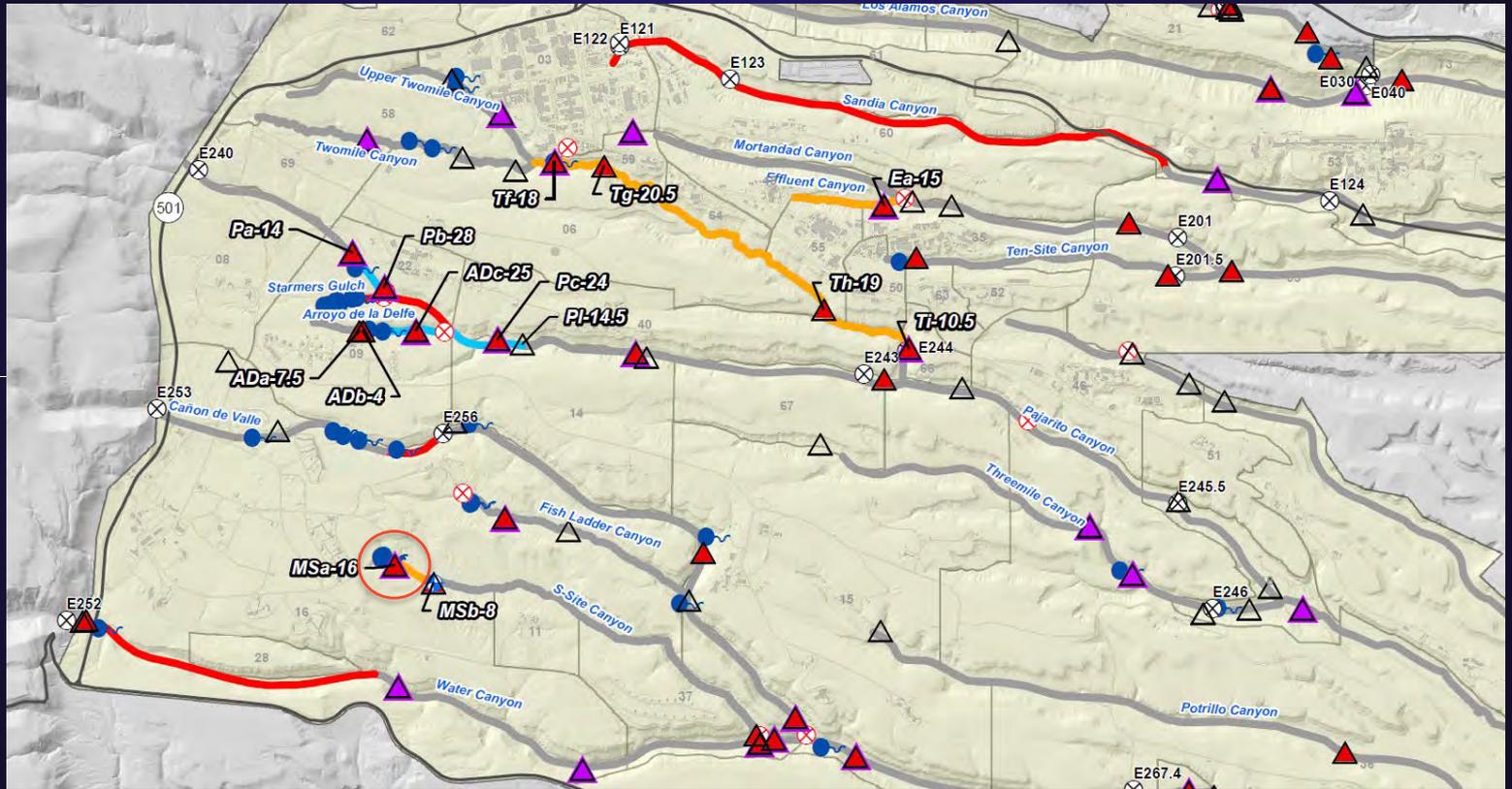


# Exhibit 41

# S-Site Canyon below Martin Spring



# HP Level 1 - S-Site Canyon below Martin Spring

*date: 2:00pm 2/18/21*

NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

*Michael DePinto 3/22 9-3-11*

Date: 2/18/2021 Stream Name: *C/N @ Martin Spring* Latitude: 36° 50' 51" N

Evaluator(s): *Leifin Schiller, Schendo Amador, Gullidon* Site ID: *467 below Martin Spring* Longitude: 106° 20' 10" W

TOTAL POINTS: *16.0* Assessment Unit: *N/A* Drought Index (12-mo. SPI Value): *0 - 1 2020 9-3-19*

WEATHER CONDITIONS

NOW:  storm (heavy rain)  rain (steady rain)  showers (intermittent)  %cloud cover  clear/sunny

PAST 48 HOURS:  storm (heavy rain)  rain (steady rain)  showers (intermittent)  %cloud cover  clear/sunny *8/27 @ 14" @ TA-dk*

OTHER: Stream Modifications  YES  NO Diversions  YES  NO Discharges  YES  NO

\*Field evaluations should be performed at least 48 hours after the last known major rainfall event.

\*Explain in further detail in NOTES section.

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Poor
1.1. Water in Channel	Flow is evident throughout the reach. Moving water is seen in riffle areas but may not be as evident throughout the runs.	Water is present in the channel but flow is barely discernible in areas of greatest gradient change (i.e. riffles) or floating object is necessary to observe flow.	Dry channel with standing pools. There is some evidence of base flows (i.e. riparian vegetation growing along channel, saturated or moist sediment, lodges, rocks, etc)	Dry channel. No evidence of base flows was found.
	6	4	2	0
1.2. Fish	Found easily and consistently throughout the reach.	Found with little difficulty but not consistently throughout the reach.	Takes 10 or more minutes of extensive searching to find.	Fish are not present.
	3	2	1	0
1.3. Benthic Macroinvertebrates	Found easily and consistently throughout the reach.	Found with little difficulty but not consistently throughout the reach.	Takes 10 or more minutes of extensive searching to find.	Macroinvertebrates are not present.
	3	2	1	0
1.4. Filamentous Algae/Periphyton	Found easily and consistently throughout the reach.	Found with little difficulty but not consistently throughout the reach.	Takes 10 or more minutes of extensive searching to find.	Filamentous algae and/or periphyton are not present.
	3	2	1	0
1.5. Differences in Vegetation	Dramatic compositional differences in vegetation are present between the stream banks and the adjacent riparian. A grid riparian vegetation corridor exists along the entire reach - riparian, aquatic or wetland species dominate the length of the reach.	A distinct riparian vegetation corridor exists along part of the reach. Riparian vegetation is interspersed with upland vegetation along the length of the reach.	Vegetation growing along the reach may occur in greater densities or grow from riparian to upland, but there are no dramatic compositional differences between the banks.	No compositional or density differences in vegetation are present between the streambanks and the adjacent uplands.
	3	2	1	0
1.6. Absence of Rooted Upland Plants in Streambed	Rooted upland plants are absent within the streambed/shalweg.	There are a few rooted upland plants present within the streambed/shalweg.	Rooted upland plants are present within the streambed/shalweg.	Rooted upland plants are present throughout the streambed/shalweg.
	3	2	1	0
SUBTOTAL (#1.1 - #1.6)				9.5

If the stream being evaluated has a subtotal  $\geq 2$  at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal  $\geq 18$  at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 2 and 18 continue the Level 1 Evaluation.

*date: 2:00pm 2/18/21*

NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

*Michael DePinto 3/22 9-3-11*

Date: 2/18/2021 Stream Name: *C/N @ Martin Spring* Latitude: 36° 50' 51" N

Evaluator(s): *Leifin Schiller, Schendo Amador, Gullidon* Site ID: *467 below Martin Spring* Longitude: 106° 20' 10" W

TOTAL POINTS: *16.0* Assessment Unit: *N/A* Drought Index (12-mo. SPI Value): *0 - 1 2020 9-3-19*

WEATHER CONDITIONS

NOW:  storm (heavy rain)  rain (steady rain)  showers (intermittent)  %cloud cover  clear/sunny

PAST 48 HOURS:  storm (heavy rain)  rain (steady rain)  showers (intermittent)  %cloud cover  clear/sunny *8/27 @ 14" @ TA-dk*

OTHER: Stream Modifications  YES  NO Diversions  YES  NO Discharges  YES  NO

\*Field evaluations should be performed at least 48 hours after the last known major rainfall event.

\*Explain in further detail in NOTES section.

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Poor
1.1. Water in Channel	Flow is evident throughout the reach. Moving water is seen in riffle areas but may not be as evident throughout the runs.	Water is present in the channel but flow is barely discernible in areas of greatest gradient change (i.e. riffles) or floating object is necessary to observe flow.	Dry channel with standing pools. There is some evidence of base flows (i.e. riparian vegetation growing along channel, saturated or moist sediment, lodges, rocks, etc)	Dry channel. No evidence of base flows was found.
	6	4	2	0
1.2. Fish	Found easily and consistently throughout the reach.	Found with little difficulty but not consistently throughout the reach.	Takes 10 or more minutes of extensive searching to find.	Fish are not present.
	3	2	1	0
1.3. Benthic Macroinvertebrates	Found easily and consistently throughout the reach.	Found with little difficulty but not consistently throughout the reach.	Takes 10 or more minutes of extensive searching to find.	Macroinvertebrates are not present.
	3	2	1	0
1.4. Filamentous Algae/Periphyton	Found easily and consistently throughout the reach.	Found with little difficulty but not consistently throughout the reach.	Takes 10 or more minutes of extensive searching to find.	Filamentous algae and/or periphyton are not present.
	3	2	1	0
1.5. Differences in Vegetation	Dramatic compositional differences in vegetation are present between the stream banks and the adjacent riparian. A grid riparian vegetation corridor exists along the entire reach - riparian, aquatic or wetland species dominate the length of the reach.	A distinct riparian vegetation corridor exists along part of the reach. Riparian vegetation is interspersed with upland vegetation along the length of the reach.	Vegetation growing along the reach may occur in greater densities or grow from riparian to upland, but there are no dramatic compositional differences between the banks.	No compositional or density differences in vegetation are present between the streambanks and the adjacent uplands.
	3	2	1	0
1.6. Absence of Rooted Upland Plants in Streambed	Rooted upland plants are absent within the streambed/shalweg.	There are a few rooted upland plants present within the streambed/shalweg.	Rooted upland plants are present within the streambed/shalweg.	Rooted upland plants are present throughout the streambed/shalweg.
	3	2	1	0
SUBTOTAL (#1.1 - #1.6)				9.5

If the stream being evaluated has a subtotal  $\geq 2$  at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal  $\geq 18$  at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 2 and 18 continue the Level 1 Evaluation.

# HP 1 - S-Site Canyon below Martin Spring

*CVE Martin Spring Cnyn 3  
below Martin Spring*

**LEVEL 1 Field Measurements**

**Pebble Count Tally Sheet**

Site Name: \_\_\_\_\_ Storet ID: \_\_\_\_\_  
Date: \_\_\_\_\_ Crew: \_\_\_\_\_

Substrate Type	Diameter Range	In-Channel COUNT	In-Channel % Composition	Out of Channel COUNT	Out of Channel % Composition
Silt/Clay	< 0.06 mm				
Sand	0.06 – 2.0 mm (gritty)				
Gravel	2.0 – 64 mm				
Cobble	64 – 256				
Boulder	> 256 mm				
Bedrock	---				

\*\*Please be sure to measure at least 50 pebbles (10 in 5 transects or 5 in 10 transects- depending on stream size) for accurate distributional representation\*\*

INDICATOR #1.8 (Floodplain and Channel Dimensions) – MEASUREMENTS & CALCULATIONS**							
Max Depth (#1)	Bankfull Stage (#2)	Maximum Depth Value (#3)	2x Maximum Depth Value (#3)	Flood-Prone Area Location (#4)	Flood-Prone Area Width (#5)	Bankfull Width (#6)	Floodplain to Active Channel Ratio (FPA Width / Bankfull Width)
8.8ft	8.56ft	0.24ft	0.48ft	8.32ft	2.45m	1.1m	2.22m

\*\*REFER to Figure 3 on page 19 for clarification

NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

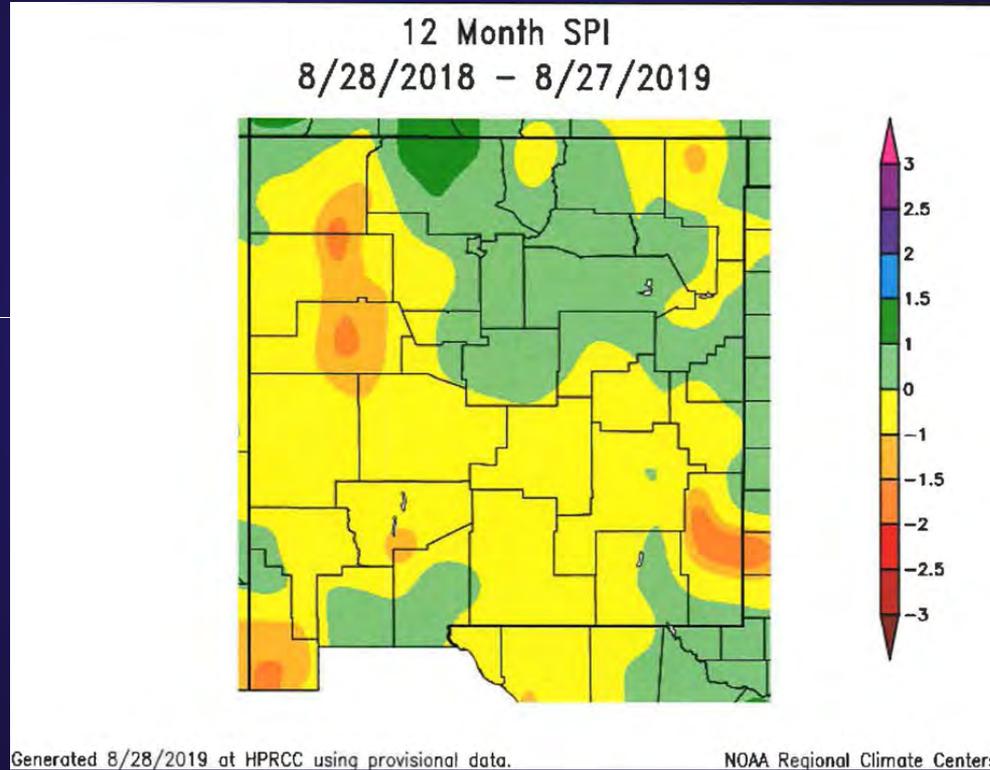
Photo Descriptions and NOTES

Photo #	Description (US, DS, LB, RB, etc.)	Notes
1	Upstream	Photos by S. Loftin
2	Left bank downstream	"
3	Right bank left bank	"
4	Right bank	"
5	Upstream	"
6	Bankline	

**NOTES:**

Flagging noted w/ purple gloves

# 12 Month Standard Precipitation Index



# Rainfall Amounts

Data is for tower ta6.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Aug 29 06:37:40 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
8	24	2019	236	0
8	25	2019	237	0
8	26	2019	238	0
8	27	2019	239	0.14
8	28	2019	240	0

Data is for tower ta49.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Aug 29 06:38:14 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
8	24	2019	236	0
8	25	2019	237	0
8	26	2019	238	0
8	27	2019	239	0.05
8	28	2019	240	0

Data is for tower ta53.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Aug 29 06:38:57 2019 MST.

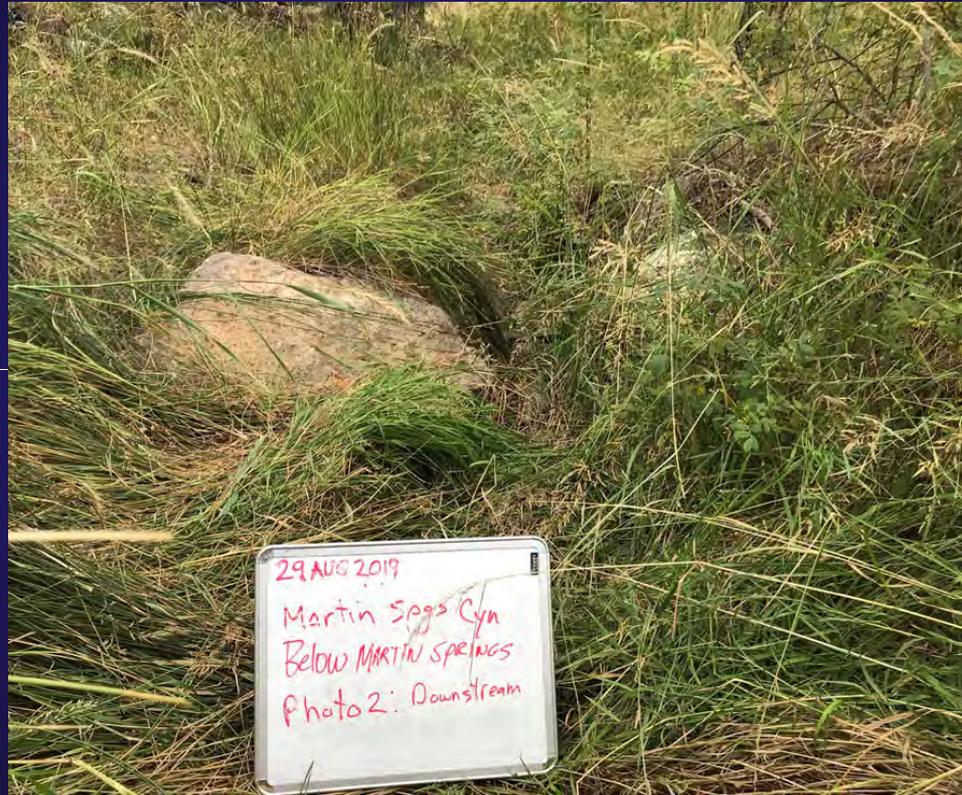
All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
8	24	2019	236	0
8	25	2019	237	0
8	26	2019	238	0
8	27	2019	239	0.02
8	28	2019	240	0

# HP 1- Upstream - S-Site Canyon below Martin Spring



# HP 1 - Downstream - S-Site Canyon below Martin Spring



# HP 1 - Right Bank – S-Site Canyon below Martin Spring



# HP 1 - Left Bank – S-Site Canyon below Martin Spring



# HP 1 - Left Bank – S-Site Canyon below Martin Spring



# HP 1 - S-Site Canyon below Martin Spring



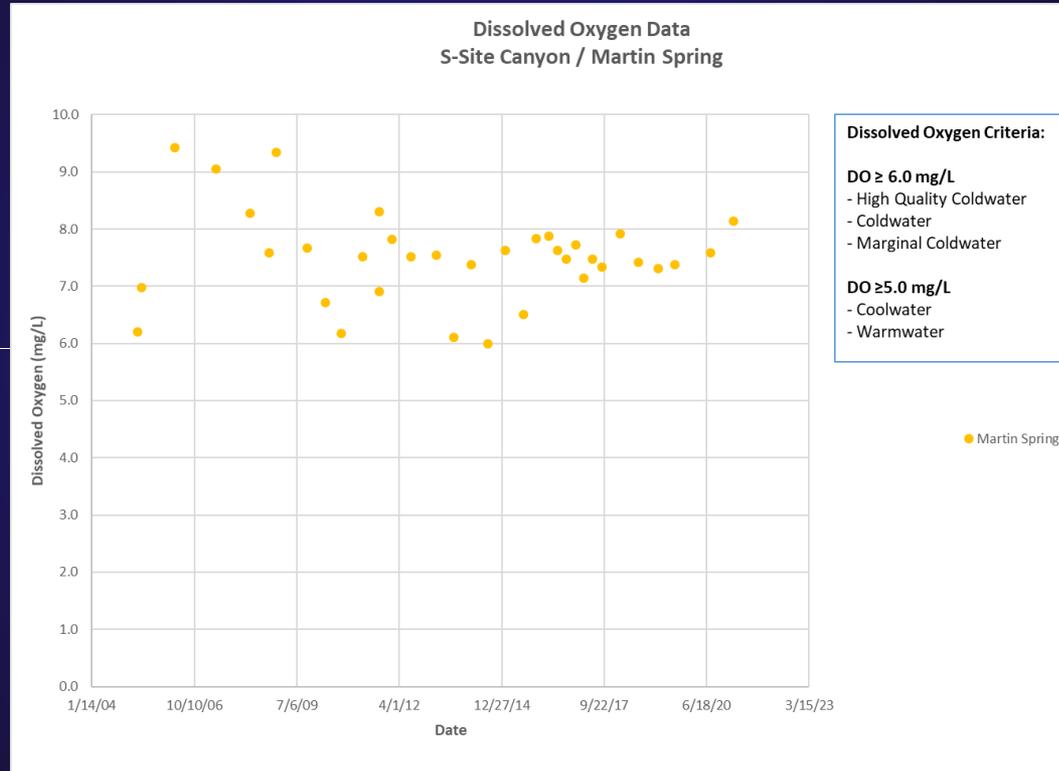
# Benthic







# DO – S-Site Canyon below Martin Spring



# HP Level 2: S-Site Canyon below Martin Spring

**NMED Surface Water Quality Bureau –  
LEVEL 2 Hydrology Determination Field Sheet  
\*\*Borderline Cases\*\***

Date: Oct. 17 2019	Stream Name: Martin Springs	Latitude: 35° 50' 31" N
Evaluator(s): BT AS	Site ID: Below Martin Springs	Longitude: 106° 20' 10" W
<b>LEVEL 1 Total Points:</b>	<b>Reach Description:</b>	<b>Drought Index (12-mo. SPI Value):</b>
1/0	Narrow channel - Spring Fed	0-1

<b>WEATHER CONDITIONS</b>	<b>NOW:</b> <input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input checked="" type="checkbox"/> 100% cloud cover <input type="checkbox"/> clear/sunny	<b>PAST 48 HOURS:</b> <input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input checked="" type="checkbox"/> 100% cloud cover <input checked="" type="checkbox"/> clear/sunny	Has there been a heavy rain in the last 48 hours? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO <small>**Field evaluations should be performed at least 48 hours after the last known major rainfall event.</small> <b>OTHER:</b> Stream Modifications <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO Diversions <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO Discharges <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO <small>**Explain in further detail in NOTES section</small>
---------------------------	--	---	---

CHECK the appropriate rating for each indicator.

LEVEL 2 INDICATORS	Stream Condition			
	Strong	Moderate	Weak	Poor
2.1. Water in Channel (OPTIONAL)	—	N/A	—	—
2.2. Hyporheic Zone/Groundwater Table	—	X	—	—
2.3. Bivalves	Present = —	—	Absent = X	—
2.4. Amphibians	Present = —	—	Absent = X	—
2.5. Macroinvertebrates (abundance/diversity)**	—	—	—	—
2.6. EPT Taxa**	Present = —	—	Absent = —	—
2.7. Fish	—	—	—	X

\*\* Macroinvertebrates and EPT Taxa should not be rated until identification and enumeration has been performed in a laboratory setting by a qualified aquatic biologist/environmental scientist.

Photo #	Description (vs. OS, LB, RB)	Notes
100-4140	Downstream point Looking Upstream	
100-4141	Midpoint Looking Downstream	
100-4142	Midpoint Looking Upstream	
100-4143	Upstream point Looking Downstream	

**NOTES:** (use back-side of this form for additional notes)

Spring water goes subsurface in a number of points. No water to be sampled within 5m of points 3 and 4.

# Summary of Benthic Data

Summary of Benthic Data for Proposed Waters 1			
Segment	Level 1-2 Locations and Scores	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absence)
Pajarito above Starmers Site 1	Pa-14	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	Pc-24	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	Weak	Present
Two Mile Canyon below Confluence	Tf-18	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	Moderate	Present
Two Mile Canyon at TA-55 Confluence	Th-19	-	-
Two Mile above E244	Ti-10.5	-	-
<sup>1</sup> . bivalves present			

# Summary of Benthic Data (cont.)

Summary of Bentic Data for Proposed Waters 2									
Segment	Level 1-2 Locations	Benthic Macroinvertebrates	Total Species Taxa Richness	EPT Taxa Richness	% EPT	Intolerant Taxa Richness	Long Lived Taxa Richness	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absent)
Pajarito above Starmers Site 1	Pa-14	N/A	N/A	N/A	N/A	N/A	N/A	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	1863.1	35.00	5.0	8.8	1.0	1.0	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	PC-24	2036.0	40.00	7.0	40.7	2.0	0.0	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	4136.0	36.00	4.0	7.5	0.0	3.0	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	1431.8	30.00	1.0	3.3	0.0	3.0	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	148.0	13.00	2.0	87.8	0.0	0.0	Weak	Present
Two Mile Canyon below Confluence	Tf-18	132.0	23.00	1.0	0.8	1.0	0.0	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	793.5	37.00	5.0	31.0	0.0	1.0	Moderate	Present
Two Mile Canyon at TA-55 Confluence	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-	-
Two Mile above E244	Ti-10.5	Not Collected	N/A	N/A	N/A	N/A	N/A	-	-

1. bivalves present

## HP 2 - Upstream S-Site Canyon below Martin Spring



Midpoint - Looking Downstream



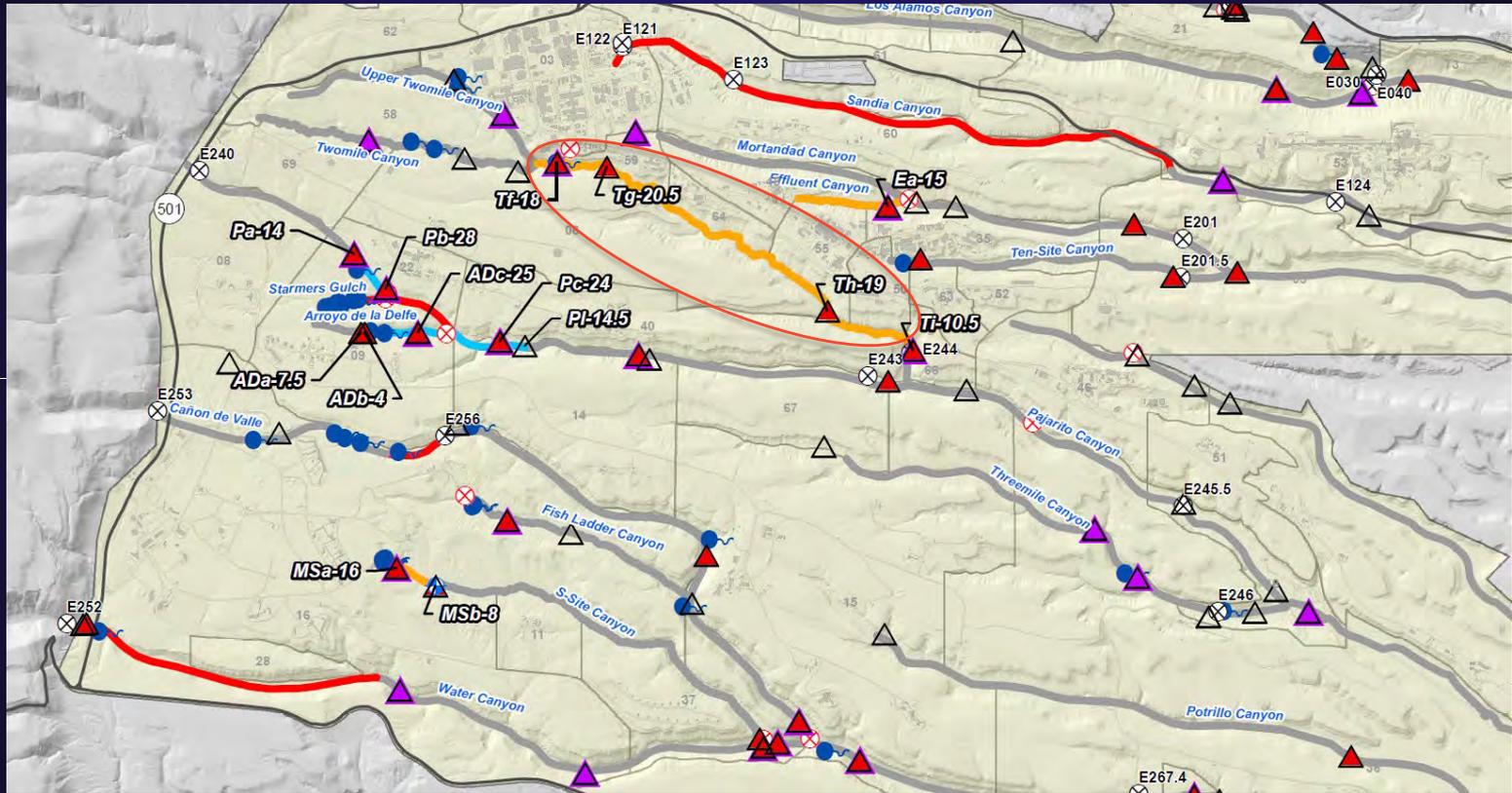
Midpoint - Looking Upstream

## HP 2 - Downstream S-Site Canyon below Martin Spring



# Exhibit 42

# Two Mile Canyon from E244 to Upper Confluence



# HP 1 – Two Mile Canyon above E244

NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

Elevation: 6,450 ft

Date: 9-12-2019 Stream Name: Two Mile Canyon Latitude: 35° 51' 21" N  
 Evaluator(s): Wesley Noyes Site ID: Abac E244 Longitude: 106° 17' 45" W

TOTAL POINTS: 10.5 Assessment Unit: NM-128, A-15 Drought Index (12-mo. SPI Value): 0-1

Stream is at least intermittent (1-2-12)

Has there been a heavy rain in the last 48 hours?  
 YES  NO

WEATHER CONDITIONS

NOW:  
 storm (heavy rain)  
 rain (steady rain)  
 showers (intermittent)  
 light/dew cover  
 clear/sunny

PAST 48 HOURS:  
 storm (heavy rain)  
 rain (steady rain)  
 showers (intermittent)  
 light/dew cover  
 clear/sunny

OTHER:  
 Stream Modifications YES  NO   
 Diversions YES  NO   
 Discharges YES  NO   
 \*\*Explain in further detail in NOTES section.

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Poor
1.1. Water in Channel	Flow is evident throughout the reach. Moving water is seen in the pools but may not be evident throughout the reach. 6	Water is present in the channel but flow is easily discernable in areas of gradual gradient change (i.e., riffles) or floating object is necessary to observe flow. 4	Dry channel with standing pools. There is some evidence of base flow (i.e., riparian vegetation growing along channel, saturated moist sediment under rocks, etc.). 2	Dry channel. No evidence of base flow was found. 0
1.2. Fish	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Fish are not present. 0
1.3. Benthic Macroinvertebrates	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Macroinvertebrates are not present. 0
1.4. Filamentous Algae/Periphyton	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Filamentous algae and/or periphyton are not present. 0
1.5. Differences in Vegetation	Dramatic compositional differences in vegetation are present between the stream banks and the adjacent uplands. A distinct riparian vegetation corridor exists along the entire reach. 3	A distinct riparian vegetation corridor exists along part of the reach. Riparian vegetation is interspersed with upland vegetation along the length of the reach. 2	Vegetation growing along the reach may occur in greater densities or grow more sporadically than vegetation in the adjacent uplands, but there are no dramatic compositional differences between the two. 1	No compositional or density differences in vegetation are present between the streambanks and the adjacent uplands. 0
1.6. Absence of Rooted Upland Plants in Streambed	Rooted upland plants are absent within the streambed/riparian. 3	There are a few rooted upland plants present within the streambed/riparian. 2	Rooted upland plants are consistently present throughout the streambed/riparian. 1	Rooted upland plants are present within the streambed/riparian. 0
SUBTOTAL (#1.1 – #1.6) 5				

If the stream being evaluated has a subtotal 5 at this juncture, the stream is determined to be EPHEMERAL.  
 If the stream being evaluated has a subtotal 2-18 at this point, the stream is determined to be PERENNIAL.  
 YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 2 and 18 continue the Level 1 Evaluation.

