NTP
National Toxicology Program

Report on the National Toxicology Program Response to the Elk River Chemical Spill

Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health

June 16, 2015

Report on the National Toxicology Program's yearlong research program into the toxicity of chemicals spilled into the Elk River in Charleston, West Virginia on January 9, 2014.

Outline

- Background on National Toxicology Program (NTP)
- Initial drinking water advisory level
- Limitations of available toxicology data
- NTP response to Elk River chemical spill
  - Goals of NTP studies
  - Description of toxicology tests
  - Findings from studies
- Conclusion

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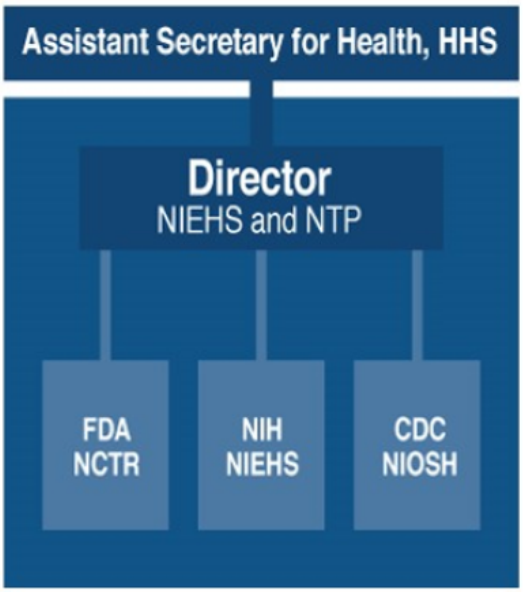
Outline of this presentation.

NTP

- NTP is an interagency program
- NTP was established in 1978 and is headquartered at the National Institute of Environmental Health Sciences (NIEHS), part of the National Institutes of Health
- Goal: to safeguard the public by identifying substances in the environment that may affect human health
  - Coordinate toxicology testing programs across the U.S. Department of Health and Human Services
  - Provide high quality data to reduce uncertainty in risk assessments



http://ntp.niehs.nih.gov

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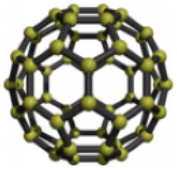
The National Toxicology Program (NTP) is headquartered at the National Institute of Environmental Health Sciences (NIEHS), part of the National Institutes of Health, in Research Triangle Park, NC.

NIEHS is one of three federal agencies that provide support for NTP activities. The other two agencies are:

- U.S. Food and Drug Administration, primarily through its National Center for Toxicological Research
- National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (CDC)

Areas for NTP Research and Testing

- Consumer products
  - Cell phone radiation, sunscreen components, flame retardants, nanomaterials, plastics, bisphenol A
- Our surroundings/environment
  - Mold, food borne toxicants/carcinogens, Elk River spill, drinking water, groundwater contaminants
- Medicines and therapeutics
  - AIDS therapeutics, dietary supplements, botanicals
- Workplace exposures
  - Butter flavorings, metal working fluids, nanomaterials



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NTP performs toxicology research and testing on a broad array of substances. For more information on NTP research, testing, and literature analysis programs visit http://ntp.niehs.nih.gov.

January 9, 2014

Residents notice a “sweet smell” (like licorice) in the air and report to the West Virginia Department of Environmental Protection.

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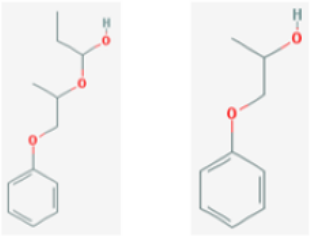
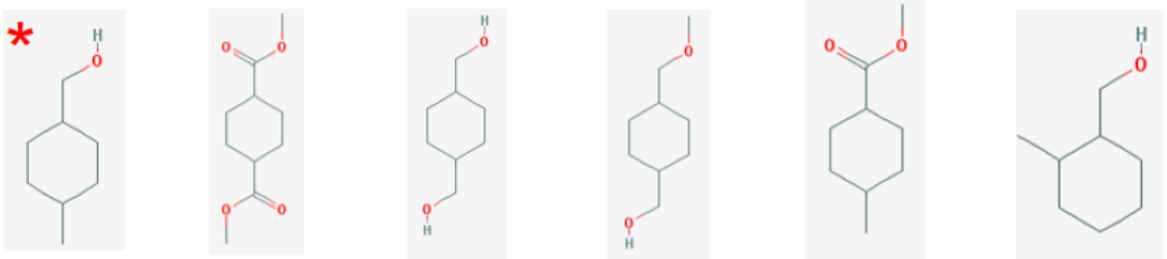
Background on the Elk River chemical spill.

