West Virginia Chemical Spill: 5-Day Rat Toxicogenomic Studies
June 2015 NTP Update*

Synopsis

The National Toxicology Program (NTP)[1] evaluated three chemicals spilled into the Elk River in West Virginia for their ability to cause toxicity or biological changes in a short-term toxicogenomic study in male rats. The primary goal of a toxicogenomic study is to monitor gene expression in selected organs as a sensitive indicator of a biological response to a foreign chemical. This type of study allows the identification of the lowest dose that produces a change in any molecular biological process (MBP; a group of genes that work together to regulate a given cellular function). The study evaluated MBP changes in the liver and kidney produced by giving 4-methylcyclohexanemethanol (MCHM), propylene glycol phenyl ether (PPH), or a commercial mixture "crude MCHM" containing primarily MCHM and lesser amounts of other spilled chemicals to male rats. Groups of rats were given one of seven doses of the chemical orally once per day for five days. For MCHM and crude MCHM, the doses ranged from 0.1-500 mg/kg/day, and for PPH the doses ranged from 1-2000 mg/kg/day.

In the liver, MCHM had effects on MBPs at doses as low as 100 mg/kg/day. Crude MCHM had effects similar to MCHM and caused MBP changes in the liver at doses slightly less than 100 mg/kg/day. The changes in MBPs caused by PPH included some of uncertain toxicological significance, which occurred at doses as low as 1-5 mg/kg/day. The effects of MCHM on MBPs occurred at doses of MCHM that showed minimal toxic effects in previous studies[2]; however, for PPH, effects on MBPs occurred at doses lower than those previously reported to cause other indications of toxicity. In addition to effects on MBPs, all three chemicals caused effects on the liver and thymus at high doses. High doses of MCHM and crude MCHM increased liver weight, decreased thymus weight, and increased levels of triglycerides, a form of fat in the blood. High doses of PPH decreased body weight, decreased thymus weight, and resulted in liver damage.

5-Day Rat Toxicogenomic Studies

Background on Toxicogenomic Studies

A toxicogenomic study is conducted to identify effects of a chemical on toxicological or biological processes in the liver or other organs. A wide range of biological processes can be evaluated at the molecular level, that is, changes in the expression of genes that are responsible for all aspects of cellular function. Genes that are expressed in a coordinated manner and enable cells to carry out a series of actions can be grouped together for analysis. Changes in expression of these groups of genes provide information on molecular biological processes (MBPs) that are affected by chemical exposure.

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*ERRATUM: An error was identified in the month of the update. This update was posted in June 2015 and not in May 2015 as originally stated. This error has been corrected in the PDF on February 9, 2016.

[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] http://www.eastman.com/Literature_Center/Misc/Pure_Distilled_MCHM-28-Day_Oral_Feeding_Study.pdf

In a toxicogenomic study, these types of molecular changes can be compared to other traditional indications of toxicity such as changes in organ weight and structure, clinical measures in the blood[3], or genetic damage. Changes at the molecular level in response to a chemical exposure often occur at lower dose levels and more quickly than other toxic effects. For this reason, such changes can give a more sensitive view of potential biological activity. Furthermore, this means that toxicological effects are less likely to occur at doses below those producing changes in MBPs.

For this study, effects on gene expression were determined by measuring the levels of messenger RNA molecules in the liver and kidney. Liver and kidney were selected because previous toxicology studies identified them as target organs for effects of the spill chemicals. A wide range of doses of the chemicals was tested to allow identification of the MBPs that are most sensitive to chemical treatment.

In order to identify the dose level where effects on MBPs start to occur, a modeling approach, referred to as benchmark dose (BMD) analysis, is used. BMD analysis of gene expression data estimates the lowest dose level where significant effects on MBPs occur. Because there are many MBPs that regulate the diverse functions of the cell and they are affected at different dose levels, the BMD analysis for each chemical yields a range of MBP BMD values.

Findings from the 5-Day Toxicogenomic Study

NTP conducted a 5-day toxicogenomic study in male rats to evaluate the toxicity of chemicals spilled into the West Virginia Elk River. The chemicals tested were the primary spill chemical 4-methylcyclohexanemethanol (MCHM), propylene glycol phenyl ether (PPH), and crude MCHM, a commercial mixture containing primarily MCHM along with lesser amounts of other spill chemicals.[4] Each chemical was mixed in corn oil and administered orally once daily for five days to separate groups of male rats at seven doses. Blood samples were obtained from the rats 24 hours after the fifth treatment for measurement of organ damage markers, biochemical changes and DNA damage.[5,6] Results from this study for effects on genetic damage in the bone marrow were reported previously.[7]

Table 1. Substances Studied in the 5-Day Toxicogenomic Study

CASRN*	Chemical Name	Dose Levels (mg/kg/day)	Notes
34885-03-5	4-Methylcyclohexanemethanol (MCHM)	0, 0.1, 1, 10, 100, 300, 500	a
NA	Crude 4-Methylcyclohexanemethanol (crude MCHM)	0, 0.1, 1, 10, 100, 300, 500	b
770-35-4	Propylene glycol phenyl ether (PPH)	0, 1, 10, 100, 500, 1000, 2000	c

*CASRN = Chemical Abstract Registry Number; [a]Major or minor constituent of the spilled liquid; [b]A commercial mixture containing >70% MCHM along with lesser amounts of five other chemicals; [c]Minor constituent of the spilled liquid.

