

**Report on the U.S. Environmental Protection Agency's
Chronic Whole Effluent Toxicity Testing
At Selected Sites in the Coalfields
Of
Kentucky and West Virginia**

By

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Evaluation of the chronic toxicity tests carried out by the U.S. Environmental protection Agency at various sites in the coalfields of Kentucky and West Virginia, USA¹.

Between December 2007 and August 2009, the United States Environmental Protection Agency (EPA) collected water quality samples for chronic whole effluent toxicity (WET) testing at twenty selected sites in the coalfields of Kentucky and West Virginia. For seventeen of these sites water samples were instream (receiving) water samples. Three sites represented discharge (or effluent) water samples (see Table 1 for details of specific sample type). Site selection criteria included: 1) the site was located just downstream or at coal mining operations, 2) no intervening pollution sources could be identified by the EPA, and 3) conductivity levels of the waters at the site of collection were greater than 1000 $\mu\text{mhos/cm}$.

Toxicity levels exceeding national EPA recommended water quality guidelines for chronic whole effluent toxicity (WET) tests were found in fourteen of the seventeen instream (receiving water) samples tested. At two of these sites toxicity levels were over fifty times acceptable levels, three further sites exceeded these levels at least twenty-fold (see Table 1). The three discharge (effluent) water samples evaluated also contained unacceptable levels of toxicity; levels high enough to trigger a review of water discharge (NPDES) permits to assure that receiving waters meet federal and state limits on toxicity.

These data were derived from EPA standard toxicity testing methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms (EPA-821-R-02-013; USEPA, 2002), which is a regulatory approved method. As water samples may contain a mixture of many toxic compounds these methods are designed to investigate the total toxicity of the water rather than the toxicity of a single compound contained within. These standard methods (also called whole effluent tests (WET); USEPA, 2000) are used to identify effluents and ambient receiving waters containing toxic materials that are present in concentrations that result in chronic toxicity. Various standard test organism species are used in these tests. These organisms have been chosen to represent field organisms; they are easy to culture in a laboratory setting and give reliable and consistent results so that toxicity comparisons between different water samples can be made. These standard laboratory methods used to test waters have been shown to correlate well with field biological assessments representing many species that are known to inhabit local streams.

To carry out these methods test waters are brought to the laboratory and a dilution series of each water sample is prepared by mixing it with control laboratory water. For a definitive WET test five sample dilutions (plus control) are recommended. Receiving water tests usually contain the 100% sample plus control. In the samples under analysis the full strength (100%) test waters and at least two dilutions (some samples used up to five) of the test waters plus controls were prepared. For example, tests contained 100, 50, 30 or 0% of the receiving or discharge water. Individuals of the daphnid (invertebrate, water flea) *Ceriodaphnia dubia*, which is ubiquitous species in freshwater streams throughout the USA, was the test species used in these tests. This standard regulatory approved test species is used to provide a benchmark assessment of the toxicity of the test waters. The use of indigenous species (at the test sites) would provide potentially a better site-specific toxicity assessment, however, many species are not amenable to laboratory testing and are not regulatory approved methods. It is quite possible that *Ceriodaphnia dubia* may be less or more sensitive to one or more toxic components in the water samples compared to resident species. For example, it appears that resident mayflies are very sensitive to high levels of conductivity (Pond et al., 2008; Pond, 2010). It is, therefore, quite

Table 1: Summary of chronic 7-day survival and reproductive *C. dubia* toxicity results (IC25 and TUc levels) and levels of conductivity, sulfate and magnesium measured in the water samples collected from the twenty stations tested.

