West Virginia Chemical Spill: NTP Research Project Plan

Background and Rationale

In January 2014, about 10,000 gallons of a liquid used to wash coal and remove impurities was spilled from a leaking tank into the Elk River in West Virginia. The spill contaminated the water supply of nearly 300,000 people within nine counties in the Charleston, West Virginia metropolitan area. Reports of licorice odors at homeowner taps and hospital admittances were signs that the population was exposed to contaminated tap water. A study by the Centers for Disease Control and Prevention (CDC) published in April 2014[1] found that one-fifth of households that received contaminated water reported health effects that they believed were related to the chemical spill. Most of the health effects involved rashes and skin irritation, although respiratory illnesses, nausea, and diarrhea were also reported.

The information available to date indicates that the major constituent of the spilled liquid was "crude MCHM," a technical product that contained primarily 4-methylcyclohexanemethanol (MCHM). The leaking tank also was reported to contain a proprietary mixture composed of predominantly dipropylene glycol phenyl ether (DiPPH) and propylene glycol phenyl ether (PPH) at less than 10 percent by weight. Based on material safety data sheets, the spilled liquid likely included lower levels of several other chemicals (noted in Table 1).

Limited toxicity data were available for the primary components of the spilled liquid and the minor constituents, so the health risks to the exposed population were uncertain, both qualitatively and quantitatively. Using the limited data available, CDC/Agency for Toxic Substances and Disease Registry (ATSDR) developed drinking water advisory levels of 1 part per million (ppm) for MCHM and 1.2 ppm for PPH. The CDC Website has summaries of the toxicology data used to develop these advisory levels.[2,3] In addition, the West Virginia Testing Assessment Project (WV TAP), an independent scientific assessment organized by the West Virginia Bureau for Public Health, convened an expert panel to review toxicity data on chemicals from the spill and develop short-term health advisory levels.[4]

In July 2014, CDC/ATSDR nominated chemicals involved in the West Virginia Elk River spill to the National Toxicology Program (NTP) for toxicological characterization. In response to this request for additional toxicology data, NTP plans to perform a number of studies of short duration to provide information relevant to the potential exposures of the Charleston residents. The chemicals of greatest concern (e.g., those for which drinking water advisory levels were developed) will be studied in rodent toxicology models; all chemicals (both major and minor constituents) will be evaluated using other model organisms and predictive computational modeling approaches.

A major focus of the toxicological characterization is the use of predictive models based on chemistry and bioinformatics. These models make it possible to rapidly (1) evaluate effects on a broad spectrum of biological processes; (2) evaluate the need for longer-term, more comprehensive toxicology studies; and (3) provide a conservative estimate of the dose levels at which health effects might occur in longer-term studies. Several assessments will evaluate effects on fetal and early postnatal development to assess the potential for acute exposures at these key time points to result in irreversible effects. Importantly, because the timing of the exposure was limited, the initial plan does not include studies to evaluate

[1] http://www.dhhr.wv.gov/News/2014/Documents/WVCASPERReport.pdf
[2] http://www.bt.cdc.gov/chemical/MCHM/westvirginia2014/mchm.asp
[3] http://www.bt.cdc.gov/chemical/MCHM/westvirginia2014/pph.asp
[4] http://www.dhsem.wv.gov/WVTAP/test-results/Documents/WV TAP Final Report.pdf

health effects after long-term exposures. The short-term studies outlined here will help determine the need for longer-term studies.

Specific Aims

1. Evaluate the teratogenic, immunotoxic, and genotoxic potential of MCHM.

2. Identify sensitive biological effects of the spill chemicals to determine the potential for low-dose effects in humans and provide additional information about levels at which there are no adverse effects.

3. Use efficient, medium- and high-throughput methods to predict qualitative and quantitative toxicological properties of all chemicals spilled into the Elk River.

Planned NTP Studies

The NTP studies that will be performed on each chemical are shown in Table 1. Studies that are the most time- and resource-intensive (e.g., the prenatal developmental toxicity assessment) are shown toward the left of the table, and those less time- and resource-intensive are shown toward the right.

Table 1. NTP Studies on Elk River Spill Chemicals and Structurally Related Compounds

	Studies								
Test Article [Abbreviation, CASRN*]	**Rat Prenatal Developmental Toxicity (Teratology)**	**Mouse Dermal Irritation and Hypersensitivity**	**5-Day Rat Toxicogenomic**	**Bacterial Mutagenicity**	**Zebrafish Developmental**	**Nematode Toxicity**	**High Throughput Screening**	**Structure-Activity Relationship Analysis**	**Notes**
4-Methylcyclohexanemethanol [MCHM, 34885-03-5]	X	X	X	X	X	X	X	X	a
Dipropylene glycol phenyl ether [DiPPH, 51730-94-0]			X	X	X	X		X	b
Propylene glycol phenyl ether [PPH, 770-35-4]			X	X	X	X	X	X	b
1,4-Cyclohexanedimethanol [CHDM; 105-08-8]				X	X	X	X	X	b
2-Methylcyclohexanemethanol [2MCHM, 2105-40-0]				X	X	X		X	b
4-(Methoxymethyl)cyclohexanemethanol [MMCHM, 98955-27-2]				X	X	X		X	b
4-Methylcyclohexanecarboxylic acid [4331-54-8]					X	X		X	c
Cyclohexanemethanol, 4-[(ethenyloxy)methyl]- [114651-37-5]					X	X	X	X	c
Cyclohexanemethanol, alpha,alpha,4-trimethyl- [498-81-7]					X	X		X	c
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC, 94-60-0]				X	X	X	X	X	b
Methyl 4-methylcyclohexanecarboxylate [MMCHC, 51181-40-9]				X	X	X		X	b
Phenoxyisopropanol [4169-04-4]					X	X	X	X	c
Technical product [“crude MCHM”]		X	X	X	X	X			d

* Chemical Abstracts Service Registry Number

[a] Major constituent of the spilled liquid (>50% of the spilled chemical mixture).

