outside the 95% confidence interval.

The regimen of methacrylate administration elicited significant effects on embryonic and fetal development. The observed incidence of adverse effects generally was dose related and compound dependent, with the more soluble compounds tending to be more active.

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FURTHER EXPERIENCE WITH THE RANGE FINDING TEST IN THE INDUSTRIAL TOXICOLOGY LABORATORY*

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THREE years ago we presented a paper (1) describing the Range Finding Test, which quickly and economically estimates the magnitude of some of the important health hazards of new chemicals. So far as we are aware and much to our surprise, there has been an absence of criticisms. We must confess we had temerity in setting forth such a simplified procedure in an age of growing refinements in techniques. A philosophy similar to ours has been admitted by Bratton (2), who describes a seven-day feeding test with mice which he employed with satisfaction for the preliminary examination of antimalarial agents. Recently one of us has had occasion to explain in some detail a program for the study of new chemicals, and to show the relation of the range finding test to this activity (3).

Far from weakening our faith, the passage of time has increased the value which we attach to this collection of abbreviated procedures. It is only by the use of short-cuts that our group has been able to accommodate in its schedule a reasonable amount of work which we consider advanced, to distinguish it from investigations of the caliber of the range finding test. The latter fills in our repertory a very real need for predicting the order of magnitude of the toxic hazards to be encountered in the production and utilization of a new chemical. The predictions may be sufficiently clear and favorable so that the compound is obviously no more hazardous to produce than a very familiar product; or so unfavorable that it is not manufactured until specially designed equipment can be obtained for synthesizing it safely; or the manufacture may be postponed because the applications which are foreseen for the product would be too hazardous in view of its physiological properties. But always it must be borne in mind that Range Finding data are in no sense final or complete, they must be supplemented by advanced studies before wide application of a chemical can be known to be safe. Our series of papers on the polyethylene glycols (4), not yet finished, represents rather concretely the concept of the many-sided advanced study.

Our earlier paper (1) stressed the speed with which sound approximations can be obtained by the range finding test. A period of three weeks was named as the time required for completion of the work. This estimate implied the existence of a staff ready to function upon receipt of a sample, and supplied at all times with suitable animals. In special situations the period elapsing before useful information is available can be much reduced. Within the past few years reassuring or cautionary statements have been issued as promptly as twenty-four hours after a sample was received, later to be amplified at greater leisure. Experience has now shown that such celerity is seldom required, and that it is uneconomical, if not actually impossible, to maintain a laboratory prepared to commence all the procedures of the range finding test the moment an express shipment is received.

Experience has further revealed that in the majority of cases some of the steps of the test may be omitted without prejudicing the applicability of the results to the immediate need. More and more as time passes, the steps performed are tailored to fit the specific need. A chemical may have such a low vapor pressure that inhalation is obviously of no concern to the industrial hygienist, a sample may be so scant that only those tests requiring minimum volumes can be performed, or the question accompanying the sample may be so specific that only one operation is required to obtain an answer. Happily this latter situation arises more often each year as those who originate requests grow more familiar with the nature of toxicological data and their own needs for information. The all-inclusive question, "What is the toxicity of such and such a compound?", which would require a life time to answer, has almost vanished from our correspondence.

There are few standardized procedures which remain unchanged over several years and the details of the range finding test have never been regarded as more than tentative. It is not sur-

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prising therefore, that if the test were to be reduced to formal specifications today, it would be found to differ in several perhaps important respects from the description given in 1944. The differences will be discussed under the head of each separate step in the collection.

Single Dose Oral Toxicity

During the war our supply of Wistar strain rats was eliminated by the drafting of a commercial breeder. Comparison of several available strains allowed us to select the Sherman as an approximation to the Wistar in resistance to the acute toxicity of some of the more important chemicals which concerned us. Accordingly, the loss of the Wistar strain did not vitiate our files of acute toxicity data.

The LD_{50} is still bracketed by the use of groups of 6 rats given dosages differing in a ratio of 10. But now the approximation is often made closer by interpolating two groups of 10 rats, each of which suffers fractional mortality. Our standard dosage series, when expressed in gm./kg., constitutes the antilogarithms of 1.0, 1.1, 1.2, etc., and for range finding tests alternate dosages in the series are employed; the larger therefore exceeds the smaller by 58 per cent. These additional animals do not materially increase the time required for the test.

It is recognized that dependence upon one species is hazardous, accordingly for advanced studies other species are added.

Sub-acute Oral Toxicity

One of the serious deficiencies of the earlier description was the absence of any criterion of chronic toxicity. This defect has been remedied by administering doses to groups of 10 rats each, 5 of each sex in one cage, for thirty days. The sample is incorporated in a diet of 1000 gms. ground whole wheat, 500 gms. dried whole milk, and 20 grams of iodized salt, freely available to the animals in modified McCollum feeding cups from which very little is spilled. Water is provided in siphon drinking bottles and rarely it, rather than the diet, is the carrier of the chemical. The diet for each cage is kept in a separate jar which is weighed before and, together with uneaten food in the cup, after the thirty-day period. Three or four dosage levels differing by a ratio of 2 or 4 are administered, together with a control diet.

The criteria of injury in this step are effects on the increase in body weight from the approximate 120-gram starting weight, reduction in appetite, death, and micropathology of adrenal, upper intestine, kidney, liver and spleen. Table 1 sets forth typical results from such a test. It is apparent from the data that dosages were selected with the aim of allowing at least one group of rats to survive without injury as judged by the limited but sensitive criteria, rather than causing many fatalities. If the latter goal is pursued many rats will voluntarily starve themselves owing to the objectionable taste of diets containing high levels of chemical, and one will be uncertain of the cause of death. Daily forced feeding would avoid this doubt but would entail too much labor to be justified in a range finding test. Usually pathological study adds little quantitative information to the other observations, and it is doubtful if the operation is worthwhile unless the thirty-day feeding is regarded as a preliminary step to a two-year study where advanced work on a chemical justified the expense.

Planning of dosage levels is simplified by the observation that, under the conditions specified, a level of 1 per cent in the diet results in an average dosage of close to 0.6 gm./kg./day and 1 per cent in the water produces 0.8 gm./kg./day.

Skin Absorption

The factor of absorption through the intact skin grows more important each year. The previous communication (1) noted a shift from the four-day poultice upon guinea pigs to the one-day rubber cuff application upon rabbits of the Food and Drug Administration (5). The change is now complete and poultice data are regarded as obsolete because the saturated pad prevents complete contact of the dose with the skin. Dosages as great as 20 ml./kg. can be retained by the cuff, or dam, as it is known to us. The numbers of animals used are similar to those given oral dosages.

Vapor Exposure

The estimation of hazard by inhalation of saturated vapors has been amplified by adding the inhalation of vapors of known concentration, aiming at a concentration which produces fractional mortality as a result of a four-hour exposure. Concentrations used fall in a series differing by a ratio of 2, and they are approximations because they are estimated from the settings of a proportioning pump and flowmeter rather than being determined analytically. The latter procedure is

TABLE 1

TYPICAL RESULTS WITH RANGE FINDING TEST FOR SUB-ACUTE ORAL TOXICITY

Materials Administered in the Food to Groups of 10 Rats for 30 Days on each Dosage Level Unless Noted Otherwise

MATERIAL STUDIED	RANGE OF DOSAGE LEVELS FED		LEAST DAILY DOSAGE CAUSING SYMPTOM				MAXIMUM DOSAGE HAVING NO EFFECT	SINGLE DOSE ORAL LD_{50} (2)
	Maximum	Minimum	Reduced growth	Reduced appetite	Micropathology (1)	Death		
	gm./kg.	gm./kg.	gm./kg.	gm./kg.	gm./kg.	gm./kg.	gm./kg.	gm./kg.
Acetophenone	0.102	0.001	—	—	—	—	>0.102	0.90 (0.81 to 1.00)
Acetylethanolamine	1.93	0.30	—	—	—	—	>1.93	27.66 (25.24 to 30.31)
Antimony trioxide	1.07	0.06	1.07	1.07	1.07	—	0.27	>20
Butyl "Carbitol" (3, 4)	1.83	0.051	—	0.094	0.65	—	0.051	6.56 (5.47 to 7.86)
Butyl phthalyl butyl glycolate	1.56	0.10	1.56	—	1.56	—	0.45	14.64 (11.73 to 18.28)
Calcium ethyl acetoacetate	0.34	0.014	—	—	0.060	—	0.014	9.93 (9.22 to 10.70)
"Carbitol" solvent (3, 4)	3.20	0.41	1.83	3.20	0.41	—	<0.41	9.05 (8.43 to 9.72)
"Carbitol" acetate (3, 4)	3.20	0.23	3.20	1.80	0.23	3.20	<0.23	11.00 (10.40 to 11.59)
Diacetone alcohol (3)	0.13	0.01	—	—	0.04	—	0.01	4.0 RF
Dibutyl "Cellosolve" phthalate	1.74	0.11	1.74	1.74	1.74	—	0.50	8.38 (6.86 to 10.23)
Diethylbutyl "Cellosolve" succinate	1.44	0.17	1.44	1.44	0.44	0.17	<0.17	3.3 RF
Diethylene glycol ethyl ether (pure) (3, 4)	3.88	0.21	1.77	0.87	1.77	—	0.49	8.69 (7.25 to 10.41)
Ethoxy triethylene glycol (3)	3.30	0.18	3.30	3.30	3.30	3.30	0.75	10.61 (9.98 to 11.28)
Ethylhexanimido diethyl di(ethyl hexanoate)	1.53	0.10	—	—	—	—	>1.53	>39.8
Glycidyl oleate	0.35	0.014	—	—	—	—	>0.35	3.52 (3.35 to 3.69)
Heptadecyl glyoxalidine hydrochloride (3)	0.26	0.06	0.16	—	0.26	0.26	0.06	1.97 (1.70 to 2.32)
Methyl Acetyl Ricinoleate	1.82	0.12	—	—	—	—	>1.82	>50
Methyl "Carbitol" (3, 4)	1.83	0.19	1.44	0.79	0.19	—	<0.19	9.21 (7.90 to 10.71)
Methyl "Cellosolve" acetyl Ricinoleate	1.50	0.11	1.50	1.50	—	—	0.45	20.27 (15.13 to 27.15)
Methyl phthalyl ethyl glycolate	1.90	0.11	—	—	1.90	—	0.48	9.06 (8.11 to 10.13)
Propylene glycol ethyl ether (beta isomer) (3)	2.14	0.16	2.14	2.14	—	—	0.68	8.93 (7.89 to 10.09)
Sodium benzoate	1.09	0.016	—	—	—	—	>1.09	4.07 (3.72 to 4.44)
Sodium sorbate	1.08	0.016	—	—	—	—	>1.08	7.16 (6.82 to 7.52)
Sorbic acid	1.27	0.011	0.048	—	0.048	—	0.011	7.36 (6.69 to 8.90)
"Tergitol" penetrant 08 (3, 4)	1.51	0.23	1.24	1.24	0.80	—	0.44	10.32 (9.05 to 11.45)
"Tergitol" penetrant 4 (3, 4)	0.73	0.22	0.35	0.35	0.22	—	<0.22	4.95 (4.42 to 5.53)
"Tergitol" penetrant 7 (3, 4)	0.74	0.25	0.55	0.36	0.41	—	0.25	5.66 (4.85 to 6.60)
Triisopropanolamine (3, 4)	1.35	0.14	1.35	1.35	0.26	—	0.14	6.50 (5.62 to 7.54)
Triethylhexyl phosphate	1.55	0.11	1.55	—	—	—	0.43	37.08 (33.70 to 40.79)

(1) Dosage level producing micropathology in liver, kidney, spleen or testis of any rat.

