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**PROBLEM DEFINITION STUDY OF
DIMETHYL SULFOXIDE (DMSO) AND INTERACTIVE
HEALTH EFFECTS WITH OTHER CHEMICALS**

PREPARED BY

Lawton H. Smith
Dennis M. Cpresko
James W. Holleman
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August 1986

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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PROBLEM DEFINITION STUDY OF DIMETHYL SULFOXIDE (DMSO) AND
INTERACTIVE HEALTH EFFECTS WITH OTHER CHEMICALS

FINAL REPORT

August 1983

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EXECUTIVE SUMMARY

Dimethyl sulfoxide (DMSO) is a versatile molecule, with interesting physical and chemical properties. It is quite stable under normal conditions, but can be oxidized, reduced, and decomposed by vigorous means. Physically, the high degree of self-association of DMSO causes it to have a high boiling point (189°C) and to be a liquid over a wide temperature range. The ability of DMSO to be polarized without dissociation is the basis for its ability to act as a solvent for a wide range of chemicals. As a chemical reactant, DMSO can act either as an electrophile (at the positive S atom) or as a nucleophile (at the negative O atom); most often it acts as the latter. Various chemical reactions are possible, including substitutions, dismutations, and others. DMSO makes dipole-dipole complexes with a number of chemical entities (e.g., nitriles and ketones), charge transfer complexes with others (e.g., iodine and iodine-halogen compounds), and coordination compounds with nearly all inorganic cations. Particularly noteworthy are the association of DMSO with transition states of reactants and its solvation of reactive species. As a "super solvent" DMSO promotes the rate of bimolecular reactions, allowing these to achieve their true rates, without the hindrance observed in other solvents. The electron-donating function of DMSO allows it to participate in H-bonding as an H-bond acceptor. This, as well as its effect on the structure of water, and its complexing ability, are the basis for its effects on biological molecules and in biological systems.

The chemical properties of DMSO allow it to pass rapidly through biological membranes, and it can easily be taken up through the skin. Accidental spillage of the solvent would not, however, pose a major health hazard, because, of itself, DMSO has a relatively low level of acute toxicity. Clinical studies indicate that cutaneous doses as high as 1 g/kg/day of a 80% DMSO gel, even if given for a 3-month-long period, would not produce signs of toxicity. Tolerance limits, however, have not been established for all exposure conditions. Higher doses, more concentrated solutions, a greater frequency of exposure, or a longer exposure period may produce one or more of the toxic effects seen in some laboratory and clinical studies; these include (1) scaling erythematous dermatitis; (2) damage to the epithelial tissue of the lungs following inhalation; (3) teratogenic effects; (4) increased frequency of chromosomal aberrations; (5) lenticular changes in the eye; (6) hemolysis; and (7) biochemical indications of hepatotoxicity and possibly nephrotoxicity, although the latter might be a secondary effect of DMSO-induced hemolysis. At present there is no clinical evidence that DMSO is teratogenic in humans or that it causes ocular changes in humans, but further study is warranted. Substantial data, however, indicate that it is not mutagenic or carcinogenic.

Laboratory studies have also shown that secondary to its toxic effects, DMSO (1) acts as a strong diuretic; (2) alters muscle tone and muscle response to stimulation in vitro and can cause vasodilation; (3) is an anti-acetylcholinesterase agent, but high concentrations may block cholinergic transmissions; (4) has analgesic properties; (5) stimulates adrenal and pituitary gland secretions in vitro but may not do so in

vivo; (6) has little effect on metabolic rate, but may influence lipolysis, protein synthesis, and some enzyme activities; and (7) increases membrane permeability and thus enhances intracellular and extracellular transfer of chemical compounds throughout the body.

Because of its effects on membrane permeability and its excellent solvent properties, DMSO has been used as a solvent vehicle for a wide variety of substances administered to humans and to several species of laboratory animals. While the broad solvent properties of DMSO have been the chief reason for its extensive application, results of many experiments demonstrate that it can modify the biological response to substances for which it has been employed as a vehicle. The observed modifications reflect rather poorly understood interactions that involve DMSO, the substance, and the target tissue.

DMSO can alter the response to many classes of substances including carcinogens, hepatotoxins, teratogens, mutagens, steroids, allergens, dyes, cytotoxins, and a wide variety of drugs. In many instances, the alteration involves an increase in the magnitude of the response, particularly in cases involving percutaneous absorption of a substance. The oral, intraperitoneal, or intravenous mode of administration of DMSO with a substance, however, often results in an unaltered response; and in some situations a reduction of response can be attributed to DMSO. Aside from their solubility in DMSO, there does not appear to be any physical or chemical characteristics common to those substances whose action is enhanced, diminished, or unchanged by this vehicle. Consequently, it is not possible at present to predict from knowledge of a substance's physical and chemical properties if and to what extent DMSO might modify its action. It would seem judicious to assume that an untested substance would penetrate the skin faster when in the presence of DMSO.

How the response to a particular substance is altered by DMSO is not clear, but generally it is thought to act as a penetrant carrier of substances through membranes at all levels of biological organization. Two important properties of DMSO as a penetrant carrier involve its ability to change conformation of proteins and to replace water; either action can alter penetration rates of substances dissolved in DMSO. Changes in penetration rates of a substance through a membrane constitute a likely basis for an altered response to that substance. Although there is some evidence for a direct chemical reaction between DMSO and its solute, e.g., hydrate formation, these kinds of reactions do not appear to play a major role in DMSO-induced alteration of the response to most substances tested.

In assessing the interactive health effects of DMSO and another liquid, a direct comparison can be made between the liquid alone and the liquid administered in conjunction with DMSO. However, it is not possible to make the same type of comparison for solid substances for which assessment can be made only relative to the solid in a vehicle other than DMSO. Therefore, DMSO diminishes, increases, or has no effect on the biologic response to a substance relative only to the response of that substance administered in another vehicle.

A most important experimental variable related to the action of DMSO on the response to a substance is the concentration of DMSO used. In general, high concentrations of DMSO are tantamount to maximum alterations of the response, especially those responses associated with percutaneous absorption.

At present there are no known restrictions in using DMSO as an industrial solvent. Although there is substantial evidence for its therapeutic value in a variety of human disorders, prior considerations of its potential toxicity have prevented its being licensed for use as a drug except in the treatment of interstitial cystitis. However, with increasing data supporting the contention that it is therapeutically effective without significant toxic side effects, it may soon be licensed for use by the U.S. Food and Drug Administration for the treatment of scleroderma and muscle-skeletal disorders.

Finally, it should be noted that little information exists on the effects of subchronic and chronic percutaneous exposure to DMSO in combination with other agents. In view of the conditions that would be present in the workplace, further studies of this kind would seem to be in order for both RDX and HMX. Agents chosen for study should be those to which personnel may be expected to be exposed.

ACKNOWLEDGMENTS

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1 INTRODUCTION

1.1 PURPOSE

The current initiative by the U.S. Army to replace cyclohexanone/acetone with dimethyl sulfoxide (DMSO) as the recrystallization solvent used in the manufacture of RDX/HMX explosives requires an evaluation of the potential health hazards associated with the proposed processes. Design and operation decisions for the new system will require consideration of potential worker exposure to DMSO at several points along the process stream. The main concern is the potential for rapid skin adsorption of DMSO contaminated with varying concentrations of RDX, HMX, and smaller amounts of other cyclic and linear nitramines such as SEX, TAX, and BSX. The objective of this report is to identify and define the known mechanisms of action and biological effects of DMSO when combined with other chemicals, and to define the similarities to nitramines of military concern, and also to briefly characterize the range of biological effects of DMSO.

1.2 APPROACH TO THE PROBLEM

The initial step to define and characterize the interactive effects of DMSO with other chemicals was a comprehensive search of the literature by both computerized and manual methods. This included a search of commercially available data bases such as DIALOG and TOXLINE as well as the holdings of the Defense Technical Information Center. Also, the proceedings of the September 1982 New York Academy of Sciences' Conference on Biological Actions and Medical Applications of DMSO was reviewed and pertinent references cited (see de la Torre 1983).

In describing and evaluating the published data relevant to the interactive effects of DMSO with other substances, certain qualifications should be considered, not the least of which is a definition of the term interaction. In the context of the present objective, interaction is considered to be a process that occurs in conjunction with administration of DMSO and another substance(s), resulting in a biologic response. In almost all cases to be described, the response measured was attributable to the substance rather than to DMSO. The substance and DMSO were usually administered as a mixture, occasionally in sequence, and by various routes.

The characteristics of the process and of the response require further comment. A process can occur at different levels, one of which involves physical and chemical reactions between DMSO and the substance before, during, or after administration to the organism. One of the most important physical interactions is reflected in the fact that DMSO is a very good solvent for a variety of polar and nonpolar substances, and it is this solvent feature which probably accounts for many of the interactive biologic effects of DMSO with another substance. Indeed, a compelling reason for studying the biologic effects of DMSO in conjunction with another agent has been its solvent capacity. Clearly, however, other modes of the process can be considered. The most important of

these is the modification of a tissue component by DMSO that results in alteration of the response to an agent.

The biologic responses resulting from administration of DMSO in conjunction with an agent are varied and in almost all cases are responses to the agent per se. Thus investigators usually have been interested in the effect of DMSO on the response to a specific agent, although conversely, some of the effects of DMSO (see Section 3 of this document) may be altered by another agent. The principal responses studied have been survival, hepatotoxic, tumorigenic, mutagenic, teratogenic, pharmacologic, physiologic, cytotoxic, biochemical, and therapeutic, some of which involve an undesirable toxic component.

DMSO can increase, decrease, or have no apparent influence on the response to an agent. In judging the influence of DMSO on the response to a liquid, the effect of DMSO can be evaluated quite readily because the control is the effect of the liquid per se. In assessing the influence of DMSO on the effect of a solid, however, results can be expressed only in relative terms (i.e., the effect of that substance administered in another vehicle). As a reference or comparison vehicle, investigators often used the least innocuous confounding or influential vehicle (water, saline, vegetable oil) or a vehicle that was an acceptable solvent for the test substance. In many of the experiments reviewed, various kinds of organic reference vehicles were used, including acetone, dimethyl formamide, and ethanol. This variable should be kept in mind in evaluating an observation that DMSO increased, decreased, or had no effect on the biologic response to a particular substance.

It is not within the scope of this document to consider those reactions that would occur under extreme conditions encountered in industrial processes. Considerations of this kind would require an evaluation of those conditions involved in the varied industrial processes in which DMSO was used, an assessment of the biologic responses to the substances generated by these processes, and determinations of the effect of DMSO on these responses.

1.3 ORGANIZATION OF REPORT

This report is divided into six sections plus appendices. Section 2 describes the physical and chemical properties and behavior of DMSO and, although somewhat technical in its presentation, sets the stage for Section 3 (DMSO Toxicity) and particularly for Section 4 (Interactive Effects of DMSO). Section 3 presents a concise yet comprehensive coverage of the range of effects of DMSO and is organized by the nature of the effect (e.g., carcinogenicity and mutagenicity). Section 4, the focal point of the report, discusses the interactive effects of DMSO with other chemicals. The Summary of Interactions Subsection (4.1) is divided into two parts: (1) nonpercutaneous administration and (2) percutaneous administration. This dichotomy, although somewhat arbitrary, was based partly on the past and current interest of investigators in the enhancement of percutaneous penetration of substances by DMSO. This interest translates to situations in the workplace where the most likely mode of human exposure to DMSO, with or without another substance, is

cutaneous. In both parts of Section 4.1, interaction is considered according to groups of substances that produce similar responses (e.g., teratogenic, pharmacologic).

Sections 3 and 4 are intended to summarize the relevant information. For more detail the reader is directed to the review and analysis of individual papers (Appendix A) and the tabular data summaries (Appendix B).

2 CHEMICAL AND PHYSICAL PROPERTIES OF DMSO AND MONITORING TECHNOLOGY

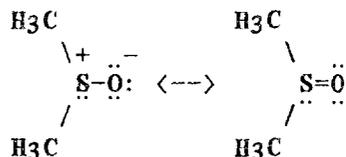
2.1 PHYSICAL CHARACTERISTICS OF DMSO

DMSO (dimethyl sulfoxide) (CAS Registry Number 67-68-5) is designated "methyl sulfoxide" in the 7th (1962-1966) and 8th (1967-1971) Collective Indexes of the Chemical Abstracts Service and "methane, sulfinylbis-" in the 9th (1972-1976) and 10th (1977-1981) Collective Indexes. The name "methylsulfinylmethane" has also been used (International Union of Chemistry, Hodgman 1947).

Some physical characteristics of DMSO, and operational parameters concerning it, are given in Tables 1-4 and Figures 1-9 taken from Rothrock et al. (1981).

2.2 CHEMICAL BEHAVIOR OF DMSO

The chemical behavior of DMSO largely reflects its makeup as a non-protic dipolar molecule. Although both the S and O atoms can act as electron donors, the greater polarizability of the nonbonding electrons at the S atom causes this "softer" (in Pearson's terminology of "hard" and "soft" acids and bases; Pearson 1968) terminal of the base to interact preferentially with a corresponding "softer" electron acceptor, or acid (Szmant 1971). Thus the S atom is generally a positive center and the O atom a negative one, giving rise to the observed polarity. In terms of resonance theory, the DMSO molecule may be represented as follows (Martin 1975):



In this representation the C-S bonds are normal single covalent bonds, while the S-O bond is a resonance hybrid of a semipolar bond and a $(p \rightarrow d)_\pi$ SO double bond. The S atom is a sp^3 -hybridized, and the SO bond is formed by an sp^3 - p_x σ overlap along a π bond of the type d_{xz} - p_z (or d_x - p_y). This kind of $(p \rightarrow d)_\pi$ overlap is not very strong, and a strongly polar SO bond results, the S being positive and the O negative, as mentioned. The question of the nature and orientation of the bonds in DMSO has been studied in detail by Guimon et al. (1973), and the partial double-bond character of the SO bond has been confirmed.

In the representation of Guimon, et al. (1973), the C, S, and two of the H atoms are in a plane, with the O and the four other H atoms projecting at angles upwards or downwards from the plane. The DMSO molecule may also be pictured as a tetrahedron (Szmant 1971), with the S occupying a central position and the CH_3 , O, and a nonbonding electron

TABLE 1. PHYSICAL PROPERTIES OF DMSO

Molecular weight	78.13
Boiling point at 760 mm Hg	189°C (372°F)
Melting point	18.55°C (65.4°F)
Specific gravity at 25°C (77°F)	1.096 (9.15 lb per gal)
Refractive index at 25°C	1.477
Vapor pressure at 25°C	0.62 mm Hg
Surface tension at 25°C	42.85 dynes per cm
Viscosity at 25°C	2.00 centipoise
Specific heat at 29.4°C	0.47 ± 0.015 cal/(gm)(°C)
Heat of vaporization at 25°C	12.64 Kcal/mole (291 Btu/lb)
at 189C	10.31 Kcal/mole (237 Btu/lb)
Heat of solution in water at 20°C	60 cal/gm
Heat of fusion	44 cal/gm
Heat of combustion at 25°C	6050 cal/gm
Flash point (TOC)	95°C (203°F)
Ignition temperature in air	300-302°C (570-575°F)
Flammability limits in air lower	3-3.5 vol %
upper	42-63 vol %
Thermal coefficient of expansion	0.00088 cc per degree C
Electrical conductivity 20°C	3 x 10 ⁻⁸ ohm ⁻¹ cm ⁻¹
80°C	7 x 10 ⁻⁸ ohm ⁻¹ cm ⁻¹
Water solubility	Completely miscible

Adapted from Rothrock et al. (1981).

TABLE 2. HEAT CAPACITY OF DMSO

Temp, °C	C _p (liquid), cal/(g)(°C)
30	0.47
60	0.47
100	0.48
150	0.52

Adapted from Rothrock et al. (1981).

TABLE 3. DENSITY OF DMSO

Temp, °C	Density, grams/cc
25	1.096
60	1.062
100	1.023
150	0.974

Adapted from Rothrock et al. (1981).

TABLE 4. VAPOR-LIQUID EQUILIBRIUM DMSO-WATER
(ONE ATMOSPHERE PRESSURE)

Temperature (°C)	Mol fraction water in liquid	Mol fraction water in vapor
100.0	1.000	1.000
100.6	0.988	0.9998
101.0	0.975	0.9997
102.0	0.945	0.9994
103.3	0.909	0.9989
105.0	0.865	0.9983
108.0	0.810	0.997
113.0	0.740	0.994
118.0	0.675	0.990
120.0	0.645	0.986
130.0	0.513	0.964
143.0	0.378	0.921
149.0	0.313	0.890
165.0	0.176	0.773
174.5	0.100	0.628
177.0	0.081	0.573
183.0	0.046	0.353
184.6	0.034	0.282
187.7	0.011	0.100
189.0	0.000	0.000

Adapted from Rothrock et al. (1981).

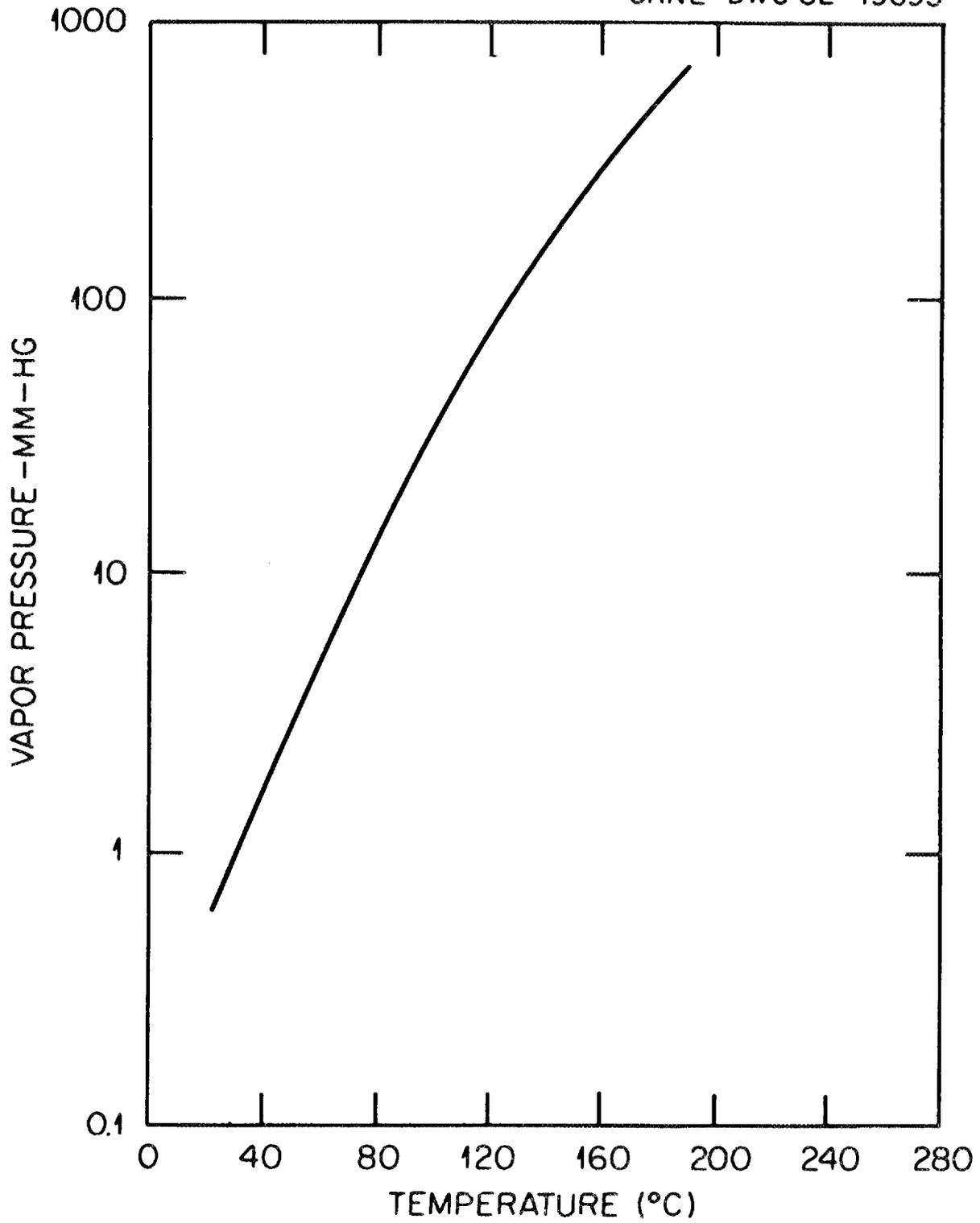


Figure 1. Vapor pressures of DMSO. Source: Adapted from Rothrock et al. (1981).

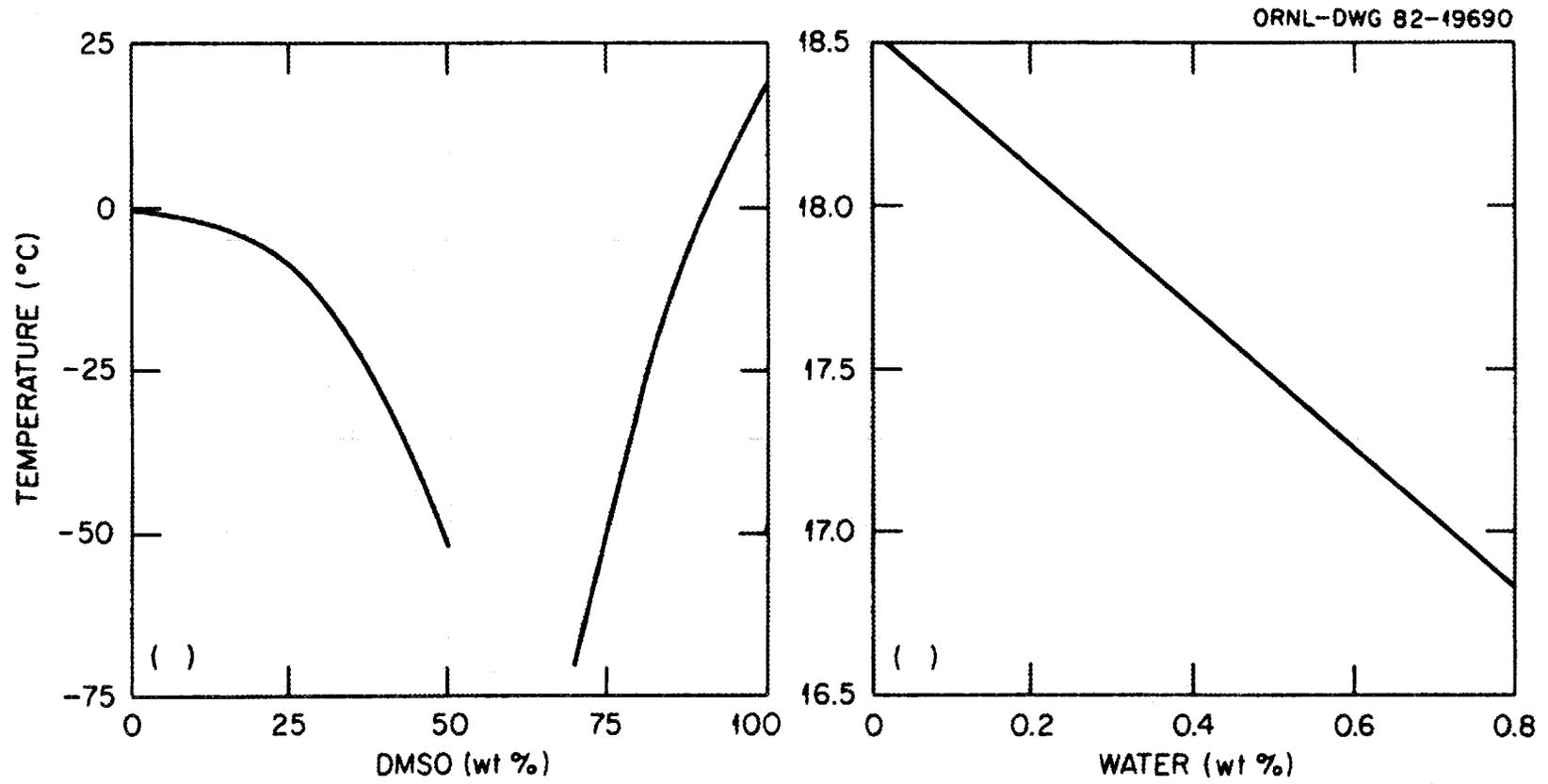


Figure 2. Freezing point curves for DMSO - water solutions. Source: Adapted from Rothrock et al. (1981).

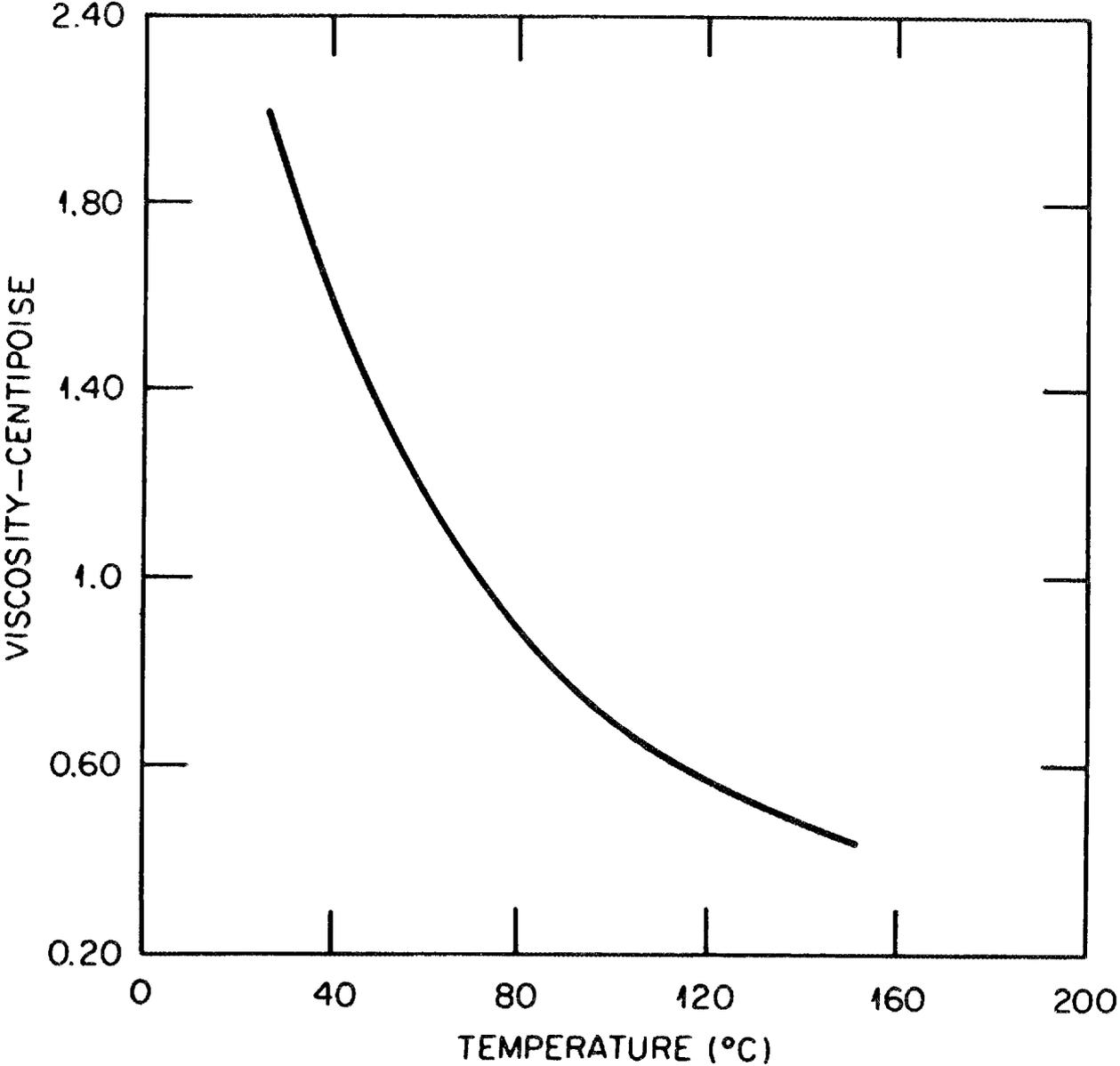


Figure 3. Viscosity of DMSO. Source: Adapted from Rothrock et al. (1981).

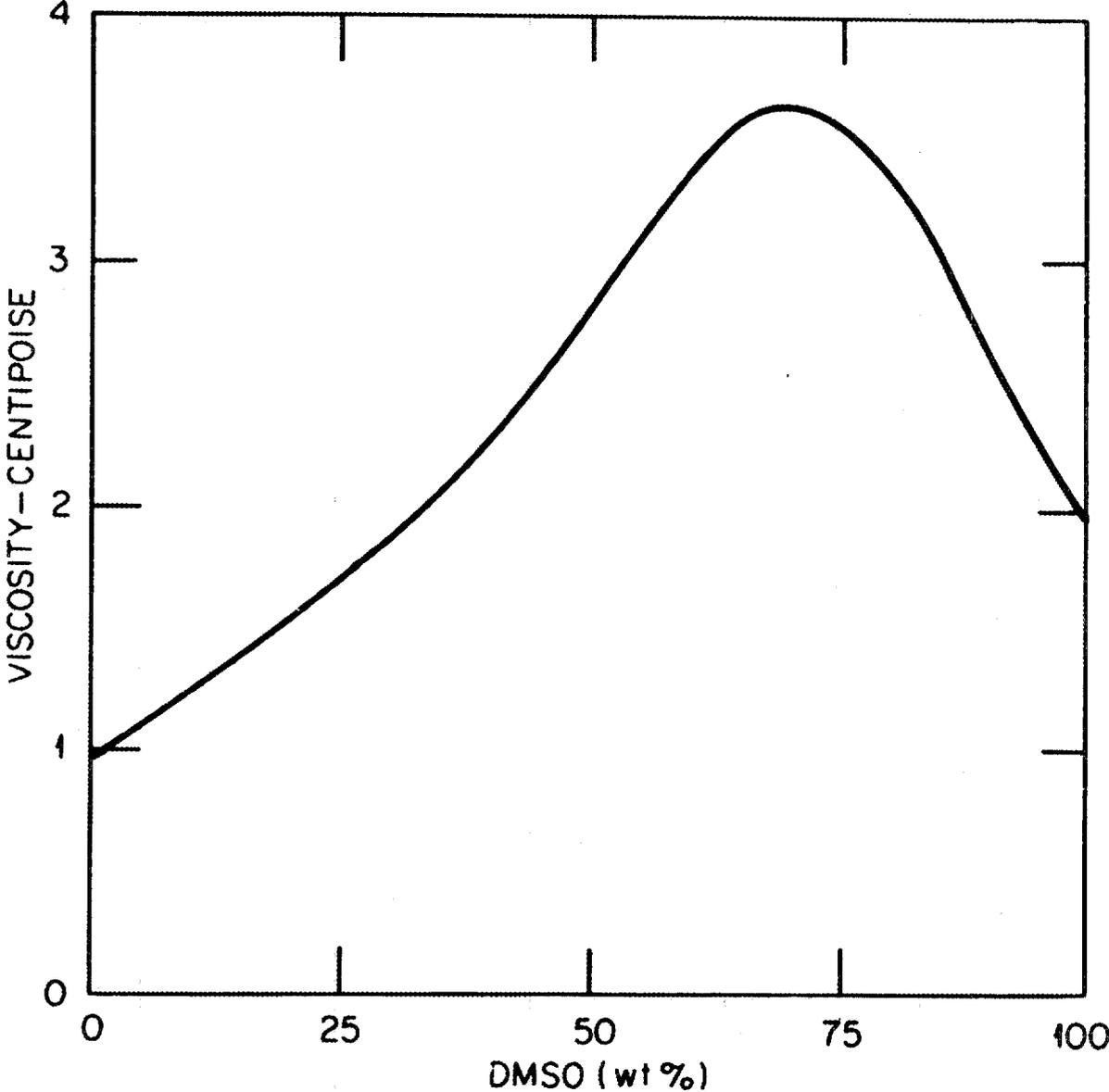


Figure 4. Viscosity of DMSO - water solutions. Source: Adapted from Rothrock et al. (1981).

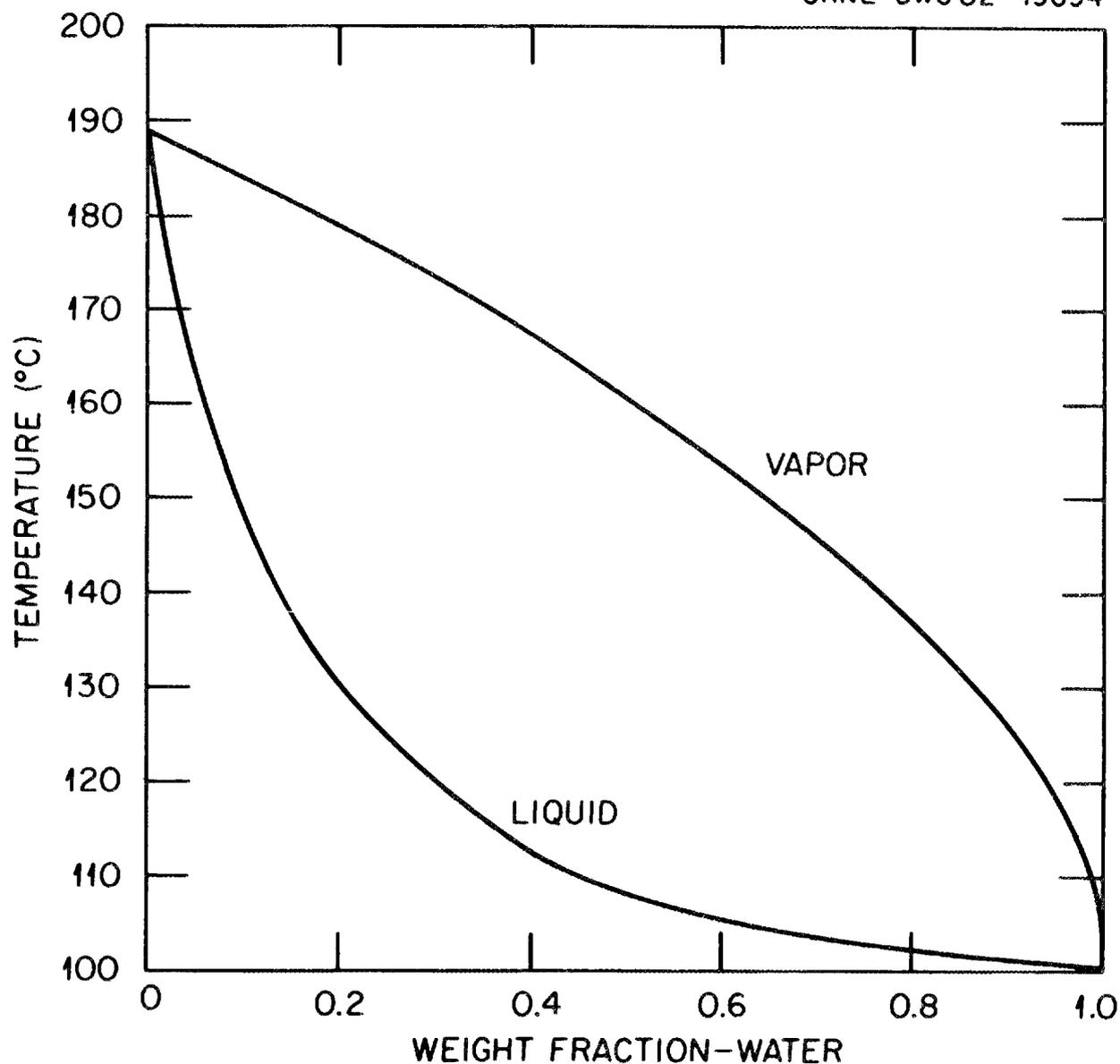


Figure 5. Boiling temperature curve for DMSO - water solutions.
 Source: Adapted from Rothrock et al. (1981).

