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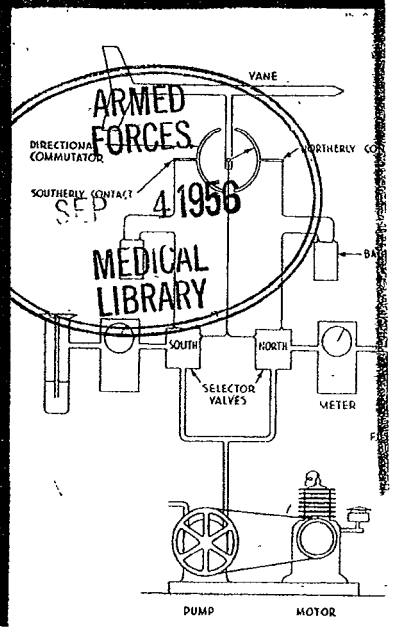

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The Toxicity of Butyl Cellosolve Solvent

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Butyl Cellosolve * ($C_4H_9OCH_2CH_2OH$ —2-butoxyethanol) is a glycol ether with excellent solvent power for many of the resins used in surface coatings. It is a useful coupling agent, because it is miscible with water and with most solvents and many oils. In surface coatings it imparts blush resistance, gloss, and good flow-out. It is also employed in metal cleaners, dry-cleaning soaps, and hydraulic fluids. Butyl Cellosolve has a specific gravity of 0.9019 at 20/20 C, a boiling point of 171.2 C, and a vapor pressure of 0.76 mm. Hg at 20 C. Vapor-saturated air at room temperature has a concentration of the order of 1000 ppm butyl Cellosolve. Its relative evaporation rate is 1, in a scale in which that of Cellosolve is 5, butyl alcohol, 7, methyl Cellosolve, 8, xylene, 10, toluene, 40, and acetone, 200.

Previous Work

Browning [1] summarized the scanty literature upon the toxicity of butyl Cellosolve and mentioned isolated complaints of eye and nose irritation and headache. She found only one report of possible systemic effect in a workman. This man twice suffered attacks of hematuria while working with both butyl Cellosolve and butyl Carbitol.

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Mellon Institute of Industrial Research.
Presented in part at the Annual Meeting of the American Industrial Hygiene Association, Philadelphia, April 23-27, 1956.

* Cellosolve is a trade-mark name, the property of Union Carbide and Carbon Corporation. It designates an ether of ethylene glycol.

The most extensive publication upo toxicity of butyl Cellosolve is the seri three papers by Werner and his asso These workers compared the effects o halation of five glycol ethers. In seven-hour inhalations by mice,[2] butyl solve was found to have an L. $C._{50}$ o ppm. Death resulted as late as the f week after inhalation. The usual sig toxic action was dyspnea. Inhalation of lethal concentrations resulted in hemog nuria. Changes were frequently seen i spleen, occasionally in the liver, lungs, kidneys. In rats inhaling 320 ppm s hours a day, five days a week for five w these workers [3] found only light rever effects, characteristic of mild hemolytic emia. In dogs inhaling 400 ppm fo weeks, they [4] found no indication of he lytic action, little damage to bone mar and little micropathologic effect afte weeks of rest. Hemoglobin, red blood count, and hematocrit were somewhat duced, and some hypochromia, polychro tophilia, and microcytosis were seen. Calc oxalate crystals were present in the ur and there was moderate retention of u It was concluded that butyl Cellosolve p duces in rodents hemolysis of red bl cells, with resulting circulation of immat forms, but that dogs are resistant to t action. Butyl Cellosolve was the most to of the five glycol ethers studied. Howev a concentration about two-thirds of satu tion was required to kill mice, and one-th of saturation inhaled repeatedly caused o mild reversible changes in rodents and do The authors concluded that industrial ha dling of the solvent is no great hazard be cause of its low volatility.

On the basis of this experimental work a threshold limit of 200 ppm has been widely accepted. On the other hand Browning,[5] Patty,[6] and Elkins [7] have each proposed that

Solvent

sive publication upon the Cellosolve is the series of Verner and his associates. mpared the effects of in- glycol ethers. In single ions by mice,[2] butyl Cello- o have an L. $C_{.50}$ of 700 ted as late as the fourth tion. The usual sign of rspnea. Inhalation of near ns resulted in hemoglobi- ere frequently seen in the y in the liver, lungs, and inhaling 320 ppm seven ays a week for five weeks, ound only light reversible stic of mild hemolytic an- nhaling 400 ppm for 12 id no indication of hemo- damage to bone marrow, athologic effect after 5 Hemoglobin, red blood cell ocrit were somewhat re- hypochromia, polychroma- ocytosis were seen. Calcium vere present in the urine, oderate retention of urea. that butyl Cellosolve pro- hemolysis of red blood ig circulation of immature dogs are resistant to this losolve was the most toxic ethers studied. However, bout two-thirds of satura- to kill mice, and one-third ıled repeatedly caused only anges in rodents and dogs. cluded that industrial han- ent is no great hazard be- volatility.

f this experimental work a 200 ppm has been widely e other hand Browning,[5] s[7] have each proposed that lower threshold limits would be more appropriate, the lowest value suggested being 25 ppm.

In view of the increasing number and variety of applications for butyl Cellosolve, work was undertaken to throw more light upon the nature of its action and to form a better estimate of the tolerated concentration.

Demonstration of a Metabolite

Butyl Cellosolve is not amenable to ready identification and estimation in body fluids. It is a reasonable postulate that this glycol ether is oxidized in the mammalian body to butoxyacetic acid. The development and use of the following analytical method verified this postulate.

Reagents.—The reagents used consisted of the following:

(1) Concentrated H_2SO_4
(2) Ten per cent sodium tungstate, 100 gm. C. P. $Na_2WO_4 4H_2O$ per liter distilled water
(3) Van Slyke protein reagent[8]
(4) Alcohol-ammonia chromatogram solvent, 99 ml. 95% EtOH + 1 ml. NH_4OH, sp. gr. 0.9
(5) Indicator, 50 mg. bromophenol blue + 200 mg. citric acid in 100 ml. distilled water
(6) NH_4OH, sp. gr. 0.9

Procedure.—(*a*) Blood: The proteins of a 5 ml. sample of venous blood were precipitated with Van Slyke reagent. After filtration the filtrate or a suitable aliquot was extracted for 24 hours with ether. The ether extract was washed with 5 ml. NH_4OH, and the alkaline solution was evaporated to dryness under an infrared lamp. The residue was taken up in 0.2 to 1.0 ml. distilled water, and 10 λ portions were spotted on chromatogram paper. The development of the chromatogram and the estimation of butoxyacetic acid were carried out by the method described by Nair[9] with use of the chromatogram solvent.

(*b*) Urine: One milliliter of the sodium tungstate solution was added to each twenty milliliters of the urine sample, and the mixture was then acidified with concentrated H_2SO_4 against Congo red paper. After ether extraction the extract was washed with NH_4OH several times. The alkaline washings were collected and diluted to 5-25 ml. in a volumetric flask, depending on the amount of urine sampled.

Ten lambda aliquots were spotted on the paper along with known amounts of butoxyacetic acid as the ammonium salt and chromatographed. At the end of the run the indicator was sprayed on the paper, and an estimation of the amount of butoxyacetic acid in the urine extracts was made. Preliminary work indicated that the extraction of the butoxyacetic acid from urine was completed with seven changes of ether per volume of aqueous liquid. A 16-hour extraction in a liquid-liquid extractor met this condition sufficiently.

The R_f value×100 of butoxyacetic acid under the conditions of the method is 55-59. The lower limit of detection is 25 γ, and the upper limit to read conveniently is 500 γ. The aliquots of the alkaline wash spotted on the paper are adjusted to keep the quantity of acid within these limits. It is important that no more than 10 λ of solution at a time be applied to the paper at any one location. If necessary, repetitive applications, alternating with drying, may be employed to concentrate sufficient material on the paper for analysis. In the analysis of urine samples a large amount of extraneous material may show up on the paper as a long streak, but the upper limit of travel will be well below the butoxyacetic acid spot.

The estimation of the amount of butoxyacetic acid in a spot on the paper is made by comparison with spots containing known amounts of butoxyacetic acid run simultaneously. The circumference and depth of color are two general criteria employed in making this comparison. At the present time there is no commercial densitometer capable of making quantitative measurements with this type of chromatogram. Consequently we are obliged to accept the inaccuracy of a visual comparison. This unavoidable uncertainty reduces the quantitative significance of butoxyacetic acid estimates.

For rigorous identification a large amount of urine was collected from rabbits inhaling 400 ppm butyl Cellosolve for four hours. By means of a silicic acid column[10] the metabolite was isolated from the urine. The melting point of the *p*-phenylphenacyl ester of the isolated metabolite was not depressed upon the addition of the same ester synthesized from an authentic sample of butoxyacetic acid.*

Table 1 presents urinary butoxyacetic acid excretion by various animal species after inhalation of butyl Cellosolve. With the exception of the dogs, a correlation between the vapor concentration and butoxyacetic acid excretion is suggested. Concurrent studies showed that most of the metabolite is excreted in 24 hours.

We were unable to demonstrate butoxyacetic acid in the blood of animals inhaling butyl Cellosolve. It was found, however, in the blood of rabbits four hours after an intravenously administered dose of butyl

* References 11 and 12.

Cellosolve and in the urine collected during the 24 hours following the dose. Accordingly it is reasonable to suppose that it is present in the blood of animals inhaling the glycol ether but at a concentration too low for detection by the present method.