(2) When LD_{50} was derived from an advanced test the range of 1.96 times the standard deviation is shown in parentheses. The letters "RF" refer to data derived from a range finding test. The symbol ">" indicates that the dosage shown is the maximum which could be administered and it killed none of 6 rats.

(3) Dose administered in the drinking water. In this case appetite is judged on the basis of ml. of water consumed.

(4) Only 5 rats to a dosage level.

TABLE 2

Typical Results with Range Finding Test

Capital Letters Designate Chemicals Having the Respective Hazard in the Same Order of Magnitude as the Material Studied, and Refer to the List Following the Table

Material Studied	Single Dose Oral Toxicity to Rats		Penetration of Rabbit Skin*		Saturated Vapor Inhalation by Rats		Inhalation of Known Vapor Concentration by Rats				Primary Skin Irritation	Eye Injury from Fluid	
	R.F. LD_{50}		R.F. LD_{50}		Maximum for no death		Concentration	Time	Mortality in 11 days			Grade	
	gm./kg.		*ml./kg.*				*PPM.*	*hrs.*					
Acetol	2.2	A	>16.0	U	—	—	2000	8	0/6	O	V	5	V
Acetonitrile	3.8	E	5.0	O	—	—	3000	4	1/6	Q	V	5	V
Acrylonitrile	0.093	N	(0.25)	N	1 min.	A′	500 1000	4 4	0/6 6/6	L	V	4	U
Allyl alcohol	0.064	N	0.053	A′	—	—	1000	1	4/6	A′	V	5	V
Allyl chloride	0.7	B	2.2	L	—	—	2000	4	1/6	A	V	2	Z
Allylidene diacetate	0.25	A′	0.32	N	—	—	8	4	4/6	T	C′	10	W
Chloropropane diol—1,3	0.11	N	0.45	B	—	—	1000	1	5/6	N	V	4	U
Chlorostyrene	5.2	O	20.0	Z	8 hr.	Z	—	—	—	—	U	2	Z
Cyanomethyl acetate	0.032	>N	0.043	A′	—	—	16 32	4 2	0/6 6/6	T	V	5	V
Diacetone alcohol	4.0	O	14.5	Z	8 hr.	Z	—	—	—	—	V	5	V
Dichloroethyl ether	0.075	N	(0.3)	N	—	—	1000	4	3/6	A′	U	4	U
Dichloroethyl formal	0.065	N	(0.17)	N	—	—	60 120	4 4	0/6 6/6	N	V	1	Z
2,3-Dichloropropanol	0.09	N	0.2	N	—	—	500	4	2/6	L	U	5	V
Dichlorostyrene	4.0	O	9.0	I	8 hr.	Z	—	—	—	—	T	5	V
Dimethyl dioxane	3.0	O	>10.0	Z	—	—	8000	4	2/6	Q	B′	5	V
Dimethyl formamide	7.0	J	5.0	O	4 hr.	I	—	—	—	—	B′	4	U
Epichlorohydrin	0.09	N	1.3	A	—	—	250	8	4/6	A′	U	4	U
Ethoxy propionaldehyde	0.9	B	1.0	A	—	—	500	4	6/6	A′	V	7	N
Ethoxy propionic acid	4.8	E	0.75	A	—	—	2000	4	0/6	A	C′	9	W
Ethyl chlorobenzene	5.0	O	17.5	Z	8 hr.	Z	—	—	—	—	B′	2	Z
Ethyl dichlorobenzene	5.5	O	14.2	Z	8 hr.	Z	—	—	—	—	C′	1	Z
Ethylene imine	0.015	>N	(0.014)	A′	—	—	50 250	8 2	5/6 3/6	T	T	7	N
Ethylidene acetone	0.32	A′	0.5	B	—	—	250	4	3/6	A′	U	8	B
Hexane diol—2,5	5.0	O	16.3	Z	8 hr.	Z	—	—	—	—	V	3	S
Isopropanol	5.84	V	16.4	Z	—	—	16000	8	4/6	Q	—	4	U
Methyl acetoacetate	3.0	O	(>10)	Z	8 hr.	Z	—	—	—	—	U	7	N
Methyl acrylate	0.3	A′	1.3	A	—	—	1000	4	3/6	L	U	4	U
Methylbenzyl "cellosolve"	2.29	A	5.0	O	4 hr.	I	—	—	—	—	—	3	S
Methyl "cellosolve" acetate	3.39	O	5.25	O	—	—	7000	4	2/6	Q	—	2	Z
Methyl diacetoacetate	1.7	A	(>10)	Z	8 hr.	Z	—	—	—	—	C′	5	V
2-Methyl pentane-2,4-diol	4.70	O	13.30	Z	8 hr.	Z	—	—	—	—	U	4	U
Morpholine ethanol	12.06	S	(2.5)	L	8 hr.	Z	—	—	—	—	V	5	V
Propylene imine	0.019	>N	(0.043)	A′	—	—	500	4	5/6	L	—	9	W
Propylene oxide	1.14	B	(8.64)	I	—	—	4000	4	6/6	A	V	5	V
Tetramethyl ethylene diamine	1.58	A	7.0	I	10 min.	L	—	—	—	—	B′	8	B
Vinyl acetate	2.92	O	2.5	L	—	—	4000	4	3/6	O	V	2	Z

* Figures in parentheses were determined by poultices on guinea pigs.

COMPARATIVE MATERIALS REFERRED TO BY LETTER CODE IN TABLE 2

The words "Cellosolve" and "Carbitol" are trade marks of Carbide and Carbon Chemicals Corporation for the solvents that are ethers of ethylene and diethylene glycol respectively. These letter codes have the same significance as in our 1944 article (1). The symbol > before a letter in the table means significantly more toxic than

A	Methyl "Cellosolve" solvent	S	Ethanol
B	Formaldehyde	T	Dimethyl sulfate
E	Lactic acid	U	Ethyl acetoacetate
I	"Cellosolve" acetate solvent	V	Acetone
J	Triisopropanolamine	W	Ammonium hydroxide (28% NH_3)
L	Mesityl oxide	Z	"Carbitol" solvent
N	Ethylene chlorhydrin	A′	Crotonaldehyde
O	"Cellosolve" solvent	B′	Benzene
Q	Cyclohexanone	C′	Diethanolamine

impractical if a new vapor is to be handled every day.

At times there is appraised the hazard of certain operations which result in the creation of a mist by bubbling air through the sample held at the arbitrary temperature of 170° C., cooling the air, and furnishing it to animals in a small chamber. Limited data obtained in this way have been published (6). An attempt is being made to standardize the creation of a fog by the use of a nebulizer, but uniform conditions have not yet been attained. This exposure would reduce the oxidation of samples, which may be important in the 170° mist test.

SKIN IRRITATION

Tests of skin irritation are very difficult to standardize and quantitate, and no further progress in this direction has been achieved.

EYE INJURY

A full description of the eye injury test has recently appeared (7) together with a large amount of data, and no time will be spent upon the procedure here.

SKIN SENSITIZATION

A second deficiency recognized in the range finding test was the absence of any test for sensitization. During the past two years the Landsteiner guinea pig skin sensitization procedure has been studied. After standardization of an intradermal technique which appeared to yield reliable results, the test was applied to some 40 chemicals of widely different compositions. For at least half of these chemicals there were available for comparison the results of human patch tests, human experience, or experience with closely similar molecules. The results were disappointing. It was found that the guinea pig test did not miss chemicals which were active human sensitizers but that it did condemn others which have been used for years upon human skins without objectionable reactions. It was found to cost as much to perform as a human patch test series done by a consulting dermatologist and to require as much time. The guinea pig test therefore offers no advantages over human application and is no longer regarded as a part of the range finding test.

Materials for which preliminary skin sensitization data are desired are assembled in groups of 8 and referred to a consulting dermatologist who applies them to 30 human subjects. The schedule is one week of contact, about two weeks for development of sensitivity, and two to three days second contact to detect sensitization.

DISCUSSION

Table 2 presents examples of the data obtained with the range finding test. Skin sensitization has not been included and sub-acute oral data are in Table 1. It will be seen that we still lean heavily upon comparisons with more familiar molecules. This part of the test is perhaps the most indefensible because of pharmacological differences between even close analogs. But it is so valuable in allowing industrial technologists to visualize the meaning of toxicological information in terms of chemicals they hand'e every day, that the comparison is indispensable.

It must be emphasized that the range finding test is not believed to yield final or complete toxicological data. Its results are sound indications, but they remain tentative until confirmed and extended. The test can condemn a particular

application of a chemical but it cannot establish its safety. It must also be emphasized that the components are separable, and that rarely are all the steps performed upon a single material. The parts selected depend upon the information desired.

Summary

There are presented elaborations of the Range Finding Toxicity Test in respect to sub-acute toxicity, vapor inhalation, and skin sensitization. Further examples of the use of the test are given.

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SAFETY in the Chemical Laboratory

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...a ChemEd feature

XLVI. Hazardous Chemicals Data* (Continued)

NATIONAL FIRE PROTECTION ASSOCIATION, 60 Batterymarch Street, Boston, Mass. 02110

Editor's Note

This issue contains further examples of chemical information recorded in "Hazardous Chemicals Data," NFPA No. 49.

Special permission was obtained to reprint these examples and the explanatory section in the previous issues, because it is our opinion that "Hazardous Chemicals Data" should be known and used as a reference in teaching, clinical, and research laboratories.

Hydrogen Peroxide (52% by weight or greater) H_2O_2

DESCRIPTION: Clear, colorless, water-like liquid; standard concentrations are 70% and 90%; concentrations to 100% may be encountered.

FIRE AND EXPLOSION HAZARDS: Powerful oxidizing agent. In concentrations above 65% H_2O_2, insufficient water is present to remove the heat of decomposition by vaporization. The adiabatic decomposition temperature of 90% H_2O_2 is 1364°F., so that most combustible materials in contact with the decomposition products will quickly burst into flame. Unstable; but commercial products normally are inhibited. An increase in temperature increases the decomposition rate of hydrogen peroxide about 1½ times for each 10°F. Violent decomposition may be caused by contact with many substances such as iron, copper, chromium, brass, bronze, lead, silver, manganese, etc., and their salts. At ordinary temperatures, hydrogen peroxide in concentrations up to about 90% does not readily detonate. Higher concentrations or elevated temperatures may facilitate detonation. Sensitive and powerful explosive mixtures are obtained on mixing many organic materials with hydrogen peroxide; organic materials, such as alcohols, glycerol, etc., soluble in peroxide, create very hazardous mixtures. Contaminated hydrogen peroxide can decompose at a rate that will exceed the capacity of the vent on the container. Its decomposition can be self-accelerating and result in an explosive pressure rupture of the container.

