

Biomonitoring: Troubleshooting a Failed Test for Plant Operators November 12, 2019

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Biomonitoring, also known as Whole Effluent Toxicity or the WET test uses two test species to generally determine wastewater plant effluent toxicity. The species *Pimephales promelas* or fathead minnow is a surrogate for all fish and *Ceriodaphnia dubia* (*C. dubia*, Daphnia) or water flea is a surrogate for all aquatic microinvertebrates.

The 40 CFR 136 approved methods are found in EPA-821-R-02-012, *Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, and EPA-821-R-02-013, *Methods for Measuring Chronic Toxicity of Effluents and Receiving Waters of Freshwater Organisms*.

As the two documents indicate, there are two types of tests. The Acute test is concerned with minnow or flea mortality and generally lasts 24 to 96 hours. The Chronic test is concerned with minnow and flea mortality and sublethal effects (growth for fathead minnow and reproduction for *C. dubia*) and is performed for seven days or three-broods, in the case of the *C. dubia*, with daily renewal of the water utilizing three freshly collected samples

The most common reasons for failure are the presence of chlorine and ammonia (EPA-/883B-99/002, Toxicity Reduction Evaluation Guidance).

Terms

Acute: This test is generally done for 96 hours or less. It involves an impact to the organisms that is rapid.

Chronic: A longer term test, generally 7 days and includes sample replenishment three times.

IC25: Inhibition Concentration causing 25% effect to the test organisms. That is a 25% reduction of survival, reproduction, or growth of the test organisms from the control group test organisms.

LC50: Lethal Concentration to kill 50% of the exposed organisms.

NOEL: No Observed Effect Level; the highest concentration of an effluent which causes no observed effect on the test organisms.

EPA included this following list of toxicants in the publication entitled, *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants*.

Table 2-1. Toxicants Identified in POTW Effluents

Toxicant Type	Level of Concern*	Potential Source	Information Needed to Assess Toxicity
Chlorine	0.05 to 1 milligram per liter (mg/L)	POTW disinfection process	TRC, temperature, and pH upon receipt of effluent sample and during toxicity test Toxicity degradation tests TIE Phase I tests†
Ammonia	5 mg/L as NH ₃ -N	Domestic and industrial sources POTW sludge processing sidestreams	Ammonia-nitrogen upon receipt of effluent sample pH, temperature, and salinity during toxicity test TIE Phase I tests†
Non-polar organics, such as organophosphate insecticides (e.g., diazinon, malathion, chlorpyrifos, and chlorfenvinphos)	Diazinon: 0.12–0.58 microgram per liter (µg/L) Chlorpyrifos: 0.03 µg/L	Homeowners, apartments, veterinarians, pest control, lawn care, and commercial businesses	High resolution analysis of organophosphate insecticides TIE Phase I tests†
Metals [e.g., cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), nickel (Ni), zinc (Zn)]	Varies	Treatment additives in POTW Industrial users	Dissolved metals, effluent hardness (mg/L as CaCO ₃), and alkalinity upon receipt of sample TIE Phase I tests†
Other treatment chemical additives such as dechlorination chemicals and polymers	Varies	Disinfection, dechlorination, sludge processing, and solids clarification in the POTW	Vendor information on toxicity of products Dosage rates Effluent characteristics that affect toxicity (e.g., pH) TIE Phase I tests†
Surfactants	Varies	Industrial users	Methylene blue active substances (MBAS) and cobalt thiocyanate active substances (CTAS) TIE Phase I tests†
Total dissolved solids (TDS)	1,000–6,000 µhos/cm depending on endpoint, species tested, and TDS constituents	Industrial users Sludge processing sidestreams	TDS, ion analysis, and anion/cation balance TIE Phase I tests†

* As referenced by USEPA (1992a) and D. Mount (personal communication, ASci Corp, Duluth, Minnesota, 1991) for chlorine; USEPA (1992a) for ammonia; TRAC Laboratories (1992), Bailey et al. (1997) for diazinon and chlorpyrifos; and USEPA (1992a) for TDS.

† The contribution of effluent constituents such as chlorine, ammonia, organic compounds, metals, and TDS to effluent toxicity can be most effectively evaluated using the TIE Phase I procedures described in Sections 3 and 4 of this guidance and the USEPA manuals (1991a, 1992a, 1996).

Sampling

EPA-821-R-02-012 guidance on sampling is loose at best. The types of samples and sample collection are identified in this section.