Station Detail	Instream or discharge sample	Conductivity (at 100%) (# (µmhos/cm)#	Magnesium (µg/l) *	Sulfate (mg/l) *	IC25 (% effluent)	TUc (Toxic unit)
Sites in Kentucky:						
Long Fork	instream	2638	283000	1610	1.81	55.2
Laurel Fork	instream	2697	278000	1820	1.85	54.1
UT Mudlick	instream	2223	243000	1210	3.3	30.3
Venters Branch ²	instream	2270	245000	1170	4.0	25.0
Venters Branch ²	instream	2468	290000	1330	4.8	20.8
Knob bottom	instream	2545	243000	1590	7.6	13.2
Jones Branch	instream	2353	231000	1250	11.1	9.0
Lick Branch	instream	1443	104000	699	25.9	3.9
Sites in West Virginia:						
Bee Run	instream	2347	216000	1230	14.4	6.9
Boardtree branch	discharge‡	2582	248000	1520	14.8	6.8
Upper Dempsey	instream	2143	33000	461	25.1	4.0
UNT to Leatherwood ^Δ	instream	2372	195000	1510	25.4	3.9
UNT to Leatherwood ^Δ	instream	2115	182000	1270	28.7	3.5
Winding Shoals	instream	2147	235000	965	31.6	3.2
Jack's Run	instream	1792	158000	932	31.8	3.1
Ballard Fork Pond	discharge‡	1446	151000	788	48.4	2.1
SugarTree	discharge‡	1383	122000	548	86.3	1.2
Grant Branch	instream	940	9570	151	>100	<1
Little Muncy ⁺	instream	1086	88700	434	>100	<1
UNT to Cow Creek	instream	1040	85600	456	>100	<1

mean of 6 samples using 100% effluent (or receiving water) samples.

* single point measurement of the 100% test water samples.

‡ no data is available regarding the potential dilution field for these effluent discharges into the receiving waters, therefore no modification regarding the TU criteria can be made.

+ no GPS data is available for this site and so it is not included in the maps.

² same site sampled at two different times.

^Δ different sites in headwaters of Leatherwood Creek.

IC25, this is the % effluent (or receiving water) which causes a 25% reduction in the mean number of young per female.

TUc is the chronic toxicity unit; a measure used to covert the IC25 % effluent values into a numerical value (TUc is calculated as 100 / IC25).

possible that *Ceriodaphnia dubia* may under-estimate the toxicity that would occur in sensitive indigenous species such as the mayfly (see Pond et al., 2008). To initiate the chronic toxicity test individual *Ceriodaphnia dubia* are added to the test waters (replicated 10 times) and their survival and reproduction over 7 days is followed. These tests provide dose-response information, expressed as the percent effluent concentration that affects survival and/or reproduction during the 7-day test.

A large amount of data is collected during these tests, including IC (inhibition concentration) levels. For example, the IC25, describes the percentage (%) effluent (or receiving water) that causes a 25% reduction in the mean number of young per female. The IC25 is the value used to calculate chronic toxicity units (TUc's). We carried out calculations to obtain these toxicity units (TU's), which are the standard numerical units used to assess and quantify the total toxicity of the test water and is used in permitting criteria. The EPA technical support document (USEPA, 1991) recommends as a national chronic criterion that for most water bodies waters should not exceed a chronic toxic unit (TUc) of 1.0. Some states set their own TUc criteria, indeed in Kentucky, the allowable instream concentration of toxic substances, or whole effluents containing toxic substances is not permitted to exceed a TUc of 1.0. All of the Kentucky sites assessed contained TUc levels above this level. In fact, in seventeen out of the total twenty test waters analyzed in this study contained TUc's above 1.0 (see Table 1).

In addition to the toxicity data some chemical water quality measurements for each sample were also provided by the U.S. Environmental Protection Agency for the test samples. Water quality measures (e.g. conductivity, pH) are monitored at the sampling site and throughout the toxicity test period in all of the sample test dilutions. Additional measurements of other analytes (e.g. metals, anions and cations) were also provided, however, these data are a single number for the 100% water sample and are statistically of limited value. As mentioned above these WET test methods look at the cumulative toxicity of the water sample as a whole i.e. it is not known what the specific causative agent or agents responsible for toxicity are. Further testing can be carried out using other methods if this is required. However, as some water quality and water chemical analyses were further provided a rudimentary attempt was made to investigate basic trends and correlations of toxicity the toxicity levels with these chemical endpoints (see below).