LIFE HAZARD: Extremely irritating to eyes, skin, and respiratory tract; can result in serious burns. Avoid inhalation of vapor and liquid contact with skin or eyes. Threshold limit value is one part per million. Wear goggles and self-contained breathing apparatus.

FIRE FIGHTING PHASES: Use water in ample quantities to dilute and wash away spills and combat fires involving this material. Other extinguishing agents will probably not be effective. Wear goggles and self-contained breathing apparatus.

USUAL SHIPPING CONTAINERS: Glass bottles of 1-quart capacity or less, aluminum drums less than 30-gallon capacity, and aluminum tank cars up to 8,000-gallon capacity. All containers must be vented.

STORAGE: Protect containers against physical damage. Store in cool, ventilated areas. Drums should not be stacked; should always be stored and handled in an upright position. Flooding or water flushing systems should be available to wash away spills. Storage should be remote from combustible materials or catalytic metals (see FIRE AND EXPLOSION HAZARDS above).

REMARKS: See Chemical Safety Data Sheet SD-53, Supplement B (Manufacturing Chemists' Association, Inc.).

Hydrogen Sulfide H_2S

Description: Colorless gas; offensive strong odor similar to rotten eggs.

FIRE AND EXPLOSION HAZARDS: Flammable gas. Forms explosive mixtures with air over a wide range. Flammable limits, 4.3% and 45%. Ignition temperature, 500°F. Heavier than air (vapor density, 1.2) and may travel considerable distance to a source of ignition and flash back. Dangerously reactive with fuming or strong nitric acid and powerful oxidizing materials.

LIFE HAZARD: Highly toxic. Irritating to eyes and respiratory tract. Threshold limit value, 10 parts per million; high concentrations cause almost immediate death. Wear goggles and self-contained breathing apparatus.

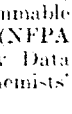
FIRE FIGHTING PHASES: Stop flow of gas. Use water to keep containers cool. Do not extinguish unless necessary to effect an immediate shutoff of flow. Dry chemical and carbon dioxide can be used to extinguish hydrogen sulfide fires. Wear goggles and self-contained breathing apparatus.

USUAL SHIPPING CONTAINERS: Steel pressure cylinders.

STORAGE: Protect against physical damage. Storage should be well isolated, detached, ventilated, and of fire-resistive construction. Store away from nitric acid, strong oxidizing materials, corrosive liquids or gases, cylinders or other containers under high pressure, and possible sources of ignition. Protect against static electricity, direct sunlight and excessive heat.

REMARKS: Electrical installations in Class I hazardous locations, as defined in Article 500 of the National Electrical Code, should be in accordance with Article 501 of the Code; and electrical equipment should be suitable for use in atmospheres containing hydrogen sulfide gas. See Explosion Venting Guide (NFPA No. 68), Static Electricity (NFPA No. 77), Fire-Hazard Properties of Flammable Liquids, Gases and Volatile Solids (NFPA No. 325M), and Chemical Safety Data Sheet SD-36 (Manufacturing Chemists' Association, Inc.).

Isopropyl Ether $(CH_3)_2CHOCH(CH_3)_2$



DESCRIPTION: Colorless, volatile liquid with ethereal odor.

FIRE AND EXPLOSION HAZARDS: Flammable liquid. Flammable limits, 1.4% and 7.9% and flammable mixtures with air are formed readily at room temperatures. Flash point, minus 18°F. (cc). Boiling point, 155°F. Ignition temperature, 830°F. Vapor-air density at 100°F. is 1.47. Vapor is heavier than air and may travel a considerable distance to a source of ignition and flash back. Unstable peroxides may form on long standing in contact with air and these may explode spontaneously or when heated.

LIFE HAZARD: The main action is anesthetic and high concentrations of the vapor in air can cause unconsciousness. Threshold limit value, 500 parts per million.

FIRE FIGHTING PHASES: Isopropyl ether is lighter than water and only slightly soluble in water; water may spread a spill fire. Use "alcohol" foam, carbon dioxide or dry chemical.

USUAL SHIPPING CONTAINERS: Glass bottles, steel drums and cans.

STORAGE: Protect against physical damage. Detached outside storage is preferred. Inside storage should be in a standard flammable liquids storage room or cabinet. Isolate from other combustible materials. Avoid direct sunlight. Protect against static electricity and lightning. For large quantity storage rooms, protect with automatic sprinkler systems and total flooding carbon dioxide systems. The

(Continued on page A314)

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reactivity hazard may be increased to 3 on long standing, due to peroxide formation.

REMARKS: Electrical equipment in Class I hazardous locations, as defined by Article 500 of the National Electrical Code, should be installed in accordance with Article 501 of the Code; and electrical equipment should be suitable for use in atmospheres of isopropyl ether. See National Electrical Code (NFPA No. 70) and Flammable and Combustible Liquids Code (NFPA No. 30).

Methyl Acrylate $CH_2{:}CHCOOCH_3$

DESCRIPTION: A colorless, volatile liquid with an acrid odor.

FIRE AND EXPLOSION HAZARDS: Flammable liquid. Vapor forms explosive mixtures with air. Flammable limits, 2.8% and 25%. Ignition temperature not established. Flash point, 27°F. (oc). Boiling point, 176°F. Specific gravity, 0.95. Vapor is heavier than air (vapor-air density at 100°F., 1.5) and may travel a considerable distance to a source of ignition and flash back. Usually contains an inhibitor to prevent self-polymerization. At elevated temperatures, as in fire conditions, polymerization may take place. If the polymerization takes place in a closed container, there is possibility of violent rupture of the container.

LIFE HAZARDS: Marked respiratory irritant. Prolonged contact with skin or eyes may cause severe damage. Threshold limit value, 10 ppm. Wear self-contained breathing apparatus.

FIRE FIGHTING PHASES: Only slightly soluble in water. Water spray may be ineffective. Use carbon dioxide, dry chemical or foam. Cool tanks or drums containing this material with hose streams to prevent polymerization. Wear self-contained breathing apparatus.

USUAL SHIPPING CONTAINERS: Bottles and cans in fiber or wooden boxes, drums, tank cars, tank trucks, tank barges.

STORAGE: Protect against physical damage. Outside or detached storage preferable. Inside storage should be in a standard flammable liquids storage room or cabinet. Avoid storage temperature in excess of 100°F. Separate from oxidizing materials. Vapors are uninhibited and may form polymers in vents or flame arrestors, resulting in stoppage of vents.

REMARKS: Electrical installations in Class I hazardous locations as defined in Article 500 of the National Electrical Code, should be in compliance with Article 501 of the Code. Electrical equipment should be suitable for use in atmospheres containing methyl acrylate vapors. See Flammable and Combustible Liquids Code (NFPA No. 30), National Electrical Code (NFPA No. 70), Static Electricity (NFPA No. 77), Fire-Hazard Properties of Flammable Liquids, Gases and Volatile Solids (NFPA No. 325M), and Chemical

(Continued on page A317)

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Safety Data Sheet SD-79 (Manufacturing Chemists' Association, Inc.).

Nitrobenzene $C_6H_5NO_2$

DESCRIPTION: Oily, pale to dark yellow or brown liquid, with a characteristic odor.

FIRE AND EXPLOSION HAZARDS: Combustible liquid; vapors form explosive mixtures with air. Flammable limits not reported. Flash point 190°F. Ignition temperature 900°F.

LIFE HAZARD: Highly toxic; fatal amounts may be absorbed through the skin, by inhalation, or ingestion. Threshold limit value, 1 part per million. Wear complete protective clothing.

FIRE FIGHTING PHASES: Use water spray, carbon dioxide, dry chemical or foam. Wear complete protective clothing.

USUAL SHIPPING CONTAINERS: Glass in fiberboard or wood boxes, 55-gallon metal drums, tank cars.

STORAGE: Protect against physical damage, freezing, and intense heat. Separate from oxidizing materials. Detached storage is preferred. Inside storage should be in a standard flammable liquids storage room or cabinet.

REMARKS: See Flammable and Combustible Liquids Code (NFPA No. 30), Fire-Hazard Properties of Flammable Liquids, Gases and Volatile Solids (NFPA No. 325M), and Chemical Data Safety Sheet SD-21 (Manufacturing Chemists' Association, Inc.).

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Sunshine, Irving

1969

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The Chemical Rubber Company, Cleveland, Ohio
Pages 628-629; 640-641

Farmakol. Toksikol. 32(1):105-107

Changes in the Content of Protein Fractions in the Blood Serum of Rabbits on Application of Methyl Acrylate to their Integument

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Source: Unknown (?) - (not given)

At present, the methyl ester of acrylic acid (methyl acrylate) is widely used in the chemical industry (in the production of nitron, plastics, rubber, etc.). There is little information on the toxicity of this product. Taking into account the property of methyl acrylate to penetrate through the epidermis and exert a resorptive action (E. I. Veling and I. N. Arkhangel'skaya), we observed the level of total protein and protein fractions in the blood serum of rabbits following application of this product to their integument.

Method

Two series of tests were carried out on 60 rabbits (30 animals in each series). Fractionation of proteins was done by the method of horizontal paper electrophoresis in a Veronal-Medinal buffer.

In the first series of tests, 1 ml pure methyl acrylate was applied twice to the left flank of the animal with a replacement of the bandage every 24 hours. Blood used in the study was taken from the marginal (limbic) ear vein prior to the test and on the 10th, 20th, and 30th day after application of the product.

In the second series of tests, 4 ml of a 1% methyl acrylate solution was applied to the skin of rabbits for 60 days with a replacement of the bandage every 24 hours. Blood used in the study was taken prior to application of the solution, on the 20th, 40th, and 60th day of the test, and 20 days after termination of the application.

Results

In the first series of tests, an insignificant increase of the albumin level ($P < 0.02$) and a decrease of α-globulins ($P < 0.001$) was noted on the 10th day. The content of δ-globulins dropped significantly ($P < 0.001$). Since, according to some authors (S. Ya. Kaplanskii and A. E. Gurvich; I. A. Oyvin), changes in the content of this globulin fraction are associated with changes in the synthesis of protective (defense) proteins, such a decrease of δ-globulins may indicate a lowering of the defense forces of the organism. A certain increase of total protein and β-globulins was found to be statistically unreliable.

On the 20th day of the test, the total protein level dropped ($P < 0.02$). The decrease in the amount of albumins was statistically unreliable. The content of α- ($P < 0.01$) and β-globulins ($P < 0.001$) increased. The level of δ-globulins still remained low ($P < 0.001$).