Sample Type

Samples can either be “grab” or “composite”. The type sample should be discussed prior to sample collection. A grab sample is just as is stated, “grab” a sample of the water to be

collected. Generally, the grab sample is collected from the surface of the water body to be sampled. The entire sample is collected within 15 minutes.

A composite sample is either a flow-weighted or time-weighted sample. This sample type utilizes numerous aliquots of samples collected over the 24-hour sampling period. A flow-weighted sample collects these aliquots of samples at a designated volume interval. The time-weighted sample is collected at a certain time interval regardless of volume of flow. Regardless of the composite sample collection type, the sample should be chilled during collection via either wet ice or a refrigerated collection device.

Sample Collection

The following steps should be following when collecting samples:

- Inspect the sample containers upon receipt to ensure the integrity of the container is not compromised (i.e., no holes in container) and container lids are in sample kit;
- Label the sample container with the Sample ID and date of collection;
- Rinse the sample container with the water to be collected (i.e., collect some water, swirl, and discard) prior to collecting the actual sample; and,
- If the lab sent sampling instructions, follow those instructions and call the lab with any questions.
- Should field parameters be required (pH, temp, TRC) these should take place within 15 minutes of sample collection. The lab will generally perform these tests, however these parameters are considered field parameters and the hold time will most likely be exceeded if not conducted in the field.

Sample Shipment

For WET samples, the samples should be shipped on wet ice that is contained either in zip-top baggies or some other container (i.e. trash bag liner filled with wet ice and sample knotted at the top). The ice should be bagged in such a way that leaks are prevented. The chain-of-custody (COC) should be completed with the Sample ID, start and end date and time of sample collection, requested type of testing, and signed/dated/timed in the relinquish section (generally at the bottom of the COC). The COC should be placed inside a zip-top bag and taped to the inside lid of the cooler. If shipping the cooler, it should be taped around to ensure it will not open during shipment.

EPA-821-R-02-012 does state “Rinse sample containers with source water before filling with sample.” This is the lab control water and is generally not easily available for plant operators who would be collecting the samples so rinse with the effluent water to be collected. Carefully follow the lab’s instruction on sampling and shipping the samples. If you are performing a retest after a failed test, everything is suspect. All containers, tubing, sampling machines, and everything near the sample should be carefully considered as a source of toxicity.

If you are retesting, be sure to test the samples for chlorine, ammonia, and pH, and properly store enough extra sample for later testing in case the retest also fails. Check with the testing lab on how to properly store the extra sample.

Most Common Toxicants

EPA reports the most common toxic materials affecting the WET tests are chlorine, ammonia, organophosphate insecticides, surfactants, metals and treatment plant additions such as chlorine, polymers and dechlorination chemicals. Ammonia toxicity is dependent on the species of ammonia present which in turn is dependent on the pH, temperature and salinity. At a pH <6.0 all ammonia is in the form of ammonium ion (NH_4^+); at pH >11.5 the species of ammonia present is NH_3 . This is ammonia gas that is dissolved in the water and is commonly called unionized or free ammonia. Between pH 6-11.5 both species of ammonia exist. The higher the pH and temperature the more unionized ammonia is present. This form of ammonia is toxic to fish and aquatic life. Generally ammonia > 5.0 mg/L is a concern. Chlorine toxicity is also dependent on the species of chlorine. Chloramines are the most toxic. Generally, chlorine >0.01 mg/L is a concern, but the DPD test method is generally only considered accurate to 0.05 mg/L.

Plant Operating Conditions

Whenever sampling or troubleshooting a failed test, plant conditions should be carefully reviewed. What are the loading conditions: hydraulic, organic (both compatible organics such as CBOD and the more complex organics such as the organophosphate insecticides, and surfactants), metals, and internal loads such as solids processing return or decant water, plant recycle streams, polymers or other coagulation/flocculation chemicals, scrubber blowdown, or chlorine and dechlorination streams. Have user discharges impacted the plant and thus the test? Does the plant receive any hauled in waste such as septage or fat-oil-and grease? Have industries, either permitted or not, affected the plant and thus the WET tests? If there have been multiple failed tests, are there common factors such as seasons or days of the week?

What are or were the plant operating conditions such as: MLSS, MCRT, SVI, return rates, recycle rates, hydraulic detention times in the various treatment units, nitrification/denitrification status, redox conditions, aeration rates, clarifier blanket levels that may impact normal operations such as BOD/CBOD, TSS, and ammonia removal? If your ammonia concentrations are the same, try checking the nitrate and nitrite levels. What is the influent, internal, and effluent pH, alkalinity, and hardness? Have there been any process changes during the sampling times? Have plant operators observed any visual changes, odors or noises? Have there been mechanical failures or changes during the sampling times? Have there been changes in the coagulants/flocculants or polymers for solids dewatering or nutrient removal (i.e., stopped using one, started using another, a change in dose or a change in feed locations)?