TABLE 1.—*Butoxyacetic Acid Found In Twenty-Four-Hour Urine Samples from Animals Inhaling Butyl Cellosolve Vapor for Four Hours*

Animals, No.	Species	Sex	Approx. Vapor Concentration, Ppm	Estimated Butoxyacetic Acid, Mg.
1	Dog	M	400	55
1	Dog	F	200	42
1	Dog	M	200	100
6	Rabbits	M	400	89–302
2	Rabbits	M	200	12–23
27	Rats	F	400	14*
17	Rats	F	200	7*
12	Rats	F	100	1*
12	Guinea pigs	M	200	5*
12	Guinea pigs	M	100	4*

* Mean value per animal calculated by dividing total milligrams in a pooled urine sample by the number of animals.

Hemolytic Effects

As early as 1938 this laboratory found that hemoglobinuria was evident in many rats dying after an oral dose of butyl Cellosolve. The report by Werner's group [3] that rodents develop hemolytic anemia during inhalation dictated that hemolytic effects should be studied in animals absorbing the glycol ether.

To determine whether the hemoglobinuria was produced by cellular destruction in the kidney or by in vivo hemolysis, eight female rats inhaled 432 ppm and were killed in pairs at two-hour intervals. Kidney sections were studied microscopically; intact erythrocytes were sought in the urine, and hemin crystals were searched for by Teichmann's method.[13] Slight cloudy swelling of the kidney was seen after two hours, and pathology had increased only to marked cloudy swelling of convoluted and loop tubules after eight hours. In vivo hemolysis was evident in two hours and severe after eight hours. Hemin crystals were found in the urine after four hours. Hemoglobinuria was evident in about three hours, but no intact erythrocytes were found in the urine time. It was concluded that the hemo nuria was produced by in vivo hem and that kidney injury was not respo

During the repeated inhalation s described later, transient hemoglob was observed in rodents. It was e during the first few hours of inhalatio was not seen at all after the third d inhalation. In a massive inhalatio vapors almost saturated at room tem ture, the urine of female rats became n black with hemoglobin. The blood h globin concentration was halved by a s hour inhalation; the erythrocyte coun reduced even further, and the leuc count was raised to the same extent.

In order to follow the course of in vivo h lysis, a test of red blood cell osmotic fragilit used. A series of stock solutions of dried s chloride in double distilled water was pre differing in concentration by 0.02% from 0 to 0.52% and by 0.04% from 0.56% to 0. One milliliter of each solution was placed separate clean 12 by 75 mm. test tube. One of whole blood was dispensed into each test from a 22 gauge hypodermic needle held a angle of 45 deg. with the long bevel down Without delay the blood was thoroughly m with the saline by inverting each tube twice. tubes were held at room temperature for hours. Initial hemolysis was designated as highest saline concentration in which the supe tant liquid first acquired a discernible yellow hue, and complete hemolysis as the highest centration in which there was no visible laye intact cells on the bottom of the tube while liquid was a transparent deep-red color.

Typical erythrocyte osmotic fragility ues for untreated rats are initial hemoly in 0.48% saline and complete in 0.40 written here as 0.48-0.40. The test is rep ducible to within one tube in the concent tion series employed.

Erythrocyte osmotic fragility values w followed on six female rats during ni daily seven-hour inhalations of 200 p butyl Cellosolve. Each day during inhala tion fragility rose as high as 0.68-0.48 an returned to normal overnight. In a simila experiment, by the fourth day the erythro cyte count had fallen by 50%, and th hemoglobin level by 25%. A five-day res

TABLE 2.—*In Vitro and In Vivo Response of Mammalian Erythrocytes to Butyl Cellosolve and to Sodium Butoxyacetate*

Animals, No. Species	Sex	In Vitro Response*				In Vivo Response	
		2.5% Butyl Cellosolve		0.1% Na Butoxyacetate			
		Max. Time Without Hemolysis, Min.	Min. Time to Hemolysis, Min.	Max. Time Without Hemolysis, Min.	Min. Time to Hemolysis, Min.	Osmotic Fragility upon Inhalation of Butyl Cellosolve	Hemoglo Inhalat Cellosol
2 Rats	F	46	60–67				
3 Rats	M			35–40	40–60	Significant increase at 62 ppm×4 hr.	Seen at 2
2 Rats	F			35–40	40–46		but not hr.
2 Mice	M	25–30	35–40				
2 Mice	M			40–45	50–60	Significant increase at least as low as 100 ppm×7 hr.	Seen at 200
1 Mouse	F			45	55		at 100 p
2 Rabbits	M	110–113	123–125				
4 Rabbits	M			60–90	80–100	Significant increase at 125 ppm×7 hr.	Not seen a
2 Rabbits	F			80–90	85–100		ppm×7 hr
1 Monkey	F			120	130	No significant increase at 200 ppm×7 hr.†	Not seen a
1 Monkey	M			103	120		ppm×7 hr
2 Dogs	F	20	40–86	89–120		No significant increase at 665 ppm×7 hr.×2 days†	Not seen a
2 Dogs	F						ppm×7 hr
1 Human	F	80	100	147–268		No significant increase at 200 ppm×8 hr.†	Not seen at hr.
2 Humans	M	60–83	80–94				
2 Guinea pigs	M	60–68	80	360		No significant increase at 665 ppm×8 hr.†	Not seen at hr.
2 Guinea pigs	M						

* These tests measured at least in part the mechanical fragility of the erythrocytes.
† These concentrations were the highest studied with these species.

TABLE 3.—*Comparison of the Effects of the Four-Hour Inhalation of Butyl Cellosolve and Some Homologues on the Osmotic Fragilities of the Erythrocytes of Groups of Six Female Rats*

Chemical	Lowest Concentration Causing Significant Fragility, Ppm	Highest Concentration Causing No Fragility, Ppm
Methyl Cellosolve	2000	1000
Cellosolve	125	62
Propyl Cellosolve	62	32
Isopropyl Cellosolve	62	32
Butyl Cellosolve	62	32
Butyl ethyl Cellosolve	125	62
Diethyl Cellosolve	2000	1000
Methyl Cellosolve acetate	32	16
Cellosolve acetate	62	32
Vinyl butyl Cellosolve	32	16
Heptyl-3 Cellosolve	—	*
2-Ethylhexyl Cellosolve	—	*
Nonyl Cellosolve	—	*
Phenyl Cellosolve	—	*

* Concentrations approached saturation at room temperature.

Quantitative Toxicity

Acute Oral Toxicity.—The Cellosolves used in these studies represent commercial material sold under the trade-mark name during the year the work was done. Wistar rats were used exclusively until 1942, and Sherman from then until June o when Carworth-Wistar found favo three lines have a like order of acu dose response to certain chemicals for comparison. The acute oral tes standardized procedure making use o fasted rats, 5 to 6 weeks of age and 120 gm. in weight, dosed at levels dif by a factor of 1.26 or 2.0 in a geo series. The Sherman and Carworth were reared in our own colony and tained from time of weaning on Roc rat diet (complete). The earlier W stock was always procured from comm breeders. Weil's [14] tables for calculatin median-effective dose (L. $D._{50}$) were In this paper L. $D._{50}$ refers to the st tically computed, most probable dosage that will result in the death of 50% of species dosed within a 14-day observati period. Any deviation from the proced is noted in tabulation or text. Table 4 a chronological listing of the acute oral t icity results for three Cellosolves.

Rabbits have an oral L. $D._{50}$ of 0.32 g

'an Erythrocytes to
cetate

In Vivo Response	
: Fragility upon ıtion of Butyl olve	Hemoglobinuria upon Inhalation of Butyl Cellosolve
ınt increase at mX4 hr.	Seen at 203 ppmX7 hr. but not at 107 ppmX7 hr.
ınt increase at as low as 100 :7 hr.	Seen at 200 ppm but not at 100 ppmX7 hr.
ınt increase at ·mX7 hr.	Not seen as high as 197 ppmX7 hr.
ificant increase ppmX7 hr.†	Not seen as high as 200 ppmX7 hr.
uficant increase · ppmX7 hr.X2	Not seen as high as 665 ppmX7 hr.
ificant increase ppmX8 hr.†	Not seen at 200 ppmX8 hr.
ificant increase ppmX8 hr.†	Not seen at 665 ppmX8 hr.

n then until June of 1952 th-Wistar found favor. All ve a like order of acute oral to certain chemicals chosen n. The acute oral test is a rocedure making use of non- to 6 weeks of age and 90 to ight, dosed at levels differing f 1.26 or 2.0 in a geometric Sherman and Carworth rats ı our own colony and main- me of weaning on Rockland ıplete). The earlier Wistar ys procured from commercial l's [14] tables for calculating the e dose (L. D.$_{50}$) were used. L. D.$_{50}$ refers to the statis- d, most probable dosage level in the death of 50% of the within a 14-day observation leviation from the procedure bulation or text. Table 4 is listing of the acute oral tox- r three Cellosolves.