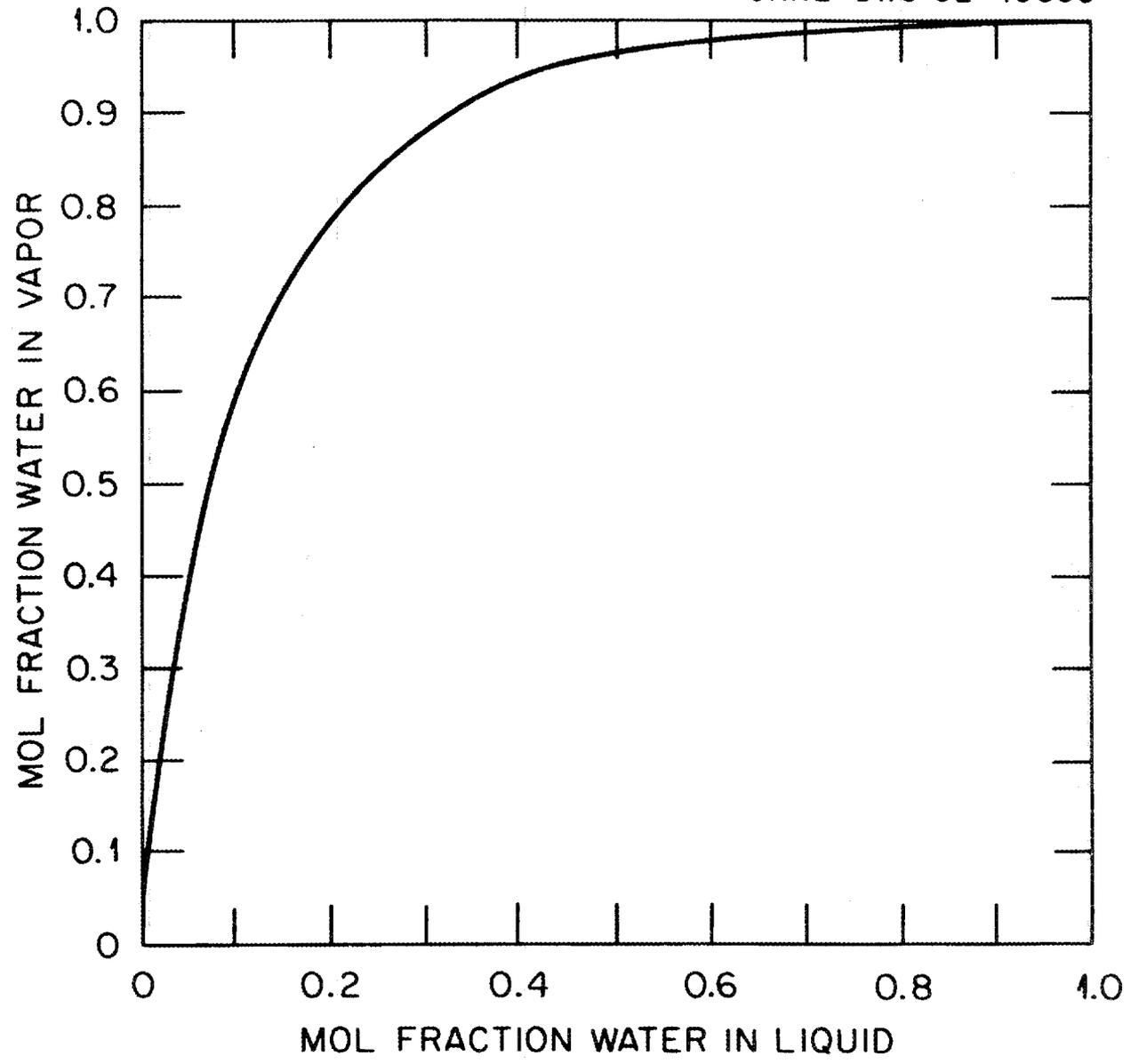


Figure 6. Vapor liquid equilibrium curve - DMSO/water at one atmosphere. Source: Adapted from Rothrock et al. (1981).

CONDITIONS: REFLUX RATIO
OVERHEAD
BOTTOMS
PRESSURE

ORNL-DWG 82-19697
1:1
500 ppm DMSO
500 ppm H₂O
1 ATMOSPHERE

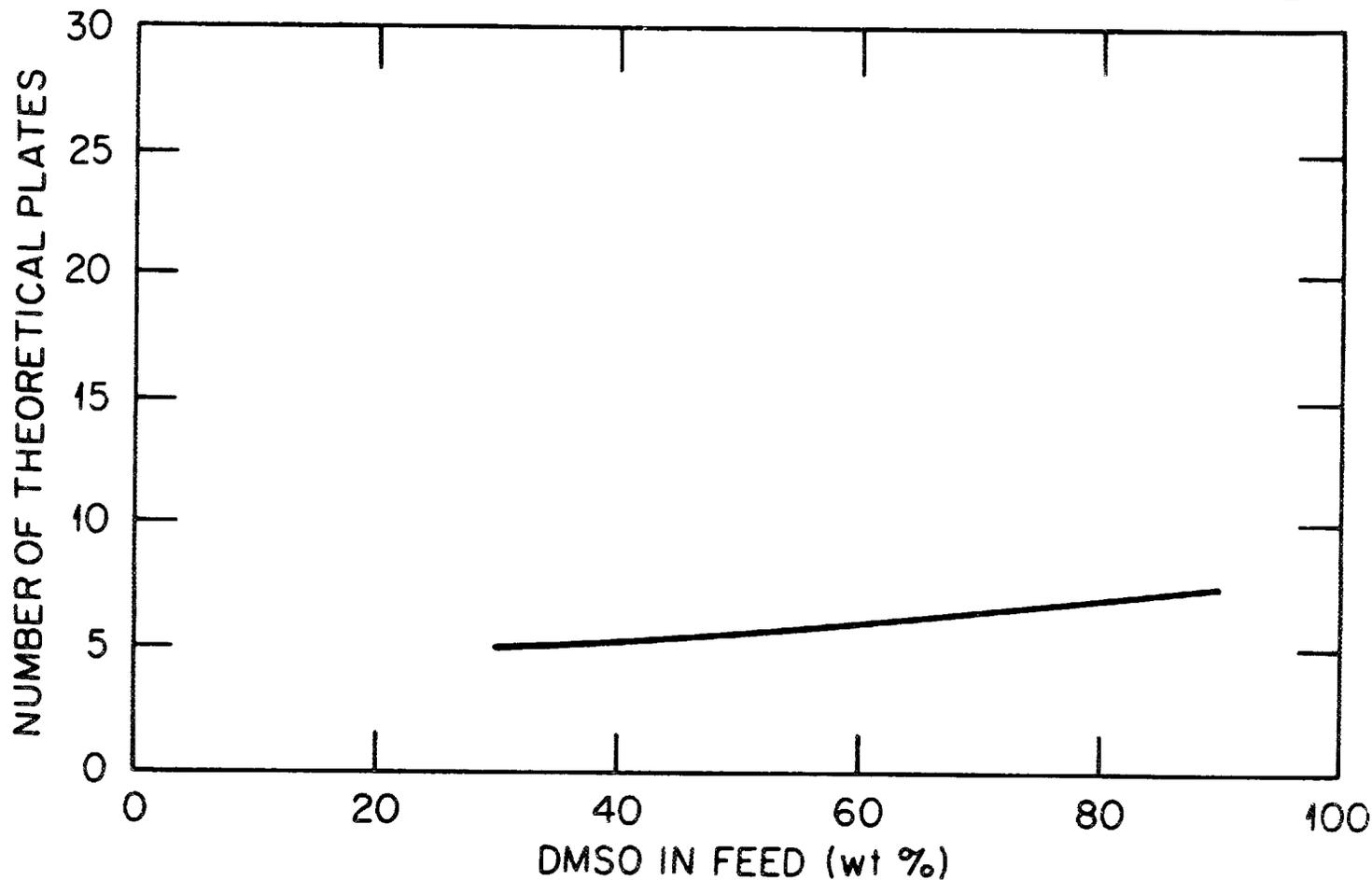


Figure 7. Theoretical plate requirement - DMSO/water system. Source: Adapted from Rothrock et al. (1981).

ORNL-DWG 82-19698

CONDITIONS: THEORETICAL PLATES 12
OVERHEAD 500 ppm DMSO
BOTTOMS 500 ppm H₂O
PRESSURE 1 ATMOSPHERE

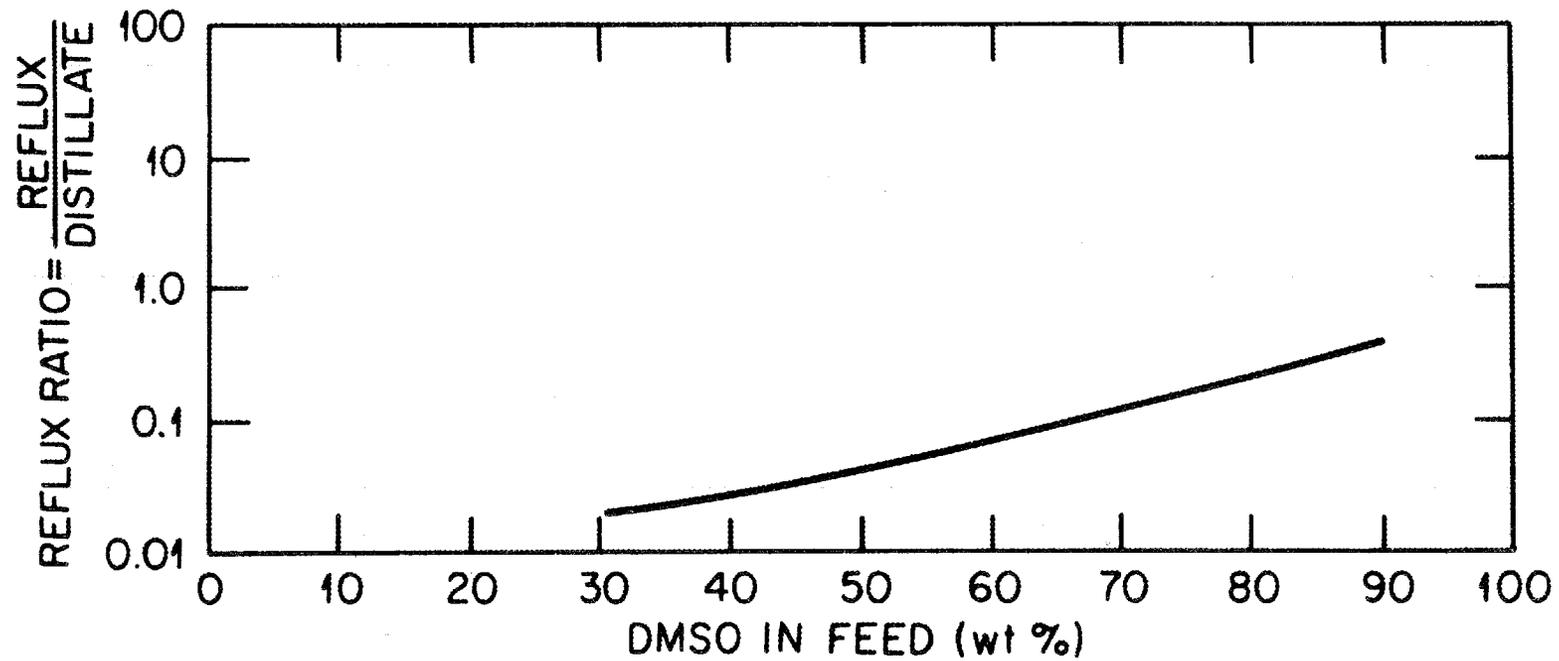


Figure 8. Reflux Requirements - DMSO/water system. Source: Adapted from Rothrock et al. (1981).

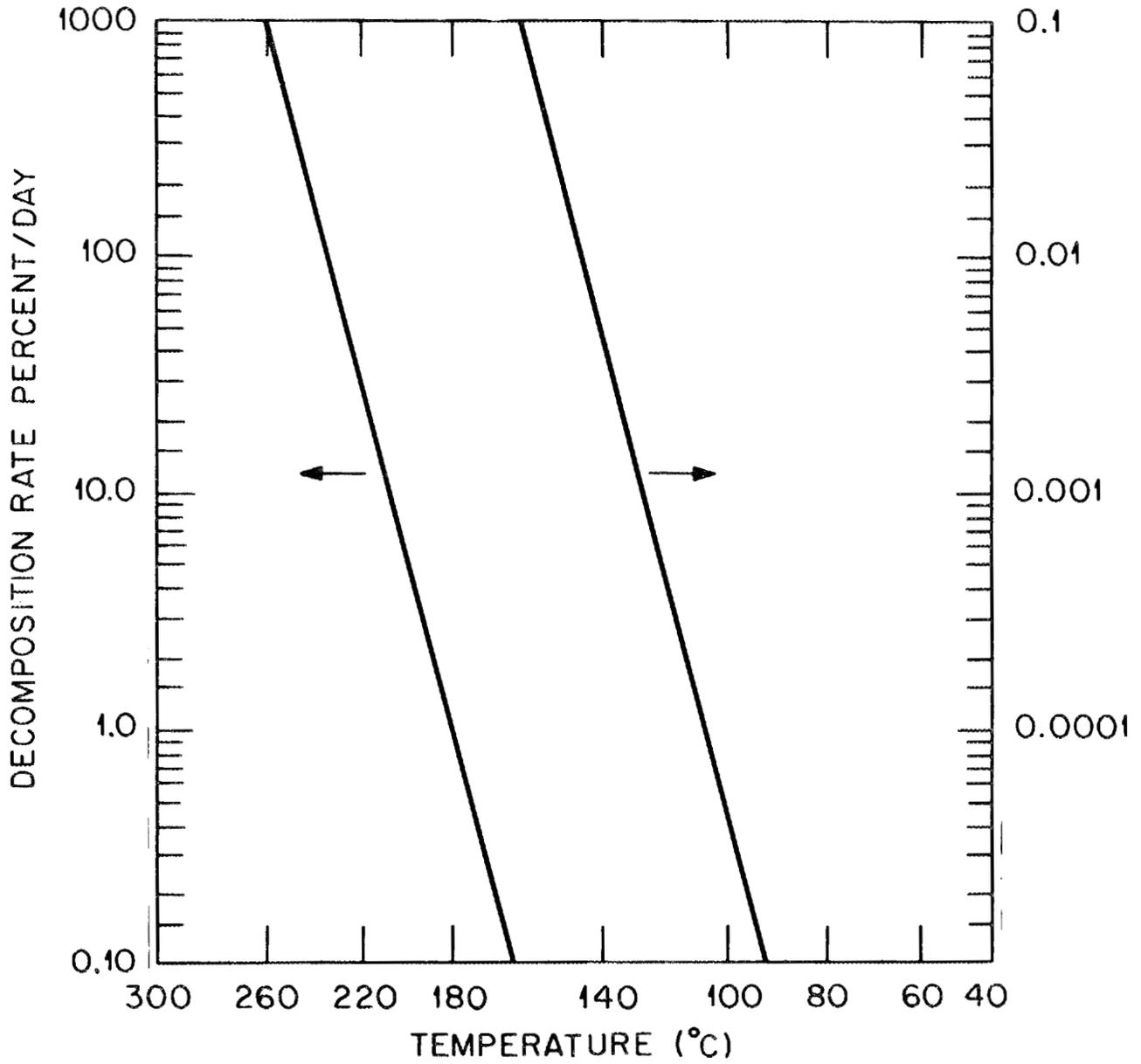


Figure 9. Thermal decomposition of DMSO. Source: Adapted from Rothrock et al. (1981).

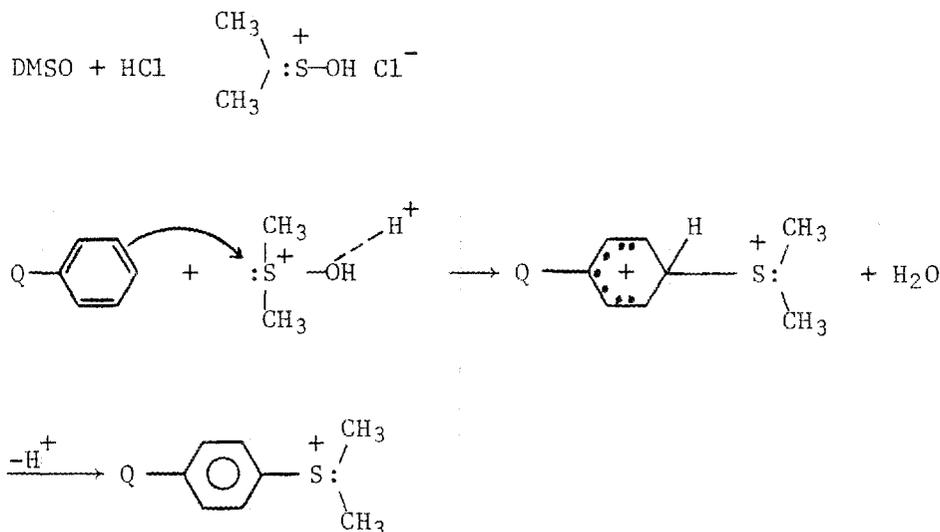
pair (arising from the S) entities being located in the apex positions. The polarity of the molecule is evident from this visualization.

As a dipolar molecule, DMSO associates strongly with itself, which results in its being a liquid over a wide temperature range (ca. 170°C), and to have a high boiling point (189°C) and a high entropy of evaporation (29.6 cal/deg/mole), higher than that of water (Martin 1975).

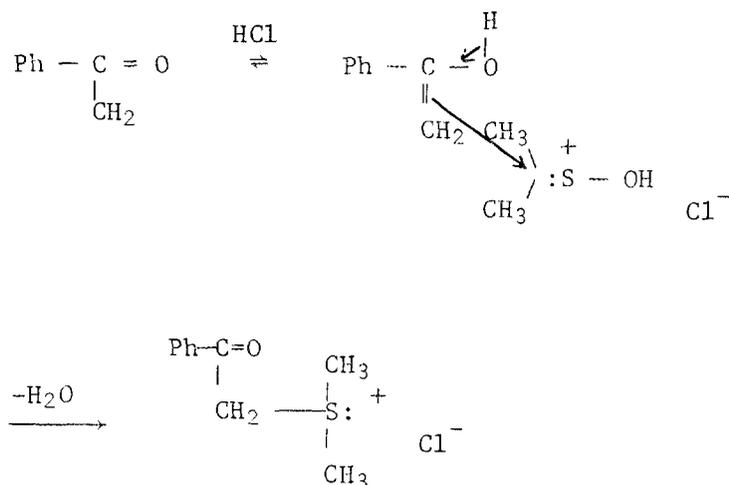
Because of its aprotic polar nature and "soft" electronic makeup, DMSO does not solvate small and "hard" anions and solvates large and polarizable anions only weakly, whereas cations and transition states with delocalized charges are highly solvated by it (Martin 1975). The electron-donating characteristic of DMSO causes it to associate strongly with protons. Compounds which are acids because they contain OH, NH, or CH groups form association complexes with DMSO. DMSO forms dipole-dipole complexes with nitriles and carbonyl compounds, charge-transfer complexes with iodine- and iodine-halogen compounds, and co-ordination compounds with nearly all inorganic cations (Martin 1975).

A striking consequence of the structure of DMSO as a "rapid" dipolar aprotic solvent is an observed enormous increase in the rates of bimolecular reactions in DMSO compared with rates of these same reactions in protic solvents (Szmant 1971; Martin 1975). Although the mechanism is complex, it may be said that in DMSO a nucleophile shows its true substitutive reactivity, whereas in protic solvents this activity is hindered. DMSO shares this characteristic with other dipolar aprotic "super solvents" such as dimethyl sulfone, dimethyl formamide, and N-methylpyrrolidone, but, in contrast with these, it can also act as an important reactant and as a pharmaceutical agent, in facilitating absorption of substances across membranes and in associating with molecules of biological interest.

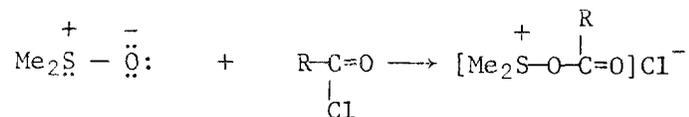
As a reactant, DMSO can act as either an electrophile or a nucleophile. Thus, under acid catalysis, DMSO can effect electrophilic substitution of a susceptible aromatic system as follows (Szmant 1971):



In the case of enolic systems, HCl catalyzes not only the formation of the electrophilic sulfonium ion center in DMSO as above, but also the formation of the nucleophilic tautomer of the ketone, and the reaction occurs as follows (Szmant 1971):



Notwithstanding the existence of the above reactions and others, such as derivatization of DMSO to enhance its electrophilicity (Szmant 1971), the electron-donating property of DMSO tends to prevail over the electron-accepting property, and DMSO reacts most commonly as a nucleophile. This predominantly electron-donating property of DMSO is responsible for numerous reactions initiated by nucleophilic attack of the "hard" O terminal upon C, P, S, and other atoms in structures containing a good leaving group. An example is the reaction of DMSO with an acid chloride (Szmant 1971):



The initial product may then react in several ways, depending on conditions. As indicated by Szmant, such reactions are often highly exothermic and unless properly controlled can be explosive.

DMSO can also act as a nucleophile at the S atom, in that case acting as a "soft" base (Pearson 1968; Szmant 1971). It can act as either a reducing or an oxidizing agent. Furthermore, the H atoms can be ionized in the presence of strong bases to give the "dimsyl" anion. At the same time, DMSO contains an excellent leaving group in the form of the methane sulfinate anion CH_3SO^- , giving rise to a series of interesting consequences in the chemistry of the dimsyl ion (Szmant 1971).

Although DMSO can be reduced by reductants such as zinc, diborane, and aluminum hydride (Szmant 1971), it is sufficiently resistant to reduction to be a useful solvent for polarographic reduction, in electrolysis, and in selective reductions (for instance, reductions with sodium borohydride). Likewise, while it is oxidized by strong oxidizing agents such as permanganate and peroxides (Szmant 1971), it is sufficiently resistant to oxidation to be a useful solvent for base-catalyzed oxidations employing molecular oxygen, or oxidation reactions employing nitrogen tetroxide, triiodide ion, periodic acid, lead tetraacetate, etc., and in electrolysis.

An important characteristic of DMSO is its action on the structure of water. As discussed by Szmant (1971), at low concentrations the effect of DMSO is to stabilize or rigidify the normally present water aggregates without causing major perturbations of the characteristic intermolecular distances. At higher concentrations of DMSO the formation of H-bonded DMSO-water complexes causes a breakdown of the structure of liquid water and inhibits the formation of hexagonal ice. This latter effect is considered to account for the cryoprotective properties of DMSO in living cells.

The effect of DMSO on water has significance with respect to its effects on proteins and nucleic acids. DMSO replaces "bound" water in these substances, and at high enough concentrations complexes with groups of the proteins and nucleic acids, resulting in a "loosening" of these structures. The ability of DMSO to exchange sites with "bound" water molecules in relatively immobile protein structures is thought to account for the transfer of DMSO across the dermal barrier without tissue damage (as discussed by Szmant 1971). The association of DMSO with the polar portion of lipids also cannot be ignored in this connection. DMSO competes with water as an H-bond acceptor in proteins and nucleic acids, and while it can hardly compete with water as an H-bond donor, it is superior to water in associations based on the induction of dipoles in aromatic rings, methyl mercapto and disulfide bonds, and other structures in proteins and nucleic acids.

DMSO can be decomposed to some degree by heat, light, acids, bases, free radicals, electron impact, and ionizing radiation, but it is, practically speaking, quite stable. For instance, refluxing DMSO at its normal boiling point of 189°C for 72 hr causes production of only 3.7% volatile products (Szmant 1971).

In conclusion, DMSO is a versatile solvent and reactant, with interesting chemical and physical properties. Its self-association causes it to be a liquid over a wide temperature range, with a high boiling point. Its dipolar aprotic nature causes it to be a solvent for a wide range of chemicals. Chemically, it can act as either an electrophile (at the S atom) or a nucleophile (at the O atom), most often the latter. Various chemical reactions are possible, including substitutions, dismutations, and cycloadditions. DMSO makes dipole-dipole complexes with a number of chemical entities, charge-transfer complexes with iodine- and iodine-halogen compounds, and coordination compounds with nearly all inorganic cations. Particularly noteworthy are the

association of DMSO with transition states of reactants and its solvation of reactive species. As a "super solvent" DMSO promotes the rate of bimolecular reactions. The electron-donating function of DMSO allows it to participate in H-bonding as an H-bond acceptor. This, together with its effect on the structure of water and its complexing ability, forms the basis for its effects on biological molecules. DMSO can be oxidized, reduced, and decomposed by vigorous means, but under normal conditions it is quite stable.

2.3 MONITORING TECHNOLOGY

2.3.1 Biological Media

No NIOSH standards or recommendations were found pertaining to monitoring of DMSO in body fluids and tissues of occupationally exposed workers. In laboratory and clinical studies a number of different analytical techniques have been used to identify and quantify DMSO, as well as its metabolites, dimethyl sulfide and dimethyl sulfone. Dimethyl sulfide has been identified by means of gas chromatography and/or mass spectrometry in samples of expired air of experimental animals injected intravenously with DMSO (DiStefano and Borgstedt 1964). Dimethyl sulfone has been extracted from urine samples, purified and then identified by its characteristic infrared spectrum (Williams et al. 1965). Isotope dilution analysis of 24 hr urine samples from a patient receiving a daily dose of 21 g of DMSO showed that 3% of the dose was excreted as dimethyl sulfone (Hucker et al. 1966). For determining DMSO in various biological samples (serum, plasma, urine, and CSF), chromatographic methods have been most often used, and a recently described gas chromatographic procedure coupled with flame ionization detection is reported to have excellent accuracy, linearity and precision (Garretson and Aitchison 1982). A laboratory method, possibly applicable for enzymatic and cytological studies utilizes mass spectrometry with a variable pH interface, which under certain conditions may eliminate the need for chromatographic separation (Weaver and Abrams 1979).

2.3.2 Environmental Media

No standards or recommended methods were found for determining DMSO levels in environmental media; however, gas chromatography appears to be the method most often used in laboratory and field studies. Seager and Stone (1971) describe a rapid chromatographic technique for detecting DMSO in aqueous solutions at concentrations as low as 0.02 weight percent. The method involves the use of isothermal gas-solid chromatography with porous polymer beads as the column packing, coupled with dual hydrogen flame ionization and dual hot wire detectors. Andreae (1980) has described a method in which the DMSO is reduced to dimethyl sulfide, separated by gas chromatography and then detected with a flame photometric sulfur detector and/or a flame ionization detector. This technique, used to measure DMSO in seawater, freshwater, rainwater, and phytoplankton culture solutions, was reported to have a detection limit of 1 ng S (DMSO) per sample, equivalent to 0.02 ug S (DMSO)/L, with a precision of 5-10%.

3 TOXICITY OF DMSO

3.1 SUMMARY OF EFFECTS

3.1.1 Lethality

The median lethal toxicity of DMSO to laboratory animals ranges from 2 to about 50 g/kg depending on species and route of administration. The LD₅₀ for intravenous injections of DMSO has been reported to be 3.1-7.6 g/kg for mice (Sommer and Tauberger 1964; Willson et al. 1965; Fishman et al. 1969); and 5.36-8.1 g/kg for rats (Sommer and Tauberger 1964; Willson et al. 1965) and 4-8 g/kg for monkeys (Mason 1971). The LD₅₀ for oral doses was found to be 16.5-21.4 g/kg for mice and 14.5-28.3 g/kg for rats (Sommer and Tauberger 1964; Willson et al. 1965; Fishman et al. 1969). Intraperitoneal injections in mice resulted in an LD₅₀ of 10.9-15.4 g/kg (Worthley and Schott 1969), and subcutaneous doses gave an LD₅₀ of 13.9 g/kg in mice and 12.0 g/kg in rats (Sommer and Tauberger 1964). The LD₅₀ for cutaneous exposures has been estimated to be about 40 g/kg for mice and 40-50 g/kg for rats (Mason 1971). In rhesus monkeys a daily oral dose of 9 mL/kg of 90% DMSO (8.8 g/kg) resulted in a 83% mortality rate after 53 weeks, but a daily dose of 3 mL/kg was not lethal (Vogin et al. 1970).

3.1.2 Mutagenicity

The mutagenicity of DMSO has been evaluated in several microbiological studies. Simmon et al. (1977) and De Flora (1981) tested the solvent in the Ames Salmonella/microsome assay, and in both cases DMSO was found to be nonmutagenic. Abbondandolo et al. (1980) exposed cell suspensions of the P1 strain of the yeast Schizosaccharomyces pombe to 0.5, 2.0, or 5% DMSO and found that forward mutation rates were similar to those of the controls in both the presence and absence of a mouse liver microsome extract. Callen and Philpot (1977), however, found that when log phase cells of the D4 strain of the yeast Saccharomyces cerevisiae were exposed to 0.5, 1.0, or 1.4 M DMSO for 4 hr at 37°C, there was a dose-related increase in gene conversions at certain loci. This was attributed to the metabolic conversion of DMSO to a genetically active compound by cytochrome P-450-dependent mixed function oxidation reactions.

In tests on cell cultures prepared from rainbow trout gonads, Kocan et al. (1982) observed no significant changes in the incidence of anaphase chromosomal aberrations after exposure to 0.5% DMSO. In vivo application of 1% DMSO to 3- and 4-day-old chick embryos resulted in no increase in chromosome breakage or sister chromatid exchanges (Bloom 1982).

In rats, daily intraperitoneal injections of 5 mL/kg of 1%, 10%, 50%, or 100% DMSO for five days resulted in an increase in chromosomal aberrations and chromosomal breaks in femoral bone marrow cells (Kapp and Eventoff 1980). Dose levels of 1 mL/kg/day, injected intraperitoneally in male rats for 10 weeks, did not induce dominant lethality in

mated females (Sheu and Green 1979). Similar results were obtained when male Swiss mice were dosed intraperitoneally with 5, 7.5, or 10 g/kg of DMSO two times at an interval of about 20 hr (Aravindakshan et al. 1975).

In tests using cultures of Chinese hamster ovary cells, Tates and Kriek (1981) found that 10% DMSO induced significant numbers of chromosome aberrations, but only when administered with rat liver microsome extract. One-percent DMSO did not cause such an effect, either with or without metabolic activation. Neither concentration changed significantly the number of observed sister chromatid exchanges.

3.1.3 Carcinogenicity

The potential carcinogenicity of DMSO has been evaluated using several in vivo and in vitro systems. Applied to hamster cheek epithelium, DMSO produced mild dyskeratosis but no carcinogenic transformations (Elzay 1967). Similarly, 2% DMSO did not cause cell transformations in cell cultures of Syrian hamster embryos (Pienta 1980) or of hamster sternal hyaline cartilage (Katoh 1977). Oral doses of 2.5 and 5.0 mL of undiluted DMSO to male Wistar rats had no effect on the number of mitoses in cells of the adrenal cortex, and this was considered as supporting evidence that DMSO is not a tumor promoter or active carcinogen (Danz and Urban 1979). A DMSO concentration of 10 mM did not induce DNA damage in vitro in cultures of Chinese hamster V79 cells, although known carcinogens did (Swenberg et al. 1976). Lohs et al. (1971) reported that rats exposed weekly to subcutaneous injections of DMSO in the presence of H₂O₂ and TiCl₃ did not show any signs of tumor formation after one year. Mondal (1971) exposed C3H mouse ventral prostate cells to 0.5% DMSO and found that 5% of the treated cells gave rise to malignant clones.

3.1.4 Teratogenicity and Embryotoxicity

DMSO has been reported to have teratogenic effects in several species of experimental animals. Developmental abnormalities including malformations of the limbs, beak and eyes, anourous embryos, and celosomia were observed by Caujolle et al. (1967) in chick embryos injected with 50% DMSO. Ferm (1966), Marin-Padilla (1966), Staples and Pecharo (1973), and Gill et al. (1981) all have described abnormalities of exencephaly, microphthalmia, fused ribs, cleft lip, and others, in hamster embryos from maternal animals injected with 2.5 g/kg or more of DMSO on day 8 of gestation. Both Caujolle et al. (1967) and Staples and Pecharo (1973) found abnormal embryos (anencephalia, malformed limbs, and celosomia) in mice injected intraperitoneally with 5 g/kg/day or more of DMSO during the second week of gestation. Juma and Staples (1967) reported that 10.25 g/kg/day of 90% DMSO given to pregnant rats on days 8-10 of gestation increased embryo mortality rates but caused no teratogenic effects; however, Caujolle et al. (1967) observed an increase in developmental abnormalities when the DMSO (5 or 10 g/kg) was administered daily from the 6th through 12th day of gestation. In rabbits daily oral or subcutaneous doses of 4-5 g/kg given on days 6-14 of gestation caused no embryoletality or teratogenicity (Caujolle et al.

1967), and subcutaneous doses of 3 g/kg on days 8-11 increased embryo mortality, but also had no teratogenic effects (Staples and Pecharo 1973).

