A NOTE ON GLYCEROL FORMAL AS A SOLVENT IN TOXICITY TESTING

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Twenty-nine organic solvents and emulsifying agents, selected for possible use as injection solvents in toxicity testing, have been submitted to a "range-finding" toxicity screening test on rats. As a result, glycerol formal was examined in greater detail. Glycerol formal is a good inert solvent for a wide range of organic chemicals. It produced no toxic effects or macroscopic pathology when 1500 mg./kg. was administered intraperitoneally to rats, or 1000 mg./kg. to mice or guinea pigs, or 4000 mg./kg. orally to rats, and at high doses the only specific effect found was narcosis. It was correspondingly innocuous subcutaneously or dermally, and was almost non-irritant to the eye surface. There was no detectable effect on the toxicity of parathion. Glycerol formal appears to be a useful addition to the range of solvents suitable for use in toxicity tests.

In toxicological investigations, the chemical under examination has usually to be dissolved in, or, if liquid, diluted with a solvent. An injection solvent for water-insoluble or water-unstable compounds should satisfy, as far as possible, the following main requirements. (*a*) Low toxicity, so that at least 1000 mg./kg. can be injected intraperitoneally without detectable toxic effect or irritancy. (*b*) Complete miscibility with water, giving a neutral solution. (*c*) Good solvent powers for a wide range of materials. (*d*) Chemical inertness. (*e*) A mode of toxic action or detoxication which would not interfere with the absorption or metabolism of a solute. (*f*) Preferably low viscosity and low volatility. (*g*) Ready availability at reasonable cost, in sufficiently pure grade. The absence of local irritative effects is particularly important for intraperitoneal injection.

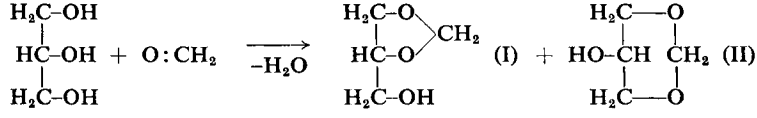
The organic solvent most commonly used in this laboratory is propylene glycol; occasionally-used solvents such as ethanol and acetone cause toxic effects and irritancy at low doses. Propylene glycol cannot be given intraperitoneally to the rat at doses greater than 1000 mg./kg., or toxic effects appear, notably marginal swelling of the liver lobes; also it is not a good solvent, and is at times troublesome to handle owing to its high viscosity.

A number of candidate materials have therefore been submitted to a simple toxicity screening test, to determine whether sufficiently large doses could be injected without detectable effects on the animal. Intraperitoneal injection to the rat was selected as being the most critical route of administration, and that most likely to show up undesirable effects. As this was intended to be merely a preliminary "sorting" test to determine the approximate maximum harmless dose, it was felt permissible to use comparatively small numbers of animals for each material.

In addition to injection, most materials have been examined for ocular irritation in the guinea pig, mainly because many of the possibly anti-cholinesterase compounds submitted to this laboratory for toxicity testing undergo measurement of miotic activity[1].

These tests have shown that glycerol formal was worthy of more detailed investigation, and further tests on this material are reported.

Glycerol formal is a condensation product of glycerol and formaldehyde, and is a mixture of the two materials 4-hydroxymethyl-1 : 3-dioxolane (I) and 5-hydroxy-1 : 3-dioxane (II) obtained by condensation involving respectively the $\alpha\beta$- and the $\alpha\alpha'$- pairs of hydroxyl groups of the glycerol (Hannay, personal communication):—



The proportions of the two compounds in the mixture depend on the reaction conditions, more of the six-membered ring (II) being formed at a lower reaction temperature[2]. The two substances cannot be separated by distillation, but can be benzoylated and the benzoates separated and hydrolysed back to the alcohols in good yield with alkali[3,4].