e an oral L. D.$_{50}$ of 0.32 gm/

TABLE 4.—*Single Oral Dose of L. D.$_{50}$'s of Cellosolves*

Year	Species	Sex	Weight, Gm.	1 Ml.=Gm. in Water	L. D.$_{50}$ and Range
				Butyl Cellosolve	
1938	Rat	M	90-120	0.10	1.48 (1.15 to 1.91) gm./kg.
1938	Guinea pig	M+F	200-300	0.10	1.20 (0.96 to 1.50) gm./kg.
1940	Rabbit	M	1500-3000	0.20	0.37 gm./kg.
1951	Rat	M	90-120	0.10	2.6 (2.3 to 2.9) gm./kg.
1951	Rat	F	90-120	0.10	2.3 (1.9 to 2.8) gm./kg.
1952	Rat	M	90-120	0.05	2.8 (2.4 to 3.3) gm./kg.
1952	Rat	F	90-120	0.05	1.6 (1.2 to 2.1) gm./kg.
1953	Mouse	M	20-30	0.10	1.23 (0.94 to 1.62) gm./kg.
1953	Rat	M	90-120	0.05	1.9 (1.3 to 2.6) gm./kg.
1954	Rat	F	90-120	0.05	1.6 (1.4 to 1.9) gm./kg.
1954	Rat	M	30-60	0.05	3.0 (2.6 to 3.4) gm./kg.
1954	Rat	F	30-60	0.05	2.3 (2.1 to 2.6) gm./kg.
1954	Rat	M	335-460	0.05	0.56 gm./kg.
1954	Rat	F	260-320	0.05	0.63 (0.38 to 0.75) gm./kg.
1954	Rat	M	90-120	0.05	2.4 (2.1 to 2.7) gm./kg.
1954	Rat	F	90-120	Undiluted	2.8 (2.2 to 3.7) ml./kg.
1955	Rat	M	90-120	0.05	2.1 (1.4 to 3.1) gm./kg.
1955	Rabbit	M	2700-3200	0.05	0.32 (0.24 to 0.43) gm./kg.
				Methyl Cellosolve	
1941	Guinea pig	M+F	200-300	0.10	0.95 (0.84 to 1.08) gm./kg.
1944	Rabbit	M	2000-3000	0.25	0.89 (0.65 to 1.23) gm./kg.
1952-55	Rat	M	90-120	0.10	3.25 gm./kg. mean of 4 assays
1953-54	Rat	F	90-120	0.10	3.4 gm./kg. mean of 2 assays
				Cellosolve	
1938	Rabbit	M	2000-3000	0.20	3.1 gm./kg.
1938	Guinea pig	M+F	200-300	0.10	1.4 gm./kg.
1952-55	Rat	M	90-120	0.10	5.0 gm./kg. mean of 4 assays
1954	Rat	F	90-120	0.10	5.4 (4.9 to 5.9) gm./kg.
1954	Rat	F	90-120	Undiluted	5.9 (5.4 to 6.4) ml./kg.

kg. and are the most sensitive to butyl Cellosolve of the species tested. Mice and guinea pigs follow with a value of 1.2 gm/kg. Young rats are four to five times more resistant to single oral doses than are one-year-old adults, as evidenced by L. D.$_{50}$ values of 3.0, 2.4, and 0.56 gm/kg. for male weanlings, 6-week-olds, and yearlings, respectively. When given undiluted, instead of as a 5%, 10%, or 20% solution in water, the 1954 L. D.$_{50}$ value of 2.8 ml/kg. (2.5 gm/kg.) for females is almost identical with the 2.4 gm/kg. mean value for the males.

Sluggishness, ruffling of coats, prostration, and narcosis followed the administration of dosages at or above the L. D.$_{50}$. Autopsies performed on rats that died revealed congested or hemorrhaged lungs, mottled livers, severely congested kidneys, and hemoglobinuria. The latter symptom is seen in males on the 3.0 gm/kg. level but in females on levels as low as 1.5 gm/kg. Early death is attributed to narcotic effect, and delayed death to lung and kidney damage.

For comparison, the means of several closely agreeing, single oral dose rat assays on methyl Cellosolve and Cellosolve are given. Inspection of Table 4 will show the order of decreasing acute oral toxicity to be butyl Cellosolve, methyl Cellosolve, and Cellosolve, with L. D.$_{50}$'s of 2.5, 3.4, and 5.5 gm/kg. The guinea pig is not as discriminating as the rat, as values of 1.2, 0.95, and 1.4 gm/kg. for the three compounds in like order indicate. Rabbits follow the rat order with L. D.$_{50}$'s of 0.32, 0.89, and 3.1 gm/kg.

Repeated Feeding.—Five randomly distributed groups of five male and five female Sherman rats, about 6 weeks of age and weighing between 100 and 150 gm., were fed Food Research Laboratory Diet 2-C in which was incorporated 2.0%, 0.5%, 0.125%, 0.03%, and 0.0% butyl Cellosolve. The latter group served as the control. The diet consisted of 60 parts of freshly ground whole wheat, 30.5 parts of dried whole milk, 1.5 parts of dried liver extract U.S.P., 5 parts of Type 50-B inactive dried brewer's yeast, 2 parts of iodized salt, and 1 part of calcium carbonate. Statistical evaluation of the several criteria of response was made.

The 90-day feeding resulted in butyl Cellosolve intakes of 1.54, 0.31, 0.076,

0.018, and 0.0 gm/kg/day for the respective concentration groups. There were no deaths attributable directly to toxic action of the compound. The fact that rats survived 1.54 gm/kg/day means that their intake was about three-fifths of an L. $D._{50}$ per day, or actually 54 L. $D._{50}$'s in 90 days. In each fatality lung infection was found attended by kidney and liver pathology, such as is coincident with death caused by pneumonia in the rat. Appetite was not affected, and no pertinent micropathology was discovered. Blood was not found in pooled urine samples from the 2.0%, 0.5%, and control groups after three and six days on the diet either by the benzidine test or by spectrophotometric measurement for hemoglobin pigments. However, the mean weight gain of the surviving rats was significantly depressed below that of the control group in the 2.0% concentration group. Liver weight as percentage of body weight was significantly increased at both the 2.0% and 0.5% levels, and kidney weight at the 2.0% level.

The highest level where significant deviation from the control was within the usually accepted range of variation, and hence not chargeable to treatment, lies between 0.5% and 0.125% in the diet or between 0.31 and 0.076 gm/kg/day or at an intake between 1/8 and 1/32 of the L. $D._{50}$ per day.

Parenteral Injection.—Undiluted butyl Cellosolve causes hemolysis of rat erythrocytes, as do all concentrations above 3% in 0.75% NaCl solutions. Tail vein injection of rats resulted in an L. $D._{50}$ of 0.38 gm/kg. for a 3% solution in 0.75% NaCl and 0.34 ml/kg. for undiluted compound. Mice were more tolerant as shown by an L. $D._{50}$ of 1.1 gm/kg. for the 3% solution. By ear vein in rabbits the L. $D._{50}$ for the 3% solution was 0.50 gm/kg. and for the undiluted material 0.28 ml/kg.

Methyl Cellosolve is hemolytic to rat erythrocytes in vitro in concentrations above 25% in 0.75% NaCl, and Cellosolve is hemolytic above a concentration of 18%. The comparative intravenous L. $D._{50}$'s for rats in the dilutions mentioned are 2.7 gm/kg. for methyl and 3.3 for Cellosolve, and for the undiluted compounds 2.2 (mean of two tests) and 2.4 ml/kg.

The L. $D._{50}$ values by intraperitoneal injection of the undiluted compound in rats are as follows: butyl, 0.55; methyl, 2.5, and Cellosolve, 2.14 ml/kg. In rats, therefore,

TABLE 5.—*L. $D._{50}$'s of Cellosolves and of Sodium Butoxyacetate by Injection*

Year	Species	Sex	Weight, Gm.	Concentration, 1 Ml.=Gm.	L. $D._{50}$ and Range
				Butyl Cellosolve	
				(Intravenous)	
1951	Rabbit	M	2500–3000	0.03 in 0.75% NaCl	0.50 (0.38 to 0.65) gm./kg.
1951	Rat	F	170–230	0.03 in 0.75% NaCl	0.38 (0.29 to 0.50) gm./kg.
1953	Mouse	M+F	15–35	0.03 in 0.75% NaCl	1.13 gm./kg.
1954	Rat	F	90–120	Undiluted	0.34 (0.30 to 0.38) ml./kg.
1955	Rabbit	M	2500–3000	Undiluted	0.28 ml./kg.
				(Intraperitoneal)	
1952	Rat	F	90–120	Undiluted	0.55 (0.32 to 0.94) ml./kg.
				Methyl Cellosolve	
				(Intravenous)	
1953	Rat	F	90–120	0.25 in 0.75% NaCl	2.70 (1.94 to 3.77) gm./kg.
1954	Rat	F	90–120	Undiluted	2.14 (1.54 to 2.99) ml./kg.
1954	Rat	F	90–120	Undiluted	2.30 (1.87 to 2.83) ml./kg.
				(Intraperitoneal)	
1954	Rat	F	90–120	Undiluted	2.46 (1.88 to 3.23) ml./kg.
				Cellosolve	
				(Intravenous)	
1953	Rat	F	90–120	0.18 in 0.75% NaCl	3.25 (2.48 to 4.26) gm./kg.
1954	Rat	F	90–120	Undiluted	2.38 (1.92 to 2.97) ml./kg.
				(Intraperitoneal)	
1955	Rat	F	90–120	Undiluted	2.14 (1.54 to 2.99) ml./kg.
				Sodium Butoxyacetate	
				(Intravenous)	
1954	Rat	F	90–120	0.03 in 0.75% NaCl	0.78 (0.59 to 1.02) gm./kg.

