West Virginia Chemical Spill: Zebrafish Photomotor Response Study
July 2016 NTP Update (Revision of the August 2015 Update)

Synopsis
The National Toxicology Program (NTP)[1] conducted a photomotor response study in zebrafish (*Danio rerio*) to evaluate the toxicity of 13 chemicals associated with the West Virginia Elk River chemical spill. This study evaluates the 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 reflect perturbations of behavior and neurological function, which are indicative of a potential neurotoxic effect.

In two identical and independent photomotor response studies,[2] three chemicals were found to have altered zebrafish movement in response to light: (1) 4-methylcyclohexanemethanol (MCHM), the main component of the spilled liquid; (2) cyclohexanemethanol, alpha,alpha,4-trimethyl-; and (3) cyclohexanemethanol, 4-[(ethenyloxy)methyl]-. Cyclohexanemethanol, alpha,alpha,4-trimethyl- and cyclohexanemethanol, 4-[(ethenyloxy)methyl]- are chemicals that are structurally similar to MCHM and were not present in the spilled liquid.

Zebrafish Developmental Toxicity Study

Background on Zebrafish

Zebrafish is a small, tropical, freshwater fish (*Danio rerio*) belonging to the minnow family 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 detailed information is available on its genetic code. Zebrafish also undergo many of the same morphological steps in embryonic development as humans, and similar genes control these steps.

The developing brains of zebrafish embryos are responsive to light. The embryos move more frequently when they are exposed to light, and this is called a "photomotor" response. If the photomotor response changes after chemical treatment, this is an indicator of neurotoxicity in the developing fish. In this study, zebrafish photomotor response was measured in the presence and absence of chemicals associated with the spill.

[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] For the August 2015 NTP update, two chemicals were found to have altered zebrafish movement in response to light: (1) 4-methylcyclohexanemethanol (MCHM), the main component of the spilled liquid and (2) cyclohexanemethanol, alpha,alpha,4-trimethyl-, a chemical that is structurally similar to MCHM and was not present in the spilled liquid. Using an updated method to evaluate the data, a third chemical was found to have altered zebrafish movement in response to light: cyclohexanemethanol, 4-[(ethenyloxy)methyl]-. This third chemical is structurally similar to MCHM and was not present in the spilled liquid. The August 2015 NTP update can be found at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/zebrafish_update_aug2015_508.pdf. The new data analysis method is described in a recent publication: Reif, D.M., et al. High-throughput characterization of chemical-associated embryonic behavioral changes predicts teratogenic outcomes. Arch Toxicol, 2015. Epub ahead of print.

Chemicals Tested in the Study

Zebrafish embryos were collected after fertilization, and the chorion, or outer membrane surrounding the embryo, was removed. Six hours post fertilization (hpf), seven concentrations (2, 18, 35, 51, 67, 84,[3] and 100 µM) of the pure chemicals or the mixture (Table 1) were added to the liquid containing individual zebrafish embryos. The seven molar concentrations of 4-methylcyclohexanemethanol (MCHM) correspond approximately to 0.3, 2.3, 4.5, 6.5, 8.5, 10.8,[3] and 12.8 ppm. At 24 hpf, zebrafish embryos were exposed to pulses of light, and their rate of movement was measured.

Table 1. Elk River Spill Chemicals Tested in Zebrafish

CASRN*	Compound Name	Notes
34885-03-5	4-Methycyclohexanemethanol (MCHM)	a
NA	Crude 4-Methycyclohexanemethanol (Crude MCHM)	b
770-35-4	Propylene glycol phenyl ether (PPH)	a
94-60-0	Dimethyl 1,4-cyclohexanedicarboxylate	a
51181-40-9	Methyl 4−methylcyclohexanecarboxylate (MMCHC)	a
98955-27-2	4−(Methoxymethyl)cyclohexanemethanol (MMCHM)	a
4331-54-8	4−Methylcyclohexanecarboxylic acid	a
2105-40-0	2−Methylcyclohexanemethanol (2MCHM)	a
105-08-8	1,4-Cyclohexanedimethanol	a
4169-04-4	Phenoxyisopropanol	c
114651-37-5	Cyclohexanemethanol, 4−[(ethenyloxy)methyl]−	c
498-81-7	Cyclohexanemethanol, alpha, alpha, 4-trimethyl-	c
NA	DOWANOL™ DiPPh	d

* CASRN = Chemical Abstracts Service Registry Number. [a]Major or minor constituent of the spilled liquid (a minor constituent is considered to be approximately 10% or less of the spilled material); [b]A commercial mixture containing >70% MCHM along with lesser amounts of five other chemicals; [c] Not a component of the spilled liquid, but included because the compound is structurally related to MCHM or PPH; [d]A proprietary commercial mixture of dipropylene glycol phenyl ether isomers.

Study Findings

Chemicals associated with the spill were evaluated in two identical and independent studies. Three chemicals altered zebrafish photomotor response in both studies. MCHM, the main component of the spilled liquid, had effects on the zebrafish photomotor response at a dose of 84 µM or 11 ppm.[4] Two chemicals that are structurally similar to MCHM and were not in the spilled liquid also altered the zebrafish photomotor response: cyclohexanemethanol, alpha,alpha,4-trimethyl- at a dose of 51 µM or 8 ppm and cyclohexanemethanol, 4-[(ethenyloxy)methyl]- at a dose of 100 µM or 17 ppm. The findings that two chemicals similar to MCHM affected zebrafish photomotor response are consistent with and provide support for the MCHM finding.

[3] A rounding error was made in the August 2015 NTP update, which stated the concentration as 83 µM or 10.6 ppm.

[4] In the August 2015 NTP update, MCHM's effects on photomotor response were reported for doses as low as 35 µM. Upon further data analysis, NTP has determined that MCHM's effects on photomotor response was observed at 84 µM.

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, 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.

Next Steps

NTP studies to evaluate the toxicity of the spilled chemicals in developing zebrafish are complete. NTP will consider the findings reported here in any future, overall assessment of the spilled chemicals.