

Summary and Overview: NIOSH Symposium on Toxic Effects of Glycol Ethers

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The papers contained in this issue of Environmental Health Perspectives were presented at a symposium sponsored by the National Institute for Occupational Safety and Health (NIOSH) on September 19–21, 1983. In his opening remarks, B. L. Johnson (NIOSH) listed four objectives for this symposium. These were: (1) to foster direct exchange between scientists who are active in glycol ether research; (2) to summarize the current state of knowledge on glycol ether toxicity; (3) to identify future research needs; and (4) to identify actions needed to protect the health of workers exposed to glycol ethers. The symposium was productive in all these areas, involving investigators from government, academic, and industrial laboratories. Speaking in behalf of the Chemical Manufacturers Association Special Project Panel on Glycol Ethers, J. P. Lyon (ICI Americas) described the Toxicology Research Task Group's testing program. Data developed as part of this industry initiative were presented throughout this symposium.

R. L. Smith (Eastman Chemical Products, Inc.) reviewed the basic uses of ethylene glycol ethers and ether esters, especially in various surface coatings. The combination of mild odor, low evaporation rate, good solvent and coupling activity, flow and leveling qualities, blush resistance ("blushing" is the formation of a hazy finish due to absorption of atmospheric moisture) and solvent release (the ability of the solvent to escape from beneath the surface film as a coating dries) were cited as a few of the factors which lead to the unique position occupied by this class of compounds. Many of the specific uses of ethylene glycol ethers are based on a combination of these characteristics and were derived through experience over many years. Product reformulation to replace one material with another therefore cannot be a simple one-for-one exchange and replacement of glycol ethers may be difficult in some applications.

Despite the widespread usage of ethylene glycol ethers, D. E. Clapp (NIOSH) described serious difficulties encountered by NIOSH when attempting to identify an appropriate population for epidemiologic study. One of these difficulties is the accurate description of worker exposure, which cannot be based solely on airborne concentrations since many of the glycol ethers penetrate the skin readily. As a potential solution to this problem, A. W. Smallwood (NIOSH) discussed the progress made toward development of methods for the analysis of glycol ethers and their metabolites in blood and urine.

A series of papers on teratogenicity testing, progressing from methyl through butyl ethers of ethylene glycol, was presented by T. R. Hanley (Dow Chemical Co.), F. D. Andrew (Syntex Research), W. J. Krasavage (Eastman Kodak Co.), J. E. Doe (Imperial Chemical Industries, PLC), B. K. Nelson (NIOSH), R. W. Tyl (Bushy Run Research Center) and B. D. Hardin (NIOSH). A consistent pattern of embryotoxic and teratogenic effects in various species and following diverse routes of exposure was described for ethylene glycol monomethyl ether (EGME) and monoethyl ether (EGEE). A similar spectrum of effects was reported for ethylene glycol monoethyl ether acetate (EGEEA). For higher ethylene glycol ethers (monopropyl ether acetate [EGPEA] and monobutyl ether [EGBE], maternal toxicity appeared to dominate, particularly in the case of EGBE. Although isolated cardiovascular and skeletal defects or variations of normal development were seen, they seemed to be associated with maternal toxicity. T. R. Hanley reported that propylene glycol monomethyl ether (PGME), which was predominantly the 1-methoxy-2-propanol isomer, had relatively low acute maternal toxicity and was not teratogenic.

A variety of *in vitro* and mutagenesis tests were reported. D. B. McGregor (Inveresk Research International) and E. D. Thompson (Proctor and Gamble Co.) each reported the apparent lack of genotoxicity in the glycols tested: EGME, diethylene glycol dimethyl ether (diEGdiME), and diethylene glycol monobutyl ether (diEGBE). *In vitro* tests that may be predictive of teratogenic activity were reported by R. Loch-Caruso (Michigan State University), F. Welsch (Chemical Industry Institute of Toxicology) and E. M. Johnson

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(Jefferson Medical College). Using V79 hamster cells in culture, Loch-Carus reported inhibition of cell-cell communication by ethylene glycol and four glycol ethers. Inhibition of intercellular communication fell and cytotoxicity increased as the molecular weight of the glycols increased. These results can be interpreted as suggesting that the lower molecular weight glycol ethers may be tumor promoting agents. The parallels between teratogenicity and activity in these tests of cell-cell communication stimulate speculation that intercellular communication may present a viable mechanistic basis for the teratogenicity of glycol ethers. Welsch also evaluated *in vitro* cell-cell communication, employing human embryonal palatal mesenchyme cells. In this system EGME inhibited intercellular communication by apparently cytotoxic means. Ethylene glycol monoisopropyl ether, on the other hand, inhibited cell-cell communication without cytotoxicity. Based on the ratios of adult toxicity to developmental toxicity (A/D ratios) in his *Hydra attenuata* system, Johnson concluded that, of the 14 compounds (glycols, glycol ethers, and ether acetates) tested in that system, EGEE warranted the greatest concern. Others, including EGME, were not identified by this system as possessing unique developmental toxicity.

A short-term *in vitro* assay was reported by R. L. Schuler (NIOSH). The results of these tests confirmed the recognized embryotoxicity of EGME, EGdME, and EGEE and suggested that mono- and dimethyl ether derivatives of diethylene and triethylene glycol should also be evaluated in detail. In similar *in vivo* studies, Doe reported that neither PGME nor diEGME had adverse effects on the outcome of pregnancy.