% in the diet or between 0.31 gm/kg/day or at an intake bend 1/32 of the L. D.50 per day.

l Injection.—Undiluted butyl causes hemolysis of rat erythro- o all concentrations above 3% JaCl solutions. Tail vein injec- resulted in an L. D.50 of 0.38 a 3% solution in 0.75% NaCl l/kg. for undiluted compound. more tolerant as shown by an .1 gm/kg. for the 3% solution. in rabbits the L. D.50 for the was 0.50 gm/kg. and for the aterial 0.28 ml/kg.

ellosolve is hemolytic to rat in vitro in concentrations above 5% NaCl, and Cellosolve is ove a concentration of 18%. itive intravenous L. D.50's for ilutions mentioned are 2.7 gm/ yl and 3.3 for Cellosolve, and uted compounds 2.2 (mean of d 2.4 ml/kg.

50 values by intraperitoneal he undiluted compound in rats : butyl, 0.55; methyl, 2.5, and 14 ml/kg. In rats, therefore,

vacetate by Injection

ution, 3m.	L. D.50 and Range
losolve ous)	
% NaCl	0.50 (0.38 to 0.65) gm./kg.
% NaCl	0.38 (0.29 to 0.50) gm./kg.
% NaCl	1.13 gm./kg
ed	0.34 (0.30 to 0.38) ml./kg.
ed	0.28 ml./kg.
neal)	
d	0.55 (0.32 to 0.94) ml./kg.
osolve us)	
NaCl	2.70 (1.94 to 3.77) gm./kg.
d	2.14 (1.54 to 2.99) ml./kg.
d	2.30 (1.87 to 2.83) ml./kg.
neal)	
d	2.46 (1.88 to 3.23) ml./kg.
e us)	
NaCl	3.25 (2.48 to 4.26) gm./kg.
l	2.38 (1.92 to 2.97) ml./kg.
eal)	
l	2.14 (1.54 to 2.99) ml./kg.
acetate is)	
NaCl	0.78 (0.59 to 1.02) gm./kg.

the Cellosolves fall in the same order of decreasing toxicity by the parenteral and oral routes. Sodium butoxyacetate with an L. D.50 of 0.78 is included in Table 5 because it is a demonstrable metabolite of butyl Cellosolve.

Skin Penetration.—Male albino New Zealand strain rabbits, 3 to 5 months of age, were immobilized during a 24-hour skin contact period. Thereafter, the Vinylite sheeting used to retain the dose in contact with the clipped skin of the trunk was removed, and the animals were caged for the remainder of the 14-day total observation period. The rabbits were procured locally and maintained on Rockland rabbit ration. Weil's [14] tables for calculating the L. D.50's were used. Table 6 presents the results.

TABLE 6.—*Skin Penetration Toxicity of Cellosolves*

Year	Species	Sex	Weight, Kg.	Concentration	L. D.50 and Range (14-day Observation Period)
			Butyl Cellosolve		
1946	Rabbit	M	2.0 to 3.0	Undiluted	0.56 (0.48 to 0.64) ml./kg.
1947	Rabbit	M	2.7 to 3.7	Undiluted	0.56 ml./kg. killed 10/10
1948	Rabbit	M	2.0 to 3.0	Undiluted	0.56 ml./kg. killed 3/6
1951	Rabbit	M	2.5 to 3.0	Undiluted	0.56 ml./kg. killed 4/6
1954	Rabbit	M	2.5 to 3.0	Undiluted	0.5 ml./kg. killed 6/8
1954	Rabbit	M	2.5 to 3.0	Undiluted	0.5 ml./kg. killed 5/8*
1955	Rabbit	M	2.5 to 3.0	Undiluted	0.45 (0.25 to 0.82) ml./kg.
1955	Rabbit	M	2.7 to 3.2	Undiluted†	2.0 ml./kg. killed 2 of 4
			Methyl Cellosolve		
1944	Rabbit	M	2.5 to 3.0	Undiluted	1.34 (1.26 to 1.43) ml./kg.
			Cellosolve		
1944	Rabbit	M	2.3 to 3.3	Undiluted	3.56 (2.24 to 5.66) ml./kg.
1944	Rabbit	M	2.3 to 3.3	Undiluted†	16.3 (13.2 to 20.2) ml./kg.

* Contact period, 4 hours; all others, 24 hours.
† Uncovered, rubbed into skin by gentle massage.

The L. D.50 for rabbits by skin penetration of undiluted butyl Cellosolve as determined in 1946 was 0.56 and in 1955, 0.45 ml/kg. Extreme congestion of the kidney, hemoglobinuria, pale liver, and engorged spleen were noted upon autopsy of the rabbits that succumbed.

In 1947, 10 rabbits were dosed at the L. D.50 (0.56 ml/kg.), and they all died within 48 hours of the application. These rabbits weighed about 3.0 kg. and were procured from Rockland Farms. Hemoglobinuria was a common finding. Again in 1948 butyl Cellosolve was applied to six rabbits from our regular local supplier; three of the six survived 0.56 ml/kg., but all had hemoglobinuria. Similarly, in 195 four of six rabbits succumbed when dose at 0.56 ml/kg.

For comparison, the rabbit skin penetra tion L. D.50 for methyl Cellosolve is 1 ml/kg. and for Cellosolve, 3.6 ml/kg. B simple inunction the L. D.50 of Cellosolve i 16.3 ml/kg. In effect this means that rabbit can tolerate four times as much Cellosolv when it is gently rubbed into the uncovere skin in one application as they can when th compound contacts the skin under an im pervious sheeting for 24 hours. The same relationship holds for butyl Cellosolve, a it has an L. D.50 of 2.0 ml/kg. by inunction and about 0.45 ml/kg. under impervious sheeting.

Rapidity of skin penetration in rabbits is demonstrated by the fact that a 4-hour and a 24-hour application of 0.5 ml/kg. of butyl Cellosolve under impervious sheeting produced comparable mortality ratios, namely, 5 of 8 and 6 of 8.

Increased erythrocyte osmotic fragility of rabbit blood one hour after a three-minute skin contact period with 0.56 ml/kg. butyl Cellosolve on 4.5% of skin area graphically demonstrates passage through the skin.

Range-Finding Inhalations.—Many of these data collected on inhalation toxicity were to guide the selection of concentration levels for repeated inhalation tests. Known concentrations were obtained by the methods described in the next section. The early

static exposures were made in an apparatus that recirculated air at room temperature over a wick saturated with the chemical during the entire period.

The concentrated vapor results of 1951 were achieved by passing air at 2.5 liters per minute through a fritted glass disc immersed in 50 ml. of the liquid held at room temperature. Judging by mortalities after eight hours, the concentrated vapor was somewhat less than 800 ppm butyl Cellosolve. Furthermore, it is again apparent that one-year-old rats are more susceptible than young, actively growing rats, as was the case with single oral doses. At 375 ppm the adults died after seven hours, while the 6-week-old rats survived eight hours at 500 ppm. The repeated exposures, 8 hours per day, served to predict that rats would not tolerate 250 ppm for 90 days.

Table 7 lists the results. Methyl Cellosolve and Cellosolve are not drastically different from each other in single inhalation; both are less toxic than butyl Cellosolve. The vapor pressures are such that saturation at room temperature would approach 1000 ppm for butyl Cellosolve, 5000 for Cellosolve, and 8000 for methyl Cellosolve. Concentrated vapor, as prepared, would be expected to approach saturation.

Repeated Inhalation Methods.—Inhalation by rats, guinea pigs, mice, dogs, and monkeys took place in chambers ranging in capacity from 200 to 7900 liters (a 6½ ft. cube). Solvent was delivered at a constant rate by means of a displacement-type proportioning pump, originally described by Irish and Adams,[15] or by a motor-driven syringe into an electrically heated tubular Pyrex evaporator, such as described by Carpenter and co-workers.[16] The resulting vapor-air mixtures were conducted to the chambers under slight negative pressure at a rate to provide a theoretical turnover of chamber air every three to five minutes.

Food and water were withheld from all animals during seven-hour exposure periods five days each week. Rats and mice were maintained on Rockland rat diet (com

TABLE 7.—*Range-Finding Inhalation Studies on Cellosolves*

Year	Species	Weight, Gm.	Sex	Concentration, Ppm	No. Exposures × Hr.	Mortality Ratio	Hemoglobinuria
				Butyl Cellosolve			
1940	Rat	70–95	M	C.V. Recirculator	1×9	4/6	
1940	Rat	75–85	M	C.V. Recirculator	1×4	2/6	
1940	Rat	88–106	M	C.V. Recirculator	1×2	0/6	
1942	Guinea pig	300–350	M+F	C.V.	1×4	1/6	0
1951	Rat	120–140	F	C.V.	1×8	1/6	+
1951	Rat	120–150	F	C.V.	1×8	0/6	+
1951	Rat	120–130	F	800	1×8	3/6	+
1951	Rat	120–130	F	800	1×4	0/6	
1951	Rat	100–120	F	500	1×8	0/6	
1951	Rat	120–130	F	500	1×4	1/6	+
1951	Rat	120–160	M	500	2×8	3/6	+
1951	Rat	140–160	F	250	4×8	1/5	+
1951	Rat	92–104	F	250	6×8	0/6	+
1952	Rat	88–98	F	125	6×8	0/6	0
1952	Rat	380–500	M	375	1×7	11/13	+
		250–330	F	375	1×7	23/23	+
				Methyl Cellosolve			
1944	Rat	115–160	M	2000	1×8	6/6	
1944	Rat	120–140	M	2000	1×4	1/5	
1944	Rat	125–150	M	2000	1×2	0/6	
1952	Rat	150–175	M	C.V.	1×4	2/6	+
1952	Rat	120–140	M	8000	1×4	4/6	
1952	Rat	125–160	M	4000	1×4	1/6	
				Cellosolve			
1945	Rat	90–130	M+F	4000	1×8	6/6	
1945	Rat	85–105	M	4000	1×4	3/6	
1945	Rat	125–160	M+F	2000	1×8	3/6	
1952	Rat	95–115	M+F	2000	1×4	0/6	
1952	Rat	130–240	M+F	C.V.	1×8	10/12	+
		120–185	M+F	C.V.	1×4	1/12	