January 9, 2014

Phenyl Ethers PPH



Crude MCHM



Freedom Industries reported a liquid (crude MCHM) used to wash coal was spilled from a leaking tank into the Elk River approximately 1.5 miles upstream of the water intake facility serving 300,000 people. Do not use water order issued. 6

Crude MCHM refers to a mixture of chemicals of similar structure shown here. MCHM or 4-methylcyclohexanemethanol is the primary chemical in the spilled liquid and is indicated with an *.

Elk River Spill

January 2014

- ~10000 gallons of crude MCHM and stripped propylene glycol phenyl ether (PPH) spilled into the Elk River
- West Virginia American Water reported levels of MCHM in the treated water as high as 3-4 ppm at the height of the spill but quickly drop below 1 ppm
- Health effects such as nausea, skin and eye irritation, and headaches were reported by residents in affected areas
- CDC developed drinking water advisory level of 1 ppm for MCHM and 1.2 ppm for PPH based on manufacturer's unpublished toxicology studies
- Initial internal NTP assessment suggested little concern for long-term health effects given transient low exposure

An advisory to not drink the water was issued on the afternoon of the spill. The spilled material overwhelmed the filtration system of the local water utility and entered the water distribution system. CDC set an initial drinking water advisory level of 1 part per million (ppm) for MCHM based on information reported on a Material Safety Data Sheet issued by the manufacturer Eastman Chemical Company. The 1 ppm level was reaffirmed later following release by the manufacturer of a more extensive 28-day, repeated-dose, rat toxicology study. A drinking water advisory level for another chemical propylene glycol phenyl ether (PPH), present in much lower quantities, was established based on a more complete database of toxicology information made available by the manufacturer Dow Chemical Company.

Based on initial evaluation of the structures of the spilled chemicals, the transient nature of the spill, and the irritating qualities of the spilled materials, NTP judged that there was low concern for any lasting health effects.

The advisory against drinking the water was lifted by the water company on January 18. CDC determined the water to be safe for drinking on January 21, but retained a caution for pregnant women.

Elk River Chemical Toxicology Data

Uncertainties

- Few toxicology studies used to support the MCHM drinking water advisory level
  - Very limited animal toxicology data set
- No studies of MCHM in developing animals
  - Are they more susceptible to the effects of the chemicals?
- Limited data on the minor components of the spill
  - Do they have distinct toxicities or are they more potent?

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At the time of the spill, there were few toxicology studies available on which to base a drinking water advisory level. Because developing animals and humans are typically considered more susceptible than adults to toxic effects of environmental chemicals, the lack of any studies in developing organisms was a concern, as was the absence of information on many of the minor spilled chemicals.

Nomination to NTP

July 2014

- The Centers for Disease Control and Prevention requests the NTP to undertake research to address lingering uncertainties
- "A research effort aimed at providing meaningful information to public health decision-makers ***over the coming year*** would be most useful."