If the plant has difficulty meeting permit limits for BOD/CBOD, ammonia or total nitrogen, or TSS, fix these problems before beginning sampling of the retest. Oftentimes a failed retest will put the plant into Toxicity Reduction Evaluation (TRE)/Toxicity Identification Evaluation (TIE) plan. Review of plant operating conditions should always be the first step in the TRE/TIE plan.

Plants that operate with a long MCRT remove toxic compounds better than those with a short MCRT.

Industrial Pretreatment

The nationwide use of industrial pretreatment programs has been very successful. Since its implementation, streams are greatly improved and treatment plants experience industrial related problems far less; but, there can still be impacts. Know your local industries, even those without permits. Be aware of what type of stored chemicals they may have on site which may somehow spill down the drain. Generally metals impact the fleas and organics impact the fathead minnow. Often biocides are used in cooling towers to control biological growth within these closed loop systems. Some non-permitted users such as schools or hospitals may have continuous blowdown or intermittent replenishment of this water.

Toxicity Guidance for Troubleshooting the WET test.

Ceriodaphnia dubia

The water flea is used as a surrogate for all other aquatic microinvertebrates. It is a general indicator of toxicity from metals and other inorganics.

- Chlorine: When at all possible collect the sample before chlorination or dechlorinate carefully without adding excessive amounts of dechlorinating chemicals.

EPA reports the Chlorine LC50 = 0.032 mg/L

TVA/EPA(1984) report LC50 = 0.017 mg/L Chlorine, but this appears to be for native Tennessee River daphnia not *C. dubia*.

TVA/EPA(1984) report Reduction in Reproduction at 0.002 mg/L Chlorine

The species of chlorine is also important with chloramines being reported as more toxic than hypochlorous acid or hypochlorite ion. Taylor (1993) reports the monochloramine LC50 = 0.012 mg/L, dichloramine LC50= 0.016 mg/L but hypochlorous acid LC50= 0.035 and hypochlorite ion LC50= 0.048 mg/L. Generally plant effluent total chlorine residuals will include chloramines.

- Ammonia: Marshal (2012) reports an ammonia toxicity level of >25 mg/L ammonia nitrogen. He did not report the pH so it is unknown whether this is ionized or unionized ammonia. At pH<6 all ammonia is in the ammonium ion state and is not toxic to fish and aquatic organisms, but above pH of 11.5 s.u. the species is unionized ammonia or dissolved ammonia gas which is toxic to fish and aquatic organisms.
- Nitrate:
 - NOEC= 21.3 mg/L (NOEC: No Observable Effect Concentration)
 - LOEC= 42.6 mg/L (LOEC: Lowest Observable Effect Concentration)

- pH.: *C. dubia* is more sensitive to low pH and low hardness in acute tests. In the chronic tests the fleas are less impacted by low pH, but low hardness did impact the test. pH range of 6-9 results in the greatest reproduction. (Belanger 1990) The pH data below is included as an example. If plant effluent or sample values were outside the standard permit limit range, there are more important issues than biomonitoring. Generally the only time a treatment plant would have a pH issue would be where high influent ammonia is paired with low sewage alkalinity to give low effluent pH.

Acute tests: *C. dubia* cultured at pH 8.0
 Suffered 100% mortality at pH 3.2-3.4
 Suffered 100% mortality at pH >10.8

48 hour LC50 = pH 4.4-4.7
 48 hour LC50 = pH 10.2-10.3

Alkalinity Mager (2010) reports lead toxicity value was less under conditions of higher alkalinity. Their research listed lead Effect Concentration-EC50= 55 ug/L at alkalinity = 30 mg/L and EC50 = 100 ug/L at alkalinity = 120 mg/L. Mager reported similar findings for Nickel and Copper, but the opposite for Zinc. This work supported the findings of Chapman et.al in 1980.

- Metals: The flea is generally more impacted by metal toxicity than the minnow. This is especially true at lower pH values such as <6.0 s.u. and lower hardness levels but each metal is different. Copper for example, is more toxic at pH range 6.0-6.5 s.u. while zinc is more toxic at pH 8.0-8.5 s.u.

Belanger & Cherry (1990) Report the following values.