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Poor
1.7. Sinuosity	Ratio > 1.4. Stream has numerous, closely spaced bends, few straight sections. 3	Ratio < 1.4. Stream has good sinuosity with some straight sections. 2	Ratio < 1.2. Stream has very few bends and mostly straight sections. 1	Ratio < 1.0. Stream is completely straight with no bends. 0
1.8. Floodplain and Channel Dimensions	Ratio > 2.5. Stream is minimally confined with a wide, active floodplain. 3	Ratio between 1.2 and 2.5. Stream is moderately confined. Floodplain is present, but may be active during major floods. 1.5	Ratio < 1.2. Stream is incised with a locally confined channel. Floodplain is narrow or absent and typically disconnected from the channel. 0	
1.9. In-Channel Structure: Riffle-Pool Sequence	Comprehended by a frequent number of riffles followed by pools along the entire reach. There is an obvious transition between riffles and pools. 3	Represented by a high frequency number of riffles and pools. Distinguishing the transition between riffles and pools is difficult. 2	Stream shows some flow but mostly has areas of pools or riffles. There is no sequence evident. 1	
SUBTOTAL (#1.1 – #1.9) 8.5				
If the stream being evaluated has a subtotal 5.6 at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal 1-21 at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 5 and 21 continue the Level 1 Evaluation.				
1.10. Particle Size or Stream Substrate Sorting	Particle sizes in the channel are in close proximity to particle sizes in the stream channel, on the streambank, and within the floodplain throughout the length of the stream. 3	Particle sizes in the channel are moderately similar to particle sizes in areas close to but not in the channel. Various sized substrates are present in the stream channel and are represented by a higher ratio of larger particles (sand/silt/clay). 1.5	Particle sizes in the channel are similar or comparable to particle sizes in areas close to but not in the channel. Substrate sorting is not readily observed in the stream channel. 0	
1.11. Hydric Soils	Hydric soils are found within the study reach. Present = 3		Hydric soils are not found within the study reach. Absent = 0	
1.12. Sediment on Plants and Debris	Sediment found readily on plants and debris within the stream channel, on the streambank, and within the floodplain throughout the length of the stream. 1.5	Sediment found on plants or debris within the stream channel although it is not prevalent along the stream. Mostly accumulating in pools. 1	Sediment is isolated in small amounts along the stream. 0.5	No sediment is present on plants or debris. 0
TOTAL POINTS (#1.1 – #1.12) 10.5				
SUPPLEMENTAL INDICATORS: The following indicators do not occur consistently throughout New Mexico but may be useful in the determination of perenniality. If the indicator is present record score below and tally with previous scores to compute TOTAL.				
1.13. Seeps and Springs	Seeps and springs are found within the study reach. Present = 1.5		Seeps and springs are not found within the study reach. Absent = 0	
1.14. Iron Oxidizing Bacteria/Fungi	Iron-oxidizing bacteria and/or fungi are found within the study reach. Present = 1.5		Iron-oxidizing bacteria and/or fungi are not found within the study reach. Absent = 0	
TOTAL plus SUPPLEMENTAL POINTS (#1.1 – #1.14) 10.5				

# HP 1 – Two Mile Canyon above E244

## LEVEL 1 Field Measurements

### Pebble Count Tally Sheet

Site Name: \_\_\_\_\_ Storet ID: \_\_\_\_\_  
 Date: \_\_\_\_\_ Crew: \_\_\_\_\_

Substrate Type	Diameter Range	In-Channel COUNT	In-Channel % Composition	Out of Channel COUNT	Out of Channel % Composition
Silt/Clay	< 0.06 mm		N/A BMT		
Sand	0.06 – 2.0 mm (gritty)		9-17%		
Gravel	2.0 – 64 mm				
Cobble	64 – 256				
Boulder	> 256 mm				
Redrock	-				

\*\*Please be sure to measure at least 50 pebbles (10 in 5 transects or 5 in 10 transects- depending on stream size) for accurate distributional representation\*\*

### INDICATOR #1.8 (Floodplain and Channel Dimensions) – MEASUREMENTS & CALCULATIONS\*\*

Max Depth (#1)	Bankfull Stage (#2)	Maximum Depth Value (#3)	2x Maximum Depth Value (#3)	Flood-Prone Area Location (#4)	Flood-Prone Area Width (#5)	Bankfull Width (#6)	Floodplain to Active Channel Ratio (FPA Width / Bankfull Width)
7.70'	7.05'	.67'	1.34'	6.36'	18.72'	12.45'	1.5

\*\*REFER to Figure 3 on page 19 for clarification

## NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

### Photo Descriptions and NOTES

Photo #	Description (US, DS, LB, RB, etc.)	Notes
1	Upstream	
2	Downstream	
3	Right Bank	
4	Left Bank	
5	Overall Channel - Downstream	

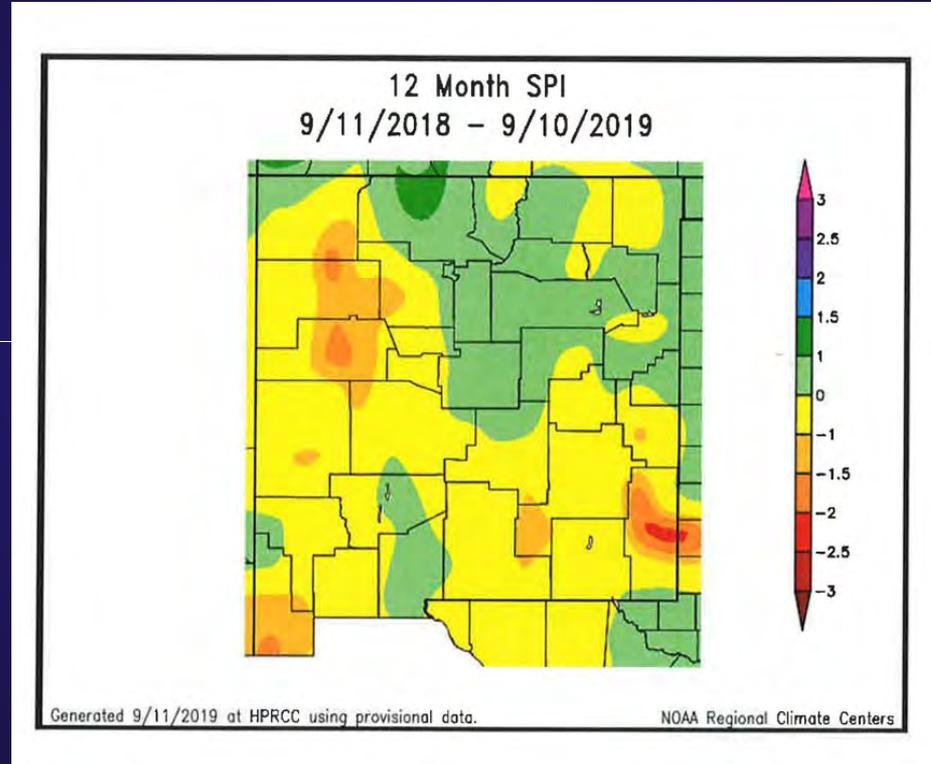
### NOTES:

Evaluators: Sam Loftin, Jennifer Fullin, Robert Gallegos

TA-54 Rain Gage recorded .06" on 9/10/19.

Vegetation present: evening primrose, orchard grass, purple loose pine, box elder, reed, red top, thistle, yarrow, New Mexico locust

# HP 1 – Two Mile Canyon above E244



# HP 1 – Two Mile Canyon above E244

Data is for tower ta6.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:53:26 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0.06
9	11	2019	254	0.03

Data is for tower ta49.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:55:52 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0
9	11	2019	254	0

Data is for tower ta53.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:56:32 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0
9	11	2019	254	0.1

Data is for tower ta54.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:57:11 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0.06
9	11	2019	254	0

# HP 1 – Two Mile Canyon above E244



# HP 1 – Two Mile Canyon above E244



# HP 1 – Two Mile Canyon above E244



# HP 1 – Two Mile Canyon above E244



# HP 1 – Two Mile Canyon above E244



# Gage E244

Summary of Gage Flow Statistics

Station #	Station Name	Analysis Period	Average Percent Days of Flow	Flow Classification <sup>1</sup> Based on Percent Days with Flow	Current NMWQCC	Proposed NMWQCC
E240	Pajarito (below SR-501)	2000 - 2019	9.6%	Ephemeral / Intermittent	20.6.4.128	No change
E241	Pajarito (above Starmers)	2000 - 2009	76.8%	Intermittent / Perennial	20.6.4.128	20.6.4.126
E242	Pajarito (Starmer's Gulch)	2000 - 2009	97.5%	Perennial	20.6.4.126	No change
E242.5	Arroyo de la Delfe (above Pajarito)	2000 - 2009	81.8%	Perennial	20.6.4.128	20.6.4.126
E244	Twomile (above Pajarito)	2003 - 2011; 2015 - 2019	34.0%	Intermittent	20.6.4.128	20.6.4.140

 Perennial flow  
 Borderline Intermittent / Perennial flow

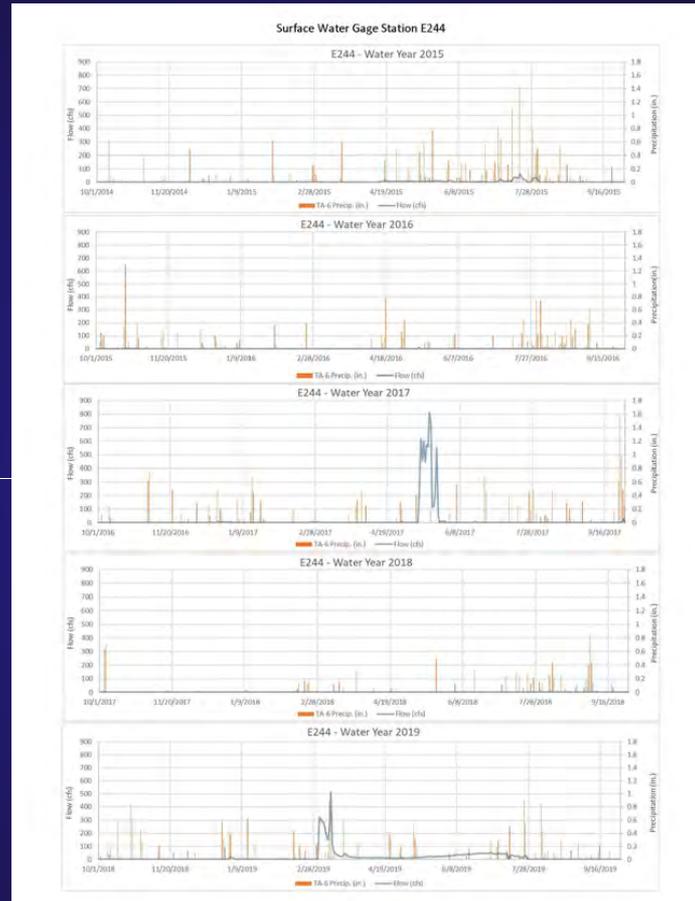
<sup>1</sup>Flow Classification based on criteria from Hedman & Osterkamp, 1982 (USGS Water Supply Paper #2193).

**Ephemeral:** measurable discharge generally occurring less than 10% of the time

**Intermittent:** measurable surface discharge between 10 and 80% of the time

**Perennial:** measurable surface discharge > 80% of the time

# Gage E244 (2014-2019)



# HP 2 – Two Mile Canyon above E244

NMED Surface Water Quality Bureau –  
LEVEL 2 Hydrology Determination Field Sheet  
\*\*Borderline Cases\*\*

Date: 10/21/14		Stream Name: Two-Mile	Latitude: 35°07.346
Evaluator(s): J. Burchett, E. Abbott		Site ID: Above 244	Longitude: 106°17.764
LEVEL 1 Total Points: 10.5	Reach Description: All Dry	Drought Index (12-mo. SPI Value): 0-1	

<b>WEATHER CONDITIONS</b>	<b>NOW:</b> <input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input type="checkbox"/> %cloud cover <input checked="" type="checkbox"/> clear/sunny	<b>PAST 48 HOURS:</b> <input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input type="checkbox"/> %cloud cover <input checked="" type="checkbox"/> clear/sunny	Has there been a heavy rain in the last 48 hours? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO <small>**Field evaluations should be performed at least 48 hours after the last known major rainfall event.</small>
	<b>OTHER:</b> Stream Modifications <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO Diversions <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO Discharges <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO <small>**Explain in further detail in NOTES section</small>		

*CHECK the appropriate rating for each indicator.*

LEVEL 2 INDICATORS	Stream Condition			
	Strong	Moderate	Weak	Poor
2.1. Water in Channel (OPTIONAL)	← NA →			
2.2. Hyporheic Zone/Groundwater Table				X
2.3. Bivalves	Present =		Absent =	X
2.4. Amphibians	Present =		Absent =	X
2.5. Macroinvertebrates (abundance/diversity)**		Absent	← X →	
2.6. EPT Taxa**	Present =		Absent =	X
2.7. Fish		Absent		X

\*\* Macroinvertebrates and EPT Taxa should not be rated until identification and enumeration has been performed in a laboratory setting by a qualified aquatic biologist/environmental scientist.

Photo #	Description (us, or, ls, r, m)	Notes
128-2598	Upstream	Looking downstream
128-2599	Mid point	Looking upstream
128-2601	Mid point	Looking downstream
128-2602	Downstream	Looking upstream

**NOTES:** (use back-side of this form for additional notes)

Assessed stream 80m up & downstream from mid point. Channel is light, rocky & very sandy. Upper portion is very damp & slightly muddy. No BMTs assessed visually by flipping rocks. Hyporheic zone assessed by digging ~15" in channel, waited 30min with no water.

# Summary of Benthic Data

Summary of Benthic Data for Proposed Waters 1			
Segment	Level 1-2 Locations and Scores	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absence)
Pajarito above Starmers Site 1	Pa-14	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	Pc-24	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	Weak	Present
Two Mile Canyon below Confluence	Tf-18	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	Moderate	Present
Two Mile Canyon at TA-55 Confluence	Th-19	-	-
Two Mile above E244	Ti-10.5	-	-
<sup>1</sup> . bivalves present			

# Summary of Benthic Data (cont.)

Summary of Benthic Data for Proposed Waters 2									
Segment	Level 1-2 Locations	Benthic Macroinvertebrates	Total Species Taxa Richness	EPT Taxa Richness	% EPT	Intolerant Taxa Richness	Long Lived Taxa Richness	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absent)
Pajarito above Starmers Site 1	Pa-14	N/A	N/A	N/A	N/A	N/A	N/A	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	1863.1	35.00	5.0	8.8	1.0	1.0	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	PC-24	2036.0	40.00	7.0	40.7	2.0	0.0	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	4136.0	36.00	4.0	7.5	0.0	3.0	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	1431.8	30.00	1.0	3.3	0.0	3.0	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	148.0	13.00	2.0	87.8	0.0	0.0	Weak	Present
Two Mile Canyon below Confluence	Tf-18	132.0	23.00	1.0	0.8	1.0	0.0	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	793.5	37.00	5.0	31.0	0.0	1.0	Moderate	Present
Two Mile Canyon at TA-55 Confluence	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-	-
Two Mile above E244	Ti-10.5	Not Collected	N/A	N/A	N/A	N/A	N/A	-	-

1. bivalves present

# HP 1 – Two Mile Canyon above E244



# HP 1 – Two Mile Canyon above E244



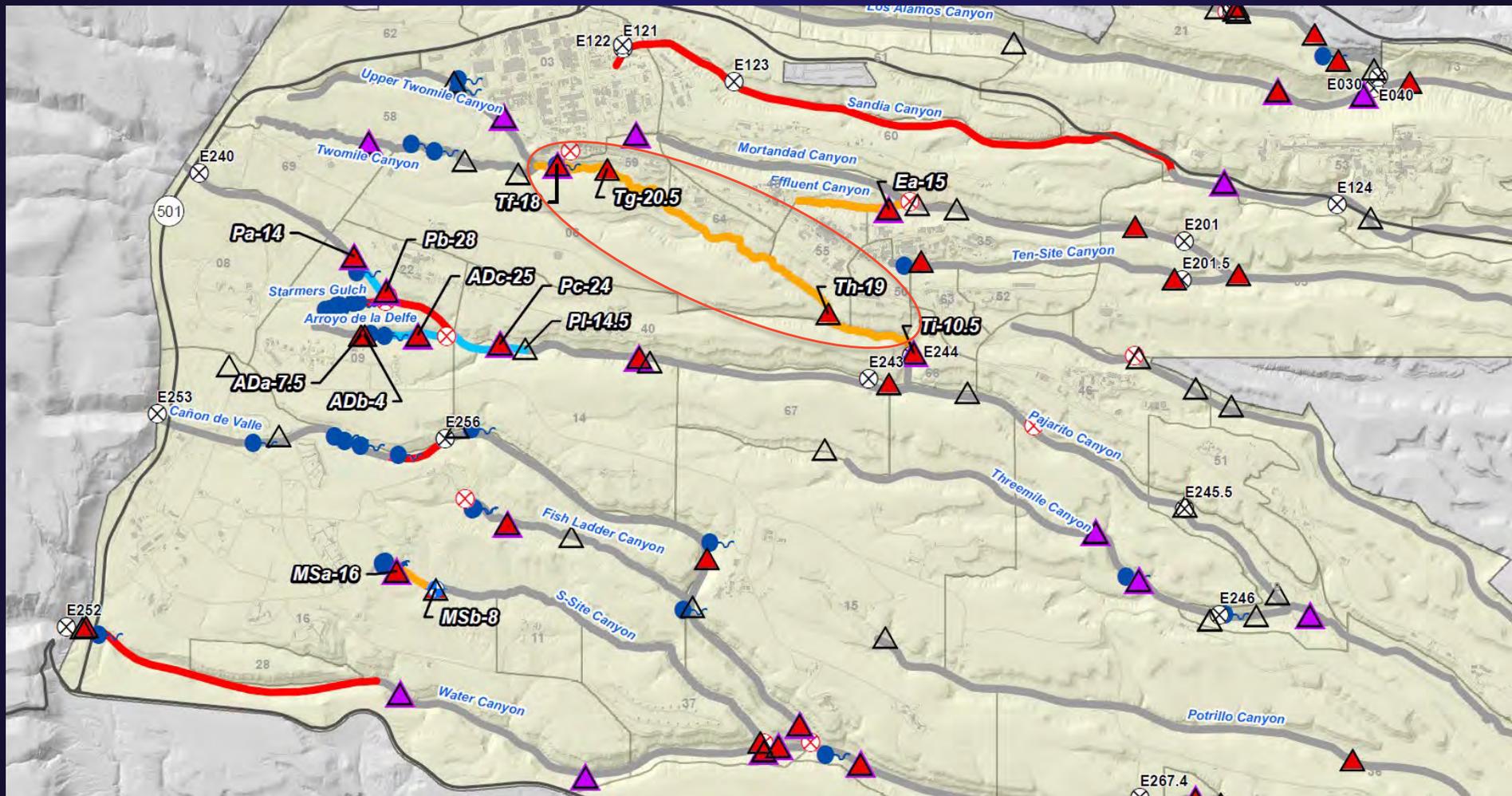
# HP 1 – Two Mile Canyon above E244



# HP 1 – Two Mile Canyon above E244



# Two Mile Canyon from E244 to Upper Confluence



# HP 1 – Two Mile Canyon at TA-55 Confluence

NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

Date: 9-12-2019 Stream Name: Two Mile Canyon Latitude: 35° 51' 30" N  
 Evaluator(s): \* See Notes Site ID: TA-55 Confluence Longitude: 106° 18' 9" W  
 TOTAL POINTS: 19 Assessment Unit: NM-128-A-15 Drought Index (12-mo. SPI Value): 0-1  
Stream is at least 12-inches deep if ≥ 1.2

Has there been a heavy rain in the last 48 hours?  
 YES  NO

WEATHER CONDITIONS  
 NOW: storm (heavy rain)  rain (steady rain)  showers (intermittent)  %cloud cover  clear/sunny   
 PAST 48 HOURS: storm (heavy rain)  rain (steady rain)  showers (intermittent)  %cloud cover  clear/sunny

OTHER:  
 Stream Modifications YES  NO   
 Diversions YES  NO   
 Discharges YES  NO   
 \*\*Explain in further detail in NOTES section

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Poor
1.1. Water in Channel	Flow is evident throughout the reach. Moving water is seen in riffle areas but may not be as evident throughout the runs. 6	Water is present in the channel but flow is barely discernible in areas of greatest gradient change (i.e. riffles) or floating object is necessary to observe flow. 4	Dry channel with standing pools. There is some evidence of base flows (i.e. riparian vegetation growing along channel, saturated or moist sediment under rocks, etc.) 2	Dry channel. No evidence of base flows was found. 0
1.2. Fish	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Fish are not present. 0
1.3. Benthic Macroinvertebrates	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Macroinvertebrates are not present. 0
1.4. Filamentous Algae/Periphyton	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Filamentous algae and/or periphyton are not present. 0
1.5. Differences in Vegetation	Dramatic compositional differences in vegetation are present between the stream banks and the adjacent uplands. A distinct riparian vegetation corridor exists along the entire reach – riparian, aquatic, or wetland species dominate the length of the reach. 3	A distinct riparian vegetation corridor exists along part of the reach. Riparian vegetation is interspersed with upland vegetation along the length of the reach. 2	Vegetation growing along the reach may occur in greater densities or grow more vigorously than vegetation in the adjacent uplands, but there are no dramatic compositional differences between the two. 1	No compositional or density differences in vegetation are present between the streambanks and the adjacent uplands. 0
1.6. Absence of Rooted Upland Plants in Streambed	Rooted upland plants are absent within the streambed/thalweg. 3	There are a few rooted upland plants present within the streambed/thalweg. 2	Rooted upland plants are consistently dispersed throughout the streambed/thalweg. 1	Rooted upland plants are prevalent within the streambed/thalweg. 0
SUBTOTAL (#1.1 – #1.6)				12
If the stream being evaluated has a subtotal ≤ 2 at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal ≥ 18 at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 2 and 18 continue the Level 1 Evaluation.				

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Poor
1.7. Sinuosity	Ratio > 1.4. Stream has numerous, closely-spaced bends, few straight sections. 3	Ratio < 1.4. Stream has good sinuosity with some straight sections. 2	Ratio < 1.2. Stream has very few bends and mostly straight sections. 1	Ratio = 1.0. Stream is completely straight with no bends. 0
1.8. Floodplain and Channel Dimensions	Ratio > 2.5. Stream is minimally confined with a wide, active floodplain. 3	Ratio between 1.2 and 2.5. Stream is moderately confined. Floodplain is present, but may only be active during larger floods. 1.5	Ratio < 1.2. Stream is incised with a noticeably confined channel. Floodplain is narrow or absent and typically disconnected from the channel. 0	
1.9. In-Channel Structure: Riffle-Pool Sequence	Demonstrated by a frequent number of riffles followed by pools along the entire reach. There is an obvious transition between riffles and pools. 3	Represented by a less frequent number of riffles and pools. Distinguishing the transition between riffles and pools is difficult. 2	Stream shows some flow but mostly has areas of pools or riffles. 1	There is no sequence exhibited. 0
SUBTOTAL (#1.1 – #1.9)				17.5
If the stream being evaluated has a subtotal ≤ 5 at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal ≥ 21 at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 5 and 21 continue the Level 1 Evaluation.				
1.10. Particle Size or Stream Substrate Sorting	Particle sizes in the channel are noticeably different from particle sizes in areas close to but not in the channel. There is a clear distribution of various sized substrates in the stream channel with finer particles accumulating in the pools, and larger particles accumulating in the riffles/runs. 3	Particle sizes in the channel are moderately similar to particle sizes in areas close to but not in the channel. Various sized substrates are present in the stream channel and are represented by a higher ratio of larger particles (gravel/cobble). 1.5	Particle sizes in the channel are similar or comparable to particle sizes in areas close to but not in the channel. Substrate sorting is not readily observed in the stream channel. 0	
1.11. Hydric Soils	Hydric soils are found within the study reach. Present = 3		Hydric soils are not found within the study reach. Absent = 0	
1.12. Sediment on Plants and Debris	Sediment found readily on plants and debris within the stream channel, on the streambank, and within the floodplain throughout the length of the stream. 1.5	Sediment found on plants or debris within the stream channel although it is not prevalent along the stream. Mostly accumulating in pools. 1	Sediment is isolated in small amounts along the stream. 0.5	No sediment is present on plants or debris. 0
TOTAL POINTS (#1.1 – #1.12)				19
<b>SUPPLEMENTAL INDICATORS:</b> The following indicators do not occur consistently throughout New Mexico but may be useful in the determination of perennality. If the indicator is present record score below and tally with previous score to compute TOTAL.				
1.13. Seeps and Springs	Seeps and springs are found within the study reach. Present = 1.5		Seeps and springs are not found within the study reach. Absent = 0	
1.14. Iron Oxidizing Bacteria/Fungi	Iron-oxidizing bacteria and/or fungi are found within the study reach. Present = 1.5		Iron-oxidizing bacteria and/or fungi are not found within the study reach. Absent = 0	
TOTAL plus SUPPLEMENTAL POINTS (#1.1 – #1.14)				19

# HP 1 – Two Mile Canyon at TA-55 Confluence

## LEVEL 1 Field Measurements

### Pebble Count Tally Sheet

Site Name: \_\_\_\_\_ Storet ID: \_\_\_\_\_  
 Date: \_\_\_\_\_ Crew: \_\_\_\_\_

Substrate Type	Diameter Range	In-Channel COUNT	In-Channel % Composition	Out of Channel COUNT	Out of Channel % Composition
Silt/Clay	< 0.06 mm		N/A BML		
Sand	0.06 – 2.0 mm (gritty)		9-17-19		
Gravel	2.0 – 64 mm				
Cobble	64 – 256				
Boulder	> 256 mm				
Bedrock	---				

\*\*Please be sure to measure at least 50 pebbles (10 in 5 transects or 5 in 10 transects depending on stream size) for accurate distributional representation\*\*

### INDICATOR #1.8 (Floodplain and Channel Dimensions) – MEASUREMENTS & CALCULATIONS\*\*

Max Depth (#1)	Bankfull Stage (#2)	Maximum Depth Value (#3)	2x Maximum Depth Value (#3)	Flood-Prone Area Location (#4)	Flood-Prone Area Width (#5)	Bankfull Width (#6)	Floodplain to Active Channel Ratio (FPA Width / Bankfull Width)
7.64'	7.25'	.39'	.78'	6.86'	10.65'	4.34'	2.45

\*\*REFER to Figure 3 on page 19 for clarification

### NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet Photo Descriptions and NOTES

Photo #	Description (US, DS, LB, RB, etc.)	Notes
1	Upstream	
2	Downstream	
3	Right Bank	
4	Left Bank	
5	Oswell Channel - Runsterm	
6	Benthic Macroinvertebrates	
7	Benthic Macroinvertebrates-2	

#### NOTES:

Evaluators: Sam Loftin, Robert Gallegos, Jennifer Falken

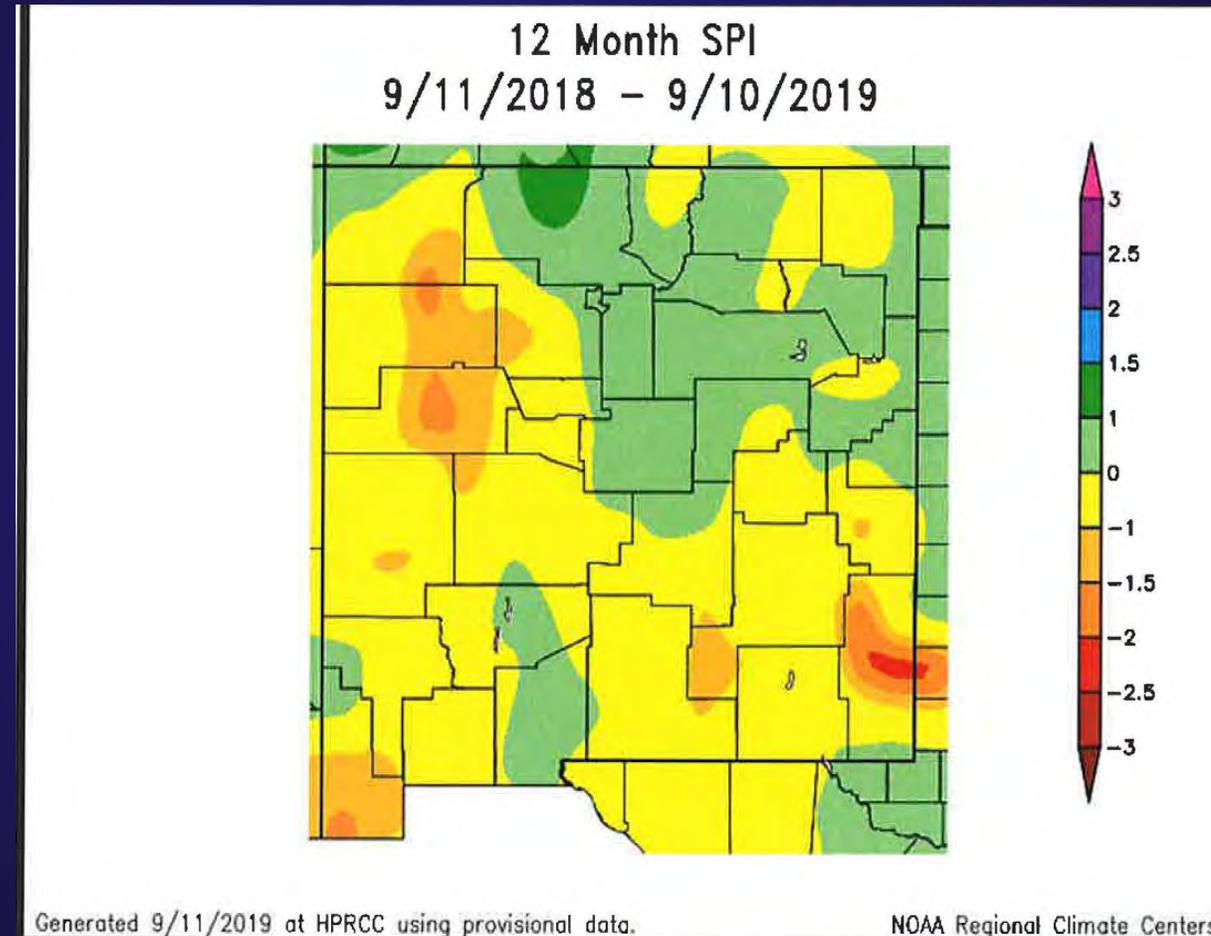
TA-54 Rain Gage recorded .06" on 9-10-19.

1.3- Caddisfly casing, worm, mayfly

1.5- oak, clover, red top, daisy, fox elder, rose, poison ivy, thistle

1.10- Limited sorting

# HP 1 – Two Mile Canyon at TA-55 Confluence



# HP 1 – Two Mile Canyon at TA-55 Confluence

Data is for tower ta6.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:53:26 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0.06
9	11	2019	254	0.03

Data is for tower ta49.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:55:52 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0
9	11	2019	254	0

Data is for tower ta53.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:56:32 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0
9	11	2019	254	0.1

Data is for tower ta54.