3.1.5 Other Effects

3.1.5.1 Skin

Studies conducted by several investigators have shown that DMSO is a skin irritant when used in sufficiently high or multiple doses. Kligman (1965b) applied 9 mL of 90% DMSO daily to 20 human volunteers over the entire area of the trunk, and found that during the first few weeks 15 subjects had a mild reaction of transient burning and stinging and 5 had transient erythema. Similar observations have been made by Zuckner et al. (1967) and Steinberg (1967). In Kligman's study, two subjects developed mild scaling diffuse erythematous dermatitis after 2-3 weeks, but this eventually disappeared. When the frequency of exposure was increased to twice daily, most subjects experienced the transient burning and stinging, about half displayed transient erythema, and two showed more severe reactions including extreme dermatitis, abdominal cramps, nausea, chills, and chest pain. Occlusive skin patch tests demonstrated that 90% DMSO would cause a mild scaling dermatitis in 15 min to 1 hr, 50% would result in epidermal alterations in 1 hr, and 38% was the threshold concentration producing local irritation in 24 hr. Biopsy samples from the most severe cases revealed mild perivascular lymphocytic infiltration, moderate acanthosis, absence of a granular layer and a parakeratotic, increased horny layer. Forty-eight-hour patches on sites previously inflamed by 10% sodium lauryl sulfate showed no contact sensitization by DMSO. Intradermal injections of 0.1 mL aqueous DMSO resulted in wheals and flares at the injection site when concentrations were 0.01% and above (Kligman 1965a), (see also Sulzberger et al. 1967). Cutaneous exposures to DMSO can also cause a wheal reaction in humans (Sulzberger et al. 1967; Frosch et al. 1980). Severity of the response was found to be dependent on concentration, exposure time, and frequency of exposure. There was considerable variation in response between individuals. No differences were observed between sexes, but whites were more sensitive than blacks. In tests on different regions of the body, it was found that the forehead was most sensitive to DMSO, followed in decreasing order by upper back, antecubital fossa, mid-ventral forearm, lower leg, and ventral wrist. Any damage to the stratum corneum intensified the wheal response, but prior treatment of the skin with adrenalin minimized the effect (Frosch et al. 1980).

Irritant effects of cutaneous exposures to DMSO disappear when solvent use is discontinued (Kligman 1965b), and the observed reactions become less severe during the course of daily repeated exposures (Kligman 1965b; Scherbel et al. 1967). Such reduced reactivity of the skin has been attributed to depletion of histamine from the mast cells and general hardening of the stratum corneum (Kligman 1965a, 1965b).

Numerous studies have demonstrated that DMSO increases skin permeability in both humans and animals (Sweeney et al. 1966; Baker 1968; Mitryukovskii 1970; Schenplein and Ross 1970; Astley and Levine 1976;

Malten and Den Arend 1978). The degree of change in permeability is a function of DMSO concentration and exposure time, and removal of the solvent results in partial or complete recovery (Kligman 1965b; Astley and Levine 1976). Turco and Canada (1969) reported that 40% DMSO plus 9% NaCl decreased electrical resistance of human skin from 150 kilohms to about 30 kilohms.

3.1.5.2 Lungs

Fishman et al. (1969) have reported that mice inhaling 1600 mg DMSO/m³ (497 ppm) for 4 hr or 2900 mg/m³ (860 ppm) for 24 hr, had edematous changes in their lungs. Daily 7-hr exposures to 200 mg/m³ (5 days per week for 6 weeks) did not cause such an effect. Six months of daily 4-hr exposures to an atmosphere saturated with DMSO resulted in vascular rearrangements, proliferative and dystrophic changes in bronchi and alveolar epithelium, hyperplasia of the septal cells, and lymphoid accumulations (Filippova and Kalimullina 1974). Atelectasis and emphysema have been observed in rhesus monkeys dosed intragastrically with 9 mL/kg of 90% DMSO (Vogin et al. 1970). The lung damage was attributed to regurgitation and/or tracheal inspiration.

3.1.5.3 Eye

At high concentrations, topical application of DMSO causes ocular irritation. In rabbits DMSO produced transient erythema and conjunctivitis (Fishman et al. 1969). In humans two drops of 50% DMSO into the conjunctival sac produced transient burning and stinging, and occasional vasodilation, but no other adverse effects (Kligman 1965b). The inflammatory response of human eyes is aggravated by 90-100% DMSO, but a concentration of 30% has antiinflammatory properties (Hanna et al. 1977). However, human corneal endothelial cells, in vitro, are damaged by concentrations less than 10% when the exposure time is 70 min (Sperling and Larsen 1979).

Several studies have shown that chronic multiple exposures to DMSO, by subcutaneous, cutaneous, oral or intravenous administration, can result in ocular abnormalities in experimental animals. The observed ocular changes involve alterations in the refractive power of the lens, leading to a myopic condition, followed in some cases by opalescence in the nucleus. Lenticular changes have been observed in rabbits after 10-15 days of 5 g/kg/day, dermally or intraperitoneally (Wood et al. 1971), and after 90 days of daily cutaneous doses of 4 mL/kg of 100% DMSO (Rubin and Barnett 1967). Similar changes occur in swine after 90 days of twice daily cutaneous doses of 8.1 mL/kg of 90% DMSO (Rubin and Barnett 1967; Noel et al. 1975), and cataracts have been observed in guinea pigs receiving cutaneous and subcutaneous doses of DMSO three times per week for 3 weeks (Rengstoff et al. 1971). Studies on dogs indicate that the ocular effects of DMSO are dose-related. Lenticular changes are apparent in 7 weeks or less when a daily subcutaneous dose of 11 g/kg is administered (Smith et al. 1969), in 9 weeks during daily oral doses of 5 g/kg, in 13 and 18 weeks during 2.5 g/kg/day orally (Rubin and Barnett 1967), and not until after two years of exposure to 1 mL/kg 5 times per week (Noel et al. 1975).

DMSO-induced ocular changes have not been observed in most studies conducted on primate species. de la Torre et al. (1981) found that 3 g/kg/day of 40% DMSO administered intravenously to rhesus monkeys for 9 days resulted in no ophthalmologic abnormalities during a 4-month post-exposure observation period. Similar negative findings for nonhuman primates were reported by Rubin and Mattis (1966) who used a daily dose level of 5 g/kg for 100 days; by Smith et al. (1969) who used a dose level of 11 g/kg/day for 185-200 days; and by Vogin et al. (1970) who used a twice daily dose level of 4.5 mL/kg (90% DMSO) for 53 weeks when given orally and for 83 weeks when given cutaneously. However, Barnett and Noel (1967) have reported that rhesus monkeys given oral doses of 9 mL/kg 5 times per week had lenticular changes similar to those seen in nonprimate species. Such changes occurred in 2 of 8 monkeys after 9 weeks and in 7 of 8 after 14 weeks. Monkeys receiving 3 mL/kg of DMSO also developed lenticular areas of increased brightness, but only after 15 weeks or more of exposure.

In one human study, 1 g/kg/day of DMSO given cutaneously for 12 weeks did not result in any ophthalmological abnormalities during the treatment period or for up to one year afterward (Hull et al. 1969). In another human study, a 3-month exposure to 1 g/kg/day of 80% DMSO, given cutaneously, did not result in any ophthalmological abnormalities during the treatment period or during a 15-month postexposure observation period (Brobyn 1975).

3.1.5.4 Blood Cells

In vitro and in vivo studies indicate that under certain conditions DMSO can cause hemolytic effects. Willson et al. (1965) observed that rats receiving 8 g DMSO/kg/day intraperitoneally six times per week became anemic with decreased hemoglobin and hematocrit values and some evidence of reticulocytosis. These values returned to normal after a 4-week recovery period. Similar effects, plus a reduced red cell count, and hemoglobinuria and hematuria were seen in dogs receiving daily intravenous doses of 2.4 g/kg/day, six times per week. DMSO-induced hemolysis has also been reported in cats exposed to DMSO by intravenous injection every 15 minutes (DiStefano and Klahn 1965). Wood et al. (1971) have reported a transient rise in serum hemoglobin in rabbits receiving daily 1 g or 5 g DMSO/kg intraperitoneally. Noel et al. (1975) observed elevations in hemoglobin, total red cell count, and packed cell volume in dogs dosed orally with 9 mL DMSO/kg/day 5 times per week for eighteen weeks. However, rhesus monkeys given this same daily dose by oral or dermal administration for 18 months showed no changes in any hematological parameters (Vogin et al. 1970). Similar negative results were reported for rhesus monkeys given daily intravenous injections of 3 g/kg for 9 consecutive days (de la Torre et al. 1981).

In one human study in which 45 volunteers received 1 g DMSO/kg/day cutaneously for 90 days, a transient eosinophilia occurred in 23 subjects and a decreased hemoglobin, hematocrit, and red blood cell count occurred in 2 subjects (Brobyn 1975). In two clinical cases of DMSO-induced toxicity, two intravenous infusions of 100 g of 20% DMSO, 24 hr apart, resulted in decreased hemoglobin concentrations, decreased white

cell counts, and shortened prothrombin time and partial thromboplastin time (Yellowlees et al. 1980).

3.1.5.5 Liver

Histomorphological and biochemical methods have been used to evaluate the potential hepatotoxicity of DMSO. Mathew et al. (1980) injected Wistar rats with single intraperitoneal doses of 3.6 or 4.8 mL DMSO/kg and found that after 12 and 24 hr there was a transient fatty infiltration of liver tissue which was associated with depletion of glycogen stores. No pathological lesions were seen. Centrilobular fatty changes of the liver have also been observed in vervet monkeys injected intraperitoneally with 0.4 mL/kg of DMSO (van der Watt and Purchase 1970). Diffuse hepatocellular fatty changes occurred after injection of 2 mL/kg. Caujolle et al. (1967) saw signs of degenerative modification of the hepatocytes and inflammation and irritation of the portal spaces in rats receiving oral DMSO doses of 5 g/kg for 45 days, and Willson et al. (1965) described cloudy swelling and granularity of the parenchymal cytoplasm in dogs injected intravenously with 0.3-2.4 g DMSO/kg six times per week for a total of 24 doses.

Several researchers have studied the effect of DMSO on liver enzymes. Feuer et al. (1965) reported that relative liver weight and activity of glucose 6-phosphatase and glucose 6-phosphate dehydrogenase were unaltered in rats receiving 11 g/kg/day of DMSO for seven days, and Fishman et al. (1969) found no changes in liver lactate levels or liver alkaline phosphatase activity in rats exposed to atmospheric DMSO levels of 2900 mg/m³ (860 ppm) for 24 hr or 2000 mg/m³ (621 ppm) for 40 hr. Gerhards et al. (1965) reported that the *in vitro* metabolism of corticosterone by rat liver slices was not affected by prior seven day intravenous exposure to 75 mg DMSO/100 g. Dobbs et al. (1980) found that bile secretion and urea synthesis were inhibited in isolated rat liver perfused with a solution of 10% DMSO (1.4 mol/L). During the 30-min exposure, and for 2.5 hr after, there was an increase in perfusate glucose levels. Aspartate aminotransferase synthesis was not altered for six hours, but after this period the rate of enzyme release from the liver increased. Tyson et al. (1980) reported that aspartate aminotransferase was not released from isolated rat hepatocytes treated with 10 mM solution of DMSO. However, in two clinical cases of DMSO toxicity, resulting from two intravenous infusions of 20% DMSO, the patients appeared jaundiced and had elevated bilirubin and aspartate aminotransferase levels in the blood (Yellowlees et al. 1980).

A recent report has indicated that a 1% DMSO solution will reduce myeloid erythropoiesis and induce the proliferation of stromal elements in human embryonic liver cultures (Makartseva 1982).

3.1.5.6 Kidney

The effects of DMSO on kidney function have been evaluated in experimental animals and human subjects. Small and Ide (1976) exposed Sprague-Dawley rats to daily intraperitoneal injections of 2 g/kg and 4 g/kg of 40% DMSO for 28 days and found no change in serum urea and

creatinine concentrations even though the higher dose was lethal to 50% of the test animals. In vitro tests on renal cortical slices taken from the exposed animals showed that DMSO had no adverse effect on the ability of the kidney to transport p-aminohippurate or N-methylnicotinamide. Baxter and Lathe (1971) reported that prior 20 min exposures to 30% DMSO had no effect on respiration of rat kidney slices, but anerobic glycolysis was markedly decreased, due, in part, to activation of fructose diphosphatase.

In tests on rhesus monkeys, Vogin et al. (1970) found no changes in blood urea nitrogen, creatinine clearance, and in urinalysis data taken during and at the end of 18 months of daily exposure to topical or intragastric doses of 1-9 mL/kg of 90% DMSO. Similar results were reported by de la Torre et al. (1981), who exposed rhesus monkeys for 9 consecutive days to daily intravenous doses of 3 g/kg of 40% DMSO. There was, however, a fourfold increase in diuresis in the test animals.

Bennett and Muther (1981) investigated the potential nephrotoxicity of DMSO in patients who had stable spinal cord injuries. The subjects received a daily intravenous injection of 1 g/kg of 10-40% DMSO for 3 consecutive days. There was no change in serum creatinine or in creatinine clearance, urine sediments were normal, and renal excretion of beta-2-microglobulin was within the normal range.

A clinical case of DMSO-induced acute tubular necrosis has been reported on by Yellowlees et al. (1980). In this instance a 77-yr-old woman, who had received DMSO therapy for arthritis 9 months earlier, was undergoing a second treatment of 100 g of 20% DMSO daily by intravenous infusion for three consecutive days. After two infusions she experienced clinical symptoms of cramps, vomiting, and drowsiness, and showed signs of jaundice and flapping tremor. Laboratory tests showed that in addition to decreases in hemoglobin, prothrombin, and partial thromboplastin time, and serum elevations in certain liver and muscle enzymes, there were elevations in blood urea (16.3 mg/dL) and creatinine (221 μ mol/L) indicative of renal tubular damage. A second patient receiving the identical treatment also had elevated urea and creatinine levels, but showed no outward clinical symptoms of toxicity.

3.1.5.7 Muscles

Sams et al. (1966) conducted in vitro studies on the effects of DMSO on isolated innervated guinea pig muscle preparations. At concentrations of 3% and 6% the response of diaphragm muscle to direct and indirect electrical stimulation was reduced significantly. The response of stomach muscle preparations was increased by 0.6-6% DMSO, and that of cardiac atrial preparations was increased at 3% (amplitude of contraction increased) and 6% (both amplitude and rate of contraction increased). Bonnardeaux (1971) demonstrated that the amplitude of spontaneous contractions of rat smooth muscle preparations (uterus, duodenum, and rectum) was reduced by a DMSO concentration of 2.5 μ g/mL, and Jackson et al. (1979) found that DMSO induced relaxation of vascular smooth muscle (rabbit aorta).

In vivo studies on dogs indicate that DMSO, at intravenous dose levels of 1-2 g/kg, will cause a transient increase in cardiac index with increased heart rate, blood pressure and pulse rate (Peterson and Robertson 1967; Hameroff et al. 1981). Lower concentrations had no effect. In vitro studies, however, have given very varying results depending on DMSO concentration, exposure time, temperature, muscle preparation, and species used (Shlafer and Karow 1975).

In one ninety-day clinical study in which 78 volunteers received a daily cutaneous dose of 1 g/kg, some subjects developed a reduced systolic blood pressure (Brobyn 1975). In a clinical case diagnosed as acute DMSO toxicity, which resulted from two 100-g intravenous infusions of 20% DMSO about 24 hr apart, the patient had a pulse rate of 84/min, blood pressure of 140/90 mm Hg, and a normal serial electrocardiogram (Yellowlees et al. 1980).

3.1.5.8 Endocrine Glands

Histomorphology of thyroid parenchyma of mice was not altered by daily oral administration of 1-2 g DMSO/kg for 49 days (Lanza et al. 1970). Fifteen percent DMSO in vitro, and 63% in vivo (0.4 mL, intraperitoneally) inhibited thyroidal uptake of iodine in mice (Hagemann and Evans 1968); however, similar effects were not observed in rats injected intraperitoneally with 63 or 85% DMSO (Goldman 1973).

Allen and Allen (1975) injected rats with 2.0 mL of 25% DMSO (0.55 mg) and found that the DMSO acted indirectly on the adrenal gland to stimulate the release of corticosterone and directly on the pituitary gland to stimulate the release of adrenocorticotrophic hormone. Increases in plasma levels of either hormone were not observed in five human subjects given an oral dose of 8.0 mL of 70% DMSO. Nagasawa (1983) reported that DMSO stimulated the synthesis of mouse pituitary growth hormone and prolactin in vitro but had the opposite effect in vivo.

3.1.5.9 Nervous System

Mice dosed with 0.5 mL of 15% DMSO intraperitoneally and 0.25 mL intravenously showed no gross or microscopic changes in brain parenchyma, but when the intraperitoneal dose was increased to 0.5-1.0 mL of 20-30% DMSO, anterior pituitary and superficial cortical hemorrhages and poorly preserved cell and organelle membranes were observed (Broadwell et al. 1982). DMSO concentrations of less than 1% inhibit acetylcholinesterase activity, but concentrations above 10% block cholinergic transmission entirely (Sawada and Sato 1975). One hundred percent DMSO failed to produce nerve block in frog sciatic nerve-gastric muscle preparation, it had no local corneal anesthetic effect in rabbits, and in mice the median effective dose for analgesia was 6.7 g/kg (Morris 1966). In rats 5.5 g/kg administered intravenously or intraperitoneally produced significant analgesia which lasted for 6-7 hr (Haigler and Spring 1981).

3.1.5.10 Physiological Effects

A DMSO concentration of 1.10 M decreased insulin-stimulated glucose oxidation and increased lipolysis by fat cells of rat adipose tissue (Wieser et al. 1977). The increase in lipolysis was attributed to an increase in cyclic AMP levels (Wieser 1983).

Kocsis et al. (1975) reported that rats exposed to DMSO exhibited a lowered body temperature, and mice showed a reduction in motor activity.

Gerhards et al. (1965) found no change in the in vitro oxygen consumption of liver, brain, and hemidiaphragm tissue of rats given an intravenous dose of 75 mg DMSO/100 g. Urease, trypsin, and chymotrypsin activities were inhibited by DMSO, but glucose-6-phosphate dehydrogenase activity was not (Gerhards et al. 1967).

In vitro studies by Gerhards et al. (1967) revealed that protein synthesis in rat liver supernatant extract was stimulated by 5-9% DMSO, inhibited by 10% DMSO and completely blocked by 30% DMSO. Fleming (1977) reported that 10% DMSO inhibited incorporation of amino acids into protein in vitro in mice brain supernatant, but 1% DMSO had no effect; however, mice given 5% DMSO at a rate of 50 g DMSO/kg/day for up to 6 months showed increased incorporation of amino acid into protein in brain, liver, and kidney.

Altland et al. (1966) administered 4.5 mg/kg of DMSO intraperitoneally to rats and observed slightly increased serum transaminase levels within 24 hr. Combined with 5 hours of exercise, DMSO produced elevations in serum transaminases, serum dehydrogenases, and urea nitrogen, significantly higher than those caused by exercise alone. Transient increases in serum levels of enzymes such as glutamic oxaloacetic transaminase, lactic dehydrogenase, and creatinine phosphokinase, have been reported in human subjects exposed to DMSO (Brobyn 1975; Yellowlees et al. 1980; Fowler 1981).

Paine et al. (1970) reported that DMSO caused a shift of water from the extracellular to the intracellular fluid in the rabbit, and Vlasova (1974) observed a generally increased permeability of the blood-tissue barrier in the heart, lungs, kidneys, and spleen, but not the liver.

3.2 DISCUSSION AND CONCLUSIONS

Laboratory studies have shown that, regardless of species tested or route of administration, DMSO has a relatively low level of acute lethal toxicity. For single exposures, the median lethal dose ranges from about 2 g/kg to about 50 g/kg (see Table B-1). The most toxic exposure route is via intravenous injections, which result in LD₅₀ values of less than 10 g/kg. Oral, intraperitoneal, and subcutaneous exposures follow, with LD₅₀ values between 10 and 30 g/kg. Cutaneous exposures are the least toxic, with median lethal levels as high as 40-50 g/kg (Mason 1971). Regardless of exposure route, the toxicity of DMSO is highest when the concentration used is at or near 100% (Worthley and Schott 1969; Mason 1971). Toxicity appears to increase only slightly when the solvent is

administered in multiple doses one or more days apart (Smith et al. 1967). This may be due to the rapid metabolism and excretion of DMSO, combined with a nonspecific and transitory binding with body tissues without any long-term bioaccumulation in a specific organ system. Multiple exposures to DMSO on a daily basis do result in an increase in DMSO serum levels, but in one human study it was shown that for oral doses of 0.5 g/kg/day, given for 14 days, serum levels reached a maximum value after 9 days and then began to decline (Hucker et al. 1967). Thus, it may be that only when exposures are close to being acutely toxic will multiple dosing have a significant cumulative effect, but this effect may still be transitory. Further study on this topic is warranted.

Extrapolation from subacute and clinical studies on humans and acute bioassays on primates, suggests that the lethal toxicity of DMSO to humans is probably similar to that observed in animal studies. An intravenous dose of 1 g/kg of 20-40% DMSO given for 3 consecutive days caused only a mild toxic reaction in humans (Bennett and Muther 1981), but 1 of 2 patients receiving 100 g/day (1.4 g/kg) intravenously for 2 consecutive days developed what appeared to be an acute reaction (Yellowlees et al. 1980). No toxic effects were reported in humans receiving an oral dose of 0.5 g/kg/day for 14 days (Hucker et al. 1967). In contrast, 8.9 g/kg/day, intragastrically, resulted in 83% mortality in rhesus monkeys (Vogin et al. 1970). Humans exposed cutaneously to 9 mL of 90% DMSO daily for 26 weeks showed no ill effects except transient erythema, but when the dose was doubled (two applications per day), 2 of 20 subjects developed a severe toxic reaction after 12-13 days (Kligman 1965b). In another clinical study a daily dermal dose of 1 g/kg of 80% DMSO for 90 days resulted in no significant toxic reactions in any of 78 volunteers (Brobyn 1975). These data indicate that, for humans, toxic levels of DMSO are above 1 g/kg/day, although they may be only slightly above in susceptible individuals receiving intravenous doses. Cutaneous doses appear to have a low level of toxicity, but this may be increased considerably when exposures occur more frequently than once per day.

Standard bioassays have indicated that DMSO is not mutagenic in bacteria (Simmon et al. 1977; De Flora 1981), or in one species of yeast (Abbondandolo et al. 1980); that it does not induce chromosomal damage in cell cultures from fish (Kocan et al. 1982) or chickens (Bloom 1982); and that it does not induce dominant lethality in mammals (Aravindakshan et al. 1975; Sheu and Green 1979). However, other studies have shown that DMSO can increase gene conversions in log phase yeast cells (Callen and Philpot 1977), and can increase chromosome aberrations in vitro and in vivo in mammals (Kapp and Eventoff 1980; Tates and Kriek 1981). The reported occurrence of chromosomal abnormalities in the absence of experimental evidence for enhanced mutagenicity might be explained by a decrease in the viability of the affected cells. In both chromosomal studies there was an increase in the number of aberrations per cell, but not an increase in the number of abnormal cells (except at 100% DMSO). Unless these data can be correlated with adverse effects on reproduction or with carcinogenicity, they may not be a cause for concern. At present, all available evidence indicates that DMSO is not carcinogenic (see Section 3.1.3), and there is some experimental data showing that DMSO can actually induce reversions in vitro in cultures of neoplastic cells (Vyadro et al. 1981).

Substantial data have been generated which show that DMSO is teratogenic in some species of animals (Ferm 1966; Caujolle et al. 1967; Staples and Pecharo 1973; Gill et al. 1981). The severity of the teratogenic effects varies with DMSO dose and concentration, exposure route and time of exposure relative to gestation period, and with the species tested. Hamsters are especially sensitive to DMSO, with the embryos exhibiting exencephaly, microphthalmia, and other abnormalities. In contrast, DMSO is not teratogenic in rabbits even at dose levels that are lethal to the embryos (Caujolle et al. 1967; Staples and Pecharo 1973). These data indicate a definite potential for DMSO to be tetraogenic in humans. Until more information is obtained, particularly from nonhuman primate studies, the solvent must be considered a possible teratogen and dealt with accordingly.

DMSO is a primary skin irritant when used in high concentrations and/or multiple doses (Kligman 1965b). The most severe reactions, resulting from exposures to DMSO concentrations of 90-100%, are typified by erythematous scaling dermatitis, and wheals and flares, and may be accompanied by signs of systemic toxicity (muscle cramps, nausea, chills, and chest pain). Such a reaction was observed at a dose level of 9 mL of 90% DMSO twice daily for 12 days. Mild attacks of erythematous dermatitis often disappear during the course of multiple exposures, due to histamine depletion and hardening of the stratum corneum. In the case of an acute exposure, the skin slowly returns to a normal state after the solvent is removed. There is no evidence that the damage from chronic or acute exposures results in long-term adverse effects.

The fine epithelial tissue of the lungs is also susceptible to damage by DMSO. A 4-hr exposure to 1600 mg/m³ caused edematous changes (Fishman et al. 1969), and six months of daily 4-hr exposures to an atmosphere saturated with DMSO caused substantial histological alterations in the lungs of mice (Filippova and Kalimullina 1974). Further studies are needed to determine minimum exposure levels causing such effects, to determine the long-term health implications, and to correlate these with actual workplace exposure levels.

Chronic exposures to DMSO have resulted in ocular abnormalities in a variety of experimental animals including rabbits, swine, guinea pigs, dogs, and monkeys (see Section 3.1.5.3). The rapidity in which the condition appears is a function of dose and length of exposure period and appears to be independent of the route of exposure. Lenticular changes occurred in dogs after only 7 weeks of subcutaneous doses of 11 g/kg/day (Smith et al. 1969), but took two years to develop in dogs exposed to only 1 mL/kg five times per week (Noel et al. 1975).

For nonhuman primates there is conflicting data as to whether DMSO produces the ocular changes seen in other animals. In two studies, doses as high as 9 mL/kg/day, orally, for 53 weeks, or 11 g/kg/day, dermally, for 185-200 days caused no ocular effects (Smith et al. 1969; Vugin et al. 1970), but in another study, 9 mL/kg/day, orally, five times per week did produce the typical lenticular changes seen in other animals (Barnett and Noel 1967).

There is no clinical or experimental data to indicate whether exposure to DMSO would cause ocular effects in humans. Exposures to low doses (1 g/kg/day) for three months or less have not resulted in lenticular changes (Hull et al. 1969; Brobyn 1975); however, it cannot be assumed from these studies that higher doses, or chronic low-level exposures over a period of years, such as might occur in a workplace environment, would not result in such changes.

Laboratory and clinical studies have shown that exposure to DMSO can produce hematological abnormalities. Decreased hemoglobin and hematocrit values, reticulocytosis, decreased blood cell counts, hemoglobinuria, and hematuria are some of the conditions observed in DMSO-dosed laboratory animals (DiStefano and Klahn 1965; Willson et al. 1965; Mason 1971) and in humans (Brobyn 1975; Yellowlees et al. 1980). Hemolytic effects of DMSO, most often seen in cases where the solvent is used at a concentration close to or at 100%, have been attributed to the osmotically induced cellular damage caused by such high concentrations. Hemolytic effects might also occur during chronic exposures. Two of 45 human volunteers developed such a condition during a 90-day exposure to 1 g DMSO/kg/day (Brobyn 1975). Hematological studies may be useful in monitoring potential worker exposure to DMSO.

Although DMSO has been shown in many studies to have no hepatotoxic effects, particularly at low dose levels, some data suggest it might be hepatotoxic at high doses. Histomorphological changes have been seen in liver tissue from animals dosed with DMSO, and these changes included fatty infiltrates (van der Watt and Purchase 1970; Mathew et al. 1980), cloudy swelling and granularity of parenchymal cytoplasm (Willson et al. 1965) and, in one case, necrosis of hepatocytes and inflammation of liver tissue (Caujolle et al. 1967). Some liver enzymes, such as glucose-6-phosphatase and glucose-6-phosphate dehydrogenase, are unaffected by DMSO (Feuer et al. 1965). Serum aspartate transferase levels, although generally unaffected by low doses of DMSO (Fishman et al. 1969; de la Torre et al. 1981), are sometimes elevated following high doses (Willson et al. 1965). Release of aspartate aminotransferase and increases in serum bilirubin levels have been observed in one clinical case, suggesting that some liver functions were inhibited by DMSO (Yellowlees et al. 1980).

Although a strong diuretic, DMSO has been found, by analysis of serum urea and creatinine levels, to have no nephrotoxic effect in experimental animals, even when doses were at lethal levels (Vogin et al. 1970; Small and Ide 1976; de la Torre et al. 1981). Similar results were obtained in one clinical study in which subjects received 1 g/kg/day for three consecutive days (Bennett and Muther 1981); however, in a second clinical study, intravenous doses of 1.4 g/kg/day for two days resulted in elevated blood urea and creatinine indicative of renal tubular damage (Yellowlees et al. 1980). Under these conditions tubular nephrosis has been attributed to the breakdown products of DMSO-induced hemolysis (Mason 1971).

In vitro studies on muscle-nerve preparations have indicated that DMSO can have direct and indirect effects on muscle activity (Sams et al. 1966; Bonnardeaux 1971; Jackson et al. 1979). The response of skeletal muscle to electrical stimulation is reduced, and that of smooth and cardiac muscle is increased. The spontaneous contractibility of smooth muscle is reduced, resulting in vasodilation. Cardiac contractile rate in vitro is reduced but contractile strength can be increased or decreased depending on concentration. Low concentrations generally cause an increase while high concentrations cause a decrease (Shlafer and Karow 1975). The indirect effects of DMSO on muscle tissue has been partially attributed to the inhibition of acetylcholinesterase activity (Sams et al. 1966). DMSO concentrations below 1% inhibit such activity but those above 10% block cholinergic transmission entirely (Sawada and Sato 1975). In laboratory animals DMSO can produce analgesia at dose levels above 5 g/kg (Morris 1966; Haigler and Spring 1981). There is little evidence that DMSO causes any direct toxic effect on the nervous system; however, in one study conducted on mice, cortical hemorrhages and poorly preserved cell and organelle membranes were seen in brain tissue following an intraperitoneal dose of 0.5-1.0 mL of 20-30% DMSO (Broadwell et al. 1982).

Although it has been reported that DMSO alters thyroid activity in mice by inhibiting iodide transport and organification (Hagemann and Evans 1968), such effects were not seen in a later study on rats (Goldman 1973), and there are no histomorphological data to indicate that DMSO is toxic to the thyroid (Lanza et al. 1970).

One laboratory study on rats found that DMSO exposure resulted in a release of corticosterone and adrenocorticotropic hormones, but, in humans, plasma levels of these hormones were not increased by an oral dose of 8.0 mL of 70% DMSO (Allen and Allen 1975).

Metabolic rates appear to be unaffected by DMSO (Gerhards et al. 1965), but insulin-stimulated glucose oxidation may be inhibited and lipolysis may be enhanced (Weiser et al. 1977). Protein synthesis may be stimulated or inhibited depending on concentration (Gerhard et al. 1967; Fleming 1977), and activity of certain enzymes, such as urease, trypsin, and chymotrypsin, may be inhibited while that of others, such as glucose-6-phosphate dehydrogenase, is not (Gerhards et al. 1967). Blood levels of some tissue enzymes increase after exposure to DMSO, but this may be partially accounted for by a generalized increase in membrane permeability (Altland et al. 1966). Increased permeability of the blood tissue barrier has been observed for the heart, lungs, kidney and spleen, but not for the liver (Vlasova 1974).

In conclusion, it can be stated that the major toxic effects of DMSO, as reported in studies on laboratory animals, include damage to the skin and lungs, lenticular changes in the eye, teratogenicity, hemolysis, and hepatotoxicity. It should be noted that large doses of highly concentrated solutions, and/or multiple doses are usually required to produce such effects. In humans, DMSO can produce scaling erythematous dermatitis and hemolysis. At acutely toxic dose levels, DMSO may be hepatotoxic and nephrotoxic, as suggested by a clinical case in which

abnormal increases in serum levels of aspartate transferase, bilirubin, creatinine, and urea were seen following two intravenous doses of DMSO. Although no evidence exists to show that DMSO causes ocular changes or is teratogenic in humans, there is insufficient data to conclude that such effects would not occur under all exposure conditions.

4 INTERACTIVE EFFECTS OF DMSO WITH OTHER CHEMICALS

4.1 SUMMARY OF INTERACTIONS

4.1.1 Nonpercutaneous Administration

4.1.1.1 Hepatotoxins

There are several reports of the effects of DMSO on a variety of aromatic hydrocarbons that cause liver damage, signs of which can be evaluated by histologic, enzymatic, urine metabolite excretion, or barbiturate sleeping time methods. Studies from two laboratories showed that DMSO enhanced the lethal potency of carbon tetrachloride (CCl₄) as reflected in the intraperitoneal LD₅₀ for rodents (Freston and Bouchier 1967; Kocsis et al. 1968a; Mancini and Kocsis 1974). Although Freston and Bouchier (1967) found histologic and serum enzyme evidence for a concomitant increase in liver injury, Mancini and Kocsis (1974) reported that DMSO reduced CCl₄-induced hepatotoxicity and that DMSO did not alter the mechanism by which CCl₄ produced its lethal effect. The lack of increased CCl₄-induced hepatotoxicity by DMSO was confirmed by Siegers (1978).

The effect of DMSO on the toxicity of other hydrocarbons in rodents has also been studied. DMSO reduced the oral toxicity of paracetamol, bromobenzene, and thioacetamide (Siegers 1978) and the intraperitoneal toxicity of dehydroheliotridine (Peterson et al. 1972) and DDT (Lewin et al. 1972), but increased the toxicity of benzene, chlorobenzene, and toluene (Kocsis et al. 1968a, 1975) and of the PCB, Arochlor 1254 (Lewin et al. 1972). Unlike the effect of DMSO on CCl₄ toxicity, the mode of toxic action of benzene or toluene can be modified by DMSO (Kocsis et al. 1975).

It was reported that the toxicity of methylchloroform in mice was increased by DMSO (Shah and Lal 1976) as was the toxicity of an iron complex of octamethylpyrophosphoramide (OMPA) (Joesten and Hill 1966). On the other hand, the toxicity of OMPA alone or complexed with sodium, magnesium, cobalt, manganese, or zinc was not appreciably altered by DMSO (Joesten and Hill 1966); in one study, OMPA toxicity was reduced by DMSO (Kocsis and Harkaway 1967).

In a series of in vitro experiments on the influence of different solvents on drug metabolism in rat liver of five toxic substances, the presence of DMSO in the assay mixtures did not have a singular effect compared to the other eight vehicles examined (Kawalek and Andrews 1980). In rat liver, metabolism of aniline and phenacetin was increased, while metabolism of ethylmorphine and benzphetamine was unchanged by DMSO (Kitda et al. 1978).

The serum levels of the herbicide 2,4,5-trichlorophenoxy acetic acid administered to rats were altered by pretreatment with DMSO, and the metabolism of 2,4-dichlorophenoxy acetic acid was slightly stimulated by DMSO (Courtney 1970).

4.1.1.2 Anticholinesterases

Several investigations have been made into the effect of DMSO on the toxicity of anticholinesterases. The oral toxicity of carbaryl in rats was not significantly affected by DMSO (Weiss and Orzel 1967) despite the observation that the relative absorption rate of this pesticide from the duodenum was high (Cambon et al. 1981). The toxicity of two other anticholinesterases, paraoxon and tetraethyl pyrophosphate, as well as OMPA was antagonized by DMSO (Kocsis et al. 1975). Pesticides other than carbaryl have been examined, i.e., thiram, dieldrin, 4-benzothrenyl N-methyl carbonate, and parathion, and their oral toxicities were not found to be modified significantly by DMSO (Weiss and Orzel 1967).