On the 30th day, the content of total protein, albumins and globulins was close to the initial content, while the discrepancies found were statistically unreliable. On the content of α-globulins remained high ($P < 0.001$).

In the second series of tests, on the 20th day after application of methyl acrylate, a lowering of the total protein level ($P < 0.002$) and of the α-globulin fraction ($P < 0.002$) was noted. There was an insignificant increase in β- and δ-globulins ($P < 0.05$). The decrease of albumins was statistically unreliable.

On the 40th day, the content of total protein and albumins dropped ($P < 0.001$). The amount of α- and β-globulins increased ($P < 0.01$ and < 0.001, respectively). The content of δ-globulins did not change significantly.

On the 60th day, there was an insignificant decrease in the amount of α-globulins, as compared with the 40th day ($P < 0.05$). The content of

β-globulins remained high ($P < 0.001$), while that of albumins was low ($P < 0.001$). There was a sharp increase in the fraction of δ-globulins ($P < 0.001$), and the amount of total protein also increased (statistically reliable in comparison with the 40th day; $P < 0.001$). Such a parallel increase of protein and δ-globulins by the 60th day of the test, with a drop in albumins, can be considered as an adaptation reaction of the organism, during which hypoalbuminemia is compensated to a certain extent and the water equilibrium between blood and tissues remains constant (I. A. Oyvin). At the same time, we should not exclude such changes in the liver, which lead to the appearance in the serum of immunologically modified proteins capable of acting as autoantigens (S. A. Kaplanskii).

Twenty days after application of methyl acrylate was terminated, the amount of total protein decreased sharply and was even lower than the original values ($P < 0.001$). In our opinion, this phenomenon can be explained as due to a lowered (reduced) biosynthesis of β- and δ-globulin fractions. The content of albumins increased (in comparison to the 60th day; $P < 0.01$), while β- and δ-globulins returned to their normal values. The albumin-globulin ratio increased, and only the level of α-globulins, as in the first series of tests, remained high ($P < 0.02$).

Conclusions

1. Methyl acrylate, by penetrating into the organism of animals through the integument, can cause changes in the protein metabolism and particularly in the ratio of individual protein fractions.
2. A twofold application of 1 ml pure methyl acrylate on the skin of rabbits results in a noticeable lowering (decrease) in the level of δ-globulins in blood serum.
3. Prolonged action of a 1% methyl acrylate solution (in doses of 4 ml) on the skin of rabbits results, on the 60th day, in an increased level of δ-globulins and a drop of the albumin-globulin ratio.

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Farmakol. Toksikol. 32 (1): 105-107, 1969

УДК 612.457.015.32:546.72

ИЗМЕНЕНИЕ СОДЕРЖАНИЯ БЕЛКОВЫХ ФРАКЦИЙ СЫВОРОТКИ КРОВИ КРОЛИКОВ ПРИ НАНЕСЕНИИ ИМ НА КОЖНЫЙ ПОКРОВ МЕТИЛОВОГО ЭФИРА АКРИЛОВОЙ КИСЛОТЫ

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Метиловый эфир акриловой кислоты (метилакрилат) в настоящее время нашел широкое применение в химической промышленности (производство нитрона, пластических масс, каучука и т. д.). Сведения о токсичности этого вещества немногочисленны. Учитывая свойство метилакрилата проникать через эпидермис и оказывать резорбтивное действие (Е. И. Велинг и И. Н. Архангельская), мы провели наблюдение за уровнем общего белка и белковых фракций сыворотки крови у кроликов после нанесения им на кожный покров этого вещества.

Методика

Были поставлены две серии опытов на 60 кроликах (по 30 животных в каждой). Фракционирование белков производили методом горизонтального электрофореза на бумаге в веронал-мединаловом буфере.

В I серии опытов чистый метилакрилат наносили двукратно по 1 мл на левый бок животного со сменой повязки через 24 часа. Кровь для исследования брали из краевой вены уха до опыта и на 10, 20 и 30-й день после нанесения препарата.

Во II серии опытов по 4 мл 1% раствора метилакрилата наносили на кожу кроликов в течение 60 дней со сменой повязки через 24 часа. Кровь для исследования брали до нанесения раствора, на 20, 40 и 60-й день опыта и через 20 дней после прекращения нанесения.

Результаты

В I серии опытов на 10-й день опыта отмечали незначительное увеличение уровня альбуминов ($P<0{,}02$) и уменьшение α-глобулинов ($P<0{,}01$). Значительно снижалось содержание γ-глобулинов ($P<0{,}001$). Так как изменения в содержании этой фракции глобулинов некоторые авторы (С. Я. Капланский и А. Е. Гурвич; П. А. Ойвин) связывают с изменениями в синтезе защитных белков, такое уменьшение γ-глобулинов может свидетельствовать о понижении защитных сил организма. Некоторое повышение общего белка и β-глобулинов было статистически недостоверно.

К 20-му дню опыта уровень общего белка снижался ($P<0{,}02$). Уменьшение количества альбуминов было статистически недостоверно. Повышалось содержание α- ($P<0{,}01$) и β-глобулинов ($P<0{,}001$). Уровень γ-глобулинов оставался все еще низким ($P<0{,}001$).

К 30-му дню содержание общего белка, альбуминов и глобулинов приближалось к исходному, а имеющиеся расхождения были статистически недостоверны. Только содержание α-глобулинов оставалось повышенным ($P<0{,}001$).

Во II серии опытов на 20-й день после нанесения метилакрилата отмечалось снижение уровня общего белка ($P<0{,}002$) и α-глобулиновой фракции ($P<0{,}002$). Имелось незначительное увеличение β- и γ-глобулинов ($P<0{,}05$). Уменьшение альбуминов статистически недостоверно.

На 40-й день начало содержание общего белка и альбуминов ($P<0{,}001$). Количество α- и β-глобулинов возрастало (соответственно $P<0{,}01$ и $<0{,}001$). Содержание γ-глобулинов существенно не менялось.

К 60-му дню опыта незначительно снижалось количество α-глобулинов (по сравнению с 40-м днем; $P<0{,}05$). Содержание β-глобулинов оставалось повышенным ($P<0{,}001$), а альбуминов — пониженным ($P<0{,}001$). Резко возрастала фракция γ-глобулинов ($P<0{,}001$) и увеличивалось количество общего белка (статистически достоверно по сравнению с 40-м днем; $P<0{,}001$). Такое параллельное увеличение к 60-му дню опыта белка и γ-глобулинов при снижении альбуминов можно расценивать как приспособительную реакцию организма, при которой до некоторой степени компенсируется гипоальбуминемия и поддерживается постоянство водного равновесия между кровью и тканями (П. А. Ойвин). При этом не исключены и такие изменения в печени, которые ведут к появлению в сыворотке иммунологически измененных белков, способных действовать как аутоантигены (С. Я. Капланский).

Через 20 дней после прекращения нанесения метилакрилата количество общего белка резко снижалось и было даже меньше исходных величин ($P<0{,}001$). Это явление, на наш взгляд, можно объяснить уменьшением биосинтеза β- и γ-глобулиновых фракций. Содержание альбуминов повышалось (относительно 60-го дня; $P<0{,}01$), β- и γ-глобулины возвращались к норме. Возрастало отношение альбуминов к глобулинам. И только уровень α-глобулинов, как и в I серии опытов, оставался повышенным ($P<0{,}02$).

Выводы

1. Метилакрилат, проникая в организм животных через кожный покров, способен вызывать изменения в белковом обмене, особенно в соотношении отдельных фракций белка.

2. Двукратное нанесение по 1 мл чистого метилакрилата на кожу кроликов приводит к заметному снижению уровня γ-глобулинов в сыворотке крови.

3. Длительное воздействие 1% раствором метилакрилата (по 4 мл) на кожу кроликов приводит на 60-й день к повышению уровня γ-глобулинов и снижению альбумино-глобулинового коэффициента.

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Поступила 16/XII 1967 г.

CHANGED LEVEL OF PROTEIN FRACTION IN THE BLOOD SERUM OF RABBITS FOLLOWING APPLICATION OF METHYL ACRYLATE TO THEIR INTEGUMENT

A. P. Suvorov

Methyl acrylate was applied to the skin of rabbits after which total protein (in gram-per cent) and its individual fractions in the blood serum were determined by paper electrophoresis. A two-fold application of a pure substance was seen to be followed by a drop of γ-globulins and a rise of β-globulins. A protracted exposure of the integument to a 1% methylacrylate solution led at the 60th day to an increase in the level of β- and γ-globulins and to a fall of the albumin-globulin ratio.

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616.633.233]-074

VARIATION OF RESIDUAL AMINO NITROGEN IN BLOOD SERUM AND AMINO NITROGEN IN URINE UNDER THE INFLUENCE OF METHYL ACRYLATE

(Izmenenie soderzhaniya ostatochnogo aminnogo azota syvorotki krovi i aminnogo azota mochi pod deistviem metilakrilata)

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The methyl ester of acrylic acid (methyl acrylate) has recently come into extensive use in the manufacture of polymers (nitron fiber, plastics and synthetic rubber).

In view of the practical importance of studying the mechanism of the toxic effect of industrial poisons, including methyl acrylate, on the organism, we carried out experimental investigations of the effect of methyl acrylate on the blood serum content of residual amino nitrogen.

Variation of amino nitrogen provides an indication of the state of protein metabolism. Blockade of the amino groups of proteins by certain industrial poisons inactivates the enzyme systems and disturbs tissue metabolism (Kokina; Souček and Madlo).

We examined workers in the Saratov chemical combine, working in the methyl acrylate department. Their blood serum was tested for residual amino nitrogen and their urine for amino nitrogen.

In comparison with donors (30 persons), the workers coming into contact with methyl acrylate (36 subjects) had a low content of residual amino nitrogen in their blood serum, 5.7 ± 0.1 and 4.6 ± 0.1 mg %, respectively ($P < 0.001$). The workers who had been employed in the methyl acrylate department for longer than 2 years had a lower concentration of residual amino nitrogen in their serum than workers with a shorter employment period, 4.0 ± 0.1 and 5.2 ± 0.4 mg %, respectively ($P < 0.05$).

Amino nitrogen was determined in the urine of 20 donors and 46 workers in occupational contact with methyl acrylate. The literature provides a wide range of data on the content of amino nitrogen in the diurnal urine of healthy subjects, for instance, 100—200 mg (Myasnikov) and 756.9—897 mg (Podrabinik), etc. In order to exclude the influence of physical stress and various physiological and emotional factors on the composition of the urine, the amino nitrogen was not determined in the diurnal, but in the morning urine. Women were investigated only during the intermenstrual period. The quantitative results for amino nitrogen were scaled to 100 ml urine for convenience. The workers' urine had a lower content of amino nitrogen than the donors, 41.7 ± 3.1 and 28.1 ± 1.3 mg, respectively, per 100 ml ($P < 0.001$).