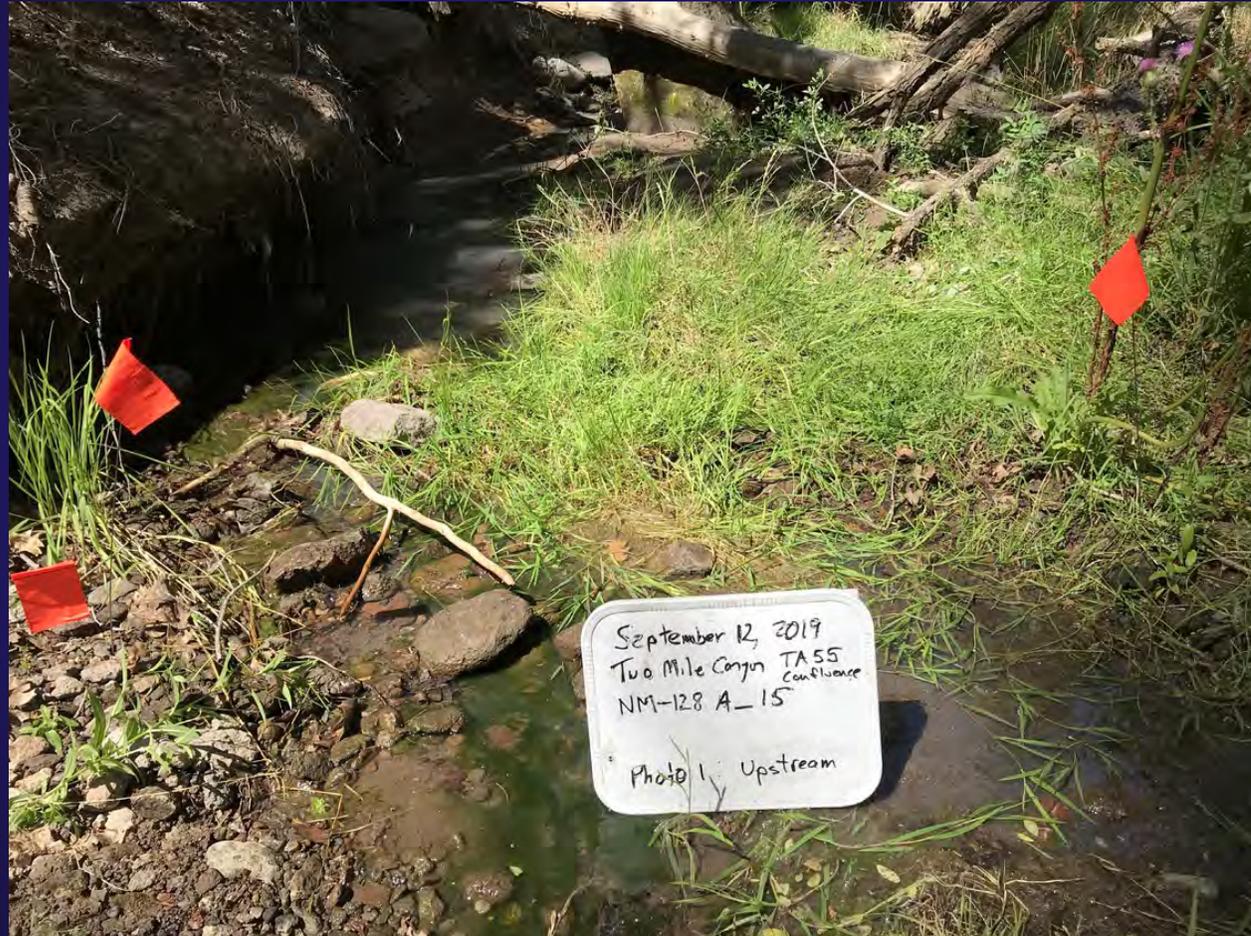
This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:57:11 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0.06
9	11	2019	254	0

# HP 1 – Two Mile Canyon at TA-55 Confluence



# HP 1 – Two Mile Canyon at TA-55 Confluence



# HP 1 – Two Mile Canyon at TA-55 Confluence



# HP 1 – Two Mile Canyon at TA-55 Confluence



# HP 1 – Two Mile Canyon at TA-55 Confluence



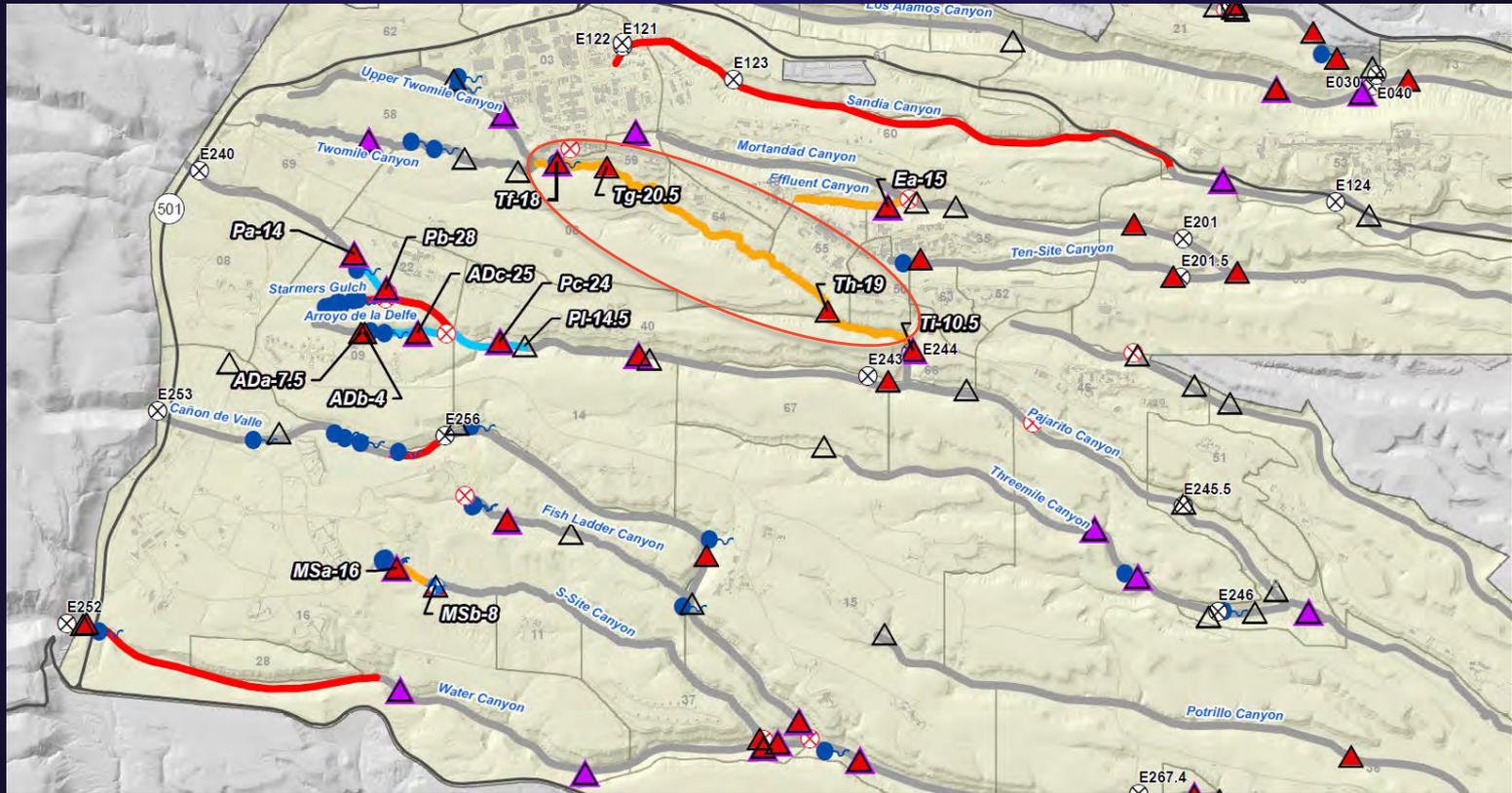
# HP 1 – Two Mile Canyon at TA-55 Confluence



# HP 1 – Two Mile Canyon at TA-55 Confluence



# Two Mile Canyon from E244 to Upper Confluence



# HP 1 – Two Mile Canyon below TA-59

NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

Date: 9-12-2019 Stream Name: Two Mile Canyon Elevation: 7,270  
 Latitude: 35° 50' 3" N  
 Longitude: 106° 19' 11" W  
 Site ID: Delta TA-59 Drought Index (12-mo. SPI) Value: C-1  
 Assessment Unit: NM-128, A-15  
 TOTAL POINTS: 20.5  
 Stream is at least perennial if ≥ 12

WEATHER CONDITIONS  
 NOW:  
 storm (heavy rain)  
 rain (steady rain)  
 showers (intermittent)  
 Nicest cover  
 clear/sunny  
 PAST 48 HOURS:  
 storm (heavy rain)  
 rain (steady rain)  
 showers (intermittent)  
 Nicest cover  
 clear/sunny  
 Has there been a heavy rain in the last 48 hours?  
 YES  NO  
 \*Field evaluations should be performed at least 48 hours after the last known major on-fall event.  
 OTHER:  
 Stream Modifications: YES  NO   
 Diversions: YES  NO   
 Discharges: YES  NO   
 \*Explain in further detail in NQ1/2/3 section

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Pool
1.1. Water in Channel	Flow is evident throughout the reach. Moving water is seen in riffle areas but may not be as evident throughout the runs. 6	Water is present in the channel but flow is barely discernible in areas of greatest gradient change (i.e. riffles) or leading edge of necessary fit observe flow. 4	Dry channel with standing pools. There is some evidence of base flows (i.e. riparian vegetation growing along channel, saturated or moist soil/mud under rocks, etc.) 2	Dry channel. No evidence of base flows was found. 0
1.2. Fish	Found easily and consistently throughout the reach. 3	Found with some difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Fish are not present. 0
1.3. Benthic Macroinvertebrates	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Macroinvertebrates are not present. 0
1.4. Filamentous Algae/Periphyton	Found easily and consistently throughout the reach. 3	Found with some difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Filamentous algae and/or periphyton are not present. 0
1.5. Differences in Vegetation	Dramatic compositional differences in vegetation are present between the stream banks and the adjacent uplands. A distinct riparian vegetation corridor exists along the entire reach – riparian: aquatic or wetland species dominate the length of the reach. 3	A distinct riparian vegetation corridor exists along part of the reach. Riparian vegetation is interspersed with upland vegetation along the length of the reach. 2	Vegetation growing along the reach may occur in greater densities or grow more vigorously than vegetation in the adjacent uplands, but there are no dramatic compositional differences between the two. 1	No compositional or density differences in vegetation are present between the streambanks and the adjacent uplands. 0
1.6. Absence of Rooted Upland Plants in Streambed	Rooted upland plants are absent within the streambed/trailway. 3	There are a few rooted upland plants present within the streambed/trailway. 2	Rooted upland plants are consistently observed throughout the streambed/trailway. 1	Rooted upland plants are prevalent within the streambed/trailway. 0
SUBTOTAL (#1.1 – #1.6) 13				
If the stream being evaluated has a subtotal ≤ 2 at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal ≥ 18 at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 2 and 18 continue the Level 1 Evaluation.				

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Pool
1.7. Sinuosity	Ratio > 1.4. Stream has numerous, closely-spaced bends, few straight sections. 3	Ratio < 1.4. Stream has good straight with some straight sections. 2	Ratio < 1.2. Stream has very few bends and mostly straight sections. 1	Ratio < 1.0. Stream is completely straight with no bends. 0
1.8. Floodplain and Channel Dimensions	Ratio > 3.5. Stream is normally confined with a wide, active floodplain. 3	Ratio between 1.2 and 2.5. Stream is moderately confined. Floodplain is present but may only be active during larger floods. 1.5	Ratio < 1.2. Stream is incised with a noticeably confined channel. Floodplain is narrow or absent and typically disconnected from the channel. 0	
1.9. In-Channel Structure: Riffle-Pool Sequence	Demonstrated by a frequent number of riffles followed by pools along the entire reach. There is an obvious transition between riffles and pools. 3	Represented by a small number of riffles and pools. Distinguishing the transition between riffles and pools is difficult. 2	Stream shows some flow but mostly has areas of pools or riffles. 1	There is no sequence exhibited. 0
SUBTOTAL (#1.1 – #1.9) 18.5				
If the stream being evaluated has a subtotal ≤ 5 at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal ≥ 21 at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 5 and 21 continue the Level 1 Evaluation.				
1.10. Particle Size or Stream Substrate Sorting	Particle sizes in the channel are noticeably different from particle sizes in areas close to but not in the channel. There is a clear distribution of various sized substrates in the stream channel with finer particles accumulating in the pools and larger particles accumulating in the riffles/runs. 3	Particle sizes in the channel are moderately similar to particle sizes in areas close to but not in the channel. Various sized substrates are present in the stream channel and are represented by a higher ratio of larger particles (gravel/cobble). 1.5	Particle sizes in the channel are similar or comparable to particle sizes in areas close to but not in the channel. Substrate sorting is not readily observed in the stream channel. 0	
1.11. Hydric Soils	Hydric soils are found within the study reach. Present = 3	Hydric soils are not found within the study reach. Absent = 0		
1.12. Sediment on Plants and Debris	Sediment found readily on plants and debris within the stream channel, on the streambank, and within the floodplain throughout the length of the stream. 1.5	Sediment found on plants or debris within the stream channel although it is not prevalent along the floodplain throughout the stream. Mostly accumulating in pools. 1	Sediment is isolated in small amounts along the stream. 0.5	No sediment is present on plants or debris. 0
TOTAL POINTS (#1.1 – #1.12) 20.5				
SUPPLEMENTAL INDICATORS: The following indicators do not occur consistently throughout New Mexico but may be useful in the determination of perennality. If the indicator is present record scores below and tally with previous score to compute TOTAL.				
1.13. Seeps and Springs	Seeps and springs are found within the study reach. Present = 1.5	Seeps and springs are not found within the study reach. Absent = 0		
1.14. Iron Oxidizing Bacteria/Fungi	Iron-oxidizing bacteria and/or fungi are found within the study reach. Present = 1.5	Iron-oxidizing bacteria and/or fungi are not found within the study reach. Absent = 0		
TOTAL plus SUPPLEMENTAL POINTS (#1.1 – #1.14) 20.5				

# HP 1 – Two Mile Canyon below TA-59

## LEVEL 1 Field Measurements

### Pebble Count Tally Sheet

Site Name: \_\_\_\_\_ Store ID: \_\_\_\_\_  
 Date: \_\_\_\_\_ Crew: \_\_\_\_\_

Substrate Type	Diameter Range	In-Channel COUNT	In-Channel % Composition	Out of Channel COUNT	Out of Channel % Composition
Silt/Clay	< 0.06 mm		N/A		
Sand	0.06 – 2.0 mm (gritty)		9 - 17%		
Gravel	2.0 – 64 mm				
Cobble	64 – 256				
Boulder	> 256 mm				
Bedrock	--				

\*Please be sure to measure at least 50 pebbles (10 in 5 transects or 5 in 10 transects- depending on stream size) for accurate distributional representation\*\*

### INDICATOR #1.8 (Floodplain and Channel Dimensions) – MEASUREMENTS & CALCULATIONS\*

Max Depth (#1)	Bankfull Stage (#2)	Maximum Depth Value (#3)	2x Maximum Depth Value (#3)	Flood-Prone Area Location (#4)	Flood-Prone Area Width (#5)	Bankfull Width (#6)	Floodplain to Active Channel Ratio – (FPA Width / Bankfull Width)
0.2'	7.65'	.55'	1.1'	7.1'	9.39'	6.1'	1.54

\*\*REFER to Figure 3 on page 19 for clarification

### NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

#### Photo Descriptions and NOTES

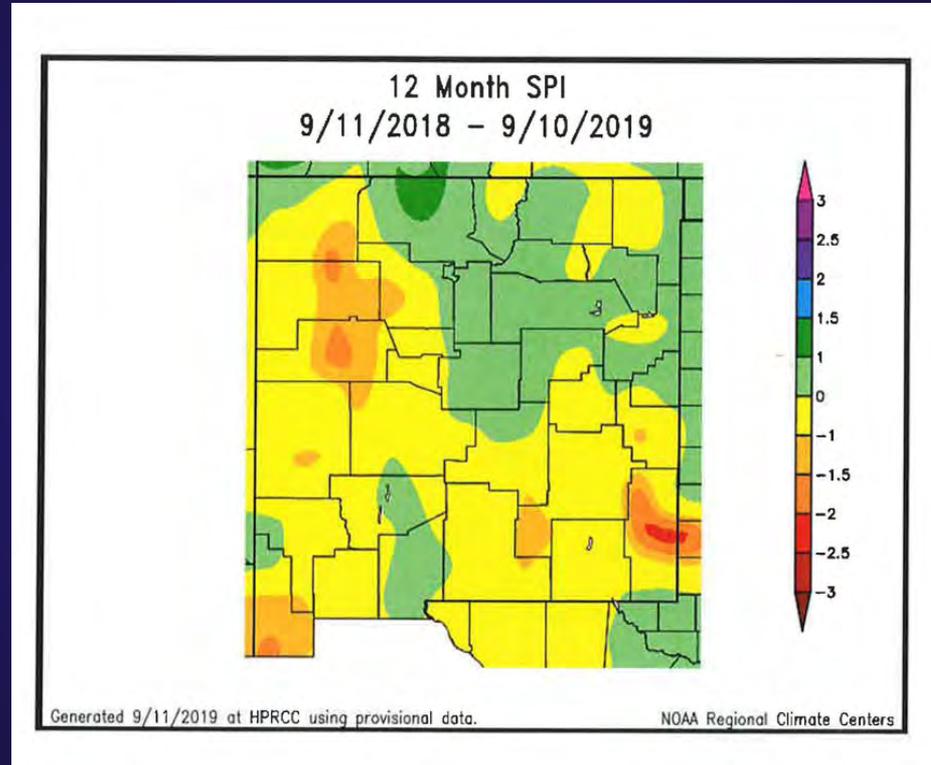
Photo #	Description (US, DS, LB, RB, etc.)	Notes
1	Upstream	
2	Downstream	
3	Right Bank	
4	Left Bank	
5	Overall Channel Upstream	

#### NOTES:

Indicators: Jason For Fuller, Sen Lofth, Robert Gilles  
 TA-6 Pan gage recorded .06" on 9/10 and .03" on 9/11.

1.3 - Macroinvertebrates - beetles, mayflies, redworm

# HP 1 – Two Mile Canyon below TA-59



# HP 1 – Two Mile Canyon below TA-59

Data is for tower ta6.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:53:26 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0.06
9	11	2019	254	0.03

Data is for tower ta49.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:55:52 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0
9	11	2019	254	0

Data is for tower ta53.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:56:32 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0
9	11	2019	254	0.1

Data is for tower ta54.

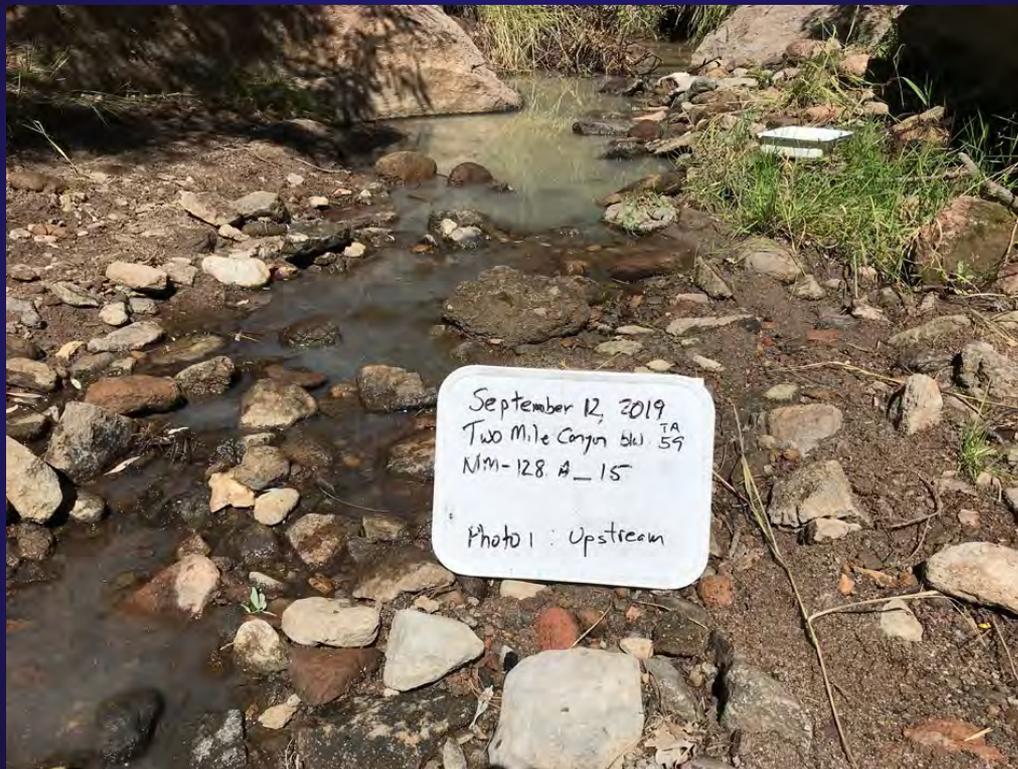
This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:57:11 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0.06
9	11	2019	254	0

# HP 1 – Two Mile Canyon below TA-59



# HP 1 – Two Mile Canyon below TA-59



# HP 1 – Two Mile Canyon below TA-59



# HP 1 – Two Mile Canyon below TA-59



# HP 1 – Two Mile below TA-59



# HP 2 – Two Mile Canyon below TA-59

**NMED Surface Water Quality Bureau –  
LEVEL 2 Hydrology Determination Field Sheet  
\*\*Borderline Cases\*\***

Date: 10-10-2019		Stream Name: Two Mile Canyon	Latitude: 35°52'3"
Evaluator(s): Jennifer C. Burk		Site ID: HP 2 Below TA-59	Longitude: -106°19'11"
LEVEL 1 Total Points: 20.5		Reach Description: Below TA-59 All wet	Drought Index (12-mo. SPI Value): 0-1

<b>WEATHER CONDITIONS</b>	<b>NOW:</b>	<b>PAST 48 HOURS:</b>	Has there been a heavy rain in the last 48 hours? _ YES <input checked="" type="checkbox"/> NO
	<input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input checked="" type="checkbox"/> without cover <input type="checkbox"/> clear/sunny	<input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input type="checkbox"/> without cover <input checked="" type="checkbox"/> clear/sunny	<small>**Field evaluations should be performed at least 48 hours after the last known major rainfall event.</small> <b>OTHER:</b> Stream Modifications <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO Diversions <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO Discharges <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO <small>**Explain in further detail in NOTES section</small>

CHECK the appropriate rating for each indicator.

LEVEL 2 INDICATORS	Stream Condition			
	Strong	Moderate	Weak	Poor
2.1 Water in Channel (OPTIONAL)	<input type="checkbox"/>	NA	<input type="checkbox"/>	<input type="checkbox"/>
2.2 Hyporheic Zone/Groundwater Table	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2.3 Bivalves	Present = <input type="checkbox"/>	<input type="checkbox"/>	Absent = <input type="checkbox"/>	<input type="checkbox"/>
2.4 Amphibians	Present = <input type="checkbox"/>	<input type="checkbox"/>	Absent = <input checked="" type="checkbox"/>	<input type="checkbox"/>
2.5 Macroinvertebrates (abundance/diversity)**	Present = <input type="checkbox"/>	<input type="checkbox"/>	Absent = <input type="checkbox"/>	<input type="checkbox"/>
2.6 EPT Taxa**	Present = <input type="checkbox"/>	<input type="checkbox"/>	Absent = <input type="checkbox"/>	<input checked="" type="checkbox"/>
2.7 Fish	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

\*\* Macroinvertebrates and EPT Taxa should not be rated until identification and enumeration has been performed in a laboratory setting by a qualified aquatic biologist/environmental scientist.

Photo #	Description (us, ds, lr, rs)	Notes
100-414	DS DS MIDPOINT	DS LOOPS US MIDPOINT
100-415	DS MIDPOINT	US LOOPS DS MIDPOINT
100-416	DS	DS LOOPS US TRANSIT R
100-417	US	US LOOPS DS TRANSIT R

**NOTES:** (use back-side of this form for additional notes)

ALL TRANSITS ARE WITH ~~ADDITIONAL~~ BIRDA <sup>EPT</sup> IN STREAM BED

COLLECTED SAMPLES AT ALL TRANSITS, BOTH UP AND DOWNSTREAM

FROM MIDPOINT Trichoptera & Ephemeroptera observed.

# Summary of Benthic Data

Summary of Benthic Data for Proposed Waters 1			
Segment	Level 1-2 Locations and Scores	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absence)
Pajarito above Starmers Site 1	Pa-14	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	Pc-24	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	Weak	Present
Two Mile Canyon below Confluence	Tf-18	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	Moderate	Present
Two Mile Canyon at TA-55 Confluence	Th-19	-	-
Two Mile above E244	Ti-10.5	-	-
<sup>1</sup> . bivalves present			

# Summary of Benthic Data (cont.)

Summary of Bentic Data for Proposed Waters 2									
Segment	Level 1-2 Locations	Benthic Macroinvertebrates	Total Species Taxa Richness	EPT Taxa Richness	% EPT	Intolerant Taxa Richness	Long Lived Taxa Richness	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absent)
Pajarito above Starmers Site 1	Pa-14	N/A	N/A	N/A	N/A	N/A	N/A	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	1863.1	35.00	5.0	8.8	1.0	1.0	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	PC-24	2036.0	40.00	7.0	40.7	2.0	0.0	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	4136.0	36.00	4.0	7.5	0.0	3.0	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	1431.8	30.00	1.0	3.3	0.0	3.0	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	148.0	13.00	2.0	87.8	0.0	0.0	Weak	Present
Two Mile Canyon below Confluence	Tf-18	132.0	23.00	1.0	0.8	1.0	0.0	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	793.5	37.00	5.0	31.0	0.0	1.0	Moderate	Present
Two Mile Canyon at TA-55 Confluence	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-	-
Two Mile above E244	Ti-10.5	Not Collected	N/A	N/A	N/A	N/A	N/A	-	-

1. bivalves present

## HP 2 – Two Mile Canyon below TA-59



Midpoint - Looking Upstream

## HP 2 – Two Mile Canyon below TA-59



Mid Point - Looking Downstream

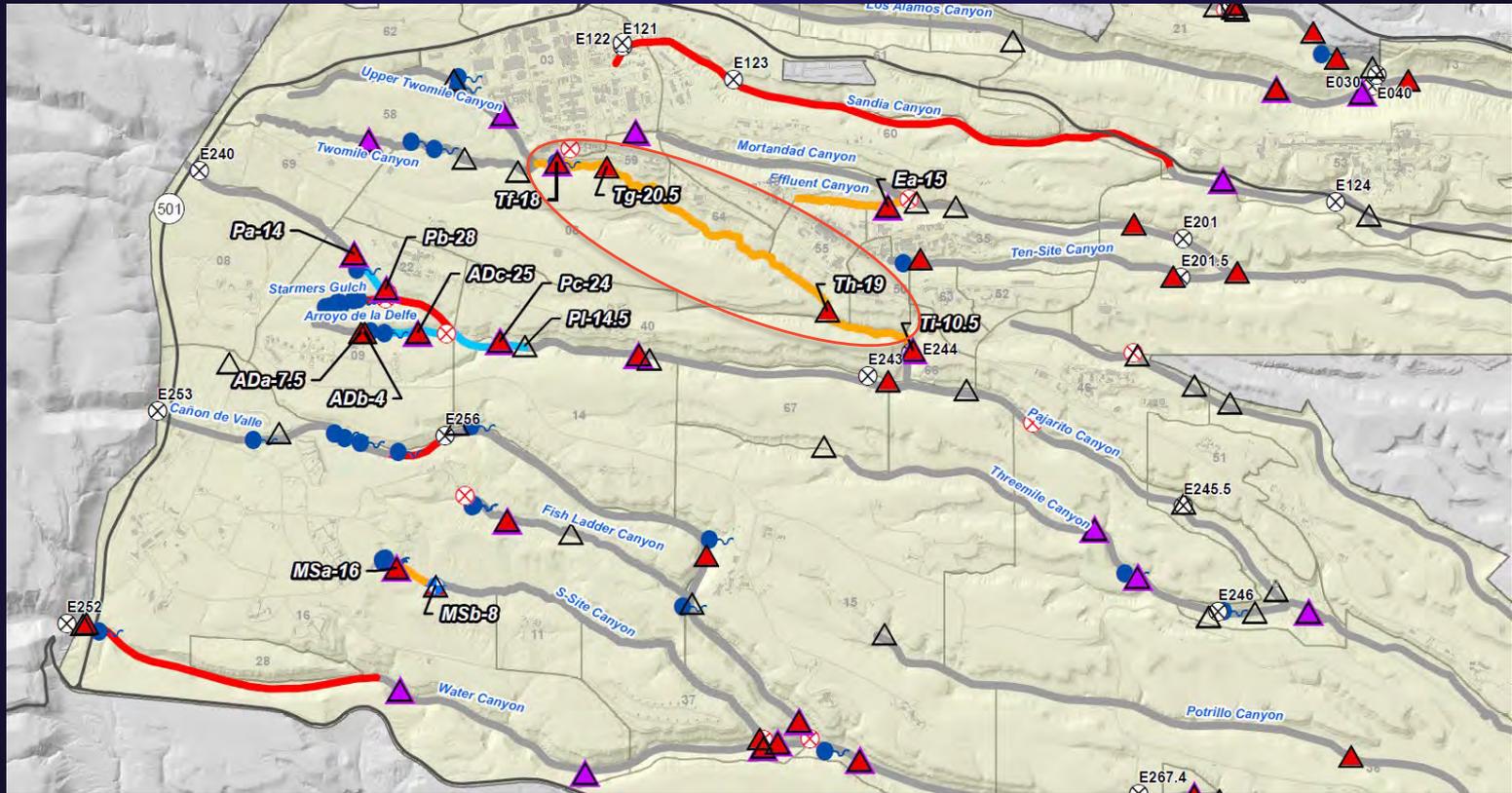
## HP 2 – Two Mile Canyon below TA-59



## HP 2 – Two Mile Canyon below TA-59



# Two Mile Canyon from E244 to Upper Confluence



# HP Level 1 – Two Mile Canyon – below Upper Confluence

NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet  
Elevation: 7340

Date: 9-12-2019  
Stream Name: Two Mile Canyon  
Latitude: 35° 52' 4" N  
Evaluator(s): K. See Nishi  
Site ID: Below Confluence  
Longitude: 106° 19' 25" W  
TOTAL POINTS: 18  
Assessment Unit: NM-128-A-15  
Drought Index (12-mo. SPI Value): 0-1

Has there been a heavy rain in the last 48 hours?  
— YES  NO

WEATHER CONDITIONS  
NOW:  
— storm (heavy rain)  
— rain (steady rain)  
— showers (intermittent)  
— cloud cover  
— clear/sunny  
— clear/sunny

PAST 48 HOURS:  
— storm (heavy rain)  
— rain (steady rain)  
— showers (intermittent)  
— cloud cover  
— clear/sunny

\*Field evaluations should be performed at least 48 hours after the last known major rainfall event.  
OTHER:  
Stream Modifications: — YES  NO  
Diversions: — YES  NO  
Discharges: — YES  NO  
\*Explain in further detail in FACIES section.