C.V.—"Concentrated" vapor generated at room temperature.

temperature would approach
butyl Cellosolve, 5000 for
8000 for methyl Cellosolve.
apor, as prepared, would be
proach saturation.

thalation Methods.—Inhala-
uinea pigs, mice, dogs, and

Cellosolves

No. Exposures × Hr.	Mortality Ratio	Hemoglobinuria
1×9	4/6	
1×4	2/6	
1×2	0/6	
1×4	1/6	0
1×8	1/6	+
1×8	0/6	+
1×8	3/6	+
1×4	0/6	
1×8	0/6	+
1×4	1/6	+
2×8	3/6	+
4×8	1/5	+
6×8	0/6	+
6×8	0/6	0
1×7	11/13	+
1×7	23/23	+
1×8	6/6	
1×4	1/5	
1×2	0/6	
1×4	2/6	+
1×4	4/6	
1×4	1/6	
1×8	6/6	
1×4	3/6	
1×8	3/6	
1×4	0/6	
1×8	10/12	+
1×4	1/12	

e in chambers ranging in
) to 7900 liters (a 6½ ft.
as delivered at a constant
a displacement-type pro-
originally described by
[15] or by a motor-driven
lectrically heated tubular
such as described by
workers.[16] The resulting
were conducted to the
ght negative pressure at
ı theoretical turnover of
three to five minutes.
were withheld from all
n-hour exposure periods
k. Rats and mice were
ckland rat diet (com-

plete),‡ guinea pigs on Rockland rabbit ration supplemented with kale, dogs on Friskies meal,§ and monkeys on Okatie Farms ‖ diet. The rodents had food and water ad libitum when not inhaling vapor, and the large animals were fed once daily.

Healthy animals whose weights differed less than two standard deviations from the group mean were distributed at random among the exposure and control groups. Control groups were carried each time for rodents but not for the dog and monkey exposures because of limitations on housing space.

All animals were weighed weekly and at the completion of the study. The livers and kidneys of the rodents were removed and weighed after disposition of the animals by section of the cervical cord and suspension by the tail for approximately five minutes. Dogs and monkeys received an overdose of sodium pentobarbital intravenously. Portions of the following tissues of dogs and monkeys were taken for histological examination: adrenals; appendix vermiformis; gall bladder; gonad; heart; intestine; kidneys; liver; lung; pancreas; spleen; stomach; thyroid; urinary bladder; uterus, and any other abnormal tissue. Usually only the liver, lung, and kidney of the rodents were studied.

Erythrocyte and leucocyte counts, hemoglobin levels, erythrocyte osmotic fragility, and hematocrit values were followed on the dogs and monkeys, using blood taken immediately upon removal from exposure. Sulfobromophthalein sodium (Bromsulphalein) retention time, sedimentation rate, icterus index, blood urea nitrogen, and plasma fibrinogen levels were measured by conventional methods. When high-level groups yielded negative results, the tests were omitted at lower exposure levels. An erythrocyte permeability test by the method of Boatman and Moses [17] was performed once on the dogs exposed at the 200 ppm level. Erythrocyte osmotic fragility tests were performed on some of the smaller animals.

The concentrations of butyl Cellosolve vapor in the exposure chambers were checked four times daily with a portable 50 cm. Zeiss interferometer. The instrument was calibrated against a bichromate oxidation method, adapted from the procedure described by Werner and Mitchell [18]; 5 ml. 0.33 N $K_2Cr_2O_7$ and 5 ml. concentrated H_2SO_4, sp. gr. 1.84, were added to a 2 liter Florence flask fitted with a 29/42 standard taper female joint and stoppered with a matching male joint sealed to a stopcock. The flask was evacuated to a pressure such that approximately a liter of the exposure atmosphere would be sampled when the stopcock was opened. For use, the stopcock was attached to a sampling tube placed centrally in the exposure chamber and opened until the flask pressure returned to atmospheric. A sample of the exposure atmosphere was read simultaneously in the interferometer. The flask was shaken for 15 minutes on a wrist-action shaker, heated on a steam bath for 90 ±5 minutes, cooled, and then 250-300 ml. distilled H_2O and 3 gm. KI were added. The contents were titrated with 0.05 N $Na_2S_2O_3$ with the addition of 4 ml. of 1% starch solution near the end-point. A blank determination was made on room air. Burette readings were converted to parts per million by means of the following equations:

$$\text{mg. butyl Cellosolve/liter air} = \frac{(\text{ml. } Na_2S_2O_3 \text{ blank} - \text{ml. } Na_2S_2O_3 \text{ sample}) \times 0.227}{\text{liters of air sample}}$$

$$\text{ppm butyl Cellosolve} = \text{mg./l.} \times 206.9$$

Repeated Inhalations by Rats and Guinea Pigs: Groups of rats and guinea pigs inhaled various concentrations of butyl Cellosolve 7 hours a day, five days a week for a total of 30 days. The initial weight of the Sherman rats was between 140 and 190 gm., and the guinea pigs weighed 435 to 580 gm.

Table 8 summarizes the results. The most notable finding was the greater susceptibility of female rats at 432 ppm. The 15 females died within three days, while 3 males survived 30 inhalations.

Gross pathology in the rats consisted of congestion and hemorrhage of the lungs and congestion of most of the abdominal viscera. Histological examination confirmed the gross findings and revealed cloudy swelling of the liver. Hemoglobinuria was observed in most of the rats at the 432 and

‡ Rockland diets are prepared by the Arcady Farms Milling Co., Chicago.

§ Friskies meal is a product of Albers Milling Co., Kansas City, Mo.

‖ Okatie Farms, Pritchardville, S. C.

TABLE 8.—*Response of Rats and Guinea Pigs to Inhalation of Butyl Cellosolve Vapor For Thirty Exposures, Seven Hours per Day, Five Days per Week*

Animals, No.	Concentration, Ppm Mean	Mortality Ratio	Body Wt.	Kidney Wt.	Liver Wt.	Gross Pathology	Micro-pathology	Erythrocyte Fragility*	Hemoglo-binuria
Rats									
15 (F)	432	15/15	—	—	—	+	+	+	+
15 (M)	432	12/15	—	—	—	+	+	+	+
15 (F)	314	15/15	—	—	—	+	+	+	+
15 (M)	203	0/15	N	0	>	0	0	+	+
14 (F)	203	0/14	N	0	>	0	0	+	+
14 (M)	107	0/14	N	>	>	0	0	+	0
15 (F)	107	0/15	N	>	>	0	0	+	0
15 (M)	54	0/15	N	N	N	0	0	+	0
15 (F)	54	0/15	N	N	N	0	0	+	0
Guinea pigs									
10 (M)	494	2/10	N	>	N	+	+	0	0
10 (M)	376	1/10	<	>	N	+	+	0	0
10 (M)	203	0/10	N	>	N	0	0	0	0
10 (M)	107	0/10	N	N	N	0	0	0	0
10 (M)	54	0/10	N	N	N	0	0	0	0

N Values not statistically different from controls (p is equal to or >0.05).
> Values statistically higher than controls ($p<0.05$).
< Values statistically lower than controls ($p<0.05$).
+ Substance or response present.
0 Substance or response absent.
— Test not performed.
* These values obtained from extra animals not included in the mortality ratios.

314 ppm levels, but in only 1 of 15 males and 10 of 15 females subjected to 203 ppm. This condition was not observed in the surviving animals after the second day of exposure. Erythrocyte osmotic fragility, proportional to the vapor concentration, was found in rats immediately after a seven-hour inhalation of 107 ppm or any higher concentration, and after some of a series of 30 inhalations of 54 ppm, with values for females usually exceeding the males. In almost every case these high fragility values returned to normal after overnight rest.

There were no statistically significant findings as regards mortality, body weight change, or tissue damage in the rats exposed at the 203, 107, and 54 ppm levels 7 hours per day for 30 days. However, significant increases in kidney and liver weights as percentage of body weight were observed in the rats at 203 and 107 ppm levels. The highest concentration level where deviation from the control is within the usually accepted range of variation, and therefore not related to exposure, is between 54 and 107 ppm. Guinea pigs were more refractory. The only criterion of response which differed significantly from the controls at the 203 ppm level was an increase in kidney weight as percentage of body weight. To characterize more completely the response of guinea pigs, groups of 10 males were exposed 30 days to 494 and 376 ppm. In addition, groups of six males and six females were exposed at these levels to detect any sex difference in susceptibility. The mean weights of the males varied from 435 to 580 gm., and the females from 351 to 369 gm.