4.1.1.3 Drugs

A variety of drugs have been tested in DMSO in view of the ability of this vehicle to modify penetration characteristics of cell membranes. Presumably, an alteration in membrane penetration of a drug could qualitatively and quantitatively affect responses of a cell to the drug.

DMSO did not alter the intraperitoneal lethality in mice of seven drugs commonly used clinically - chlorpromazine, curare, insulin, morphine, ouabain, penicillin, pentobarbital (Dixon et al. 1965). Other LD₅₀ studies with mice showed that the toxicity of tetracycline, tula-zid, novocainamide, and arecoline was decreased by DMSO; however, the solvent increased the acute toxicity of norsulfazole, streptocide, theophylline, and oxytetracycline (Berezovskaya and Rudzit 1976).

Ten quarternary ammonium salts comprising two representatives each of ganglionic blocking agents, muscle relaxants, antispasmodics, parasympathomimetics, and cationic germicides were tested in rodents to determine the effect of DMSO as a vehicle for these substances. Oral LD₅₀ data showed that in most cases DMSO increased the toxicity of these substances, but there were no instances in which this vehicle reduced toxicity (Rosen et al. 1965). Although the cardiovascular effects of the cardiac glycosides ouabain and proscillaridin were somewhat amplified in cats by DMSO, these effects were transient, and none appeared to be life threatening (Melville et al. 1968). There is evidence that DMSO facilitated transport of drugs like L-dopa and toxogonin across the blood brain barrier (Rump et al. 1969; de la Torre 1970).

In studies by Mallach (1967, 1971), it appeared that the lethal effect of ethanol given orally to mice was increased by DMSO especially if given before or after ethanol administration. In humans it was found that DMSO applied percutaneously affected the blood levels of ingested ethanol, the exhaled level of dimethylsulfone, nerve conduction rate, and mental performance (Mallach 1967, 1971).

4.1.1.4 Antineoplastics

The therapeutic efficacy of the alkylating agent cyclophosphamide (CYC) was found to be enhanced by DMSO administered to humans with

malignant neoplasms (Garrido and Lagos 1975) and to rats bearing a transplantable mononuclear cell leukemia (Warren et al. 1975). In the latter study it was shown that amplification of the effect by DMSO was not associated with an increase in CYC activation by liver microsomes. Results of these two studies indicate that DMSO rendered the organism more sensitive to the antiproliferative effect of CYC, but results from other laboratories showed that DMSO was not effective in augmenting the antitumor property of CYC in patients with lung carcinoma (Fuks et al. 1981). Negative results were also reported for DMSO used in conjunction with other antineoplastic agents (ifosfamide and daunomycin) in rodents (Von Ardenne and Reitnauer 1975; Marian and Mathkovich 1982).

4.1.1.5 Carcinogens

A few studies have demonstrated that DMSO can affect certain characteristics of the actions of known potent carcinogens administered orally or intraperitoneally. In the presence of DMSO, the pattern of 3-methylcholanthrene or benzo(a)pyrene binding to subcellular components of rat liver homogenates was altered such that a greater fraction was bound to microsomes (Levine 1972, 1975). Other studies at the cellular level showed that 3-methylcholanthrene or Clophen A-50 used as inducers of drug-metabolizing enzymes were more potent in DMSO than in olive oil (Hietanen et al. 1980). DMSO did not enhance metabolism or adduct binding to DNA of dimethylbenzanthracene in primary cultures of epidermal cells, but it prevented reduction in metabolism and binding of this carcinogen during an extended culture period (Yuspa et al. 1976).

On the other hand, the acute toxic effects of 7,12-dimethylbenzanthracene in rodents were not altered by the use of DMSO as a vehicle (Schmid et al. 1967; Somogyi and Kovacs 1970). Likewise, DMSO did not significantly affect the influence of diethylnitrosamine, acetylaminofluorene, benzo(a)pyrene, or methylcholanthrene on mitosis of the rat adrenal cortex (Amlacher et al. 1974), a presumptive test for carcinogenesis. However, the mitotic effect of trinitroso-trimethylene-triamine was enhanced by DMSO (Amlacher et al. 1974), and there is marginal evidence for increased tumorigenicity of this chemical when administered with DMSO (Urban et al. 1975). The urinary excretion of the hepatocarcinogen N,N-diethyl-4-aminobenzene or its methyl derivative was delayed and diminished in rats when given orally with DMSO (Danz et al. 1978). The potential tumorigenicity of methyl and methane sulfonyl radicals resulting from the interaction of DMSO with hydrogen peroxide in the presence of a metal salt of titanium was not realized in experiments with rats (Lohs et al. 1971).

4.1.1.6 Viral Oncogens

In experiments with mice and quail, injections of DMSO before infection with sarcoma virus decreased incubation time for tumor appearance, increased viral titers, and decreased spontaneous regression of tumors (Warren et al. 1973). Stewart et al. (1971) reported that in the presence of DMSO and 5-iododeoxyuridine, virus production in a human tumor cell line was greatly increased and that this combination activated a human virus. Enhancement of viral transformation and

infectivity by DMSO have also been reported for the polyoma virus (Kisch 1969). In these and many studies with DMSO and viruses, it is not clear whether the effect is on the cell or on the virus.

4.1.1.7 Teratogens

Several studies have been conducted on the effect of recognized teratogens on embryonic and fetal abnormalities in rodents. The teratogenesis of a single injection of the antimalarial pyrimethamine or the antitumor agent 6-mercaptopurine to pregnant rats on day 13 of gestation was reduced by pretreatment with DMSO (Barilyak et al. 1978). But in another study, DMSO appeared to have little modifying effect on the teratogenic action of pyrimethamine (Anderson and Morse 1966). The incidence of fetal malformations resulting from an injection of the tranquilizer diazepam into pregnant rats on day 8 of gestation was increased considerably by DMSO (Gill et al. 1981). Multiple injections of the insecticide dieldrin (days 6-14) into pregnant mice resulted in maternal and fetal toxicity that was increased when DMSO was used as a vehicle (Dix et al. 1977). Embryonic and fetal toxicity induced by the fungal metabolite secalonic acid, however, was reduced somewhat by DMSO (Reddy et al. 1981). Fetal mortality and abnormalities in hamsters given the insecticide thiram or disulfiram orally on day 7 or 8 of gestation were increased by DMSO (Robens 1969).

Seven diverse substances were tested in chicken embryos to ascertain what effects DMSO might have on developmental abnormalities and on mortality (Landauer and Salam 1972). The chemicals, tested on 4-day-old embryos, were 3-acetylpyridine, 6-aminonicotinamide, Bidrin, sulfanilamide, 3-amino-1,2,4-triazole, physostigmine, and nicotine. DMSO either protected against or had no effect on mortality of embryos, and the teratogenic effects of each substance were increased (sulfanilamide), decreased (3-acetylpyridine, 6-aminonicotinamide, 3-amino-1,2,4-triazole), or unchanged (physostigmine and nicotine) by DMSO.

Clearly, no generalizations of any kind can be drawn about the influence of DMSO on the action of teratogens because of the differences in experimental procedures and the types of abnormalities scored.

4.1.1.8 Mutagens

Mammalian Studies. Limited information is available concerning the influence of DMSO on known mutagens in laboratory animals. There is evidence that DMSO protected rats against chemically induced chromosome abnormalities (Barilyak et al. 1978). Rats were given DMSO 30 min before an injection of the antimalarial pyrimethamine or the antineoplastic 6-mercaptopurine. Two days later the bone marrow was examined, and it was found that DMSO protected against chromosome aberrations induced by these mutagens. Other studies with rats suggested that pretreatment with DMSO protected against hepatic DNA strand breaks induced by the hepatocarcinogen dimethylnitrosamine but not by the carcinogen nitrosourea (Sosnowski et al. 1976). It is of interest that the former requires metabolic activation to induce carcinogenicity while the latter does not. Thus, DMSO may have inhibited activation of the amine. In the

presence of DMSO, cytostatic agents such as colchicine were more potent in causing chromosome aberrations and giant cell formations in epidermal cells of the mouse (Stjernvall 1969).

Nonmammalian Studies. Difficulties in finding solvents that are not toxic for use in bacterial mutagen assay systems have led several investigators to test DMSO as an appropriate solvent for their assays. Because of its solvent properties, its relatively low toxicity to bacteria, and its nonreactivity with microsomal enzymes, DMSO has been found to be most useful. Nonetheless, some problems with its use in the bacterial assay system have led to conflicting results (Maron et al. 1981). An early report indicated that 2.5% DMSO had no effect on survival or on mutation frequency in E. coli exposed to N-nitroso-N-methylurea (Vasil'eva 1975). However, the mutagenicity of two other agents, 2-amino-anthracene and benzo(a)pyrene was enhanced in S. typhimurium exposed to a low concentration of DMSO (Hermann et al. 1978; Anderson and McGregor 1980), while higher concentrations of DMSO (14%) inhibited the mutagenic effect of N,N-dimethylnitrosamine in this organism (Yahagi et al. 1977). These kinds of contrasting results may be related to the length of time and conditions under which the mixture of the mutagen and DMSO has been standing. A fresh solution of DMSO and p-phenylenediamine was not mutagenic in S. typhimurium but became active on standing for 4 hours (Burnett et al. 1982). DMSO mixtures of dimethylcarbaryl or diethylcarbaryl, however, lost their mutagenic activity upon standing (Hermann et al. 1978). The influence of standing may involve a reaction between DMSO and the chemical, as in the case of the mutagen hexachloroacetone, which reacts with water in DMSO solutions to form the non-mutagen hexachloroacetone hydrate (Zochlinski and Mower 1981).

The mutagenic activity of N-nitroso-N-methylbiuret in Penicillium chrysogenum was increased by DMSO (Zakhorova et al. 1974).

In Drosophila melanogaster, the frequency of sex-linked lethal mutations induced by ethyl methane sulfonate was increased by DMSO (Sharma et al. 1973).

There is evidence both for (Bhatia 1967; Sharma et al. 1973; Singh et al. 1979) and against (Khalatkar 1976) enhancement of mutation frequency of mutagens by DMSO in green plants.

4.1.1.9 Miscellaneous

The effect of DMSO on the response to a wide variety of agents was identified. The anaphylactic reactions of rats to dextran was inhibited by administration of DMSO daily for 11 days (Rodriguez et al. 1966). Orally administered DMSO enhanced the immunosuppressive effect of imuran in mice (Aronov and Radionar 1979), and the potency of Salmonella typhimurium injection in mice was increased by administration of DMSO (Klein and Kunze 1971).

DMSO enhanced the toxicity of snake venom (Tiru-Chelvam 1974), accelerated the onset of effects of alpha-glucochloralose (Braude and

Monroe 1965), increased the radioprotective effect of mercaptoethylamine (Roerig et al. 1973), cysteamine, and S,2-aminoethylisothiourea (Ashwood-Smith 1962), and enhanced the toxicity of mercaptoethylamine (Roerig et al. 1973).

Modifications of responses to hormones by DMSO in rats have been reported. DMSO provided some protection against epinephrine-induced tissue damage (Highman and Altland 1969), and, compared to the vehicle propylene glycol, DMSO enhanced toxicity of diethylstilbestrol (Klaassen 1973).

A potentially practical use of DMSO involves tissue oxygenation by hydrogen peroxide. This chemical in a perfusate can be used as a source of oxygen in maintaining or resuscitating the anoxic heart, and the addition of DMSO to the perfusate may enhance oxygenation by the peroxide (Finney et al. 1967).

Pretreatment of rabbits with DMSO intensified the febrile-inducing response to the exogenous pyrogens sodium nucleate or purified bacterial lipopolysaccharide; however, pretreatment did not affect the response to endogenous pyrogen (van Miert and van Duin 1976).

The in vitro hemolysis of rabbit erythrocytes by the antibacterial agent chlorhexadine diacetate was reduced almost to zero in the presence of 15% DMSO (Ansel 1967). In a related type of study, DMSO was found to enhance the ability of carbamyl phosphate to inhibit sickling of human erythrocytes in vitro (Smith and Allen 1975). Damage to mitochondrial and lysosomal membranes by butylated hydroxyanisole - an antioxidant used in food - was increased in the presence of DMSO (Sgaragli et al. 1975).

The efficiency of fusion of human diploid cells by polyethylene glycol was greatly enhanced by addition of DMSO to the cultures (Norwood et al. 1976).

Extensive studies in mice have shown that the addition of DMSO to vaccines did not improve the effectiveness of bacterial or viral vaccinations by oral, nasal, or rectal routes (Raettig 1971).

4.1.2 Percutaneous Administration

4.1.2.1 Carcinogens

Several aspects of the effects of DMSO on tumor induction by potent skin carcinogens in rodents have been reported. Repeated applications of dimethylbenzanthracene (DMBA) in DMSO to the hamster cheek pouch reduced the mean latent period for tumor appearance (Dachi et al. 1967; Elzay 1967; Lalonde 1969); and evidence for altered tumorigenesis was presented (Elzay 1967; Shklar et al. 1969; Siegel and Shklar 1969). In the rat, however, DMSO as a vehicle, resulted in a decrease in DMBA-induced skin tumors (Stenback 1970). For two-stage skin carcinogenesis in mice treated with DMBA and a promoter, repeated applications of 10% DMSO in acetone during the promotion stage resulted in a significant

inhibition of tumors (Belman and Troll 1974), but with neat DMSO, there was no effect on tumor parameters (Hozumi et al. 1972). Likewise, neat DMSO failed to affect tumorigenesis when applied to mouse skin during the initiation stage with DMBA (Stenback and Garcia 1975). Somewhat different results were obtained in experiments with repeated applications of another potent carcinogen, benzo(a)pyrene, on mouse skin, the results of which showed that DMSO as a vehicle reduced the total number of skin tumors but tumor incidence was not altered (Stenback and Garcia 1975).

With five applications of 2-methylcholanthrene (MC) to hairless mice, DMSO as a vehicle increased mortality and had an inhibitory effect on MC-induced skin carcinogenesis (Iversen et al. 1981). More frequent applications of MC with DMSO may have somewhat shortened the latent period of tumor development (Finogenova 1974).

4.1.2.2 Steroids

An important therapeutic approach to the management of human skin lesions has involved the carrier penetrant quality of DMSO as a means of increasing percutaneous absorption of steroids. Studies from several laboratories have shown that in humans DMSO enhanced the percutaneous penetration of testosterone and hydrocortisone (Stoughton and Fritsch 1964; Kligman 1965a; Munro and Stoughton 1965; Feldman and Maibach 1966; Maibach and Feldmann 1967; Feldmann and Maibach 1968; Munro 1969). DMSO also increased penetration of topically applied prednisolone, estradiol, triamcinolone acetonide, and fluocinolone acetonide (Stoughton and Fritsch 1964; Kligman 1965a; Lafille and Sagon 1969; Munro 1969).

Experiments have been conducted on the effect of DMSO on steroid absorption through surface epithelium. Unlike the enhanced penetration of fluocinolone acetonide by DMSO through human skin (Stoughton 1965), the effect of this steroid applied directly to the eye was not altered by the presence of DMSO (Wood et al. 1967). In rats, topical application of estradiol or cortisone in DMSO resulted in biological changes, viz. organ weights, comparable in magnitude to those achieved with subcutaneous administration of these steroids in an aqueous vehicle (Djan and Gunberg 1967). No change in blood level kinetics of corticotropin resulted from the use of DMSO as a vehicle for this steroid applied to rat skin (Kastin et al. 1966).

Preliminary results from patients with cervical carcinoma or dysplastic lesions indicated that application of 0.01% dexamethasone in a DMSO gel brought about regressive cytologic changes in these lesions that progressed to a more normal cellular architecture (Ayre and LeGuerrier 1967). These normalizing changes were not observed with DMSO alone. In the same study, it was also reported that application of a DMSO-barium chloride mixture to the cervix led to reversible epithelial cell dysplasia not unlike early changes indicative of preneoplasia.

4.1.2.3 Salicylic Acid

Principally because of the antiinflammatory properties of salicylic acid, there has been some interest in the use of DMSO as a penetrant

carrier vehicle for the topical application of this drug. Investigators from two laboratories have shown that DMSO enhanced the penetration of salicylic acid through rabbit skin as judged by appearance of salicylate in the blood (Stelzer et al. 1968; Marcus et al. 1970). Results obtained from an in vitro method, in which the penetration of substances through a silicone rubber membrane were measured, indicated that enhanced penetration of salicylic acid by DMSO may be due to facilitated release of the drug (Nakano and Patel 1970). In another study, DMSO enhanced penetration of sodium salicylate through the urinary bladder of the dog (Jacob et al. 1964). In a rat model designed to evaluate the antiinflammatory and antiarthritic potential of topically applied substances, vehicle mixtures containing DMSO appeared to best augment the therapeutic efficacy of copper salicylates (Beveridge et al. 1982).

4.1.2.4 Cytotoxic Agents

The influence of DMSO on percutaneous penetration of the antineoplastic drug, methotrexate, through human skin in vitro has been evaluated. Penetration of this folic acid antagonist was not altered when applied together with DMSO (McCullough et al. 1976). Preliminary findings with the clinical use of 5-fluorouracil, podophyllin, or 5-iododeoxyuridine suggest that DMSO may be useful in increasing the effectiveness of these drugs in the treatment of skin dyscrasias (Goldman et al. 1967). Enhanced penetration of these cytotoxic drugs may well account for their increased effectiveness.

4.1.2.5 Dyes

Because of the ease of following the movement of a colored or fluorescent substance, the influence of DMSO on the percutaneous penetration of several dyes has been examined. DMSO was found to increase the penetration of fluorescein (human, in vitro), tetrachlorsalicylanilide, demethylchlortetracycline (demeclocycline), and methylene blue (human, in vivo) (Kligman 1965a), rhodamine B in fish (Narula 1967), and Evans blue in the dog urinary bladder (Jacob et al. 1964). There is suggestive evidence that DMSO did not enhance penetration of tetrachlorsalicylanilide through the human finger nail (Kligman 1965a).

4.1.2.6 Allergens

The action of 2,4-dinitrochlorobenzene, a chemical which causes contact sensitization in humans and laboratory animals, was enhanced when applied with DMSO to the skin of guinea pigs (Heise et al. 1969) and of rats (Vakilzadeh et al. 1973).

DMSO as a vehicle for tuberculin antigen abolished the skin reaction in tuberculin sensitive humans, and the dermatitis manifested by topical trypsin was prevented when applied in DMSO (Kligman 1965a). In the same series of studies, it was reported that for humans who were demonstrably contact sensitive to penicillin, streptomycin, neomycin, nickel, chromium, or cobalt, DMSO sometimes increased and sometimes diminished 25-hour allergic responses to the allergens. Reductions were

more common with nickel, chromium, or cobalt which may signify the formation of a DMSO-metal complex.

4.1.2.7 Miscellaneous

The percutaneous penetration of some organophosphorous compounds can be influenced by DMSO. The penetration of the insecticide butonate through cattle skin was increased by DMSO but not the penetration of trichlorofon or dimethoate (Dedek et al. 1975). The potent anticholinesterase, soman, penetrated the skin of guinea pigs at a faster rate in the presence of DMSO (McDermot et al. 1967). One of the antidotes for anticholinesterase poisoning is 1-methyl-2-hydroxy-iminomethylpyridinium methane sulfonate, which has been shown to penetrate guinea pig skin faster when applied with DMSO (McDermot et al. 1965).

The effect of DMSO has been studied on the response of mice to eight topically applied drugs whose actions could be readily monitored visibly. In general, the response to the following drugs was most pronounced when DMSO was used as a vehicle: d-amphetamine, thiosemicarbazide, chlorpromazine, reserpine, d-tubocurarine, pheniprazine with intraperitoneally administered reserpine, and metrozol (Horita and Weber 1964). Absorption of vasopressin by rat skin was slightly increased by DMSO (Kastin et al. 1966). Penetration of the vasoconstrictor naphazoline through human skin was also increased by DMSO (Stoughton and Fritsch 1964).

The percutaneous penetration of mercuric chloride in the guinea pig (Wahlberg and Skog 1967), iron chloride in the pig (Oliver et al. 1969), hexachlorophene in the rat (Nakane and Bukler 1976), and penicillin in the cow (Walser 1966) was increased by DMSO as vehicle while penetration of phenol appeared to be hindered (Roberts and Anderson 1975). There is evidence that penetration of three antiperspirants, benzoyl scopalamine, aluminum chloride, and hexopyrroonium, was accelerated in humans in the presence of DMSO (Stoughton and Fritsch 1964; Kligman 1965a; Goldman et al. 1967). Absorption of a wide variety of substances through the mucosa of the dog urinary bladder was increased by DMSO: heparin, insulin, sodium salicylate, Evans blue dye, sulfadiazine, aminophylline, and triethylene thiophosphoramidate (Jacob et al. 1964).

In a rat model system, DMSO appeared to enhance the ability of alpha tocopherol to protect against adriamycin-induced skin ulcerations (Svingen et al. 1981).

4.2 DISCUSSIONS AND CONCLUSIONS

Several generalizations emerge from the results of the experiments reviewed herein. In studies in which substances were administered with DMSO, either orally, intravenously, or intraperitoneally, the effect of, or response to the agents was increased by DMSO in about 40% of the cases, reduced in 20%, and was unchanged in about 40%. Different proportions were observed for substances applied percutaneously; the responses to about 79% were increased, 19% were inhibited, and only 2% were unaffected. Thus, in many instances, DMSO was found to be an

enhancer of a biologic response to a substance, especially after percutaneous applications. While it appears that in about all experiments reviewed the altered biologic response reflected a synergistic action of DMSO with another substance, there are some cases in which an observable effect of DMSO itself was influenced by another agent. For example, the teratogenic effect of DMSO (Robens 1969; Landauer and Salam 1972; Gill et al. 1981) was enhanced by the tranquilizer diazepam (Gill et al. 1981).

Whether a response is enhanced or diminished, DMSO is capable of modifying the actions of a wide variety of polar and nonpolar substances. An examination of the substances reviewed in this section indicates that there are no apparent physical or chemical properties common to those classes for which DMSO enhanced, diminished, or had no effect on biologic responses. However, regardless of whether or to what extent the effect of a substance was modified, it was soluble in DMSO, and in many of the cases cited, solubility was the sole reason for using this vehicle. The solubility factor was particularly relevant to the use of many kinds of nonpolar organic chemicals such as carcinogens (e.g., see Somogyi and Kovacs 1970), hepatotoxins (e.g., see Danz et al. 1978), insecticides (e.g., see Weiss broad solvent characteristics of DMSO result from its ability to form solvates or solvent-solvate associations by hydrophobic interactions (Rammler 1971; see also Section 2).

The so-called penetrant carrier capacity of DMSO (Jacob et al. 1964) has been the subject of many kinds of investigations involving percutaneous administration, especially in humans (Kligman 1965a, 1965b). The penetrant concept not only serves as a working basis for the DMSO-enhanced percutaneous absorption of topically applied substances, but also may be implicated in altering body distribution of substances and in their transport across cell membranes (Misch and Misch 1975; Volden et al. 1980). These changes in distribution and transport kinetics could very well alter the response to a substance, qualitatively as well as quantitatively. Although it may be more logical to consider that the penetrant carrier concept would increase the biologic action of a substance, a basis for a diminished effect may also involve changes in membrane penetration. Thus, a case can be made that rather than allowing more substances to penetrate a membrane, DMSO broadens distribution of the substance in the body to the extent that the target tissue is exposed to less substance had DMSO not been present.

It is difficult to escape the conclusion that the penetration characteristics of DMSO are closely related to the interaction of DMSO with water and proteins. Some of these characteristics have been considered in detail (Rammler and Zaffaroni 1967) and principally involve complex formation with water, i.e., hydrate formation, and replacement of protein-bound water by DMSO, leading to reversible conformational changes in the protein. These interactions would be expected to influence the biologic response to any agent if only because of the importance, abundance, and widespread distribution of water and protein in living systems. It was also suggested that DMSO might associate with many other kinds of biologic molecules (e.g., nucleic acids) and thereby

provide a wider basis for altered biologic responses to substances (Szmant 1975).

To what degree, if any, chemical reactions between DMSO and a substance could occur in vivo or under ambient in vitro conditions and consequently modify the biologic response to that substance remains largely speculative. As cited, there is evidence for hydrate formation in a mixture of DMSO and the mutagen hexachloracetone upon standing; and the resulting hexachloracetone hydrate was found not to be mutagenic for bacteria (Zochlinski and Mower 1981). It was also suggested that DMSO could form complexes in vivo with carcinogens such as methylcholanthrene and benzo(a)pyrene (Levine 1975).

Another aspect of DMSO interaction with tissues that could affect the biologic response to a substance involves a reservoir phenomenon. It was shown that DMSO could enhance the persistence of a storage pool in the outer layer of the skin for topically applied glucocorticosteroids (Stoughton 1965). Thus, for some substances, an epidermal reservoir would provide a slow release type of administration that possibly could alter dermal or even systemic effects of a substance.

Two parameters of DMSO-chemical interaction studies require further comment, the temporal aspects of administration and the concentration of DMSO. In most studies cited, DMSO was administered simultaneously (i.e., as a vehicle for the substance). In several studies, however, DMSO was injected several minutes before administration of the test substance (e.g., Courtney 1970; Sosmowski et al. 1976; Barilyak et al. 1978). The temporal modes of administration are cogent to problems of potential interaction (Witschi and Hakkinen 1982), but no documents were found in which a comparison was made between simultaneous or sequential administration of DMSO and another substance. In studies whereby substances are given in sequence, it is possible to begin to sort out the mechanisms of how an observable tissue response to one agent is altered by another agent. When two agents are administered simultaneously by the same route, they may reach a target tissue at the same time, thereby making it more difficult to provide an explanation for a change in response resulting from interactions. The latter point is especially applicable to DMSO, which may help keep its solute in a form that has access to a target tissue.

Regarding DMSO concentration, it was found that for most experiments the test substance was dissolved in undiluted DMSO. In general, it appears that high concentrations of DMSO were tantamount to a maximal alteration in response. This feature was clearly demonstrated by some human data which showed that the average penetration rate of topical steroids was fastest at DMSO concentrations of 60% or greater (Kligman 1965a). The concentration factor is essentially confined to percutaneous absorption, and presumably has less relevance to oral or intraperitoneal administration because of rapid dilution when given by these routes. On the other hand, the concentration of DMSO in body fluids, which is dependent on the amount given orally or intraperitoneally, presumably could determine whether and to what extent a substance administered with DMSO elicits a biological response.

From a toxicological standpoint there are numerous deficiencies in the literature concerning the interactive effects of DMSO with other substances. These deficiencies are no more numerous, however, than present in other cases where toxicologic factors involving interaction between two or more substances are expected to be encountered. Nonetheless, a meaningful evaluation of the potential hazards to humans involved with RDX and HMX production would benefit from more information about effects of subchronic and chronic percutaneous exposure to DMSO in combination with other agents including these explosives. The choice of other agents would be predicated on those to which personnel may be expected to be exposed, accidentally or otherwise.

The detailed study by McNamara et al. (1974) provides a firm basis for further toxicologic evaluation of HMX and RDX. Their broad base investigations established in laboratory animals the acute toxicities for each of the 2 explosives administered in DMSO. These data do not permit comparative assessment of DMSO with other vehicles for HMX and RDX because, with one minor exception, different concentrations of the explosives had to be used. It is not possible, therefore, to determine whether DMSO had any influence on the toxicity of HMX or RDX relative to effects in other vehicles.

The following conclusions can be drawn from data in the documents reviewed:

1. DMSO enhanced the biologic response of many kinds of substances regardless of the route of administration. With topical application, the effect of some substances was reduced while very few were unaffected. When given orally or by injection, the biologic responses of a large number of substances were unaffected while some were reduced.
2. There does not appear to be a common physical or chemical characteristic of those substances whose action is enhanced, diminished, or unchanged by DMSO. Consequently, it is not possible at present to predict whether or to what degree DMSO would influence the action of a substance from its physical or chemical characteristics. That the penetration of almost 80% of the substances applied topically was increased by DMSO dictates that until shown otherwise, it would be prudent to assume that any untested substance would penetrate the skin faster in the presence of DMSO.
3. Interaction effects of DMSO have been demonstrated for agents other than chemicals, e.g., bacteria.
4. DMSO appears to alter the biological response to a substance by virtue of its ability to act as a penetrant carrier through biologic membranes.

5 REGULATORY ACTIONS

DMSO was first used commercially as an industrial solvent in 1953 (Brown 1982). The potential pharmacological value of the solvent was recognized several years later, and clinical studies were initiated and continued until 1965 when the U.S. Food and Drug Administration (FDA) terminated all human studies because of evidence that DMSO caused ocular abnormalities in experimental animals (USFDA 1965). Specific requirements were established for testing the drug on humans, and in the clinical studies that followed, no DMSO-induced ocular changes were observed. In 1970 the FDA approved the veterinary use of DMSO for treating acute musculo-skeletal injuries in horses, and in 1972 this use was extended to dogs (see Brown 1982). In 1978 the FDA approved a New Drug Application (NDA) for the use of a 50% solution of DMSO in the treatment of interstitial cystitis, and in 1980 the restrictions governing the clinical testing of the drug on humans were revoked (USFDA 1980). Currently, 33 clinical studies are being conducted on DMSO under the Investigational New Drug Regulations of the FDA (Karusaitis 1982, personal communication). It is anticipated by the FDA that in the near future NDAs pertaining to the use of DMSO in the treatment of scleroderma and musculo-skeletal disorders will be submitted by the pharmaceutical industry for agency approval (Karusaitis 1982, personal communication).

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APPENDIX A-1

**REVIEW AND ANALYSIS OF
PERTINENT REFERENCES ON DMSO TOXICITY**

• Abbondandolo, A., S. Bonatti, C. Corsi, G. Corti, R. Fiorio, C. Leporini, A. Mazzaccaro, R. Nieri, R. Barale, and N. Loprieno. 1980. The use of organic solvents in mutagenicity testing. Mutat. Res. 79:141-150.

Review:

Cell suspensions of the yeast Schizosaccharomyces pombe (P1 strain) were mixed with 0.5, 2.0 and 5% DMSO (v/v) and the effects on cell survival were evaluated after 1 hr and after 24 hr. Relative survival was 63-67% for 0.5% DMSO, 57-66% for 2.0% DMSO, and 29-33% for 5% DMSO, indicating a moderate degree of toxic action. Sixty-minute exposure to 5% DMSO resulted in a forward mutation rate of <1.10 mutants/ 10^4 colonies in S. pombe as compared with 0.74 for the control. In the presence of a mouse-liver microsomal extract, the mutation rate was 0.61 as compared with 0.66 for the control. A separate study showed that 5% DMSO had no direct inhibitory effect on the mouse liver enzyme system. Under in vitro conditions the DMSO had no effect on aminopyrine demethylase activity.

A second series of cytological toxicity tests were conducted using cultures of V79 Chinese hamster cells. Monolayer cultures and free cell suspensions were exposed to 0.5, 2.0, 5.0 and 10% DMSO for 1 hr at 37°C. Relative survival values were 79 and 100% at 0.5% DMSO, 102 and 103% at 2.0% DMSO, 89 and 74% at 5.0% DMSO, and 75% at 10% DMSO.

Analysis:

This study is valuable in providing data on the mutagenicity and cytotoxicity of DMSO. Although the results seen here may not necessarily apply to other cell systems or in vivo conditions, they do contribute to the evidence suggesting that DMSO is nonmutagenic and only mildly cytotoxic.

• Bennett, W.M. and R.S. Muther. 1981. Lack of nephrotoxicity of intravenous dimethyl sulfoxide. Clin. Toxicol. 18(5):615-618.

Review:

Nephrotoxicity of DMSO was examined in seven patients who had stable spinal cord injuries. DMSO was given intravenously, 1 g/kg in 5% dextrose water, daily for three consecutive days. Concentrations ranged from 10 to 40% with infusions lasting 30 min to 24 hr. All patients receiving 20-40% DMSO showed signs of hemoglobinuria within minutes of the start of the infusions. Hemolysis was evidenced by depletion of haptoglobin and pink serum. Serum creatinine and creatinine clearance did not change. Urine sediments were normal and without protein, casts, crystals, or tubular cell debris. Renal excretion of beta-2-microglobulin was not out of the normal range.

Analysis:

This study shows that normal renal tubular function, in terms of reabsorption of beta-2-microglobulin, is not impaired by the hemolysis induced by short-term intravenous administration of DMSO.

† Caujolle, F.M.E., D.H. Caujolle, S.B. Cros, and M.-M.J. Calvet. 79:141-150. 1967. Limits of toxic and teratogenic tolerance of dimethyl sulfoxide. Ann. N.Y. Acad. Sci. 141:110-126.

Review:

Standard acute toxicity tests were conducted on Swiss mice (25 ± 1 g), Wistar rats (200 ± 10 g), L. Fawn Burgundy rabbits (average weight 2.88 kg), and dogs. The doses were administered as a 50% DMSO solution in 0.9% sodium chloride. In mice the 24-hr LD₅₀ values were: 11.0 g/kg, iv; 20.1 g/kg, ip; and 16.0 g/kg, sc. In rats the 24-hr LD₅₀ values were 13.7 g/kg, ip, and 13.7 g/kg, sc. Rabbits were given a 40% solution of DMSO intravenously at a rate of 90 mL/hr. The mean survival time was 92 min and corresponded to an average dose of 19.2 g/kg. Dogs were given an intravenous perfusion of 50% DMSO. Lethal effects were observed only when doses reached 30-40 g/kg.

In chronic toxicity studies, mice and rats were given 50% DMSO subcutaneously, intraperitoneally, or orally six times per week for 3 to 12 consecutive weeks. Daily oral doses of 5 g/kg to mice caused an initial loss of weight, and 2.5 g/kg over a 5-week period resulted in a slower rate of growth (data not given). Histological studies revealed that 2.5 g/kg given intraperitoneally caused tubulonephritis and some damage to organs in the area of the injection (liver, pancreas, and spleen). Growth rates of young rats (120-140 g) were reduced by intraperitoneal injections of 5 or 10 g/kg, and oral doses of 5 g/kg for 45 days caused slight weight loss (data not given) and damage to the liver (degenerative modification of the hepatocytes) with inflammation and irritation of the portal spaces.

Teratogenic studies were conducted on chick embryos, mice, rats, and rabbits. The chick embryos were injected with 50% DMSO in saline at 72 or 96 hr of incubation. The LD₅₀ values were 10.3 mg for the 72-hr embryos, and 12.2 mg for the 96-hr embryos. Toxic doses of DMSO caused developmental abnormalities including malformations of the limbs, beak, and eyes, anourous embryos, and celosomia. Mice were given 50% DMSO orally or intraperitoneally from the 6th to the 12th day of gestation. Oral doses of 5 to 12 g/kg caused no embryonic malformations, but intraperitoneal injections produced four abnormal fetuses (anencephalia, malformed limbs and celosomia) in animals given 12 g/kg and three abnormal ones in animals given 5-10 g/kg. A total of 100 fetuses were examined.