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Poor
1.1. Water in Channel	Flow is evident throughout the reach. Moving water is seen in riffle areas but may not be as evident throughout the runs. 6	Water is present in the channel but flow is barely perceptible in areas of greatest gradient change (i.e. riffles) or forcing object is necessary to observe flow. 4	Dry channel with standing pools. There is some evidence of base flows (i.e. riparian vegetation growing along channel, saturated moist sediment and/or rocks, etc.) 2	Dry channel. No evidence of base flows was found. 0
1.2. Fish	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Fish are not present. 0
1.3. Benthic Macroinvertebrates	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Macroinvertebrates are not present. 0
1.4. Filamentous Algae/Periphyton	Found easily and consistently throughout the reach. 3	Found with little difficulty but not consistently throughout the reach. 2	Takes 10 or more minutes of extensive searching to find. 1	Filamentous algae and/or periphyton are not present. 0
1.5. Differences in Vegetation	Dramatic compositional differences in vegetation are present between the stream banks and the adjacent uplands. A distinct riparian vegetation corridor exists along the entire reach – riparian, aquatic, or wetland species dominate the length of the reach. 3	A distinct riparian vegetation corridor exists along part of the reach. Riparian vegetation is intermingled with upland vegetation along the length of the reach. 2	Vegetation growing along the reach may occur in present densities or grow more vigorously than vegetation in the adjacent uplands, but there are no dramatic compositional differences between the two. 1	No compositional or density differences in vegetation are present between the streambanks and the adjacent uplands. 0
1.6. Absence of Rooted Upland Plants in Streambed	Rooted upland plants are absent within the streambed throughout the reach. 3	There are a few rooted upland plants present within the streambed throughout the reach. 2	Rooted upland plants are consistently dispersed throughout the streambed throughout the reach. 1	Rooted upland plants are prevalent within the streambed throughout the reach. 0
SUBTOTAL (#1.1 – #1.6) 11				
If the stream being evaluated has a subtotal ≤ 2 at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal ≥ 18 at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 2 and 18 continue the Level 1 Evaluation.				

LEVEL 1 INDICATORS	STREAM CONDITION			
	Strong	Moderate	Weak	Poor
1.7. Sinuosity	Ratio > 1.4. Stream has numerous, closely spaced bends. See straight sections. 3	Ratio < 1.4. Stream has good sinuosity with some straight sections. 2	Ratio < 1.4. Stream has very few bends and mostly straight sections. 1	Ratio = 1.0. Stream is completely straight with no bends. 0
1.8. Floodplain and Channel Dimensions 6.07	Ratio > 2.5. Stream is minimally confined with a wide, active floodplain. 3	Ratio between 1.2 and 2.5. Stream is moderately confined. Floodplain is present, but may only be active during larger floods. 1.5	Ratio < 1.2. Stream is incised with a not clearly confined channel. Floodplain is narrow or absent and typically unaccessed throughout the year. 0	Ratio = 1.2. Stream is incised with a not clearly confined channel. Floodplain is narrow or absent and typically unaccessed throughout the year. 0
1.9. In-Channel Structure: Riffle-Pool Sequence	Demarcated by a frequent number of riffles followed by pools along the entire reach. There is an obvious transition between riffles and pools. 3	Represented by a less frequent number of riffles and pools. Distinguishing the transition between riffles and pools is difficult. 2	Stream shows some flow but mostly has areas of pools (i.e. riffles). 1	There is no sequence exhibited. 0
SUBTOTAL (#1.1 – #1.9) 12-15				
If the stream being evaluated has a subtotal ≤ 5 at this juncture, the stream is determined to be EPHEMERAL. If the stream being evaluated has a subtotal ≥ 21 at this point, the stream is determined to be PERENNIAL. YOU MAY STOP THE EVALUATION AT THIS POINT. If the stream has a subtotal between 5 and 21 continue the Level 1 Evaluation.				
1.10. Particle Size or Stream Substrate Sorting	Particle sizes in the channel are noticeably different from particle sizes in areas close to but not in the channel. There is a clear distribution of various sized substrates in the stream channel with finer particles accumulating in the pools, and larger particles accumulating in the riffles. 3	Particle sizes in the channel are moderately similar to particle sizes in areas close to but not in the channel. Various sized substrates are present in the stream channel and are represented by a higher ratio of larger particles (gravel/cobbles). 1.5	Particle sizes in the channel are similar or comparable to particle sizes in areas close to but not in the channel. Substrate sorting is not readily observed in the stream channel. 0	
1.11. Hydric Soils	Hydric soils are found within the study reach. Present = 3		Hydric soils are not found within the study reach. Absent = 0	
1.12. Sediment on Plants and Debris	Sediment found readily on plants and debris within the stream channel, and within the floodplain throughout the length of the stream. 1.5	Sediment found on plants or debris within the stream channel although it is not prevalent along the stream. Mostly accumulating in pools. 1	Sediment is isolated in small amounts along the stream. 0.5	No sediment is present on plants or debris. 0
TOTAL POINTS (#1.1 – #1.12) 13-15 16.5				
SUPPLEMENTAL INDICATORS: The following indicators do not occur consistently throughout New Mexico but may be useful in the determination of perennality. If the indicator is present record scores below and tally with previous score to compute TOTAL.				
1.13. Seeps and Springs	Seeps and springs are found within the study reach. Present = 1.5		Seeps and springs are not found within the study reach. Absent = 0	
1.14. Iron Oxidizing Bacteria/Fungi	Iron oxidizing bacteria and/or fungi are found within the study reach. Present = 1.5		Non-oxidizing bacteria and/or fungi are not found within the study reach. Absent = 0	
TOTAL SUPPLEMENTAL POINTS (#1.1 – #1.14) 18				

# HP Level 1 – Two Mile Canyon – below Upper Confluence

## LEVEL 1 Field Measurements

### Pebble Count Tally Sheet

Site Name: \_\_\_\_\_ Store ID: \_\_\_\_\_  
 Date: \_\_\_\_\_ Crew: \_\_\_\_\_

Substrate Type	Diameter Range	In-Channel COUNT	In-Channel % Composition	Out of Channel COUNT	Out of Channel % Composition
Silt/Clay	< 0.06 mm		N/A Bmt 9/17/11		
Sand	0.06 – 2.0 mm (gritty)				
Gravel	2.0 – 64 mm				
Cobble	64 – 256				
Boulder	> 256 mm				
Bedrock	—				

\*Please be sure to measure at least 50 pebbles (10 in 5 transects or 5 in 10 transects- depending on stream size) for accurate distributional representation\*\*

### INDICATOR #1.8 (Floodplain and Channel Dimensions) – MEASUREMENTS & CALCULATIONS\*\*

Max Depth (#1)	Bankfull Stage (#2)	Maximum Depth Value (#3)	2x Maximum Depth Value (#3)	Flood-Prone Area Location (#4)	Flood-Prone Area Width (#5)	Bankfull Width (#6)	Floodplain to Active Channel Ratio (FPA:VADR) (Bankfull Width)
23.87'	23.80'	.07'	.14'	23.73'	9.10'	8.45'	1.07

\*\*REFER to Figure 3 on page 19 for clarification

## NMED Surface Water Quality Bureau – LEVEL 1 Hydrology Determination Field Sheet

### Photo Descriptions and NOTES

Photo #	Description (US, DS, LB, RB, etc.)	Notes
1	Upstream	
2	Downstream	
3	Right Bank	
4	Left Bank	
5	Overall Channel	Downstream
6	Bottom Macroinvertebrates	

### NOTES:

Evaluators: Jenifer Fuller, Sam Loftis, Robert Gellies

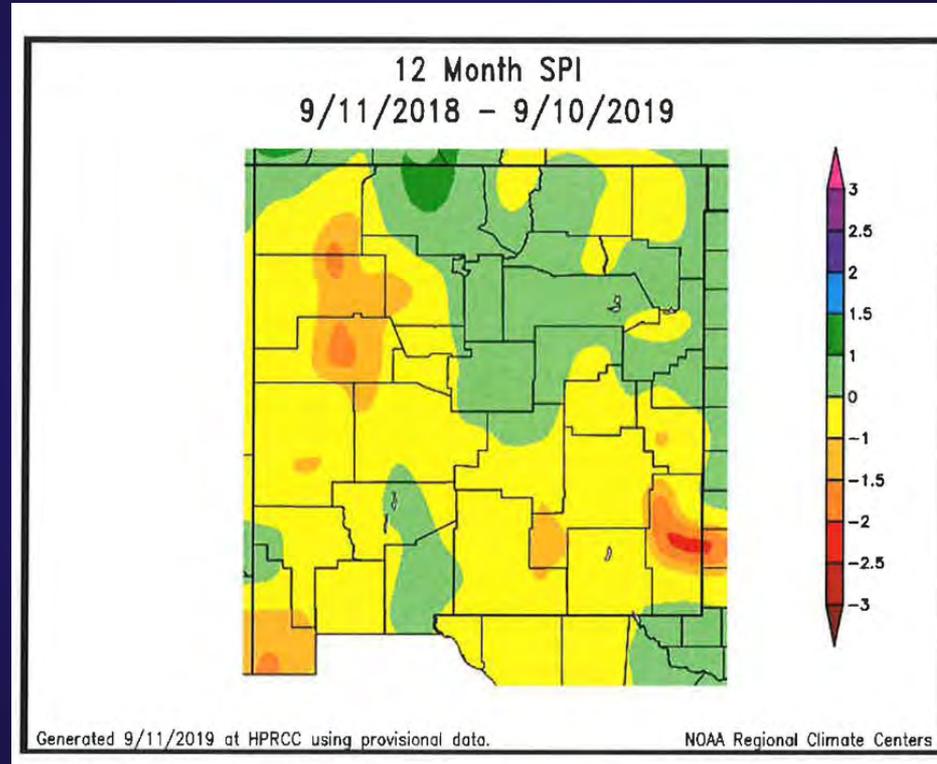
TA-6 San Geronimo recorded .06" on 9/10 and .05" on 9/11.

1.7 - Stream is inhibited by narrow canyon

1.10 - narrow channel - no difference between channel / floodplain

Vegetation present - chukchuck, grasses, forsythia, red top

# HP Level 1 – Standard Precipitation Index



# HP 1 Level – Rainfall Amounts

Data is for tower ta6.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:53:26 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0.06
9	11	2019	254	0.03

Data is for tower ta49.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:55:52 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0
9	11	2019	254	0

Data is for tower ta53.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:56:32 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0
9	11	2019	254	0.1

Data is for tower ta54.

This file was obtained from the LANL Weather Machine, <http://weather.lanl.gov>.

Request made on Thu Sep 12 06:57:11 2019 MST.

All data times are MST.

month	day	year	doy	tprecip
mm	dd	yyyy	ddd	in
9	9	2019	252	0
9	10	2019	253	0.06
9	11	2019	254	0

# HP Level 1 – Two Mile Canyon – below Upper Confluence - Upstream



# HP Level 1 – Two Mile Canyon – below Upper Confluence - Downstream



# HP Level 1 – Two Mile Canyon – below Upper Confluence – Right Bank



# HP Level 1 – Two Mile Canyon – below Upper Confluence – Left Bank



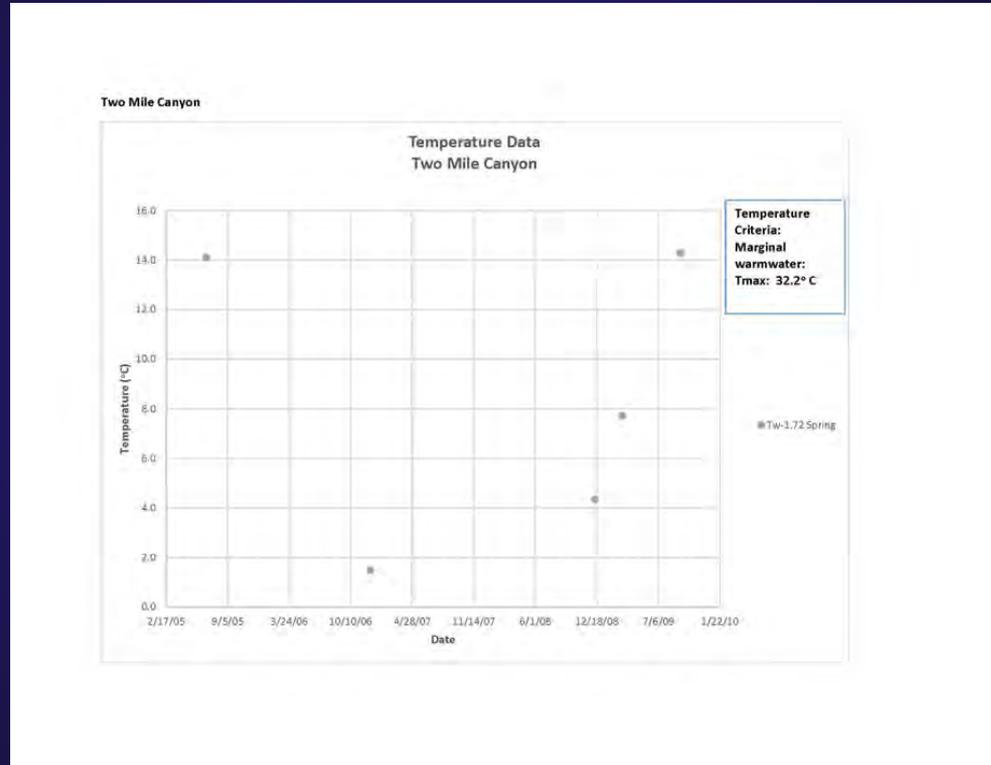
# HP Level 1 – Two Mile Canyon – below Upper Confluence - Downstream



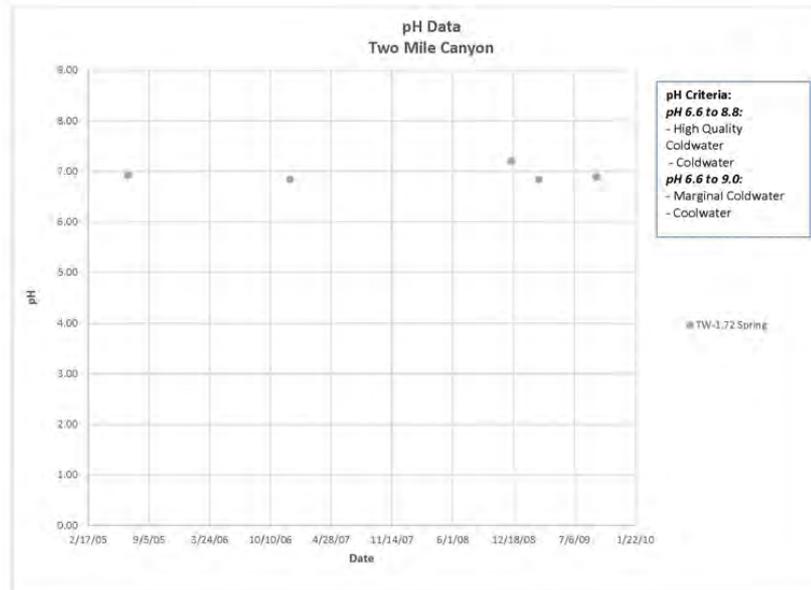
# HP Level 1 – Two Mile Canyon – below Upper Confluence - Benthics



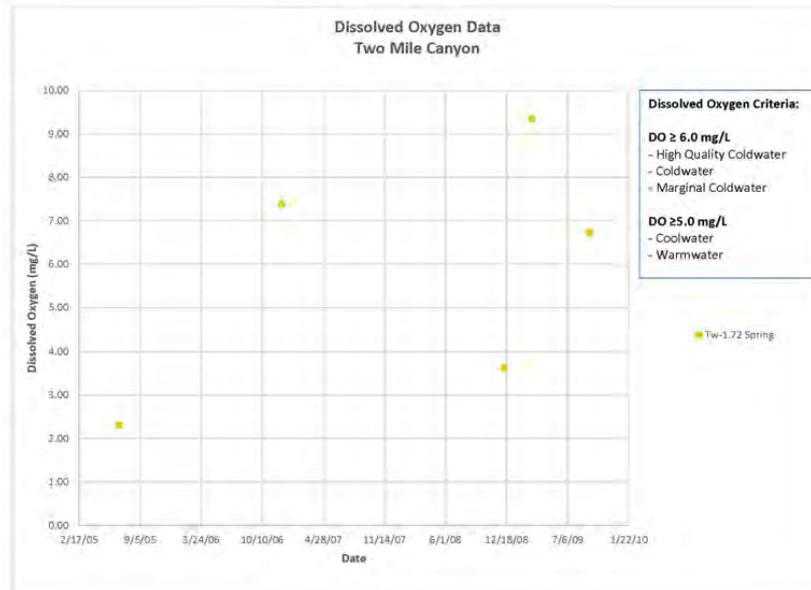
# Temperature – TW-1.72 – Below Confluence



# pH – TW-1.72 – Below Confluence



# DO – TW-1.72 – Below Confluence



# HP Level 2: Two Mile below Upper Confluence

**NMED Surface Water Quality Bureau –  
LEVEL 2 Hydrology Determination Field Sheet  
\*\*Borderline Cases\*\***

Date: 12/08/19 1:04 PM		Stream Name: Two Mile Canyon	Latitude: 35°52'41"
Evaluator(s): Shannon G. Wood Dana Thompson		Site ID: B/LW (affluent to 128)	Longitude: -106°19'25"
LEVEL 1 Total Points: 18		Reach Description: All wet	Drought Index (12-mo. SPI Value): 0-1

<b>WEATHER CONDITIONS</b>	<b>NOW:</b> <input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input type="checkbox"/> % cloud cover <input type="checkbox"/> clear/sunny	<b>PAST 48 HOURS:</b> <input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input type="checkbox"/> % cloud cover <input checked="" type="checkbox"/> clear/sunny	Has there been a heavy rain in the last 48 hours? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO <small>**Field evaluations should be performed at least 48 hours after the last known major rainfall event.</small> <b>OTHER:</b> Stream Modifications <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO Diversions <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO Discharges <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO <small>**Explain in further detail in NOTES section</small>
---------------------------	--	---	---

CHECK the appropriate rating for each indicator.

LEVEL 2 INDICATORS	Stream Condition			
	Strong	Moderate	Weak	Poor
2.1 Water in Channel (OPTIONAL)	←		NA	→
2.2 Hyporheic Zone/Groundwater Table	X			
2.3 Bivalves	Present =		Absent =	
2.4 Amphibians	Present =		Absent =	X
2.5 Macroinvertebrates (abundance/diversity)**				
2.6 EPT Taxa**	Present =		Absent =	
2.7 Fish				X

\*\* Macroinvertebrates and EPT Taxa should not be rated until identification and enumeration has been performed in a laboratory setting by a qualified aquatic biologist/environmental scientist.

Photo #	Description (us, ca, la, r#)	Notes
128-2565	Downstream point	looking upstream
128-2566	midway point	looking downstream
128-2567	mid reach point	looking upstream
128-2568	upstream point	looking downstream

**NOTES:** (use back-side of this form for additional notes)

from center point (coordinates listed above) went 20m downstream, started downstream and collected benthic macroinvertebrates at 9 equidistant (18 m apart) transects. PMI parameters to be filled in after results are received from Ecolabysis.

# Summary of Benthic Data

Summary of Benthic Data for Proposed Waters 1			
Segment	Level 1-2 Locations and Scores	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absence)
Pajarito above Starmers Site 1	Pa-14	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	Pc-24	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	Weak	Present
Two Mile Canyon below Confluence	Tf-18	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	Moderate	Present
Two Mile Canyon at TA-55 Confluence	Th-19	-	-
Two Mile above E244	Ti-10.5	-	-
<sup>1</sup> . bivalves present			

# Summary of Benthic Data (cont.)

Summary of Benthic Data for Proposed Waters 2									
Segment	Level 1-2 Locations	Benthic Macroinvertebrates	Total Species Taxa Richness	EPT Taxa Richness	% EPT	Intolerant Taxa Richness	Long Lived Taxa Richness	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absent)
Pajarito above Starmers Site 1	Pa-14	N/A	N/A	N/A	N/A	N/A	N/A	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	1863.1	35.00	5.0	8.8	1.0	1.0	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	PC-24	2036.0	40.00	7.0	40.7	2.0	0.0	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	4136.0	36.00	4.0	7.5	0.0	3.0	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	1431.8	30.00	1.0	3.3	0.0	3.0	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	148.0	13.00	2.0	87.8	0.0	0.0	Weak	Present
Two Mile Canyon below Confluence	Tf-18	132.0	23.00	1.0	0.8	1.0	0.0	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	793.5	37.00	5.0	31.0	0.0	1.0	Moderate	Present
Two Mile Canyon at TA-55 Confluence	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-	-
Two Mile above E244	Ti-10.5	Not Collected	N/A	N/A	N/A	N/A	N/A	-	-

1. bivalves present

## HP 2 – Two Mile below Upper Confluence



## HP 2 – Two Mile below Upper Confluence



## HP 2 – Two Mile below Upper Confluence



## HP 2 – Two Mile below Upper Confluence



# Exhibit 43

## Arroyo de la Delfe

Segment	Triennial Review Change	HP Assessment Site	Map Legend	NMED Present	Level I Overall Score	Water in Channel	Macroinvertebrates Level I	Level II	Hyphoreic Zone Value	Macroinvertebrates (abundance/diversity)/EPT Taxa	Gage	Gage Data pH/DO/Temperature Average (Range)	Spring	Spring Data pH/DO/Temperature Average (Range)
From Pajarito Canyon upstream to Kieling Spring	From 20.6.2.128 NMAC to 20.6.2.126 NMAC	Upstream from Kieling Spring	ADb-4	Yes	N/A	0	0	N/A	N/A	N/A	E242.5 (Inactive) -Dates Active: 2003 to 2009  -Average % days of flow: 90.5 %	pH: 7.1 (6.4 to 8.5)  DO: 6.5  Temp: 11.9 (10.2 to 13.5)	Kieling Spring	Kieling Spring: pH: 6.9 (6.1 to 7.7) DO: 8.5 (5.0 to 13.5) Temp: 10.4 (8.6 to 12.8)
		Downstream of Bulldog Stream	ADc-25	Yes	25	6	3	Yes	Strong	Strong/Present			Bulldog Spring	Bulldog Spring: pH: 7.3 (5.4 to 8.0) DO: 8.6 (5.8 to 14.1) Temp: 10.3 (6.7 to 14.2)

## Pajarito Canyon

Segment	Triennial Review Change	HP Assessment Site	Map Legend	NMED Present	Level I Overall Score	Water in Channel	Macroinvertebrates Level I	Level II	Hyphoreic Zone Value	Macroinvertebrates (abundance/diversity)/EPT Taxa	Gage	Gage Data pH/DO/Temperature Average (Range)	Spring	Spring Data pH/DO/Temperature Average (Range)
Upper Section – From existing perennial reach upstream to Homestead Spring	From 20.6.2.128 NMAC to 20.6.2.126 NMAC	Pajarito above Starmers Site 1	Pa-14	Yes	14	1	0	Yes	Poor	Not Collected	E241 (Inactive) -Dates Active: 2003 – 2009  - Average % days of flow: 73.0 %	pH: 7.1 (6.4 to 8.2)  DO: 8.6  Temp: 14.7 (13.2 to 16.2)	Homestead Spring	pH: 6.4 (5.7 – 8.0)  DO: 6.7 (3.3 to 10.6)  Temp: 10.5 (8.0 to 16.7)
		Pajarito above Starmers Site 2	Pb-28	Yes	28	6	2	Yes	Moderate	Strong/Present				

## Pajarito Canyon

Segment	Triennial Review Change	HP Assessment Site	Map Legend	NMED Present	Level I Overall Score	Water in Channel	Macroinvertebrates Level I	Level II	Hyphoreic Zone Value	Macroinvertebrates (abundance/diversity)/EPT Taxa	Gage	Gage Data pH/DO/Temperature Average (Range)	Spring	Spring Data pH/DO/Temperature Average (Range)
Lower Section - 0.5 Miles below Arroyo de La Delfe (PI-14.5) upstream to existing perennial reach	From 20.6.2.128 NMAC to 20.6.2.126 NMAC	Pajarito above Two Mile Site 1	Pc-24	Yes	24	6	3	Yes	Strong	Strong/Present	N/A	N/A	N/A	N/A
		Pajarito Below Deos	PI-14.5	No	14.5	2	0	--	--	--				

## Effluent Canyon

Segment	Triennial Review Change	HP Assessment Site	Map Legend	NMED Present	Level I Overall Score	Water in Channel	Macroinvertebrates Level I	Level II	Hyphoreic Zone Value	Macroinvertebrates (abundance/diversity)/EPT Taxa	Gage	Gage Data pH/DO/Temperature Average (Range)	Spring	Spring Data pH/DO/Temperature Average (Range)
Mortandad Canyon confluence upstream to Effluent Canyon headwaters	From 20.6.2.128 NMAC to 20.6.2.140 NMAC	Below Outfall 051	Ea-15	Y	16.5	2	1	Yes	Poor	Strong/Present	N/A	N/A	N/A	N/A

## Two Mile Canyon

Segment	Triennial Review Change	HP Assessment Site	Map Legend	NMED Present	Level I Overall Score	Water in Channel	Macroinvertebrates Level I	Level II	Hyporheic Zone Value	Macroinvertebrates (abundance/diversity)/ EPT Taxa	Gage	Gage Data pH/DO/Temperature Average (Range)	Spring	Spring Data pH/DO/Temperature Average (Range)
From LANL Stream Gage E244 upstream to its confluence with upper Two Mile Canyon	From 20.6.2.128 NMAC to 20.6.2.140 NMAC	Below Confluence	Tf-18	Y	18	4	2	Yes	Strong	Strong/Present	E244 -Dates Active: Historical – 2005 to 2011 Recent – 2015 to 2019  - Average % days of flow: Historical – 37.8% Recent – 21.6 %	pH: No data  DO: No data  Temp: No data	TW-1.72	pH:6.9 (6.8 – 7.2)  DO: 5.9 (2.3 to 9.4)  Temp: 8.4 (1.5 to 14.3)
		Below TA-59	Tg-20.5	Y	20.5	4	2	Yes	Strong	Strong/Present				
		TA-55 Confluence	Th-19	Y	19	4	2	No	N/A	N/A				
		Above E244	Ti-10.5	Y	10.5	0	0	Yes	Poor	Poor/Absent				

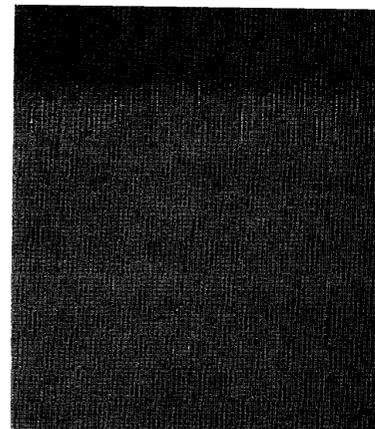
## S-Site Canyon / Martin Spring

Segment	Triennial Review Change	HP Assessment Site	Map Legend	NMED Present	Level I Overall Score	Water in Channel	Macroinvertebrates Level I	Level II	Hyporheic Zone Value	Macroinvertebrates (abundance/diversity)/ EPT Taxa	Gage	Gage Data pH/DO/Temperature Average (Range)	Spring	Spring Data pH/DO/Temperature Average (Range)
From Alluvial Groundwater Well MSC 16-06293 upstream to Martin Spring	From 20.6.2.128 NMAC to 20.6.2.140 NMAC	Below Martin Springs	MSa-16	Yes	16	2	2	Yes	Moderate	Strong/Present	N/A	N/A	Martin Spring	pH: 7.0 (5.7 to 7.8)  DO: 7.5 (6.0 to 9.4)  Temp: 11.6 (7.4 to 18.7)
		Martin Springs Canyon 3 at MSC16-06293	MSb-8	Yes	8	1	0	No	N/A	N/A				

# Exhibit 44

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# Streamflow Characteristics Related to Channel Geometry of Streams in Western United States



United States  
Geological  
Survey  
Water-Supply  
Paper 2193

Prepared in cooperation  
with the U.S. Bureau  
of Land Management



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# Streamflow Characteristics Related to Channel Geometry of Streams in Western United States

By E. R. Hedman and W. R. Osterkamp

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U.S. GEOLOGICAL SURVEY WATER-SUPPLY PAPER 2193

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# Streamflow Characteristics Related To Channel Geometry of Streams in Western United States

By E. R. Hedman *and* W. R. Osterkamp

## Abstract

Assessment of surface-mining and reclamation activities generally requires extensive hydrologic data. Adequate streamflow data from instrumented gaging stations rarely are available, and estimates of surface-water discharge based on rainfall-runoff models, drainage area, and basin characteristics sometimes have proven unreliable. Channel-geometry measurements offer an alternative method of quickly and inexpensively estimating stream-flow characteristics for ungaged streams. The method uses the empirical development of equations to yield a discharge value from channel-geometry and channel-material data. The equations are developed by collecting data at numerous streamflow-gaging sites and statistically relating those data to selected discharge characteristics.

Mean annual runoff and flood discharges with selected recurrence intervals can be estimated for perennial, intermittent, and ephemeral streams. The equations were developed from data collected in the western one-half of the conterminous United States. The effect of the channel-material and runoff characteristics are accounted for with the equations.

## INTRODUCTION

The judicious management of land resources requires knowledge of the hydrologic characteristics of an area and how those characteristics may be modified by various land uses. Recently renewed emphasis on the recovery of coal resources from both public and private lands of the United States has produced an awareness that hydrologic information for most coal-resource areas is inadequate. The problem is acute in some parts of the western United States where the development of coal resources is limited by the availability of water. In these areas, coal-mine development is dependent partly on reasonable assessments of the useable surface-water and ground-water resources, as well as the means to discharge excess (unuseable) water from

the mine area during periods of both normal and peak streamflow. Discharge information, however, may be completely lacking.

For areas where gaged streamflow data are unavailable, various indirect methods have been developed to estimate total runoff and flood-discharge characteristics (flow rates for specified recurrence intervals). Initial attempts were applied largely to humid and subhumid areas. They included the transfer of streamflow records from gaged to adjacent or nearby ungaged basins, and the estimation of runoff from drainage area and precipitation. More recent methods, designed to be more universally applicable than the earlier efforts, relied on numerous basin characteristics and multiple-regression analysis to estimate discharge characteristics (Thomas and Benson, 1970). Further development of these techniques led to various models relating rainfall (precipitation) and runoff. Although some of these newer techniques have worked well for relatively moist areas and some limited areas with arid or semiarid climates, they also have proven to be ineffective for general use in dry regions having complex patterns of topography, vegetation, and hydrology (Riggs, 1978). The sophisticated techniques, such as rainfall-runoff models, potentially can yield realistic estimates of discharge characteristics regardless of climate, but they are hampered by the need for extensive input data. Some of these data can be difficult to collect, and others, such as soil moisture, are variable with time.

An alternative method of indirectly estimating streamflow, using channel geometry as a modification of the hydraulic-geometry concept (Leopold and Maddock, 1953), was reported by Moore (1968) in Nevada and by Hedman (1970), in California. The method has the advantage of being easily applied, and the estimates are based on channel characteristics formed by the water and sediment discharge of a stream. Thus, the size of an alluvial channel is indicative of the water conveyed through that channel, and the shape of the channel is largely the result

of the sediment transported by the stream. The data commonly used to estimate discharge characteristics by this technique, therefore, are measurements of geometry (principally width) and the particle-size distributions of the material forming the channel perimeter.

Results of studies undertaken to develop channel-geometry relations applicable to the western United States are presented in this report. The purposes of the study are: (1) to provide a general description of the channel-geometry technique, (2) to present equations useful for the determination of streamflow characteristics in areas generally lacking hydrologic data, and (3) to extend the technique of active-channel geometry to intermittent and ephemeral streams of semiarid and arid regions.

## CHANNEL-GEOMETRY TECHNIQUE

The basis of all channel-geometry relations is the continuity equation for discharge (water) of a stream:

$$Q_i = WDV, \quad (1)$$

where

$Q_i$  = instantaneous discharge, in cubic feet per second;

$W$  = water-surface width, in feet;

$D$  = mean depth of water, in feet; and

$V$  = mean velocity of water, in feet per second.