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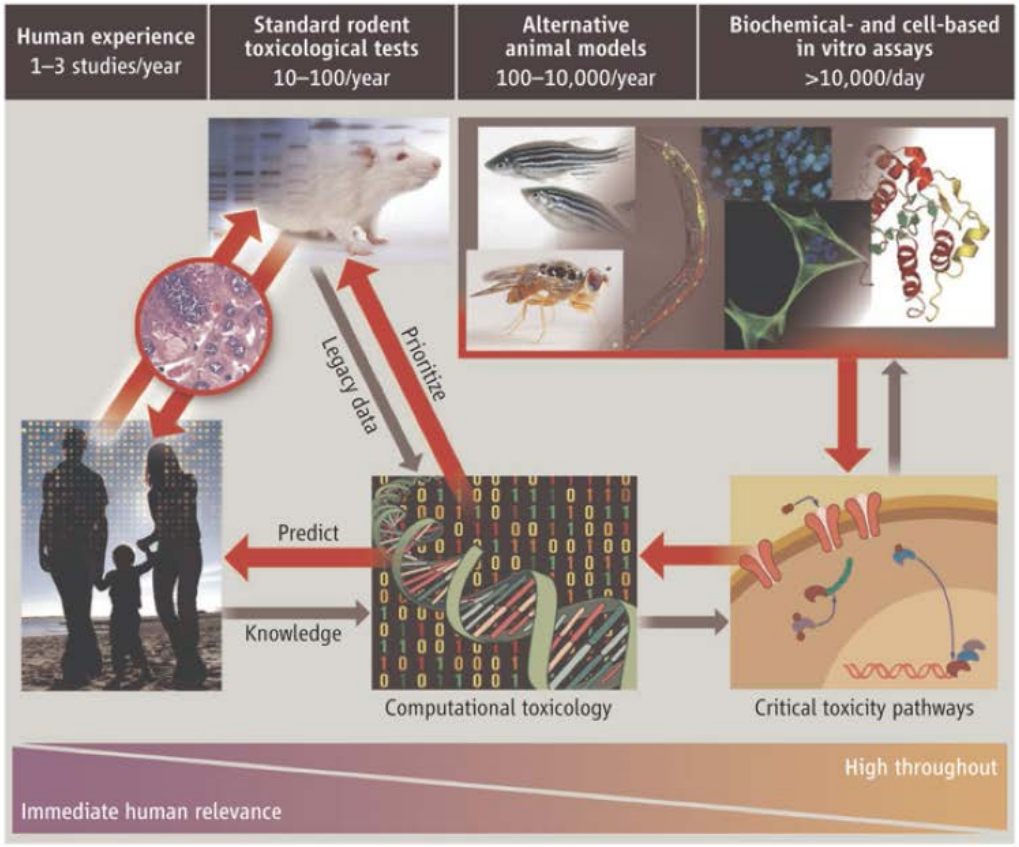
CDC nominated the Elk River spill chemicals to NTP for further studies in July 2014 (http://ntp.niehs.nih.gov/testing/noms/search/summary/nm-n21408.html).

Toxicology Models and Human Relevance



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Many different experimental models are available to study the toxic effects of chemicals. The slide depicts some of them. These range from in silico approaches, involving only computerized searches of toxicology databases, through studies in cells in vitro, to bacteria, flies, worms, fish, rodents, and in rare cases even non-human primates. They differ widely in their capacity to predict human health effects, as well as in the time and costs associated with their performance. However, many of the molecular pathways involved in a response to a toxicant are similar across different life forms, and studying a broad selection of assays can provide predictions that provide more confidence than studies in one or just a few models. Because of the desire to produce usable data relatively quickly, NTP chose to design and perform studies using a variety of toxicology models in assays of relatively short duration, which are believed to represent a wide spectrum of biology.

Goals of NTP Studies

- Strengthen the science base on primary spill chemicals and reduce uncertainty around information used to develop the drinking water advisory levels
  - Evaluate doses or concentrations of MCHM, crude MCHM, and PPH that produce toxicity or biological effects
- Determine if there are hazards for sensitive life stages (e.g., development) from exposure to MCHM
  - Address concerns over the extended time that pregnant women were advised to not consume the water
- Examine minor components of the spilled liquid to determine if they produce effects that are distinct from MCHM

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There were three primary goals of the NTP studies. All were focused on determining the adequacy of the initial drinking water advisory levels that were established by CDC at the time of the spill.

Information about the NTP response to the West Virginia Elk River chemical spill is available on the on the NTP Website at http://ntp.niehs.nih.gov/results/areas/wvspill/index.html.

Types of NTP Studies Selected

Rapid predictive screens

- Structure activity relationship – computer searches of known toxicology information for chemicals of similar structure
- High throughput screens – expose human cells to chemicals to monitor toxicity
- Nematode (roundworm) toxicity – expose nematodes to chemicals to monitor effects on reproduction, growth, and behavior
- Zebrafish embryo toxicity – expose zebrafish embryos to chemicals to monitor effects on structural and functional development
- Bacterial mutagenicity – expose bacteria to chemicals to monitor for mutations in genes

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The spilled chemicals were studied in five types of toxicity screening assays in human cells in vitro and in lower life forms. Computerized structure activity relationship screens were performed to extend those performed in the months immediately following the spill.