Significant tissue damage, which consisted of lung congestion and cloudy swelling of the convoluted and loop tubules of the kidneys, was found only in the two fatalities at 494 ppm and the one at 376 ppm. The mean weights of both groups of animals fell slightly by the third exposure, but only at the 376 ppm level were they significantly below the controls after 30 exposures. A significant elevation in kidney weight as percentage of body weight occurred in both groups The mixed groups showed essentially the same mortality ratios as did the all-male groups and evidenced no significant difference in sex response. The above data indicate that guinea pigs tolerate a concentration below 203 ppm but above 107 ppm butyl Cellosolve. The latter concentration was without statistically significant deviation from controls in any of the criteria of toxic response followed in this study.

Repeated Inhalation by Mice: Groups

of Butyl Cellosolve Vapor
Days per Week

Micro-pathology	Erythrocyte Fragility*	Hemoglo-binuria
+	+	+
+	+	+
+	+	+
0	+	+
0	+	+
0	+	0
0	+	0
0	+	0
0	+	0
+	0	0
+	0	0
0	0	0
0	0	0
0	0	0

, groups of 10 males were
ys to 494 and 376 ppm. In
)s of six males and six fe-
)osed at these levels to detect
ence in susceptibility. The
)f the males varied from 435
id the females from 351 to

issue damage, which con-
congestion and cloudy swell-
voluted and loop tubules of
/as found only in the two
4 ppm and the one at 376
n weights of both groups of
ghtly by the third exposure,
e 376 ppm level were they
low the controls after 30 ex-
nificant elevation in kidney
entage of body weight oc-
groups. The mixed groups
ılly the same mortality ratios
ale groups and evidenced no
rence in sex response. The
cate that guinea pigs tolerate
below 203 ppm but above
Cellosolve. The latter con-
without statistically signifi-
from controls in any of the
c response followed in this

halation by Mice: Groups

of 10 mature male C3H mice, obtained from the Shalom Research Farms, Mars, Pa., were exposed without controls to 494 and 376 ppm butyl Cellosolve for 30 days, with resultant mortality ratios of 7/10 and 2/10.

For more definitive data, groups of 70 male mice were exposed to metered concentrations of 400, 200, and 100 ppm. Serial examinations were made on 15 mice from each group after 30 and 60 exposures, on 10 more after 90 exposures, and on groups of 11 to 16 after 90 exposures followed by a 42-day rest period. Ten mice from each group were used for serial determination of erythrocyte osmotic fragility on blood obtained by section of the cervical vessels and cord. Table 9 lists the results.

Statistically significant alteration of liver weights occurred in the mice exposed to 400 ppm for 30, 60, and 90 days but not in those rested 42 days after 90 exposures. Why liver weights were increased after 60 days but not after 30 or 90 days at 200 ppm is not understood. No significant mortality or gross pathology was observed. Histological examination of kidneys from 80 mice from various exposure levels revealed no significant tissue changes.

Table 10 shows that the number of mice which had bloody urine immediately after exposure was proportional to the vapor concentration. No hemoglobinuria was found after the third exposure, but increased erythrocyte osmotic fragility occurred at all concentrations. The increase appeared to be as great after the first exposure as it was after the 89th exposure, and in all instances was normal after a 17-hour rest. The response of mice and guinea pigs at 400 ppm for 30 days was similar, but the former are much more sensitive from the standpoint of osmotic fragility.

Repeated Inhalation by Dogs: A female mongrel dog inhaling 617 ppm butyl Cellosolve died after a total of 13½ hours of exposure during two days. Emesis and extreme weakness were observed on both days. Autopsy revealed moderate congestion of the kidneys and lungs but no hemoglobinuria. Findings of this sort would be expected following a narcotic death. Death occurred so quickly that there was no opportunity to repeat preexposure blood chemistry and hematological tests.

A male and a female basenji hybrid inhaled 385 ppm repeatedly. Hematological tests were performed daily on both dogs, with blood chemistry on the male after 26 and 27 exposures. Analysis of the urine of the male for possible butoxyacetic acid excretion was performed after the 25th exposure. The female died after 8 days and the male after 28 days of exposure. Both dogs showed similar symptomatology and physio-

TABLE 9.—*Response of Male Mice to Repeated Seven-Hour Inhalations of Butyl Cellosolve Vapor*

Animals, No.	Concentration, Ppm	Exp. Days	Mortality Ratio	Body Wt.	Kidney Wt.	Liver Wt.	Gross Pathology	Micro-pathology	Erythrocyte Fragility	Hemoglo-binuria
15	112±4	30	0/15	N	N	N	0	0	+	0
15	111±5	60	0/15	N	N	N	0	0	+	0
10	112±5	90	0/10	N	N	N	0	0	+	0
16	112±5	90*	0/16	N	N	N	0	0	0	0
15	203±4	30	0/15	N	N	N	0	0	+	+
15	200±6	60	0/15	N	N	>	0	0	+	+
10	201±6	90	0/10	N	N	N	0	0	+	+
14	201±6	90*	0/10	N	N	N	0	0	0	0
15	396±10	30	0/15	N	N	<	0	0	+	+
15	399±9	60	0/15	<	N	>	0	0	+	+
10	401±9	90	0/10	N	N	>	0	0	+	+
11	401±9	90*	0/11	N	N	N	0	0	0	0

* Plus 42 days rest before examination.
N Values not statistically different from controls ($p>0.05$).
> Values statistically higher than controls.
< Values statistically lower than controls.
+ Substance or response present.
0 Substance or response absent.

TABLE 10.—*Erythrocyte Osmotic Fragility Values and Hemoglobinuria in Mice After Inhalation of Butyl Cellosolve Vapor*

Approx. Concentration, Ppm	No. of 7-Hr. Exposures	Fragility Immediately After Exposure	Fragility 17 Hr. After Exposure	Ratio of Mice Which Ex Bloody Urine Imm After Exposu
100	1	0.60–0.52	0.52–0.44	0/60
100	2	0.64–0.52	—	0/60
100	3	0.64–0.52	—	0/60
100	29	0.60–0.52	0.52–0.44	—
100	88	0.60–0.52	0.52–0.44	—
100	89	0.60–0.52	—	—
200	1	0.68–0.64	0.48–?	9/60
200	2	—	—	0/60
200	3	0.68–0.62	—	0/60
200	29	0.68–?	0.50–0.42	—
200	89	0.60–0.50	0.54–0.40	—
400	1	0.68–0.58	0.50–0.42	26/60
400	2	0.75–0.60	—	4/59
400	3	0.68–0.60	—	0/59
400	29	0.68–0.60	0.50–0.42	—
400	88	—	0.52–0.38	—
400	89	0.60–0.50	—	—
0.0 (Air controls)	1	0.50–0.42	—	0/60
	2	0.50–0.42	—	0/60
	3	—	—	0/60
	88	—	0.50–0.40	—
	88	—	0.52–0.40	—
	89	0.46–0.38	—	—
	89	0.54–0.46	—	—

logical response, although toxic manifestations appeared more slowly in the male. Loss of weight, transitory increase in erythrocyte osmotic fragility, nasal and ocular infection, generalized weakness, apathy, anorexia, and increased leucocyte count were observed in both animals. Emesis occurred in the female several times during the first four days of exposure. A significant decrease in erythrocyte count and hemoglobin level occurred in the male. It was of note that the fragility value of the male reached a maximum of 0.54-0.42 in 7 days and fell to 0.32-0.20 after 27 days, demonstrating that all susceptible erythrocytes had been removed from this animal's blood stream. Analysis of a 16-hour urine sample from the male after the 25th exposure indicated the presence of 55 mg. of butoxyacetic acid. The only positive results obtained from the blood chemistry tests were elevated plasma fibrinogen levels of 1.75 and 2.26 gm/100 ml. in the male dog after the 26th and 27th exposures, respectively. These values were several times higher than the accepted normal value of 0.52 gm/100 ml.[19]. It might be presumed that these elevated fibrinogen values were caused by dehydration in the dog prior to death, but this possibility was elim by the relatively low hemoglobin conc tions and erythrocyte counts. The striking findings at autopsy consist moderate to severe congestion and h rhage of the lung, congestion of the and congestion of both kidneys in th male. Histological examination conf the gross observations, with the addi discovery of a severe subcapsular h rhage in one adrenal in the female dog

Male and female basenji litter mates exposed to a mean concentration o ppm butyl Cellosolve for 31 days. He logical tests were performed weekly blood chemistry tests immediately afte 15th, 22d, and 29th exposures and b the 28th exposure. An erythrocyte p ability test was performed on the ne the last day of exposure, using ra dine.[17] The corneas were inspected the aid of fluorescein dye before the exposure and after the 31st. There slight evidence of toxic effect in the after 31 days of exposure. Erythro osmotic fragility values of the male in creased slightly, and leucocyte counts doubled. The fragility value of the female also rose slightly, while the erythrocyte

emoglobinuria in Mice
apor

ıgility Hr. ıfter posure	Ratio of Mice Which Excreted Bloody Urine Immediately After Exposure
2–0.44	0/60
—	0/60
—	0/60
2–0.44	—
2–0.44	—
—	—
8–?	9/60
—	0/60
—	0/60
)–0.42	—
4–0.40	—
0–0.42	26/60
—	4/59
—	0/59
0–0.42	—
2–0.38	—
—	—
—	0/60
—	0/60
—	0/60
)–0.40	—
2–0.40	—
—	—
—	—

s possibility was eliminated
ly low hemoglobin concentra-
throcyte counts. The more
igs at autopsy consisted of
evere congestion and hemor-
ung, congestion of the liver,
ı of both kidneys in the fe-
gical examination confirmed
rvations, with the additional
ı severe subcapsular hemor-
drenal in the female dog.
nale basenji litter mates were
mean concentration of 200
osolve for 31 days. Hemato-
ere performed weekly, and
y tests immediately after the
29th exposures and before
ure. An erythrocyte perme-
s performed on the next to
of exposure, using radioio-
orneas were inspected with
rescein dye before the first
after the 31st. There was
of toxic effect in the dogs
of exposure. Erythrocyte
ty values of the male in-
ly, and leucocyte counts
fragility value of the female
ıtly, while the erythrocyte

count and hemoglobin level fell slightly. The erythrocyte permeability of both dogs was numerically higher than that for similar control dogs, but the increase was not statistically significant. Blood chemistry tests revealed no abnormal value. At autopsy there appeared to be some slight capillary engorgement or breakdown in the lungs of both dogs, but these findings were not confirmed by histological examination. Fluorescein staining of the eyes indicated no damage. Butoxyacetic acid was present to the extent of 100 and 42 mg. in 16-hour urine samples from the male and the female after the 14th exposure.