Considering numerous stream sites of various flow characteristics, the simplifying assumption is made that the rates of change of  $W$ ,  $D$ , and  $V$  with  $Q_i$  are constant and, therefore, can be expressed by a multiple regression equation. This assumption requires that  $Q_i$  represents the same flow frequency (flow duration or recurrence interval) at all sites considered.

$$Q_i = kW^b D^f V^m, \quad (2)$$

where  $k$  is a coefficient and  $b$ ,  $f$ , and  $m$  are exponents. The multiple regression form of the continuity equation (equation 2) can be expressed as three simple functions:

$$Q_i \sim W^b \quad (3)$$

$$Q_i \sim D^f \quad (4)$$

$$Q_i \sim V^m \quad (5)$$

The practical use of relations 3, 4, and 5 requires that water-surface width, mean water depth, and mean water velocity be measured for the same flow frequency at all sites considered. This requirement cannot be met because: (1) stream stage cannot be related to a flow-duration value if the site is ungaged, and (2) the three parameters cannot be measured at many or most flow durations for intermittent and ephemeral streams.

To avoid the necessity of water-related measurements, therefore, the channel-geometry method relies on measurements obtained from a geomorphic reference feature recognizable at all channel sites. When using the level

of a geomorphic feature as a basis of evaluating flow characteristics, velocity, of course, cannot be measured (relation 5). Mean channel depth generally is measured and related to discharge (relation 4), but variability of channel profiles and the capacity for measurement error commonly lead to unreliable results. Thus, most channel-geometry relations include or are limited to channel-width measurements as an independent variable and yield a specified measure of discharge as the dependent variable. Expanding relation 3 to equation form gives:

$$Q_v = aW^b, \quad (6)$$

where "a" is a coefficient, and  $Q_v$  is a measure of streamflow, such as mean discharge or a flood discharge of specified recurrence interval.

Implicit in equation 6 is the assumption that similar hydraulic and sorting processes produce similar channel features at each site and, therefore, that measurements taken from those features are comparable. Equal widths of perennial- and ephemeral-stream channels may yield similar estimates for the discharge of floods with selected recurrence intervals, but that width might correlate with greatly different values for the mean annual runoff of the two streams. Even though the widths may be the same, the mean annual runoff will be much greater for a perennial stream that flows most of the time than for an ephemeral stream that flows for a short period of time. Thus, separate equations for annual runoff are presented for channels having perennial, intermittent, and ephemeral streamflow. Ephemeral streams are further subdivided into three groups depending on the number of flow days per year.

Difficulty might arise when trying to determine which equations are applicable to an ungaged site. Normally, however, consideration of channel appearance, riparian vegetation, and regional climate dictate which group of equations is appropriate.

Separate equations are provided for the differences in channel shapes that result from variations in the sediment-discharge characteristics. These equations for annual runoff are based on particle sizes of the material forming the channel perimeter.

Channel-geometry relations generally are developed using measurements taken from one of three geomorphic reference points, as shown in figure 1. These points define the depositional-bar level (A-A'), the active-channel level (B-B'), and the bankfull level (C-C'). Where feasible, data for this study were collected from each of the three reference levels in order to ascertain which feature would yield the best estimates of discharge characteristics.

The lowest of the three levels, defined by the surfaces of depositional bars, is described by R. F. Hadley in Hedman, Moore, and Livingston, (1972, p. 4) as a "...longitudinal, in-channel feature formed along the borders of a stream channel at a stage of the flow regime

when the local competence of the stream is incapable of moving the sediment particles on the submerged surface of the bar "(shown as reference level A-A' in figure 2). Experience has shown that the depositional-bar level is a useful feature for channels with a well-graded sediment supply. If the channel material is predominantly sand and fine gravel, however, which is the case for numerous ephemeral and intermittent streams of the western United States, depositional bars may be poorly formed or absent. Hence, the depositional-bar data collected for this study produced inconsistent results and are not provided.

The active-channel level, shown as reference level B-B' of figure 1, was used by Hedman, Kastner, and Hejl (1974) to determine flood-frequency discharge and later described by Osterkamp and Hedman (1977, p. 256) as "...a short-term geomorphic feature subject to change by prevailing discharges. The upper limit is defined by a break in the relatively steep bank slope of the active channel to a more gently sloping surface beyond the channel edge. The break in slope normally coincides with the lower limit of permanent vegetation so that the two features, individually or in combination, define the active channel reference level. The section beneath the reference level is that portion of the stream entrenchment in which the channel is actively, if not totally, sculptured by the normal process of water and sediment discharge. "

At most perennial and intermittent streams the active-channel level is exposed between 75 and 94 percent of the time. The active-channel level of many ephemeral

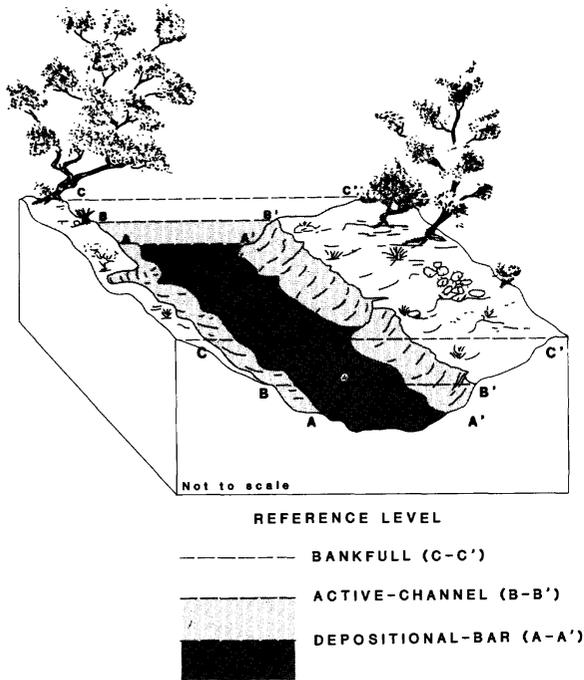


Figure 1. Commonly used reference levels.

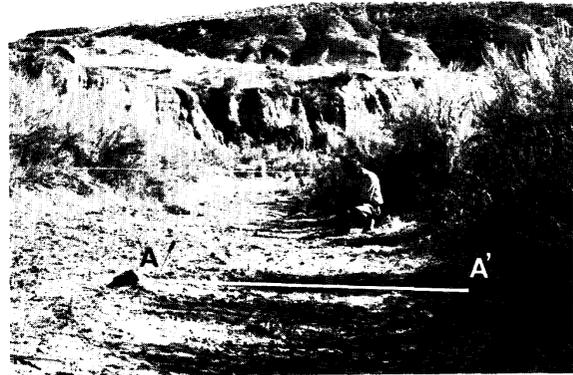


Figure 2. Reference points for bar geometry (A-A') in a reach of an ephemeral-stream channel in Wyoming.

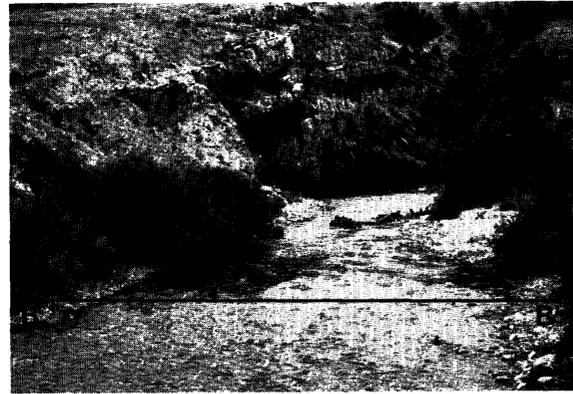


Figure 3. Reference points for active-channel geometry (B-B') in a reach of a perennial-stream channel in Montana.

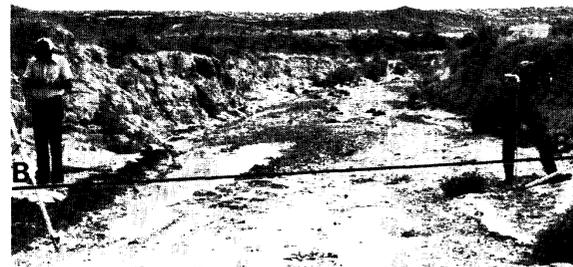


Figure 4. Reference points for active-channel geometry (B-B') in a reach of an ephemeral-stream channel in New Mexico.

streams may be exposed more than 99 percent of the time. The stage corresponding to mean discharge of most perennial streams approximates that of the active-channel level, shown as reference level B-B' in figure 3, but is lower than the active-channel level of the highly ephemeral stream channels, shown as reference level B-B' in figure 4.

The highest reference level at which channel-geometry measurements commonly are made is bankfull stage, as shown by reference level C-C' in figure 5. Bankfull stage is defined as the level of the active flood plain and, therefore, is the stage at which overbank flooding occurs (Wolman, 1955, p. 29). The bankfull level of many perennial-stream channels approximates the stage of a flood with a recurrence interval ranging from 1.5 to 3 years. Thus, the bankfull level of these channels is exceeded a very small percentage of the time. Flow at bankfull stage in ephemeral-stream channels generally is more infrequent than that in perennial or intermittent stream channels.

A disadvantage of the bankfull reference level is that its use requires the recognition of a flood-plain level or a bench, features that are not easily recognized in incised channels as shown in figure 6. In addition, changes in bankfull geometry generally occur much more slowly than do those of the active channel and may not be representative of prevailing conditions of water and sediment discharge. Bankfull-geometry data routinely were collected for this study, but, owing to the difficulties described above, the data did not yield suitable results.

### ACTIVE-CHANNEL GEOMETRY

The geometry of the active channel can be identified and measured at selected sites in virtually all alluvial stream channels although the significance of the measurements at some streams, such as those with a braided channel pattern, may be questioned. Because the active channel is identifiable at almost all channel reaches and because it is indicative of relatively recent conditions of water and sediment discharge, relations presented in this paper are based on active-channel geometry.

### Collection and Compilation of Data

Channel surveys were made at continuous-record streamflow-gaging stations with relatively stable channels where channel-geometry reference levels could be identified and where streamflow records provided good estimates of streamflow characteristics. The width and the average depth were measured in feet.

At most sites, samples of bed and bank material were collected from the perimeter of the active channel. Three composite samples were collected, one from portions of



Figure 5. Reference points for bankfull level (C-C') in a reach of an ephemeral-stream channel in New Mexico.



Figure 6. An incised channel in southeastern New Mexico with well-defined active-channel reference points (B-B') but lacking a defined bankfull level.

material taken at equal intervals across the channel bed and one each taken at intervals up each bank to the reference point.

Streamflow data for this study were tabulated from published records for various gaging stations operated by the U.S. Geological Survey. Values of mean annual runoff for gaged sites are based on the most recent 10 years of streamflow records; flood discharges of specified recurrence interval necessarily were calculated from longer term records. For mean annual runoff in particular, records for the past 10 years can produce results quite different than are obtained from long-term records owing to changes in land- and water-use practices or variation in precipitation patterns. As an example, figure 7 illustrates changes in runoff during 67 years (represented by 38 years of runoff records) for Rio San Jose at Grants, New Mexico. The records show that the mean annual runoff during the 1970 through 1979 water years is less than 4 percent of the

mean annual runoff for the long-term (38 years) period of record. The channel width at the Grants site presently reflects recent runoff rates rather than the longer term average (fig. 7).

In the initial regression analyses, a digital computer was used to relate the mean annual runoff and each of the flood-frequency discharges to the channel characteristics. Only the active-channel width provided useable relations, so the data were grouped according to channel-material, and regional-runoff characteristics (whether streamflow is perennial, intermittent, or ephemeral), and reanalyzed. The data were analyzed using a program developed by the University of California School of Medicine (Dixon, 1965). The program provides linear-regression equations with statistical summaries and residuals for the individual input values. The equations for flood discharges provide estimates of discharge rates in cubic feet per second. This unit loses meaning, however, when applied to mean discharges of intermittent and ephemeral streams. Thus, mean annual runoff is used and expressed in acre-feet per year, the depth (in feet) to which the average annual dis-

charge of a stream would cover an area of 1 acre. The coefficients and exponents of some equations were adjusted slightly to provide simplicity and ordered variation among the equations.

### Advantages and Disadvantages

Equations for estimating discharge by the channel-geometry technique are defined by data collected from numerous gaged stream sites. The accuracy of the method, therefore, varies with the overall accuracy of the records from which the equations are computed. Both precision and accuracy also depend on the type of stream measured, the discharge parameter being estimated, the regional conditions of climate, geology, and topography, and the experience of the person collecting and applying the data.

Wahl (1977) reported on a test that was made in northern Wyoming to determine how consistently individuals could measure channel geometry for the three different reference levels. Seven participants independently

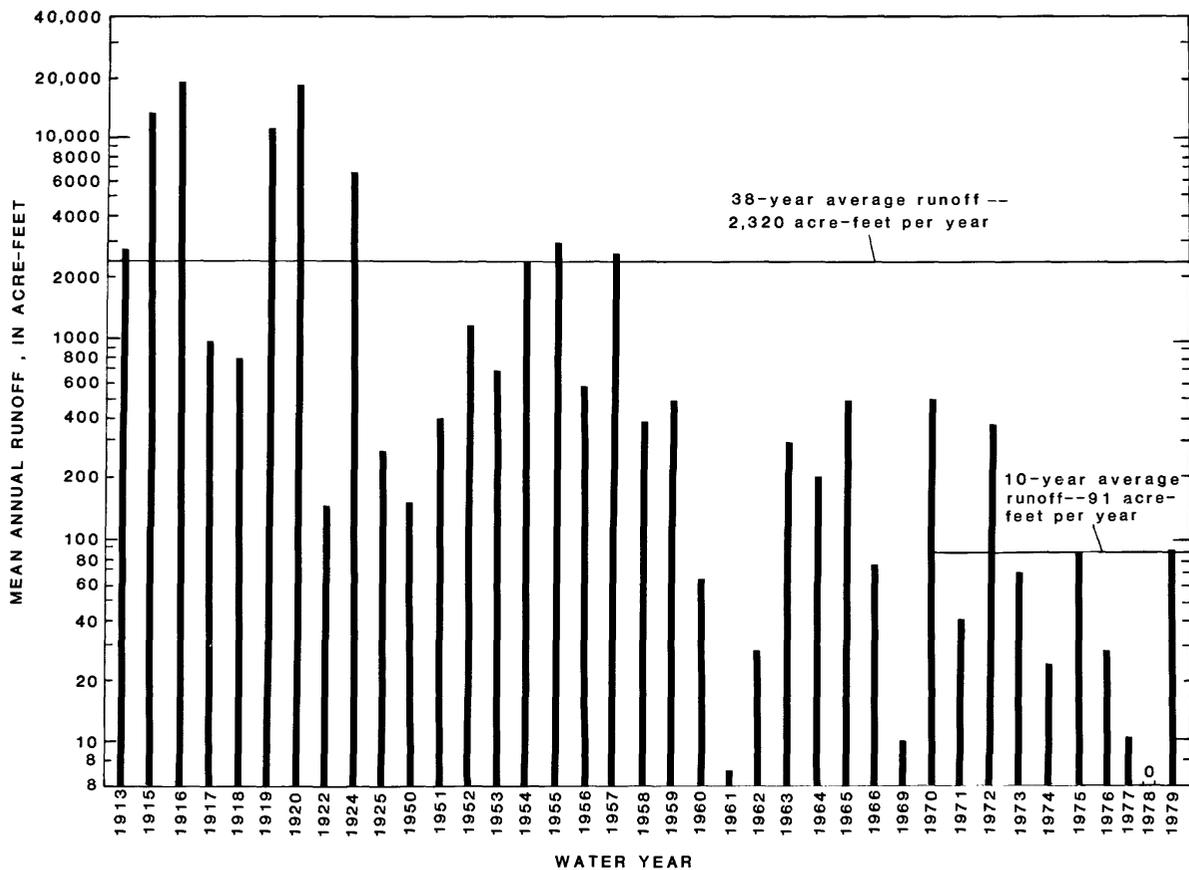


Figure 7. Changes in mean annual runoff, Rio San Jose at Grants, New Mexico.

visited 22 sites and measured the geometry in sections of their choosing. An average standard error for discharge of about 30 percent was attributed to differences in width measurements alone.

The method, as indicated by standard errors of estimate and other statistical measures, is most accurate when applied to perennial streams with stable banks. Examples are upland streams with coarse material (armor) protecting the bed and banks from erosion, and valley streams with well-vegetated banks formed largely of cohesive silt and clay. Conversely, the use of channel geometry probably is least accurate when applied to streams of flashy or erratic discharge (including ephemeral streams) that have sandy, noncohesive banks, and lack of well-developed growth of riparian vegetation.

## TYPES AND GROUPINGS OF DATA

The data upon which this study is based are listed in table 1. Each data set is identified by the number and name of the streamflow-gaging station as assigned by the U.S. Geological Survey; the location of each station is indicated in figure 8. Data shown for each station generally include active-channel width, active-channel depth, and sediment characteristics of the channel bed and banks. Discharge characteristics were obtained from the streamflow- and basin-characteristics file of the U.S. Geological Survey, and channel gradients were computed from topographic maps.

All relations provided herein express mean annual runoff ( $Q_A$ ) or a flood discharge for a specified recurrence interval ( $Q_n$ ) as the dependent variable. A 5-year flood discharge ( $Q_5$ ), for example, is the discharge rate which is expected to be equaled or exceeded an average of once every 5 years or has a 20-percent chance of occurring during 1 year. The independent variable for the relations is active-channel width ( $W_{AC}$ ). All other data provided in table 1 were used in the original multiple-regression analyses and in the final analyses either to classify and group the width-discharge data or to evaluate the reliability of the data.

Owing to the purposes of this study, most data (table 1) pertain to channel sites in arid to semiarid parts of the western United States. Most of the gage sites are located on channels of ephemeral or intermittent streams. Some perennial-stream data are included in table 1, but most of these data were collected in mountainous and other upland areas where snowmelt and relatively large precipitation rates sustain perennial streamflow in an otherwise water-deficient region. The data of table 1, therefore, are used primarily to define width-discharge relations for channels of ephemeral and intermittent streams.

The width of an alluvial stream channel is a function of the geology and climate of the basin that the channel drains. Because the geologic and climatic conditions of the

western United States have wide ranges, the relations of active-channel width with a variable of discharge ( $Q_2$ , for example) likewise show large ranges. To permit reasonable estimates of discharges from width, therefore, it is necessary to group data according to the characteristics of climate and geology. The groupings of data for this study rely on differences of flow frequency, channel-material characteristics, and runoff characteristics as reflected in potential evapotranspiration.

## Grouping by Flow Frequency

All channel data for this study (table 1) were separated into groups representing perennial, intermittent, or ephemeral streamflow to define annual runoff. Ephemeral streamflow was further subdivided depending on number of flow days per year. Although the percentage of days that streamflow occurs in these channels was a major criterion for grouping, the terms perennial, intermittent, and ephemeral, when related to streamflow, are qualitative and cannot be applied precisely. For the purposes of classifying streamflow, the following definitions (modified from Meinzer, 1923, p. 57-58) were used:

*A perennial stream*—or stream reach, has measurable surface discharge more than 80 percent of the time. Discharge is at times partly to totally the result of springflow or ground-water seepage because the streambed is lower than surrounding ground-water levels.

*An intermittent stream*—or stream reach, has surface discharge generally between 10 and 80 percent of the time. Because an intermittent-stream channel is at or near the water-table surface, discharge can be the result of a discontinuous supply from springs or ground-water seepage, a discontinuous supply from surface sources, including runoff of rainfall and seasonal snowmelt, or both. If a channel has sustained periods of no streamflow interrupted by a seasonal period of continuous streamflow, at least 1 month in length, the stream or streams is intermittent.

*An ephemeral stream*—or stream reach, is one that flows only in direct response to precipitation; measurable discharge generally occurs less than 10 percent of the time. It receives no long-continued supply from melting snow or other surface sources. Because an ephemeral-stream channel is at all times above the water table, it also receives no water from springs or sustained ground-water seepage.

The data sets of table 1 were divided according to the above definitions. The channels of the ephemeral group were divided further into groups having discharge approximately 6 to 9 percent of the time, 2 to 5 percent of the time, and 1 percent of the time or less. Relations between active-channel width and mean discharge were developed for each group. Channels with steady, perennial discharge are shaped by limited discharge ranges, and commonly are narrow relative to mean discharge. An ephemeral stream

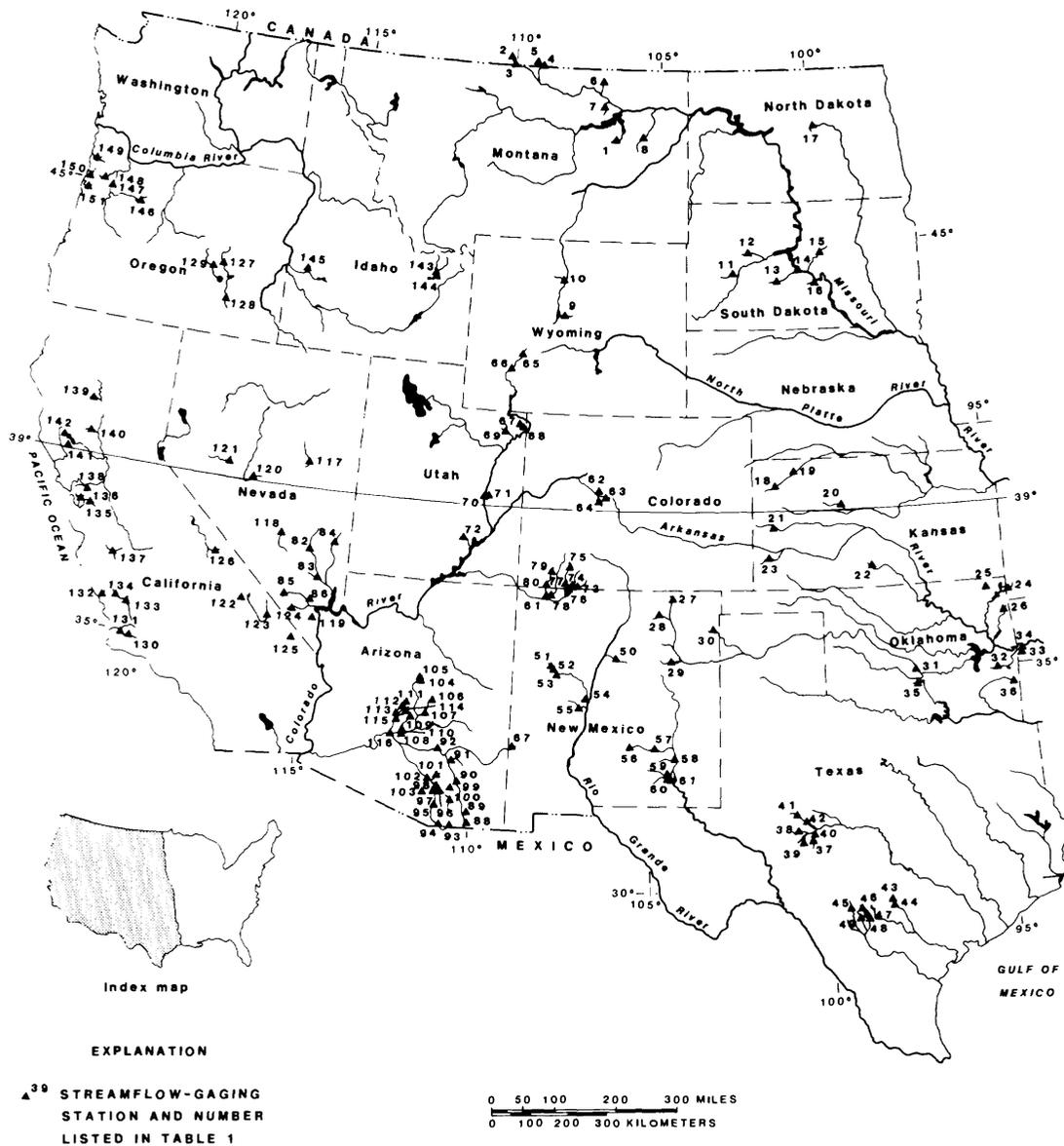


Figure 8. Location of measurement sites.

channel with discharge occurring less than 1 percent of the time, however, is shaped by flow that might occur less frequently than once per year. Owing to the extended no-flow periods, the mean discharge of these channels is very small, and channel widths generally are large relative to mean discharge. The infre-

quent discharges, however, help shape channels regardless of whether a channel has perennial, intermittent, or ephemeral streamflow. Therefore, it was not necessary to develop separate relations between channel width and flood discharges for the flow-frequency groups.

**Table 1. Channel and streamflow characteristics at selected gaging stations.**

WAC, WIDTH OF ACTIVE CHANNEL, IN FEET; DAC, DEPTH OF ACTIVE CHANNEL, IN FEET; BWS, BED SILT-CLAY, IN PERCENT; D50, MEDIAN PARTICLE SIZE OF BED MATERIAL, IN MILLIMETERS; BSH, BANK SILT-CLAY-HIGH, IN PERCENT; NF, NO-FLOW DAYS, IN PERCENT; RL, RECORD LENGTH, IN WATER YEARS; DA, DRAINAGE AREA, IN SQUARE MILES; GRA, CHANNEL GRADIENT, IN FEET PER FOOT.

MAP NO	STATION NO	STATION NAME	WAC	DAC	BWS	D50	BSH	NF	RL	DA	GRA
1	06131000	BTG DRY CR NR VAN NORMAN, MT	91	1.70	6	1.0	44	21	34	7554	.00230
2	06135500	SAGE CR AT O RANCH NR WILD HORSE, ALBERTA	9.0	1.58	14	.78	60	76	44	175	
3	06136000	SAGE CR AT INTERNAT BOUNDARY	15	1.26	57	.05	61	74	33	270	
4	06150500	F FK RATTLE CR NR INTERNAT BOUNDARY	15	2.86	6	3.5	60	90	50	89.5	
5	06151000	LYONS CR AT INTERNAT BOUNDARY	16	1.61	24	.32	54	89	52	66.7	
6	06170200	WILLOW CR NR HINSDALE, MT	37	3.05	41	.08	68	68	8	283	.00075
7	06174000	WILLOW CR NR GLASCO, MT	20	1.98	59	.04	76	56	24	538	.00060
8	06177500	REDWATER R AT CIRCLE, MT	9.4	2.60	27	.14	68	29	39	547	.00089
9	06256900	DRY CR NR BONNEVILLE, WY	14	.79				73	11	52.6	.00810
10	06268500	FIFTEEN MILE CR NR WYLAND, WY	28	2.49				70	21	518	.00260
11	06425500	ELK CR NR FLM SPRINGS, SD	34	.85	89	.06	6	45	28	540	.00160
12	06439000	CHEPBY CR NR PLAINVIEW, SD	44		6	5.8	83	65	32	1196	.00240
13	06441000	BAD K AT MIDLAND, SD	42	2.56	3	7.4	94	51	32	1460	.00120
14	06441500	BAD K NR FORT PIERRE, SD	63	2.94				49	48	3107	.00097
15	06442000	MEDICINE KNOLL CR NR BLUNT, SD	14	.89	7	2.5	8	82	27	317	.00247
16	06442500	MEDICINE CR AT KENNEDY, SD	20	1.94	27	.12	56	77	23	465	.00044
17	06467600	JAMES R NR WAREHO, ND	6.0	.65	40	100	30	60	14	253	.00047
18	06444700	S FK SAPPA CR NR BROWSTER, KS	28	1.35				98	11	74.0	.00098
19	06444900	S FK SAPPA CR NR ACHILLEFS, KS	19	2.28				69	14	446	.00120
20	06463900	N FK BIG CR NR VICTORIA, KS	12	1.80	26	2.6	97	63	16	54	.00120
21	07138650	WHITEWATER CR NR LEDIT, KS	26	.98	37	.54	86	98	12	750	.00130
22	07144850	S FK S FK MINNESOTA R NR PRATT, KS	44	1.44	55	.05	58	93	17	21	.00190
23	07156220	BFAR CR NR JOHNSON, KS	51	1.03	43	.08	69	97	12	835	.00180
24	07188500	LOST CR AT SENECA, MO	31	1.79	3	25	39	0	11	42	.00210
25	07190600	HIG CAHIN CR NR PYRAMID CORNERS, OK	23	2.07	17	2.0	36	25	9	71.1	.00077
26	07196000	FLINT CR NR KANSAS, OK	72	1.95	0	16	30	0	19	110	.00180
27	07199000	CANADIAN R NR HEBRON, NM	71			.20		6	31	229	.00540
28	07207000	CHARRON R NR CHARRON, NM	17	1.19		50		0	27	294	.01410
29	07222500	CONCHAS R NR VARIADERO, NM	62	1.85		.20		28	41	523	
30	07227200	TRAMPFROS CR NR STEAD, NM	76	1.50				72	11	556	.00240
31	07229300	WALNUT CR AT PURCELL, OK	77	2.37	0	.34	78	0	10	202	.00100
32	07247500	FOURCH MALINE NR RED OAK, OK	35				78	2	37	122	
33	07249400	JAMES FK NR HACKETT, AR	58	5.00			76	2	19	147	.00057
34	07249500	COVE CR NR DEF CR, AR	45	1.99		50	15	4	20	35.3	.00360
35	07329500	RUSH CR NR MAYSVILLE, OK	32	1.73	2	.31	75	10	21	206	.00140
36	07335700	KIAMICHI R NR BIG CEDAR, OK	69	3.02		50	23	6	10	40.1	
37	08128000	S CONCHO R AT CHRISTSTOVAL, TX	25	1.90	2	10	25	0	47	409	.00150
38	08128400	M CONCHO R AB TANKERSLEY, TX	72	3.20	1	7.0	56	22	16	2436	.00140
39	08130500	LOVE CR AT KNICKERBOCKER, TX	23	2.80	2	11	70	0	17	229	.00200
40	08131400	PECAN CR NR SAN ANGELO, TX	14	.82	0	50	17	61	16	83.2	
41	08133500	N CONCHO R AT STERLING CITY, TX	16	1.34	6	6.2	31	72	38	605	
42	08134000	N CONCHO R NR CARLSBAD, TX	33	1.94	2	3.8	46	69	53	605	.00180
43	08184000	CITRUS CR NR RUIVERDE, TX	42	1.47	5	4.0	25	94	19	198	.00130
44	08185000	CITRUS CR AT SELMA, TX	46	2.27				87	31	274	.00190
45	08198000	SAHINAL R NR SAHINAL, TX	49	2.81				0	35	206	.00210
46	08200000	HONDU CR NR TARDIFY, TX	85	1.90	0	14	28	0	25	86.2	.00200
47	08200500	HONDU CR NR HONDU, TX	62	1.00				85	12	132	.00250
48	08200700	HONDU CR AT KING WATERHOLE NR HONDU, TX	50	1.84	0	50	17	44	17	142	
49	08202700	SFCO CR AT RUWE RANCH NR O'HAMIS, TX	72	1.02		10		94	16	168	.00170
50	08318000	GALSTEIN CR AT DUMINGO, NM	154	0.70	0	.66		72	26	640	.00310
51	08343000	RIO SAN JOSE AT GRANTS, NM	5.2	.65	53	.06	54	96	34	1020	.00500
52	08343100	GRANTS CANYON AT GRANTS, NM	12	.52	15	.18	37	98	14	13.0	.00560
53	08343500	RIO SAN JOSE NR GRANTS, NM	15					0	39	2300	.00500
54	08353000	RIO PIERCE NR BERKADO, NM	63	.66	37	.09	57	69	35	7350	.00098
55	08355300	ARROYO DE LA MATANZA NR SOCORRO, NM	28	2.19				65	6	46.0	.01600
56	08387000	RIO RUIZOSO AT HOLLYWOOD, NM	16	1.50				0	22	120	.00430
57	08390500	RIO HONDU NR ROSWELL, NM	16	2.00				75	36	947	.00330
58	08394500	RIO FFLIX NR HAGERMAN, NM	42	2.50				87	36	932	.00270
59	08398500	RIO PENASCO AT DAYTON, NM	24	1.00				89	24	1060	.00360
60	08400000	FOURMILE CREEK NR LAKEWOOD, NM	22	1.00				99	24	265	.00350
61	08401200	S SPYEN RS NR LAKEWOOD, NM	20	1.00				98	12	220	.00450
62	09073400	ROARING FK R NR ASPEN, CO	39	1.50	0	1.3		0	13	108	.01090
63	09074800	CASTLE CR AB ASPEN, CO	23	1.30	0	30		0	8	32.2	.03330
64	09075700	MARQUON CR AB ASPEN, CO	31	2.00				0	8	35.4	.02600
65	09215000	PACTIC CR NR FARSON, WY	18	.80				69	19	500	
66	09216000	HIG SANDY R BL FDFN, WY	51	2.23				0	24	1610	
67	09235600	POI CR AB DIVISIONS, NP VERNAL, UT	5.8	1.43	34	.16	59	34	19	25	.00540
68	09235800	POT CR NR VERMILION, UT	6.0	.77				72	51	106	.00540
69	09270500	DRY FK AT MOUTH, NR DRY FK, UT	60					0	22	116	.02400
70	09315500	SALFRATHS WASH AT GREEN R, UT	30	1.93	2	.57	55	34	22	180	.00350

**8 Streamflow Related to Channel Geometry, Western U.S.**

**Table 1. Channel and streamflow characteristics at selected gaging stations—Continued**

QA, AVERAGE ANNUAL RUNOFF, IN ACRE-FEET; QN, FLOOD DISCHARGE OF SPECIFIC RECURRENCE INTERVAL; N EQUALS 2, 5, 10, 25, 50, OR 100 YEARS, IN CUBIC FEET PER SECOND; PA, AVERAGE ANNUAL PRECIPITATION, IN INCHES; P2-24, 2-YEAR, 24-HOUR PRECIPITATION, IN INCHES.

