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Acute Toxicity of Monacetin, Diacetin and Triacetin.

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Experimental studies of the toxic effects of "industrial solvents" are of considerable importance since some have been used as vehicles or solvents for medicinal substances in recent years. The glycols and their related compounds have been studied extensively.[1-5] On the other hand, the acetins have not received the attention they deserve. Diacetin or glyceryl diacetate has been used as a solvent for camphor[6] and more recently monacetin of glycol for vitamin K.[7] Latven and Molitor[8] have found the LD_{50} of diacetin to be 3.5 cc per kilo on subcutaneous injection in white mice. The narcotic effects[9] of monacetin and triacetin, as well as diacetin, were studied in 1899 by Meyer. Quantitative studies on their comparative toxicity are lacking, however. On account of ease of preparation and of their possible usefulness as substitutes for other organic solvents, a study of the acute toxic effects of these compounds was made in rats and mice.

Monacetin, diacetin and triacetin were prepared according to the methods of Schuette and Sah[10] and Schuette and Hale.[11] The purity of the compounds was established by comparison of their physical constants with those given in standard references.[12]

Inbred albino rats weighing usually from 200 to 350 g and inbred

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white mice* weighing usually from 17 to 21 g were used as test objects. At least 10 animals were used for each dose level. For injection into rats each acetin was mixed with equal volumes of distilled water to which 2% by volume of a 3 M phosphate buffer solution[18] was added to make the pH approximately 7 (bromthymol blue). In this medium diacetin and triacetin were not completely soluble. Care was taken to insure an even mixture before each injection and not more than 1 cc was given at one site. Mice were injected with acetins in 1:5 or 1:10 dilutions. These contained 1% by volume of the buffer solution. In all animals injections were given subcutaneously. Rats were observed for 15 days and mice for 5 days after a single injection.

Animals dying during the period of observation were examined and the following organs were removed for microscopic study: lungs, heart, liver, spleen and kidney. Gross observations of animals poisoned by monacetin revealed dilatation of the heart, diffuse congestion of the lungs, and in 2 rats, a small amount of fluid in the pleural cavity. In animals dying as a result of the toxic effects of diacetin and triacetin hemorrhagic areas in the lungs appeared frequently. Preliminary studies of sections of tissues showed some cloudy swelling of the convoluted tubules of the kidney and in some cases the lumen was filled with casts. Hydropic degeneration and necrosis of the tubules was noted in some areas. The liver appeared to be congested.†

The results of this study of the acute toxicity of the acetins in

TABLE I.
Acute Toxicity of Monacetin, Diacetin and Triacetin in Inbred Rats and Mice.

	Rats			Mice		
	Dose, cc/kg	No. of animals	No. died	Dose, cc/kg	No. of animals	No. died
Monacetin	3.0	10	0	2.0	10	2
	5-6	10	5	4.0	10	6
	7.5-10	10	10	6.0	10	10
Diacetin	2-3	15	0	2.0	10	0
	3.5	10	4	2.5	10	6
	4.0	10	5	3-4	10	9
	5-10	10	10			
Triacetin	2.0	10	1	1.0	10	2
	3.0	10	6	2.0	10	2
	4-10	10	10	2.5	10	7
				3.0	10	10

* This strain of inbred mice was kindly supplied by Dr. A. B. D. Fortuyn.

[18] Butler, A. M., and Montgomery, H., *J. Biol. Chem.*, 1932, **99**, 173.

† We are indebted to Dr. C. H. Hu who confirmed these findings.

rats and mice are summarized in Table 1. The order of toxicity of the acetins appears to increase with the degree of acetylation and is approximately in the ratio of 1.0:1.4:2.0 in rats and of 1.0:1.4:1.5 in mice. Comparative studies of the subcutaneous toxicity of ethylene glycol indicate that monacetin is less toxic for rats and mice. Diacetin would appear to be approximately as toxic and triacetin apparently is more toxic than ethylene glycol.

Animals receiving fatal doses usually died in from 20 minutes to 3 or 4 hours after injection. Symptoms of marked depression, weakness, prostration and in some animals, labored respiration just before death were noted. Animals in which death was delayed for 24 hours or more generally showed no symptoms except slight transient depression soon after injection.

Occasionally animals receiving monacetin and diacetin exhibited local irritation of the skin at the site of injection. In the eye of the rabbit, 50% monacetin caused only a slight degree of transient injection of conjunctival vessels, but diacetin and triacetin in similar concentrations caused marked congestion and moderate edema.

The effect of the acetins on red blood corpuscles of the dog was studied. Dilutions of monacetin in 0.9% NaCl solution of from 1:500 to 1:4,000 did not cause hemolysis over 24 hours. At 18 hours slight hemolytic effects were noted with diacetin and triacetin in from 1:500 to 1:2,000 dilutions. Spectroscopic examination of these samples did not show the characteristic band for acid hematin.

Aqueous solutions of acetins were distinctly acid. The pH of solutions of 50% monacetin and of diacetin was 3.88 as determined with the quinhydrone electrode. In 1:14 dilution the pH for monacetin, diacetin and triacetin was 4.52, 4.18 and 3.88, respectively. Monacetin was miscible with an equal volume of dog's serum, while diacetin precipitated serum, triacetin and serum produced an emulsion. The pH of such serum mixtures was 7.45, 7.16 and 6.43, respectively as determined with the glass electrode.

Summary. A study of the toxicity of the acetins reveals that the subcutaneous LD_{50} for monacetin is approximately 5.5 cc per kilo for inbred albino rats, and 3.5 cc per kilo for inbred white mice. The LD_{50} dose for diacetin is 4.0 cc and 2.5 cc per kilo, respectively, and for triacetin 2.8 cc and 2.3 cc per kilo, respectively. Slight irritation to mucous membranes and occasionally to cutaneous tissues was noted with monacetin. Monacetin did not hemolize blood corpuscles in dilutions of 1:500 or greater. Blood serum and monacetin are compatible with a resulting pH of 7.45. Diacetin and triacetin are more irritating to tissues, are slightly hemolytic and are relatively incompatible with blood serum.