Male and female wire-haired terrier litter mates, 8 months of age, were exposed for 90 days to a mean concentration of 100 ppm. Routine blood chemistry tests were performed on both dogs before the first exposure and after the 90th. Hematocrit values and icterus indices were determined in addition to the regular hematological studies performed weekly. Analysis for urinary butoxyacetic acid was done on specimens from the male and the female. The dogs did not appear to be affected appreciably by exposure to 100 ppm, although some alterations were observed in the hematological picture. There was a transitory doubling of the leucocyte count in both dogs midway in the 90-day period. The female's count returned to the preexposure level, but that of the male remained approximately 50% higher at the end of the period. An appreciable drop in the hematocrit values of the male, from 43.0% packed red cells before the first exposure to 34.5% after 90 exposures, was observed. Twenty-four-hour urine samples from the male and female dogs contained 100 and 94 mg. of butoxyacetic acid.

Repeated Inhalation by Monkeys: Two monkeys inhaled 100 ppm butyl Cellosolve for 90 days. Erythrocyte osmotic fragility rose on several occasions, higher in the female than in the male, but returned to normal by the end of the period. Erythrocyte counts fell briefly but returned to normal. After the 42d inhalation the female excreted 30 mg. of butoxyacetic acid in a 48-hour period, but only trace amounts were found from the male and from the female later in the inhalations. At autopsy tuberculosis in both animals was found—sufficient to obscure possible effects of the inhalation.

A rhesus monkey inhaled 210 ppm for 30 days. After the fourth inhalation the erythrocyte osmotic fragility was found to be elevated. It returned to normal overnight. For the balance of the exposure period this cycle was substantially repeated. After 30 inhalations erythrocyte count and hemoglobin level had been reduced to half their initial values. After 14 inhalations plasma fibrinogen was found to be 1.22 gm/100 ml., four times the normal concentration. Emesis occurred four times during the latter part of the period. At autopsy the organs appeared normal except for a suggestion of pulmonary tuberculosis. There was nothing noteworthy in the histopathology.

Human Inhalations

The human inhalation studies are summarized in Table 11. In the first trial two men and six rats simultaneously inhaled 113 ppm butyl Cellosolve for four hours in a 1250 cu. ft. room. None of the erythrocyte osmotic fragility values of the men deviated from their preexposure values, while the values for the rats rose appreciably. Human symptoms, which were secretly recorded, included nasal and ocular irritation, disagreeable metallic taste, slight increase in nasal mucous discharge, and occasional eructation. Four to six hours after exposure one man complained of feeling as though he had "smoked too many cigarettes," although none had been used.

About a year later the same two men, Subjects C and P, and one woman, Subject N, inhaled 195 ppm butyl Cellosolve for two 4-hour periods separated by a 30-minute recess for lunch. Exposure took place in a 6½ ft. cube (7900 liters) through which air was drawn at the rate of approximately 1300 liters per minute. Blood pressure and pulse rate were determined for

TABLE 11.—*Responses of Humans to Butyl Cellosolve Vapor*

Concentration, Ppm	Hr. of Exposure	Chamber	Subject	Sex	Age, Yr.	Erythrocyte Fragility†	Blood Pressure†	Pulse Rate†
195	8	278 cu. ft. chamber	N	F	24	0.44–0.36	98/64	75
			C	M	44	0.42–0.36	114/74	83
			P	M	34	0.42–0.36	114/71	74
113	4	1250 cu. ft. room	C	M	44	0.42–0.36	—	—
			P	M	34	0.42–0.36	—	—
98	8	278 cu. ft. chamber	L	F	34	0.40–0.36	116/70	86
			K	F	24	0.42–0.36	108/68	80
			W	M	37	0.40–0.36	120/84	84
			C	M	44	0.42–0.36	112/74	76

* Milligrams per 24-hour sample.
† These values taken near end of the exposure periods.
— Tests not performed.
‡ Sample analyzed twice to verify low range reported.

each subject three times during the exposure day, and erythrocyte osmotic fragility tests were determined four times. Urinalyses for glucose and albumin were conducted the following morning, and the butoxyacetic acid levels determined on urine samples collected during the 24 hours following completion of the exposure. Subjects N and P excreted considerable amounts of butoxyacetic acid in the 24-hour period following exposure, but Subject C, for some unknown reason, excreted only trace amounts of the metabolite. The remainder of the tests on humans were negative, although the erythrocyte fragility values of three female rats, concurrently exposed, increased steadily during the exposure. The privately recorded response of all three subjects included immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste. The woman, who excreted the largest amount of metabolite, also acquired a headache which lasted about 24 hours.

Based on the response of the humans inhaling 195 ppm for eight hours, the woman appeared to be more sensitive than either of the two men. All agreed that 195 ppm butyl Cellosolve was too high for comfort when continually breathed. Other volunteers inhaled a concentration of approximately 100 ppm for eight hours. Three of these subjects, two women and one man between 24 and 37 years of age, had had no previous contact with the vapor. Subject C was included because only trace am of urinary butoxyacetic acid were after the 195 ppm inhalation, and the ity of this result required a check. Th ppm inhalation took place 20 days aft 195 ppm experiment and included m the tests performed previously, excep urinary glucose and albumin in the hu and erythrocyte osmotic fragility in were omitted. The mean concentrati butyl Cellosolve was 98 ppm, and the chamber temperature, 26.2 C, with ext of 23.0 and 29.2 C. The only objective ing of significance was the urinary exc of butoxyacetic acid. Following the sure, Subject C, who had not excrete nificant quantities of the metabolite the 195 ppm exposure, eliminated 75 in 24 hours. The urinary butoxyacetic level of the other three subjects was si to that found at the 195 ppm exposure. subjective response of the humans inh 98 ppm may have been almost as gre that elicited at the 195 ppm level. Th male subject (L) apparently experie greater distress during and after exp than did the others. Subject L experie emesis after seven hours in exposure several times the following day, while jects K and W complained of headaches next day. The former subject stated high temperature often caused emesis that she was convinced that the pres episode was caused by the relatively h chamber temperature in the afternoon.

solve Vapor

·cyte ity†	Blood Pressure†	Pulse Rate†	Urinary Butoxy-hcetic Acid*
).36	98/64	75	300
).36	114/74	83	19–38‡ 6–12‡
).36	114/71	74	175
).36	—	—	—
).36	—	—	—
).36	116/70	86	183
.36	108/68	80	100
.36	120/84	84	250
.36	112/74	76	75

because only trace amounts oxyacetic acid were found ɔm inhalation, and the valid- t required a check. The 100 took place 20 days after the ment and included most of med previously, except that and albumin in the humans osmotic fragility in rats The mean concentration of was 98 ppm, and the mean ature, 26.2 C, with extremes C. The only objective find- ce was the urinary excretion acid. Following the expo- who had not excreted sig- es of the metabolite after cposure, eliminated 75 mg. e urinary butoxyacetic acid r three subjects was similar the 195 ppm exposure. The ase of the humans inhaling ve been almost as great as he 195 ppm level. The fe-) apparently experienced during and after exposure ers. Subject L experienced en hours in exposure and following day, while Sub- omplained of headaches the former subject stated that e often caused emesis and onvinced that the present sed by the relatively high ature in the afternoon. It

remains uncertain whether or not these phenomena should be charged to inhalation of vapors.

Comment

Butyl Cellosolve has been a commercially available solvent for at least 30 years. Since 1945 the generally accepted hygienic standard of inhalation has been 200 ppm, and little or no attention has been directed to the ease with which the liquid penetrates the skin, although by this route it is a moderately toxic material. Based upon the response of experimental animals to repeated inhalation of butyl Cellosolve, one might suppose 50 ppm or even less to be an appropriate hygienic standard of inhalation. In view of these facts, it is not idle to inquire why there have been no reported instances of human injury, beyond one uncertain observation of hematuria.

One factor undoubtedly is low volatility. The hygienic standard of inhalation has been one-fifth of saturation at ordinary temperatures. It is most unlikely that any workman actually inhales this much for more than a brief period during a working day. This factor is not important in the case of skin penetration. In the handling of hydraulic fluids there is no doubt that skin penetration accounts for more absorption than does inhalation.

A second factor is the nature of injury from repeated inhalation of low concentrations of butyl Cellosolve. It is a slow drain upon the erythrocyte producing organs. With the existing great capacity of the bone marrow to replace erythrocytes, so long as only those in the circulation are affected, a relatively enormous, long-acting drain is required to result in any clinical symptoms or in any actual injury.

