

# West Virginia Chemical Spill: Zebrafish Developmental Toxicity Study

## July 2015 NTP Update

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### Synopsis

The National Toxicology Program (NTP)<sup>1</sup> used zebrafish (*Danio rerio*) to evaluate the toxicity of six additional chemicals associated with the West Virginia Elk River chemical spill. This assessment evaluated effects on embryonic and larval development and behavior. None of the six chemicals resulted in developmental toxicity in zebrafish. In a June 2015 update, NTP reported zebrafish developmental toxicity findings for seven chemicals, including the main component of the spilled liquid, 4-methylcyclohexanemethanol (MCHM).<sup>2</sup> None of those chemicals, with the exception of a minor component of the spilled liquid, were toxic to developing zebrafish.<sup>2</sup>

### 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. In these studies, physical development, growth, and behavior were evaluated during the embryonic and larval period, which occurs over a period of five days.

#### 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, 83, and 100  $\mu$ M) of the pure chemicals or the mixture (Table 1) were added to the liquid containing individual zebrafish embryos. At 24 and 120 hpf, the embryos were evaluated for specific endpoints related to embryonic and larval development and behavior (Table 2).

Table 1. Elk River Spill Chemicals Tested in Zebrafish

CASRN*	Compound Name	Notes
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
114651-37-5	Cyclohexanemethanol, 4-[(ethenyloxy)methyl]-	b
NA	DOWANOL™ DiPPH	c

\*CASRN = Chemical Abstracts Service Registry Number. <sup>a</sup>Major or minor constituent of the spilled liquid (a minor constituent is considered to be approximately 10% or less of the spilled material); <sup>b</sup>Not a component of the spilled liquid, but included because the compound is structurally related to MCHM or PPH; <sup>c</sup>A proprietary commercial mixture of dipropylene glycol phenyl ether isomers.

<sup>1</sup> 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/>.

<sup>2</sup> The June 2015 update for the zebrafish developmental toxicity study can be found at [http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/zebrafish\\_update\\_508.pdf](http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/zebrafish_update_508.pdf).

Table 2. Endpoints Evaluated in Zebrafish

<b>Endpoints evaluated 24 hours post fertilization (hpf)</b>	
<b>Endpoint</b>	<b>Evidence of an adverse effect</b>
Mortality	Fish dies
Spontaneous movement	Fish exhibits no spontaneous movement
Progression of Development	Fish exhibits delayed development
Notochord	Notochord (embryonic anatomy structure that defines the center line of the body) is malformed (wavy notochord)
<b>Endpoint evaluated 120 hours post fertilization (hpf)</b>	
<b>Endpoint</b>	<b>Evidence of an adverse effect</b>
Mortality	Fish dies between 24 and 120 hpf
Axis	Axis (centerline of the body in larval fish) is curved or bent axis in either direction
Brain	Brain is malformed or tissue is dead
Caudal fin	Caudal fin is malformed or missing
Circulation	Fish exhibits reduced or no circulation or blood flow
Eye	Eyes are malformed, missing, or smaller/larger than normal
Heart	Fish has pericardial edema (fluid around the heart)
Jaw	Jaw is malformed
Otic	Auditory system is malformed or missing
Pectoral fin	Pectoral fin is malformed or missing
Pigmentation	Fish lacks pigmentation or is over pigmented
Snout	Snout is shortened or malformed
Somite	Somites (body sections) are malformed, disorganized, or missing
Swim bladder inflate	Swim bladder fails to inflate
Touch response	Fish is not responsive to touch
Trunk	Fish has short, malformed, or missing trunk
Yolk sac edema	Fish has fluid around the yolk sac

**Study Findings**

Chemicals associated with the spill were evaluated in two identical and independent studies. All six chemicals evaluated were inactive in both studies. Previously, NTP evaluated seven chemicals and found that a minor component of the spilled liquid (dimethyl 1,4-cyclohexanedicarboxylate) was toxic to developing zebrafish. The rest of the chemicals were inactive, including 4-methylcyclohexanemethanol (MCHM), the main component of the spilled liquid. Thus, 12 of the 13 chemicals evaluated did not result in developmental toxicity in 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.