A NOTE ON THE TOXICITY AND SOLVENT PROPERTIES OF DIMETHYL SULPHOXIDE

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Dimethyl sulphoxide has good solvent properties for a wide range of chemicals, and a low toxicity to mammals. The acute oral LD50 for rats is about 20 ml./kg. and for chickens about 12·5 ml./kg. Rats tolerate up to 10 daily doses of 10 ml./kg. weight. Dimethyl sulphoxide is therefore a useful solvent for chemical compounds under toxicological evaluation.

The selection of a solvent for use in toxicological investigations requires considerable care. On the one hand, solvents with desirable physicochemical characteristics may be sufficiently toxic to affect significantly the results obtained with only slightly toxic solutes either as a result of the toxicity of the solvent *per se*, or as a result of an increase in the rate of absorption of the solute through the alimentary tract or via the skin. On the other hand, physiologically inert substances may have poor solvent properties. Sanderson (1959) has discussed the desirable characteristics of a solvent to be used in toxicity testing and recommended glycerol formal as a satisfactory solvent with a low toxicity. However, many compounds under development for use in the plastics industry and in agriculture have very low acute toxicities and consequently large doses of the compounds must be administered. The solubility of some of these compounds in glycerol formal is low and it is difficult to prepare solutions of the necessary concentration for LD50 determinations. We are, therefore, continually searching for compounds with good solvent properties combined with low toxicity (LD50$>$20 ml./kg.). One such compound of great promise is dimethyl sulphoxide.

Physical Characteristics

Dimethyl sulphoxide, $Me_2S:O$, is the simplest member of the homologous series of organic sulphoxides. It is prepared by oxidation of dimethyl sulphide commercially available from both the petroleum and sulphite-pulp industries.

Pure dimethyl sulphoxide (DMSO) is a colourless liquid, with a slight odour and a slightly bitter taste; the commercial material, 99·5 per cent purity, has a strong characteristic odour of sulphur compounds. It is very hygroscopic, absorbing over 70 per cent of its own weight of water from air at 20° and 65 per cent relative humidity. The physical properties are summarised in Table I.

DMSO is a dipolar aprotic solvent in which many classes of compound are soluble. Thus, the gases acetylene, ammonia and sulphur dioxide and most organic compounds are soluble. At 20° DMSO mixes in all proportions with methanol, octanol, glycerol, acetaldehyde, acetone, propionic acid, formic acid, diethylether, ethyl acetate, triacetin, dioxane,

pyridine, benzene, toluene and xylene. The chlorinated hydrocarbon insecticides, many proteins, and polynuclear hydrocarbons, e.g., chrysene and benzpyrene, are also soluble in DMSO.

TABLE I

PHYSICAL PROPERTIES OF DIMETHYL SULPHOXIDE

Melting-point	18·4°
Boiling-point (760 mm. Hg)	189°
Vapour pressure at 10°	0·417 mm. Hg
Vapour pressure at 50°	3·07 mm. Hg
Density (20°)	1·100
Refractive index n_D^{20}	1·4783
Viscosity (20°)	2·14 centipoise
Dielectric constant (20°)	48·9
Dipole moment	4·3 Debye

Organic compounds that are almost insoluble in DMSO include paraffin hydrocarbons, glycerides of higher fatty acids and other compounds with a highly paraffinic moiety, and plastic materials such as nylon, polystyrene and polyethylene.

DMSO is a good solvent for many inorganic compounds. It is miscible in all proportions with water. Lithium chloride, ferric chloride and ammonium thiocyanate are very soluble, whereas mercurous chloride, lead chromate and potassium ferrocyanide are almost insoluble. More detailed information concerning the solubility of inorganic and oganic compounds in DMSO has been published by Ranky and Nelson (1961) and Schläfer and Schaffernicht (1960).

EXPERIMENTAL

Rats derived from Carworth Farm strains, Carworth Farm No. 1 and AH (hairless) mice and Pirbright strain guinea-pigs were obtained from the Tunstall Laboratory Animal Breeding Unit. White Leghorn chickens aged 8–9 weeks were obtained from an accredited dealer.

The rats and mice were maintained at an environmental temperature of 73° F $\pm$ 3° F and a relative humidity of 45 per cent. Guinea-pigs were kept in a building where the temperature ranged from 60–70° F during the experimental period.

All animals were fasted overnight before weighing, and dosing using a ball point needle. Water was allowed *ad libitum* throughout the experiment and food was also always available after dosing. DMSO (British Drug Houses) Laboratory Reagent Grade was used throughout these experiments.

RESULTS

Oral Toxicity

Acute oral toxicity in rats. 20 ml./kg. killed one of four animals in a group of male and also in a group of female rats. Below this dose there were no deaths. This indicates that the LD50 is over 20 ml./kg. Above this dose it is considered that mechanical factors such as gastric distension play an important part in determining toxicity. A parallel

experiment was also made with rats maintained in an unheated room at about 50° F and the results were similar to those reported above. (2/8 deaths at 17·5 ml./kg., 1/8 at 20·0 ml./kg.). It is therefore considered that the ambient temperature is unlikely to materially affect the results.

