

West Virginia Chemical Spill: Bacterial Mutagenicity Study

July 2015 NTP Update

Synopsis

The National Toxicology Program (NTP)¹ tested two additional chemicals spilled into the Elk River in West Virginia for their ability to cause mutations, or permanent changes in DNA sequence, using the bacterial mutagenicity or Ames test. Specifically, the Ames test assesses the ability of a chemical to induce mutations in any of several different strains of bacteria. A positive test in any strain indicates the chemical is mutagenic and, therefore, has the potential to cause cancer. In a June 2015 update, NTP reported Ames test findings for eight spilled chemicals, including 4-methylcyclohexanemethanol (MCHM), the primary component of the spilled liquid. None of the eight chemicals caused mutations in the Ames test.² The two new chemicals tested are dimethyl 1,4-cyclohexanedicarboxylate and 1,4-cyclohexanedimethanol, which are minor constituents of the spilled liquid. NTP found that dimethyl 1,4-cyclohexanedicarboxylate caused mutations in two of the bacterial strains that were used in the test.

Bacterial Mutagenicity Study

Background on the Bacterial Mutagenicity Study

Bacterial mutagenicity (Ames) tests have been used widely for many years to determine if a chemical has the potential to cause mutations, which are permanent changes in the DNA sequence of the bacteria. These tests are performed regularly in the chemical and pharmaceutical industries and are accepted by regulatory agencies as a standard method of assessing the mutagenic potential of chemicals.

The Ames test employs several different strains of bacteria. NTP routinely uses three strains of bacteria in the test: two strains of *Salmonella typhimurium* and one strain of *Escherichia coli*. Each strain may react differently to chemical exposure, so using multiple strains increases the opportunity for detecting a mutagenic chemical. Chemicals are tested using five or more widely spaced concentrations that are determined by preliminary trials in each bacterial strain.

Study Findings

NTP tested the two chemicals listed in Table 1 in the Ames test to determine their ability to mutate bacterial DNA. Both chemicals are estimated to be 2 percent or less of the spilled material.³ Each chemical was tested at a minimum of five concentrations using standard procedures.⁴ One of the chemicals, a minor constituent of the spilled mixture (dimethyl 1,4-cyclohexanedicarboxylate), was mutagenic in two of the three strains used in the test. 1,4-Cyclohexanedimethanol did not cause mutations in any of the bacterial strains that were used.

The finding that dimethyl 1,4-cyclohexanedicarboxylate is mutagenic in bacteria does not establish that

¹ NTP is a federal, interagency program whose goal is to safeguard the public by identifying substances in the environment that may affect human health. NTP is headquartered at the National Institute of Environmental Health Sciences, which is part of the National Institutes of Health. For more information about NTP and its programs, visit <http://ntp.niehs.nih.gov/>.

² The June 2015 NTP update for the bacterial mutagenicity study can be found at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/bacterial_mutagenesis_update_508.pdf.

³ http://www.eastman.com/literature_center/misc/q_and_a_west_virginia_spill.pdf

⁴ Information about the test is available on the NTP Website at <http://ntp.niehs.nih.gov/testing/types/genetic/invitro/sa/index.html>.

its presence in the spilled liquid would result in DNA mutations in humans. Factors such as the amount and duration of exposure and differences in how the human body handles the chemical compared with bacteria would determine whether similar effects might occur in humans.

Table 1. Elk River Spill Chemicals Tested for Bacterial Mutagenicity

CASRN*	Chemical Name	Doses (ug/plate)	Notes
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. ^aMinor constituent of the spilled liquid (a minor constituent is considered to be approximately 10% or less of the spilled material).

Next Steps

The studies to evaluate the mutagenicity of the spilled chemicals in bacteria are complete. NTP will consider the findings reported here in any future, overall assessment of the spilled chemicals.