PASS/FAIL METHODOLOGY FOR DETERMINING ACUTE TOXICITY IN A SINGLE EFFLUENT CONCENTRATION

North Carolina Department of Natural Resources and Community Development Division of Environmental Management Water Quality Section

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This procedure has been approved for release

4 December 1987



PASS/FAIL METHODOLOGY FOR DETERMINING ACUTE TOXICITY IN A SINGLE EFFLUENT CONCENTRATION

It is the intent of this document to provide a method by which significant mortality in a single effluent concentration may be determined. It may be applied to any concentration of effluent but is specifically designed to test higher effluent concentrations where a measured LC50 may not necessarily protect for acute toxicity. This would occur in situations where the receiving stream or mixing zone is almost entirely effluent. In these instances it is important that short term acute effects not occur. The standard methodology specified in the EPA Guidance Document (Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms EPA/600/4-85/013) does not cover situations where a statistical determination of acute Mortality in a specific effluent concentration is to be determined. This document is intended to specify exact procedural modifications of the above cited methodology for performing analyses of this type. Unless specifically modified by this procedure document, all other test conditions and requirements will be as specified in the EPA Document.

Methodology

The procedure shall be performed as an acute static non-renewal toxicity examination using either the Fathead Minnow (*Pimephales promelas* less than three weeks old), *Daphnia pulex* or *Ceriodaphnia* sp. There will be two concentrations utilized in this procedure. The control population will be specified as treatment one and the effluent treatment will be specified as treatment two. Typically, the effluent concentrations utilized for this test procedure will be 90%. The actual effluent concentration at which the test is to be performed and test duration will be specified in the NPDES Permit or by Administrative Letter.

Each treatment will be tested using four identical test vessels each containing ten test organisms for a total of eight vessels and 80 test organisms. At the end of the test all organisms will be identified as alive or dead and recorded on the appropriate laboratory forms. If all chemical and physical protocols have been met then data analysis can proceed. If testing protocols have not been followed or met then the test must be repeated.

Data Analysis

Analysis of the data from this test procedure is performed using a Standard Students t test. This analysis determines if mortality in the effluent



treatment (treatment two) is significantly different than the control population. If mortality in the control population exceeds 10% then the test is considered invalid. All statistical analyses are performed using arc sign square root transformed data (see referenced EPA document) and tested for significance at a 90% confidence level. Should treatment two mortality exceed that of the control population and the absolute value of the calculated t value exceed the tabular t value then the effluent treatment is considered as having significant acute effects on the test organisms. This would be considered a "FAIL".

If all test vessels have exactly the same response then a t value is not calculable. If all vessels in each treatment have the same response but at different levels between treatments then a t statistic is not calculable. In these instances if the response is identical between treatments then the test is considered a "PASS". If the response in treatment two is greater than treatment one (control) then the test is considered a "FAIL".

Data obtained by use of this methodology should be entered on the attached form AT-2 and sent to the designated address. Additionally the results of this testing should be entered as a PASS or FAIL using the appropriate parameter code on the MR-1 forms for self-monitoring purposes if required part of an NPDES Permit.



PLAN TO COME BACK.

METHODS

SITE REF FOR PROCEDURE + SAMPLING METHODS EPA 600 4-85-013

REG WE ARE IN VOLINTION.

1:30

NOV LETTER COMING ?

- COMMENT ON LETTER

15 NRAC 028 .0214 (L)

REGAMPLES -

TAPS ON LINE



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FEB 87	4.5	3.9	3-0	820	7.29	0	
MAR	4.0	3.9	3.0	10	1.41	0	
APR	4.0	3.9	3.0	4	1.13	0	
MAY	4.0	3.7	3.0	6	1.28	٥	
MUL	4.0	3.7	3.0	70	1.43	0	
JUL	5.0	3.9	2.0	70	2.31	0	
Aug	4.0	3.7	3.0	16	1.44	0	
SEP	6-0	3.4	1.5	10	1.41	0	
OCT	3.0	1.9	1.0	550	3.10	0	
Nov	2.5	2.1	2.0	28	2-96	٥	
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MAY	5.0	4.0	3.0	/0	1.12	0	
JUN	. 4.0	3.9	3.0	6	1.16	0	
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Auc	4.0	4.0	4.0	62	2.55	0	
SEP	4.0	4.0	4.0	820	2.51	0	
Ост	4.0	4.0	4-0	310	1.74	o	
Nov	4.0	3.9	2.0	12	1.38	0	
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JANUARY 87	4.5	3.9	2.0	16	1.63	0	
FEB	5.0	4.1	3.0	480	1.46	0	
MAR	5.0	4.2	3.0	6	1.16	0	
APR	5.0	4.3	4.0	4	1.25	z	
MAY	5.0	4.1	4.0	4	1.23	0	
AUL	5.0	3.9	2.5	12	1.16	0	
JOL	5.0	4.1	3.0	28	1.56	0	
Aug	6.0	4.0	3.0	12	1.61	0	
SEP	5.0	4. 0	2.5	140	2.42	0	
Oct	5.0	4.3	3.5	20	2.02	0	
Nov	5.0	4.1	4.0	440	2.08	0	
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CAMP JOHNSON	Real Control						
JAN	6.0	4.0	1.5	6	1.77	0	
FEB	5.0	4.0	0.2	0	0	0	
MAR	8.0	4.1	o	4	1.30	0	
APR	8.0	4.4	2.0	2	1.09	0	
MAY	6.0	4.2	2.5	6	1.29	0	
JUN	6.0	3.8	0.4	Z	1.08	0	
JUL	60	4.1	2.0	2	1.09	6	
Auc	5.0	3.9	0.8	6	1.36	0	
SEP	4.0	4.0	1.5	0	0	υ	
Oct	8.0	4.3	3.0	228	3.04	0	
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JAN 87	5.0	3.8	2.5	34	19.7 *	10	
FEB	5.0	3.8	2-0	30	9.64 \$	2	
MAR	5.0	3.9	2.0	4	1.32.	0	
APR	5.0	4.0	3.0	0	0	0	
May	5.0	3.8	2.0	0	0	0	
JUN	5.0	3. 8	2.5	560	6.45	0	
JUL	6.0	4.0	2.0	o	0	0	
Auc	6.0	3.5	0	0	o	0	
Ser	4.5	3.6	2.5	0	o	0	
Oct	5.0	4.0	2.0	0	0	õ	
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JAN 87	6.0	4,3	3.0	0	0 *	0	
FEB	4.0	4.5	6.0	0	0 #	0	
MAR	5.0	4.1	2.0	12,500	10.57	0	
APR	5.0	3.8	2.0	10	1.78	o	
MAY	6.0	4.6	3.0	0	C	0	
JON	8.0	5.1	4.0	. 0	o	0	
JUL	6.0	4.7	4.0	0	O	0	
Auc	6.0	4. Z	z.0	0	0	0	
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JAN 87	6.0	4.7	3.0	ь	2.88	6			
FEB	6.0	5.3	4.0	90	6.51 +	0			
MAR	8.0	5.3	3.0	10	2.11	0			
APR	8.0	4.6	2.0	0	٥	0			
MAY	5.5	4. Z	2.0	0	0	O			
ANT	8.0	4.8	4.0	26	2.96	٥			
JUL	6.0	4.4	2. 0	60	2.61	٥			
Auc	6.0	4.4	0.5	٥	0	0			
SEP	6.0	4.7	2.0	٥	٥	0			
Ост	6.0	4.6	2.0	0	0	o			
Nov	6.0	3.9	2.0	0	0	0			
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