[3] http://ntp.niehs.nih.gov/testing/types/clin/index.html
[4] Dipropylene glycol phenyl ether was also studied and those results will be reported in a later NTP Update.
[5] http://ntp.niehs.nih.gov/testing/types/clin/index.html
[6] http://ntp.niehs.nih.gov/testing/types/genetic/invivo/mn/index.html
[7] http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/micronucleus_wvfeb2015_508.pdf (ERRATUM: An error was identified in the original URL. The URL has been corrected in the PDF on February 9, 2016.)

MCHM

MBP Findings. In the liver, the expression of a small number of genes was changed in response to exposure to MCHM indicating an overall weak toxicogenomic response. The MBP BMD values in liver for MCHM ranged from 107 mg/kg/day to 495 mg/kg/day (Table 2). These changes in MBPs in liver occur at doses similar to those causing toxicological effects in rats as reported in a previous study.[8] In kidney, there were no significant changes in gene expression related to MCHM exposure; therefore, it was determined that there was not enough signal in the data to reliably identify MBP BMDs.

Other Findings. There was no effect of MCHM on body weight at any dose level. A slight increase in liver weight was observed in the group of rats receiving the highest dose level, and decreased thymus weight was observed in the groups of rats receiving the two highest dose levels. An effect on thymus weight is commonly observed in animals experiencing general toxicological stress. An increase in triglycerides, a type of fat found in the blood, in the 500 mg/kg/day dose group was the only significant effect of MCHM on clinical chemistry and hematology.

Crude MCHM.

MBP Findings. In the liver, the expression of a small number of genes was changed in response to exposure to crude MCHM, indicating an overall weak toxicogenomic response. The MBP BMD values in liver ranged from 63 mg/kg/day to 479 mg/kg/day (Table 2). These changes in MBPs occur at doses similar to those causing toxicological effects in rats as reported in a previous study of MCHM.[9] In kidney, there were no significant changes in gene expression; therefore, it was determined that there was not enough signal in the data to reliably identify MBP BMDs.

Other Findings. There was no effect of crude MCHM on body weight at any dose level. An increase in liver weight was observed in the groups of rats receiving the two highest dose levels. Decreased thymus weight also occurred in the groups of rats receiving the two highest dose levels and is likely attributable to general toxicological stress. The only significant effect of MCHM on clinical chemistry and hematology measurements attributable to crude MCHM was an increase in triglycerides in the 300 and 500 mg/kg/day dose groups.

PPH

MBP Findings. In the liver and kidney, the expression of a small number of genes was changed in response to PPH exposure, indicating an overall weak toxicogenomic response. In liver, the MBP BMD values ranged from 0.6 to 985 mg/kg/day (Table 2). In the kidney, the MBP BMD values ranged from 4 to 998 mg/kg/day (Table 2). Some MBP changes in liver and kidney occurred at doses 10-20 fold lower than those causing toxicological effects in rats as reported in previous studies.[10] The toxicological significance of these changes in MBPs occurring at lower dose levels will require further investigation.

Other Findings. The group of rats administered PPH at the top dose level of 2000 mg/kg exhibited signs of acute toxicity and were removed from the study before it ended. In addition, one death occurred in the 1000 mg/kg/day dose group, and there was a slight decrease in body weight in the group of rats receiving 1000 mg/kg/day. Decreased thymus weights were found in groups of rats receiving 500 and 1000 mg/kg/day and are likely attributable to general toxicological stress. The only significant effect of

[8] http://www.eastman.com/Pages/Eastman-Crude-MCHM-Studies.aspx and http://emergency.cdc.gov/chemical/MCHM/westvirginia2014/pdf/MCHM-Summary-Report.pdf

[9] *ibid*.

[10] http://emergency.cdc.gov/chemical/MCHM/westvirginia2014/pdf/DiPPH-PPH-calculation.pdf

PPH on clinical chemistry and hematology measurements was a slight increase in the levels of the enzyme ALT, a marker of liver damage, in the 500 and 1000 mg/kg/day dose groups.

Table 2. Molecular Biological Process Benchmark Dose Ranges

Chemical	Liver Molecular Biological Processes	Kidney Molecular Biological Processes
MCHM	107-495 mg/kg/day	ND*
Crude MCHM	63 - 487 mg/kg/day	ND
PPH	0.6-985 mg/kg/day	4-998 mg/kg/day

*ND = not determined because significant change in gene expression in response to chemical treatment was not observed.

Next Steps

The 5-day toxicogenomic studies of MCHM, crude MCHM, and PPH to evaluate potential toxicity and identify the level at which biological effects occurred in liver and kidney are finished. NTP will consider the findings from these studies in any future, overall assessment of the spilled chemicals.