Maternal rats were given 5 and 10 g/kg of DMSO orally, or 5, 8, or 10 g/kg of DMSO intraperitoneally on days 6 through 11 of gestation. Intraperitoneal doses of 8 and 10 g/kg resulted in an increase in the percentage of aborted fetuses, a decrease in fetal weight, and an

increase in developmental malformations. Eleven of 729 fetuses were abnormal. Malformations involved the nervous system, limbs, jaw, tail bud, abdominal wall and edema.

Ten pregnant rabbits were given daily oral (5 g/kg) or subcutaneous (4 g/kg) doses of 50% DMSO on days 6 through 14 of gestation. Of 83 fetuses examined (20-24 day of gestation) only one was found to be abnormal with celosomia. There were no differences in weight or number of aborted fetuses between treated and control animals.

Analysis:

The median lethal toxic doses of DMSO to mice and rabbits as found in this study are similar to those reported by other investigators. The chronic exposure studies were incompletely described and analyzed. Reported histopathological damage resulting from intraperitoneal injections may have been due to direct injury rather than systemic toxic effects of the DMSO. The frequency and severity of the hepatic injury in orally-dosed rats requires more detailed analysis. An increased number of developmental abnormalities were seen in chick embryos dosed with DMSO and in the fetuses of mice and rats given multiple doses of DMSO. The data were not analyzed statistically, and in most cases toxic dose levels were required to produce the teratogenic effect. In a comment reported in a discussion section at the end of this paper, it was noted that the DMSO solutions used were not stable and thus impurities may have caused some of the effects seen.

♦ de la Torre, J.C., J.W. Surgeon, T. Ernest, and R. Wollmann. 1981. Subacute toxicity of intravenous dimethyl sulfoxide in rhesus monkeys. J. Toxicol. Environ. Health 7:49-57.

Review:

This study evaluates the subacute toxicity of DMSO in male rhesus monkeys weighing 3-6 kg. Four monkeys received 3 g/kg (40% DMSO solution in saline), one received 2 g/kg (40% DMSO solution in saline), and one control received 3 mL/kg of 0.9% saline. The iv doses were administered daily for nine consecutive days. All but one test animal were monitored for four months, at the end of which time period they were sacrificed and examined for gross and microscopic pathology. The sixth monkey was sacrificed on day 4 of the DMSO administration. Blood and urine analysis were conducted on samples taken 1 hr before the first exposure, 1 hr after the last exposure and 18 weeks after the study began. For all test animals, blood chemistry (serum electrolytes, CO₂, creatinine, urea nitrogen, glucose, total protein, albumin, calcium, phosphorus, cholesterol, uric acid, total bilirubin, alkaline phosphatase, lactic dehydrogenase, and serum glutamic-oxaloacetic transaminase); hematology (red, white, and total cell counts, hemoglobin, and hematocrit); and urinalysis (pH, specific gravity, protein, glucose, ketones, bilirubin, blood, urobilinogen, and turbidity) were not significantly different from those of the control animal. No neurological or ophthalmologic abnormalities observed in any of the animals, and gross and histological

examination of body organs, muscle, lymph nodes, salivary glands, and peripheral nerves revealed no pathological condition. The DMSO animals, however, did show a fourfold increase in diuresis, a slight increase in cardiac rate, and a significant increase in respiratory rate.

Analysis:

This work is a thorough analysis of the subacute toxicity of intravenously administered DMSO in rhesus monkeys. The results show that for the exposure levels used in these tests, there were no significant toxic effects. Similar experiments using higher or more frequent exposures and different exposure routes would contribute substantially to delineating no effect levels.

♠ Ferm, V.H. 1966. Congenital malformations induced by dimethyl sulphoxide in the golden hamster. J. Embryol. Exp. Morphol. 16(1):49-54.

Review:

The teratogenicity of DMSO was evaluated in golden hamsters. On the 8th day of gestation females were injected with 50-8250 mg DMSO/kg in saline (0.5 mL/100 g body weight). Doses of 50, 250, 500, 1000, 2500 and 5500 mg/kg were given intravenously (the largest dose was diluted to 1.0 mL to avoid the lethal effects caused by the more concentrated solution). In a second test series, doses of 5500 or 8250 mg/kg were given intraperitoneally. Test animals receiving 2500 mg/kg or higher iv showed signs of generalized muscular tremors for about 1 min after the injection, and the ip dosed animals had some abdominal muscle rigidity for the same postinjection period.

On day 11 of gestation the maternal animals were sacrificed, and the embryos were examined for developmental malformations. In each test group there were five or six females exposed to DMSO and the total number of embryos examined ranged from 57 to 77. At dose levels of 1000 mg/kg or less there were not more than 2 abnormal embryos in each group; at 2500 mg/kg there were 4; at 5500 mg/kg, iv, 12; at 5500 mg/kg, ip, 25; and at 8250 mg/kg, ip, there were 18. Exencephaly was the most common malformation. Rib fusions, microphthalmia, limb abnormalities, and cleft lip were also present in some embryos. The embryocidal or teratogenic effects of a dose level of 1000 mg/kg or less was reported to be no different than that seen in several hundred nonspecific saline-injected control animals examined over several months.

Analysis:

Although the data were not analyzed statistically, the tenfold to twentyfold increase in embryonic malformations seen at 5500 and 8250 mg/kg is indicative of a significant teratogenic effect. The high dose levels required may have been toxic to the maternal animals and thus could have contributed to the observed developmental abnormalities.

♦ Feuer, G., L. Goldberg, and J.R. LePelley. 1965. Liver response tests. I. Exploratory studies on glucose 6-phosphatase and other liver enzymes. Fd. Cosmet. Toxicol. 3:235-249.

Review:

Undiluted DMSO was administered to male and female Carworth rats (80-120 g) in daily doses of 0, 1100, 3300, or 11000 mg/kg for seven days. The animals were sacrificed 24 hr after the last dose, and the liver was removed, weighed, and processed for enzyme analysis. Relative liver weight and activity of glucose 6-phosphatase and glucose 6-phosphate dehydrogenase for all test groups (6 animals each) were not significantly different from those of the control group.

Analysis:

DMSO did not produce the increase in relative liver weight and the decrease in glucose 6-phosphatase activity which are often caused by known hepatotoxic chemicals. However, the effects of DMSO on other hepatic enzyme systems, and on histomorphology of liver tissue, were not examined in this study.

♦ Fishman, E.G., L.J. Jenkins, Jr., R.A. Coon, and R.A. Jones. 1969. Effects of acute and repeated inhalation of dimethyl sulfoxide in rats. Toxicol. Appl. Pharmacol. 15:74-82.

Review:

This study consisted of three different series of toxicity tests. The first series involved preliminary dermal and ocular irritant tests conducted on 20 New Zealand albino rabbits. Topical application of DMSO (dose-level not reported) resulted in only slight localized erythema. Ocular application (dose level not given) resulted in slight conjunctivitis during the initial 24-hr observation period. This effect disappeared by 48 hr.

Acute lethal toxicity tests were conducted on Sprague-Dawley rats and Swiss mice. The oral LD₅₀ for the rats was 14.5 g/kg, and the iv LD₅₀ for the mice was 3.1 g/kg.

The third part of the study involved inhalation toxicity tests on male Sprague-Dawley rats (250-325 g). The test animals were exposed to an aerosol of DMSO (98% of particles less than 8 microns in diameter) in a 30-liter exposure chamber. Eight rats were exposed to 2900 mg/m³ for 24 hr, another group of 8 to 2000 mg/m³ for 40 hr, and a group of 24 to 1600 mg/m³ for 4 hr. In the last mentioned group, 8 animals were sacrificed immediately at the end of the exposure, another eight after 24 hr and the last eight after two weeks. In addition, another 32 test animals were exposed to 200 mg/m³ for 7 hr per day, 5 days per week, for a total of 30 exposures. Control animals were exposed to normal chamber conditions. Hematologic and biochemical parameters (microhematocrit, hemoglobin, cell counts, serum urea nitrogen, serum glutamic-pyruvic

transaminase, serum glutamic-oxaloacetic transaminase, liver lactate, and liver alkaline phosphatase), in samples taken at the end of the exposure periods, were not abnormal (statistical analysis of data not given) for any of the treated animals except those exposed to 2900 mg/m³ for 24 hr; these rats had elevated serum urea nitrogen concentrations (31.5 ± 8.6 mg/100 mL as compared to 16.6 ± 2.3 mg/100 mL for the controls). Histological examinations revealed that some rats exposed to single acute doses and some control animals had areas of hemorrhage in the lungs and focal and diffuse collections of clear pneumocytes within the lung alveoli. Areas of pulmonary edema were seen in some of the animals exposed to 1600 mg/m³ for 4 hr and sacrificed 2 weeks later, in those exposed to 2900 mg/m³ for 24 hr, and in those exposed to 2000 mg/m³ for 40 hr. Similar edematous changes were not noted in the control animals. Nonspecific inflammatory changes in the liver occurred in about one-fourth of all acutely exposed rats and in the controls.

In rats exposed to repeated 200 mg/m³ doses, there were no biochemical or hematologic abnormalities (statistical analysis of data not given), and there were no histopathological conditions except for nonspecific inflammatory changes in the lungs and livers of nearly all animals, including controls.

Analysis:

The dermal and ocular tests conducted in this study provide only a limited amount of information on the irritant effects of DMSO because dose levels and concentrations of the test solutions were not given. The acute lethal toxicity tests conducted on rats and mice gave median lethal dose levels which are, in general, in agreement with those found by other researchers.

Interpretation of the results of the inhalation tests is complicated by the fact that exposure levels may have been modified by varying respiratory activity of the test animals, and by potential dermal uptake of the DMSO. The occurrence of liver and lung abnormalities in the control animals may have increased the susceptibility of the test animals to the edematous effects seen in the higher dosed groups.

♦ Haigler, H.J. and D.D. Spring. 1981. DMSO (dimethyl sulfoxide), morphine and analgesia. Life Sci. 29:1545-1553.

Review: Male albino Sprague-Dawley rats (250-400 g) were treated with DMSO and then tested for analgesic effect using the hot plate and tail flick tests. The DMSO was administered either intraperitoneally (0.55, 2.75, or 5.5 g/kg); topically (100% DMSO on back, or on feet and ventral portion of the body); intravenously (2.75 or 5.5 g/kg); and orally (1.5-2 cc). DMSO administered at high doses iv and ip (5.5 g/kg) and topically produced significant analgesia comparable to that produced by morphine although its duration of 6-7 hr was longer than that of morphine (< 2 hr). The analgesic effect was not blocked by the narcotic antagonist, naloxone, indicating that the mechanism of action was different from that of morphine.

Analysis:

Statistical analysis of the experimental data verified that DMSO had a definite analgesic effect. There was no evidence that DMSO produced a general anesthetic effect.

◊ Juma, M.B. and R.E. Staples. 1967. Effect of maternal administration of dimethyl sulfoxide on the development of rat fetuses. Proc. Soc. Exp. Biol. Med. 125:567-569.

Review:

Female Sprague-Dawley rats were given daily subcutaneous injections of 10.25 g/kg (90% aqueous DMSO solution) on day 8, days 8 and 9, or days 8, 9, and 10 of gestation. Controls received distilled water. All animals were sacrificed on the 19th day of gestation, and reproductive status and the condition of the fetuses were determined. No maternal deaths occurred, and there were no significant changes in maternal weight gain or in the weight of the live fetuses obtained on day 19. Only in the group receiving three injections was there a significant decrease in litter size (9.8 ± 1.1 as compared with 13.8 ± 0.7 for the control group). However, only 2 of the 127 live young obtained from this group were grossly malformed (slight umbilical hernias). No skeletal malformations were seen. A dead and macerated fetus, with an abnormally shaped head, was found in the group of maternal rats that received two injections of DMSO, but all other fetuses, in treated and control groups, were normal.

Analysis:

The daily dose used in this study was calculated to be one-half the LD₅₀ for adult rats. Even though no toxic effect was seen in the maternal animals, even after three injections, the dose may have been sufficiently high to the embryos to cause the observed reduction in litter size. There was no evidence to suggest that any of the resorbed embryos were malformed, and the cases of slight umbilical hernias in two fetuses could not be attributed solely to the effects of DMSO.

◊ Kapp, R.W., Jr. and B.E. Eventoff. 1980. Mutagenicity of dimethylsulfoxide (DMSO): In vivo cytogenetics study in the rat. Teratogen. Carcinogen. Mutagen. 1(2):141-145.

Review:

The potential mutagenicity of DMSO was evaluated in an in vivo series of assays using 50 male albino Sprague-Dawley rats. The 8- to 10-week-old rats were divided into groups of ten, and each group received a daily 5 mL/kg, intraperitoneal injection of DMSO (1%, 10%, 50%, or 100%) for five consecutive days. Dilutions were made with distilled water, and the control group received distilled water only. On day 6 the animals were sacrificed, and femoral bone marrow was collected and prepared for

microscopic examination to determine the degree of chromosomal alterations. Fifty metaphasic cells per test animal were analyzed. The incidence of aberrant cells (percent per animal) ranged from 10% in the 1% DMSO group to 68.7% in the 100% DMSO group; it was 4% in the control group. Comparative analyses, based on the Wilcoxon nonparametric test, showed that (1) there was no significant differences in the incidence of chromosomal breaks between all test groups and the controls, (2) total chromosomal aberrations and chromosomal markers (exchanges, rings and dicentrics) in all test groups were significantly different from that of the control group, (3) incidence of chromatid breaks in all but the 10% DMSO group were significantly different from that of the control group, and (4) only in the 100% DMSO group was the incidence of severely damaged cells significantly different from that of the control group.

Analysis:

Although the study indicates that the incidence of various chromosomal aberrations in rat bone marrow cells does increase after intraperitoneal injections with DMSO, the mean percent aberrant cells per test animal for three of the four test groups (1, 10, and 50% DMSO) was only slightly higher than that of the control group (10.0-19.2 as compared with 4.0; statistical significance not reported). Thus, the cytogenetic effect of low doses of DMSO may be to increase the number and variety of chromosomal aberrations in cells which are predisposed to show such effects. Furthermore, because all test animals were sacrificed on day 6 of the study, the degree to which the bone marrow cells would have returned to normal cytogenetic state after a longer postexposure time interval, could not be determined.

The very high incidence of aberrant cells per animal and the significantly higher incidence of severely damaged cells in the 100% DMSO group, as compared to those of the control group, could be the result of the acute toxicity of that dose level, which was also evidenced by the 30% mortality rate in the group.

♣ Kligman, A.M. 1965b. Topical pharmacology and toxicology of dimethyl sulfoxide - Part II. J. Am. Med. Assoc. 193(11):923-928.

Review:

In addition to microbiological tests which demonstrated that DMSO is weakly bactericidal and fungicidal, this study also examined chronic and acute toxicity of DMSO in human volunteers. In chronic exposures, 9 mL of 90% DMSO were applied to the entire trunk of 20 men once daily for 26 weeks. At 0, 2, 4, 8, 12, 16, and 24 weeks, samples were taken for blood count, urinalysis, thymol turbidity, serum glutamic oxaloacetic transaminase, sodium sulfobromophthalein, fasting blood sugar, and blood urea nitrogen. These values remained essentially normal throughout the test period (data not given). Symptoms of DMSO exposure included malodorous breath, transient erythema in about one-fourth of the subjects during the first two weeks, and transient burning and stinging in about 75% of the subjects during the first few weeks. Two subjects developed

mild scaling diffuse erythematous dermatitis after 2-3 weeks, but this eventually disappeared. Skin biopsies of the backs of six subjects at the conclusion of the study revealed no abnormal conditions. During the test period no sedative or tranquilizing effects of DMSO could be seen in any of the subjects.

In acute exposure tests, 9 mL of 90% DMSO were applied twice daily to the entire trunk of 20 subjects for three weeks. At the end of the treatment period hematologic, biochemical, and urinalysis values were normal (data not given). Most patients experienced stinging and burning during exposure, and about one-half displayed a transient erythema. On day 12 one subject developed a toxic reaction to the DMSO: diffuse erythematous, scaling rash accompanied by severe abdominal cramps. These symptoms disappeared after DMSO exposure was discontinued. On day 13 a second subject developed a similar rash and reported slight nausea, chills, and chest pains; all symptoms abated during continued treatment. Biopsies of both subjects showed a mild perivascular lymphocytic infiltrate, moderate acanthosis, absence of the granular layer, and a parakeratotic, increased horny layer. Similar, but milder, cutaneous effects were seen in biopsy samples, taken at three weeks from three subjects who did not show outward signs of toxicity. Intradermal injections of 0.1 mL of aqueous DMSO into the backs of human volunteers produced wheals and flares when concentration were above 0.01%. Concentrations of 50% and above produced an inflammatory papule at 24 hr. Biopsies showed edema, intense lymphocyte infiltration, and vasodilation, but little epidermal change. Conjunctival toxicity was assessed by instilling two drops of increasingly concentrated DMSO in the conjunctival sacs of groups of adult men. Except for transient burning and stinging at concentrations of 50% and higher, and occasional vasodilation, there were no abnormal changes in the eye (methods of examination not given).

Contact sensitization was evaluated in 25 human subjects. Ninety percent DMSO was applied in five 48-hr occlusive patches to sites inflamed by 10% sodium lauryl sulfate two weeks earlier. None of the subjects showed signs of contact allergy. Twenty-four-hour occlusive patch tests were used to calculate a DMSO threshold irritating concentration of 38%. One-hour occlusive patch tests showed that the histologically observable threshold for epidermal alterations caused by DMSO occurred at a concentration of 50%. At 90% DMSO a papulovesicular reaction occurred within 15 min to 1 hr which resulted in the mild scaling dermatitis.

Analysis:

Although this study contains a large amount of data on DMSO toxicity, it is deficient in terms of experimental technique and presentation of the data. No controls were used in any of the tests, and descriptions of hemotological and biochemical methods and results were incomplete and could not be adequately evaluated. However, the study does provide useful data on chronic and acute dermal DMSO toxicity in humans, particularly in providing an estimate of the minimum DMSO exposures causing histological and clinical signs of toxicity. Descriptions

of histopathology and recovery of DMSO exposed cutaneous tissue are also useful in evaluating potential effects of chronic exposures.

✦ Marin-Padilla, M. 1966. Mesodermal alterations induced by dimethyl sulfoxide. Proc. Soc. Exp. Biol. Med. 122:717-720.

Review: Twelve pregnant hamsters were treated with 5500 mg/kg of DMSO (concentration not given) on the eighth day of gestation. Groups of animals were sacrificed 10 hr, 12 hr, and 24 hr after dosing, and the embryos were examined for developmental abnormalities. Histological studies showed mesodermal alterations in the cephalic region of the embryos. The mesodermal cells appeared to be collapsed, and there was an increase in the intercellular space, possibly due to the accumulation of edema fluid. The collapse of the cephalic mesoderm results in failure of the neural tube to close and cranioschisis in the surviving fetuses.

Analysis:

It was noted by the author that the teratogenic effect produced by DMSO in hamsters is morphologically similar to that induced by hypervitaminosis A. Previous studies on hamsters have shown that doses of DMSO of 1000 mg/kg or less, ip, do not produce teratogenic effects.

✦ Mathew, T., R. Karunanithy, M.H. Yee, and P.N. Natarajan. 1980. Hepatotoxicity of dimethylformamide and dimethylsulfoxide at and above levels used in some aflatoxin studies. Lab. Invest. 42(2):257-262.

Review:

Hepatotoxicity of DMSO was studied in 5 to 6-week-old male Wistar albino rats receiving single intraperitoneal injections (1.2, 2.4, 3.6, or 4.8 mL/kg) of doubly distilled laboratory grade solvent. Thirty to thirty-two animals were included in each test group allowing for sampling 12, 24, 48, 72, and 120 hr after dosing. A control group of 30 rats, receiving either 1.0 or 2.0 mL/kg distilled water, was sacrificed at 120 hr.

Only minor morphologic changes occurred in the liver of rats exposed to DMSO, and these occurred mainly in rats exposed to the two highest doses. These changes consisted of transient fatty infiltration seen at 12 and 24 hr and were associated with depletion of glycogen stores; however, the condition returned to normal at 48 hr. No inflammatory changes, cellular atypism, or cell death were observed at any dose level.

Analysis:

This study verifies that single ip exposures to DMSO do not cause significant morphological changes in the liver of rats. The possible long-term effects of multiple exposures was not evaluated in this study.

♦ Rengstorff, R.H., J.P. Petrali, and V.M. Sim. 1971. Cataracts Induced in Guinea Pigs by Acetone, Cyclohexanone, and Dimethyl Sulfoxide. Technical Report 4550. U.S. Army Medical Research Laboratory, Edgewood Arsenal, MD.

Review:

Nine to eighteen week old albino guinea pigs were exposed to pure or technical grade DMSO three times per week for 3 weeks. Ocular examinations were made up to 6 months after the initial exposure to determine the extent of lens alteration. The DMSO was applied either cutaneously (0.05 mL) or subcutaneously (0.05 mL of either a 1:1 solvent:saline solution or a 5% solution in saline). Control animals received no treatment. Of 12 test animals exposed to pure DMSO cutaneously, only one developed cataracts. The cataracts were seen in this animal in months 3 and 4 but not in months 5 and 6. Of 12 test animals given technical grade DMSO cutaneously, three developed extensive cataracts in months 5 and 6. Subcutaneous injection of technical grade DMSO (1:1 solvent:saline) did not produce cataracts in any of four guinea pigs, and pure DMSO, administered in the same way, produced only minor, transitory, vacuolated areas in the lens (during months 2 and 3) in 2 of 4 test animals. Subcutaneous injection of a cutaneous injection of a 5% DMSO solution resulted in major cataracts occurring throughout the entire 6-month period in 3 of 12 animals given pure DMSO and in 2 of 12 animals given technical grade DMSO. In addition, 2 of the 12 hamsters given technical grade DMSO developed minor cataracts in months 2 and 3, but these disappeared in subsequent months.

Analysis:

The data were not analyzed statistically to determine levels of significance of the observed effects, and no saline-only control group was used in any of the tests. The results, however, do corroborate the findings of other studies showing ocular abnormalities in various species of rodents following DMSO exposure.

♦ Sams, W.M., Jr., N.V. Carroll, and P.L. Crantz. 1966. Effect of dimethylsulfoxide on isolated-innervated skeletal, smooth, and cardiac muscle. Proc. Soc. Exp. Biol. Med. 122:103-107.

Review:

The effects of DMSO on the response of muscle tissue to electrical stimulation was evaluated on isolated-innervated diaphragm, stomach, and cardiac atrial preparations obtained from adult guinea pigs. The tissues were exposed to DMSO concentrations of 0.6 to 6%. DMSO depressed the response of the diaphragm to both muscle and nerve stimulation and caused spontaneous skeletal muscle fasciculations. It increased the response of the smooth muscle of the stomach to both muscle and nerve stimulation. The amplitude of atrial contraction was increased by DMSO, but the rate of contraction was not altered. In vitro tests demonstrated that DMSO was a cholinesterase inhibitor (7.8% DMSO caused 85%

inhibition), and this may account for the observed effects on muscle tissue.

Analysis:

The experimental data support the contention that DMSO is a cholinesterase inhibitor.

⊕ Small, A. and R.S. Ide, 1976. Failure to detect nephrotoxicity of chronically administered dimethyl sulfoxide (DMSO) in rats. Cryobiology 13:328-333.

Review:

Nephrotoxicity of DMSO was evaluated in 21-39-day-old male and female Sprague-Dawley rats. The test animals, divided into groups of ten, received intraperitoneal injections of saline (controls); 2 g DMSO (40% solution in saline)/kg/day for 28 days, or 4 g DMSO/kg/day for 28 days. Five of the rats receiving the higher DMSO dose, and one receiving the lower dose, died during the test period. Both groups of rats showed impaired growth. In samples taken at the end of the test period, serum urea and creatinine concentrations were not higher than those of the controls, and in vitro tests on renal cortical slices taken from the test animals showed that the DMSO had no adverse effect on the ability of the kidney to transport p-aminohippurate or N-methylnicotinamide.

Analysis:

This study is similar to others in demonstrating that chronic DMSO exposures have no adverse effects on kidney function.

⊕ Smith, E.R., M.M. Mason, and E. Epstein. 1969. The ocular effects of repeated dermal applications of dimethyl sulfoxide to dogs and monkeys. J. Pharmacol. Exp. Ther. 170(1):364-369.

Review:

The effects on DMSO on hematology, hemochemistry, urinalysis, and on lenticular changes in the eye were studied in dogs and rhesus monkeys. The dogs (beagles) were divided into groups of two animals each and were exposed to a daily dermal dose of 1.1, 3.3, or 11.0 g/kg (90% aqueous solution) for 118 days. The monkeys were divided into groups of eight animals each and received the same dose levels as the beagles, but for 185 to 200 consecutive days. In both species the 11.0 g/kg daily dose was divided in half and applied in the morning and afternoon. Both species exhibited malodorous breath and desquamation at the application site; both conditions disappeared after treatment stopped. In the dogs, a dose-related myopia developed during treatment and intensified in the 71- to 92- day- period after the last dose was given. The two animals exposed to the highest dose developed a haze or opalescence in the nucleus of the lens of the eye during the 118 day dosing period, and 92

days after the last dose was given, 5 of the 6 dogs exhibited lenticular changes. Hematologic and hemochemical tests, conducted on samples taken before, during, and after the treatment period, revealed no abnormal values (numeric data not given). Urinalysis showed that pH and specific gravity were within reasonable limits, and no sugar, protein, or abnormal number of blood cells appeared in the urine. However, in the animals receiving the two highest doses, the 24-hr urine volume and 24-hr total hydroxyproline values tended to be higher during treatment. The data were not analyzed statistically.

No ocular abnormalities were observed in any of the monkeys given DMSO, and all hematologic and biochemical parameters were within normal limits. There was, however, drug-related increases in 24-hr urine output and hydroxyproline excretion and, in some cases, also lowered urinary pH and specific gravity. Gross anatomical examinations of monkeys sacrificed at the end of the test period revealed no pathological condition except pleural adhesions or granulomata indicative of lung mites.

Analysis:

The most significant finding of this study was the absence of ocular abnormalities in rhesus monkeys following chronic dermal administration of DMSO. The DMSO-induced diuresis and elevated hydroxyproline excretion have been reported by other researchers.

♦ Staples, R.E. and M.M. Pecharo. 1973. Species differences in DMSO-induced teratology. Acta Univ. Carol. Med. Monogr. 61:131-133.

Review: The teratogenicity of DMSO was evaluated in hamsters, rats, mice, and rabbits.

Hamsters received 3.3, 5.5, or 8.25 g/kg of DMSO (concentration not given) intraperitoneally on day 8, days 7 through 9, or days 7 through 11 of gestation. Single exposures of 5.5 and 8.25 g/kg resulted in exencephaly, cranioschisis, and cleft lip seen on day 11, and exencephaly, hemangioma, absence of kidney and ureter and fused ribs seen on day 15. Multiple exposures produced, additionally, microphthalmia, pug nose, shortened maxilla, altered digits and limbs, heart and vessel malformations, and pelvic alterations. The use of either high dose on day 10 of gestation did not elevate the incidence of gross, visceral, or skeletal malformations above the control level, and single ip injections of 3.3 g/kg did not appear to be embryotoxic or teratogenic.

In rats subcutaneous doses of DMSO of 10.25 g/kg/day on days 8 through 10 of gestation were lethal to the embryos (specific data not given) but were not teratogenic.

In the CF₁S strain of mice, DMSO doses of 11 g/kg administered intra peritoneally on day 8, 9, 10, or 11 of gestation were embryo-lethal, and very teratogenic. The embryos exhibited gross, visceral, and skeletal malformations. Significant skeletal abnormalities were also seen at a dose level of 5.5 g/kg.

Subcutaneous injections of 90% DMSO at a dose level of 3.0 g/kg/day in the Dutch Belted rabbit on days 8 through 11 of gestation resulted in the death of 3 of 26 maternal animals, embryoletality, as determined on animals sacrificed on day 28 of gestation, but no teratogenic effects.

Analysis:

This analysis shows the considerable difference in teratogenic effects of DMSO from species to species, and consequently, until studies are conducted on primate species, no reasonably definitive extrapolation can be made as to the potential teratogenicity of DMSO in humans.

⊕ Tates, A.D. and E. Kriek. 1981. Induction of chromosomal aberrations and sister chromatid exchanges in chinese hamster cells in vitro by some proximate and ultimate carcinogenic arylamide derivatives. *Mutat. Res.* 88:397-410.

Review:

One percent and ten percent DMSO were used as solvent controls in tests evaluating the chromosomal alterations caused by carcinogenic arylamide derivatives. The assays were carried out on cultures of Chinese hamster ovary cells in the presence and absence of an enzyme activating system prepared from rat liver tissue (S9 mix). Exposures were for 1 hr at 37°C. Treated cells were analyzed for chromosomal aberrations and sister chromatid exchanges. Ten percent DMSO induced significant numbers of chromosomal aberrations, but only when administered with S9 mix (85 aberrations per 100 cells with S9 mix, and 8 aberrations per 100 cells without S9 mix). The number of sister chromatid exchanges (SECs) per cell did not change significantly (13.9 ± 4.5 with S9 mix, and 8.9 ± 3.1 without). Metabolic activation did not increase the frequency of chromosomal aberrations at the 1% DMSO level, and the number of SECs was similar to that occurring in 10% DMSO.

Analysis:

An increase in chromosomal aberrations following DMSO exposure has been reported in a number of studies. In some cases metabolic activation was not required for the observed effect. However, in this study an S9 mix control would have been appropriate to verify that the reported effect was due entirely to DMSO.

⊕ van der Watt, J.J. and I.F.H. Purchase. 1970. The acute toxicity of retrorsine, aflatoxin and sterigmatocystin in vervet monkeys. *Br. J. Exp. Pathol.* 51:183-190.

Review:

As a solvent control in studies on the hepatotoxicity of sterigmatocystin, two vervet monkeys were injected intraperitoneally with 0.4 mL/kg or 2.0 mL/kg of DMSO (concentration not given). Both monkeys

survived for a 10-day-post exposure period and were then sacrificed. Histological examination revealed that liver tissue from the animal exposed to 0.4 mL/kg showed distinct centrilobular fatty changes, and tissue from the monkey receiving 2.0 mL/kg had diffuse hepatocellular fatty changes.

Analysis:

Four animals that received only methyl cellulose (5.0 mL) served as a second control group and none of these showed any microscopic alteration in liver tissue. The significance of the fatty changes seen in the DMSO animals was not evaluated in terms of liver function and enzyme activity.

• Vogin, E.E., S. Carson, G. Cannon, C.R. Linegar, and L.F. Rubin. 1970. Chronic toxicity of DMSO in primates. Toxicol. Appl. Pharmacol. 16:606-612.

Review:

Eighteen-month-long chronic DMSO toxicity studies were conducted on rhesus monkeys. Daily doses of 1, 3, or 9 mL/kg body weight of a 90% aqueous DMSO solution were administered topically, on the abdomen, or intragastrically in equally divided doses each morning and afternoon. Control animals received 9 mL/kg of water. Four or six test animals (male and female) were used in each test group.

Five of the six monkeys receiving 9 mL/kg per day orally died between weeks 15 and 53. These animals had emphysema and atelectasis which were the pathopneumonic causes of death. It was noted that it was impossible to exclude the probability that some regurgitation and or tracheal inspiration of the DMSO may have occurred. These animals were emetic and anorexic throughout the test period and exhibited considerable weight loss. Monkeys receiving smaller oral doses showed sporadic signs of emesis and ptyalism and weighed slightly less than controls at the end of the study.

Animals tested topically with DMSO exhibited localized scaling and flaking of the skin, but only during the initial phases of the study. Erythema of the skin occurred in some monkeys in all treated groups.

Physical examinations, conducted on all treated animals during weeks 1, 4, 7, 12, 24, 37, 51, and 73, revealed no DMSO-related changes in mean systolic blood pressure, heart rate, respiratory rate, body temperature, 48 hr water consumption, neurological reflexes, and electrocardiogram.

Ocular examinations revealed that one monkey (in the group receiving 1 mL/kg topically) had a unilateral complete retinal detachment and syneresis of the vitreous humor. None showed any signs of lenticular changes.

Hematologic tests, conducted at six-month intervals, and including blood cell counts, hemoglobin, hematocrit, and prothrombin, and biochemical tests such as blood urea nitrogen, glucose, serum glutamic-pyruvic transaminase, and serum alkaline phosphatase, were not significantly different from those of the control group. There were also no differences in erythrocyte sedimentation rate, BSP retention, creatinine clearance, urinalysis, and absolute or relative organ weights.

Histomorphologic examinations were made on liver, spleen, stomach, small intestine, large intestine, pancreas, kidneys, bladder, adrenalin glands, thyroid, pituitary, thymus, salivary glands, lymph nodes, heart, lungs, femoral bone marrow, skin, skeletal muscle spinal cord, brain, gallbladder, epididymis, seminal vesicles, prostate, uterus, aorta, larynx, trachea, peripheral nerve, diaphragm, and lacrimal glands, and no pathological lesions were observed in any of these organs.

Analysis:

Statistical analysis of the hematologic, biochemical, and urinalysis data would have been appropriate, but this may have been precluded by the small number of animals in each test group. Also, the time periods between dosing and blood and urine sampling were not given, and the possibility exists that transitory changes in hemotologic and/or biochemical parameters may have been overlooked. However, the most significant finding of this study was the absence of any pathological change in any organ system, even after eighteen months daily exposure to DMSO. Particularly important was the absence of hepatotoxicity, adverse erythropoietic activity, or lenticular abnormalities.

⊕ Willson, J.E., D.E. Brown, and E.K. Timmens. 1965. A toxicologic study of dimethyl sulfoxide. Toxicol. Appl. Pharmacol. 7:104-112.

Review:

Acute lethal toxicity assays were conducted on male and female albino mice (19-26 g) and Carworth CFN rats (112-140 g). Intravenous dose levels were 2.5, 5.0, or 10 g/kg. Oral dose levels were 10, 20, or 40 g/kg. The LD₅₀ values were 5.75 g/kg, iv, and 21.4 g/kg, oral, for mice and 5.36 g/kg, iv, and 28.3 g/kg, oral, for rats. Chronic exposure studies were conducted on groups of ten Carworth CFN rats receiving daily intraperitoneal injections of 0.5, 1.0, 2.0, 4.0, or 8.0 g/kg of undiluted DMSO six times per week for a maximum of 24 injections. Controls received normal saline. Half the animals were sacrificed at the end of the treatment period and half after a 4-week recovery period. Eight of the animals exposed to 8 g/kg/day died during the treatment period. Animals in this group were anemic (decreased hemoglobin and hematocrit values), and some exhibited reticulocytosis; however, the surviving animals had normal hematologic values after the 4-week recovery period.