In addition to the clinical investigations, we performed two series of experiments on 80 chinchilla rabbits aged 8 months, maintained on identical rations, with 40 animals per series (10 of them serving as controls). In view of the capacity of methyl acrylate to penetrate through the intact skin, with a subsequent systemic effect (Veling and Arkhangel'skaya), we chose

percutaneous administration of the substance for our experiments. Methyl acrylate was applied by Pravdin's method, in a manner preventing its evaporation. The fur on the animals' skin area was shorn before application. The exposure time was 5 hr.

In the first series of experiments, 1 ml pure methyl acrylate was applied on the skin of the rabbits' left side twice, with a 24-hr interval. The second application was made to take advantage of the enhanced skin permeability produced by the dermal hyperemia caused by the first application. Blood was sampled from the marginal aural vein prior to feeding, before the experiment, and 10, 20 and 30 days after the application.

In the second series of experiments, 4-ml portions of 1% methyl acrylate solution were applied to the rabbits' skin daily for 60 days. Blood samples were taken prior to the experiment, on the 20th, 40th and 60th days of the experimental period, and 20 days after the last application (the 80th day of the experiment).

Residual amino nitrogen in the blood serum was determined by Uzbekov's method, and the content of amino nitrogen in the urine by the method of Pope and Stevenson (Predtechenskii).

The application of pure methyl acrylate produced profound degenerative and necrotic changes in the rabbits' skin. A necrotic crust appeared on the application site after 10 days, and sloughing had begun by the 20th day of experiment. Simultaneously with these skin changes, there was a decrease of residual amino nitrogen in the blood serum, in comparison with the controls ($P < 0.001$). The affected skin area recovered (complete sloughing of the necrotic lesion, shrinking of the zone of depilation) and the content of residual amino nitrogen in the blood serum increased towards the 30th day of experiment.

The results of the determinations of residual amino nitrogen in the animals' blood serum are summarized in Table 1.

TABLE 1. Content of residual amino nitrogen in the animals' blood serum (1st series of experiments)

Statistical index	Rabbits	Time of investigation			
		before experiment	10th day of experiment	20th day of experiment	30th day of experiment
Mean ± S. E.	Experimental	5.7 ± 0.2	3.6 ± 0.1	3.1 ± 0.2	4.9 ± 0.3
	Control	5.8 ± 0.3	5.7 ± 0.4	6.1 ± 0.4	5.9 ± 0.6
P		> 0.05	< 0.001	< 0.001	> 0.05

Repeated applications of 1% methyl acrylate solution likewise produced a necrotic crust at the application site. A large depilated zone had formed around the necrotic focus by the 60th day of the experiment. The blood serum of the experimental rabbits had a lower residual amino nitrogen concentration than that of the controls. Twenty days after the last application, when there was complete sloughing of the necrotic lesion and renewed growth of hair on the application site, the residual amino nitrogen content in the blood serum increased.

The results are summarized in Table 2.

TABLE 2. Content of residual amino nitrogen (mg%) i[illegible]nals' blood serum (2nd series of experiments)

Statistical index	Rabbits	Time of investigation				
		before experiment	20th day of experiment	40th day of experiment	60th day of experiment	80th day of experiment
Mean ± S. E.	Experimental	5.6 ± 0.1	4.1 ± 0.1	3.4 ± 0.2	4.6 ± 0.1	5.1 ± 0.2
	Control	5.7 ± 0.5	5.6 ± 0.3	5.9 ± 0.3	5.8 ± 0.5	5.9 ± 0.3
p		> 0.05	< 0.001	< 0.001	< 0.05	> 0.05

Thus, workers in occupational contact with methyl acrylate and animals subjected to percutaneous poisoning with this substance exhibit a decreased residual amino nitrogen content in the blood serum. Furthermore, the urine of workers engaged in the manufacture of methyl acrylate has a reduced content of amino nitrogen. These changes possibly reflect the disturbances in protein metabolism, produced by methyl acrylate poisoning, as described by us in an earlier paper (Suvorov).

Uzbekov's method of determining residual amino nitrogen in the blood serum can be recommended for use in the detection of early methyl acrylate poisoning during periodic medical examinations. The method is effective and can readily be used in a factory medical office or workers' prophylactorium.

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Variation in the Content of Ascorbic Acid and Glutathione in the Blood of Rabbits on Application of Methyl Acrylate to the Skin

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Source: Farmakologiya i Toksikologiya, 1971, No. 5, pp. 593-4.

Summary

When 1 ml pure methyl acrylate was applied twice to the skin of rabbits, the content of ascorbic acid and reduced glutathione in the blood was lowered. At the same time, the absorption of ascorbic acid (in %) by blood proteins and the content of oxidized glutathione increased. Thirty days after application of methyl acrylate, reduced and oxidized glutathione was restored to the values prior to the test, while the content of ascorbic acid increased. The percentage absorption of ascorbic acid by blood proteins was reduced.

In 40 rabbits, 10 of which served as controls, before and after application on the skin of methyl acrylate (after 10, 20, and 30 days) the content of ascorbic acid in the blood was investigated by Eidelman and Gordon's method, and that of glutathione by the micromethod of M. S. Chulkova. Methyl acrylate in a dose of 1 ml was applied on a 3 X 3 cm^2 cloth twice every 24 hours. The duration of exposure was 5 hours.

Application of methyl acrylate to the skin of rabbits was followed by the appearance of extensive dystrophic and necrobiotic processes. On the 10th day, a necrotic scab was fromed at the site of application, which started to tear off (disengage) on the 20th day of observation. On the 30th day of the test, only an area of alopecia (baldness) remained at the site of action of the compound.

Ten days after application of methyl acrylate, the content of ascorbic acid in the blood of test rabbits decreased ($P < 0.001$) and its absorption by blood proteins increased ($P < 0.01$), as compared to control animals. On the other hand, the level of the reduced glutathione fraction decreased ($P < 0.001$), while that of the oxidized fraction increased ($P < 0.001$). The amount of total glutathione was also higher ($P < 0.02$).

On the 20th day of the test, there was no substantial change in the content of ascorbic acid and reduced glutathione in the blood of test animals. However, absorption of ascorbic acid by blood proteins continued to increase, while the content of total glutathione and its oxidized fraction continued to decrease.

On the 30th day of the test, the content of total glutathione and its fractions and also the absorption of ascorbic acid by blood proteins in the test animals did not differ from the analogous indices in control rabbits ($P < 0.05$). However, although the level of ascorbic acid in the blood of test animals was still lower than that of controls ($P < 0.002$), it already exceeded the values noted on the 20th day of observation ($P < 0.001$).

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ИЗМЕНЕНИЕ СОДЕРЖАНИЯ АСКОРБИНОВОЙ КИСЛОТЫ И ГЛЮТАТИОНА В КРОВИ КРОЛИКОВ ПРИ НАНЕСЕНИИ НА КОЖУ МЕТИЛОВОГО ЭФИРА АКРИЛОВОЙ КИСЛОТЫ

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Двукратное нанесение на кожу кроликов чистого метилакрилата по 1 мл приводило к снижению содержания в крови аскорбиновой кислоты и восстановленного глютатиона. При этом поглощение аскорбиновой кислоты и восстановленного глютатиона белками крови и содержание окисленного глютатиона повышались. Через 30 дней после нанесения метилакрилата восстановленный и окисленный глютатион возвращался к данным до опыта, а содержание аскорбиновой кислоты повышалось. Процент поглощения аскорбиновой кислоты белками крови снижался (Фармакол. и токсикол., 1971, № 5, с. 593).

На 40 кроликах, 10 из которых служили контролем, до и после нанесения им на кожу метилакрилата (через 10, 20 и 30 дней) изучали содержание в крови аскорбиновой кислоты (по методу Эйдельмана и Гордона) и глютатиона (по микрометоду М. С. Чулковой). Метилакрилат в дозе 1 мл наносили на салфетке размером 3×3 см² дважды через 24 часа. Экспозиция равнялась 5 часам.

После нанесения метилакрилата в коже кроликов наступали глубокие дистрофические и некробиотические процессы. К 10-му дню на месте аппликации формировался некротический струп, который начинал отторгаться на 20-й день наблюдения. К 30-му дню опыта на месте воздействия препаратом сохранялась только зона облысения.

В крови подопытных кроликов по сравнению с контрольными животными через 10 дней после нанесения метилакрилата уменьшалось содержание аскорбиновой кислоты ($P<0{,}001$) и возрастало поглощение ее белками крови ($P<0{,}01$). Уровень же восстановленной фракции глютатиона снижался ($P<0{,}001$), а окисленной повышался ($P<0{,}001$). Повышенным было также количество общего глютатиона ($P<0{,}02$).

К 20-му дню опыта содержание аскорбиновой кислоты и восстановленного глютатиона в крови подопытных животных существенно не изменилось. Поглощение же аскорбиновой кислоты белками крови продол-

жало нарастать, а содержание общего глютатиона и его окисленной фракции — снижаться.

На 30-й день опыта содержание общего глютатиона и его фракций, а также поглощение аскорбиновой кислоты белками крови у подопытных животных не отличались от аналогичных показателей у контрольных кроликов ($P>0{,}05$). Уровень же аскорбиновой кислоты в крови подопытных животных хотя еще и оставался ниже, чем у контрольных ($P<0{,}002$), но уже превышал данные 20-го дня наблюдения ($P<0{,}001$),

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THE TOXICITY OF METHYL AND ETHYL ACRYLATE*

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I. INTRODUCTION

NFORMATION on the toxicologic properties of methyl acrylate and ethyl acrylate has come to be necessary because these unsaturated esters are being used in the manufacture of plastics. Some of their physical properties, as reported by the manufacturer, are given in table 1. Maglio (2) lists their more common solvents. The penetrating, unpleasant and somewhat sweetish odor of the compounds could be detected readily in the lowest concentrations employed in these experiments (methyl acrylate, 31 p.p.m.; ethyl acrylate, 25 p.p.m.). The extreme persistence of this odor is shown by the fact that metal containers in which the esters had been kept still retained the characteristic odor after having been soaked in kerosene for some months.

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II. EXPERIMENTAL METHODS

Oral Administration of a Single Dose

In order to determine the immediate toxicity of these substances, measured amounts of the undiluted material were introduced into the stomachs of healthy female rabbits through a rubber catheter (F12) passed through the esophagus. Some of the lower doses were given in the form of a 10 per cent W/V solution of the respective esters in propylene glycol. In seeking to determine whether the administration of a series of small doses of either ester would give rise in rabbits to cumulative effects, a 10 per cent solution was given in like fashion on each of 5 consecutive days of each week.