I and II have very similar boiling point : pressure relationships, with boiling points at 760 mm. Hg respectively 194 and 193°, and approximate vapour pressures at 20° respectively 0·22 and 0·25 mm. Hg[3–6]. Mixtures boil at 192–5° at 760 mm. Hg[7], so there is probably no azeotrope formation. The densities of I and II are respectively 1·2113 and 1·2256 at 20°, and 1·2008 and 1·2200 at 25°[3,4]. There is a difference in refractive indices, n_D^{20} being 1·4477 for I and 1·4533 for II, and n_D^{25} 1·4469 for I and 1·4527 for II[3,4]; in the absence of other gross impurities, it should therefore be possible to determine the approximate composition of a mixture of these components by the refractive index[3]. Both I and II are miscible with water and chemically stable, resisting hydrolysis by hot aqueous potassium hydroxide[3,4,6,7]. No peroxide formation in contact with air has been reported.

No toxicity tests have been reported on glycerol formal or either of its components. However, glycerol formal has been used industrially on the ton scale under the name of Sericosol N as a solvent for cellulose acetate, without special precautions, and has as yet produced no detected hazards (Hannay).

Experimental Methods

In the screening tests, doses of each compound were administered intraperitoneally, undiluted, to groups of semi-adult albino rats (130–200 g.) of either sex, and of Wistar or Glaxo-Wistar strain. Initial tests were on single animals at each dose, which were then made up to groups of 3–4 near the threshold of effects. Animals were observed for mortality and toxic effects for seven days, this being the normal observation period for acute toxicity tests in this laboratory. Survivors were

killed by decapitation and examined macroscopically. Ocular irritancy was tested by placing a 10 μl. standard drop on the corneal surface of one eye of a guinea pig and comparing with the untreated eye. Average lethal dose values were estimated non-statistically, and maximum ineffective doses were the highest at which none of the animals showed any detectable toxic effects. Similar but more extensive methods were used in the detailed tests on glycerol formal.

Most materials screened were of normal laboratory grade, and were checked for gross impurity by measuring the pH change when 10 per cent was added to water, and by comparing the refractive index with values quoted in the literature. Purification was only found necessary with trimethyl and triethyl phosphates; this was done by shaking with anhydrous sodium bicarbonate, drying with anhydrous sodium sulphate, and filtering. The glycerol formal and 1:3-dioxolane were experimental samples supplied by Messrs. Brotherton and Co., Leeds. Of the surface active agents, Lissapol NXA was the standard I.C.I. product, the "Sorpol" products are non-ionic emulsifying agents of Japanese manufacture supplied by Internationale Crediet-Handels-Vereeniging, Rotterdam, and the "Tween" products are polyoxyethylene sorbitan esters supplied by Messrs. Honeywill-Atlas Ltd.

Results

Screening tests. The results of the main screening tests are summarised in Table I. In order to indicate the scope and nature of the screening methods used, a typical test, on diethylene glycol monomethyl ether, is given in greater detail in Table II.

As a result of these tests, a more detailed examination was made on glycerol formal.

Glycerol formal. The material tested was a colourless liquid with no appreciable odour, slightly viscous but considerably less so than propylene glycol. It was readily and completely miscible with water, and a 10 per cent aqueous solution had pH between 5·0 and 5·5. The refractive index was 1·4519 at 20°, indicating, on the basis of the literature values[4,5], that it probably contained about 75 per cent II and 25 per cent I; a second sample gave a value of 1·4513, corresponding to about 70 per cent II. The material ignited with difficulty only after warming, and burnt slowly with a non-smoky blue flame. A test for peroxide with ferrous ammonium sulphate and ammonium thiocyanate gave only a very faint positive reaction. A test for free aldehyde with Schiff's reagent was very faintly positive, suggesting that a trace of formaldehyde might have been present.

A series of non-quantitative test-tube solubility experiments were carried out with a range of organic pesticidal chemicals. The results are shown in Table III.