MAP NO	STATION NO	QA	Q2	Q5	Q10	Q25	Q50	Q100	PA	P2-24
1	06131000	36880	2840	8290	14100	24400	34400	46400	11.0	1.30
2	06135500	5520	579	1470	2330	3740	5020	6490	13.0	1.60
3	06136000	730	29	43	52	63	71	80	13.0	1.60
4	06150500	2120	329	782	1190	1800	2330	2910	12.0	1.60
5	06151000	1040	220	512	773	1170	1510	1890	12.0	1.60
6	06170200	15720							12.0	1.60
7	06174000	51510	2690	7170	11800	19700	27400	36500	12.0	1.80
8	06177500	9930	758	3460	7080	14300	21900	31400	13.0	1.40
9	06256900	2210	209	598	1020	1800	2570	3550	6.9	1.00
10	06268500	7900	1090	1790	2340	3130	3780	4490	7.2	1.20
11	06425500	18480	1290	3060	4880	8130	11400	15500	16.9	2.00
12	06439000	33760	1470	3670	6120	10300	14300	19300	13.3	2.00
13	06441000	35070	2550	5060	7420	11400	15200	19900	15.9	2.20
14	06441500	93460	6120	13900	21700	35100	48200		16.3	2.20
15	06442000	2930	73	442	1110	2950	5510	9600	17.4	2.30
16	06442500	10360	539	1600	2880	5490	8400	12400	17.5	2.30
17	06467600	2760	103	310	525	887	1220	1610	16.6	1.80
18	06844700	127	65	265	548	1180	1930	3000	19.5	2.30
19	06944900	2530	456	1440	2620	4930	7410	10700	19.5	2.30
20	06863900	3160	464	1380	2440	4480	6640	9460	24.0	2.60
21	07138650	659	661	1600	2530	4100	5580	7360	17.0	2.40
22	07144850	3040	519	1730	3200	6050	9070	13000	24.0	3.00
23	07156220	2120	829	2000	5230	10100	15400	22500	16.0	2.40
24	07188500	20340	956	3270	5970	11000	16100	22500	42.5	4.00
25	07190600	22750	4720	9010	12300	17100	20900	25000	41.0	4.00
26	07196000	82590	4560	11300	17600	27600	36600	46700	44.5	4.10
27	07199000	3590	2660	6400	10100	16400	22300	29500	14.0	2.35
28	07207000	14490	349	773	1160	1790	2360	3030	20.0	2.00
29	07222500	6570	3630	8860	14400	24300	34300	47000	15.0	2.00
30	07227200	3170	652	6710	22400	90200	182000	378000	16.0	2.26
31	07229300	39340	9400	15100	19200	24500	28600	32700	33.0	3.75
32	07247500	107950	6360	12300	17200	24200	30000	36200	43.9	4.10
33	07249400	94910	7080	13300	18100	24700	30000	35500	43.0	4.00
34	07249500	27460	5530	10600	14700	20300	24800	29600	49.0	4.00
35	07329500	38470	7340	13500	18400	25200	30700	36500	34.0	3.75
36	07335700	54050	9630	14800	18300	22700	25900	29100	52.0	4.25
37	08128000	26520	2850	16600	37300	92000	131000	194000	16.5	3.30
38	08128400	17460	1990	5870	10100	17900	25700	35300	20.0	3.50
39	08130500	19560	2370	6310	10320	17220	23780	31630	18.0	3.30
40	08131400	1530	458	1580	3140	6670	11000	17500	18.0	3.80
41	08133500	2610	2110	5630	9200	15300	21000	27800	18.0	3.00
42	08134000	8620	6250	21600	38100	66200	91800	121000	20.0	3.10
43	08184000	7740	2510	8610	15800	29600	43600	61300	32.5	3.90
44	08185000	11300	4140	21300	36500	53800	63600	71000	28.5	3.90
45	08198000	62090	4040	14100	26100	48900	72200	102000	25.0	3.70
46	08200000	38470	6490	18000	29700	49900	69000	91700	32.6	3.80
47	08200500	12020	5210	17900	33100	62200	92400	131000	28.0	3.80
48	08200700	15360	9490	18600	27500	41100	52700	65700	25.0	3.80
49	08202700	9420	3000	9460	17700	35100	55200	83700	25.0	3.80
50	08318000	7390	6310	11000	14700	19900	24300	29000	13.0	1.51
51	08343000	188							10.0	1.50
52	08343100	101	327	762	1190	1900	2570	3380	10.0	1.50
53	08343500	4670	231	581	942	1580	2200	2970	10.0	1.50
54	08353000	26520	4420	7960	10800	14900	18300	22000	10.0	1.22
55	08355300	315	478	1400	2450	4410	6440	9030	10.0	1.50
56	08387000	12030	215	417	590	853	1080	1340	25.0	1.87
57	08390500	7610	2950	8480	14900	27200	40200	57500	18.0	1.92
58	08394500	8170	5030	15800	25100	37900	47200	56000	16.0	1.98
59	08398500	3330	2770	7970	13800	24700	36000	50500	18.0	2.02
60	08400000	2930	470	3650	9620	25200	45000	74100	14.0	2.00
61	08401200	2330	2610	11900	26100	60400	104000	168000	14.0	2.00
62	09073400	60400	733	970	1110	1280	1390	1500	20.0	1.40
63	09074800	27310	340	390	417	447	466	484	20.0	1.40
64	09075700	42460	508	592	638	688	721	751	20.0	1.40
65	09215000	3620	258	555	808	1180	1500	1840	8.7	1.00
66	09216000	42600	500	840	1080	1390	1620	1850	10.0	1.50
67	09235600	2910	66	136	193	274	340	409	20.0	1.00
68	09235800	1440	49	117	178	272	354	443	20.0	1.00
69	09270500	25290	463	945	1350	1950	2460	3020	20.0	1.50
70	09315500	2170	2490	4770	6620	9290	11500		7.5	.97

Table 1. Channel and streamflow characteristics at selected gaging stations—Continued

WAC, WIDTH OF ACTIVE CHANNEL, IN FEET; DAC, DEPTH OF ACTIVE CHANNEL, IN FEET; BDS, BED SILT-CLAY, IN PERCENT; D50, MEDIAN PARTICLE SIZE OF BED MATERIAL, IN MILLIMETERS; BSH, BANK SILT-CLAY-HIGH, IN PERCENT; NF, NO-FLOW DAYS, IN PERCENT; RL, RECORD LENGTH, IN WATER YEARS; DA, DRAINAGE AREA, IN SQUARE MILES; GRA, CHANNEL GRADIENT, IN FEET PER FEET

MAP NO	STATION NO	STATION NAME	WAC	DAC	BDS	D50	BSH	NF	RL	DA	GRA
71	09316000	BROWNS WASH NR GREEN R, UT	46	1.34	21	.19	53	95	19	75	.00700
72	09334000	N WASH NR HANKSVILLE, UT	63	1.50	4	3.6	29	54	20	136	.01200
73	09346400	SAN JUAN R NR CARACCAS, CO	133	4.10	1	25	46	0	14	75.3	.00340
74	09349800	PEIDRA R NR ARBOLES, CO	79	2.16	0	25	79	0	15	674	.00430
75	09352900	VALLFCITO CR NR BAYFIELD, CO	64	1.97	0	100		0	15	72.1	.02600
76	09354500	LOS PINOS R AT LA BOCA, CO	65	1.64	0	20	50	0	27	510	.00680
77	09355000	SPRING CR AT LA BOCA, CO	37	1.95	6	.25	56	0	27	58	.00720
78	09364500	ANIMAS R AT FARMINGTON, NM	160	3.85				0	64	1360	.00430
79	09365500	LAPLATA R AT HESPERUS, CO	24	.82		20		0	61	37	.01600
80	09366500	LA PLATA R AT CO-NM STATE LINE	26	1.41				2	57	331	.00530
81	09367500	LA PLATA R NR FARMINGTON, NM	28	1.46	3	.43	43	5	37	583	.00540
82	09415600	PARAGUT VALLEY TRIB NR HIND, NV	16	.91	10	2.3	12	100	14	17	.01200
83	09416000	MIDDY R NR MUSA, NV	24	3.50	16	.09	54	0	39	3820	.00400
84	09418500	MEADOW VALLEY WASH NR CALIENTE, NV	21	1.44	1	70	61	0	21	1670	.00900
85	09419610	LE CANYON NR CHARLESTON PEAK, NV	26	1.15	0	18	33	0	14	9.2	.00700
86	09419650	LAS VEGAS WASH AT N LAS VEGAS, NV	76	.41	12	.15	72	90	15	1300	.00500
87	09444000	SAN FRANCISCO R NR GLENWOOD, NM	64	1.80				0	48	1653	.00620
88	09470500	SAN PEDRO R AT PALOMINAS, AZ	64	1.65	2	.45	74	18	35	741	.00140
89	09471000	SAN PEDRO R AT CHARLESTON, AZ	91	1.52	2	.51	74	0	66	1219	.00240
90	09472000	SAN PEDRO R NR REDINGTON, AZ	72	2.53	1	1.4	34	41	23	2939	.00380
91	09473000	ARAVATPA CR NR MAMMOTH, AZ	93	1.35	0	14	40	0	22	541	.00590
92	09474000	GILA R AT APILVIN, AZ	91	2.50	0	.84	52	0	66	8011	.00230
93	09480000	SANTA CRUZ R NR LOCHIELD, AZ	51	1.09	2	1.4	43	6	28	82.2	.00390
94	09480500	SANTA CRUZ R NR NOGALES, AZ	350		1	1.1	80	24	58	533	.00390
95	09482000	SANTA CRUZ R AT CONTINENTAL, AZ	99	2.18			78	89	32	1662	.00810
96	09482400	AIRPORT WASH AT TUCSON, AZ	26	.83			41	95	12	23	.00620
97	09482500	SANTA CRUZ R AT TUCSON, AZ	46	1.59	4	.50	67	85	72	2222	.00310
98	09483000	TUCSON ARROYO AT VINE AVE., TUCSON, AZ	21	1.07	2	1.2	64	82	21	8.2	.00750
99	09483100	TANQUE VERDE CR NR TUCSON, AZ	21	1.27	1	1.8	29	48	11	43.0	.00300
100	09484560	CIENEGA CR NR PANTANO, AZ	93	1.22	11	2.0	57	94	8	289	.00370
101	09486300	CANADA DEL ORO NR TUCSON, AZ	70	.75	7	.88	19	97	12	250	.00720
102	09486500	SANTA CRUZ R AT CORTARO, AZ	101	.67	3	.54	61	11	34	3503	.00260
103	09486800	ALTA WASH NR THREE POINTS, AZ	185	1.20	5	.82	76	93	10	463	.00440
104	09505250	RED TANK DRAW NR RIMROCK, AZ	35	1.34	2	200	50	56	20	49.4	.02100
105	09505350	DRY BEAVER CR NR RIMROCK, AZ	95	1.97	0	15	4	72	17	142	.01100
106	09510100	E. FK SYCAMORE CR NR SUNFLOWER, AZ	22	1.30	1	10	18	27	16	4.49	.03700
107	09510200	SYCAMORE CR NR FORT McDONALD, AZ	58	.85	0	3.3	64	3	16	164	.00970
108	09512200	SALT R TRIB AT PHOENIX, AZ	29	.44	2	2.8	52	99	16	1.75	.01600
109	09512300	CAVE CR NR CAVE CR, AZ	70	1.40	1	3.1	47	96	7	121	.00830
110	09512400	CAVE CR AT PHOENIX, AZ	32	2.75	1	.71	43	97	20	252	.00490
111	09513780	NFW R NR ROCK SPRINGS, AZ	55	2.40	0	1.6	4	71	12	67.3	.00850
112	09513800	NFW R AT NEW RIVER, AZ	145	3.00	2	.76	8	79	16	83.3	.00520
113	09513835	NFW R NR PEORIA, AZ	162	2.00	1	.70	60	98	10	187	.00460
114	09513860	SKUNK CR NR PHOENIX, AZ	56	1.65	7	.26	25	98	10	64.6	.00570
115	09513910	NFW R AT GLENDALE, AZ	460	2.50	9	.35	27	96	6	323	.00320
116	09513970	AGUA FRIA R AT AVONDALE, AZ	430	2.50	2	.39	31	0	9	2013	.00120
117	10245800	NEWARK VALLEY TRIB NR HAMILTON, NV	7.7	.43	63	.04	87	97	15	157	.00970
118	10247860	PENNYER VALLEY TRIB NR TEMPIUTE, NV	12	.94	5	2.1	27	0	12	1.48	.02000
119	10248510	ELDORADO VALLEY TRIB NR NELSON, NV	17	.32		6.1		0	12	1.41	.03700
120	10249300	S TWIN R NR ROUND MOUNTAIN, NV	12	1.15	0	30	40	0	12	70	.03500
121	10249411	CAMPBELL CR TRIB NR EASTGATE, NV	2.8	.28	0	15	75	76	14	2.14	.01300
122	10250600	WILDOUSE CR NR WILDOUSE STATION, CA	26	1.22	0	7.6	61	0	10	23.7	.06300
123	10251300	AMARGOSA R AT TROPICA, CA	7.9	2.00	47	.06	90	21	15		.00900
124	10251980	LOWELL WASH NR BLUE DIAMOND, NV	15	.75	0	14	8	96	11	52.8	
125	10252300	CHINA SPRING CR NR MOUNTAIN PASS, CA	8.6	.54	2	2.1	13	0	11	.94	
126	10282480	MAZOURKA CR NR INDEPENDENCE, CA	90	1.00		20		0	10	15.6	.08000
127	10393500	SILVER R NR BURNS, OR	52	5.00				0	64	934	.00649
128	10396000	DONNER UND RITZEN R NR FRENCHGLEN, OR	56	4.00				0	47	200	.00260
129	10403000	SILVER CR NR RILEY, OR	26	1.34				0	26	228	
130	11139000	LAHREA CR NR SISQUOC, CA	42	1.00	6	.58		79	22	93.8	.00800
131	11140000	SISQUOC R NR GAREY, CA	265	1.50	2	.41	13	83	36	471	.00310
132	11142500	ARROYO DE LA CRUZ NR SAN SIMON, CA	78	2.78	1	10	33	54	27	41.2	.00340
133	11147800	CHOLAME CR NR SHANDON, CA	55	1.10	2	.42	71	92	12	227	.00230
134	11148500	ESTRELLA R NR ESTRELLA, CA	185	1.80	4	.30	53	69	23	922	.00270
135	11176000	ARROYO MOCHO NR LIVERMORE, CA	7.1	.96		5.0	18	25	32	38.2	.00640
136	11180500	DRY CR AT UNION CITY, CA	25	3.09	2	2.7	47	63	21	9.39	.00420
137	11255500	PANOUCHE CR BL SILVER CR, NR PANOUCHE, CA	40	.75	4	.56	27	58	11	293	.00640
138	11337500	MARKS CR NR BYRON, CA	28	1.37	0	14	56	67	22	42.6	.00510
139	11378800	RED BANK CR NR RED BLUFF, CA	99	2.18	0	12	78	52	17	93.5	
140	11390672	STONE CORRAL CR NR SITES, CA	16	1.88	10	.36	54	62	17	38.2	.00310

10 Streamflow Related to Channel Geometry, Western U.S.

**Table 1. Channel and streamflow characteristics at selected gaging stations—Continued**

QA, AVERAGE ANNUAL RUNOFF, IN ACRE-FEET; QN, FLOOD DISCHARGE OF SPECIFIC RECURRENCE INTERVAL;  
 N EQUALS 2, 5, 10, 25, 50, OR 100 YEARS, IN CUBIC FEET PER SECOND; PA, AVERAGE ANNUAL  
 PRECIPITATION, IN INCHES; P2-24, 2-YEAR, 24-HOUR PRECIPITATION, IN INCHES.

MAP NO	STATION NO	QA	Q2	Q5	Q10	Q25	Q50	Q100	PA	P2-24
71	09316000	688	1700	3550	5130	7500	9530	11800	7.5	1.00
72	09334000	869	1180	3070	5000	8340	15400	20100	10.0	1.15
73	09346400	384710	3800	5940	7470	9510	11100	12700	30.0	1.80
74	09349800	251400	2290	3940	5140	6740	7960	9200	27.0	1.70
75	09352900	98530	1370	2110	2660	3370	3890	4410	46.0	2.60
76	09354500	157200	1340	2330	3090	4170	5050	5990	12.0	1.40
77	09355000	23470	335	638	882	1240	1550	1880	12.0	1.39
78	09364500	566600	6120	9190	11400	14200	16400	18700	29.0	1.50
79	09365500	27460	452	772	1000	1310	1550	1800	35.0	2.20
80	09366500	23330	766	1560	2240	3240	4100	5040	35.0	1.60
81	09367500	17170	1250	2220	2990	4090	5000	5990	29.0	1.50
82	09415600	1.4							10.0	1.30
83	09416000	29420	209	548	916	1600	2300	3190	6.3	1.30
84	09418500	8550	474	1060	1610	2530	3380	4380	7.5	1.30
85	09419610	13	24	169	464	1360	2740	5130	19.5	1.70
86	09419650	643	180	1150	3040	8550	16700	30400	6.0	1.40
87	09444000	57310	2540	5110	7310	10700	13600	16800	17.6	1.80
88	09470500	21080	6370	10200	12900	16500	19300	22200	17.9	1.90
89	09471000	33760	6920	12500	17800	26700	35300	45900	16.5	1.90
90	09472000	25860	8710	16800	23400	33100	41400	50400	15.5	1.90
91	09473000	15360	4560	8820	12300	17200	21300	25700	16.2	2.00
92	09474000	267300	21400	45600	66600	98400	126000	156000	20.0	2.50
93	09480000	1930	1700	3510	5040	7330	9270	11400	18.2	1.90
94	09480500	20580	4320	7930	10900	15200	18800	22900	18.7	2.00
95	09482000	12530	4460	8630	12000	17000	21100	25500	18.1	2.10
96	09482400	329	320	572	764	1030	1240	1470	10.8	1.80
97	09482500	13040	5140	8820	11600	15600	18800	22100	16.9	2.10
98	09483000	650	1000	2300	3300	4400	5500	6900	11.0	1.80
99	09483100	6430	1040	2020	2810	3970	4920	5950	17.0	2.00
100	09484560	1700	920	2690	4600	8000	11300	15400	16.6	1.90
101	09486300	6590	2180	5450	8610	13800	18600	24200	16.4	2.00
102	09486500	41730	8340	13700	16600	21000	24200	27500	16.3	2.00
103	09486800	5400	5700	10100	13500	18100	21800	25600	15.6	2.20
104	09505250	6220	507	2340	5130	11700	19700	31400	21.6	2.40
105	09505350	28040	2880	8550	14900	26700	38700	53900	23.1	2.50
106	09510100	484	30	158	362	850	1460	2340	24.5	3.00
107	09510200	18480	1660	5650	10400	19600	29100	41400	21.2	2.70
108	09512200	4.4	35	181	418	1010	1760	2900	9.0	1.60
109	09512300	2770	2010	4900	7730	12500	16900	22200	15.7	2.30
110	09512400	3280	417	1200	2040	3540	5030	6870	9.0	1.60
111	09513780	7140	1580	5760	11100	22300	34700	51500	20.0	2.40
112	09513800	7610	2100	7220	13600	26400	40300	58800	19.5	2.30
113	09513835	5750	1470	5110	9650	18800	28800	42100	15.6	1.90
114	09513860	1130	1200	4750	9600	20100	32100	48900	12.2	1.90
115	09513910	8190	2390	8380	15900	31100	47600	69600	13.8	1.80
116	09513970	6670	249	2400	7550	24900	53000	103000	16.3	1.70
117	10245800	120	23	95	202	448	749	1190	10.3	1.20
118	10247860	1.4	.5	8.2	37	185	524	1340	8.0	1.10
119	10248510	5.8	2.8	62	370	1820	5620	15500	6.0	1.30
120	10249300	4950	38	79	116	175	228	289	15.4	1.60
121	10249411	55	3.5	20	51	135	253	447	16.0	1.50
122	10250600	20	8.2	166	705	3000	7290		8.0	1.10
123	10251300	1990	234	972	2050	4540	7580	12000	4.0	1.10
124	10251980	198	40	411	1390	5130	11900	25400	9.0	1.70
125	10252300	.6	3.0	22	47	100			7.0	1.10
126	10282480	61							6.0	1.40
127	10393500	134000	1280	2130	2760	3620	4310	5030	19.0	1.00
128	10396000	94190	1270	2030	2570	3270	3810	4350	14.0	1.00
129	10403000	31590	552	1030	1430	2020	2530	3090	20.0	1.00
130	11139000	2930	169	951	2350				23.0	3.20
131	11140000	45720	1290	5130	10500	22200	36000	54400	20.0	3.50
132	11142500	42240	7590	14600	19800	26600	31700	36700	31.0	3.50
133	11147800	4880	113	1190	3740	12000	24600	46100	10.0	1.50
134	11148500	40140	387	2810	7020	17000	28800	44900	13.0	1.90
135	11176000	3780	167	574	1010	1760	2450	3220	16.0	3.50
136	11180500	2480	136	568	1100	2090	3050	4200	22.0	2.90
137	11255500	1340	290	1670	3750	8220	13100	19400	14.0	2.00
138	11337500	6570	459	1650	2970	5250	7370	9810	16.0	2.30
139	11378800	42380	4180	7080	9240	12200	14500	17000	26.0	3.00
140	11390672	4380	1180	2450	3540	5160	6550	8080	20.0	2.50

**Table 1. Channel and streamflow characteristics at selected gaging stations—Continued**

WAC, WIDTH OF ACTIVE CHANNEL, IN FEET; DAC, DEPTH OF ACTIVE CHANNEL, IN FEET; BDS, BED SILT-CLAY, IN PERCENT; D50, MEDIAN PARTICLE SIZE OF BED MATERIAL, IN MILLIMETERS; BSH, BANK SILT-CLAY-HIGH, IN PERCENT; NF, NO-FLOW DAYS, IN PERCENT; RL, RECORD LENGTH, IN WATER YEARS; DA, DRAINAGE AREA, IN SQUARE MILES; GPA, CHANNEL GRADIENT, IN FEET PER FOOT

MAP NO	STATION NO	STATION NAME	WAC	DAC	BDS	D50	BSH	NF	RL	DA	GPA
141	11448500	ADURE CR NR KELSEYVILLE, CA	22	.99	0	15	59	35	22	6.36	.00440
142	11449100	SCOTTIS CR NR LAKEPORT, CA	42	2.76	0	8.6	32	46	16	55.2	.00210
143	13112000	CAMAS CR AT CAMAS, ID	31	1.57				29	50	400	
144	13114000	BFAVER CR AT CAMAS, ID	15	1.10				85	49	510	
145	13207000	SPRING VALLEY CR NR EAGLE, ID	9.0	.60				48	16	20.9	
146	14179000	BRETTENRUSH R AR CANYON CR, NR DETROIT, OR	123	2.60		50		0	45	106	.01200
147	14192000	MILL CR AT SALEM, OR	45	2.01				0	38	110	.00160
148	14193000	WILLAMINA CR NR WILLAMINA, OR	64	2.77				0	43	64.7	.00350
149	14301500	WILSON R NR TILLAMOOK, OR	125	3.10		25		0	47	161	.00120
150	14303600	NESTUCCA R NR BFAVER, OR	150	3.61				0	13	180	.00370
151	14305500	STLETZ R AT STLETZ, OR	130	4.32		10		0	58	202	.00160

**Grouping by Channel-Material Characteristics**

Channel-geometry studies for the Rocky Mountain States and the Missouri River basin (Osterkamp and Hedman, 1977; 1982) indicate that width-discharge relations of perennial-stream channels vary measurably with the channel-material characteristics. In general, streams that transport predominantly fine-grained material (silt and clay) form relatively narrow and deep channel sections with cohesive banks of fine material. Predominantly sandy channels tend to be wide and shallow, the banks lacking the cohesiveness necessary to resist erosive discharges and maintain a stable, well-defined shape. Channels armored with increasingly larger material sizes (gravel through boulders) tend to have the narrow shape, relative to mean annual runoff, of the fine-grained channel sections. Armored streams (generally alpine streams in these studies) have relative narrowness and pronounced stability because the material forming the channel perimeter is immobile except during uncommonly large flows. The armor, that is the coarse-material sizes, provides the same stabilizing effect for these channels as does the cohesiveness of silt-clay channels.

The data collected for this study are sufficient to define three groups of channels: (1) silt-clay channels—those with a median-particle size ( $d_{50}$ ) of the bed material of less than 0.1 mm (millimeter) or a bank-material silt-clay content of at least 70 percent and a  $d_{50}$  of the bed material of no greater than 5.0 mm; (2) sand channels—those with a  $d_{50}$  of the bed material ranging from 0.1 to 5.0 mm and silt-clay contents of the banks of less than 70 percent; and (3) armored channels—those with  $d_{50}$  of the bed material greater than 5.0 mm.

Separate relations between active-channel width and annual runoff were developed for each of the channel-material groups. The basic equations developed by regression analyses for each flow-frequency group and geographic area were used to define approximately the coefficients

and exponents. The separate relations were then developed graphically for the channel-material groups. This procedure was necessary because there were not enough data sets for a regression analysis of each group. Because equivalent standard errors could not be determined for the graphical analyses, the approximate standard errors shown are for the basic regression equations. It is assumed that the standard errors for the separate relations are at least equal to and probably less than those shown. The use of channel-material groups to define relations between active-channel width and flood discharges showed minimal statistical significance, and therefore separate channel-material relations are not included to estimate the flood discharges.

**Grouping of Runoff Characteristics**

Different groupings of the data sets were made depending upon whether the intended relations estimated mean annual runoff or flood discharge. The intermittent-stream data were divided into northern and southern groups for the purpose of relating width to mean-annual runoff. The two groups are approximately separated by a latitude 39° N. (fig. 9). To develop equations yielding flood-discharge estimates, each data set in table 1 was placed in one of four groups. The first includes alpine and pine-forested drainage areas. The other three groups are defined similarly to those of the mean annual runoff data of intermittent streams. Thus, latitude 39° N. again separates the plains that are east of the Rocky Mountains. A fourth group includes the intermontane areas that are west of the Rocky Mountains.

Regression analyses were made of various groupings of the data in table 1 to yield equations that estimate mean annual runoff and flood discharges. The results provided here represent the groupings of data that appeared to produce the most consistent and statistically significant re-

**Table 1. Channel and streamflow characteristics at selected gaging stations—Continued**

QA, AVERAGE ANNUAL RUNOFF, IN ACRE-FeET; QN, FLOOD DISCHARGE OF SPECIFIC RECURRENCE INTERVAL; N FOYIALS 2, 5, 10, 25, 50, OR 100 YEARS, IN CUBIC FEET PER SECOND; PA, AVERAGE ANNUAL PRECIPITATION, IN INCHES; P2=24, 2-YEAR, 24-HOUR PRECIPITATION, IN INCHES.

MAP NO	STATION NO	QA	Q2	Q5	Q10	Q25	Q50	Q100	PA	P2=24
141	11448500	8980	956	1330	1560	1850	2060	2270	41.0	4.50
142	11449100	62450	4390	7970	10700	14600	17800	21100	30.0	3.00
143	13112000	35650	380	690	910	1180	1390	1590	10.0	1.20
144	13114000	13110	110	170	210	250	290	310	10.0	1.20
145	13207000	1850	52	130	204	326	438	568	14.0	1.30
146	14179000	455700	6260	8890	10700	13100	14900	16700	77.0	3.70
147	14192000	96360							40.0	3.00
148	14193000	205800	3850	5240	6210	7500	8490	9530	87.5	4.90
149	14301500	907100	17400	22600	26000	30100	33200	36300	102.5	5.50
150	14303600	844800	14500	20100	24000	28900	32600	36300	110.0	5.80
151	14305500	1159000	20900	26600	30200	34300	37300	40200	117.7	5.70

sults. In order to develop easily applied equations of general utility, however, the data groupings are intentionally broad and necessarily different for the mean annual runoff and flood-discharge equations.

Users of the equations need to realize that latitude 39° N. and the edges of the Rocky Mountains (fig. 9) are not exact boundaries. These divisions need to be considered transition zones. Because the computed discharge

**Table 2. Equations for determining mean annual runoff for streams in western United States.**

Flow frequency	Areas of similar regional-runoff characteristics <u>a/</u>	Percentage of time having discharge	Channel-material characteristics <u>b/</u>	Equation <u>c/</u>	Standard error of estimate (percent)	Equation number
Perennial	Alpine	More than 80	Silt-clay and armored	$Q_A = 64W_{AC}^{1.88}$	28	(7)
Intermittent	Plains north of latitude 39°N.	10 to 80	Silt-clay and armored	$Q_A = 40W_{AC}^{1.80}$	50 <u>d/</u>	(8)
			Sand	$Q_A = 40W_{AC}^{1.65}$	50 <u>d/</u>	(9)
	Plains south of latitude 39°N.	10 to 80	Silt-clay and armored	$Q_A = 20W_{AC}^{1.65}$	50 <u>d/</u>	(10)
			Sand	$Q_A = 20W_{AC}^{1.55}$	50 <u>d/</u>	(11)
Ephemeral	Northern and southern plains	6 to 9	Silt-clay and armored	$Q_A = 10W_{AC}^{1.55}$	<u>e/</u>	(12)
			Sand	$Q_A = 10W_{AC}^{1.50}$	<u>e/</u>	(13)
	and intermontaine areas	2 to 5	Silt-clay and armored	$Q_A = 4.0W_{AC}^{1.50}$	40 <u>d/</u>	(14)
			Sand	$Q_A = 4.0W_{AC}^{1.40}$	40 <u>d/</u>	(15)
	Deserts of the Southwest	1 or less	Silt-clay and armored	$Q_A = 0.04W_{AC}^{1.75}$	75 <u>d/</u>	(16)
Sand			$Q_A = 0.04W_{AC}^{1.40}$	75 <u>e/</u>	(17)	

a/ Areas of climatic characteristics shown in figure 9.

b/ Silt-clay channels--bed material  $d_{50}$  less than 0.1 millimeter or bed material  $d_{50}$  equal to or less than 5.0 millimeters and bank silt-clay content equal to or greater than 70 percent.