Cutaneous

The undiluted esters were applied on the intact skin of rabbits in 1 to 5 ml. portions at intervals of 5 to 20 minutes. In experiments that were completed on one day the duration of contact ranged from 2.67 to 6.0 hours, but in others, in which the material was applied on each of two successive days, it was permitted to remain on the skin for but 1 hour on each day. In 2 instances methyl acrylate was applied on the intact skin and allowed to evaporate freely. In all other instances the volatilization of the compound was minimized by means of a glass cover placed over the area of application and retained by means of a resilient bandage applied around the body of the animal, the esters being introduced by means of a pipette the tip of which was inserted through a small hole in the cover. Regardless of the manner of application, all animals were kept in a ventilated hood with their heads directed toward the stream of influent air. At the end of the desired period of contact the skin was washed with water and a sulfonated oil, and dried, and then a wide collar was placed about the neck of the animal to prevent it from licking the skin.

Inhalation

In experiments in which animals were exposed to the two highest concentrations of methyl acrylate they were confined in a 223-liter cylindrical steel cage coated on the inside surface with baked plastic (1). By means of a special syringe (1), the plunger of which was operated by a synchronous motor, methyl acrylate was delivered at a slow rate into a glass tube upon a layer of warmed glass wool. There it was vaporized into a stream of air which had been dried by being passed through calcium chloride and which was moving at the rate of 10 liters per minute as measured by a rotameter.

In all other experiments with either material the animals were confined in a 600-liter insulated plywood chamber (4). When an atmospheric concentration of the ester ranging from 0.817 to 4.83 mg. per liter was desired, the ester was metered by a Bosch injection pump through a Bosch Spray nozzle into a stream of air conditioned with respect to temperature, humidity and dust (4). A stream of about 500 liters of air per minute, measured by

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an inclined manometer that had been calibrated against a Pittsburgh Equitable Meter, entered the chamber through an Anemostat located in the ceiling and was expelled into a stack from an outlet located in the rear and near the bottom.

In experiments in which lower concentrations were desired, a small stream of air (425 ml. in the case of methyl and 120 ml. in that of ethyl ester) was passed through a volume of ester contained in a coarse-mesh, fritted-glass bubbler and maintained at a constant level and constant temperature of either 25° or 0°C., depending on the concentration desired in the air. The air bearing the ester was then passed into a larger stream of conditioned air (640 liters per minute) which was measured and passed into the chamber in the manner previously described.

TABLE 1

PROPERTIES OF ETHYL AND METHYL ACRYLATES

	METHYL ACRYLATE	ETHYL ACRYLATE
Formula	$CH_2 = CHCO_2CH_3$	$CH_2 = CHCO_2C_2H_5$
Molecular weight (1941)	86.09	100.11
Density at 25°C. (gm./cc.)	0.949	0.917
Freezing point (°C.)	−75	−70
Boiling point (°C.)	80	99
Vapor pressure at 25°C. (mm. Hg.)	83	43
Flash point, open cup (°F.) (2)	26.6	48.2
Refractive index	1.400	1.404
Solubility in H_2O (%)	<6	<2

TABLE 2

MORTALITY AMONG RABBITS GIVEN AN ORAL DOSE OF METHYL OR ETHYL ACRYLATE

DOSAGE	METHYL ACRYLATE		ETHYL ACRYLATE	
	Number of animals that died/number given ester	Length of survival	Number of animals that died/number given ester	Length of survival
gm./kg.		hr.		hr.
1.00	1/1	2.8	—	—
0.94	—	—	1/1	3.6
0.62	1/1	4.1	1/1	6.4
0.42	1/1	4.0	2/2	12–89
0.28	2/2	5.3–5.8	0/4	—
0.18	0/4	—	0/3	—
0.12	0/2	—	0/1	—

Determination of the Esters in the Air

The method by which the methyl or ethyl acrylate present in air was determined was based on that by which Werner and Mitchell (5) determined the monoalkyl ethers of ethylene glycol. The ester was oxidized with a solution of chromic acid in strong sulfuric acid, the excess of chromic acid remaining after the completion of the reaction being determined iodimetrically. Methyl acrylate was found to be oxidized stoichiometrically to carbon dioxide and water, each milliliter of N/20 potassium dichromate consumed being equivalent to 0.2391 mg. of methyl acrylate. Although under the conditions employed the oxidation of ethyl acrylate was only 91 per cent complete, the uniformity of the results in this respect was sufficient to justify the employment of the method. In this case it was necessary to make use of an empirical rather than a stoichiometrical relationship of the volume of the chromic acid solution consumed and the amounts of ethyl acrylate originally present. As actually determined, 1 ml. of N/20 chromic acid was found to be equivalent to 0.3419 mg. of ethyl acrylate instead of 0.3129 mg. as demanded stoichiometrically.

Air from the chamber was drawn through a series of two fritted-glass bubblers (coarse mesh) at the rate of 100 to 250 ml. per minute as measured by a vertical flowmeter. Each of the bubblers contained 50 ml. of standardized dichromate in a solution of sulfuric acid. Depending on the concentration in the chamber, the duration of sampling ranged from 30 to 120 minutes, and the normality of the dichromate used ranged from N/20 to N/4. Two or more samples were collected daily from each chamber.

Twenty-five milliliter portions of chromic acid from each of the two bubbling towers were pipetted into separate glass-stoppered Pyrex test tubes (140 x 22 mm.) and immersed in boiling water for 20 minutes. After being cooled in an ice-water bath for 4 minutes, the solutions were titrated in the presence of potassium iodide with sodium thiosulfate, which was standardized daily against a solution of chromic acid in sulfuric acid.

At 745 mm. of mercury and at 25°C. the concentration of 1 mg. of methyl acrylate per liter of air is equivalent to 289.9 parts per million (p.p.m.) by volume, and 1 mg. of ethyl acrylate per liter of air to 249.3 p.p.m. by volume.

Samples of uncontaminated air (unmixed with either ester) drawn from the chamber while ani-

mals were housed therein did not reduce chromic acid.

III. Results

Effects of Oral Administration of One Dose

From the data in table 2 it is apparent that the minimum lethal dose of methyl acrylate administered orally to rabbits lies between 0.18 and 0.28 gm. per kilogram of body weight, while the corresponding value for ethyl acrylate is between 0.28 and 0.42 gm. per kilogram.

The signs of intoxication observed in animals poisoned fatally by either compound included lethargy and distention of the veins of the ear during the early stage of intoxication, and, later and shortly before death, running movements of the legs, tremors, spasms of the diaphragm, labored respiration and cyanosis. The length of survival of animals poisoned fatally by either compound, with one exception, ranged between the limits of 2.8 to 12 hours and varied roughly in inverse relation to the size of the dose.

The body temperatures of the rabbits decreased gradually after the administration of the ester, the decrements being greater in fatally poisoned animals than in survivors. The packed cell volume of the blood tended to increase, the extent of the increase varying directly with the size of the dose administered (table 3).

TABLE 3

The Effects of the Oral Administration of Methyl and Ethyl Acrylate in One Dose upon the Packed Cell Volume (Hematocrit) of the Blood and upon the Rectal Temperature of Rabbits

Dose	Methyl acrylate				Ethyl acrylate			
	Percentage change in packed cell volume after administration	Hours elapsed between administration and withdrawal of blood sample	Drop in rectal temperature following administration		Percentage change in packed cell volume after administration	Hours elapsed between administration and withdrawal of blood sample	Drop in rectal temperature following administration	
			°F.	Hours elapsed between administration and observation			°F.	Hours elapsed between administration and observation
gm./kg.								
0.94					+67.0	3.6	0.2 1.2 2.8 4.8	0.7 1.7 2.8 3.5
0.42	+51.8	4	1.5 1.8 2.9 3.5	0.8 2.0 3.3 3.8	+27.5	6.5	1.2 1.4 2.4	0.9 2.5 6.3
0.28	+27.4	5.3	0.5 2.3 3.3 4.4	0.7 1.7 2.7 4.7	+8.0	3.8	1.5 2.2 1.4 1.4	0.5 1.7 3.0 3.0
0.28	+28.9	5.8	1.6 1.7 2.7 4.1 2.8	1.1 2.1 3.0 4.0 5.5				
0.18	+8.2	5.0	0.9 0.9 1.2 1.2	1.0 2.1 3.5 4.8	0.0	4.9	1.9 1.9 1.7 1.3	1.3 2.6 4.0 4.9
0.18	−6.9	3.3	0.2 0.3	1 3.3				

Transient losses of weight and decreases of body temperature were the only signs of intoxication noted in any of the rabbits given a sublethal dose of either ester.

The odor of these compounds was noticeable in all of the tissues of fatally poisoned rabbits and it was especially strong in the stomach. Deep congestion of the stomach, duodenum and upper jejunum, with small hemorrhagic extravasations, gave evidence of severe irritant action. The mucosa of the stomach, duodenum and upper jejunum was edematous, with an unusual amount of mucus in the duodenum and jejunum. In some instances, gelatinous edema was seen in the gastric mucosa, and the fundic mucosa was partially or completely eroded. Early acute toxic degenerative changes were present in heart, liver and kidneys. Central cellular degeneration was well advanced in the liver in some animals. Congestion and cloudy swelling were observed in the kidneys, while the spleen exhibited congestion and reticulo-endothelial edema and hyperplasia. Other alterations consisted of hypostatic congestion and atelectasis of the lungs and moderate congestion and edema of the brain or the meninges.

Gross and microscopic examination of animals killed 2 months after the administration of a sublethal dose disclosed only slight to moderately severe central hepatocellular degeneration and minimal degeneration of the renal tubules.

To determine the extent to which the unsaturated nature of the esters contributed to their toxicity, comparable observations were made upon the corresponding saturated compounds. The minimum lethal oral dose of methyl propionate (Eastman) was found to lie between 2.55 and 3.2 gm. per kilogram of body weight, and that of ethyl propionate between 3.2 and 3.95 gm. per kilogram of body weight. Thus the saturated esters possess but one-tenth to one-thirteenth the toxicity of the corresponding unsaturated compounds. Following the administration of a lethal dose of either saturated ester, rabbits exhibited ataxia, prostration, labored and convulsive respiration, and hypothermia (as much as 7°F.). The pathologic findings were essentially similar to those observed in the tissues of animals that had been given unsaturated compounds.

Effects of Repetitive Oral Administration

Each of 2 rabbits was given an oral dose of 0.023 gm. (approximately one-tenth the lethal dose) of methyl acrylate per kilogram of body weight on each of 5 days per week until 24 doses had been given to each over a period of 33 days. Each of 2 other rabbits was given an oral dose of 0.0315 gm. of ethyl acrylate per kilogram on each of 5 days per week until 25 doses had been given over a period of 35 days.

Other than retardation of growth or small losses of weight during the period of administration, no signs of intoxication were evident. The animals grew at a normal rate during the interval of 2 months between the time of administration of the last dose and that at which they were sacrificed. In view of these facts and because no significant changes were observed in gross and microscopic examination of the tissues of these animals, it would appear that no cumulative effect resulted from the administration of the esters in this manner.