[b] Minor constituent of the spilled liquid (<10% of the spilled chemical mixture).

[c] Not a component of the spilled liquid, but included because the compound is structurally related to MCHM or PPH.

[d] The commercial product present in the leaking tank; a mixture of MCHM, MMCHM, MMCHC, DMCHDC, CHDM, and methanol.

Rat Prenatal Developmental Toxicity (Teratology) Study. A potential concern of acute exposures is the effect on the developing fetus. The occurrence of birth defects often depends upon the developmental stage of the fetus at the time of chemical exposure. The goal of a teratology study is to determine if a chemical has the potential to adversely affect fetal survival or development or cause birth defects at any stage of fetal development. This study will address, in part, concern over spill-related exposures to

pregnant women in the Charleston area. MCHM was selected for this assessment because it is the primary chemical in the spilled liquid and the effects of MCHM on fetal development are unknown.

Mouse Dermal Irritation and Hypersensitivity Study. An assay examining skin irritation and cell proliferation in the lymph nodes evaluates the ability of a compound to cause skin inflammation by directly damaging cells and causing irritation, or by inducing an immune response known as allergic hypersensitivity or contact allergy. This study will focus on MCHM because it is the major constituent of the spilled liquid. Available data suggest that MCHM does not cause hypersensitivity but is a known irritant. This study will provide information about the dose level needed to cause irritant effects and a no-effect dose level for irritancy. The study will also assess crude MCHM to determine if additional constituents of the mixture might alter the effects of MCHM.

5-Day Rat Toxicogenomic Study. A 5-day toxicogenomic study in rats identifies subtle effects of a chemical on molecular processes in the liver and kidney and examines toxic effects in blood (e.g., clinical chemistry, hematology) and damage to DNA (genetic toxicity). In addition, computational approaches and information from similar studies on a wide range of other chemicals will help identify effects of a chemical on disease-related processes in humans. This study will examine a wide range of doses of the spill chemicals to help identify biological processes that are most sensitive to their effects. In addition, data will be used to identify the no-effect dose level. MCHM, PPH, and DiPPH will be studied because they are the chemicals for which drinking water advisory levels were developed, and crude MCHM will be included to determine if additional constituents of the mixture might alter the effects of MCHM.

Bacterial Mutagenicity Studies. This set of short-term, *in vitro* tests evaluates DNA damage (genetic toxicity) in the bacteria *S. typhimurium* and *E. coli* caused by exposure to a chemical. The results of these tests show a good correlation with studies of carcinogenicity in rodent models.[5] DNA mutagenesis, or the process where genetic information in an organism is changed, is an irreversible process and can occur after short-term exposure to a chemical. The limited data currently available suggest the spill chemicals have a low potential to cause genetic toxicity. All chemicals in Table 1 that were components of the spilled material will be evaluated for their ability to cause mutations in several bacterial species and strains.

Zebrafish (*Danio rerio*) Developmental Study. Studies in zebrafish provide a rapid screen to identify developmental effects in vertebrates and have been extensively used in basic biomedical research for many years. These studies measure more than 20 developmental and behavioral effects that a chemical may have on developmental stages. All chemicals in Table 1 will be evaluated using this assay.

Nematode (*Caenorhabditis elegans*) Toxicity Study. The nematode worm *C. elegans* is used as a model organism to rapidly identify a variety of effects at different life stages. Four primary measurements (feeding, growth, reproduction, and locomotion) are evaluated in the life cycle toxicity assessment. All chemicals in Table 1 will be evaluated in this assay to provide insight into their toxicological potency relative to many other chemicals that NTP has already studied in this model system previously.

High Throughput Screening (HTS) Studies. The federal Tox21 HTS Program[6] is evaluating 10,000 chemicals in cellular and molecular-based assays to identify potential toxicological/biological properties. Four of the spill chemicals listed in Table 1 (MCHM, CHDM, DMCHDC, and PPH) are included in the

[5] http://ntp.niehs.nih.gov/go/9407
[6] http://www.niehs.nih.gov/health/assets/docs_p_z/tox21_transforming_environmental_health_508.pdf

10,000 chemicals library, so HTS data are available for these chemicals. Comparison of their HTS data with other chemicals with known toxicities will determine if these chemicals have biological effects related to specific, well-studied toxicological processes.

Structure-Activity Relationships (SAR) Analysis. SAR analysis uses a chemical's structure to predict its toxicological or biological properties. This computational analysis will evaluate the specific chemicals in Table 1 using a variety of SAR software platforms across a wide range of toxicological outcomes.

Significance and Expected Outcomes

The short-term studies outlined here will provide information for addressing critical toxicological concerns surrounding the chemicals spilled into the Elk River. The project leverages emerging technologies to provide a large body of data that can be used by federal and state agencies for risk assessment and determine if longer-term, resource-intensive definitive studies are needed. The prenatal developmental toxicity study results are expected to be available in 9-12 months, and results from the less resource-intensive studies in 3-6 months.