Sand channels--bed material  $d_{50} = 0.1-5.0$  millimeters and bank silt-clay content less than 70 percent.

Armored channels--bed material  $d_{50}$  greater than 5.0 millimeters.

c/ Active-channel width,  $W_{AC}$ , in feet; discharge,  $Q_A$ , in acre-feet per year.

d/ Approximate--standard error of estimate of the basic regression equation.

e/ Standard error of estimate not determined; graphical analyses.

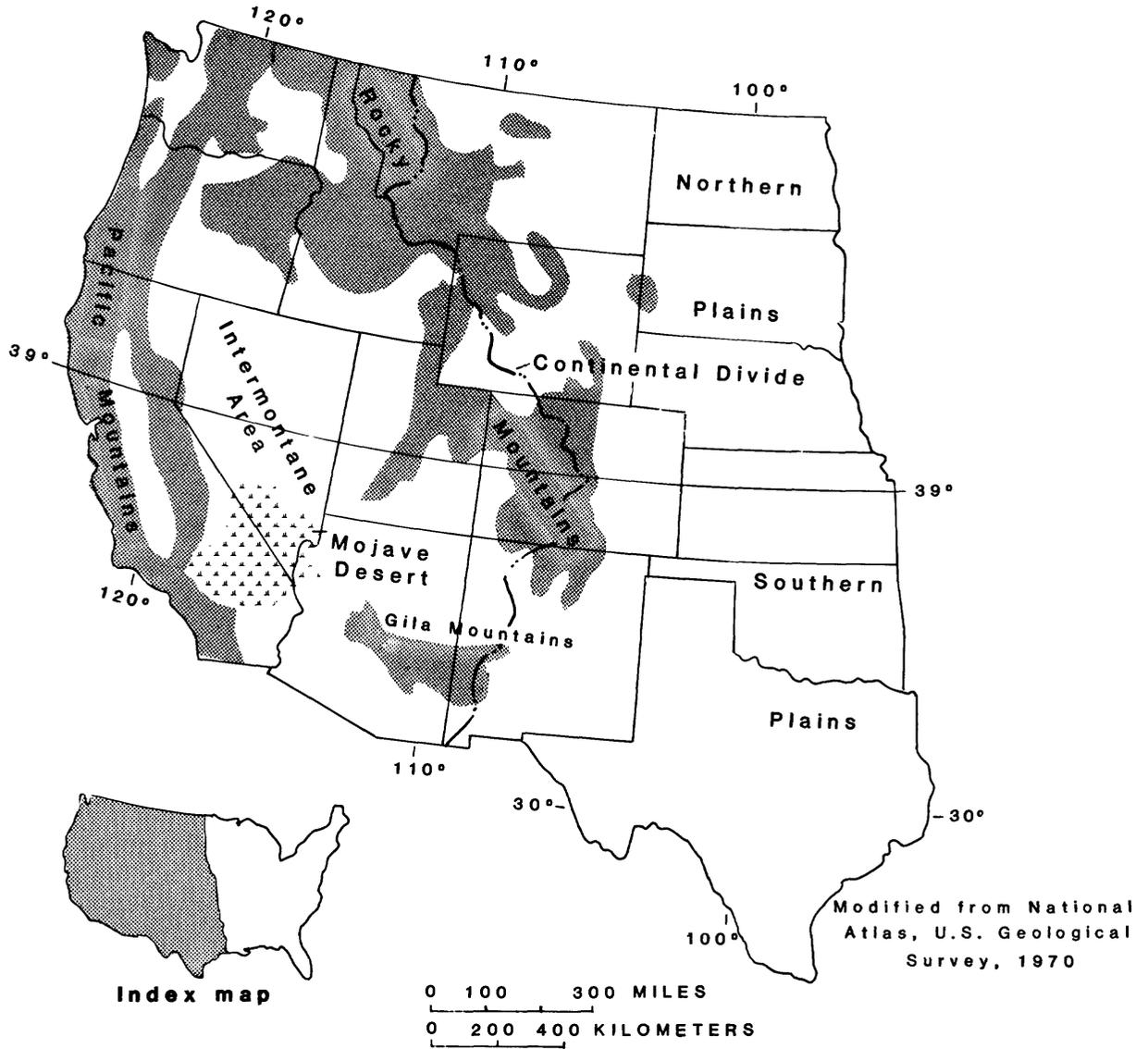


Figure 9. Areas of similar hydrology and channel geometry.

values can differ as much as 100 percent from one area to another, it may be necessary to compute discharge values with both equations if the drainage areas of the stream are separated by one of the boundaries. The discharge values then need to be adjusted on the basis of that part of the drainage basin which is in each area (table 2).

## APPLICATION OF THE METHOD

### Collection of Channel-Geometry Data

A reach of channel for which the discharge characteristics are desired needs to be thoroughly investigated to lo-

cate at least three cross sections, one or more stream widths apart, that are representative of the channel. Care needs to be taken not to select cross sections upstream or downstream from tributaries that would significantly change the drainage area. At cross sections where the reference points for the active-channel width are adequately defined, a tape or graduated tag line needs to be stretched tightly across and perpendicular to the channel, as shown by line B-B' in figure 1. The width is measured between the reference points and recorded. A photograph of the cross section with the tape in place needs to be taken to show the location and for possible review at a later date. Detailed procedures for collecting channel-geometry data

are given by Hedman and Kastner (1977).

Field training and experience are necessary for effective selection of the active-channel reference levels. Unusually shaped channel cross sections need to be avoided. Relatively straight or stabilized reaches of meandering channels need to be selected where active bank cutting or deposition is not in the process of changing the channel width. Braided reaches need to be avoided, as well as reaches in the channel that indicate the channel has been widened or realigned by an extreme flood or by construction work and has not had time to readjust. Likewise, reaches with banks that cannot be rapidly sculptured by the water (that is, banks composed of resistant material, such as bedrock, and reaches lined with riprap or concrete that have abnormally narrow widths) need to be avoided. Reaches with large pools or steep inclines also need to be avoided.

### Channel-Material Sampling Procedures

At least one set of samples of bed-and bank-material need to be collected at each site at which the channel-geometry technique is used. Samples of bed and bank material should be collected from the perimeter of the active channel. Three composite samples should be collected, one from portions of material taken at equal intervals across the channel bed, and one each taken at intervals up each bank to the reference point. Because fluvial sorting processes are different for the bed and banks, care should be exercised to insure that the bed samples are not contaminated with bank material, or the reverse. Specific sampling procedures at channel-geometry sites are described by Osterkamp (1979, p. 87–88). A particle-size analysis (Guy, 1969) is made for each of the three channel-material samples, with the results being expressed as percent of the sample finer than the various specified sizes.

### COMPUTATION OF MEAN ANNUAL RUNOFF

Mean annual runoff for various types of streams in the western United States can be computed from equations given in table 2. The equations are separated on the basis of flow frequency, runoff, and channel-material characteristics.

#### Perennial Streams

Mean annual runoff for all perennial streams with silt-clay or armored channel can be computed with equation 7. This is an easily recognized class of stream. The active-channel reference level is well developed, easy to identify, and the equation has a minimum standard error of estimate.

#### Intermittent Streams

Mean annual runoff for intermittent streams can be computed with equations 8–11. This is a broad group of streams with regard to the flow frequency (10 to 80 percent), and identification will require thorough knowledge of the area and the climate. To be classified intermittent, the stream should have flow 10 to 80 percent of the days. Most of the streams with drainage areas greater than 500 square miles, except those in the arid southwest, will generally have discharge for more than 10 percent of the time due to prolonged snowmelt in the northern States and due to larger and more frequent precipitation events in the southern States, generally east of New Mexico. The areas of the northern and southern plains and intermontane areas are approximately separated by latitude 39° N. (fig. 9).

#### Ephemeral Streams

Mean-annual runoff for ephemeral streams can be computed with equations 12–17. To be classified ephemeral, the streams should have flow on the average of less than 10 percent of the days. Ephemeral streams are further separated into those that have flow 1 percent or less of the days, 2 to 5 percent of the days, and 6 to 9 percent of the days.

Identification of the streams that are ephemeral and the groups within the ephemeral classification again will require thorough knowledge of the area and the climate. All available hydrologic, geologic and climatic information should be used to determine the flow frequency of ungaged streams. All gage records should be examined because streams within large general areas commonly have about the same flow frequency. Local residents can provide valuable information on the number of low events. Inspection of channel and flood-plain debris and vegetation will give clues on the frequency of flow events. The channel material and basin soil types should be investigated. Streams with sandy channels and sandy drainage basins will have fewer runoff events than those with fine material sizes.

### COMPUTATION OF FLOOD-FREQUENCY DISCHARGE

Flood-frequency discharge, in cubic feet per second, for the indicated recurrence intervals in years can be computed with the equations in table 3. The equations are given for four separate groups—alpine streams, including streams with pine-forested drainage areas, and three geographic areas to account for the variation in runoff characteristics (fig. 9). The equations are applicable for all three flow-frequency groups (ephemeral, intermittent, and perennial).

**Table 3.** Equations for determining flood-frequency discharge for streams in western United States.

Areas of similar climatic characteristics a/	Equation b/	Standard error of estimate (percent)	Equation number
Alpine and pine-forested	$Q_2 = 1.3W_{AC}^{1.65}$	44	(18)
	$Q_5 = 2.8W_{AC}^{1.60}$	37	(19)
	$Q_{10} = 4.4W_{AC}^{1.55}$	38	(20)
	$Q_{25} = 7.0W_{AC}^{1.50}$	42	(21)
	$Q_{50} = 9.6W_{AC}^{1.45}$	45	(22)
Northern plains and intermontane areas east of Rocky Mountains	$Q_{100} = 13W_{AC}^{1.40}$	50	(23)
	$Q_2 = 4.8W_{AC}^{1.60}$	62	(24)
	$Q_5 = 24W_{AC}^{1.40}$	42	(25)
	$Q_{10} = 46W_{AC}^{1.35}$	40	(26)
	$Q_{25} = 61W_{AC}^{1.30}$	44	(27)
Southern plains east of Rocky Mountains (subject to intensive precipitation events)	$Q_{50} = 130W_{AC}^{1.30}$	51	(28)
	$Q_{100} = 160W_{AC}^{1.25}$	58	(29)
	$Q_2 = 7.8W_{AC}^{1.70}$	66	(30)
	$Q_5 = 39W_{AC}^{1.60}$	57	(31)
	$Q_{10} = 84W_{AC}^{1.55}$	56	(32)
Plains and intermontane areas west of Rocky Mountains	$Q_{25} = 180W_{AC}^{1.50}$	57	(33)
	$Q_{50} = 270W_{AC}^{1.50}$	59	(34)
	$Q_{100} = 370W_{AC}^{1.50}$	62	(35)
	$Q_2 = 1.8W_{AC}^{1.70}$	120	(36)
	$Q_5 = 7.0W_{AC}^{1.60}$	73	(37)
	$Q_{10} = 14W_{AC}^{1.50}$	60	(38)
	$Q_{25} = 22W_{AC}^{1.50}$	62	(39)
	$Q_{50} = 44W_{AC}^{1.40}$	71	(40)
	$Q_{100} = 59W_{AC}^{1.40}$	83	(41)

a/ Areas of runoff characteristics shown in figure 9.

b/ Active-channel width,  $W_{AC}$ , in feet; discharge,  $Q_n$ , in cubic feet per second, where n is the recurrence interval, in years.

Flood-frequency discharge for alpine streams, including all streams with pine-forested drainage areas, can be computed with equations 18–23. These streams have small floods in relation to total discharge and to active-channel width. Much of the precipitation is stored and released later as springflow or ground-water seepage.

Flood-frequency discharge for all other streams (excluding alpine and those with pine-forested drainage areas) can be computed with equations 24–41.

## CONCLUSIONS

Active-channel geometry measurements can be used to determine mean annual runoff and flood-frequency discharges for streams in the western United States. The method offers an alternative for estimating streamflow characteristics for ungaged streams. The equations yield discharge values from active-channel width and channel-material data. The principal advantage is that the discharge values can be determined quickly and inexpensively.

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**UNITS AND CONVERSION FACTORS**

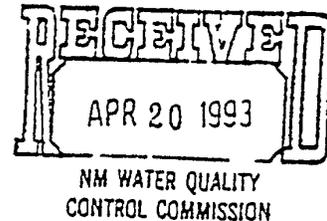
For those readers who may prefer to use metric units rather than inch- pound units, the conversion factors for the International System (SI) of Units used in this report are as follows:

<i>Multiply inch-pound units</i>	<i>By</i>	<i>To obtain SI units</i>
inch	25.4	millimeter
foot	0.3048	meter
mile	1.609	kilometer
acre	0.4047	square hectometer
square mile	2.590	square kilometer
cubic foot per second	0.02832	cubic meter per second
acre-foot per year	0.001233	cubic hectometer per year

# Exhibit 45

BEFORE THE NEW MEXICO WATER QUALITY CONTROL COMMISSION

IN RE: CONDITIONAL CERTIFICATION )  
OF DRAFT NATIONAL POLLUTANT )  
DISCHARGE ELIMINATION SYSTEM )  
(NPDES) PERMIT NO. NM0028355 )  
THE REGENTS OF THE UNIVERSITY OF )  
CALIFORNIA and the UNITED STATES )  
DEPARTMENT OF ENERGY, )  
Petitioners. )



SETTLEMENT AGREEMENT

The United States Department of Energy, The Regents of the University of California (collectively, the "Petitioners"), and the New Mexico Environment Department ("NMED"), agree:

1. Recitals. On October 14, 1992, Petitioners filed a Petition for Review with the New Mexico Water Quality Control Commission ("Commission") appealing the conditional certification dated September 11, 1992, by NMED (the "Conditional Certification") of the draft NPDES Permit published May 16, 1992 (the "1992 Draft NPDES Permit") by the United States Environmental Protection Agency ("USEPA"). Pursuant to an order of the Hearing Officer, the parties met on March 17, 1993 for purposes of negotiation of a possible settlement of this proceeding. At the settlement conference, the parties agreed to certain points of settlement and agreed to continue settlement negotiations. Settlement negotiations have been ongoing since that date, and an agreement in principle with respect to settlement of this matter has been reached.

2. Purpose. The purpose of this agreement is to set forth all of the terms and conditions of the settlement among Petitioners and NMED in this proceeding.

3. Conditional Certification. NMED will withdraw the Conditional Certification and issue a new certification certifying the 1992 Draft NPDES Permit based upon effluent limitations that protect livestock and wildlife watering, as set forth in Section 3-101 and other applicable sections of the New Mexico Water Quality Standards for Interstate and Intrastate Streams in New Mexico ("The New Mexico Water Quality Standards") and other applicable state and federal laws and regulations. The effluent limitations in the certification shall be those set forth in Exhibit 1 to this agreement. Exhibit 1 to this agreement is incorporated into this agreement as if fully set forth in this agreement. The new certification shall provide for a term of the 1992 NPDES Permit of five years from the date issued and shall provide for a reopener clause containing the provisions set forth in paragraph 4 below.

4. Reopener Clause. The 1992 NPDES Permit shall contain a reopener clause to allow the permit to be modified, as required, under the following circumstances:

- (A) to reflect any applicable changes to the New Mexico Water Quality Standards;
- (B) to impose new or additional permit limitations as allowed by law or regulation that

arise as a result of the information obtained from the study referred to below in Section 6;

(C) as provided by law. For the purpose of this paragraph 4C, Petitioners will provide NMED with copies of its annual environmental surveillance reports, the addition and deletion of new outfalls, its waste stream characterization final studies, and its NPDES discharge monitoring reports.

5. Voluntary Dismissal of Petition for Review and Withdrawal of Motions. Petitioners shall file a voluntary dismissal of their Petition for Review and the parties shall withdraw all pending motions after NMED has withdrawn the Conditional Certification and issued the new certification.

6. Study. A study shall be conducted for the purpose of identifying the stream uses associated with the watercourses in the canyons into which Petitioners discharge waters subject to NPDES regulation. The study shall be prepared by a neutral, unbiased, third party who shall be selected as provided under the New Mexico Procurement Code for the provision of services by professional consultants. A four-person selection committee composed of two representatives of Petitioners and two representatives of NMED shall be established. The selection committee shall prepare a request for proposals ("RFP"), including a statement of work, and select the consultant to conduct the study. The parties shall have the right to fully

participate in drafting the RFP, including the scope of workplans and required studies necessary to accomplish the purpose of the study and to review all drafts of the study and provide comments on all drafts.

If the selection committee cannot agree on any matter within its responsibility, the matter shall be referred to a dispute resolution committee whose members shall be the Secretary or Deputy Secretary of NMED, the Associate Director for Operations of the Los Alamos National Laboratory and the Manager of the Los Alamos Area Office of the Department of Energy. The dispute resolution committee shall make a good faith effort to resolve the matter. If the dispute resolution committee cannot unanimously agree on a resolution of the matter, the Secretary of NMED shall make the final decision concerning the matter.

7. NMED Review of Data and Studies. After NMED issues the new certification, the parties shall have the right to submit data and studies, including water quality, hydrological and ecological data and studies, to the consultant selected under the RFP only after prior NMED determination that the water quality data for use by the consultant adheres to the methods authorized under 40 C.F.R. § 136 and Section 1-103 of the New Mexico Water Quality Standards, to the extent that 40 C.F.R. § 136 and Section 1-103 are applicable to the data being submitted. Copies of any data or studies provided to the consultant by NMED shall be provided to Petitioners.

8. Access to Data. The parties shall have the right to access and copy, during normal business hours, all raw and validated data associated with any data or studies submitted to or prepared by the consultant for purposes of conducting the study.

9. Cost of the Study. Petitioners shall contribute up to \$180,000 for fees and costs of the consultant that conducts the study described in paragraph 6.

10. Approval by Commission. Pursuant to paragraph 12 of the Procedural Order entered by the Commission in this proceeding, this agreement is subject to approval of the Commission.

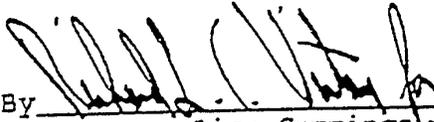
11. Entire Agreement - Binding Effect. This agreement constitutes the entire agreement of the parties and the obligations hereunder shall be binding on the parties and their successors jointly and severally after approval by the Commission.

DATED: April 20, 1993.

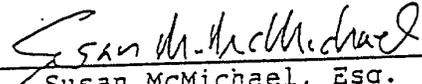
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UNITED STATES DEPARTMENT OF  
ENERGY

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4-20-13

NEW MEXICO ENVIRONMENT DEPARTMENT

By   
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Counsel for the New Mexico  
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P.O. Box 26110  
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APPROVED:

William R. Hendley  
Hearing Officer

APPROVED:

NEW MEXICO WATER QUALITY  
CONTROL COMMISSION

By \_\_\_\_\_  
Chairperson

We hereby certify that we have mailed a copy of the foregoing pleading to the following persons this 21<sup>st</sup> day of April, 1993, *except as noted below*

Ms. Gloria Miller *by hand delivery on April 20*  
Hearing Clerk  
New Mexico Environment Department  
P. O. Box 26110  
Santa Fe, NM 87501

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VIRTUE, WILSON & NAJJAR

By   
Richard L. C. Virtue

stlmmat.agr

April 20, 1993

Parameter <sup>1</sup>	Adjusted WQS/Effluent Limit <sup>2</sup>
Aluminum	5.0 mg/l
Arsenic	0.04 mg/l
Boron	5.0 mg/l
Cadmium	0.2 mg/l
Chromium	5.1 mg/l
Cobalt	1.0 mg/l
Cooper	1.6 mg/l
Lead	0.4 mg/l
Mercury	0.01 mg/l
Radium 226 + 228	30.0 pCi/l
Selenium	0.05 mg/l
Tritium <sup>3</sup>	3X10 <sup>-3</sup> µCi/ml (3,000,000 pCi/l)
Vanadium	0.10 mg/l
Zinc	95.4 mg/l
fecal coliform bacteria <sup>4</sup>	500/100 ml
Chemical Oxygen Demand <sup>5</sup>	125 mg/l
pH <sup>6</sup>	between 6.0 and 9.0 S.U.

All values based upon Water Quality Standards for Interstate and Intrastate Streams in New Mexico (WQS) §3-101.K, unless otherwise noted. All values are expressed as "total." Federal regulation 40 CFR 122.45(c) requires effluent limit values for metals to be expressed as "total." In order to make the transition from dissolved WQS to total effluent limits, the WQS values are translated to "total" utilizing partition coefficients from the EPA document entitled Technical Guidance Manual for Performing Wasteload Allocations, Book II Streams and Rivers Chapter 3 Toxic Substances, EPA-440/4-84-022, June 1984. For parameters with no coefficient in the cited document, the total value is considered to be the same as the dissolved.

<sup>2</sup>Standards adjusted as necessary to TSS=15 mg/l, where partition coefficients are available. TSS value represents average of ambient TSS data collected by NMED May 5-7, 1992.

<sup>3</sup>Based upon WQS §1-102.G. Applicable where meets definition of "pollutant" at 40 CFR 122.2.

<sup>4</sup>In accordance with Work Element 6 of the NM Water Quality Management Plan. Applies only to sanitary outfalls.

<sup>5</sup>As delineated in the July 16, 1992 State certification enclosure pg. 3, ¶ 3, attached hereto as exhibit A.

<sup>6</sup>In accordance with Work Element 6 of the NM Water Quality Management Plan. Applies at

State Certification  
NPDES Permit # KX0023153  
Los Alamos National Laboratory  
July 16, 1992

The fecal coliform limit for these outfalls must be 500/100ml daily maximum.

It is understood that LANL was supposed to eliminate all sanitary outfalls by July, 1992, with the exception of 05S and 11S. However, this has not occurred and fecal coliform limitations apply to all discharges of treated domestic wastewater in New Mexico. Compliance with these limitations can be addressed in the permittee's Federal Facility Compliance Agreement (FFCA) or through a compliance schedule developed by EPA's Enforcement Branch. A waiver for sources without chlorination shall not be written into the permit as currently proposed by the permit writer; especially one that includes a schedule which terminates on a date that violates the permittee's current FFCA and Administrative Order. (See endnotes: 1, 2 & 3).

3. A Chemical Oxygen Demand (COD) effluent limitation of 125 mg/l shall be included in the permit for those outfall categories which exhibited COD values in excess of this value in samples taken either for the permit application or for past Discharge Monitoring Reports. These categories should include, but are not limited to, 051, 045, 045, 095 and all other categories which have a probability of exceeding this value. This limit for these outfalls is necessary in order for conditions of this permit to be compatible with appropriate State regulation which may be found at § 2-101 of the New Mexico Water Quality Control Commission Regulations, as amended through August 17, 1991. (See endnotes: 2 & 3)
4. Mass based effluent limits for Biochemical Oxygen Demand (BOD5) and Total Suspended Solids must be included at outfall 12S. Mass-based effluent limits are required for NPDES permits at 40 CFR 122.45. Mass-based limits should be calculated using 'long term daily average' and 'design maximum' flows at this facility. (See endnote: 3)
5. Limitations and monitoring requirements for radium, tritium, or other naturally occurring and accelerator produced radiological contaminants contributed to the wastewater treatment facilities at TA-50 (outfalls 050 and 051) and TA-53 (outfall 09S) should be included in the permit. We agree with the draft permit that tritium needs to be limited at TA-53; however, we feel the discharge limitation should be 20,000 pCi/l (see above table of WCS). This number should also be applied at Outfalls 050 and 051. (See endnotes: 1, 2 & 3).

# Exhibit 46

BEFORE THE NEW MEXICO WATER QUALITY CONTROL COMMISSION

IN RE: CONDITIONAL CERTIFICATION )  
OF DRAFT NATIONAL POLLUTANT )  
DISCHARGE ELIMINATION SYSTEM )  
(NPDES) PERMIT NO. NMOO28355 )  
THE REGENTS OF THE UNIVERSITY OF )  
CALIFORNIA and the UNITED STATES )  
DEPARTMENT OF ENERGY, )  
Petitioners. )

RECEIVED  
JAN 22 1996  
USFWS - NMESSO

AMENDMENT TO SETTLEMENT AGREEMENT

The United States Department of Energy, The Regents of the University of California (collectively, the "Petitioners"), and the New Mexico Environment Department ("NMED"), (collectively the "Parties") agree:

1. Recitals. The Parties in this matter entered into a Settlement Agreement dated April 20, 1993 (the "Settlement Agreement"). The New Mexico Water Quality Control Commission ("Commission") and the Hearing Officer in this matter subsequently approved that Settlement Agreement. In association with furthering the goals of the Settlement Agreement, the Parties have reached an agreement in principal with respect to certain amendments to the Settlement Agreement.

2. Purpose. The purpose of this Amendment is to modify certain terms and conditions of the Settlement Agreement among Petitioners and NMED in this proceeding.

3. Paragraph 6 of the Settlement Agreement shall be deleted in its entirety and the following language shall be substituted in its place:

6. Study. A study shall be conducted for the purpose of identifying the stream uses associated with the watercourses in the canyons into which Petitioners discharge waters subject to NPDES regulation. The study shall be prepared by the Fish and Wildlife Service of the United States Department of Interior ("U.S. Fish and Wildlife"). The parties believe that U.S. Fish and Wildlife is the most cost effective and technically qualified organization to conduct this study because of its technical expertise, its experience in conducting similar studies for other state and federal agencies, its knowledge of the subject matter covered by the scope of this study and its familiarity with the facility and the surrounding area.

The parties shall have the right to fully participate in and approve the statement of work, scope of workplans and required studies necessary to accomplish the purpose of the study to be conducted by U.S. Fish and Wildlife. If the parties cannot agree upon any of these matters, the dispute shall be referred to a dispute resolution committee whose members shall be the Secretary or Deputy Secretary of the NMED, the Director or Deputy Director of the ESH Division of the Los Alamos National Laboratory and the Manager of the Los Alamos Area Office of the Department of Energy. The dispute resolution committee shall make a good faith effort to resolve the matter. If the dispute resolution committee cannot unanimously agree on a resolution of the matter, the Secretary of NMED shall make the final decision concerning the matter. The parties shall also have the right to review and comment on all drafts of the study prepared by U.S. Fish and Wildlife.

4. Paragraph 7 of the Settlement Agreement shall be deleted in its entirety and the following language shall be substituted in its place:

7. NMED Review of Data and Studies. After NMED issues the new certification, the parties shall have the right to submit data and studies, including water quality, hydrological and ecological data and studies, to U.S. Fish and Wildlife only after prior NMED determination that the water quality data for use by the consultant adheres to the methods authorized under 40 C.F.R. S 136 and Section 1103 of the New Mexico Water Quality Standards, to the extent that 40 C.F.R. S 136 and Section 1103 are applicable to the data being submitted. Copies of any data or studies provided to U.S. Fish and Wildlife by NMED shall be provided to Petitioners.

5. Paragraph 9 of the Settlement Agreement shall be deleted in its entirety and the following language shall be substituted in its place:

9. Cost of the Study. Petitioners shall pay to U.S. Fish and Wildlife up to \$180,000 for the fees and costs of conducting the study described in Paragraph 6 of the Settlement Agreement, as said paragraph is modified by Paragraph 3 of this Amendment to the Settlement Agreement.

6. Approval by Commission. Pursuant to paragraph 12 of the Procedural Order entered by the Commission in this proceeding, this Amendment is subject to approval of the Commission.

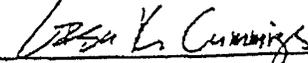
7. Entire Agreement - Binding Effect. The Settlement Agreement, as modified by this Amendment to Settlement Agreement, constitutes the entire agreement of the Parties and the obligations hereunder shall be binding on the Parties and their successors jointly and severally after approval by the Commission.

Los Alamos National Laboratory

By 

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U.S. Department of Energy

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APPROVED:

New Mexico Water Quality  
Control Commission

By *Jim Pate*  
Chairperson

# Exhibit 47

STATE OF NEW MEXICO  
WATER QUALITY CONTROL COMMISSION

IN THE MATTER OF THE PETITION TO AMEND  
20.6.4 NMAC - STANDARDS FOR INTERSTATE AND  
INTRASTATE SURFACE WATERS, THE TRIENNIAL REVIEW



ORDER AND STATEMENT OF REASONS FOR AMENDMENT OF STANDARDS

I. INTRODUCTION

A. Clean Water Act

1. The federal Clean Water Act (CWA), 42 U.S.C. Section 1251(a), states its objective as the restoration and maintenance of the chemical, physical and biological integrity of the Nation's waters.
2. The CWA achieves this objective by ensuring "wherever attainable, water quality which provides for the protection and propagation of fish, shellfish and wildlife, and provides for recreation in and on the water be achieved."
3. CWA Section 1313(c) establishes the purpose of water quality standards ("WQS" or "standards") as "serv[ing] the purposes of the Clean Water Act." The WQS should fulfill the objectives, goals and policies of the CWA.
4. The Environmental Protection Agency's (EPA's) *Water Quality Standards Handbook* (Handbook) provides more specific guidance. To "serve the purposes of the Clean Water Act", WQS must (a) include provisions for restoring and maintaining chemical, physical, and biological integrity of state waters; (b) wherever attainable, achieve a level of water quality that provides for the protection and propagation of fish, shellfish and wildlife, and recreation in and on the water; and (c) consider the use and value of state waters for public water supplies, propagation of fish and wildlife, recreation, agriculture and industrial purposes, and navigation.
5. WQS serve two important purposes: (a) to "define the goals for a water body, or portion, thereof, by designating the use or uses to be made of the water, by setting criteria necessary to protect the uses"; and (b) to "serve as the regulatory basis for the establishment of water-quality-based treatment controls and strategies beyond



**B. Criteria:**

~~[(1) In any single sample: pH within the range of 6.6 to 8.8 and temperature 20°C (68°F) or less.]~~ The use-specific numeric criteria set forth in 20.6.4.900 NMAC are applicable to the designated uses ~~[listed above in Subsection A of this section.~~

~~[(2) The monthly geometric mean of E. coli bacteria 126 cfu/100 mL or less; single sample 410 cfu/100 mL or less (see Subsection B of 20.6.4.14 NMAC)].~~

~~[20.6.4.127 NMAC.- N, 05-23-05, A, XX-XX-XX]~~

367. The Commission adopts the Department's proposal to restructure subsection B for the reasons given in section 101.

**20.6.4.128 RIO GRANDE BASIN - Ephemeral and intermittent portions of watercourses within lands managed by U.S. department of energy (DOE) within LANL, including but not limited to: Mortandad canyon, Cañada del Buey, Ancho canyon, Chaquehui canyon, Indio canyon, Fence canyon, Potrillo canyon and portions of Cañon de Valle, Los Alamos canyon, Sandia canyon, Pajarito canyon and Water canyon not specifically identified in 20.6.4.126 NMAC. (Surface waters within lands scheduled for transfer from DOE to tribal, state or local authorities are specifically excluded.)**

**A. Designated Uses:** livestock watering, wildlife habitat, limited aquatic life and secondary contact.

**B. Criteria:**

~~[(1) The use-specific criteria in 20.6.4.900 NMAC [except the chronic criteria for aquatic life] are applicable [for] to the designated uses [listed above in Subsection A of this section]. except that the following segment-specific criteria apply: the acute total ammonia criteria set forth in Subsection K of 20.6.4.900 NMAC (salmonids absent).~~

~~[(2) The monthly geometric mean of E. coli bacteria 548 cfu/100 mL or less; single sample 2507 cfu/100 mL or less (see Subsection B of 20.6.4.14 NMAC).~~

~~[(3) The acute total ammonia criteria set forth in Subsection K of 20.6.4.900 NMAC (salmonids absent) are applicable to this use.]~~

368. The Commission adopts the Department's proposal to strike the phrase "except the chronic criteria for aquatic life" because chronic criteria are not applicable to the limited aquatic life use in section 900.H.