Acute oral toxicity in mice. A small experiment indicated that the acute oral toxicity of dimethyl sulphoxide to mice was about the same as that of rats, 1/4 animals dying at a dose rate of 17·5 ml./kg. and 2/4 at 20·0 ml./kg. weight.

Acute oral toxicity in guinea-pigs. No deaths occurred with doses up to 10 ml./kg. body weight. Higher doses were not investigated.

Acute oral toxicity in chickens. Chickens gave results for 10·0 ml., 12·5 ml. and 15 ml. of 0/3, 2/3 and 3/3 respectively for cocks and 1/3, 1/3 and 3/3 respectively for hens. Thus the LD50 for this species is therefore approximately 12·5 ml./kg.

Repeated oral dosing to rats. Experiments were made in groups of 5 of each sex in which the rats were dosed 5 days a week for two successive weeks at dose levels of 1, 3·5, 5 and 10 ml./kg. Control groups, 10 animals of each sex, and groups dosed with water, 1 and 3·5 ml., were also included to determine the effect on weight which might be caused by recaging and other manipulatory procedures. The only animals that died were 2 males on the 5 ml./kg. and 1 male and 1 female on the 10 ml./kg. dose. The deaths were due to injuries caused by dosing.

Animals were weighed before dosing and during the first week of dosing the regrouping and caging of both control and treated animals was found to produce an adverse effect on weight. Losses of from 0·1 to 1·2 per cent were recorded for water dosage and with the solvent, as little as 1·0 and as much as 17 per cent loss was recorded in the first week. This was largely recovered during the 6th and 7th days, when no dosing took place, and the subsequent changes during the second week were not so marked. The animals were killed about 10 days after exposure ceased and at that time no changes in the formed elements of the blood of the treated animals were noted.

Dermal Irritation and Sensitisation

Sensitisation. 5 male and 2 female adult guinea-pigs had a course of intradermal injections of 0·1 ml. of a 10 per cent v/v aqueous solution of DMSO, given on alternate days for 3 weeks. Two weeks after the last injection a further intradermal injection of 0·1 ml. of the same solution was given but no signs of sensitisation were noticed. The experiment was then repeated on the same animals, but again no sensitisation was seen.

Irritation. 28 daily applications of undiluted DMSO to the clipped backs of guinea-pigs produced no macro- or microscopic signs of injury. DMSO was also painted on to the dorsal scapula region of 5 male AH (hairless) mice twice a week for 30 weeks. At the end of this period there was no discernable effect on the skin of the treated animals.

Although DMSO has been used in a large number of dermal toxicity experiments in rats as a solvent for other materials under investigation there has been no evidence of dermal injury.

Ocular damage. Undiluted DMSO was dropped into the conjunctival sac of the right eye of 6 adult New Zealand White rabbits. Two rabbits had the eye irrigated immediately with warm water and a further 2 rabbits were irrigated after a delay of 2–3 min. The eyes of the remaining 2 rabbits were not irrigated. No ill effects were noted in any of the treated eyes.

Intraperitoneal Toxicity

Both adult rats and guinea-pigs tolerated doses of 5 ml. DMSO per kg. weight. Eight male rats were given four successive daily intraperitoneal injections of DMSO at a dose of 7·5 ml./kg. weight. One rat died 2 days after the last injection but the remaining animals remained healthy during an observation period of 10 days after the last dose.

Pathology

No specific micro- or macroscopic evidence of toxicity has been noted at the autopsies of exposed animals. In a few animals some fatty degeneration and congestion of the sinusoids was seen in the liver. Where rats were given repeated intraperitoneal injections of large doses of DMSO there was evidence of rounding of the liver margin and of fibrinous strands within the peritoneum.

DISCUSSION

It is plausible to argue that the ability of a compound to dissolve large amounts of a wide range of chemical compounds excludes the possibility of its biological inertness. The formulation of chemical compounds for toxicity testing can therefore be a very real problem. A volume of 20 ml. solution/kg. weight may be regarded as the maximum tolerated oral dose. Thus a saturated solution containing 10 per cent w/v of toxicant cannot be used to give a dose greater than 2 g./kg. Sometimes it is possible to overcome this difficulty by administering the material as a suspension in water; however, the preparation of stable suspensions containing more than 10 per cent w/v of the material may be difficult, particularly with solids which have high elasticity.

Dimethyl sulphoxide fulfils most of the criteria proposed by Sanderson for the ideal solvent in toxicity tests. It is completely miscible with water, it not known to interfere with absorption or metabolism, has good solvent properties and a low viscosity and volatility. However, while large doses may be tolerated orally, dermally or intraperitoneally, even small doses may cause transient fall in body weight. After the administration of a large dose of DMSO animals may become depressed, but do not lose consciousness. DMSO causes denaturation of blood proteins and is therefore not suitable for intravenous injection.

Furthermore, in view of its dipolar aprotic nature, the possibility exists that a compound dissolved in dimethyl sulphoxide may undergo a chemical

change. Thus certain trivalent phosphorus compounds react with dimethyl sulphoxide, and two organophosphorus compounds showed colour changes of their solutions in dimethyl sulphoxide after 24 hr.

Dimethyl sulphoxide has two main disadvantages. It is more expensive than many other more readily available solvents and it has a characteristic odour which may be considered obnoxious.

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