Types of NTP Studies Selected

Studies using rodents

- 5-Day toxicogenomic study – chemicals given to rats for 5 days to monitor liver and kidney for evidence of a biological response; in this case, changes in the expression of genes known to be associated with responses to toxic chemicals
- Mouse dermal irritation and hypersensitivity studies – chemicals applied to mouse skin to assess potential to cause irritation and allergic responses
- Rat prenatal toxicity studies – chemicals given to pregnant rats to determine effects on their offspring

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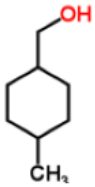
There were three types of studies performed in rodents. These involved measurements of the irritant and potential sensitizing effects on mouse skin, studies of the development of rat fetuses after dosing pregnant females, and very sensitive studies of changes in the expression of genes involved in toxic responses in the livers and kidneys of rats following repeated oral exposures.

Structure Activity Relationship (SAR)

Description

- Predict chemical toxicity of the spilled chemicals based on their structure
- Used six computer software platforms containing ~200 SAR models that cover many toxicological endpoints
- Rapidly identifies potential toxicological hazards



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Structure activity relationship (SAR) assessments use computerized models that compare the chemical structures of substances of interest with existing toxicology databases of findings from studies of chemicals with similar chemical structures. They vary widely in their predictive accuracy depending on the extent of toxicology information and the variety of chemical structures included in the models. Therefore, use of many models increases confidence in the predictions.

Structure Activity Relationship (SAR)

Findings

- MCHM and other chemicals that look like MCHM
  - Positive predictions of moderate to high confidence
    - Developmental toxicity and irritancy
    - Predictions do not take into account doses required to produce effects
- Phenyl Ethers (PPH and DiPPH)
  - Positive predictions of moderate to high confidence
    - None

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Findings from a comprehensive battery of SAR models suggested that MCHM would likely be an irritant and may produce toxicity to developing organisms. It is important to note that the models do not take into account the doses required to produce effects and that many of the models have a number of limitations that reduce confidence in the predictions.

The NTP update with findings from the SAR analysis is available at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/sar_wvupdate_dec2014_508.pdf.

High Throughput Screening

Description

- Evaluate effects of chemicals on signaling pathways of toxicological concern in human cells in vitro
- Used 27 different human cell-based screening assays
- Tested at concentrations up to 92 μM
- Determine if a chemical has the potential to affect biological processes related to toxicity

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The studies on the Elk River spill chemicals are part of a larger, high throughput screening effort where over 8000 chemicals in commerce are being evaluated for their ability to activate biological targets related to toxicity in human cells and cell lines.

High Throughput Screening

Findings

- All tested chemicals, including MCHM, were inactive at concentrations up to 92 µM (~10-20 ppm)

Chemical	Call
4-Methylcyclohexanemethanol [MCHM]	Inactive
Propylene glycol phenyl ether [PPH]	Inactive
1,4-Cyclohexanedimethanol [CHDM]	Inactive
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC]	Inactive

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All chemicals were inactive in the assays performed to date. Verification of the actual concentrations of chemicals in these assays is ongoing. The NTP update with findings from the high throughput screening assays is available at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/hts_wvupdate_508.pdf.

Nematode Toxicity

Description

- Growth, feeding, and reproduction are measured in the nematode (a small roundworm) following chemical treatment
- Screening level study to determine if a chemical is toxic in a multicellular organism

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The nematode (*Caenorhabditis elegans*) is a multicellular organism that is well characterized as to its genetics and normal developmental patterns. It provides a rapid screening assay that has been used extensively to identify chemicals that may disrupt development, feeding behavior, and reproduction.

Nematode Toxicity

Findings

- All tested chemicals, including MCHM, were inactive up to 100 µM (~20-40 ppm)

Chemical	Call
4-Methylcyclohexanemethanol [MCHM]	Inactive
Technical product [crude MCHM]	Inactive
Dipropylene glycol phenyl ether [DiPPH]	Inactive
Propylene glycol phenyl ether [PPH]	Inactive
1,4-Cyclohexanedimethanol [CHDM]	Inactive
4-(Methoxymethyl)cyclohexanemethanol [MMCHM]	Inactive
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC]	Inactive
Commercial product [Dowanol DiPPh glycol ether]	Inactive

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All chemicals tested were inactive in this assay. The NTP update with findings from the nematode toxicity study is available at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/celegans_wvupdate_march2015_508.pdf.