Intraperitoneal injection of undiluted glycerol formal to rats gave the mortality and toxic effects shown in Table IV. At 2000 mg./kg. and above, narcosis occurred. At 2000 mg./kg. this was only moderate and lasted about three hours, while at 4000 mg./kg. weakness persisted until death. No macroscopic post-mortem abnormalities were found in any

TABLE I

SUMMARISED RESULTS OF INTRAPERITONEAL SCREENING TESTS

Material	Rat sex	Estimated approx. rat average lethal dose, mg./kg.	Estimated max. symptomless dose, mg./kg.	Estimated max. dose without macroscopic pathology, mg./kg.	Ocular irritation	Effect*
Methanol	M	1500	500	350		NWUI
Ethanol	M	500	300	300		NWPUI
Acetone	F	500	<500	500		NWPUI
Ethylene glycol monomethyl ether	F	1200	<500	<500	+	NWPUDRLI
Ethylene glycol monoethyl ether	F	1200	500	<500	+	NWUDRLI
Ethylene glycol monomethyl ether mono-acetate	F	1200	700	500	±	WRIX
Diethylene glycol monomethyl ether	M	3000	1500	750	−	NWUDI
Diethylene glycol monoethyl ether	M	3000	1000	750	−	NWURI (? K)
Propylene glycol	M/F	>8000	2000	1000	−	NI
Hexylene glycol	M	1500	500	500	±	NUI
Ethyl glycollate	F	1500	500	500	+	NWRIX
Methyl lactate	F	>2000	500	500	−	NRI
Ethyl lactate	F	1000	750	<500		WRIX
Trimethyl phosphate, tech.	F	1000	750	500		NWUI
Trimethyl phosphate, purified	F	1500	850	750		NI
Triethyl phosphate, tech.	M	500–1000	<500	<500		NWUDI (? L)
Triethyl phosphate, purified	M	800	<500	<500		NI
NN-Dimethylformamide	F	1500	1000	750	±	NWUIL
NN-Dimethylacetamide	F	>2000	1000	<500	±	NPDI
Dioxan	F	1500	<500	<500	++	NWDLKI
Tetrahydrofurfuryl alcohol	M/F	1000	750	750	±	NUR
1:3-Dioxolane	M	500–1000	<500	<500	+	NDU
Glycerol formal	M/F	3000	1500	1500	−	N
2:2-Dimethyl-4-hydroxymethyl-1:3-dioxolane	M	3000	750	750	±	NUI
Emulsifying agents:—						
Lissapol NXA	M	200	100	25		WUI
Sorpol 144	F	700	<500	<500		WRUI
Sorpol 200	F	<500	<500	<500		WCRI
Tween 20	F	3000	1000	<500		WIL
Tween 40	F	>4000	1000	500		WUIL
Tween 60	F	>4000	2000	500		WIL
Tween 80	F	>4000	2000	500		UIL

> indicates highest, and < lowest dose tested.

*Abbreviations: C = convulsions
D = diarrhoea
I = peritoneal adhesions, etc.
K = kidney damage
L = liver damage
N = narcosis
P = pain
R = respiratory distress
U = urinary incontinence
W = weakness
X = congestion, cyanosis, "rubbery" liver, pale contracted gut (acute deaths only).

TABLE II

INTRAPERITONEAL RAT SCREENING TEST ON DIETHYLENE GLYCOL MONOMETHYL ETHER

Dose, mg./kg.	7 day mortality	Survivors affected	Survivors showing macroscopic pathology	Time of onset	Time of death	Effects*
500	0/1	0/1	0/1	—	—	—
750	0/1	0/1	0/1	—	—	—
1000	0/4	0/4	1/4	—	—	I
1500	0/4	0/4	3/4	—	—	I
2000	0/1	1/1	1/1	7 min.	—	NDWI
4000	1/1	—	—	5 min.	3 days	NUW

* Abbreviations as Table I.

TABLE III

SOLUBILITIES OF PESTICIDE ACTIVE INGREDIENTS IN GLYCEROL FORMAL

Solute	Solubility
Parathion	Completely miscible
Malathion	Completely miscible
Hercules 528 ("Delnav")	Completely miscible
Rogor	Very soluble
Dinitrocresol (free acid)	Very soluble
Pentachlorophenol	Moderately soluble, very soluble warm
DDT	Very soluble
Benzene hexachloride	Moderately soluble
Aldrin	Sparingly soluble, very soluble warm
Dieldrin	Sparingly soluble, very soluble warm