Effects of Maintaining the Esters in Contact with the Skin of Rabbits

Data pertaining to the effects of applying methyl and ethyl acrylates on the skin of rabbits over short periods (1 to 2 days) are presented in table 4. The minimum quantity of ethyl acrylate that was lethal when applied in a series of small doses at short intervals for 1 day lies between 44.4 and 54.3 ml. per kilogram of body weight. The methyl ester was not applied in comparable dosage. Inflammation of varying severity and intense edema of the skin were manifested by all of the rabbits, and in some instances hemorrhagic areas appeared. The condition of the skin became progressively worse after applications were made on a second day, but in all cases it returned to normal within a week after the last application. Only small transitory losses of weight occurred.

Microscopic examination of the skin of a rabbit that was sacrificed on the day following a period of contact with methyl acrylate disclosed edema and cloudy swelling of the rete and supporting connective tissue, with scattered collections of neutrophilic polymorphonuclear cells. There were also a few focal intraepithelial abscesses. Pathological examination of those animals that died subsequent to contact of their skin with ethyl acrylate (table 4) disclosed severe early acute inflammatory changes in the skin and subcutaneous tissues at the site of contact. The epidermis was almost completely destroyed, and there was abundant edema of the corium with congestion of the regional vessels. There were also alterations in the viscera, consisting of cloudy swelling and congestion of the heart, the liver and the kidneys and generalized edema and congestion of the lungs.

Gross and microscopic examination of animals subjected to contact with either compound and sacrificed some months later revealed no significant abnormalities.

The left ear of a rabbit was immersed for 1 minute in methyl acrylate in a beaker. Erythema resulted within a few minutes, and white, dried flakes appeared on the epidermis. On the next day the ear was warm, somewhat red and definitely swollen. By the third day the erythema had subsided, but the swelling persisted for 6 days.

TABLE 4

THE EFFECTS OF THE APPLICATION OF UNDILUTED METHYL OR ETHYL ACRYLATE UPON THE INTACT SKIN OF RABBITS

AREA COVERED	AMOUNT APPLIED EACH TIME	INTERVAL BETWEEN SUCCESSIVE APPLICATION	NUMBER OF APPLICATIONS PER DAY	NUMBER OF DAYS ON WHICH APPLICATIONS WERE MADE	DURATION OF CONTACT EACH DAY	TOTAL DOSAGE		FATE	LENGTH OF SURVIVAL AFTER REMOVAL FROM SKIN
						ml./kg.	gm./kg.		
Methyl acrylate									
sq. in.	*ml.*	*min.*			*hr.*				
30	5	10	16	1	2.67	34.4	32.6	Survived	8 weeks; sacrificed
30	5	10	18	1	3.0	29.9	28.4	Survived	1 day; sacrificed
4.5*	1	10	6	2	1.0	4.5	4.3	Survived	2 months; sacrificed
Ethyl acrylate									
4.5*	5	5 and 10	38†	1	6.0	75.3	69.1	Died	16 hours
4.5*	5	10	30	1	5.0	54.3	49.8	Died	7.5 hours
4.5*	5	10	24	1	4.0	44.4	40.7	Survived	53 days; sacrificed
4.5*	5	20	3	2	1.0	11.7	10.7	Survived	3 months; sacrificed
4.5*	3	20	3	2	1.0	5.9	5.4	Survived	3 months; sacrificed

* Evaporation was reduced to a minimum since the material was applied beneath a glass cover.

† The first four applications were made at 5 minute intervals; the next 34 applications, at 10 minute intervals.

Effects of Inhalation of the Vapors

The essential signs of intoxication observed during and after the exposure of animals to the vapors of methyl or ethyl acrylate are summarized, together with mortality data, in tables 5 and 6, respectively.

Rabbits, guinea pigs, rats and a monkey survived throughout a period of 185 days during which they were subjected to 130 seven-hour periods of exposure to methyl acrylate in air in a concentration of 0.107 mg. per liter (31.0 p.p.m.). This was the only concentration of methyl acrylate to which a monkey was exposed. All rabbits, rats and guinea pigs that were exposed for 7 hours on each of 50 days over a period of 70 days to methyl acrylate in air at the concentration of 0.328 mg. per liter (95.1 p.p.m.) survived, but when, in another experiment, the concentration of vapor was 0.817 mg. per liter (236.8 p.p.m.) all of the rabbits had died by the end of the 11th period of exposure, and all of the guinea pigs by the end of the 12th, but 2 rats survived throughout and after the 12 seven-hour periods. On the other hand, rats succumbed when exposed to the concentration of 1.99 mg. per liter (577.8 p.p.m.) for 7 hours per day on successive days, one on the second day following the third day of exposure, and the other at the end of the seventh.

No fatalities occurred among rabbits, guinea pigs, rats and a monkey throughout a period during which they were exposed to ethyl acrylate in the air in the concentration of 0.099 mg. per liter (24.5 p.p.m.) for 7 hours on each of 130 days over a period of 199 days. (The total period of exposure was shorter in some instances. Cf. table 6.) Furthermore, no fatalities resulted among rabbits, guinea pigs and rats exposed for 7 hours on each of 50 days over a period of 72 days to the vapor in the air in a concentration of 0.300 mg. per liter (74.8 p.p.m.), but 4 rabbits (of 4 subjected to exposures) and 1 of 2 guinea pigs died when exposed repeatedly to the concentration of 1.09 mg. per liter (272 p.p.m.). The rabbits died during or after the 8th,

TABLE 5

MORTALITY, WEIGHT CHANGES, AND SIGNS OF INTOXICATION INDUCED IN ANIMALS BY INHALED METHYL ACRYLATE

CONCENTRATION		DURATION OF EXPOSURE	NUMBER OF VARIOUS SPECIES EXPOSED	NUMBER OF ANIMALS THAT DIED	AVERAGE CHANGE IN WEIGHT DURING EXPOSURE	SIGNS OF INTOXICATION
mg./l.	p.p.m.	hr.			gm.	
9.04	2,620.7	2.75	4 rabbits	4	—	Distention of ear veins, conjunctival irritation, lacrimation, sneezing, nasal irritation, salivation, coughing, labored respiration, prostration, convulsions, trembling, jerking of body, cyanosis
8.70	2,522.1	1	4 rabbits	2	−146	Distention of ear veins, conjunctival irritation, contraction of pupils of eyes, lacrimation, sneezing, rubbing and twitching of nose, nasal irritation, coughing, salivation, labored respiration, rales, prostration, convulsive kicking
1.99 ±0.07	577.8	2 × 7	4 rabbits	4	−311	Excitation, distention of ear veins, closure of eyes, lacrimation, twitching and reddening of nose, salivation, labored respiration, rales, lethargy, running-like movements of legs, opisthotonos, cyanosis
		3 × 7	2 guinea pigs	2	−99	Closure of eyes, slight lacrimation, nasal and oral irritation, salivation, labored respiration, lethargy, few convulsive movements
		7 × 7	2 rats	2	−68	Closure of eyes, conjunctival irritation, nasal irritation, salivation, labored respiration, lethargy
0.817 ±0.038	236.8	11 × 7	5 rabbits	5	−609	Closure of eyes, conjunctival irritation, lacrimation, rubbing of nose, sneezing, nasal irritation, coughing, salivation, labored respiration, lethargy
		12 × 7	2 guinea pigs	2	−228	Closure of eyes, nasal irritation, salivation, labored respiration, lethargy
		12 × 7	2 rats	0	−6	Eyes closed, nasal irritation, lethargy
0.328 ±0.003	95.1	50 × 7	4 rabbits	0	+419	Slight conjunctival irritation, slight nasal irritation
		50 × 7	2 guinea pigs	0	+110	Not remarkable
		50 × 7	2 rats	0	+120	Not remarkable
0.107 ±0.007	31.0	130 × 7	4 rabbits	0	−299	Not remarkable
		130 × 7	2 guinea pigs	0	−39	Not remarkable
		130 × 7	2 rats	0	+63	Not remarkable
		130 × 7	1 monkey	0	−1251	Not remarkable

12th, 16th and 17th days of exposure, respectively, and the guinea pig after the 28th. Two rats and a monkey survived throughout 28 periods of exposure to this concentration (1.09 mg. per liter) over a period of 38 days. Only 1 of 2 rats exposed to the concentration of 2.01 mg. per liter died (4th

TABLE 6

MORTALITY, WEIGHT CHANGES AND SIGNS OF INTOXICATION INDUCED IN ANIMALS BY INHALED ETHYL ACRYLATE

CONCENTRATION		DURATION OF EXPOSURE	NUMBERS OF VARIOUS SPECIES EXPOSED	NUMBER OF ANIMALS THAT DIED	AVERAGE CHANGE IN WEIGHT DURING EXPOSURE	SIGNS OF INTOXICATION
mg./l.	p.p.m.					
		hr.			*gm.*	
4.83	1,204	7	4 rabbits	4	−159	Coughing, salivation, rales, conjunctival and nasal irritation, prostration, ataxia, convulsive movements, spasmodic respiration, diarrhea
		7	2 guinea pigs	2	−38	Hiccoughing, salivation, rales, conjunctival and nasal irritation, prostration, convulsive movements, spasmodic respiration
		2 × 7	2 rats	2	−29	Salivation, conjunctival and nasal irritation, prostration, convulsive movements, spasmodic respiration
		2.2 × 7	1 monkey	1	−627	Salivation, conjunctival and nasal irritation, prostration, convulsive movements, spasmodic respiration
2.01 ±0.034	501	4 × 7	4 rabbits	4	−308	Salivation, rales, conjunctival and nasal irritation, prostration, gasping, convulsive movements, diarrhea
		13 × 7	2 guinea pigs	2	−79	Salivation, rales, conjunctival and nasal irritation, lethargy, gasping
		13 × 7	2 rats	1	−18	Salivation, conjunctival and nasal irritation, lethargy, labored respiration
1.09 ±0.019	272	(8 to 17) × 7	4 rabbits	4	−478	Moderate conjunctival and nasal irritation, lethargy, gasping, convulsive movements
		28 × 7	2 guinea pigs	1	−72	Moderate conjunctival and nasal irritation, lethargy, moderate gasping
		28 × 7	2 rats	0	−29	Slight salivation, slight conjunctival and nasal irritation, lethargy, diarrhea
		28 × 7	1 monkey	0	−476	Slight irritation of mucous membranes, slight lethargy
0.300 ±0.002	74.8	50 × 7	4 rabbits	0	+891	None
		50 × 7	2 guinea pigs	0	+81	None
		50 × 7	2 rats	0	+27	None
0.105 ±0.001	26.2	130 × 7	2 guinea pigs	0	+322	None
		(62 to 130) × 7	2 rats	0	+25	None
		130 × 7	1 monkey	0	+336	None
0.099 ±0.007	24.5	(108 to 130) × 7	4 rabbits	0	+1058	None
		130 × 7	2 guinea pigs	0	+93	None
		(87 to 130) × 7	2 rats	0	+65	None
		130 × 7	1 monkey	0	−401	None

period of exposure), the other surviving throughout 13 such periods of exposure.