Zebrafish Developmental Toxicity

Description

- Chemical is added to water containing developing fish
- Fish are monitored for effects on behavior and development

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The zebrafish is a vertebrate that offers many advantages in toxicity screening. The study is performed by adding the chemicals to the water in which the zebrafish embryo lives and its development is monitored. As can be seen in the slide, the zebrafish larva is essentially transparent and can be visually scored for a variety of structural defects. Also, its response to touch can be measured and used as a way to evaluate neurological development.

Zebrafish Developmental Toxicity

Findings

- MCHM, crude MCHM, and PPH produced no effects at concentrations up to 100 µM (~10-20 ppm)
- Minor spill component (<1%), dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC), produced developmental abnormalities at a concentration of ~13 ppm

Chemical	Call
4-Methylcyclohexanemethanol [MCHM]	Inactive
Technical product [crude MCHM]	Inactive
Propylene glycol phenyl ether [PPH]	Inactive
1,4-Cyclohexanedimethanol [CHDM]	Inactive
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC]	Active

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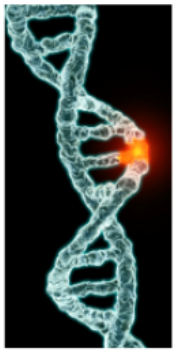
The spill chemicals were inactive in causing structural malformations or mortality in the zebrafish embryo assay with one exception. The minor spill component dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC) was active in this assay, causing several common structural malformations at a concentration of 13 ppm and above, and mortality at approximately 17 ppm. Although one might wish to compare the water concentrations in these assays with the drinking water advisory level of 1 ppm, it is important to remember that both the nematode and zebrafish embryo assays are screening level assays intended to reveal the potential for developmental effects. Comparisons of doses or concentrations that might cause heath effects in the exposed human population are more appropriately derived from the rodent prenatal dosing study and the 5-day toxicogenomic study. The NTP update with findings from the zebrafish developmental toxicity study is available at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/zebrafish_update_508.pdf.

Bacterial Mutagenesis

Description

- DNA mutation rates are measured after treatment of bacteria with chemical
- Identifies chemicals that mutate DNA, which tend to have a greater potential to cause cancer and developmental effects



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The bacterial mutation assay is commonly known as the Ames assay. Three different strains of bacteria are exposed to the chemicals and monitored for mutations or permanent changes in DNA sequence.

Bacterial Mutagenesis

Findings

- None of the chemicals from the spill tested to date, including MCHM, crude MCHM, and PPH, caused DNA mutations

Chemical Name	Call
4-Methylcyclohexanemethanol [MCHM]	Inactive
Technical product [crude MCHM]	Inactive
Propylene glycol phenyl ether [PPH]	Inactive
Dipropylene glycol phenyl ether [DiPPH]	Inactive
Commercial Product [Dowanol DiPPh glycol ether]	Inactive
Methyl 4-methylcyclohexanecarboxylate [MMCHC]	Inactive
4-(Methoxymethyl)cyclohexanemethanol [MMCHM]	Inactive
2-Methylcyclohexanemethanol [2MCHM]	Inactive

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None of the chemicals were found to cause mutations in these studies. The NTP update with findings from the bacterial mutagenicity study is available at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/bacterial_mutagenesis_update_508.pdf.

5-Day Toxicogenomic

Description

- Chemical is administered in wide dose range to rats for 5 days
- Sensitive molecular endpoints are measured to determine the lowest dose where biological change is observed
- Global screen that helps to identify a biological NOEL (no observed effect level), which typically occurs at lower doses than toxicity

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In this study, the chemicals were given orally, once a day, for five days, to rats. At the end of the study, the animals were humanely killed, and the liver and kidneys were evaluated at the molecular level for evidence that the animals sensed and responded to the chemical treatment.

5-Day Toxicogenomic

Findings

- MCHM
  - Lowest biological effect level was a dose that is approximately >3000 times higher than estimated human adult exposure, assuming 1 ppm in the drinking water
- Crude MCHM
  - Lowest biological effect level was a dose that is approximately >2000 times higher than estimated human adult exposure, assuming 1 ppm in the drinking water
- PPH
  - Lowest biological effect level was a dose that is approximately >2000 times higher than estimated human exposure, assuming 0.01 ppm in the drinking water

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This study showed that the rats sensed the tested chemicals at the higher doses, but at much higher doses than would have possibly been encountered by persons consuming the water at or above the drinking water advisory level of 1 ppm for MCHM. The specifics and assumptions supporting the statements in the slide follow on the next page. Rats receiving PPH did show evidence of a biological response of unknown toxicological significance in the liver at a dose lower than that required for MCHM or crude MCHM. The NTP update with findings from the 5-day toxicogenomic study is available at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/micronucleus_update_508.pdf.

