

West Virginia Chemical Spill: Nematode (*Caenorhabditis elegans*) Toxicity Study March 2015 NTP Update

Synopsis

The National Toxicology Program (NTP)¹ evaluated the toxicity of six chemicals that were spilled into the West Virginia Elk River, four structurally related chemicals, and two chemical mixtures to the nematode roundworm *Caenorhabditis elegans* (*C. elegans*). These assays evaluated effects on growth and development, feeding, and reproduction at different developmental stages in *C. elegans*' lifecycle. NTP found that none of the chemicals affected growth and development, feeding, or reproduction of *C. elegans*.

The chemicals tested included the primary chemical in the spilled liquid 4-methylcyclohexanemethanol (MCHM); five other minor components of the spilled liquid namely dipropylene glycol ether (DiPPH), propylene glycol phenyl ether (PPH), 1,4-cyclohexanediemethanol, dimethyl 1,4-cyclohexanedicarboxylate, and 4-methoxymethylcyclohexanemethanol; four chemicals related in structure to MCHM or PPH; a commercial mixture "crude MCHM" containing primarily MCHM and smaller amounts of other chemicals; and a proprietary commercial mixture (DOWANOL™ DiPPH) containing different forms of DiPPH.

Background on the *C. elegans* Lifecycle Assays

Caenorhabditis elegans (*C. elegans*) is a roundworm about 1mm in length that lives freely in soil and feeds on bacteria. It is a useful test model for evaluating the effects of chemicals at different development stages because it has a short life cycle, detailed information is available on its genetic code and developmental processes, and its reported molecular and biological changes in response to chemical exposure appear similar to those seen in studies with rodents.

Larval Development Assay

During development, *C. elegans* mature through the embryonic stage, four larval stages (L1-L4), and adulthood. In the growth assay, *C. elegans* in the first larval stage, L1, were exposed to varying concentrations of each of the 12 spilled chemicals for 48 hours, which is the time required for untreated nematodes to mature to the L4 stage. After exposure to the chemicals, the size of the nematodes in each treatment group was measured and compared with the size of untreated nematodes to determine if treatment with any of the spilled chemicals affected *C. elegans* development.

Feeding Assay

C. elegans feed via coordinated contractions of two pharyngeal bulbs that take in suspended food (bacteria) to the back of the pharynx, trap the bacteria and pump it into the intestine, and expel excess liquid out through the mouth. These rhythmic contractions are controlled by a self-contained nervous system that can be affected by environmental conditions, such as food availability and exposure to chemicals. For the feeding assay, adult nematodes were exposed to each of the 12 spilled chemicals for 24 hours. The feeding activities of nematodes in each treatment group were then assessed and

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/>.

compared with untreated nematodes to determine if treatment with any of the spilled chemicals affected *C. elegans* feeding.

Reproduction Assay

C. elegans egg-laying rates and embryonic survival may be affected by environmental conditions including salt concentration and chemical exposures. In the reproduction assay, *C. elegans* in the fourth larval stage, L4, were exposed to each of the 12 spilled chemicals for 48 hours, yielding a population of adults, embryos, and larvae in stages L1 and L2. The number of adults and their offspring in each treatment group was measured and compared to untreated nematodes to determine if treatment decreased *C. elegans* reproduction.

Chemicals Tested in the *C. elegans* Lifecycle Assays

The 12 chemicals tested in the *C. elegans* assays (see Table 1) were added to the liquid where the *C. elegans* were living and tested at four concentrations (50, 75, 100, and 200 μ M). In the case of MCHM, the four molar concentrations correspond to 6.4, 9.6, 12.8, and 25.6 ppm.

Table 1. Elk River Spill Chemicals and Structurally Related Compounds Tested in *C. elegans* Assays

CASRN*	Compound Name	Notes
34885-03-5	4-Methylcyclohexanemethanol (MCHM)	a
51730-94-0	Dipropylene glycol phenyl ether (DiPPH)	a
770-35-4	Propylene glycol phenyl ether (PPH)	a
105-08-8	1,4-Cyclohexanedimethanol	a
98955-27-2	4-Methoxymethylcyclohexanemethanol	a
4331-54-8	4-Methylcyclohexanecarboxylic acid	b
114651-37-5	Cyclohexanemethanol, 4-[(ethenoxy)methyl]-	b
498-81-7	Cyclohexanemethanol, alpha, alpha, 4-trimethyl-	b
94-60-0	Dimethyl 1,4-cyclohexanedicarboxylate	a
4169-04-4	Phenoxyisopropanol	b
NA	Crude 4-Methylcyclohexanemethanol (Crude MCHM)	c
NA	DOWANOL™ DiPPH	d

* CASRN = CAS Registry Number; ^aMajor or minor constituent of the spilled liquid; ^bNot a component of the spilled liquid, but included because the compound is structurally related to MCHM or PPH; ^cA commercial mixture containing >70% MCHM along with lesser amounts of five other chemicals; ^dA proprietary commercial mixture of DiPPH isomers

C. elegans Lifecycle Assay Findings

Across the concentrations tested, there were no effects of the chemicals on *C. elegans* with regard to growth and development, feeding, or reproduction. No physical abnormalities between treated and untreated nematodes were observed during examination of nematodes by microscopy in any of the *C. elegans* assays.

Next Steps

The studies to evaluate the toxicity of the chemicals in *C. elegans* are finished. NTP will consider the findings from these studies in any future, overall assessment of the spilled chemicals.