# NTP Research Program on Chemicals Spilled into the Elk River in West Virginia

# Final Update


## Summary

The U.S. National Toxicology Program (NTP) completed a yearlong research program to evaluate the potential toxicity of chemicals spilled into the Elk River in Charleston, West Virginia. The collected findings of the NTP studies support the adequacy of the drinking water screening level concentrations recommended by the Centers for Disease Control and Prevention (CDC) at the time of the spill. Exposure at or below the screening level is considered not likely to be associated with any adverse health effects.

The results of the NTP studies also identified a potential health effect of the main spilled chemical 4-methylcyclohexanemethanol (MCHM). When given to pregnant rats, at concentrations in their drinking water well above the screening level, NTP found lower weights in rat fetuses due to MCHM exposure. Based on NTP's findings, the West Virginia Department of Health and Human Resources performed a birth weight study. This study analyzed the prevalence of children with low birth weights born during the period of the chemical spill in the nine affected counties. The results were published on June 1, 2016.

The NTP studies strengthened our knowledge about the toxicity of MCHM and other spilled chemicals, and reduced uncertainty about the drinking water screening levels set at the time of the spill.


## Background on the Spill and Nomination to NTP for Studies

On January 9, 2014, a liquid used to wash coal was spilled into the West Virginia Elk River, a primary municipal water source serving about 300,000 people in the Charleston area. On the afternoon of the spill, West Virginia American Water issued an advisory to not drink the water. The spilled material overwhelmed the filtration system of the local water utility and entered the water distribution system. Crude 4-methylcyclohexanemethanol (crude MCHM) was the main liquid in the storage tank that leaked into the Elk River. As shown in Table 1, it is a commercial product that contained primarily MCHM; thus, MCHM was the main component of the spill. In addition to crude MCHM, the same storage tank contained a proprietary mixture primarily composed of propylene glycol phenyl ether (PPH) and dipropylene glycol phenyl ether (DiPPh). This mixture represented less than 10 percent by weight of what was in the tank.

The Centers for Disease Control and Prevention (CDC) recommended an initial drinking water screening level of 1 part per million (ppm) for MCHM based on available scientific information at the time. A level of 1 ppm corresponds to approximately 0.1 milligram (mg) of MCHM per kilogram (kg) of body weight per day. For PPH, a minor component of the spill, CDC recommended a drinking water screening level of 1.2 ppm, which corresponds to a dose of approximately 0.04 mg/kg/day.

At the time of the spill, there were few toxicological studies available on which to base a drinking water screening level. The lack of any studies in developing animals and humans was a

concern, because developing organisms are typically considered more susceptible than adults to the toxic effects of environmental chemicals. There was also concern about the absence of information on many chemicals that were minor components of the spill. CDC/Agency for Toxic Substances and Disease Registry nominated the Elk River spill chemicals to NTP for further study in July 2014.

**Table 1: Spilled Chemicals Studied by NTP (see Table of Chemicals Evaluated in NTP Studies for the full list).**

| CHEMICAL | ALSO KNOWN AS | REASON SELECTED FOR STUDY |
|---|---|---|
| Crude 4-methylcyclohexanemethanol | Crude MCHM | Commercial product present in the leaking tank; a mixture of MCHM, MMCHM, MMCHC, DMCHDC, CHDM, and methanol. |
| 4-Methylcyclohexanemethanol | MCHM | Major component of crude MCHM and the spilled liquid (more than 50% by weight of crude MCHM) |
| 1,4-Cyclohexanedimethanol | CHDM | Minor components of crude MCHM and the spilled liquid |
| Dimethyl 1,4-cyclohexanedicarboxylate | DMCHDC | |
| 4-(Methoxymethyl)cyclohexanemethanol | MMCHM | |
| Methyl 4-methylcyclohexanecarboxylate | MMCHC | |
| Propylene glycol phenyl ether | PPH | A proprietary mixture primarily composed of PPH and DiPPh was in the same leaking tank as the crude MCHM. This mixture is estimated to be less than 10% by weight of the total amount of liquid in the tank. |
| Dipropylene glycol phenyl ether | DiPPh | |

## NTP's Response to the Nomination

Many different experimental approaches are available to study the toxic effects of chemicals. These range from computational assessments that predict toxicological properties to studies in cells and organisms of increasing complexity, such as bacteria, worms, fish, and rodents. While these approaches differ in their capacity to predict human health effects, they all offer valuable biological and toxicological information. Thus, conducting studies using a broad range of approaches can yield predictions with greater confidence than studies that only use a few.