TABLE IV

ACUTE INTRAPERITONEAL TOXICITY OF GLYCEROL FORMAL TO RATS

Rat sex	Dose, mg./kg.	7 day mortality	Survivors affected	Time of onset	Time of death
M	500	0/1	0/1	—	—
	750	0/1	0/1	—	—
	1000	0/4	0/4	—	—
	1500	0/4	0/4	—	
	2000	0/1	1/1	1 min.	—
	4000	1/1	—	1 min.	1½ days
F	1000	0/4	0/4	—	—
	1500	0/10	0/10	—	—

TABLE V

INTRAPERITONEAL TOXICITIES TO FEMALE RAT OF SOLUTIONS OF PARATHION

Solvent	Dose of parathion mg./kg.	7 day mortality	Survivors affected	Time of onset	Time of death	Approx. LD50, mg./kg.
Propylene glycol ..	1·5	0/4	4/4	2½ hr.	—	—
	3·0	1/4	3/3	15–20 min.	6–21 hr.	—
	6·0	4/4	—	10–13 min.	¼–21 hr.	4
Glycerol formal ..	1·5	0/4	4/4	2½–5½ hr.	—	—
	3·0	1/4	3/3	20–30 min.	6–21 hr.	—
	6·0	4/4	—	7–10 min.	¼–21 hr.	4

animals, including two groups of three female rats killed three and twenty hours after 1500 mg./kg., and the rate of weight gain was unaffected in survivors.

Groups of four female rats were given 1000, 2000 and 4000 mg./kg. orally. All were apparently unaffected, gained weight normally, and showed no macroscopic pathology.

A group of four female rats was given 1000 mg./kg. subcutaneously. They were completely unaffected, and showed no evidence of local irritation at the injection site.

A group of four female rats was given 1000 mg./kg. on the clipped skin of the back, which was then covered with waterproof adhesive plaster. This test method is the standard technique of this laboratory for dermal toxicity testing. The solvent was removed after 24 hours by washing with soap and water. The rats were completely unaffected, and showed no evidence of local irritation at the application site.

Intraperitoneal injection of 1000 mg./kg. to two male guinea pigs caused no apparent toxic effects or macroscopic pathology. Similar administration of 1000 mg./kg. to six male albino mice was equally innocuous.

Administration of a single drop (10 μl.) to the eye of a guinea pig caused only slight temporary irritation without pupillary constriction or obscuration.

An experiment was then made to compare the toxic effects of intraperitoneal injection of female rats with 0·6 per cent v/v solutions of the organophosphorus insecticide parathion in glycerol formal and propylene glycol. The results are shown in Table V. There were no detectable differences between the effects of the two solutions, and no macroscopic post-mortem abnormalities.

Discussion

The screening test results summarised in Table I suggested that propylene glycol was a solvent of low toxicity; its most obvious effect at higher non-narcotic intraperitoneal doses, marginal liver lobe swelling probably due to local irritation, can often be allowed for at post-mortem. However, if this swelling be taken into account, glycerol formal could apparently be given in higher doses than propylene glycol without causing macroscopic pathology.

Glycerol formal appeared from Table III to be a useful solvent for a number of classes of compound, and considerably better in this respect than propylene glycol. There are no data on the degree of polarisation of the hydroxyl groups of the components, but they would probably be less polar than in the primary aliphatic alcohols, and therefore less reactive. There was no indication during this work of chemical instability or reactivity.

The toxicity of glycerol formal appeared to be low; average lethal dose values to the rat were estimated from these results as approximately 3000 mg./kg. intraperitoneally, and greater than 4000 mg./kg. orally. The only effect observed was narcosis; there was no evidence of the

mild irritant effects found after injection of propylene glycol. Hence the maximum dose producing no detectable effect of any type, 1500 mg./kg. intraperitoneally in the rat, was greater than with propylene glycol. The absence of more than slight ocular irritation indicated further that the material would be suitable for miosis testing of phosphate esters[1].

The fact that glycerol formal is a mixture of possibly variable composition is not necessarily a disadvantage, in view of the low toxicity. Gross changes in composition could be detected by measuring the refractive index. This solvent appears to be a useful addition to the range of solvents suitable for use in acute toxicity tests. It is now in regular use in this laboratory.

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