No signs of intoxication other than a slight irritation of the eyes and nose, occurring in rabbits, were observed among animals exposed to 0.328 mg. or less of methyl acrylate per liter. In the case of ethyl acrylate no conjunctival irritation appeared when the concentration was 0.300 mg. or less per liter, but became evident when it was 1.09 mg. or more. Rabbits and guinea pigs suffered lethargy and labored respiration, when exposed to air containing 0.817 mg. of methyl acrylate per liter, while rats presented similar symptoms when exposed to 1.99 mg. per liter. Rabbits and guinea pigs had like symptoms when subjected to inhalation of air containing 1.09 mg. of ethyl acrylate per liter. The concentration of 2.01 mg. of ethyl acrylate per liter was required to induce comparable behavior in rats.

Convulsions occurred among animals of various species when these were subjected to the inhalation of air containing either compound in adequate concentration, and they increased in intensity and in speed of onset as the concentration was increased.

The length of time required for the onset of the various signs of intoxication may be illustrated by describing an experiment in which animals were exposed to the concentration of 4.83 mg. of ethyl acrylate per liter. Almost as soon as the animals were introduced into the chamber they began to sneeze, rub their noses with their forefeet, close their eyes and become excited. Within 5 to 15 minutes they exhibited slight dyspnea, coughing, salivation, reddening of the nostrils and a nasal discharge, conjunctival injection and lacrimation, and within another 15 to 30 minutes they became lethargic with rapid and labored respiratory movements. After about two hours they were weak, lying on their sides and exhibiting certain effects of irritation of the central nervous system, such as extensive tremors, incoördinated and convulsive movements and spasmodic breathing. These effects subsided and recurred in varying intensity at irregular intervals. When the animals were removed from the respiratory chamber at the end of 7 hours the most prominent signs of intoxication were prostration, ataxia, opisthotonos, convulsions, hiccoughing and respiratory sounds indicative of the presence of fluid in the respiratory passages.

Gross and microscopic examination of the tissues of fatally poisoned animals revealed edema and congestion of the entire respiratory tract and in some cases bronchopneumonia. The lungs were deeply congested, with areas of hemorrhagic extravasation, small scattered areas of atelectasis, diffuse emphysema and increased secretion of mucus. The right chambers of the heart were dilated, while the left ventricle was contracted and empty. Edema, congestion, cloudy swelling and granular degeneration of the myocardium were observed. The liver was edematous, congested; hepatocellular degeneration was accompanied in a few instances by fatty changes. The liver cords were edematous, and granular degeneration, with nuclear pyknosis and karyolysis, was prominent in the central zones. Cloudy swelling, edema and congestion of the kidney were noted. The spleen was congested, and the reticuloendothelial cells were prominent. Cerebral and meningeal congestion and edema were present, as well as pyknosis and chromatolysis of many of the neurons throughout the brain. The latter changes were presumed to have been related to cerebral anoxia.

No significant changes were found in the tissues of animals which survived after exposure and were sacrificed two months later.

The capacity of methyl acrylate to injure the tissues of animals of various species when inhaled as vapor is shown in the following statement in which the first value represents the highest atmospheric concentration of methyl acrylate which failed to give microscopic evidence of injury, while the second represents the least concentration which gave rise to injury: rabbits and guinea pigs, 0.328 and 0.817 mg. per liter; rats, 0.817 and 1.99 mg. per liter. No changes were observed in the tissues of a monkey exposed to a concentration of 0.107 mg. per liter, the only one to which a monkey was subjected. In the case of ethyl acrylate the corresponding values are: rabbits and guinea pigs, 0.300 and 1.09 mg. per liter; rats, 1.09 and 2.01 mg. per liter; monkeys, 1.09 and 4.83 mg. per liter.

No consistent or general trend was apparent with respect to changes in the formed elements of the peripheral blood of animals exposed to either of these esters. The time required for clotting of blood of exposed rabbits as measured by the prothrombin method of Kato remained within normal limits. No significant alteration occurred in the relationship of inorganic to total sulfates in the urine of rabbits.

Discussion

Rats appear to be somewhat more resistant than guinea pigs or rabbits to the effects of being exposed to the vapor of either of these esters in air.

Although a comparison of the minimum lethal dosages of methyl and ethyl acrylate for rabbits indicates that the latter is slightly less toxic than the former when ingested, little difference could be detected between the two materials when they were inhaled in concentrations within the range of 0.300 to 0.328 mg. per liter. However, at slightly higher concentrations the rabbits exposed to the methyl ester died more promptly than those exposed to comparable concentrations of the ethyl compound. For example, when the concentration of methyl acrylate was 0.817 mg. per liter of air, all of the exposed rabbits died before or during the 11th 7-hour period of exposure, whereas with ethyl acrylate 18 periods of exposure to the concentration of 1.09 mg. per liter of air were required in order to kill all the rabbits. Furthermore, all of the rabbits died before or after the 2nd 7-hour exposure to air containing 1.99 mg. of methyl acrylate per liter, but with ethyl acrylate a comparable result was observed only after the fourth 7-hour exposure to air containing 2.01 mg. per liter. Likewise, at these higher concentrations, guinea pigs and rats exposed to the methyl ester succumbed more promptly than did those exposed to the ethyl homolog.

In experiments of 6 months' duration neither compound induced signs of intoxication when inhaled in concentrations of 0.099 to 0.107 mg. per liter.

Smyth and his associates (3) have obtained results which are in good agreement with those described above, in experiments in which rabbits and rats were exposed to ethyl acrylate in concentrations of approximately 1,000, 500, 250 and 63 p.p.m.

In equilibrium at 745 mm. of mercury and 25° C., the concentration of vapor in air reaches 384.3 mg. of methyl acrylate per liter or 231.5 mg. of ethyl acrylate per liter. Therefore, dangerous concentrations can readily occur under ordinary climatic conditions of handling in still air or in enclosed spaces.

In view of the foregoing experimental observations it might be presumed, tentatively, that men may breathe air containing concentrations of the order of 75 p.p.m. (0.26 mg. of methyl acrylate per liter or 0.30 mg. of ethyl acrylate per liter at 25° C. and 745 mm. of mercury) for periods of several hours per day without any considerable likelihood of injurious effects. Obviously this presumption should be accepted cautiously in practice, and men who may be subjected to the vapors should be given careful medical supervision until such time as the facts with reference to human susceptibility can be established.

Summary

1. Oral administration of 0.28 gm. or more of methyl acrylate per kilogram or 0.42 gm. of ethyl acrylate per kilogram resulted in rabbits in fatal poisoning, characterized in its terminal stages by dyspnea, cyanosis and convulsive movements, accompanied by hypothermia and an increase in the packed cell volume of the blood. Severe local irritation of the gastroenteric tract and toxic degenerative changes of cardiac, hepatic, renal and splenic tissues were observed.

2. The presence of the double bond in these esters caused an increase of ten to thirteen fold in their toxicity.

3. No evidence of cumulative effects was observed in rabbits following the oral administration of either 23 sublethal doses of methyl acrylate or 25 sublethal doses of ethyl acrylate (23 mg. of the former or 31.5 mg. of the latter per kilogram on each of 5 days per week over total periods of 33 and 35 days, respectively).

4. When applied on the intact skin of rabbits the methyl and the ethyl ester caused marked local irritation, erythema, edema, thickening and vascular damage.

5. The results observed when animals of various species inhaled methyl acrylate for 7 hours per day on a series of days are expressed in terms of (1) the highest concentration in parts per million which failed to kill animals and (2) the least corresponding concentration which induced death in animals, as follows: rabbit and guinea pig, (1) 95.1, (2) 237; rat, (1) 237, (2) 578; monkey, (1) 31. With respect to ethyl acrylate, the corresponding data are: rabbit and guinea pig, (1) 74.8, (2) 272; rat, (1) 272, (2) 501; monkey, (1) 272, (2) 1,204.

6. Animals subjected to the higher atmospheric concentrations of these esters suffered irritation of the mucous membranes of their eyes, nose and mouth, as well as lethargy, dyspnea and convulsive movements. Microscopic changes occurred in the form of pulmonary congestion, edema and toxic degenerative changes in the heart muscle, liver and kidneys.

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Toxicology Studies

ETHYL ACRYLATE

This is a summary of single exposure studies on animals. The data indicate the relative degree of hazard in handling the product. Increasing degrees of hazard are expressed by these terms: slight, moderate, definite, serious. It must be remembered that results of experiments on animals cannot be numerically translated to probable human response.

The National Research Council defines toxicity as the capacity of a substance to produce injury. Hazard is the probability that injury will result from the handling or use of the substance in the quantity, frequency and manner proposed.

Toxicity is only one factor important in determining the degree of hazard in handling a chemical or in a proposed use. Physical properties of the chemical together with extent and frequency of exposure are equally important.

The term LD_{50} has been adopted as a uniform expression of single dose toxicity for comparing one chemical with another. It refers to that quantity of chemical which kills 50 per cent of exposed animals. For further uniformity, quantities are expressed in grams or milliliters of chemical per kilogram of animal body weight.

Single skin penetration refers to a covered 24-hour skin contact with the liquid chemical.

Single inhalation refers to continuously breathing a certain concentration of chemical vapors for a specified period of time.

Primary irritation refers to the skin response following uncovered skin contact. A covered contact can be expected to have a more severe effect.

Eye injury refers to surface damage produced by contact of the eye with the chemical.

Legal responsibility is assumed only for the fact that all studies reported here, and all opinions, are those of qualified experts.

Single Oral Dose In Rats: Moderate Hazard.

LD_{50} - 2.08 grams per kilogram body weight.

For comparison, this compound is quantitatively about as toxic as a 10% solution of acrylic acid which has an LD_{50} value of 2.5 grams per kilogram.

Single Skin Penetration In Rabbits: Moderate Hazard.

LD_{50} - 1.95 grams per kilogram body weight.

This result suggests that skin penetration in harmful amounts might occur after massive or prolonged skin contact.

Single Inhalation By Rats: Definite Hazard.

Vapor Pressure: 29.3 mm. Hg. at 20°C.

Breathing the vapors in a state approaching saturation in air was fatal to all animals in a 15 minute exposure. Breathing a concentration of 1,000 parts per million for four hours killed none of six animals, but a 2,000 parts per million concentration killed five of six animals after the same exposure time.

Threshold Limit Value: 25 ppm.

(OVER)

For Further Information Write To:

Industrial Medicine and Toxicology Department

UNION CARBIDE CORPORATION

270 Park Avenue, New York, N.Y. 10017

Skin Irritation, Rabbit Belly: Slight Hazard.

The undiluted chemical caused no reaction on the tender skin of the rabbit belly greater than a faint redness of short duration.

Eye Injury To Rabbits: Moderate Hazard.

One average sized drop caused minor injury in the rabbit eye.

This chemical produces injury no more severe than that seen with liquid hand soaps. In case of eye contact, the eye should be given a 15 minute emergency wash.

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Berries and Pineapples; Pineapple Juice
Raspberries

Agricultural Statistics
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