Toxicity values at pH 6.0 & hardness 100 mg/L

Zinc 48 hour LC50 = 0.07 mg/L

Copper 48 hour LC50 = 0.014 mg/L

Toxicity values at pH 9.0 & hardness 185 mg/L

Zinc 48 hour LC50 = 0.153 mg/L

Copper 48 hour LC50 = 0.093 mg/L

Mager (2011) reports that copper toxicity decreases as TSS, dissolved organic matter (DOM), and humic acid increases. Kim (2001) confirms this citing decreased copper toxicity with increased pH, natural organic substances (humic acid and dissolved organic matter) and suspended particles. The greater the naturally occurring elements such as carbon and suspended solids, the less bioavailable the metals are to the organisms. Kim (2001) also cites several others who report less metal toxicity with increasing hardness, alkalinity, and pH.

A listing of metal toxicity values is shown below. It shows silver as the most toxic followed by cadmium, copper, mercury, zinc, nickel, and selenium. The reference does not mention pH or hardness of the water.

ACUTE TOXICITY OF SELECTED COMPOUNDS (96-hr LC₅₀)^a
 (Source: Lankford and Eckenfelder, 1990)

	Units	Fathead Minnow	Daphnia	Rainbow Trout
Metals^b				
Arsenic	µg/l	15,600	5,278	13,340
Chromium, hexavalent	µg/l	43,600	6,400	69,000
Cadmium	µg/l	58.2	0.29	0.04
Copper	µg/l	3.29	0.43	1.02
Lead	µg/l	158.00	4.02	158.00
Mercury	µg/l	--	5.00	249.00
Nickel	µg/l	440.00	54.00	--
Selenium	µg/l	1,460.00	710.00	10,200
Silver	µg/l	0.012	0.00192	0.023
Zinc	µg/l	169.00	8.89	26.20

- Ions: *C. dubia* are extremely sensitive to increasing salinity and the inhibitory effect on reproduction is made worse by ionic imbalance (Moore 2006). Conductivity and total dissolved solids (TDS) are parameters which are used to describe the salinity level. Acute tests showed no toxicity. The chronic lethal endpoint, similarly to the acute test, also demonstrated no toxicity. However, the chronic sublethal (i.e. reproduction) endpoint was affected. There was a reduction in reproduction observed in the test. Moore suggests TDS should be kept <1200 mg/L. The conductivity test is used as a quick surrogate for Total Dissolved Solids (TDS). These results give a strong indication of the amount of dissolved ions within the test sample, but they are not a robust predictor of toxicity. The constituents and their contributions to the TDS is more relevant to toxicity than overall TDS concentrations. Common ions of suspicion include sodium, potassium, magnesium, calcium, sulfide, and chloride. USEPA Region 3 reports the relationship of conductivity to TDS is based on the following equation. (Conductivity* 0.721)-28.661=TDS.
- Another toxicity source to keep in mind is cooling tower blowdown or replenishment. Cooling towers often use a variety of biocides to prevent biological fouling within these closed loop systems.

Pimephales promelas

The common name is fathead minnow, and they are used as a surrogate for all other fish and generally are sensitive to ammonia and organics.

- Ammonia: EPA lists a level of 5.0 mg/L NH₃-N as a level of concern. It is assumed that this is at neutral pH so the predominant species of ammonia represented is the ammonium ion, NH₄⁺. This form of ammonia is not toxic to fish. But at higher pH and temperature values more NH₃ or unionized ammonia (free ammonia) is formed which is toxic.

Armstrong (2012) reports an estimated NOEC for minnow reproduction for Total Ammonia at 2.19 mg/L and for unionized ammonia at 0.025 mg/L.

Thurston (1986) reported unionized ammonia at pH 8.0 and 24.2°C:

NOEC for growth or survival of parent minnows at 0.44 mg/L

NOEC for egg growth at 0.37 mg/L

NOEC for growth of larvae at 0.36 mg/L, and

Effects on adult growth and survival and egg growth at 0.91 mg/L

Note at pH 8.0 and temperature of 24.4° C, 6% of the total ammonia present will be in the unionized form. So a total ammonia value of 6 mg/L would have 0.36 mg/L unionized ammonia and be toxic according to Thurston's work.

Wang reports a total ammonia EC50 at 12.5 mg/L, and

Dwyer (2005) reports ammonia IC 25 at 7.2 mg/L with a range of 2.4->17mg/L

If the pH drifts upward during the test, ammonium can change to toxic unionized ammonia during the test. Regulators have been known to allow pH adjustment to that of the receiving stream in such cases (EPA 1999).