NTP performed a broad range of studies of relatively short duration to provide data and information for those affected by the chemical spill. These studies evaluated MCHM, minor

components of the spill, and chemicals that are similar in structure to MCHM (see Table of Chemicals Evaluated in NTP Studies for the full list). NTP used computational models that predict potential toxicities of MCHM and other chemicals to help determine what types of studies would be most appropriate to perform. Overall, all NTP studies on the spilled chemicals focused on determining the adequacy of the drinking water screening levels recommended by CDC at the time of the spill.

NTP studies were designed to evaluate the following health and biological effects:

- **Development and growth**: Evaluated effects of MCHM on fetal and early life development in rats, and effects of MCHM and other spilled chemicals on growth and development over the lifespan of other lower animal species, including fish and worms.
- **Skin irritation and hypersensitivity**: Examined the ability of MCHM to cause skin irritation and hypersensitivity in mice.
- **Motor behavior**: Evaluated the ability of the spilled chemicals to change the movement of fish in response to light, an indication of potential neurotoxic effects.
- **DNA mutation and genetic damage**: Evaluated the ability of the spilled chemicals to cause mutations or permanent changes in DNA of bacteria and genetic damage in red blood cells of rats.
- **Molecular effects on biological processes**: Used cellular components, cells, and tissues to identify biological processes that are sensitive to effects of the spilled chemicals.

Throughout a year of conducting these toxicity tests, NTP regularly informed the public and other federal agencies on study findings through Updates. NTP has also released supporting data files that served as the basis for the Updates. External experts have peer reviewed all NTP data and results.[1] This Final Update serves as NTP's overall interpretation of its studies on the spilled chemicals.

## Development and Growth

### *Prenatal development in rats*[2]

When pregnant rats were given MCHM orally for two weeks, they experienced minimal toxicological effects and there were no effects on fetal survival. The prenatal developmental toxicity study is standard for testing the effects of chemicals on normal fetal development in a pregnant rat. Pregnant rats are given the chemicals orally during the period of pregnancy when the skeleton and organs of the fetus are undergoing rapid growth and development.

While there were no effects on rat fetal survival, there were other effects at doses that were thousands of times higher than what a pregnant woman would have been exposed to by drinking water containing MCHM at the screening level. At the higher doses in the study (200 and 400 mg/kg/day), the rat fetuses were found to be of lower weight than expected. Some malformations in the fetuses were seen at the very highest dose tested (400 mg/kg/day).

[1] The list of peer reviewers are available at http://ntp.niehs.nih.gov/go/792686
[2] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=734

### *Development in fish*[3]

In developing zebrafish, MCHM had no effect on embryonic and larval development and behavior. Zebrafish is a small, tropical, freshwater fish that has been used extensively in biological research. It is useful for evaluating the effects of chemicals on developmental outcomes. It is a vertebrate, it has a short life cycle, and its embryonic development is similar to humans. In the NTP studies, physical development, growth, and behavior were evaluated during the embryonic and larval period, which occurs over a period of five days. With the exception of one minor spill constituent, all chemicals evaluated were without effect on development.

The minor spill component dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC) caused several structural malformations at a concentration of 13 ppm and above, and mortality at approximately 17 ppm. These concentrations are significantly higher than the 1 ppm water screening level for the main component of the spill, MCHM, and DMCHDC was likely in the spill at concentrations much lower than 1 ppm. The finding that this minor spill component is toxic to developing zebrafish does not by itself establish that it would cause similar effects in humans at similar high concentrations.