MCHM and crude MCHM calculations

Drinking water level of 1 ppm = 1 mg/L

70 kg adult drinks 2.0 L per day; therefore, the dose received is 2.0 mg/70 kg = 0.029 mg/kg/day

107 mg/kg/day (MCHM lowest BMD)/0.029 mg/kg/day = margin of exposure of 3689

63 mg/kg/day (crude MCHM lowest BMD)/0.029 mg/kg/day = margin of exposure of 2172

MCHM - In order to get a 7490 mg dose/day (=107 mg/kg/day MCHM in a 70 kg adult) at 1 mg/L of MCHM in the water, an adult would have to drink 7490 L of water or 1978 gallons per day

Crude MCHM - In order to get a 4410 mg dose/day (=63 mg/kg MCHM in a 70 kg adult) at 1 mg/L of crude MCHM in the water, an adult would have to drink 4410 L of water or 1165 gallons per day

PPH calculations

Highest level of PPH found was about 0.01 ppm in the water (most measurements did not detect any PPH)

Drinking water level of 0.01 ppm = 0.01 mg/L

70 kg adult drinks 2.0 L per day; therefore, the dose received is 0.02 mg/70 kg = 0.00029 mg/kg/day

0.6 mg/kg/day (PPH lowest BMD)/0.00029 mg/kg/day = margin of exposure of 2100

PPH - In order to get a 42 mg dose/day (=0.6 mg/kg PPH in a 70 kg adult) at 0.01 mg/L of PPH in the water, an adult would have to drink 4200 L of water or 1110 gallons per day

Statements about required consumption to achieve biological effect dose

MCHM - Assuming 1 ppm MCHM in the drinking water, a person would need to consume >1000 gallons of water per day to achieve a dose level that produced biological changes in the rats

Crude MCHM - Assuming 1 ppm crude MCHM in the drinking water, a person would need to consume >1000 gallons of water per day to achieve a dose level that produced biological changes in the rats

PPH - Assuming 0.01 ppm PPH (highest detected level in water) in the drinking water, a person would need to consume >1000 gallons of water per day to achieve a dose level that produced biological changes in the rats

BMD = benchmark dose - a concept commonly used risk assessment to indicate a dose or concentration that produces a predetermined change in response rate of an adverse effect compared to background

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* BMD = benchmark dose - a concept commonly used risk assessment to indicate a dose or concentration that produces a predetermined change in response rate of an adverse effect compared to background

Dermal Irritancy and Hypersensitivity

Description

- Chemical is applied to skin of a mouse and localized skin swelling and immune response are monitored to determine if the chemical is an irritant or can induce sensitization (skin allergy)

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This is a commonly used test, formally known as the Local Lymph Node Assay. Chemicals are applied to the skin and various measures are made to evaluate both the ability of the substances to cause local irritation, as well as sensitize the mouse to respond to repeated applications with an allergic reaction.

Dermal Irritancy and Hypersensitivity

Findings

- MCHM
  - Caused mild irritation at concentrations ~200,000 times higher than the estimated concentration that humans were exposed (1 ppm)
  - Did not cause an allergic response
- Crude MCHM
  - Caused mild irritation at concentrations ~750,000 times higher than the estimated concentration that humans were exposed (1 ppm)
  - Caused a weak allergenic response at concentrations ~400,000 times higher than the estimated concentration that humans were exposed (1 ppm)

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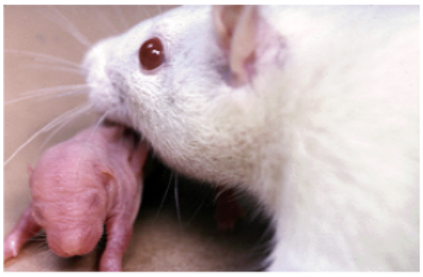
This study found that pure MCHM was a mild skin irritant, and that crude MCHM was both an irritant and sensitizer. However, the concentrations of the chemicals applied to the skin, which were required to produce these effects, were quite high. The NTP update with findings from the mouse dermal irritancy and hypersensitivity study is available at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/mouse_dermal_wvupdate_508.pdf.