Chronic exposure studies were also conducted on mongrel dogs. Groups of three or four animals received daily iv injections of 0.3,

0.6, 1.2, or 2.4 g/kg of pure or diluted DMSO (diluted in 5% dextrose) 6 times a week for a maximum of 24 doses. About half the animals were sacrificed 24 hr after the last dose and the remainder after a 3-4-week recovery period. All test animals survived doses as high as 1.2 g/kg. At 2.4 g/kg both males died during the first injection, and the females died at the 17th and 21st injection. The iv injection resulted in perivascular inflammatory reactions and intravascular thrombi at all four dose levels. Anemia, characterized by reduced red blood cell count, hemoglobin and hematocrit values, and accompanied by reticulocytosis, was generally dose correlated (data not given). The bone marrow appeared normal histologically, but showed signs of erythroid hyperplasia. Hemoglobinuria and hematuria were observed during the treatment period. Cloudy swelling and granularity of the parenchymal cytoplasm of the liver occurred at all dose levels, and biliary thrombi were occasionally seen at the 1.2 g/kg dose level. Serum bilirubin values and prothrombin times did not indicate any evidence of hepatic disease; however, bilirubinuria was frequently noted and serum transaminase values (primarily glutamic-oxalacetic) were slightly elevated in some dogs at the highest dose levels (data not given). Histological examination revealed accumulations of yellowish, brown pigment in sections of the liver, spleen, kidney, and at the injection site, at all dose levels.

Analysis:

Although not all the hematologic data were given in detail and although there was no statistical analysis supporting the conclusions of the authors, the reported hemolytic effects of high doses of DMSO are not in conflict with those of other studies. The reversibility of this condition has also been noted by other researchers.

APPENDIX A-2

REVIEW AND ANALYSIS OF
PERTINENT REFERENCES ON THE EFFECTS OF
THE INTERACTION OF DMSO WITH OTHER CHEMICALS

♣ Ansel, H.C. 1967. Hemolysis of erythrocytes by antibacterial preservatives. IV. Hemolytic activity of chlorhexidine diacetate. J. Pharm. Sci. 56:616-619.

Review:

DMSO was tested for its effect on chlorhexidine diacetate-induced hemolysis of erythrocytes. Heart blood of rabbits was collected, and washed erythrocyte preparations were incubated with 110 µg/mL of chlorhexidine for 45 min at 37°C. At the end of this period, the degree of hemolysis was determined colorimetrically. The effect of chlorhexidine-induced hemolysis was determined in the presence of various concentrations of DMSO (0.4-30%) and compared with agent-induced hemolysis in saline.

At a chlorhexidine concentration of 110 µg/mL, there was a progressive decline in the hemolysis of erythrocytes with increasing concentrations of DMSO up to 10%. A further increase in DMSO concentration reduced the protection because DMSO itself caused hemolysis with higher concentrations.

Analysis:

These results demonstrate the protection of the erythrocyte membrane by DMSO against the hemolyzing action of chlorhexidine at a concentration that otherwise would hemolyze 100% of the erythrocytes. Although the mode of protection was not elucidated from these studies, the author concluded that DMSO interferes with cellular mechanisms leading to hemolysis by chlorhexidine.

♣ Barilyak, I.R., L.V. Neumerzhitskay, and A.N. Turkevich. 1977. Antimutagenic and antiteratogenic properties of dimexide (DMSO). Cytol. Genetic 12:45-50.

Review:

This is a study in rats of the teratogenic and embryolethal properties of the antimalarial drug chloridin, and the antitumor drug 6-mercaptopurine (6 MP), and the modification of their effect by DMSO. Randomly bred white rats were given 50 mg/kg of chloridin ***** (2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine), or 60 mg/kg of 6-mercaptopurine po on day 13 of gestation. A group of these animals was injected ip with 5 mL/kg of DMSO 30 min before administration of chloridin or 6 MP. The rats were sacrificed on day 20 of gestation and the number of live fetuses and implantation sites recorded. Some of the fetuses were evaluated for the existence of external and internal malformations, i.e., cerebral, skeletal, limb, paw, and genito-urinary anomalies. In another series of experiments the same doses of drugs and of DMSO were given to young adult male rats. They were sacrificed 48 hr later and an evaluation of chromosome morphology in bone marrow was made.

The embryotoxicity (number of implantation sites), fetotoxicity (number of live fetuses), and teratogenicity were significantly reduced in the group of rats given DMSO before administration of the drugs. The percentage of fetal defects was reduced from 100 to almost zero in the DMSO treated animals. Results of the cytogenetic studies showed that the percentage of structural chromosome aberrations, e.g., aneuploidy, induced by chloridin or 6 MP was significantly reduced in rats pre-treated with DMSO.

Analysis:

DMSO given ip 30 min before po administration of chloridin or 6 MP reduced the embryolethal and teratogenic effects and the incidence of chromosome aberrations resulting from these drugs. Reduction of the effects on embryos was dramatic while protection against chromosome anomalies was moderate. The number of rats used per group and the number of cells scored for aberrations were adequate, but control groups given saline in place of DMSO were not included.

⊕ Beveridge, S.J., M.W. Whitehouse, and W.R. Walker. 1982. Lipophilic copper (II) formulations: Some correlation between their composition and anti-inflammatory/anti-arthritis activity when applied to the skin of rats. Agents and Actions 12:225-231.

Review:

Several copper complexes were tested for their ability to reduce inflammation or arthritic conditions in rats using various vehicle combinations of DMSO, glycerol, and ethanol. Inflammation was induced in the paws of male Wistar rats by an injection of hydroxylapatite or carageenan, and paw thickness was measured 2, 4, and 6 hr later. An arthritic condition was induced in the paws of PVG X DA male rats by an injection of arthritogenic adjuvant into the base of the tail. Twelve days later, the copper complexes in each of the vehicles were applied daily for 4 days to the skin over the swollen joints, and the reduction of paw thickness and the extent of skin lesions were recorded.

Of the various combinations of DMSO, glycerol, and ethanol tested as vehicles for copper salicylate, the most potent antiinflammatory and antiarthritic topical preparation contained DMSO with 20% glycerol. This preparation also produced minimal dermatotoxicity as judged by skin lesions.

Analysis:

The authors were convinced that because of the solvent and penetrating properties, DMSO is a preferred vehicle for the therapeutic use of copper complexes such as copper salicylate. The principal thrust of the paper, however, was the antiinflammatory and antiarthritic properties of many kinds of copper complexes rather than the effects of different vehicles.

A statistical analysis of the data was not presented, and the number of animals used was not specified in some experiments. Despite the fact that the data are principally of a qualitative nature, it appears safe to conclude that, compared to ethanolic formulations of copper salicylate, DMSO-glycerol preparations of the copper complex appeared to be more potent topically as an antiinflammatory and antiarthritic agent in rats.

◊ Cambon, C., Y. Fernandez, M. Falzon, and S. Mitjavila. 1981. Variation of the digestive absorption kinetics of carbaryl with the nature of the vehicle. Toxicology 22:45-51.

Review:

Studies were made of the effect of DMSO on the acute appearance and disappearance rate in blood of the insecticide carbaryl from gastric and intestinal sites of young adult female Wistar rats. The abdominal cavity of anesthetized animals was opened, and ¹⁴C carbaryl in DMSO (33% in water), olive oil, 2% gum tragacanth, or milk was injected intragastrically or intraduodenally. To measure the kinetics of carbaryl absorption from these sites, ¹⁴C and acetylcholinesterase determinations in the blood were made at hourly intervals.

When carbaryl was administered in the DMSO solution, the rate of absorption from the duodenum was 14 times faster than from the stomach. Absorption rates from the stomach and intestine were greater if carbaryl was given with DMSO than with tragacanth or milk. The rate of absorption of the insecticide from the duodenum was an order of magnitude higher when given in DMSO than in olive oil, milk, or tragacanth.

Over the 5-hr period studied, blood levels of carbaryl remained at the maximum attained levels and did not appear to be influenced by route of administration or vehicle. Thus, although absorption rates may be vehicle dependent, the final amount of carbaryl delivered to the blood was the same for all vehicles.

Analysis:

The data demonstrate differential absorption rates of carbaryl in DMSO from the stomach and intestine. This differential is not surprising in view of the fact that little absorption occurs from the stomach. The extremely rapid absorption of carbaryl from the duodenum in the presence of DMSO, compared to other vehicles, may reflect the ability of DMSO to enhance permeability of the intestinal epithelium to the insecticide. It appears, however, that the vehicle used had no effect on the maximum concentration and amount of carbaryl delivered to the blood.

◊ Dachi, S.F., J.E. Sanders, and E.M. Urie. 1967. Effects of dimethylsulfoxide on dimethylbenzanthracene-induced carcinogenesis in the hamster cheek pouch. Cancer Res. 27:1183-1185.

Review:

The purpose of these experiments was to determine the effect of DMSO on dimethylbenzanthracene-induced tumor parameters. DMSO was compared to mineral oil as a vehicle for dimethylbenzanthracene (DMBA) which was used to produce carcinomas in the cheek pouch of the male Syrian hamster. Seven groups of 16 animals (9 weeks old) each were used, and DMBA at concentrations of 0.005, 0.05, 0.1, or 0.5% was painted on the cheek pouch either twice or thrice weekly. Some animals were sacrificed 2, 5, 10, or 15 weeks after the appearance of tumors while others were sacrificed when impending death was obvious. The onset time of tumor appearance, the number and size of tumors, and survival time were recorded as well as a necropsy and histologic evaluation of each animal.

The incidence of squamous cell carcinoma was 100% in all groups that received DMBA except in the low dose DMBA-mineral oil group for which the incidence was zero. The same low dose applied in DMSO yielded 100% tumors.

In the groups that received either 0.1 or 0.5% DMBA, the mean latent period was significantly reduced by about 64% when DMSO was used as a vehicle. The mean survival time of animals with tumor was about the same whether DMSO or mineral oil was used as a vehicle.

In the group that received 0.5% DMBA in DMSO, 8 of the 10 surviving hamsters also had tumors on the contralateral cheek pouch while none were noted at this location in the DMBA-mineral oil group.

Regardless of which vehicle was used, there were no morphologic differences in the tumors induced by DMBA nor were any pathologic changes observed in other organs of any group, e.g., metastatic lesions.

Analysis:

Results of these studies conclusively demonstrate the augmentation of DMBA-induced carcinoma by DMSO. The protocols and data are presented unambiguously, and the conclusions are firmly supported by the results. A sufficient number of animals and an appropriate statistical analysis of the data were used. It should be noted that although tumor numbers were determined, no mention of them was made.

Compared to mineral oil, the principal effects of DMSO on DMBA-induced tumorigenesis were (1) a reduction in the latent period, (2) a 100% tumor incidence at a low dose of DMBA that in mineral oil yielded no tumors, and (3) the appearance of contralateral tumors. A minimal discussion of the results was presented.

⊕ Dixon, R.L., R.H. Adamson, M. Ben, and D.P. Rall. 1965. Apparent lack of interaction between dimethyl sulfoxide (DMSO) and a variety of drugs. Proc. Soc. Exper. Biol. Med. 118:756-759.

Review:

These experiments were designed to determine whether the toxicities of a variety of drugs commonly used clinically were altered by DMSO (25% in saline) as a vehicle compared to saline. As a measure of toxicity, LD50s and barbiturate sleeping time in male Swiss mice were determined for these drugs administered ip or orally. Using at least four dose levels and ten mice per dose, the LD50 of the following drugs given ip were determined in 25% DMSO or in saline: chlorpromazine, curare, insulin, morphine, ouabain, penicillin, pentobarbital, vincristine, and 1,3-bis(2-chlorethyl)-1-nitrosourea. Aspirin and penicillin were also tested po. In addition the effect on sleeping time of DMSO solution (2.5 mg/kg) administered sc 1 hr, 24 hr, 7 days, or daily for 7 days before ip injection of 100 mg/kg of barbiturate was determined.

Results of these tests showed that for each drug, the LD50 in DMSO solution was essentially the same as that in saline. Likewise, none of the drugs changed the barbiturate sleeping time.

Analysis:

The tabular data presented in this article show that DMSO solution as a vehicle for ten commonly used drugs in human medicine does not alter their toxicity in mice. The authors state also that none of the drugs changed barbiturate sleeping time, but data are not presented. This finding would suggest that DMSO had no detectable effect on barbiturate detoxification by the liver. The administration route for some of the drugs was omitted and some of the results are simply stated without including tables or graphs of data.

• Elzay, R.P. 1967. Dimethylsulfoxide and experimental oral carcinogenesis in the hamster pouch. Arch. Path. 83:293-297.

Review:

DMSO and mineral oil were compared as vehicles for 7,12-dimethylbenzanthracene-induced (DMBA) oncogenesis in the hamster cheek pouch. Five-week old golden Syrian hamsters were divided into 3 groups of 25 animals each. DMBA (0.5%) in DMSO or mineral oil was applied to the cheek pouch 3 times a week for 11 weeks, and the animals were observed for an additional 12 weeks. The hamsters were sacrificed, the tumors counted and graded, and tissues prepared for histologic evaluation.

The mean latent period for grade 1 and 2 papillomas was 127 days in the DMBA-mineral oil group and 114 days in the DMBA-DMSO group. Therefore, DMSO as a vehicle did not significantly alter the mean time of appearance of papillomas. However, based on clinical and histologic evidence, the author concluded that DMSO augmented DMBA-induced carcinogenesis in the cheek pouch epithelium. This conclusion was based on two observations. First, although 100% tumor incidence was observed for DMBA in each vehicle, the DMBA-DMSO group had more grade 1 and 2 papillomas and fewer nodules; second, carcinomatous transformations and carcinoma

incidence were higher in the DMBA-DMSO group. Mortality figures were tabulated and amounted to 41% in the DMBA-DMSO group and 60% in the DMBA-mineral oil group. An evaluation of this difference cannot be made in terms of the vehicle effect because the author stated that deaths appeared to result from wet tail, which is a consequence of an infectious disease in hamsters.

Analysis:

This is a study which emphasized the histologic characteristics of tumors induced by DMBA administered either in DMSO or mineral oil. The results indicate that while the mean latent period for appearance of papillomas was not changed by DMSO, the histologic evidence supports the view that DMSO augmented DMBA carcinogenesis compared to mineral oil as a vehicle.

♦ Feldmann, R.J. and H.I. Maibach. 1966. Percutaneous penetration of ¹⁴C hydrocortisone in man. Arch. Derm. 94:649-651.

Review:

A study was made in vivo of the effect of vehicles on the penetration of hydrocortisone and testosterone through skin of the human forearm. Each vehicle was prepared as a 25% solution in acetone, and 60 µg of each radiolabeled steroid was applied in a volume of 0.1 mL to the skin. After the acetone had evaporated, the residual steroid concentration amounted to a 0.24% solution. Urine was collected over the next 5 days and analyzed daily for radioactivity. Percutaneous penetration of the skin by the steroids was judged by their cumulative excretion in the urine. Four or five subjects were used for each experiment.

The cumulative 5-day urine excretion of hydrocortisone applied in DMSO was fourfold higher than when applied in acetone only (i.e., hydrocortisone alone because acetone evaporated shortly after application). The penetration rate of hydrocortisone was greater from the DMSO solution than from solutions of dimethylformamide, dimethylacetamide, propylene glycol, or mineral oil. The same kind of results were obtained with testosterone. From the data presented, only about 1% of the applied hydrocortisone and 12% of the testosterone had been excreted in 5 days.

Analysis:

This study is one of the few reviewed that demonstrated in humans the enhancement of skin penetration of substances by DMSO in vivo. The limitations of using urinary excretion of a substance applied topically to assess skin penetration kinetics are not well defined, but the technique does represent a first approach to in vivo studies in humans.

♦ Finogenova, M.A. 1974. Effect of dimethyl sulfoxide as the carcinogen solvent on induced carcinogenesis of the skin in mice. Bull. Exper. Biol. Med/ 77:167-168.

Review:

Mice were used to study the effect of DMSO on the induction of papillomas and carcinomas by 20-methylcholanthrene (MC). Adult male (CBA X C57BL)F₁ mice were exposed percutaneously to MC in DMSO or benzene. Solutions (20 µL) were applied to the clipped skin of animals once a week presumably for the entire observation period. Two concentrations of MC were tested, 0.25% and 0.5% in DMSO and in benzene; 39 to 49 mice were used in each group.

In the groups exposed to MC in DMSO, the onset of papillomas and carcinomas was 1 to 3 weeks earlier and the mean period of development was 0.4 to 1 week earlier than observed for the benzene-MC group. There were no differences in the mean number of papillomas between the two groups.

Analysis:

In all cases, the data show that differences in onset time and mean period of development of tumor were slight despite the fact that some were stated to be significant at the 5% level. A more conservative appraisal of the data suggests that compared to benzene as a vehicle for MC, DMSO had no influence on skin tumor parameters in mice.

♣ Freston, J.W. and I.A.D. Bouchier.***** Potentiation of carbon tetrachloride toxicity by dimethyl sulfoxide.

Review:

The purpose of this study was to elucidate the effect of DMSO on carbon tetrachloride (CCl₄) toxicity in fasting female Sprague-Dawley rats. CCl₄ in an equal volume of paraffin was given po simultaneously with an ip injection of DMSO at 2 mL/kg of a 90% solution. Control rats received saline rather than DMSO.

Toxicity was evaluated in terms of the LD₅₀, changes in liver lipid and serum enzymes, and the serum and liver kinetics following absorption of radioactive (¹⁴C) CCl₄. The latter measures were made to determine if DMSO enhanced gastrointestinal absorption or hepatic uptake of CCl₄.

The LD₅₀ of CCl₄ was reduced from 4.26 mL/kg to 2.22 mL/kg when administered with DMSO (number of doses and rats not specified). CCl₄ with DMSO also enhanced CCl₄ toxicity in regard to total liver lipid and centrilobular liver necrosis and exaggerated the mean and range of serum aspartate transaminase level associated with hepatotoxicity. Blood kinetics of labeled CCl₄ were not altered by DMSO, but CCl₄ in the liver was retained at higher levels longer in DMSO-treated animals.

Analysis:

These results demonstrate that DMSO given ip increased the toxicity of CCl₄ in terms of lethality and as judged by hepatic injury as

reflected in histologic criteria by CC14 retention by the liver, and by serum levels of an enzyme associated with hepatotoxicity. However, the reduction in LD50 for the CC14-DMSO group was quite small and may be of borderline significance despite the fact that the 95% confidence intervals did not overlap. Similarly, the increase in liver injury parameters was moderate at best in the rats given CC14 and DMSO.

⊕ Gill, T.S., M.S. Guram, and W.F. Geber. 1981. Comparative study of the teratogenic effects of chlordiazepoxide and diazepam in the fetal hamster. Life Sciences 29:2141-2147.

Review:

One purpose of this series of experiments was to compare the teratogenicity of diazepam in different vehicles following administration to pregnant animals. Syrian golden hamsters (10 per group) were given a single ip injection of diazepam in undiluted DMSO, carboxymethylcellulose, (0.5% in water) or sorbitol (70% in water) on day 8 of gestation. The animals were sacrificed on day 12 to examine the fetuses for external malformations.

The percentage of external malformations (e.g., atrophy, exteriorized organs, abnormal cranial features), in fetuses of hamsters given 130 or 370 mg/kg of diazepam was highest when DMSO was the vehicle. The incidence of malformations was almost 90% in the diazepam-DMSO group compared to less than 11% in the diazepam-carboxymethylcellulose or diazepam-diazepam-sorbitol group.

Analysis:

These results show that of the three substances tested as vehicles for diazepam, DMSO resulted in the highest incidence of external abnormalities in fetal hamsters. Therefore, the synergistic effect of diazepam teratogenicity of DMSO was established. A more complete analysis of the potentiation would have included documentation of implantation and resorption sites as well as an evaluation of internal malformations, such as skeletal abnormalities, and a statistical evaluation.

⊕ Highman, B. and P.D. Atland., 1969. Effect of dimethyl sulfoxide on serum enzymes and tissues of rats given epinephrine and cortisone. Life Sciences 8:673-683.

Review:

Experiments with young adult male Sprague-Dawley rats were conducted to examine the influence of DMSO on tissue changes induced by epinephrine and/or cortisone. DMSO was injected ip at 4.5 g/kg immediately before sc injection of the adrenal hormones. At intervals up to 48 hr after treatment, cardiac blood was obtained and analyzed for glutamic oxalacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT),

aldolase (AL), alkaline phosphatase (ALP), lactic dehydrogenase (LDH), malic dehydrogenase (MDH), urea nitrogen (N), and glucose. Major organs were processed for histologic examination, especially for lipid and glycogen changes.

The epinephrine-induced increases in the serum enzymes and glucose were either unchanged or moderately increased by DMSO administration. The epinephrine-induced "fatty acid changes" in the heart (but not liver or muscle) and lipid depletion in the adrenals were diminished by DMSO. Also, DMSO reduced the severity of liver necrosis produced by epinephrine-cortisone administration.

Analysis:

The effect of DMSO on tissue damage resulting from epinephrine and/or cortisone are incidental to the main theme of this report. Nonetheless, the results support the conclusion that DMSO given before epinephrine affords some protection against tissue injury as reflected by the appearance of enzymes in the serum associated with that injury. DMSO also appeared to provide partial protection against epinephrine-induced fatty accumulation in the heart and lipid depletion in the adrenals as well as against epinephrine-cortisone-induced liver necrosis.

♦ Iversen, O.H., E. Thorud, and G. Volden. 1981. Inhibition of methylcholanthrene-induced skin carcinogenesis in hairless mice by dimethyl sulfoxide. Carcinogenesis 2:1129-1133.

Review:

This study was made to determine whether the addition of DMSO to acetone as a vehicle for 20-methylcholanthrene (MC) effected a change in oncogenesis in mice. Male and female hairless Oslo strain mice were given five topical applications of 470 n moles of MC in 0.2 mL at one week intervals. A 50/50 mixture of DMSO and acetone was used as a vehicle for MC in one group of 56 animals while MC in acetone was used as a vehicle for a control group of 47 mice. The animals were observed over a 75-week period during which time development of papillomas and carcinomas was recorded.

The results showed that the group treated with MC in DMSO/acetone had higher mortality than the acetone-MC group. On day 75, only 50% of the DMSO/acetone-MC group were alive compared to 68% of the acetone-MC mice. In the DMSO/acetone-MC group, the onset of tumor appearance was delayed 4 weeks and the subsequent rate of appearance of all tumors was less than in the acetone-MC group. On day 75, the tumor incidence in the DMSO/acetone-MC group was significantly lower (63%) than in the acetone-MC group (81%). There was also a lower incidence of carcinoma in the DMSO/acetone-MC mice, but it was of borderline significance.

Analysis:

The data from the experiments indicate that DMSO in the DMSO/acetone vehicle for MC had an inhibitory effect on MC-induced skin carcinogenesis in mice. It also appeared that DMSO rendered MC more toxic as reflected in the higher mortality in that group of mice. Effects of DMSO on MC metabolism or delivery of MC to target tissues were considered as possible explanations for these effects.

⊕ Joesten, M.D. and R.A. Hill. 1966. Toxicity of metal complexes of octamethylpyrophosphoramidate in water and dimethylsulfoxide. J. Agr. Food Chem. 14:512-514.

Review:

One purpose of this study was to determine the effect of DMSO on the toxicity of octamethylpyrophosphoramidate (OMPA) and of metal salts and their complexes with OMPA. Adult Ha ICR Swiss Webster male mice were used to determine LD50s (16 to 41 mice per substance) and to record observable toxic signs for the test agents administered ip in 0.2 mL of DMSO or water.

According to the LD50s and toxic signs, the toxicities of $\text{NaClO}_4 \cdot \text{H}_2\text{O}$, or $\text{FeCl}_3 \cdot \text{H}_2\text{O}$, were about the same whether given in DMSO or water, i.e., about 1150 and 300 mg/kg, respectively. OMPA alone was somewhat more toxic in DMSO than in water. Of the 6 metal-OMPA complexes studied (i.e., Fe, Na, Mg, Mn, Co, Zn), only iron in a FeCl_4 form was more toxic in DMSO (LD50 4.8 mg/kg) than in water (LD50 21 mg/kg); and the LD50s of the other complexes were not significantly different from those in water. However, it was observed that compared to water as a vehicle, the metal complexes administered in DMSO extended the time range over which deaths occurred.

Analysis:

The minimum acceptable number of mice per dose and number of doses tested were employed in this study. The data however would appear to show that for mice, an ip injection of Na, Mg, Mn, Co, or Zn complexes of OMPA were no more or less toxic in DMSO than in water as a vehicle. The iron complex was about 4 times more toxic in DMSO than in water. The extended length of time over which deaths occurred when the vehicle was DMSO suggests an altered mode of toxicity.

⊕ Kastin, A.J., A. Arimura, and A.V. Schally. 1966. Topical absorption of polypeptides with dimethylsulfoxide. Arch. Derm. 93:471-473.

Review:

The purpose of these studies was to determine whether DMSO compared to saline altered topical absorption of antidiuretic hormone or adrenocorticotropin in the rat and melanocyte stimulating hormone in the frog.

Young adult Sprague-Dawley rats were prepared in a way that allowed determinations of the rate of urine flow following application of antidiuretic hormone (ADH) to shaved abdominal skin. Two units of ADH were applied either in saline or in a 70% solution of DMSO in saline. In a second series of experiments, 5 units of corticotropin in saline or 70% DMSO were applied to the shaved skin of rats, and, at intervals, blood was analyzed for the concentrations of the adrenal steroid. In a third series, the skin of hypophysectomized adult male Rana pipiens was exposed to from 400 to 4000 units of melanocyte-stimulating hormone (MSH) and the degree of darkening of melanin pigment granules in dermal melanocytes of the web of the foot was determined 1 hr after application.

Only 1 of 6 rats in the DMSO-ADH group showed a clearly positive response to ADH (reduced urine flow) that was greater than observed for animals in the saline-ADH group. Therefore, results of this series were inconclusive. Over a 2-hr interval studied in groups of 5 to 15 rats, the plasma concentrations of corticosterone (derived from percutaneous corticotropin) at four intervals were essentially the same whether the steroid was applied with saline or with 70% DMSO. Thus, DMSO appeared not to alter the percutaneous penetration of corticotropin.

In 3 of 5 experiments utilizing 4 to 22 frogs per group, the MSH-DMSO animals manifested a melanocyte response significantly greater than that of the MSH-saline group. For reasons not stated, the authors concluded that the small increase in response (20 to 70%) was probably insignificant.

Analysis:

Of the three responses studied, the results with ADH are weak because only 6 rats were used, and no meaningful conclusions can be drawn from the data. Clearly, DMSO did not enhance penetration of corticotropin through rat skin. In view of the data and the authors' conclusions, the influence of DMSO on penetration of MSH in frog skins can only be considered questionable. It could be concluded from these experiments that DMSO did not appreciably alter the penetration of topically applied ADH, corticotropin, or MSH.

♦ Klaassen, C.D. 1973. The effect of altered hepatic function on the toxicity, plasma disappearance and biliary excretion of diethylstilbestrol. Toxicol. Appl. Pharmacol. 24:142-149.

Review:

One of the purposes of this investigation was to determine the toxicity of the synthetic steroid diethylstilbestrol (DES) in DMSO, ethanol, and propylene glycol (PG). Adult male Sprague-Dawley rats were used to determine the 24 hr LD50 for intact and bile-duct-ligated (BDL) animals. The LD50s for DES in DMSO (10 mL/kg), ethanol (2.5 mL/kg), and PG (5 mL/kg) were, respectively, 100, 34, and 530 mg/kg. Comparable LD50s in BDL rats were reduced to 0.75, 0.47, and 99 mg/kg.

respectively. In animals given DES in DMSO, deaths usually occurred in 1 hr while deaths from DES in PG occurred 18-24 hr after dosing.

Analysis:

Results of these experiments show that of the three vehicles used for oral administration of DES, this steroid was most toxic in ethanol and least toxic in PG with DMSO intermediate. Although the same order of toxicity for DES in the three vehicles was observed in animals with blocked biliary excretion (BDL), the LD₅₀ of DMSO was 140 times less in BDL than in intact rats. Why DMSO rendered DES much more toxic in BDL rats than in normal animals probably resulted from faster absorption of DES by intestinal epithelium, which may have accounted for the rapid rate of deaths in the BDL group.

The small numbers of rats per group (5) used in these experiments reduces the impact of the results; yet the large differences in LD₅₀s indicate that the qualitative aspects of the data are valid.

♦ Kocsis, J.J., S. Harkaway, M.C. Santoyo, and R. Snyder. 1968. Dimethyl sulfoxide: Interactions with aromatic hydrocarbons. Science 160:427-428.

Review:

Rats (Wistar males) and mice were used to study the effects of DMSO on the toxicity of benzene, chlorobenzene, and toluene. By using 24-hr urinary excretion of taurine as a direct measure of toxicity, the authors showed that more of this metabolite was excreted if any of the hydrocarbons are injected ip together with DMSO. In another study, all 8 rats died when given 5 mL/kg of DMSO followed by 1 mL/kg of benzene ip whereas only 2 of 18 rats died if the same amount of diluted (25%) DMSO was given before the benzene. Even 5 mL/kg of benzene did not kill any of the 20 rats injected.

In further experiments with mice, the LD₅₀ of benzene was determined with and without DMSO. The LD₅₀ for benzene alone was 0.34 mL/kg; when given with DMSO it was reduced to 0.092 mL/kg. The slopes of the survival curves for the two treatments were also different, suggesting that the mechanism of toxicity of benzene was different from that of benzene plus DMSO.

Experiments were carried out in vitro with rat liver homogenates to determine the effect of DMSO on conversion of benzene to phenol. Data showed that regardless of how the DMSO was given (po, ip, sc, or dermal) the amount of phenol and its conjugates was increased in rats given DMSO 24 hr before benzene injection. Daily application of DMSO for 7 days prior to benzene injection further increased liver metabolism of benzene. There was an indication that the enhanced toxicity of benzene by DMSO was reduced if benzene was given sc or iv, suggesting that enhancement of benzene toxicity involved access to the liver.

Analysis:

These investigations provide much important work about benzene-DMSO interactions. Some of the experiments are well documented while data of others are simply stated. There is no reason to believe that the qualitative aspects of the data are not valid. The small size of some of the experiments may leave the quantitative validity questionable. The results provide firm evidence for enhancement of benzene toxicity by DMSO.

♣ Kocsis, J.J., S. Harkaway, and R. Snyder. 1975. Biological effects of the metabolites of dimethyl sulfoxide. Ann. N.Y. Acad. Sci. 243:104-109.

Review:

Rats and mice (type not stated) were used to determine the effect of DMSO or two of its metabolites, dimethylsulfone (DMSO₂) and dimethyl sulfide (DMS), on toxicity induced by hydrocarbons. Toxicity was assessed by urinary excretion of taurine and by LD₅₀ determinations. Additional studies were made of the anticholinesterase toxicity of paraoxon, tetraethyl pyrophosphate (TEPP), and octamethyl pyrophosphamide (OMPA).

The results showed that pretreatment of rats ip with 5 mL/kg of DMSO or 5 g/kg of DMSO₂ shortly before giving benzene or chlorobenzene ip markedly increased the urinary excretion of taurine. However, DMS did not enhance taurine excretion. In LD₅₀ experiments with mice, DMSO given ip at 5 mL/kg mixed with benzene, chlorobenzene, carbon tetrachloride, or toluene potentiated (up to a factor of 10) the toxicity of these hydrocarbons. Neither DMSO₂ nor DMS potentiated the toxicity of benzene. In one series of experiments, DMSO at 5.5 g/kg or DMSO₂ at 5 g/kg antagonized the lethal effects of all three anticholinesterases whereas DMS did not.

Analysis:

The data show that DMSO and one of its metabolites, DMSO₂, but not another metabolite, DMS, enhanced the toxic effects of benzene or chlorobenzene as reflected by an increase in the urinary excretion of taurine. The lethality resulting from these hydrocarbons was also potentiated by DMSO but not by DMSO₂ or DMS (data not presented). In contrast, DMSO antagonized the lethal effects of three anticholinesterases. This is a good series of experiments, some of the results of which were published in previous documents.

♣ Landauer, W. and N. Salam. 1972. Aspects of dimethyl sulfoxide as solvent for teratogens. Dev. Biol. 28:35-46.

Review:

The toxicity and teratogenicity in chicken embryos of seven diverse teratogens were compared in DMSO and water. The substances tested were 3-acetylpyridine (5 mM), 6-aminonicotinamide (0.041 mM), sulfanilamide (7.5 mM), 3-amino-1,2,4-triazol (47.6 mM), 3-hydroxy-N,N-dimethyl-cis-crotonamide dimethyl phosphate (0.21 mM) (Bidrin), physostigmine (0.46 mM), or nicotine (15.4 mM). These substances were injected into the yolk sac of 4-day-old embryos, and the eggs were incubated for another 15 days. The embryos were removed from the shell and were examined for viability and for a variety of morphologic abnormalities.

Compared to water, DMSO as a vehicle for the substances had the following effects on chicken embryos:

1. For 3-acetylpyridine, mortality and muscle abnormalities were reduced.
2. For sulfanilamide, mortality was reduced but the incidence of beak and limb abnormalities was increased.
3. For physostigmine or nictotine, mortality was reduced and there was no effect on the incidence of abnormalities.
4. For 6-aminonicotinamide, there was no effect on mortality but the incidence of beak abnormalities was reduced.
5. For Bidrin, there was no effect on mortality or on the incidence of beak and limb abnormalities, but there was a decrease in the incidence of neck abnormalities.
6. For 3-amino-1,2,4-triazole, there was no effect on mortality, but there was an increase in the incidence of beak abnormalities.

Analysis:

The results of these studies show that compared to water, DMSO as a vehicle either protected against the lethal effects of each substance or had no effect. In no case did DMSO increase the lethality of the substances. DMSO increased, reduced, or had no effect on the teratogenicity of each substance. No broad generalizations can be drawn from these data but they do underline the varied nature of the effects of DMSO as a vehicle for a group of diverse teratogens.

♦ Levine, W.G. 1975. Effect of dimethylsulfoxide on the hepatic disposition of chemical carcinogens. Ann. N.Y. Acad. Science 243:185-193.

Review:

The authors studied the influence of DMSO on hepatic binding of the chemical carcinogens methylcholanthrene (MC) and benzo(a)pyrene (BP). They studied biliary excretion rates of the carcinogens and binding of

the carcinogens to subcellular liver fractions in young adult female Wistar rats injected iv with labeled MC or BP.

Biliary excretion of metabolites of MC and BP was faster when DMSO was the vehicle rather than 1% albumin. In liver distribution studies, DMSO compared to 1% albumin enhanced binding of MC and BP to the microsomal fraction. The data indicate that a DMSO-carcinogen complex can be formed that has binding characteristics different from that of carcinogen per se. A change in binding characteristics occurs only if the carcinogen is dissolved in DMSO because a BP-DMSO complex was not formed in vitro.