### *Development and growth in worms*[4]

NTP found that neither MCHM nor the other chemicals tested affected growth and development, feeding, or reproduction of nematode worms (*Caenorhabditis elegans*). These roundworms are about 1 mm in length, live in soil, and feed on bacteria. The nematode worm is a useful test model for evaluating the effects of chemicals at different developmental stages because it has a short life cycle and detailed information is available on its genetic code and developmental processes.

Overall, the NTP studies in rodents and lower animal species resulted in two main findings. First, one of the more sensitive responses to MCHM was a decreased weight of the fetuses in pregnant rats given the chemical during gestation. However, this effect was observed at doses many thousands of times higher than any expected exposure of pregnant women to MCHM in drinking water following the spill. Second, a minor component of the spill, DMCHDC, might be more toxic to developing zebrafish than MCHM. However, the toxicity in zebrafish produced by DMCHDC occurred at doses higher than any expected exposure of pregnant women to the chemical. As a minor component, it was present at less than 10 percent of the spilled liquid.

## Skin Irritation and Hypersensitivity[5]

Rashes and skin irritation were among the health effects reported by households following the chemical spill. NTP evaluated MCHM and crude MCHM for their ability to irritate the skin or cause skin sensitization (hypersensitivity).

NTP study results indicated that MCHM is a skin irritant at a concentration well above (more than 100,000 fold) what a person would be exposed to at the 1 ppm drinking water screening level. MCHM did not induce skin sensitization or hypersensitivity, meaning it did not cause an

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[3] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=726

[4] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=727

[5] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=728

allergic response in the skin. Crude MCHM caused skin irritation and sensitization at a concentration much higher (more than 100,000 fold) than what a person would be exposed to on their skin when showering or bathing in water containing crude MCHM at the screening level.

Overall, this study found that MCHM is a skin irritant, and that crude MCHM is both a skin irritant and sensitizer in mice. However, the concentrations of the chemicals applied to the skin to produce these effects were quite high. Notably, even concentrations that did not produce skin irritation or hypersensitivity in mice in this study were higher than what a person would be exposed to at the screening level.

## Motor Behavior[6]

NTP conducted a photomotor response study in zebrafish to evaluate the toxicity of the spilled chemicals. This study evaluated changes in movement of zebrafish in response to light, also known as the photomotor response, in the presence and absence of chemicals associated with the spill. Changes in the photomotor response after chemical treatment potentially reflect changes in behavior and neurological function, which are indicative of a potential neurotoxic effect.

MCHM altered photomotor response at a concentration of about 11 ppm, which is over 10 fold higher than the 1 ppm screening level. Two chemicals that are structurally similar to MCHM and were not present in the spilled liquid also altered zebrafish motor behavior in response to light.

The finding that MCHM altered the photomotor response in zebrafish does not establish that the spilled liquid would be neurotoxic to adult or developing humans. Many factors determine whether similar effects might occur in humans, such as the amount and duration of exposure to MCHM, whether the biological basis for the effect in zebrafish is the same as in humans, and differences in how the human body handles the chemical compared to zebrafish.

For comparison, in NTP's prenatal developmental toxicity study, effects likely related to neurotoxicity were observed in rats exposed to MCHM at doses more than 1000 fold higher than exposure at the water screening level. Similarly, an Eastman Kodak Company study found similar effects in rats exposed to MCHM at a dose that is far greater than exposure at the water screening level.

At doses that a person would be exposed to at the MCHM screening level, both zebrafish and rats showed no signs of motor behavior changes.

## DNA Mutation and Genetic Damage

### *DNA mutation in bacteria*[7]

NTP tested the ability of the spilled chemicals to cause DNA mutations in bacteria. Three different strains of bacteria were exposed to these spilled chemicals and monitored for mutations or permanent changes in DNA sequence. Chemicals that mutate DNA, with prolonged exposures, tend to have a potential to cause cancer and/or developmental effects.