MCHM Rat Prenatal Developmental Toxicity

Description

- Pregnant rats are exposed to chemical to determine if there are effects on the developing fetus
  - Approximately two weeks of exposure



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The prenatal developmental toxicity study is a standard study designed to test whether chemicals have the ability to affect normal fetal development in a pregnant rat. Pregnant rats are given the chemicals orally during the period of pregnancy when the skeleton and organs are undergoing rapid growth and development. The rats are humanely killed just prior to the expected time of birth, and the fetuses undergo a detailed evaluation.

MCHM Rat Prenatal Developmental Toxicity

Findings

- Minimal toxicity observed in pregnant rats
- No effects on fetal survival
- Effects on birth weight were observed at doses ~6000 times higher than would be achieved by a pregnant woman
  - Pregnant woman would have to drink 3900 gallons of water containing 1 ppm of MCHM to achieve the dose of MCHM that produced effects on birth weight in developing rats
- Developmental defects observed at doses ~12000 times higher than estimated human exposure
  - Pregnant woman would have to drink 7800 gallons of water containing 1 ppm of MCHM to achieve the dose of MCHM that produced developmental effects in rats

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The findings from this study showed minimal evidence of toxicity to the pregnant rats. 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, and some malformations in the fetuses were seen at the very highest dose (400 mg/kg/day). As can be seen in the slide, these doses were thousands of times higher than a pregnant woman would have been exposed to by ingesting the water containing MCHM at the drinking water advisory level. The NTP update with findings from the prenatal developmental toxicity study is available at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/prenatal_wvupdate_june2015_508.pdf.

Assumptions for margin of exposure of 6000

- Water levels of MCHM were 1 ppm (1 mg/L)
- Pregnant woman drinks 2.5 L (0.66 gallons) of water per day
- Pregnant woman weighs 75 kg (165 lbs)
- Dose in a pregnant woman drinking 2.5 L of water containing 1 ppm MCHM (1 mg/L) is 0.033 mg/kg/day
- Lowest effect level on rat fetus is 200 mg/kg/day
- 200 mg/kg/day/0.033 mg/kg/day = a margin of exposure of 6060

Results in Context of NTP Study Goals

- Strengthen the science base on MCHM and reduce uncertainty around information used to develop the drinking water advisory levels
  - Rat Prenatal Toxicity Study and 5-Day Toxicogenomic Study confirm NOEL (no observed effect level) of approximately 100 mg/kg/day for MCHM, similar to Eastman 28-day toxicity study
  - PPH produced changes in biological activity (gene expression in liver) starting at approximately 1 mg/kg/day; however, the toxicological implications of these findings are uncertain
  - Concentrations of MCHM and crude MCHM required to produce skin irritation and sensitization are quite high
  - Lack of genotoxic potential of the spilled chemicals minimizes concern for long-term effects such as carcinogenicity

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The lowest doses at which there was evidence for toxicity in the collected NTP studies were similar to those in the studies used by CDC to establish the drinking water advisory level at the time of the spill.

Results in Context of NTP Study Goals

- Determine if there are hazards for sensitive life stages from exposure to MCHM
  - The fetus is more sensitive to toxicity than the pregnant adult
  - Toxicity was observed at doses that approximate the no-effect level used to derive the drinking water advisory level for MCHM (100 mg/kg/day)
  - Toxicity occurred well in excess of the drinking water advisory level that was derived by CDC

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The rat prenatal toxicity study found that one of the more sensitive responses to MCHM was a decreased weight of the fetuses in pregnant rats given the chemicals during gestation. However, this effect was observed at doses many thousands of time higher than any expected exposures of pregnant women following the Elk River chemical spill.

Results in Context of NTP Study Goals

- Screen minor components of the mixture to determine if any are significantly more toxic than MCHM
  - There are minimal differences in potency or toxicity between most of the minor constituent chemicals and MCHM, and between MCHM and crude MCHM
  - A very minor spill component (DMCHDC) may be more toxic to developing organisms (zebrafish study finding) than MCHM
  - A minor, yet unidentified, component of crude MCHM may be a sensitizer

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Dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC), a minor spill component, has been found to be of low toxicity in a rat reproductive toxicity study, suggesting the findings in zebrafish are of minimal concern. An unknown component of the spill may have the capacity to cause skin sensitization, but at very high concentrations.

The collected findings of the NTP studies support the adequacy of the drinking water advisory levels established at the time of the spill.