An unexpected observation in the *in vivo* assay reported by Schuler was evidence that ethylene glycol (EG) might itself be embryotoxic. Subsequent EG studies, reported by J. C. Lamb (National Institute for Environmental Health Sciences), in fact confirmed the teratogenicity of EG. These studies involved both conventional teratology in mice and rats, as well as a new screening assay in which breeding pairs of mice are cohabited for up to 120 days while being exposed to the chemical in the drinking water. Remarkably similar skeletal defects were reported in both species and in both experimental protocols. The continuous breeding protocol was also reported by Lamb to reveal antifertility effects of EGEE not only in males, as would have been predicted, but also in females.

Studies of the testicular toxicity of a large number of glycol ethers were summarized by K. Nagano (Japan Bioassay Laboratory), whose 1979 publication stimulated many of those in attendance to investigate the glycol ethers. Declining testicular toxicity with increasing substituent alkyl chain length was revealed by these studies, as was the molar equivalence of two acetates (EGMEA and EGEEA) with their corresponding glycol ethers (EGME and EGEE). Both Nagano and Doe presented evidence that EGPE and EGBE did not have adverse effects on the testis. P. M. D. Foster (British

Industrial Biological Research Association) and R. E. Chapin (National Institute of Environmental Health Sciences) presented detailed evaluations of the earliest stages of testicular pathology. Using EGME as a model compound, they were able to demonstrate the remarkable sensitivity of meiotic spermatocytes. Equivalent doses of methoxy and ethoxy acetic acids were reported to produce similar effects. Effects on Sertoli cell function were not detected. H. Zenick (University of Cincinnati) reported wide-ranging studies of the effects of EGEE on reproductive performance of male rats. Observations included unusual abnormalities of sperm head morphology caused by EGEE treatment.

The metabolism of ethylene glycol ethers was discussed by R. R. Miller (Dow Chemical) and H. B. Plotnick (NIOSH), who have shown that metabolism probably proceeds primarily by way of the alcohol/aldehyde dehydrogenase system to yield alkoxyacetic acid as the primary metabolite. Plotnick presented data showing that cleavage of the ether linkage occurs as a minor metabolic pathway and documented that urinary excretion of ethoxyacetic acid and its glycine conjugate accounts for the majority of an administered dose. PGME, on the other hand, was shown by Miller to be demethylated and excreted in urine primarily as propylene glycol. Foster demonstrated on the histologic level that the *in vivo* effects of methoxyacetic acid parallel those of EGME and showed that inhibition of alcohol dehydrogenase protected against the testicular toxicity of EGME, while inhibition of aldehyde dehydrogenase did not prevent testicular toxicity.

The ability of glycol ethers to penetrate the skin in toxic amounts has long been recognized. That rats can absorb teratogenic doses of EGEE and EGEEA by this route was reported by B. D. Hardin (NIOSH). Quantitative studies of skin penetration by various glycol ethers were presented by D. Guest (Eastman Kodak Co.) and P. Dugard (Imperial Chemical Industries, PLC). Radio-labeled EGEEA and EGPEA were tested in beagle dogs by Dr. Guest, who noted very similar rates of percutaneous absorption. Dugard employed an *in vitro* human skin preparation in which he was able to determine with precision the kinetics of skin penetration. In both the ethylene and diethylene glycol series, the relative rate of absorption declined from methyl to ethyl to butyl ether derivatives. Diethylene glycol ethers were absorbed more slowly than the corresponding ethylene glycol ethers.

The acute and subchronic toxicity of EGEE, EGPE and EGBE was reviewed by C. C. Conaway (Texaco, Inc.), G. V. Katz (Eastman Kodak Co.) and T. Tyler (Union Carbide Corporation). Reviews focused on toxic effects and target organ specificity of these glycols (kidneys, liver, hematopoietic system and red blood cells). For EGBE it was suggested that current occupational exposure limits are adequate for protection of human health.

Finally, there were several papers reviewing the subchronic and chronic toxicity of several glycol ethers.

News of the chronic studies of EG and EGEE being sponsored by the National Toxicology Program were anxiously awaited. Histopathologic evaluations were not complete, so R. L. Melnick (National Institute of Environmental Health Sciences) was unable to report conclusions regarding carcinogenicity. He did note, however, that some gross lesions (of the spleen, pituitary and testis and in the mammary gland region of females) often seen at necropsy of F344 rats were less common in EGEE-treated animals. This observation is intriguing in light of the report by D. P. Houchens (Battelle Columbus Laboratories) that EGME- and EGEE-treated mice survived challenges with L1210 leukemia cells that were fatal to untreated mice. These unexpected results were tentatively interpreted as suggesting an enhancement by these glycol ethers of immune function, since a direct cytotoxic effect on tumor cells was not detected.

Overall, the symposium was a stimulating opportunity for a profitable discussion of current research activities. Some general concepts can be developed, e.g., toxicologic equivalence on a molar basis of alkoxy ethyl ether acetates with the corresponding alkoxy

ethyl ether; reproductive toxicity inversely related to length of the substituted alkoxy group; biologic activation of alkoxy ethyl ethers by metabolism to alkoxy acetic acid. However, many questions remain unanswered, such as the basic mechanisms by which the cell-specific testicular effects are produced; the role, if any, of cell-cell communication in the embryotoxicity and teratogenicity of glycol ethers; the basis for and consequences of the apparent enhancement of immune function in glycol-treated mice.

Questions of a more immediate and practical nature face the many users of glycol ethers. Exposures must be controlled to acceptable levels. Where exposure controls are not feasible substitutes will be necessary. Alternative solvents with appropriate physical, chemical, and solvent properties but lacking the reproductive toxicity of EGME or EGEE must be found. The growth of knowledge regarding biological activity has been rapid in the past two to three years and promises to accelerate. Studies reported at this symposium will go far toward providing a basis for the design of future studies and for making the best-informed business decisions possible on exposure or substitutions.