Analysis:

Although the data presented are not extensive, these were critical experiments, the results of which show that DMSO can alter microsomal binding of MC and BP and thereby enhance the biliary excretion of these carcinogens.

♦ Mallach, H.J. 1971. Interaction of DMSO and alcohol. In: S.W. Jacob, E.E. Rosenbaum, and D.C. Wood, eds. Dimethyl Sulfoxide. pp. 281-293. Marcel Dekker, New York.

Review:

Studies in mice and humans were made to investigate the possible enhancement of ethanol toxicity by DMSO.

Animal studies - Young adult male and female white mice (20 per group) were given DMSO and ethanol orally. When both agents were administered together at a constant ethanol dose, mortality increased with increasing doses of DMSO. At about 0.8 of the DMSO LD50 and at an LD55 dose of ethanol, mortality was almost 100%. Thus, the toxic effect of the two agents was slightly more than additive. In contrast, mortality was greatly increased if ethanol was given 60 min after DMSO; and mortality was 100% at all doses of DMSO if ethanol was administered 60 min before DMSO. These results showed that at a nonlethal dose of DMSO and an LD50 dose of ethanol, toxicity of ethanol was increased when there was a 60-min interval between administration of the two substances.

Human studies - Young men were used to study the effects of topical DMSO (50 mg/kg) on oral ethanol (0.75 g/kg) as judged by blood ethanol levels, dimethyl sulfide (DMS) levels in expired air, urine flow, and mental functions. In one group of 15 men ethanol was administered concomitantly with DMSO applied percutaneously to the back. In another group of 16 men, DMSO was applied 1 hr before ethanol consumption; and a third group of 16 received ethanol only. Over a 6-hr period, blood samples were obtained and analyzed for ethanol. Results of this series showed that the decrease in blood levels of ethanol was slightly faster in men given DMSO than in men given ethanol only.

Gas chromatograms of expired air indicated that DMS concentration was higher in men given DMSO 60 min before ethanol than in men given DMSO and ethanol concomittantly. It was suggested that ethanol could have demethylated DMSO giving rise to DMS.

There were no perceptable differences in urine flow of men given ethanol and DMSO than in the group given ethanol only. Therefore, DMSO did not alter ethanol-induced diuresis.

Mental function tests were performed that measured attention focus and concentration, reliability and speed of reaction, and adaptability and stress capacity. The results demonstrated that deterioration in mental performance was significantly greater after administration of DMSO and ethanol than after ethanol alone.

Analysis:

This book chapter deals with important aspects of possible interaction between DMSO and ethanol in mice and humans. The report is one of the few of its kind carried out with humans and as such is a useful reference. The mortality data from about 1000 mice demonstrated that the toxicity of ethanol was increased by a nonlethal dose of DMSO given 1 hr before or after ethanol, but given together, lethality of the two agents was essentially additive. Mention was made of additional experiments with about 3000 mice wherein further support for potentiation was obtained, but data were not presented.

Principally because of the manner in which the human data are presented, it was difficult to evaluate the experiments in which the effect of DMSO on blood ethanol levels was determined. Hourly decline rates were given, and, despite the small differences in rates among the groups, the author states that there was a more rapid decline in blood ethanol when DMSO was given with or before ethanol administration. Mention is made of preliminary results showing effects of DMSO on ethanol alterations in motor function, but details are not presented.

The human data point to an unidentified relationship between DMSO and ethanol as reflected by changes in DMS levels in expired air and to diminished ethanol-induced cognitive functions in men treated simultaneously with DMSO.

These extensive but seemingly preliminary results from human experiments are of value because they underline several possible modes by which DMSO may influence ethanol toxicity; however, they do not provide definitive information about the interaction between DMSO and ethanol.

⊕ Mancini, R.E. and J.J. Kocsis. 1974. Dimethylsulfoxide increases the lethality of CCl₄ in rats but decreases its hepatotoxicity. Toxicol. Appl. Pharmacol. 27:206-209.

Review:

The investigators used young male Sprague-Dawley rats to determine the effect of DMSO on CCl₄ lethality and hepatotoxicity. For lethality studies, LD₅₀s were obtained; for hepatotoxicity, barbiturate sleeping time, accumulation of liver triglycerides, and plasma BSP retention were measured.

When mixtures of DMSO and CCl₄ were administered ip, the LD₅₀ was reduced to about 5% that obtained with CCl₄ alone. The LD₅₀ of CCl₄ alone was 3.5 mL/kg (2.5-4.9); with DMSO it was 0.16 mL/kg (0.067-0.38). The slope for both was about 1.86.

In rats given 0.08 mL/kg of CCl₄ together with 5 mL/kg of DMSO ip, there was a decrease in sleeping time, in triglyceride accumulations in the liver, and in plasma retention in BSP compared to rats given CCl₄ only.

The results demonstrate that in rats, DMSO increased lethality of CCl₄ but reduced hepatotoxicity according to results of the three liver function tests. The similarity of slopes for the mortality dose-response to CCl₄ and CCl₄-DMSO mixture indicates action of a single toxicant species, i.e., CCl₄. The divergence of effect of CCl₄-DMSO mixture on lethality and liver functions suggests that lethality of CCl₄ may not be the result of hepatotoxicity.

Analysis:

The impact of these important observations are somewhat offset by lack of data (i.e., presentations for the LD₅₀ determinations), but the hepatotoxic data are conclusive.

♦ Melville, K.I., B. Klingner, and H.E. Shister. 1968. Effects of dimethylsulfoxide (DMSO) on cardiovascular responses to ouabain, proscillaridin and digitoxin. Arch. Int. Pharmacodyn. Therap. 174:277-293.

Review:

The purpose of these experiments was to evaluate the influence of DMSO on the cardiovascular response to drugs known for their action on the heart. Male and female cats were anesthetized and rigged for cardiac monitoring. Continuous blood pressure and electrocardiographic recordings were maintained to determine the character of the effects of ouabain, proscillaridin, and digitoxin with and without DMSO. DMSO and various doses of the drugs were injected iv, alone or mixed.

Results showed that a single injection of 0.5 mL of 50% DMSO in saline potentiated the onset of bradycardia and arrhythmias and development of ventricular fibrillation induced by ouabain or proscillaridin. DMSO did not increase the blood pressure reducing effects of these drugs nor did DMSO alter the cardiac effects of digitoxin.

Continuous infusion of ouabain or proscillaridin mixed with DMSO reduced the onset time of bradycardia but not of arrhythmias or fibrillation. Results of timed, sequential, and multiple injection studies indicated that the cardiac effects of ouabain or proscillaridin in the presence of DMSO are less than additive. DMSO did not enhance accumulation of any of the three glycosides studied. The potentiating effects of DMSO observed in this series of studies were found to be transient.

Analysis:

The carefully conducted and documented studies show that with non-fibrillating single doses of two cardiac glycosides, prior injections of DMSO enhanced the onset of bradycardia, arrhythmias, and ventricular fibrillation; augmentation of digitoxin action was not observed. Continuous injection with DMSO and ouabain or proscillaridin did not alter any facet of cardiac dysfunction. In contrast, repeated injections of DMSO following ouabain or proscillaridin led to outbursts of arrhythmias and hypotension. The data also indicate that DMSO does not result in a change in rate of uptake or loss of glycosides in the heart.

Despite the demonstrated effects of these glycosides on cardiovascular functions, none appear to be life threatening and are transient. It should also be noted that the enhancing effects of DMSO occurred at low but not high doses of the cardiac glycosides and, indeed, were absent or minimal for digitoxin.

♠ McCullough, J.L., D.S. Snyder, G.D. Weinstein, A. Friedland, and B. Stein. 1976. Factors affecting human percutaneous penetration of methotrexate and its analogues in vitro. J. Invest. Dermatol. 66:103-107.

Review:

In this study various vehicles were tested to determine their effect on percutaneous absorption of methotrexate (MTX) by excised human skin. Tritiated MTX was deposited on skin, which was mounted in special chambers, and the preparations were incubated at 37°C for 20 hr. The fluid below and in constant contact with the skin and the skin itself were analyzed for radioactivity and the results expressed as the percentage of radioactivity deposited on the skin.

Compared to water as a vehicle, 80% DMSO in water increased the penetration of MTX (0.05% solution) through the skin by a factor of 1.8 during the 20-hr incubation period. The concurrent accumulation of MTX in the skin was only half that observed with water as a vehicle.

Analysis:

From their data, the authors concluded that enhancement of MTX penetration by DMSO was minimal (i.e., factor of 1.8) compared to water. The fact that an in vitro system was used is important in drawing conclusions about penetration rate because the skin used was not viable and was not supplied by a subcutaneous network of drainage vessels. In addition, increasing the total flux of MTX across the skin by a factor of 1.8 in 20 hr could be considered a significant alteration in transport.

♣ McDermot, H.L., A.J. Finkbeiner, W.J. Wills, and R.M. Heggie. 1967. The enhancement of penetration of an organophosphorous anticholinesterase through guinea pig skin by dimethyl sulfoxide. Can. J. Physiol. Pharmacol. 45:299-303.

Review:

These studies were carried out to determine whether and to what degree DMSO altered the penetration of an anticholinesterase through the skin. Guinea pigs were clipped or shaved 12 hr before application of the preparations. In one series of experiments, the stratum corneum was removed by stripping with cellulose tape. Pinacolyl methylphosphorofluoridate (soman) was mixed with DMSO in different proportions and was applied to the skin in 1- μ L drops. Penetration of soman was assessed by the mortality resulting from its anticholinesterase characteristic and expressed as the LD₅₀/24 hr.

With a 50/50 mixture of soman and DMSO applied to the intact skin, the LD₅₀ was 2.16 mg/kg compared to 12.8 mg/kg with soman alone. With the stratum corneum removed, the comparable LD₅₀s were 0.35 and 1.33 mg/kg. In both experiments, DMSO enhanced penetration of soman to about the same extent.

In another series, the effect of 90, 50, 25, and 0% DMSO with soman on the LD₅₀ was assessed. With increasing proportions of DMSO, the LD₅₀ of soman was progressively decreased, and at a 90/10 ratio of DMSO/soman, the LD₅₀ was 0.85 mg/kg compared to 12.8 for soman alone.

Analysis:

This is a concise document evaluating the effect of DMSO on percutaneous penetration of soman. The results are based on an adequate number of animals used to estimate the LD₅₀ and 95% confidence limits. The data clearly show that DMSO enhanced percutaneous penetration of soman and that the extent of enhancement was a direct function of DMSO concentrations. Enhancement occurred about equally through intact skin and skin from which the stratum corneum had been removed. No mention was made of the effect of DMSO on the time of onset of symptoms of soman poisoning, which might have given an additional indication of the percutaneous penetration rates of soman in the presence and absence of DMSO.

♣ McDermot, H.L., G.W. Murray, and R.M. Heggie. 1965. Penetration of guinea pig and rabbit skin by dimethyl sulfoxide solutions of a quaternary oxime. Can. J. Physiol. Pharmacol. 43:845-848.

Review:

The purpose of this study was to determine the effect of DMSO on the percutaneous penetration of the quaternary salt 1-methyl-2-hydroximino-methylpyridinium methane sulfonate (2PS). Guinea pigs and

rabbits were clipped at least 12 hr before application of 2PS in DMSO at a concentration of 150 mg/mL or in water to all of the skin except the head and legs. The average dose of 2PS was estimated to be 1.1 g/kg for guinea pigs and 0.5 g/kg for rabbits. At intervals after application, blood samples were obtained, and the concentrations of 2PS in the plasma were measured spectrophotometrically.

Results with guinea pigs showed that following percutaneous application of 2PS in DMSO, the blood concentration of the salt increased rapidly, peaked at about 2 hr (40 μ g/mL), and declined over the next 10 hr to low levels. In contrast, when water was used as a vehicle, 2PS could not be detected in the blood at any time. Similar results were obtained with rabbits except that small amounts of 2PS (15 μ g/mL) applied to the skin in water appeared in the blood during the first 5 hr.

That the absorbed 2PS was pharmacologically active was demonstrated by the observation that death of most animals given supralethal doses of the anticholinesterase sarin could be prevented by topical application of 2PS in DMSO in concert with im injections of atropine.

Analysis:

Despite the small number of animals used in this study, the data clearly show that, compared to water, DMSO as a vehicle greatly enhanced the penetration of 2PS in a pharmacologically active form through the skin of guinea pigs and rabbits. Although an approximation of the total amount of 2PS applied to the skin was known, the data do not allow for an estimate of the fractional absorbed dose. The persistence of 2PS in the blood for at least 12 hr suggests that the agent may have been released continuously from the skin at low levels.

⊕ McNamara, B.P., H.P. Averill, E.J. Owens, J.F. Callahan, D.G. Fairchild, H.P. Ciuchta, R.H. Rengstroff, and R.K. Biskup. 1974. The Toxicology Cyclotrimethylenetrinitramine (RDX) and Cyclo-tetramethylenetetranitramine (HMX) Solutions in Dimethylsulfoxide (DMSO), Cyclohexanone, and Acetone. EB-TR-73040, Edgewood Arsenal, AD 780010.

Review:

This report describes an extensive series of experiments with mice, guinea pigs, rabbits, and dogs aimed at determining the toxicity of RDX and HMX administered in DMSO, acetone, or cyclohexanone. The iv LD₅₀s for both explosives were determined with DMSO as a vehicle, but not with the other solvents. As judged by physiological responses in dogs and blood levels of the explosives in rabbits, RDX and HMX did not penetrate skin to a significant degree. Cardiovascular and central nervous system effects resulted from HMX and RDX given with DMSO iv, respectively; but neither compound was examined for these effects when injected in acetone or cyclohexanone. There was no indication that RDX or HMX caused

delayed-type allergic hypersensitivity in guinea pigs when administered in any of the three vehicles.

The only series of experiments in which the same doses of RDX or of HMX were tested at the same concentrations in each of the three vehicles was the one involving cataract induction in guinea pigs. These results demonstrated that RDX or HMX given percutaneously or intradermally were no more injurious than the vehicles themselves.

Analysis:

Because of solubility limitations and vehicle toxicity, it was not possible to test the two explosives at comparable doses in the same concentrations of all three vehicles for any of the responses evaluated except for cataract induction in guinea pigs. Therefore, the toxicity of RDX and HMX relative to the vehicles in which they were administered cannot be ascertained from these studies.

♦ Nakae, H.S. and D.R. Buhler. 1976. Percutaneous absorption of hexachlorophene in the rat. Toxicol. Appl. Pharmacol. 35:381-391.

Review:

Rats were used to study several parameters of hexachlorophene penetration through skin including the effects of different vehicles on the kinetics of penetration. Young adult male and female Wistar albino rats were used. Known amounts of ¹⁴C hexachlorophene in acetone, corn oil, ethanol, 1% aqueous sodium lauryl sulfate, or DMSO were applied to small areas of shaved abdominal skins. At different intervals after application, the marked areas of skin were removed, placed in scintillation vials, and assayed for radioactivity. Thus, the amount of hexachlorophene that penetrated into the body was calculated from the residual amount of radioactivity measured in the piece of skin. The effect of each vehicle on hexachlorophene penetration was tested on four pieces of skin from each of three or four rats.

At 24 hr, 55% of the hexachlorophene in DMSO had been absorbed while absorptions in acetone, corn oil, ethanol, or lauryl sulfate were about 38, 34, 25, and 21%, respectively. Therefore, of the five vehicles tested, DMSO resulted in the greatest amount of hexachlorophene absorbed. With DMSO as the solvent, there was a linear increase in absorption of hexachlorophene over a 24 hr period.

Analysis:

The study of the effect of vehicles on hexachlorophene penetration through the skin was a small part of this series of experiments. Nonetheless, the results show that of the broad class of solvents examined, the penetration of hexachlorophene 24 hr after percutaneous application was greatest when DMSO was used as a vehicle.

◆ Reddy, C.S., R.V. Reddy, and A.W. Hayes. 1981. Teratogenicity of secalonic acid D in mice. J. Toxicol. Environ. Health 7:445-455.

Review:

Groups of 8 to 16 pregnant CD1 mice were used to determine the teratogenicity and fetotoxicity of secalonic acid D in a mixture of 10% DMSO, and 5% NaHCO₃ in water or in 5% NaHCO₃ in water. Secalonic acid D (SAD) was administered ip at doses of 5, 10, or 15 mg/kg on days 7 through 15 of gestation. Dead mice and 19 day survivors were examined for the number of dead fetuses and early and late resorptions, and fetuses were scored for visceral and skeletal abnormalities.

At equal doses of SAD, the number of implants and live fetuses were higher and the number of resorptions lower in pregnant mice given SAD in the DMSO solution than in the NaHCO₃ solution. For the induction of skeletal abnormalities and cleft palate more SAD was required when the vehicle was the DMSO solution than the NaHCO₃ solution.

Analysis:

The protective effect of DMSO against SAD-induced embryotoxicity and fetotoxicity was demonstrated by the results of these experiments. The teratogenic data showing the effects of SAD in each of the two vehicles do not permit definitive conclusions. At most, the data suggest that DMSO offered some protection against SAD-induced skeletal malformations and possibly against cleft palate.

◆ Roerig, D.L., K.C.Z. Chen, A.T. Hasegawa, and R.I.H. Wang. 1973. Potentiation of the effects of mercaptoethylamine by dimethyl sulfoxide. Arch. Int. Pharmacodyn. 203:251-258.

Review:

These investigators studied the effects of DMSO on toxicity and absorption of mercaptoethylamine (MEA). Adult Swiss female mice were given MEA (175 mg/kg), DMSO (4.5 g/kg), or a mixture of the two substances ip. At short intervals after injection, spleen, liver, brain, and plasma samples were obtained and analyzed for sulfhydryl activity (μ mole SH/g tissue). The LD₅₀s for MEA in saline or DMSO were also determined.

The most significant result from this study is the potentiation of MEA toxicity by DMSO by a factor of about 1.5 according to the LD₅₀s. There is also suggestive evidence that DMSO temporarily enhanced accumulation of nonprotein SH groups in the spleen and plasma.

Analysis:

This is a reasonably complete study the main point of which was to elucidate the enhanced radioprotective characteristics of MEA by DMSO. A sufficient number of mice were used in most experiments, and the

statistical analysis of the data was adequate. From their data, the authors concluded that the increased toxicity of MEA in the presence of DMSO involved a drug interaction but that the increased toxicity of MEA in the presence of DMSO was not related to an increase in nonprotein SH brain levels.

♦ Rosen, H.R., A. Blumenthal, R. Panasevich, and J. McCallum. 1965. Dimethyl sulfoxide (DMSO) as a solvent in acute toxicity determinations. Proc. Soc. Exper. Biol. Med. 120:511-514.

Review:

Oral toxicity of ten quaternary ammonium salts in rats and mice was tested using water and DMSO (50% in water) as vehicles. The animals used were CD-1 male and female mice and male Sprague-Dawley rats. Ten animals per dose with four to seven doses were used to determine LD50s. The salts tested were chosen to cover a range of pharmacokinetic substances, i.e., pentolinium tartrate, hexamethonium bitartrate, decamethonium iodide, tubocurarine chloride, homatropine methylbromide, atropine methylnitrate, neostigmine bromide, carbachol, cetylpyridinium chloride, and benzethonium chloride.

When a DMSO solution was used as a vehicle, the LD50 in rats for eight of the drugs was decreased significantly, but toxicity of the two antispasmodics was not altered. Only two drugs, neostigmine (a parasympathomimetic in rats) and homatropine (an antispasmodic in mice), caused a significant change in the slope of the response when DMSO solution was used as a vehicle.

For mice, the DMSO solution decreased the LD50 significantly for 5 of the 10 drugs. Parallel decreases in LD50 occurred in both species for 7 of the 10 drugs.

Analysis:

Several firm conclusions can be drawn from these well-documented results. The data show that for half the drugs tested in mice and for 8 of the 10 drugs tested in rats, a 50% DMSO solution in water as vehicle increased their oral toxicity. In no case did the DMSO solution reduce toxicity of these quaternary ammonium salts. The slopes of the LD50 responses clearly show that with two exceptions the basis for toxicity was not different when the substances were administered in the DMSO solution than when given in water.

Finally, the results suggest that vehicle-dependent effects on the toxicity of a drug in one species are reasonably useful in predicting vehicle effects on the toxicity of the same substance in a closely related species.

♦ Schmid, F.A., R.C. Pena, W. Robinson, and G.S. Tarnowski. 1967. Toxicity of intraperitoneal injection of 7,12-dimethylbenz(a)anthracene in inbred mice. Cancer Res. 27:558-562.

Review:

A study was made of the effect of DMSO, sesame oil, hexadecane, or saline on the toxic effects of 7,12-dimethylbenz(a)anthracene (DMBA) on two strains of mice (young adult AKR and C57BL/6). The preparations were given as a single ip injection at a DMBA concentration of from 0.75 to 3 mg/mouse. Animals were sacrificed 5-20 days after injection, and the effects on the peritoneal cavity and on body, spleen, and thymus weight were recorded.

Toxicity of DMBA as reflected by mortality, peritoneal inflammation, and organ weight was not a function of the vehicle used.

Analysis:

The vehicle effects are based on a few mice because the main emphasis of this report is on mouse strain variations in response to DMBA rather than on vehicle effects. Nonetheless, there appears to be enough data to draw the conclusion that DMSO does not alter the systemic toxicity of DMBA compared to other vehicles.

♦ Shad, H.C. and H. Lal. 1976. Effects of 1,1,1-trichloromethane administered by different routes and in different solvents on barbiturate hypnosis and metabolism in mice. J. Toxicol. Environ. Health 1:807-816.

Review:

Young adult male Swiss mice were used to study the influence of vehicle and route of administration on lethality and hepatic function following exposure to trichloromethane (TCM). Hepatic function was judged by hypnosis time induced by barbiturate. TCM alone or in DMSO (1:99) or olive oil was injected ip or applied to an unshaved abdominal area.

It was found that TCM administered in DMSO markedly enhanced barbiturate-induced hypnosis time compared to TCM in olive oil. Results of mortality studies showed that at a constant dose of TCM, 24-hr survival decreased with increasing amounts of DMSO as vehicle. In addition, when 10 mL/kg of DMSO was given followed by 1 mL of TCM, 48-hr mortality was higher (60%) than in controls given 10 mL/kg of DMSO only (10%). In contrast, three topical applications of TCM diluted 1:1 with DMSO reduced barbiturate hypnosis time by 36%.

The results show that in mice (1) toxicity of TCM ip was increased by using a high concentration of DMSO as a vehicle when judged by mortality and hepatic function, (2) direct access to the liver is necessary for TCM and DMSO to increase hepatic injury because application of DMSO plus TCM to the abdominal area of mice did not result in hepatic malformation in terms of its ability to detoxify barbiturate, and (3) enhancement of rapid ip absorption of TCM in the presence of DMSO may not be the basis for increased mortality because DMSO potentiated TCM toxicity even after a 2-hr interval between DMSO and TCM injections.

Analysis:

The lack of hepatotoxicity after percutaneous application of TCM and DMSO is a significant finding in terms of exposure hazards. From the practical standpoint, the augmenting effect of DMSO on hepatotoxicity following ip administration may not be a very important aspect of DMSO toxicity. It would have been better to apply TCM and DMSO to shaved skin rather than to hair because the presence of hair may have inhibited percutaneous penetration of one or both chemicals.

♣ Siegers, C.P. 1978. Antidotal effects of dimethylsulfoxide against paracetamol-, bromobenzene-, and thioacetamide-induced hepatotoxicity. J. Pharmacy Pharmacol. 30:375-377.

Review:

Adult male NMRI mice were used to determine the effects of DMSO on liver damage induced by paracetamol (PA), bromobenzene (BR), thioacetamide (TH), or carbon tetrachloride (CCl₄). Liver damage was assessed by the levels of serum enzymes (two aminotransferases and sorbitol dehydrogenase). Liver glutathione was also determined as an indication of the level of conjugated metabolites of PA or BR. The chemicals and DMSO were given simultaneously either po or ip. Blood was obtained 24 hr after administration and was analyzed for enzymatic activities.

As judged by elevation of serum enzyme levels, treatment of mice with DMSO caused a dose-dependent reduction of PA-induced liver damage, and at 1 g/kg of DMSO, serum enzymes were almost in the normal range. DMSO alone did not alter serum enzyme levels. Protection against PA-induced hepatotoxicity was also observed when DMSO was given 1 hr after PA administration, and DMSO po was protective against PA given ip. Protection against liver toxicity induced by BR or TH was also achieved by DMSO administration, but not against CCl₄. The PA- or BP-induced decrease in liver glutathione stores was partially offset in mice given DMSO at 1 g/kg.

The author suggests that the protective effect of DMSO may involve inhibition of microsomal oxidation of the chemicals to toxic products. The effect of DMSO on liver glutathione also indicates reduction in the metabolism of the hepatotoxic chemicals.

Analysis:

The results clearly show that in mice DMSO protected the liver from certain toxic agents but not CCl₄. The data are sufficient to support the conclusions although some of the procedures are not clear.

♣ Somogyi, A. and K. Kovacs. 1970. Dimethyl sulfoxide, a convenient solvent of 7,12-dimethylbenz(a)anthracene for intravenous injection. Cancer Res. 30:1958-1962.

Review:

Young adult female Sprague-Dawley rats were used to study the effects of DMSO as a solvent for 7,12-dimethylbenz(a)anthracene (DMBA). Young rats were injected iv with 2, 4, or 6 mg of DMBA in DMSO or in a fatty emulsion. Three days later, the rats were sacrificed and their adrenal glands examined grossly and histologically for DMBA-induced adrenocorticolytic. Other rats were also injected with the DMBA preparation and examined weekly for mammary tumors.

As judged by its ability to induce adrenal necrosis and mammary tumors, DMBA had the same activity in DMSO as in the fatty emulsion.

Analysis:

A very clear cut and sound series of experiments were carried out by the investigators. The results show that DMSO neither inhibited nor potentiated systemic and carcinogenic effects of DMBA.

♦ Sosnowski, S.A., S. Rajalakshmi, and D.S.R. Sarma. 1976. Protection by dimethylsulfoxide of strand breaks in hepatic DNA induced by dimethylnitrosamine. Chem.-Biol. Interactions 15:101-104.

Review:

Young adult Wistar rats were used to study the effects of methylnitrosourea (MNU) or dimethylnitrosamine (DMN) with or without DMSO pretreatment on DNA strand breaks in regenerating rat liver. Animals received either DMSO (0.1 mL/100 g) or saline ip 45 min before DMN or MNU and were sacrificed 4 hr later. Liver DNA was isolated, and sedimentation analysis of DNA was carried out to estimate strand breaks.

The results showed that compared to saline, DMSO inhibited DNA strand breaks caused by DMN but not by MNU. The authors concluded from preliminary data that DMSO inhibited DMN-induced DNA strand breaks by inhibiting metabolic activation of DMN.

Analysis:

The inhibitory effect of DMSO on DMN-induced DNA strand breaks is based on results of one experiment in which liver preparations were used in the experimental group. The standard errors are quite large, yet the inhibitory effect is probably real but the extent is questionable. Since scant data are given for the basis of the inhibition, the conclusions that the inhibitory effect is related to metabolic action of DMN is open to question.

♦ Stelzer, J.M., J.L. Colaizzi, and P.J. Wurdack. 1968. Influence of dimethyl sulfoxide (DMSO) on the percutaneous absorption of salicylic acid and sodium salicylate from ointments. J. Pharm. Sci. 57:1732-1737.

Review:

This report describes a study in which young adult New Zealand white rabbits were used to compare the effects of DMSO in each of four bases on percutaneous penetration of salicylic acid and sodium salicylate. Mixtures of the two substances were prepared in 15% DMSO incorporated in a base of a hydrophilic ointment USP XVII, a hydrophilic ointment petrolatum USP XVII, polyethylene glycol ointment USP XVII (PEG), or a polyoxyethylene (20) stearyl ether gel system (PE20). The effect of DMSO on penetration of salicylic acid and sodium salicylate from these bases was compared to penetration in the absence of DMSO. The preparations were applied to clipped skin and remained in percutaneous contact under an occlusive bandage for a period of 8 hr during which periodic blood samples were obtained and analyzed colorimetrically for salicylate concentration.

The presence of DMSO in the hydrophilic ointment or petrolatum enhanced the percutaneous absorption of salicylic acid (10%) in terms of rate and total amount absorbed during the 8-hr period. In contrast, the relatively low absorption of salicylic acid from PEG or PE20 was little enhanced by the presence of DMSO. The penetration of sodium salicylate (11.6%) was tested only in the two hydrophilic bases with or without DMSO. The results showed that DMSO significantly decreased percutaneous absorption of sodium salicylate from the hydrophilic ointment while no effect of DMSO was observed with the hydrophilic petrolatum.

Analysis:

This series of experiments is straightforward, and the conclusions were predicated on the use of a sufficient number of observations and their statistical analysis. It was suggested that for salicylic acid, DMSO acts as a penetrant carrier by altering the lipoidal characteristics of the cell membrane, thereby facilitating penetration of the lipid-soluble substance, salicylic acid. On the other hand, the hydroscopic nature of DMSO hindered penetration of water soluble sodium salicylate by holding it in the hydrophilic base on the surface of the skin.

♦ Stenback, F. and H. Garcia. 1975. Studies on the modifying effect of dimethyl sulfoxide and other chemicals on experimental skin tumor induction. Ann. N.Y. Acad. Sci. 243:209-227.

Review:

Experiments with mice were conducted to compare tumorigenesis of 3,4-benzo(a)pyrene (BP) in benzene with tumorigenesis in DMSO, and to determine if topical DMSO affected the initiation of tumors by 7,12-dimethylbenzanthracene (DMBA) and croton oil.

Female Swiss mice 8 weeks old in groups of 40 or 50 were used. Hair was clipped from the back, and substances were applied to the skin. Four groups were treated twice a week for 50 weeks with one of the following preparations: 13.3 µg of BP in benzene, 13.3 µg of BP in DMSO, or one of

the two vehicles. In another series, the effect of DMSO on initiation of tumorigenesis by 100 µg of DMBA and repeated applications of croton oil as a promoter was studied.

As judged from the total number of tumors and the average tumor per mouse, DMSO compared to benzene as a vehicle caused a reduction in BP-induced skin tumors, although the percentage of mice bearing tumors was about equal for BP in the two vehicles. In the other series of experiments, daily applications of 20 µL of DMSO 2 days prior and 3 days after induction with DMBA and promotions by croton oil failed to effect the initiating phase of carcinogenesis in terms of the total number of tumors and average tumor per animal.

Analysis:

These experiments were carried out with a sufficient number of mice and appropriate control groups. Although the reduction in tumor yield in groups treated with BP in DMSO appeared to be significant, a statistical analysis of the data was not presented. The results clearly show that, in the two-stage skin carcinogenesis model with DMBA and croton oil, DMSO failed to modify carcinogenesis.

♦ Warren, J., M.R. Sacksteder, H. Jarusz, B. Wasserman, and P.E. Andreotti. 1975. Potentiation of antineoplastic compounds by oral dimethyl sulfoxide in tumor bearing rats. Ann. N.Y. Acad. Sci. 243:194-208.

Review:

These experiments were designed to determine if DMSO (1-2% in drinking water) altered the toxicity and antineoplastic effects of cyclophosphamide (CTX). The effects of CTX alone or in combination with DMSO was ascertained by measuring peripheral WBC counts, tumor diameter, survival, and the appearance of the CTX metabolite, cytoxylamine, in blood and urine and in liver homogenates. Young Fisher 344 male and female rats were used.

The following results were obtained:

1. In normal rats, single or repeated doses of CTX ip resulted in greater mortality and in a more severe leucopenia when DMSO was in the drinking water. If CTX was given po to normal rats, these toxic effects of DMSO were less severe and also less definitive than when CTX was given ip.
2. When CTX was given ip to leukemic rats supplied with DMSO in drinking water, only a weak potentiation of the antineoplastic capacity of CTX by DMSO was observed.
3. When CTX and DMSO were present in drinking water for leukemic animals, all 10 rats survived with normal WBC counts while only 1 of 10 rats supplied with CTX in water survived.

4. DMSO in drinking water enhanced the antineoplastic capacity of CTX given ip against a lymphosarcoma. When incorporated in drinking water with DMSO, CTX suppressed tumor growth.
5. DMSO did not increase the enzymatic conversion of CTX ip to CTX-amines in vivo or in vitro, but blood levels of these metabolites were 2-3 times the water-fed controls.

Analysis:

From these results, it is reasonable to conclude that rats continuously exposed to low levels of DMSO in drinking water were more sensitive to the toxic antineoplastic effects of CTX than water-fed controls. Furthermore, the evidence showed that the therapeutic effect of CTX on tumors is potentiated in rats supplied with drinking water containing DMSO. Results from the in vitro experiments indicate that potentiation of CTX toxicity in normal rats or of the therapeutic efficacy of CTX in tumor-bearing animals by continuous exposure to DMSO was not associated with an increase in activation rate by liver microsomes.

This report describes a variety of experiments performed with a minimal number of animals. The qualitative aspects appear valid, but the magnitude of some of the effects may be inaccurate.

♣ Weiss, L.R. and R.A. Orzel. 1967. Some comparative toxicologic and pharmacologic effects of dimethyl sulfoxide as a pesticide solvent. Toxicol. Appl. Pharmacol. 11:546-557.

Review:

The purpose of this study was to determine whether DMSO as a vehicle for several pesticides altered their toxicologic or pharmacologic properties. Young adult male Osborne-Mendel rats were used together with the following pesticides: carbaryl, thiram, dieldrin, and parathion. LD50s were determined for all pesticides. Brain cholinesterase activity was measured in rats given carbaryl; and the effect of DMSO on barbiturate sleeping time was assessed.

Compared to water as a vehicle, DMSO did not significantly alter the oral LD50s of any of the pesticides tested. Likewise, DMSO did not increase the inhibitory effect of carbaryl on brain cholinesterase. When DMSO was given to rats at 10 mL/kg po 24 hr before administration of barbiturate, there was no alterations of sleeping time.

Analysis:

The conclusion that DMSO as a vehicle does not alter the toxicity of carbaryl, thiram, dieldrin, or parathion nor the anticholinesterase activity of carbaryl compared to water or corn oil is well established by these experiments. Lack of an effect of DMSO on barbiturate-induced sleeping time is also proved.

† Yuspa, S.H., H. Hennings, P. Dermex, and D. Michael, 1976. Dimethyl sulfoxide-induced enhancement of 7,12-dimethylbenz(a)anthracene metabolism and DNA binding in differentiating mouse epidermal cell cultures. Cancer Res. 36:947-951.

