West Virginia Chemical Spill: Bacterial Mutagenicity

Materials and Methods

The National Toxicology Program (NTP) evaluated 10 Elk River spill chemicals, structurally related chemicals, and related chemical mixtures (Table 1) for their ability to cause mutations, or permanent changes in DNA sequence, using the bacterial mutagenicity or Ames test.

Table 1. Elk River Spill Chemicals Tested for Bacterial Mutagenicity

CASRN*	Chemical Name	Doses (ug/plate)	Notes
34885-03-5	4-Methylcyclohexanemethanol (MCHM)	200 - 1000	a
NA	Crude 4-Methycyclohexanemethanol (Crude MCHM)	200 - 1000	b
770-35-4	Propylene glycol phenyl ether (PPH)	300 - 5000	a
51730-94-0	Dipropylene glycol phenyl ether (DiPPH)	300 - 5000	a
NA	Dowanol DiPPh glycol ether	300 - 5000	c
51181-40-9	Methyl 4-methylcyclohexanecarboxylate	15.6 - 3000	a
98955-27-2	4-(Methoxymethyl)cyclohexanemethanol	500 - 5000	a
2105-40-0	2-Methylcyclohexanemethanol	100 - 2000	a
94-60-0	Dimethyl 1,4-cyclohexanedicarboxylate	500 - 5000	a
105-08-8	1,4-Cyclohexanedimethanol	500 - 5000	a

* CASRN = Chemical Abstracts Service Registry Number. [a]Major or minor constituent of the spilled liquid (a minor constituent is considered to be approximately 20% or less of the spilled material); [b]A commercial mixture containing >70% MCHM along with lesser amounts of five other chemicals; [c]A proprietary commercial mixture of DiPPH isomers.

Bacterial mutagenicity assays were conducted according to Zeiger *et al.* (1992)[1] with slight modifications. Samples of each test article were sent to the testing laboratory and coded to ensure that samples were tested blind. Each test article was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 and the *Escherichia coli* strain WP2 *uvrA*/pKM101 either in buffer or 10% S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine (for the *S. typhimurium* strains) or tryptophan (for the *E. coli* strain) and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine- or tryptophan-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of the test article. The high dose was either limited by toxicity of the test article or by assay design to 5,000 µg/plate. All trials were repeated.

Bacterial mutagenicity test data are not subjected to statistical analysis. Rather, a positive response is defined as a reproducible, dose-related increase in histidine- or tryptophan-independent (revertant) colonies in any single strain/activation combination. An equivocal response is defined as an increase in

[1] Zeiger, E. et al. Salmonella Mutagenicity Tests. V. Results from the Testing of 311 Chemicals. Environ Mol Mutagen. 1992;19:2-141.

revertants that is not dose-related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are dose-dependent and at least twofold over background.