- Chlorine. Dwyer (2005) lists the Chlorine IC25 for fathead minnows at 0.565 mg/L with a range of 0.254-0.673 mg/L. Wilde (1983) reports the Total Residual Chlorine 96hr LC50 = 0.08 mg/L for juvenile fathead minnows, and 0.35 mg/L for adult fathead minnows.
- Organophosphate insecticides are toxic to many organisms. EPA lists a concern level for Diazinon at 0.12-0.58 ug/L. This residential use of diazinon was outlawed in the U.S. in 2004 but it is still approved for agricultural uses. Dwyer (2005) lists the diazinon IC25 at 1.176 mg/L with a range of four values being 0.413-2.261 mg/L. EPA also lists Chlpryifos with a concern level at 0.03 ug/L. This is a class of compounds that include the products Dursban and Lorsban. These are some of the most widely used agricultural insecticides. Prior to the 2001 prohibition of residential use, this was also widely used in homes. Malathion is the number one used organophosphate insecticide. It has low toxicity to humans and is widely used in residential areas including mosquito spraying. One SDS for a product that was 57% malathion listed a 48 h, EC50: 0.72 mg/L for *Daphnia magna*.
- D-Limonene. MSDS reports list the 96 hour LC50 for minnows at 0.619-0.769 mg/L in a flow through test. D-Limonene is a degreasing product sometimes used in collection system lift stations but also in industrial and home cleaning. It has a very nice citrus odor.
- Cationic polymers can be toxic to the minnows. Hall (1991) reports LC50 for Cationic polymer to be <14 mg/L and Anionic polymer >20 mg/L. The tested cationic polymers had a toxicity range of 0.4 mg/L to >170 mg/L and the anionic range was 37-85 mg/L.

He concluded that the polymers would clog the gills of the minnows and that products with higher charge density were more toxic. Alum and ferric sulfate were less toxic.

- Elevated cations can also affect the minnows. Grothe (1996) reports elevated conductivity (10,000-25,000 micromhos/cm, containing Na^+ of 1020 mg/, Ca^+ of 3000 mg/L and Cl^+ of 7310 mg/L) was toxic to the minnows.
- Mount (1997) reported that the minnows tolerated Total Dissolved Solids (TDS) up to 15,000 mg/L in Saskatchewan water high in Na^+ and SO_4^+ but did not tolerate Nebraska water with TDS at 2,000 mg/L containing Na^+ , K^+ , and CHO_3^- . Ionic toxicity in decreasing order was listed as $\text{K}^+ > \text{Mg}_2^+ \sim \text{CHO}_3^- > \text{Cl}^- > \text{SO}_4^{2-} > \text{Na}^+ > \text{Ca}^+$. Na and Ca are not clearly identified as toxic by themselves and elevated Mg and Ca hardness decreased toxicity to salt to *Daphnia Magna*. Mount reports the following LC50's:
 - $\text{K}^+ = 600 \text{ mg/L}$
 - $\text{Mg}_2^+ = 1800 \text{ mg/L}$
 - $\text{CHO}_3^- = 2100 \text{ mg/L}$
 - $\text{Cl}^- = 4300 \text{ mg/L}$
 - $\text{SO}_4^{2-} = >5000 \text{ mg/L}$

Common source of salts in treatment plants are industrial processes, exhaust air scrubbers, road salts runoff to the sanitary sewer, and food processing.

- Surfactants. Nonylphenol Etholates (NPE) surfactants are reported toxic at $>0.5 \text{ mg/L}$ in high hardness (high TDS) effluent. The toxicity may be due to the Nonylphenol after the Ethoxylate was stripped from the molecule. NPE is reported to be more toxic at lower pH and temperatures (Wayment, 2004). Surfactant chemistry is extraordinarily complex and varied. NPE surfactants are one small group of dozens of groups. They fall into the general class of nonionic surfactants and should be captured in the CTAS test for surfactants. Anionic surfactants are captured in the MBAS test. Nonionic surfactants are also known to deflocculate activated sludge more than others. The CTAB test is used for quantifying some cationic surfactants.
- Bis-(2-ethylhexyl)phthalate, State of New Jersey (1996-1999):
 - Threshold Effect Level (TEL) = 182 ug/kg (Marine Sediment)
 - Upper Effects Threshold (UEL) = 750 ug/kg (Freshwater).Tennessee operators have experienced repeated WET failures during times of cured-in-place-pipe (CIPP) collection system repairs which were attributed to phthalate from the pipe resin.
- Pathogens. The Littleton/Englewood Colorado treatment plant has reported numerous failed minnow tests due to pathogens. These are generally warm weather occurrences. The *C. dubia* is not affected and toxicity does not correlate with the dose (percent dilution) of the test water. (Russell)

- Dissolved Oxygen. Brungs (1971), in a carefully controlled long-term study, found that the growth of fathead minnows was reduced significantly at all dissolved oxygen concentrations below 7.9 mg/L. The method oxygen minimum is 4.0 mg/L and aeration is not recommended where DO values are above that value.