Review:

A study was made of the effects of DMSO on the ability of newborn mouse (Balb/c) epidermal cells in short-term culture to metabolize and bind 7,12-dimethylbenz(a)anthracene (DMBA). Over the 3- to 10-day culture period, these cells show a decrease in their ability to metabolize DMBA as reflected in hydroxylase levels and a decrease in DMBA binding to DNA. The additions of 1.25% DMSO to the culture medium eliminated the decrease in metabolism and binding.

The basis for the decrease was not established but appeared not to be related to hydroxylase induction by DMSO or enhancement of cell penetration by DMSO. Since DMSO prolongs life of these cells in culture, the authors suggest that the effect of DMSO on DMBA interaction may result from facilitations of nutrient and waste transport which, in turn could affect carcinogen processing.

Analysis:

This is a straightforward, concise set of experiments principally designed to enhance culture life of cells. Whether or not the results from cell culture experiments of this kind relate to in vivo conditions is questionable.

APPENDIX B

TABLE B-1. TABULAR SUMMARY OF LETHALITY OF DMSO

Species	Dose ^a	Route	Effects	Reference
Monkey	4-8	iv	LD50	Mason 1971
	1 and 3 ^b	po	No mortality	Vogin et al. 1970
	9 ^b	po	100% mortality by 53rd week	Vogin et al. 1970
	1, 3, 9 ^b	dermal	No mortality	Vogin et al. 1970
Mouse	3.1 ^c	iv	LD50	Fishman et al. 1969
	5.75	iv	LD50	Willson et al. 1965
	7.6	iv	LD50	Sommer and Tauberger 1964
	10.9	ip	LD50	Worthley and Schott 1969
	15.4 ^d	ip	LD50	Worthley and Schott 1969
	16.5	po	LD50	Sommer and Tauberger 1964
	21.4	po	LD50	Willson et al. 1965
	13.9	sc	LD50	Sommer and Tauberger 1964
	40	dermal	LD50	Mason 1971
Rat	5.36	iv	LD50	Willson et al. 1965
	8.1	iv	LD50	Sommer and Tauberger 1964
	14.5 ^c	po	LD50	Fishman et al. 1969
	19.7	po	LD50	Sommer and Tauberger 1964
	28.3	po	LD50	Willson et al. 1965
	12.0	sc	LD50	Sommer and Tauberger 1964
	40-50	dermal	LD50	Mason 1971

^ag/kg of 100% DMSO except where noted.

^bml/kg/day for 18 mo, 90% DMSO.

^cConcentration not given.

^d25% DMSO.

TABLE B-2. TABULAR SUMMARY OF GENETIC TOXICITY OF DMSO

Species	Dose/ concentration	Route	Effects and comments	Reference
Bacteria	Toxic level or 5 mg/plate maximum	-	Not mutagenic	Simmon et al. 1977
	1.4 x 10 ⁶ nmoles/ plate (100% DMSO)	-	Not mutagenic	De Flora 1981
Chicken	10-50 µL/embryo (0.5-1% DMSO)	-	Not genotoxic	Bloom 1982
Fish	0.5% DMSO for 24, 48 hr	-	Incidence of anaphase aberrations in cultured ovary cells not significantly increased above that of untreated cells	Kocan et al. 1982
Hamster	0.1 mL (1% DMSO)	-	No effect in cultured ovary cells	Tates and Kriek 1981
	0.1 mL (10% DMSO)	-	Increased chromosomal aberrations with microsomal activation	Tates and Kriek 1981
Rat	1 mL/kg/day for 10 wk	ip	No effect on dominant lethality	Sheu and Green 1979
	5 mL/kg/day for 5 days	ip	Disrupts chromosomes at concentrations of 1-100% DMSO	Kapp and Eventoff 1980
Yeast	1.4 M for 4 hr	-	Promutagenic	Callen and Philpot 1977
	5% DMSO for 60 min	-	Not mutagenic with microsomal activation; 50% survival	Abbondandolo et al. 1980

TABLE B-3. TABULAR SUMMARY OF CARCINOGENICITY OF DMSO

Species	Dose/ concentration	Route	Effects and comments	Reference
Hamster	2% DMSO	-	No cell transformations in embryo cell cultures	Pienta 1980
	2% DMSO	-	No cell transformations in cell cultures of hyaline cartilage	Katoh 1977
	100% DMSO	topical	No tumorigenesis after application to cheek pouch epithelium	Elzay 1967
Mouse	0.5% DMSO	-	5% of cultured ventral prostate cells gave rise to malignant clones	Mondal 1971
Rat	18% DMSO, biweekly for 20 wk	sc	No tumors after 1 yr. Treatment included H ₂ O ₂ and/or TiCl ₃	Lohs et al. 1971

TABLE B-4. TABULAR SUMMARY OF TERATOGENICITY AND EMBRYOTOXICITY OF DMSO

Species	Dose/ concentration	Route	Effects and comments	Reference
Chicken	Toxic level up to 10.3 mg/72 or 96 hr-old embryo (50% DMSO)	injection	Malformations of the limbs, beak and eyes, anourous embryos, celo-somia	Caujolle et al. 1967
Hamster	0.05-1 g/kg on 8th day of gestation	iv	No significant teratogenic effects	Ferm 1966
	2.5-8.25 g/kg on 8th day of gestation	iv, ip	Exencephaly, rib fusions, microphthalmia, limb abnormalities and cleft lip	Ferm 1966
	5.5 g/kg on 8th day of gestation	ip	Impaired development of brain	Marin-Padilla 1966
	3.3 g/kg on 8th day of gestation	ip	No teratogenic effect	Staples and Pecharo 1973
	3.3 g/kg/day on 7th-9th days of gestation	ip	Slight increase in visceral and skeletal abnormalities	Staples and Pecharo 1973
	5.5-8.25 g/kg/day on 8th day, or 7th-9th day of gestation	ip	Cranioschisis, exencephaly, cleft lip, hemangioma, fused ribs, absence of kidneys	Staples and Pecharo 1973
	0.7 mL of 100% DMSO given to 115-125 gm animals on 8th day of gestation	ip	Exencephaly and other abnormalitis	Gill et al. 1981

TABLE B-4. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
Mouse	5-12 g/kg/day on 6th-12th day of gestation (50% DMSO)	oral	No effect	Caujolle et al. 1967
	5-12 g/kg/day on 6th-12th day of gestation (50% DMSO)	ip	Anencephalic, malformed limbs, and celosomia	Caujolle et al. 1967
	5.5-11 g/kg on 8th, 9th, 10th or 11th day of gestation	ip	Visceral and skeletal malformations	Staples and Pecharo 1973
Rabbit	5 g/kg/day on 6th-14th day of gestation (50% DMSO)	po	No teratogenic effect	Caujolle et al. 1967
	4 g/kg/day on 6th-14th day of gestation (50% DMSO)	sc	No teratogenic effect	Caujolle et al. 1967
	3 g/kg/day on 8th-11th day of gestation (90% DMSO)	sc	No teratogenic effect	Staples and Pecharo 1973
Rat	5-10 g/kg/day on 6th-12th day of gestation (50% DMSO)	po, ip	Malformations of nervous system, limbs, jaw. Celosomia and edema	Caujolle et al. 1967
	10.25 g/kg/day on 8th-10th day of gestation (90% DMSO)	sc	Decreased live litter size, increased resorp- tions, but no gross malformations	Juma and Staples 1967
	10.25 g/kg/day on 8th-10th day of gestation	ip	Not teratogenic	Staples and Pecharo 1973

TABLE B-5. TABULAR SUMMARY OF OTHER EFFECTS OF DMSO*

Species	Dose/ concentration	Route	Effects and comments	Reference
<u>Adrenal Gland:</u>				
Human	8.0 mL (70% DMSO)	po	No significant change in plasma corticosterone level	Allen and Allen 1975
Rat	2.0 mL (2.5% DMSO)	ip	Significant increase in plasma corticosterone level	Allen and Allen 1975
	2.0 mL (25% DMSO)	ip	Significant increase in plasma corticosterone level	Allen and Allen 1975
<u>Eye:</u>				
Human	2 drops (50% DMSO)	contact	Transient burning and stinging and occasional vasodilation	Kligman 1965b
	90-100% DMSO	contact	Aggravation of inflammatory response	Hanna et al. 1977
	30% DMSO	contact	Antiinflammatory	Hanna et al. 1977
	10% DMSO for 70 min	<u>in vitro</u>	Corneal epithelial cells damaged	Sperling and Larsen 1979
	1 g/kg/day for 12 wk	dermal	No ophthalmological abnormalities	Hull et al. 1969
Dog	1 g/kg/day for 3 mo. (80% DMSO)	dermal	No ophthalmological abnormalities	Brobyn 1975
	11 g/kg/day (90% DMSO) 1, 3, 9 mL/kg/day, 5 days/wk for 2 yr	sc po	Lenticular changes in 7 wk or less Changes in retractive index and vitreous humor, equatorial opacities, nuclear opalescence	Smith et al. 1969 Noel et al. 1975
Guinea pig	0.5 mL 3 times/wk for 3 wk (tech. grade DMSO)	dermal	Cataracts appeared in 3 of 12 animals in 5-6 mo.	Rengstoff et al. 1971
	0.05 mL 3 times/wk for 2 wk (5% DMSO)	sc	Cataracts appeared in 3-4 of 12 animals	Rengstoff et al. 1971
Monkey	11 g/kg/day for 185-200 days (90% DMSO)	dermal	No ocular changes	Smith et al. 1969
	4.5 mL/kg twice/day for 53 wk (90% DMSO)	po	No ocular changes	Vogin et al. 1970
	3-9 mL/kg, 5 times/wk (50% DMSO)	po	Lenticular changes occurred in 9-15 wk	Barnett and Noel 1967
Pig	8.1 mL/kg, twice daily, 5 times/wk (90% DMSO)	dermal	Lenticular changes occurred after 90 days	Noel et al. 1975
Rabbit	5% DMSO in drinking water	po	Lenticular changes occurred after 24 wk	Wood et al. 1971
	1 g/kg/day (100% DMSO)	ip, dermal	No lenticular changes	Wood et al. 1971
	5 g/kg/day (100% DMSO)	ip, dermal	Lenticular changes in 10-15 days	Wood et al. 1971

TABLE B-5. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
<u>Hematology:</u>				
Human	1 g/kg/day for 90 days	dermal	23 of 45 subjects exhibited transient eosinophilia, 2 had decreased hemoglobin, hematocrit and RBC	Brobyn 1975
	100 g/day for 2 days (20% DMSO)	iv	Decreased hemoglobin, decreased WBC and shortened prothrombin and partial thromboplastin times	Yellowlees et al. 1980
Dog	0.3-2.4 g/kg/day 6 times/wk for 4 wk	iv	Anemia with reduced hemoglobin, hematocrit and RBC. Reticulocytosis	Willson et al. 1965
Monkey	3 g/kg/day for 9 days (40% DMSO)	iv	No change in blood chemistry	de la Torre et al. 1981
	9 mL/kg, 5 times/wk for 18 mo	po, dermal	No hematological effects	Vogin et al. 1970
Rat	8 g/kg/day, 6 times/wk	ip	Anemia, with decreased hemoglobin and hematocrit values	Willson et al. 1965
<u>Kidney:</u>				
Human	1 g/kg/day for 3 days (10-40% DMSO)	iv	No nephrotoxicity	Bennett and Muther 1981
	100 g/day for 2 days (20% DMSO)	iv	Elevations in blood urea and creatinine indicative of renal tube damage	Yellowlees et al. 1980
Monkey	1-9 mL/kg/day for 18 mo. (90% DMSO)	po, dermal	No impairment of renal function	Vogin et al. 1970
	3 g/kg/day for 9 days (40% DMSO)	iv	Fourfold increase in diuresis, but no nephrotoxicity	de la Torre et al. 1981
Rat	2 and 4 g/kg/day for 28 days (40% DMSO)	ip	No change in serum urea or creatinine levels	Small and Ide 1976
<u>Liver:</u>				
Human	100 g/day for 2 days (20% DMSO)	iv	Jaundiced appearance and elevated bilirubin and aspartate aminotransferase	Yellowlees et al. 1980
	1% DMSO	<u>in vitro</u>	Reduction in myeloid erythropoiesis in embryonic tissue cultures	Markartseva 1982
Dog	0.3-2.4 g/kg, 6 times/wk for 4 wk	iv	Cloudy swelling and granularity of parenchymal cytoplasm	Willson et al. 1965

TABLE B-5. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
Monkey	a) 0.4 mL/kg b) 2.0 mL/kg	ip	a) Centrilobular fatty changes b) Diffuse hepatocellular fatty changes	van der Watt and Purchase 1970
Rat	3.6-4.8 mL/kg	ip	Transient fatty infiltration of tissue	Mathew et al. 1980
	5 g/kg/day for 45 days	po	Degeneration of hepatocytes and inflammation and irritation of portal spaces	Caujolle et al. 1967
<u>Lungs:</u>				
Mouse	200 mg/m ³ for 7 hr daily (5 days/wk for 6 wk)	inhalation	No histopathological changes	Fishman et al. 1969
	1600 mg/m ³ for 4 hr, 2900 mg/m ³ for 24 hr	inhalation	Edematous changes in lungs	Fishman et al. 1969
	Saturated atmosphere for 2 hr	inhalation	Nontoxic	Filippova and Kalimullina 1974
	Saturated atmosphere 4 hr daily for 6 mo	inhalation	Histological changes	Filippova and Kalimullina 1974
Monkey	9 mL/kg (90% DMSO)	intra-gastric	Atelectasis and emphysema possibly due to regurgitation and tracheal inspiration	Vogin et al. 1970
<u>Muscles:</u>				
'Human	1 g/kg/day for 90 days	dermal	Reduced systolic blood pressure in several of 78 test subjects	Brobyn 1975
	100 g/day for 2 days (20% DMSO)	iv	Normal serial electrocardiogram, 84/min pulse, 140/90 mm Hg BP	Yellowlees et al. 1980
Dog	1-2 g/kg	iv	Transient increase in cardiac index with increased BP, heart and pulse rates	Peterson and Robertson 1967
Guinea Pig	0.6-6% DMSO	<u>in vitro</u>	Response to electrical stimulation was decreased in diaphragm, and increased in stomach and cardiac muscle	Sams et al. 1966
Rabbit	0.70-2.10 M DMSO	<u>in vitro</u>	Concentration dependent induced relaxation of aortic strips	Jackson et al. 1979
Rat	2.5 µg/mL	<u>in vitro</u>	Amplitude of spontaneous contractions of duodenum, uterus and rectum was reduced	Bonnardeaux 1971

TABLE B-5. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
<u>Nervous System:</u>				
Aplysia	1% DMSO	<u>in vitro</u>	Acetylcholinesterase activity inhibited in isolated nerve cells	Sawada and Sato 1975
	>10% DMSO	<u>in vitro</u>	Cholinergic transmission blocked in isolated nerve cells	Sawada and Sato 1975
Mouse	0.5, 0.25 mL (15% DMSO)	ip, iv	No gross or microscopic changes in brain parenchyma	Broadwell et al. 1982
	0.5-1.0 mL (20-30% DMSO)	ip	Histological alterations in brain	Broadwell et al. 1982
	6.7 g/kg	ip	Median effective dose for analgesia	Morris 1966
Rabbit	0.25 mL (30-100% DMSO)	contact	Had no immediate corneal anesthetic effect when applied to conjunctival sac	Morris 1966
Rat	5.5 g/kg	iv, ip	Significant level of analgesia	Haigler and Spring 1981
<u>Physiology:</u>				
Mouse	1-10% DMSO	<u>in vitro</u>	10% DMSO inhibited amino acid incorporation into protein in brain supernatant	Fleming 1977
	50 g/kg/day for 6 mo (5% DMSO)	po	Increased incorporation of amino acids into protein of brain, liver, and kidney	Fleming 1977
Rat	5-30% DMSO	<u>in vitro</u>	Protein synthesis in liver extract stimulated at 5-10%, inhibited at 10% and blocked at 30%	Gerhards and Gibian 1967
	5.5 g/kg	ip	Decrease in body temperature	Kocsis et al. 1975
	1.10 M DMSO	<u>in vitro</u>	Decreased insulin stimulated glucose oxidation and increased lipolysis by fat cells	Wieser et al. 1977
	75 mg/100 g	iv	No effect on subsequent <u>in vitro</u> oxygen consumption. Urease, trypsin and chymotrypsin activities inhibited	Gerhards et al. 1965
	4.5 mg/kg	ip	Slight increase in serum transaminase levels in 24 hr	Altland et al. 1966
<u>Pituitary:</u>				
Human	8.0 mL (70% DMSO)	po	No significant change in plasma ACTH levels	Allen and Allen 1975
Mouse	0.1% DMSO (2 days)	<u>in vitro</u>	Synthesis of GH and prolactin stimulated in anterior pituitary culture	Nagasawa 1983
	0.05 mL twice daily for 2 days	sc	No increase in synthesis of GH or prolactin in pituitary culture	Nagasawa 1983

TABLE B-5. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
Rat	2.0 mL (2.5% DMSO)	ip	No change in plasma ACTH levels	Allen and Allen 1975
	2.0 mL (25% DMS)	ip	Significant increase in plasma ACTH	Allen and Allen 1975
<u>Skin:</u>				
Human	9 mL/day (90% DMSO)	dermal	Transient burning and stinging Several cases of transient erythema	Kligman 1965b
	9 mL 2/day (90% DMSO)	dermal	10 of 20 subjects developed tran- sient erythema and 2 exhibited extreme dermatitis and toxic shock	Kligman 1965b
	38% DMSO	dermal	Threshold concentration producing local irritation in occlusive patch test	Kligman 1965b
	90-100% DMSO	dermal	Whealing reaction which varied in intensity between individuals and on different regions of the body	Frosch et al. 1980
	20-100% DMSO	dermal	Increased skin permeability	Kligman 1965a
	0.9-9% DMSO	<u>in vitro</u>	Concentration dependent increase in water permeability	Astley and Levine 1976
Mouse	20-100% DMSO	<u>in vitro</u>	Concentrations of >50% DMSO resulted in an increase in water penetration of the epidermis	Sweeney et al. 1966
<u>Thyroid:</u>				
Mouse	1-2 g/kg/day for 49 days	po	No histomorphological alterations in thyroid parenchyma	Lanza et al. 1970
	15% DMSO	<u>in vitro</u>	Inhibition of iodine uptake by thy- roid following 15 min incubation	Hagemann and Evans 1968
	0.4 mL (63% DMSO)	ip	Transient inhibition of iodine uptake in vivo	Hagemann and Evans 1968
Rat	0.5 mL (63% and 85% DMSO)	ip	No effects on thyroidal iodine uptake	Goldman 1973

TABLE B-6. TABULAR SUMMARY OF THE EFFECTS OF INTERACTIONS OF
NON-PERCUTANEOUSLY ADMINISTERED DMSO WITH OTHER CHEMICALS

Species	Dose/ concentration	Route	Effects and comments	Reference
<u>Anti-Cholinesterases:</u>				
Rat	99% DMSO	ip, po	LD50 of insecticides in DMSO same as that in corn oil or water	Weiss and Orzel 1967
	~66% DMSO	po	Absorption rate of carbaryl from duodenum greatly enhanced	Cambon et al. 1981
Rat, Mouse		ip	DMSO and DMSO ₂ but not DMS protected against anticholinesterases	Kocsis et al. 1975
<u>Antineoplastics:</u>				
Human	Not given	iv	Antitumor effect of cyclophosphamide potentiated by DMSO and side effects reduced	Garrido and Lagos 1975
	5 L of 5 or 6% DMSO over 3 days	po	DMSO had no effect on antitumor activity, but caused a decrease in urinary excretion	Fuks et al. 1981
Mouse	1 g/kg	ip	DMSO had no effect on survival time or tumor development in daunomycin-treated mice with P-333 leukemia	Marian and Matkovic 1982
Rat	2% in drinking water for 300 days	po	DMSO enhanced the therapeutic effect of cyclophosphamide on lymphatic leukemia	Warren et al. 1975
	0.6-2.4 g/kg	iv	DMSO slightly enhanced the therapeutic effect of ifosfamide on carcinosarcomas. High doses decreased effectiveness	Von Ardenne and Reitnauer 1975
<u>Carcinogens:</u>				
Mouse	as vehicle	ip	DMSO did not alter toxicity of dimethylbenzanthracene as compared with sesame oil or hexadecane	Schmid et al. 1967
	1.25% DMSO	<u>in vitro</u>	DMSO affects enzyme synthesis and carcinogen binding in epidermal cell cultures	Yuspa et al. 1976

TABLE B-6. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
Rat	as vehicle	po	DMSO altered P-450 in liver, delayed and diminished urinary excretion of, and increased adrenal cortex mitotic activity in N,N-diethyl-4-amino-azobenzene (DEAB)-treated rats compared to DEAB in sunflower oil	Danz et al. 1978
	as vehicle	<u>in vivo</u>	DMSO altered binding characteristics of benzopyrene and methylcholanthrene with respect to liver organelles	Levine 1972, 1975
	as vehicle	ip	Methylcholanthrene and a polychlorinated biphenyl were more potent as drug-metabolizing enzyme inducers when dissolved in DMSO compared with olive oil.	Hietanen et al. 1980
	as vehicle	po, ip	DMSO as vehicle for one carcinogen increased mitotic activity of adrenal cortex more than vegetable oil; no difference with 3 other carcinogens	Amlacher et al. 1974
<u>Drugs:</u>				
Human	50 mg/kg	dermal	DMSO with ethanol (0.75 g/kg), or 60 min before ethanol, caused a slight decrease in blood ethanol level, reduced motor nerve conduction rate, and adversely affected mental performance	Mallach 1971
Cat	0.5 mL/kg (50% DMSO)	iv	Pharmacologic actions of glycosides potentiated	Melville et al. 1968
Mouse	10-80% of LD50 (24 g/kg)	po	Lethality of DMSO and ethanol lowest when given simultaneously with low DMSO doses, and highest when DMSO given 1 hr after ethanol	Mallach 1971
	25% DMSO	po	Toxicity of 11 drugs not affected by DMSO	Dixon et al. 1965
	50% DMSO	po	The toxicity of 5 of 10 drugs, including a muscle relaxant, antispasmodic, parasympathomimetic and cationic germicide were increased by DMSO	Rosen et al. 1965

TABLE B-6. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
Rabbit	1 mL/kg	iv	DMSO enhanced penetration of toxogonin through blood-brain barrier	Rump et al. 1969
Rat	50% DMSO	po	Toxicity of 8 of 10 drugs, including ganglionic blockers, muscle relaxants, parasympathomimetics and cationic germicides increased by DMSO	Rosen et al. 1965
<u>Hepatotoxins:</u>				
Mouse	0.25-3 g/kg	ip, po	DMSO protected against the toxicity of paracetamol, bromobenzene, and thioacetamide but not that of carbon tetrachloride	Siegers 1978
	10-90% DMSO and 10 mL DMSO/kg 100% DMSO as vehicle	iv	DMSO enhanced toxicity of methylchloroform	Shah and Lal 1976
		ip	Toxicity of Fe salt-octamethylpyrophosphoramid complex greater with DMSO as vehicle than with water	Joesten and Hill 1966
	5.5 g/kg	ip	DMSO decreased the toxicity of octmethylpyrophosphoramid (20 mg/kg) given 1 hr later	Kocsis and Harkaway 1967
	100% DMSO as vehicle	ip	DMSO significantly lowered the LD ₅₀ value for benzene	Kocsis et al. 1968a
	5 mL/kg as vehicle	ip	DMSO significantly increased toxicity of chlorobenzene, carbon tetrachloride and toluene	Kocsis et al. 1975
	Rat	100% DMSO as vehicle	ip	DMSO reduced toxicity of the antimitotic agent dehydroheliotridine
2.0 mL/kg (90% DMSO)		ip	DMSO potentiated the hepatotoxicity of oral doses of carbon tetrachloride	Freston and Bouchier 1967
5 mL/kg		ip	DMSO potentiated carbon tetrachloride toxicity without increasing heptatotoxicity	Mancini and Kocsis 1974

TABLE B-6. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
<u>Hepatotoxins:</u>				
Rat	5 mL/kg (25% and 100% DMSO)	ip	DMSO potentiated the lethal effects of benzene, and increased urine taurine levels	Kocsis et al. 1968a
	50 µL (100% DMSO)	<u>in vitro</u>	Liver microsomal metabolism of aminopyrine, 7-ethoxycoumarin, and benzo[a]pyrene enhanced by DMSO	Kawalek and Andrews 1980
	not given	not given	Pretreatment with DMSO altered serum levels of 2,4,5-T and slightly stimulated 2,4-D metabolism	Courtney 1970
	not given	<u>in vitro</u>	Metabolism of aniline and phenacetin increased; that of ethylmorphine and benzphetamine unchanged	Kitada et al. 1978
<u>Mutagens:</u>				
Bacteria	as vehicle	Ames test	Fresh DMSO plus p-phenylenediamine is not mutagenic, but when mixture stands for 4 hours, it becomes highly mutagenic	Burnett et al. 1982
	as vehicle	Ames test	Heating of mixture of DMSO and hexachloracetone altered toxicity and mutagenicity characteristics of hexachloracetone	Zochlinski and Mower 1981
Drosophila	1% DMSO as vehicle	intra-abdominal	DMSO enhanced mutagenicity of ethylmethane sulfonate	Sharma et al. 1973
Green plant	5% DMSO	seedling	DMSO doubled mutagenicity of ethylmethanesulfonate	Bhatia 1967
Penicillium			DMSO enhanced mutagenicity of N-nitroso-N-methylbiuret	Zakhorova et al. 1974
Rat	5 mL/kg	ip	DMSO reduced number of chromosomal aberrations induced by pyrimethamine and 6-mercaptopurine	Barilyak et al. 1978
	1 mL/kg	ip	DMSO prevented strand breaks in hepatic DNA induced by dimethylnitrosamine	Sosnowski et al. 1976

TABLE B-6. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
<u>Teratogens:</u>				
Chicken	as vehicle	injection	DMSO reduced teratogenicity of 3-acetylpyridine, 6-aminonicotinamide, and 3-amino-1,2,4-triazole; that of sulfanilamide was increased, and that of physostigmine and nicotine unchanged when compared with water as solvent	Landauer and Salam 1972
Hamster	0.7 mL on 8th day of gestation	ip	DMSO potentiated the teratogenicity of diazepam	Gill et al. 1981
Mouse	10% DMSO	ip	DMSO reduced teratogenic, embryocidal, and fetotoxic potency of secalonic acid	Reddy et al. 1981
Rat	5 mL/kg	ip	DMSO reduced teratogenic effect of chloridin and 6-mercaptopurine	Barilyak et al. 1978
<u>Miscellaneous:</u>				
Human	0-20% DMSO	<u>in vitro</u>	5-15% DMSO enhanced polyethylene glycol mediated cell fusion	Norwood et al. 1976
	not given	<u>in vitro</u>	DMSO enhanced the inhibitory effect of carbamyl phosphate on red blood cell sickling	Smith and Allen 1975
Mouse	0.5 mL/day (15% DMSO)	po	DMSO enhanced immunosuppressive effect of imuran	Aronov and Radionov 1979
	not given	not given	DMSO accelerated onset of action of glucochloralose but not its toxicity	Braude and Monroe 1965
	4.5 g/kg	ip	DMSO potentiated the toxicity of mercaptoethylamine	Roerig et al. 1973
	as vehicle	ip, sc	DMSO increased toxicity of snake venom compared with saline	Tiru-Chelvam 1974
Rabbit	2 mL/kg (25% DMSO)	iv	DMSO potentiated febrile response to exogenous pyrogen	van Miert and van Duin 1976
	5-30% DMSO	<u>in vitro</u>	15% DMSO caused a maximum reduction in the hemolytic effect of chlorohexidine acetate	Ansel 1967

TABLE B-6. (CONTINUED)

Species	Dose/ concentration	Route	Effects and comments	Reference
Rat	as vehicle	ip	DMSO potentiated toxic effects of diethylstilbestrol in rats with altered hepatic function	Klaassen 1973
	6 mL/day for 22 days	not given	DMSO inhibited the anaphylactoid reaction produced by butylated hydroxyanisole dextran	Rodriguez et al. 1966
	not given	<u>in vitro</u>	Membrane damage by butylated hydroxyanisole was greatest with substances with large dielectric constants like DMSO	Sgaragli et al. 1975
	0.08-2.57 M	<u>in vitro</u>	DMSO can potentiate action of substances which stimulate production of adenylyl cyclase	Hynie and Klenerova 1980
	as vehicle	<u>in vitro</u>	DMSO solutions of cigarette smoke condensate (CSC) were not as inhibitory to respiration of brown fat cells <u>in vitro</u> as were ethanol solutions of CSC	Pettersson 1980

TABLE B-7. TABULAR SUMMARY OF THE EFFECTS OF INTERACTIONS OF PERCUTANEOUSLY ADMINISTERED DMSO WITH OTHER CHEMICALS

Species	Dose/ concentration	Effects and comments	Reference
<u>Allergens:</u>			
Guinea pig	1%	DMSO enhanced sensitization to dinitrochlorobenzene	Heise et al. 1969
Rat	as vehicle	DMSO reduced time to produce contact sensitization when used as a vehicle for 2,4-dinitrochlorobenzene	Vakilzadeh et al. 1973
<u>Carcinogens:</u>			
Hamster	as vehicle	DMSO reduced latent period for squamous cell carcinoma induced by dimethylbenzanthracene compared to mineral oil	Dachi et al. 1967
	as vehicle	DMSO applied 3 times per week for 11 weeks enhanced carcinogenicity of dimethylbenzanthracene	Elzay 1967
	as vehicle	DMSO enhanced tumorigenicity of dimethylbenzanthracene	Lalonde 1969
	70% DMSO as vehicle	Presence of DMSO as vehicle given 3 times per week for 10 weeks retarded dimethylbenzanthracene induced skin carcinogenesis	Siegel and Shklar 1969

TABLE B-7. (CONTINUED)

Species	Dose/ concentration	Effects and comments	Reference
Mouse	as vehicle	DMSO slightly shortened the appearance time of 20-methylcholanthrene induced tumors	Finogenova 1974
	50% DMSO: 50% acetone	Compared to acetone as solvent, DMSO-acetone had moderate inhibitory effect on 20-methylcholanthrene induced skin carcinomas	Iverson et al. 1981
Rat	as vehicle	DMSO increased latency period and reduced number of carcinomas compared to acetone as a vehicle	Stenback 1970
<u>Cytotoxic Agents:</u>			
Human	80% DMSO	DMSO had no effect on penetration of methotrexate	McCullough et al. 1976
	as vehicle	DMSO enhanced effectiveness of 5-fluorouracil and 5-iododeoxyuridine	Goldman et al. 1967
<u>Salicylic Acid:</u>			
Rabbit	15% DMSO	DMSO enhanced penetration of salicylic acid, especially at low pH	Marcus et al. 1970
	15% DMSO	DMSO in ointments enhanced rate of absorption into blood of salicylic acid	Stelzer et al. 1968

TABLE B-7. (CONTINUED)

Species	Dose/ concentration	Effects and comments	Reference
<u>Steroids:</u>			
Human	not given	DMSO enhanced penetration of the steroids hydrocortisone and testosterone 3 to 4 fold	Feldmann and Maibach 1966
Rat	as vehicle	DMSO favored penetration of prednisolone but not its ester	Lafille and Sagon 1969
	70% DMSO	DMSO had no effect on penetration of corticotropin	Kastin et al. 1966
<u>Miscellaneous:</u>			
Cow	as vehicle	Applied on the udder, DMSO promoted absorption of penicillin into milk and serum compared with water as vehicle	Walser 1966
Guinea pig	as vehicle	DMSO enhanced penetration of 1-methyl-2-hydroximinomethylpyridinium	McDermot et al. 1965
	10-90% DMSO	DMSO enhanced penetration of soman as indicated by LD50 values	McDermot et al. 1967

TABLE B-7. (CONTINUED)

Species	Dose/ concentration	Effects and comments	Reference
	100% DMSO	DMSO increased absorption and mortality of mercurous chloride 7- and 4-fold respectively	Wahlberg and Skog 1967
Rat	as vehicle	DMSO slightly enhanced penetration of vasopressin	Kastin et al. 1966
	as vehicle	In DMSO hexachlorophene penetration was 55% of dose in 24 hr	Nakaue and Buhler 1976
	10-100% DMSO	With increasing concentrations of DMSO, phenol penetration through excised rat skin was reduced	Roberts and Anderson 1975
	90% DMSO	DMSO plus alpha-tocophenol reduced ulcerogenic activity of adriamycin	Svingen et al. 1981

APPENDIX C

DMSO REFERENCES REVIEWED BUT NOT CITED

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LIST OF ABBREVIATIONS

AMP	adenosine S'-monophosphate
BSX	1,7-diacetoxy-tetramethylene-2,4,6-trinitramine
Btu/lb	British Thermal units per pound
C	carbon
°C	degrees Celsius
cal/deg/mol	calories per degree per mol
cal/gm	calories per gram
CCl ₄	carbon tetrachloride
cc per degree C	cubic centimeters per degree Celsius
CH	methyl group
CH ₃	methyl group
cm	centimeter
Cp	molecular heat
CSF	cerebrospinal fluid
CYC	cyclophosphamide
DDT	2,2 bis(4-chlorophenyl)-1,1-dichloroethane
DIALOG	Lockheed online data retrieval system
DMBA	dimethylbenzanthracene
DMSO	dimethyl sulfoxide
dx-Py	type of molecular orbital bonding
dxz-Pz	type of molecular orbital bonding
°F	degrees Farenheit
FDA	Federal Drug Administratin
g	gram

gal	gallon
g/kg	grams per kilogram
H	hydrogen
H ₂ O	water
H ₂ O ₂	hydrogen peroxide
HCl	hydrochloric acid
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
hr	hour
ip	intraperitoneal
iv	intravenous
kcal/mol	kilocalories per mole
L	liter
lb	pound
LD ₅₀	lethal dose - 50% of test animals
MC	2-methylcholanthrene
mg/cu m	milligrams per cubic meter
mg/kg	milligrams per kilogram
µg	microgram
µmol/L	micromoles per liter
min	minute
mL	milliliter
mL/dL	milliliters per deciliter
mL/kg	milliliters per kilogram
mM	millimoles
mmHg	millimeters of mercury
mol/L	moles per liter

N	nitrogen
ng	nannogram
NH	amino group
NIOSH	National Institute of Occupational Safety and Health
O	oxygen
OH	hydroxyl group
ohm ⁻¹ cm ⁻¹	ohms per centimeter
OMPA	octamethylpyrophosphoramide
%	percent
p	para
P	phosphorus
PCB	polychlorinated biphenyls
PM	benzene
pH	hydrogen ion activity
π	pi
po	per oral
ppm	parts per million
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
S	sulfur
SEX	1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine
σ	sigma
SO	sulfur-oxygen bond
sp ³	molecular orbital
sp ³ -px	type of molecular orbital bonding
TAX	1-acetylhexahydro-3,5-dinitro-1,3,5-triazine
TiCl ₃	titanium trichloride

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