High levels of conductivity are often reported to cause declines in the numbers of resident stream organisms, particularly invertebrate species (Pond et al., 2008). This is thought to occur because of an ionic imbalance and disruption of osmoregulation. High conductivity levels, often greater than 1000 $\mu\text{mhos/cm}$ are seen in areas of mining activities. Natural streams are normally around 100 $\mu\text{mhos/cm}$. For example, Pond et al (2008) found that conductance levels in West Virginia streams ranged from 13-253 $\mu\text{S/cm}$ while valley fill stream levels were 502-2540 $\mu\text{S/cm}$. We found generally, a correlation between high conductivity and high TUc's are seen in this study. The two sites containing the highest TUc levels were also those that had the highest conductivity levels measured (at the 100% test water concentration). The three sites with the lowest chronic toxicity units (TUc's <1) recorded the lowest measured conductivity levels of the study (again using only the 100% test water concentration).

The concentrations of many metals and other contaminants were also measured although (apart from sulfate and magnesium) there appeared to be no obvious patterns with any of these levels and the TUc's levels found. However, the waters containing the highest concentrations of sulfates were the two top TUc level sites and the sites containing the three lowest TUc levels correlated to the three lowest sulfate levels measured. High sulfate levels are often seen in areas

of high mining activities (often 30-40 fold increases over ambient levels; Pond et al., 2008) and can be highly correlated with conductivity levels (WVDEP, 2008). These relationships are so well established that the 2008 WVDEP West Virginia Integrated Water Quality Monitoring and Assessment Report suggested that sulfate concentrations $>50 \text{ mg L}^{-1}$ could be used as an indicator of mining activity (WVDEP, 2008).

Magnesium ions are known to be elevated in mining activity area receiving streams (WVDEP, 2008). The five samples containing the highest TUC levels contained the three highest magnesium levels. Additionally the sites containing the lowest TUC levels contained over three-fold less concentrations of magnesium and were sites listed within the four lowest magnesium level samples.

It appears that high conductivity, sulfate and magnesium concentrations may be contributors to the toxicity observed at these sites. It is well established that increased levels of these three water quality parameters occur downstream of mining activities and correlations of these levels with observed higher toxicity values suggest mining activity may be contributing to the observed toxicities at these sites.

References:

Pond, G.J. 2010. Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA). *Hydrobiologia*, 641, 1, 185-201.

Pond, G.J., J.E. Bailey, B. Lowman (2008). West Virginia GLIMPSS (genus-level index of most probable stream status): a benthic macroinvertebrate index of biotic integrity for West Virginia's wadeable streams. West Virginia Department of Environmental Protection, Division of Water and Waste Management, Watershed Assessment Branch, Charleston, WV.

USEPA, 1991. Technical support document for water quality-based toxic controls. Office of Water enforcement and Permits and Office of Water Regulations and Standards. U.S. Environmental Protection Agency, Washington, D.C., 20460. EPA/505/2-90/001.

USEPA, 2000. Method guidance and recommendations for whole effluent toxicity (WET) testing (40 CFR Part 136). Office of Water, U.S. Environmental Protection Agency, Washington D.C. 20460. EPA/821/B-00/004.

USEPA, 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 4th Edition. Office of Water, U.S. Environmental Protection Agency, Washington D.C. 20460. EPA/821/R-02/013.

WVDEP, 2008. 303d and 305b Integrated Water quality Monitoring and Assessment Report, p21.

¹ Data was obtained through Freedom of Information Act requests to EPA by the Appalachian Center for the Economy and the Environment. All toxicity (and water quality) test data was provided in multiple Comprehensive Environmental Toxicity Information System (CETIS) data reports.