Additional Published Toxicity Values.

ACUTE TOXICITY OF SELECTED COMPOUNDS (96-hr LC₅₀)^a
(Source: Lankford and Eckenfelder, 1990)

	Units	Fathead Minnow	Daphnia	Rainbow Trout
<u>Organics^b</u>				
Benzene	mg/l	42.70	35.20	38.70
Carbon Tetrachloride	mg/l	17.30	15.20	14.50
Chorobenzene	mg/l	13.20	11.60	11.10
1,1-Dichloroethane	mg/l	120.00	96.40	113.00
1,1,2-Trichloroethane	mg/l	88.70	72.60	81.10
2-Chlorophenol	mg/l	21.60	18.60	18.40
1,4-Dichlorobenzene	mg/l	3.72	3.46	2.89
1,2-Dichlorobenzene	mg/l	87.40	71.10	80.50
2,4-Dinitrophenol	mg/l	5.81	5.35	4.56
4,6-Dinitro-o-cresol	mg/l	2.79	2.65	2.10
Pentachlorophenol	µg/l	170.00	--	--
Ethylbenzene	mg/l	11.00	9.97	9.47
Methylene Chloride	mg/l	326.00	249.00	325.00
Toluene	mg/l	31.00	26.00	27.40
Trichloroethylene	mg/l	55.40	46.20	49.50
Phenol	mg/l	39.60	33.00	35.40
1,4-Dinitrobenzene	mg/l	1.68	1.61	1.24
2,4,6-Trichlorophenol	mg/l	5.91	5.45	4.62
2,4-Dichlorophenol	mg/l	9.27	8.35	7.49
Naphthalene	mg/l	5.57	5.07	4.44
Nitrobenzene	mg/l	118.00	95.40	110.00
1,1,2,2-Tetrachloroethane	mg/l	31.10	26.70	26.70

EPA 1976, Toxicity to Fathead Minnows

MINNOWS IN LAKE SUPERIOR WATER

Test chemical	LC50 concentration (in mg/l.)				
	1-hr	24-hr	48-hr	72-hr	96-hr
<u>Acids</u>					
Acetic ^b	>315	122	92	88	88
Adipic ^b	>300	172	114	97	97
Caproic ^b	140	88	88	88	88
Oleic ^a	>1,000	285	252	205	205
Valeric ^b	>100	>100	77	77	77
<u>Alcohols</u>					
Benzyl	770	770	770	480	460
1-Butanol	1,950	1,950	1,950	1,950	1,910
Cyclohexanol	1,550	1,033	1,033	1,033	1,033
Ethanol ^a	>18,000	>18,000	13,480	13,480	13,480
Isopropanol	11,830	11,160	11,130	11,130	11,130
<u>Hydrocarbons</u>					
Cyclohexane	95	93	93	93	93
Indan	39	14	14	14	14
1-Methylnaphthalene	39	9	9	9	9
m-Nitrotoluene	43	30	30	30	30
Quinoline	78	46	46	46	46
Styrene	100	32	32	32	32
Styrene ^c	40	30	29	29	29
Xylene	46	42	42	42	42
<u>Ketones and Aldehydes</u>					
Acetophenone	>200	>200	163	158	155
d-Camphor	145	112	111	110	110
Furfural	>50	48	37	32	32
Vanillin-Test 1 ^a	>173	131	123	121	121
Vanillin-Test 2 ^a	370	125	116	112	112
<u>Phenols</u>					
p-Cresol ^a	>30	26	21	21	19
Eugenol	24	24	24	24	24
Pentachlorophenol	8	0.6	0.6	0.6	0.6
Phenol ^b	>50	>50	>50	33	32
3,4 Xylenol	>20	>20	15	14	14

^aDissolved oxygen measured ≤ 4.0 mg/l. during test.

^bpH measured ≤ 5.9 units during test.

^cAcetone added to diluent water.

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