There is a third, and apparently a more important, factor explaining why there have been no reported injuries. Butyl Cellosolve is one of the few industrial materials to which the human is more resistant than are the usual experimental animals. A greater contact with butoxyacetic acid is required to cause osmotic fragility of human red blood cells than is required to hemolyze rat, mouse, rabbit, dog, or monkey erythrocytes. Inhalation of 200 ppm for eight hours does not result in fragile human erythrocytes, but as little as 62 ppm for four hours does affect rat cells. For this reason a hygienic standard of inhalation for butyl Cellosolve should not be based solely upon repeated inhalations by susceptible animals. The current hygienic standard of 200 ppm for inhalation of butyl Cellosolve appears, on the basis of evidence and experience, to be low enough to prevent human injuries. However, discomfort will be experienced by some human subjects inhaling this concentration for eight hours. In this instance, 200 ppm refers to the time-weighted average concentration throughout a working day. Detectable injury from high concentrations apparently cannot occur in less than an hour at saturation—1000 ppm and discomfort from this concentration will make prolonged voluntary inhalation unlikely.

Summary and Conclusions

Inhalation of a high concentration of butyl Cellosolve, of the order of 500 ppm, half of saturation, for several hours may injure by respiratory tract irritation and narcosis, with slighter damage to kidney and liver. Death may be prompt, due to narcosis, or it may be delayed several days, due to pneumonitis added to kidney injury.

Butoxyacetic acid is a metabolite of butyl Cellosolve in the rat, rabbit, guinea pig, dog, rhesus monkey, and man. It can be estimated semiquantitatively in the urine. This excretion is the first detectable evidence of absorption of butyl Cellosolve. Men inhaling 100 ppm for 8 hours excrete 100 to 200 mg. of butoxyacetic acid within the next 24 hours, with considerable individual variation.

Butyl Cellosolve, and to a greater extent its metabolite, butoxyacetic acid, increases the osmotic fragility and presumably the mechanical fragility of the erythrocyte. This action is greatest in the rodents—rats, mice, rabbits. It is less in the guinea pig, the dog, the rhesus monkey, and the human. Other

glycol ethers act similarly, with quantitative differences. Increased fragility leads to destruction of erythrocytes and excretion of their hemoglobin in the urine as such or as hemin. The destroyed erythrocytes are promptly replaced by new cells, and if insult is prolonged, eventually immature forms may enter the circulation. Repeated excessive inhalation of butyl Cellosolve by rodents and dogs was found to result eventually in reduction of the blood hemoglobin concentration and the total erythrocyte count to the point of anemia. Increased osmotic fragility of erythrocytes was not found in man during inhalations in which simultaneously exposed rats were so affected. It was found in vitro with human erythrocytes, and hence is to be expected after inhalation of some concentration higher than the 200 ppm to which men did not respond.

In repeated inhalations by animals the significant effects were lung irritation, cloudy swelling of renal loop and convoluted tubules, heavy kidneys, low hemoglobin and erythrocyte counts, hemoglobinuria, and fragile erythrocytes. Females were susceptible to lower concentrations than were males. Rats were not affected by 30 seven-hour inhalations of 54 ppm, guinea pigs by 30 inhalations of 100 ppm, dogs by 90 inhalations of 100 ppm. Mice, rats, and monkeys inhaling 100 ppm for 90 days had only fragile erythrocytes. Humans are more resistant to hemolytic anemia, the injury characteristic of small animal response to inhalation of low concentrations of butyl Cellosolve. Therefore, a safe working concentration for humans cannot properly be based solely upon rodent response.

During an eight-hour inhalation of 200 ppm butyl Cellosolve there was no objective effect upon three humans, although butoxyacetic acid in their urines proved considerable absorption. Subjectively the concentration was judged too high for comfort, with eye, nose, and throat irritation evident. During eight hours at 100 ppm four humans likewise showed a similar qualitative response.

The present work interprets the accepted hygienic standard of 200 ppm for inha tion of butyl Cellosolve as a concentrat which rodents cannot tolerate, which slightly injurious to dogs, and which appe not to injure humans. Our human w was not sufficiently extensive to demonstr that 200 ppm will not eventually result some degree of hemolytic anemia. Beca of this prudence dictates that 100 ppm a more appropriate level to maintain workroom atmospheres. Even at this le there may be occasional complaints of comfort from extremely sensitive perso

No matter how butyl Cellosolve may en the circulation, it is a relatively toxic terial. It penetrates the skin readily. to its low volatility, there are many ap cations where prevention of excessive p longed skin contact is more important health than is prevention of inhalation.

REFERENCES

1. Browning, E.: Toxicity of Industrial Org Solvents, London, H. M. Stationery office, p. 355.

2. Werner, H. W.; Mitchell, J. L.; Mi J. W., and von Oettingen, W. F.: Acute Toxi of Vapors of Several Monoalkyl Ethers Ethylene Glycol, J. Indust. Hyg. & Tox 25:157-163, 1943.

3. Werner, H. W.; Nawrocki, C. Z.; Mitc J. L.; Miller, J. W., and von Oettingen, W. Effects of Repeated Exposures of Rats to Va of Monoalkyl Ethylene Glycol Ethers, J. In Hyg. & Toxicol. 25:374-379, 1943.

4. Werner, H. W.; Mitchell, J. L.; Mi J. W., and von Oettingen, W. F.: Effects of peated Exposure of Dogs to Monoalkyl Ethy Glycol Ether Vapors, J. Indust. Hyg. & Tox 25:409-414, 1943.

5. Browning, E.: Toxic Solvents, Lon Edward Arnold & Co., 1953, p. 136.

6 Patty, F. A., editor: Industrial Hyg and Toxicology, New York, Interscience lishers, Inc., 1949, Vol. 2, pp. 965-966.

7. Elkins, H. B.: Chemistry of Indus Toxicology, New York, John Wiley & Sons, 1950, pp. 229-230.

8. Peters, J. P., and Van Slyke, D. Quantitative Clinical Chemistry, Baltimore, W liams & Wilkins Company, 1932, Vol. 2, p. 66.

9. Nair, J. H., III Paper Chromatography some Alkoxy Acids, Analyt. Chem. 25:1912, 19

10. Ramsay, L. L., and Patterson, W. Separation and Determination of the Straig Chain Saturated Fatty Acids C_5 to C_{10} by Parti

ard of 200 ppm for inhala-
Cellosolve as a concentration
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us to dogs, and which appears
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ntact is more important for
prevention of inhalation.

REFERENCES

E.: Toxicity of Industrial Organic
, H. M. Stationery office, 1953,

I. W.; Mitchell, J. L.; Miller,
Oettingen, W. F.: Acute Toxicity
Several Monoalkyl Ethers of
, J. Indust. Hyg. & Toxicol.

W.; Nawrocki, C. Z.; Mitchell,
W., and von Oettingen, W. F.:
ted Exposures of Rats to Vapors
hylene Glycol Ethers, J. Indust.
25:374-379, 1943.
I. W.; Mitchell, J. L.; Miller,
Oettingen, W. F.: Effects of Re-
of Dogs to Monoalkyl Ethylene
pors, J. Indust. Hyg. & Toxicol.

E.: Toxic Solvents, London,
Co, 1953, p. 136.
A., editor: Industrial Hygiene
New York, Interscience Pub-
, Vol. 2, pp. 965-966
. B.: Chemistry of Industrial
York, John Wiley & Sons, Inc.,

P., and Van Slyke, D. D.:
ical Chemistry, Baltimore, Wil-
Company, 1932, Vol. 2, p. 66.
, III: Paper Chromatography of
ds, Analyt. Chem. 25:1912, 1953.
L., and Patterson, W. I.:
Determination of the Straight-
Fatty Acids C_5 to C_{10} by Partition

Chromatography, J. A. Off. Ag. Chem. 31:139-150, 1948.

11. Shriner, R. L., and Fuson, R. C.: Systematic Identification of Organic Compounds, Ed. 2, New York, John Wiley & Sons, Inc., 1945.

12. Hann, R. M.; Reid, E. E., and Jamieson, G. S.: Phenacyl, *p*-Chlorophenacyl and *p*-Bromophenacyl Esters of Some Higher Fatty Acids, J. Am. Chem. Soc. 52:818-820, 1930.

13. Hawk, P. B.; Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, Ed. 13, New York, Blakiston Company (Medical Division of McGraw-Hill Book Co., Inc.), 1954.

14. Weil, C. S.: Tables for Convenient Calculation of Median-Effective Dose (LD_{50} or ED_{50}) and Instructions in Their Use, Biometrics 8:249-263, 1952.

15. Irish, D. D., and Adams, E. M.: Apparatus and Methods for Testing Toxicity of Vapors, Indust. Med., Indust. Hyg Sect. 1:1-5, 1940.

16. Carpenter, C. P.; Smyth, H. F., Jr., and Pozzani, U. C.: Assay of Acute Vapor Toxicity and Grading and Interpretation of Results on 96 Chemical Compounds, J. Indust. Hyg. & Toxicol. 31:343-346, 1949.

17. Boatman, J. B., and Moses, C.: Role of Erythrocyte in Blood Iodine Transport Using Radioiodine I^{131}, Am. J. Physiol. 164: 783-785, 1951.

18. Werner, H. W., and Mitchell, J. L.: Determination of Monoalkyl Ethers of Ethylene Glycol, Indust. Eng. & Chem., Analyt. Ed. 15:375-376, 1943.

19. Albritton, E. C., editor: Standard Values in Blood, Philadelphia, W. B. Saunders Company, 1952, p. 96.