[6] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=726

[7] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=790

Of the spilled chemicals tested, only DMCHDC, a minor component of the spill that also produced effects in the zebrafish, caused DNA mutations in bacteria. This finding does not establish that this minor component in the spilled liquid would cause 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.

### *Genetic damage in rats*[8]

NTP found that none of the three chemicals tested caused genetic damage after oral treatment of male rats for five days. The three spilled chemicals tested were MCHM, crude MCHM, and PPH. This evaluation for genetic damage used an experimental approach that measures the induction of micronuclei in rat red blood cells. Micronuclei contain small fragments of genetic material, and their presence in red blood cells is an indication of the capacity of a chemical to cause damage to chromosomes.

## Molecular Effects on Biological Processes

### *Molecular level effects on biological processes in liver and kidney of rats*[9]

NTP evaluated MCHM, crude MCHM, and PPH for their effect on molecular processes as determined by changes in gene expression in the liver and kidney of rats. Gene expression is the process by which the information coded in the gene is used to make a gene product, such as a protein. The most sensitive changes in gene expression often indicate the dose where an initial adaptive response to chemical stress occurs; thus, these studies provide an approximation of the lowest dose where any biological or toxicological changes would occur. Liver and kidney were studied because chemicals commonly affect these organs. The chemicals were given orally to rats for five days. At the end of the study, the liver and kidney were evaluated at the molecular level for evidence that the rats were affected by the chemical treatment. Changes in gene expression were measured to determine the lowest dose where molecular level effects were observed.

For MCHM, changes in gene expression in the liver occurred at doses as low as 6-99 mg/kg/day, and there was no effect on gene expression in the kidney. Crude MCHM changed gene expression in the liver starting at doses between 5 and 7 mg/kg/day, and there was no effect in the kidney. A person drinking water at the screening level recommended for MCHM by CDC following the spill would be exposed to a dose of 0.1 mg/kg/day, which is much lower than the level where gene expression changes were observed in rats. Thus, it is unlikely that sensitive molecular changes found in rats would be observed in humans at the screening level.

PPH caused gene expression changes in the range of 3-4 mg/kg/day in liver and 4-26 mg/kg/day in kidney. The drinking water screening level for PPH recommended by CDC was 0.04 mg/kg/day. It is unlikely that sensitive molecular changes found in rats would be observed in humans at the screening level.

[8] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=736

[9] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=736

### *Biological processes in human cells*[10]

NTP evaluated the effects of the spilled chemicals on signaling pathways of toxicological concern in human cells by performing high throughput screening assays. High throughput screening assays determine if a chemical has the potential to affect biological processes related to toxicity. NTP used 27 different human cell-based screening assays and tested chemicals at concentrations up to 92 µM, which is about 10-20 ppm. All chemicals, including MCHM, were not active in the assays performed to date.

## Conclusions[11]

The findings of the NTP studies on the spilled chemicals support the adequacy of the drinking water screening levels recommended by CDC at the time of the spill. Most of the spilled chemicals had no effect in the studies that were performed. When chemicals did produce effects, they occurred at dose levels that were considerably higher than either the drinking water screening levels recommended by CDC (MCHM and PPH) or the estimated levels of the minor components in the drinking water based on the reported tank contents. In the rat prenatal developmental toxicity study, NTP found lower weights in rat fetuses due to MCHM exposure. Based on NTP's findings, the West Virginia Department of Health and Human Resources performed a birth weight study to analyze the prevalence of children with low birth weights born during the period of the chemical spill in the nine affected counties. The birth weight study results were published on June 1, 2016.

The NTP studies increased our knowledge about the toxicity of MCHM and other spilled chemicals. The results from the NTP studies reduced uncertainty about the information used to develop the drinking water screening levels.

[10] Supporting files are available at http://tools.niehs.nih.gov/cebs3/wvspill/index.cfm?action=main.dataReview&bin_id=751

[11] All NTP Updates and supporting files are available at http://ntp.niehs.nih.gov/go/792827