369. The Commission adopts the Department's proposal to revise the first sentence in subsection B to read "applicable to the designated uses" for consistency with other sections and to restructure subsection B for the reasons given in section 101.

370. The Commission does not adopt Amigos Bravos' proposal to replace limited aquatic life use with aquatic life use because this segment was created and designated uses were assigned in the last triennial review; Amigos Bravos presented no new evidence regarding current water quality conditions that would support a change in the standards.

371. A UAA was completed and approved by EPA for this segment. The UAA noted that the 2002 study referenced by Amigos Bravos "provide[s] information from numerous sources indicating that ephemeral and intermittent streams in the Jemez Mountains

support aquatic life that includes aquatic invertebrates and perhaps amphibians, but not fish." Amigos Bravos relies on information that the Commission already considered in assigning the limited aquatic life use.

372. EPA approved this provision based on the hearing record and the UAA submitted by the Department, and has not indicated any problem with that decision.

373. The UAA for this segment acknowledges the presence of aquatic invertebrates, and even amphibians, but not fish, and therefore concludes that the waters cannot attain the CWA section 101(a)(2) goal of water quality providing for the "protection and propagation of fish, shellfish and wildlife."

**20.6.4.129 RIO GRANDE BASIN - Perennial reaches of the Rio Hondo.**

**A. Designated Uses:** domestic water supply, high quality coldwater aquatic life, irrigation, livestock watering, wildlife habitat and ~~[secondary]~~ primary contact.

**B. Criteria:**

~~[(1) In any single sample: specific conductance 400  $\mu$ mhos/cm or less, pH within the range of 6.6 to 8.8, total phosphorous (as P) less than 0.1 mg/L and temperature 20°C (68°F) or less.]~~ The use-specific numeric criteria set forth in 20.6.4.900 NMAC are applicable to the designated uses ~~[listed above in Subsection A of this section], except that the following segment-specific criteria apply: specific conductance 400  $\mu$ S/cm or less and phosphorus (unfiltered sample) less than 0.1 mg/L.~~

~~[(2) The monthly geometric mean of E. coli bacteria 126 cfu/100 mL or less; single sample 410 cfu/100 mL or less (see Subsection B of 20.6.4.14 NMAC).]~~

[20.6.4.129 NMAC - N, 05-23-05; A, XX-XX-XX]

374. The Commission adopts the Department's proposal to change secondary contact to primary contact for consistency with the assigned criteria for the reasons explained in section 101, change  $\mu$ mhos/cm to  $\mu$ S/cm for the reasons given in section 7.A, replace "total" preceding phosphorus and delete the parenthetical "(as P)" for the reasons given in section 109, and restructure subsection B for the reasons given in section 101.

**20.6.4.130 RIO GRANDE BASIN - The Rio Puerco from the Rio Grande upstream to Arroyo Chijuilla, excluding the reaches on Isleta, Laguna and Cañoncito Navajo pueblos. Some waters in this segment are under the joint jurisdiction of the state and Isleta, Laguna or Cañoncito Navajo pueblos.**

**A. Designated Uses:** irrigation, warmwater aquatic life, livestock watering, wildlife habitat and primary contact.

**B. Criteria:**

(1) The use-specific numeric criteria set forth in 20.6.4.900 NMAC are applicable to the designated uses.

(2) At mean monthly flows above 100 cfs. the monthly average concentration for: TDS 1,500 mg/L or less, sulfate 500 mg/L or less and chloride 250 mg/L or less.

[20.6.4.130 NMAC - N, XX-XX-XX]

J. United States environmental protection agency. [1989] 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Environmental monitoring systems laboratory, Cincinnati, Ohio. ([2nd] 4th Ed., EPA [600/4-89/001] 821-R-02-01). [250] 335 p.

566. The Commission adopts the Department's proposal to correct the edition because a later edition has been issued.
567. The Commission directs the Department to prepare the amended surface water standards in a format acceptable to Records and Archives for filing as part of the New Mexico Administrative Code. This preparation may include re-numbering and re-lettering of existing sections of the standards and the correction of errata consistent with the findings above.



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CHAIR, WATER QUALITY CONTROL COMMISSION

# Exhibit 48

**STATE OF NEW MEXICO  
BEFORE THE WATER QUALITY CONTROL COMMISSION**

**IN THE MATTER OF THE TRIENNIAL REVIEW  
OF STANDARDS FOR INTERSTATE AND  
INTRASTATE SURFACE WATERS, 20.6.4 NMAC**

**WQCC No. 14-05(R)**

**REBUTTAL TESTIMONY OF MICHAEL T. SALADEN  
LOS ALAMOS NATIONAL SECURITY, LLC.**

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1    **I.     INTRODUCTION**

2           I have prepared the following rebuttal testimony in response to the direct testimony of  
3 Rachel Conn and Jon Klingel, submitted on behalf of Amigos Bravos. *See* Amigos Bravos’  
4 Notice of Intent to Submit Technical Testimony (“Amigos Bravos NOI”) (filed Dec. 12, 2014);  
5 Witness Statement of Rachel Conn Submitted on Behalf of Amigos Bravos (“Conn Direct”);  
6 Witness Statement of Jon Klingel Submitted on Behalf of Amigos Bravos (“Klingel Direct”).  
7 Amigos Bravos proposes to change the designated aquatic life use for Stream Segment  
8 20.6.4.128 (“Segment 128”) from “limited aquatic life” to “marginal warmwater aquatic life.”

9           In support of this change, Amigos Bravos’ witnesses assert three central points: (1)  
10 intermittent waters on Los Alamos National Laboratory (“LANL”) property are given weaker  
11 protections than other intermittent waters in New Mexico; (2) the uses for Segment 128 have not  
12 been reassessed for more than 10 years, and are therefore past due for reassessment under 40  
13 C.F.R. § 131.20(a); and (3) the Use Attainability Analysis supporting the current designated  
14 aquatic life use for Segment 128 was inadequate. As explained in my Direct Testimony, filed on  
15 December 12, 2014, the current designated aquatic life use for Segment 128 was adopted by the  
16 New Mexico Water Quality Control Commission (“WQCC”) in the 2004 Triennial Review of  
17 Surface Water Quality Standards, and was approved by the United States Environmental  
18 Protection Agency (“EPA”) in 2007 based on a Use Attainability Analysis (the “2007 UAA”)  
19 prepared by the New Mexico Environment Department (“NMED”) with technical assistance by  
20 EPA. The WQCC rejected a challenge by Amigos Bravos to the current designated aquatic life  
21 use during the 2009 Triennial Review based on similar arguments raised here, finding that the  
22 current designated use for Segment 128 was appropriate, and no change was warranted.

1 In its testimony in the current proceeding, Amigos Bravos has not put forth any new  
2 information or data indicating that a change to the existing designated aquatic life use for  
3 Segment 128 is appropriate.

#### 4 **II. RESPONSE TO RACHEL CONN**

##### 5 **A. Intermittent Waters on LANL Property are Provided Adequate Protections**

6 In her direct testimony, Ms. Conn asserts that the current designated aquatic life use for  
7 Segment 128 is inappropriate because the presence of invertebrates in this segment indicates the  
8 presence of Clean Water Act 101(a)(2) uses requiring protections under a “marginal warmwater  
9 aquatic life” designation for intermittent waters. Conn Direct at 4. She thus suggests that the  
10 presence of invertebrates automatically requires classification of Segment 128 as an intermittent,  
11 as opposed to an ephemeral, water, for which a marginal warmwater aquatic life designation is  
12 required. On this basis, Ms. Conn also criticizes the lack of a distinction between intermittent  
13 and ephemeral waters in the 2007 UAA.

14 Ms. Conn made this same argument in the 2009 Triennial Review. *See* Witness  
15 Statement for Rachel Conn, at 4-5 (August 27, 2009), attached hereto as Rebuttal Exhibit A,  
16 (arguing it is improper to apply the “limited aquatic life use to both ephemeral and intermittent  
17 waters” in Segment 128). However, as was the case in the previous Triennial, the WQCC’s own  
18 regulations provide that a limited aquatic life designated use is appropriate for both ephemeral  
19 *and* intermittent waters. Specifically, 20.6.4.7(L)(2) NMAC states:

20 Limited aquatic life as a designated use, means the surface water is capable of  
21 supporting only a limited community of aquatic life. This subcategory includes  
22 surface waters that support aquatic life selectively adapted to take advantage of  
23 naturally occurring rapid environmental changes, *ephemeral or intermittent*  
24 *water*, high turbidity, fluctuating temperature, low dissolved oxygen content or  
25 unique chemical characteristics.

26  
27 Emphasis added. Thus, the classification of a stream segment as intermittent or ephemeral is not  
28 in itself determinative of whether a limited aquatic life designation is appropriate. Ms. Conn does

1 not, and cannot, contend that the limited aquatic life designation may not be applied to  
2 intermittent waters. Nor does she offer any reasons, data, or explanation as to why limited  
3 aquatic life is not an appropriate designation for Segment 128, beyond simply restating the long-  
4 acknowledged fact that there exists some macroinvertebrate life in that segment, which has  
5 already been considered by the WQCC. WQCC Order and Statement of Reasons for  
6 Amendment of Standards, October 14, 2010, at 81, ¶ 371 (“Amigos Bravos relies on information  
7 [regarding aquatic invertebrates] that the Commission already considered in assigning the limited  
8 aquatic life use.”).

9 With regard to Ms. Conn’s suggestion that the presence of invertebrates indicates the  
10 presence of Clean Water Act 101(a)(2) uses requiring protections under a “marginal warmwater  
11 aquatic life” designation, such protections are not required when, as here, a UAA demonstrates  
12 that attaining that designation is not feasible. A UAA is a scientific study conducted to examine  
13 the factors affecting the attainment of a use. The CWA and WQCC regulations allow a UAA to  
14 be conducted in order to evaluate and assign the appropriate use for any stream segment,  
15 including ephemeral and intermittent streams, if appropriately justified. *See* 40 C.F.R. §  
16 131.10(g); NMAC 20.6.4.15(A)(1). As discussed below in response to Jon Klingel’s direct  
17 testimony, the 2007 UAA was properly prepared and approved, and is sufficient to support the  
18 current designated aquatic life use for Segment 128.

19 **B. LANL Waters are Assessed on a Continuous Basis**

20 Ms. Conn points to 40 C.F.R. § 131.20(a), which requires that water body segments that  
21 do not meet CWA § 102(a)(2) uses must be reexamined every three years, and then suggests that  
22 this regulation has not been followed because “it has been more than 10 years since the waters  
23 subject to 20.6.4.128 NMAC have been afforded 101(a)(2) protections.” Conn Direct at 3. As

1 an active participant in all matters relating to LANL waters, Amigos Bravos is well aware that  
2 Ms. Conn's suggestion that Segment 128 has not been reexamined in over 10 years is incorrect.

3 All stream segments at LANL are assessed on an essentially continuous basis through a  
4 combination of an extensive gage network that is monitored *daily*, and field teams that routinely  
5 walk canyons and observe stream conditions. Moreover, Segment 128 and its designated uses  
6 have been addressed in every Triennial since that segment was adopted. Indeed, Amigos Bravos  
7 has submitted substantively identical petitions regarding Segment 128 in 2004, 2009, and in this  
8 Triennial. Additionally, each assessment unit within Segment 128 is addressed every two years  
9 in NMED's CWA Section 303/305 Integrated Report, available at  
10 <http://www.nmenv.state.nm.us/swqb/303d-305b/>. A map depicting assessment units on LANL  
11 property is attached hereto as Rebuttal Exhibit B.

12 In 2014, LANL field teams photographed gaging station sites, evaluated whether there  
13 was water in the channel, looked for evidence of base flows, identified if benthic  
14 macroinvertebrates were present, and evaluated vegetative cover. Based on information gathered  
15 during these field visits, it was determined that, of the 73 miles of Segment 128, approximately  
16 71 miles are ephemeral and approximately two miles are intermittent (97% ephemeral and 3%  
17 intermittent).

18 Segment 128 has been evaluated in line with, and indeed beyond, the requirements of 40  
19 C.F.R. § 131.20(a). All LANL monitoring information, Triennial documents, and reports are  
20 publicly available. None of this information reveals any changes or concerns warranting a  
21 different designated aquatic life use for Segment 128.

### 22 **III. RESPONSE TO JOHN KLINGEL**

#### 23 **A. LANL Agrees that Intermittent and Ephemeral Streams are Important and** 24 **Need to be Protected**

25

1 Mr. Klingel's testimony contains a lengthy discussion of the importance of ephemeral  
2 and intermittent stream drainages in providing increased primary productivity (food and cover);  
3 increased plant diversity (increased wildlife diversity); increased plant density (food and cover);  
4 recharge of ground water (wells and springs); and periodic surface water for wildlife drinking  
5 and reproduction. Klingel Direct at 2-6. LANL agrees that ephemeral and intermittent streams  
6 are important and need to be protected. LANL maintains that the current designated aquatic life  
7 use for Segment 128, as supported by the 2007 UAA, as well as LANL's and NMED's continued  
8 monitoring and evaluation activities, is appropriate and protective of aquatic life in that segment.

9 **B. The Current Classification of Segment 128 is Appropriate**

10 Mr. Klingel points to what he views as five "serious problems" with the designation of  
11 Segment 128: (1) Segment 128 does not define the location of perennial waters; (2) there is little  
12 documentation of biotic communities found in intermittent streams; (3) the limited aquatic life  
13 designated use does not contain chronic criteria; (4) shell fish have been reported as existing in  
14 Pajarito, Water, Los Alamos and Valle Canyons; and (5) the presence of people bathing and  
15 drinking downstream suggests that "secondary contact" is not appropriate. Klingel Direct at 6-7.

16 Mr. Klingel is correct in that Segment 128 does not provide locations of perennial waters  
17 on LANL property; however, those locations are expressly defined in Segment 126, which  
18 identifies specific geographic landmarks of all perennial LANL segments. *See* 20.6.4.126  
19 NMAC

20 As to documentation of biotic communities in intermittent streams, numerous benthic  
21 studies were conducted by NMED, the United States Fish and Wildlife Service and LANL.  
22 These studies are referenced in the 2002 Use Study prepared by the U.S. Fish and Wildlife  
23 Service ("2002 Use Study"), *see* Saladen Direct at 3, and testimony from previous Triennial  
24 Reviews.

1 Mr. Klingel correctly notes that the limited aquatic life use does not contain chronic  
2 criteria. This is, presumably, because the WQCC recognizes that chronic criteria are not  
3 appropriate for the type of waters with the limited aquatic use. Indeed, during the last Triennial  
4 Review, the WQCC considered the question whether the water quality criteria associated with  
5 the limited aquatic life use were sufficiently protective, given that EPA does not consider that  
6 designation a CWA Section 101(a)(2) use. The Commission confirmed the appropriateness of  
7 the criteria when it adopted the definition in the 2004 Triennial Review and affirmed that  
8 conclusion when it rejected Amigos Bravos' attempt to strike the limited aquatic life use in 2009.  
9 WQCC Statement of Reasons for Amendment of Standards, May 13, 2005; WQCC Order and  
10 Statement of Reasons for Amendment of Standards, October 14, 2010, at 81, ¶ 370. (“[t]he  
11 Commission does not adopt Amigos Bravos’ proposal to replace limited aquatic life with aquatic  
12 life use because this [Segment 128] was created and designated uses were assigned in the last  
13 triennial review; Amigos Bravos presented no evidence regarding current water quality  
14 conditions that would support a change in the standards.”).

15 The shellfish discussed by Mr. Klingel are located in Segment 126 waters, and are  
16 afforded appropriate protections. Mr. Klingel provides no support for his speculation that these  
17 shellfish “possibly” occur in some ephemeral streams on DOE lands. *See supra* at 4 (97% of  
18 Segment 128 is ephemeral). Nor, in my opinion, does Mr. Klingel’s speculation satisfy the  
19 requirement in § 74-6-4.D that water quality standards be “based on credible scientific data and  
20 other evidence appropriate under the Water Quality Act.”

21 Finally, both the 2002 Use Study and the 2007 UAA concluded that recreational  
22 use/primary contact is highly unlikely and, because of the flash-flood nature of any flow, would  
23 be unreasonably hazardous. Moreover, the particular sections where Mr. Klingel speculates that

1 people bathe and otherwise have primary contact (i.e. Pajarito springs drainage) are located in  
2 Segment 20.6.4.98. See Klingel Direct at 6.

3 **C. The 2007 UAA Was Properly Prepared and Approved**

4 As set forth in LANL's direct testimony, the 2007 UAA was prepared by NMED and  
5 approved by EPA. Amigos Bravos does not contend otherwise. Instead, Mr. Klingel argues that  
6 2007 UAA is flawed in a number of respects. Mr. Klingel's arguments regarding the problems  
7 with the 2007 UAA either were, or should have been, made when the UAA was prepared by  
8 NMED and adopted by EPA in 2007. Regardless, Amigos Bravos does not point to any  
9 significant changes with respect to Segment 128 that would warrant any further action or change  
10 in designated uses.

11 **IV. CONCLUSION**

12 In my opinion, the current designated aquatic life use for Segment 128 is appropriate, and  
13 Amigos Bravos has not put forth anything in their direct testimony that would indicate a change  
14 is warranted to that use.



STATE OF NEW MEXICO  
WATER QUALITY CONTROL COMMISSION

\_\_\_\_\_)  
IN THE MATTER OF THE TRIENNIAL REVIEW )  
OF STANDARDS FOR INTERSTATE AND ) WQCC No.08-13 (R)  
INTRASTATE SURFACE WATERS, 20.6.4 NMAC )  
\_\_\_\_\_)

WITNESS STATEMENT FOR RACHEL CONN

*Submitted on Behalf of Amigos Bravos  
August 27, 2009*

Estimated Time for Direct Testimony: 35 minutes

Please Note: Proposed materials to be deleted are indicated by bold strikethrough (red in color copies) and proposed new language is indicated by bold underlining (blue in color copies). NMED's proposed changes are included here as non-bolded (and non-colored) underlined and strikethrough text.

Rachel Conn is the Clean Water Circuit Rider for Amigos Bravos, a non-profit river conservation organization dedicated to protecting the ecological and cultural richness of the Rio Grande and other wild rivers in New Mexico. Ms. Conn has a BA in Environmental Biology from Colorado College. She has worked for the past 11 years in the environmental field. She worked for the Massachusetts Department of Environmental Protection as a consultant assessing the data management needs of the various bureaus in the department. Ms. Conn also worked for a non-profit in Colorado assessing and addressing water quality problems associated with gold mining. For the past seven years she has worked for Amigos Bravos on water quality issues. She is a Clean Water Act trainer and in this capacity gives trainings around the state on water quality standards, TMDLs, and other Clean Water Act topics. As Clean Water Circuit Rider for Amigos Bravos Ms. Conn helps New Mexico communities learn about and then use the Clean Water Act to clean up their rivers.<sup>1</sup>

**1. COMPLIANCE WITH WATER QUALITY STANDARDS**

Currently section 20.6.4.12 states, "The following provisions apply to determining compliance for enforcement purposes; they do not apply for purposes of determining attainment of uses." Because this section is entitled "Compliance With Water Quality Standards" it is assumed that

<sup>1</sup> A resume is attached to this testimony.

AMIGOS BRAVOS  
TECHNICAL TESTIMONY  
RACHEL CONN

PAGE 1 OF 7

SALADEN REBUTTAL  
EXHIBIT A

the enforcement purposes are related to enforcing water quality standards. Compliance with water quality standards is inextricably linked to attainment of uses. In fact, water quality standards are designated uses. As an experienced Clean Water Act trainer, I have given many trainings on the components of water quality standards. These components include designated uses, criteria and antidegradation. These are the basic requirements, as set out by the Clean Water Act, for setting water quality standards. Amigos Bravos urges the Commission to revise this section to accurately reflect the relationship between complying with water quality standards and the attainment of use.

*Amigos Bravos' proposal:*

#### 20.6.4.12 - Compliance with Water Quality Standards

**20.6.4.12 COMPLIANCE WITH WATER QUALITY STANDARDS:** The following provisions apply to determining compliance with **20.6.4 NMAC**. ~~for enforcement purposes they do not apply for purposes of determining attainment of uses. The department has developed assessment protocols for the purpose of determining attainment of uses that are available for review from the department's surface water quality bureau.~~

## **2. FLOW CRITERIA**

In many stretches of river in New Mexico, the applicable criteria are not adequately protecting the designated uses because of lack of flow. To ensure that New Mexico's standards are ensuring that state's criteria protect the state's designated uses (a required component of water quality standards) it is recommended that the state consider including a general criterion for flow in the standards to meet designated uses. Implementation of this general criterion will take some work and guidelines will need to be developed to identify the appropriate adequate flow for each use. For example, to meet the designated use of irrigation, water only needs to be flowing during irrigation season and to meet the wildlife habitat use, flow may not be necessary year round as long as there are pools remaining to provide drinking water to wildlife. EPA regulations require that states set criteria that are "necessary to protect the uses". 40 C.F.R. § 131.2. Seasonal flow is essential to attain the use of irrigation and thus flow is "necessary to protect the uses." Many other states have implemented flow criteria to protect the designated uses of their waters. For example, both the states of Washington and Minnesota have adopted flow criteria.

*Amigos Bravos' proposal:*

#### 20.6.4.13.N – Flow

**N. Flow: If waters of the state are not attaining designated uses due to lack of adequate flow they shall be considered impaired and appropriate planning documents and steps shall be taken.**

### 3. PRIMARY CONTACT

The policy of having secondary contact listed as a designated use and then have site-specific primary contact standards should be stopped. Waters that have primary contact as an existing use should also have it as a listed designated use. The former policy causes undue confusion to the public, and I would assume to the regulators and policy makers as well. This practice makes it especially difficult to review the 303(d) list because there is no indication what is meant when a segment says that secondary contact is "fully supported". There is no way for the public to know if the primary contact criterion is being supported. This has come up time and time again in the trainings and work I have done across the state. Numerous people have come to me saying that they are concerned because their river is not protected for swimming and their family, kids, or neighbors are immersing themselves in the water. Upon closer inspection many of these rivers are indeed protected for primary contact but people are confused because it states secondary contact under the designated uses. In implementing the policy of having waters that are protected by primary contact criteria have a designated use of primary contact, care must be taken to ensure that if there is segment specific criteria that applied previously that was more protective than the criteria that are associated with primary contact, those more protective criteria continue to apply. For example, 20.6.4.115 currently has a designated use of secondary contact but has segment specific criteria for E.coli (monthly geometric mean of 126cfu/100mL or less; single sample 235cfu/100mL or less) that is more protective than the criteria associated with the primary contact use (monthly geometric mean of 120cfu/100mL or less; single sample 410 cfu/100mL). Downgrading of criteria can only occur if a UAA is performed. Care must be taken to ensure that section 20.6.4.115 and any other segment that has more protective criteria than those associated with primary contact maintain the more protective segment specific criteria.

#### *Amigos Bravos' proposal:*

20.6.4.115 RIO GRANDE BASIN - The perennial reaches of Rio Vallecitos and its tributaries, and perennial reaches of Rio del Oso and perennial reaches of El Rito creek above the town of El Rito.

A. Designated Uses: domestic water supply, irrigation, high quality coldwater aquatic life, livestock watering, wildlife habitat and ~~secondary~~ primary contact; public water supply on the Rio Vallecitos and El Rito creek.

B. Criteria:

~~[(1) In any single sample: specific conductance 300 µmhos/cm or less, pH within the range of 6.6 to 8.8 and temperature 20°C (68°F) or less.]~~ The use-specific numeric criteria set forth in 20.6.4.900 NMAC are applicable to the designated uses ~~[listed above in Subsection A of this section],~~ except that the following segments specific criterion criteria applies apply: specific conductance 300 µS/cm or less; the monthly geometric mean of E.coli 126 cfu/100mL or less; single sample of 235 cfu/100mL or less

~~[(2) The monthly geometric mean of E. coli 126 cfu/100 mL or less; single sample 235 cfu/100 mL or less (See Subsection B of 20.6.4.14NMAC.)]~~

#### 4. CONTACT STANDARDS FOR PERENNIAL / INTERMITTENT WATERS

One of the key aspects of the Clean Water Act (CWA) that I always include in my trainings is the Clean Water Act requirement to provide fishable and swimmable waters. This requirement has been clearly expressed by EPA in their comments on New Mexico's water quality standards. As stated by EPA, a use attainability analysis is required before a downgrading of uses from these baseline standards is permitted.

#### 5. KLAUER SPRING

As Clean Water Circuit Rider for Amigos Bravos I have been approached by concerned citizens about the lack of appropriate standards for Klauer Spring, a small spring located about 20 yards from the banks of the Rio Grande near the Taos Junction Bridge. This spring is used by many Taos County residents as their drinking and domestic water supply (see photos attached as Exhibit 1). Clean Water Act regulations require that existing uses be protected (40 CFR 131.10(h) and 40 CFR 131.12(a)(1)). Because domestic water supply is an existing use as demonstrated by the photos, it should be included as a designated use.

*Amigos Bravos' proposal:*

##### 20.6.4.114- Klauer Spring

20.6.4.114 RIO GRANDE BASIN - The main stem of the Rio Grande from the ~~[headwaters of]~~ Cochiti ~~[reservoir]~~ pueblo boundary upstream to Rio Pueblo de Taos, Embudo creek from its mouth on the Rio Grande upstream to the ~~[junction of the Rio Pueblo and the Rio Santa Barbara]~~ Picuris Pueblo boundary, the Santa Cruz river ~~[below]~~ from the Santa Clara pueblo boundary upstream to the Santa Tesuque and Pojoaque pueblos, and the Pojoaque river [below Nambu dam] from the San Ildefonso pueblo boundary upstream to the Pojoaque pueblo boundary, and Klauer Spring.

A. Designated Uses: irrigation, livestock watering, wildlife habitat, marginal coldwater aquatic life, primary contact and warmwater aquatic life; domestic water supply on Klauer Spring and public water supply on the main stem Rio Grande.

#### 6. LOS ALAMOS INTERMITTENT AND EPHEMERAL WATERS

All intermittent waters on LANL property are given weaker protections (those associated with the limited aquatic life use) than all other intermittent waters in the state (which receive the aquatic life use). If EPA had issues with applying limited aquatic life to ephemeral waters in section 20.6.4.97, then they certainly would have a problem with applying the limited aquatic life use to both ephemeral and intermittent waters as is done in section 20.6.4.128. The standards

should be consistently applied unless a UAA has been conducted for a specific segment. If a UAA analysis is conducted that shows that the aquatic life use is not attainable in some ephemeral waters under this segment then a separate segment should be created for those waters. At this point, without an UAA for segment 20.6.4.128, to ensure that all waters are given “fishable/swimmable” protections, an “aquatic life” (rather than a “limited aquatic life” use) is necessary for all waters in 20.6.4.128. There is data that indicates that both intermittent and ephemeral streams on LANL property deserve protection of both the chronic and acute criteria. The US Fish and Wildlife provided testimony in the 2004 Triennial Review that showed many species of aquatic life thrived in these stretches. (Testimony attached as Exhibit 2). In addition, a 2002 study conducted by USFW and USGS found that “[b]ased on location, measure of air and water temperatures, and the presence of coldwater indicator species of aquatic life, these intermittent streams were considered coldwater in nature.” (Study attached at Exhibit 3) The four intermittent streams on LANL property that were studied included Los Alamos Canyon, Sandia Canyon, Pajarito Canyon and Valle Canyon.

*Amigos Bravos’ proposal:*

20.6.4.128 - Los Alamos Intermittent and Ephemeral Waters

20.6.4.128 RIO GRANDE BASIN - Ephemeral and intermittent portions of watercourses within lands managed by U.S. department of energy (DOE) within LANL, including but not limited to: Mortandad canyon, Cañada del Buey, Ancho canyon, Chaquehui canyon, Indio canyon, Fence canyon, Potrillo canyon and portions of Cañon de Valle, Los Alamos canyon, Sandia canyon, Pajarito canyon and Water canyon not specifically identified in 20.6.4.126 NMAC. (Surface waters within lands scheduled for transfer from DOE to tribal, state or local authorities are specifically excluded.)

A. Designated Uses: livestock watering, wildlife habitat, limited aquatic life and secondary contact.

**7. COOLWATER CRITERIA**

The current water quality standards allow for five categories of temperature criteria: high quality coldwater, coldwater, marginal coldwater, warmwater, and marginal warmwater. Adding more categories brings up that waters will be placed into whatever category it presently fits rather than classifying for the appropriate designated use, i.e. its historical or appropriate use, and then working toward achieving that condition. In particular, as climate change causes New Mexico’s waters to become more limited, and thus more susceptible to temperature change, there is a risk that the addition of another category will enable the categorizing what are appropriately coldwater streams as coolwater.

## 8. LIMITED AQUATIC LIFE

The designated use of "limited aquatic life," set forth at 20.6.4.900(H)(7), is ambiguous and confusing. The standards would be clearer and more in line with the goals of the Clean Water Act if there was a return to the pre-2005 policy of setting segment specific uses in the rare case where the other aquatic life uses are not attainable. For instance, in the case of Sulphur Creek, Section 20.6.4.124 it would be simple to say under paragraph B(3) that, "except for subsections I and J of 20.6.4.900, the chronic aquatic life criteria do not apply." The limited aquatic life use adds one more layer of confusion to the standards requiring members of the public to flip back and forth between the segment and the back of the standards. In addition, the limited aquatic life use could be abused to lower water quality standards. It is more appropriate to make segment specific changes in cases where the natural conditions have resulted in an impairment associated with either the chronic or acute aquatic life criteria. This method would allow for more fine tuned standards. For example, in some cases it may be that none of the chronic life criteria are attainable, and therefore all the criteria could be listed as not applying, but, in some other cases, it may be that only a couple of the chronic life criteria do not apply and in those cases these constituents could be listed individually. Returning to the pre-2005 policy also ensures that water quality standards are applied equitably and that standards are modified only when natural conditions necessitate such changes. Getting rid of the limited aquatic life use would not require a large overhaul to the standards as presently only three segments have the limited aquatic life designated use.

EPA's disapproval of the use of the limited aquatic life use for ephemeral waters is consistent with this point. EPA noted that "this limited use does not 'serve the purposes of the [CWA], as defined in CWA sections 101(a)(2) and 303(c)." See Discussion Draft, § 20.6.4.97 NMAC, Basis for Change. Although NMED has addressed this concern in part by requiring that ephemeral waters shall be classified as such by a hydrology protocol, it did not address the concern that such waters automatically include a limited aquatic life use, when they may qualify for a more protective standard. Organisms in ephemeral waters are often especially sensitive to changes, and thus ensuring that chronic life criteria are applied can be crucial to the survival of those species. As such, a separate limited aquatic life designation is inappropriate. At most, the criteria specified in the limited aquatic life designation should be applied on a segment-specific basis.

*Amigos Bravos' proposal:*

### 20.6.4.900(H)(7) - Limited Aquatic Life Use

~~{(6)}(7) Limited Aquatic Life: Criteria shall be developed on a segment-specific basis. The acute aquatic life criteria of Subsections I and J of this section shall apply to this subcategory. Chronic aquatic life criteria do not apply unless adopted on a segment-specific basis. Human health-organism only criteria apply only for persistent pollutants unless adopted on a segment-specific basis.~~

## **9. HARDNESS TABLE FOR ACUTE AND CHRONIC CRITERIA FOR METALS**

The Department's proposal of a hardness table for acute and chronic criteria for metals (20.6.4.900.I) will greatly increase the public's ability to understand the standards. This addition will also help me, as a Clean Water Act Trainer, to help people understand the standards.

## **10. DOMESTIC WATER SUPPLY CRITERIA**

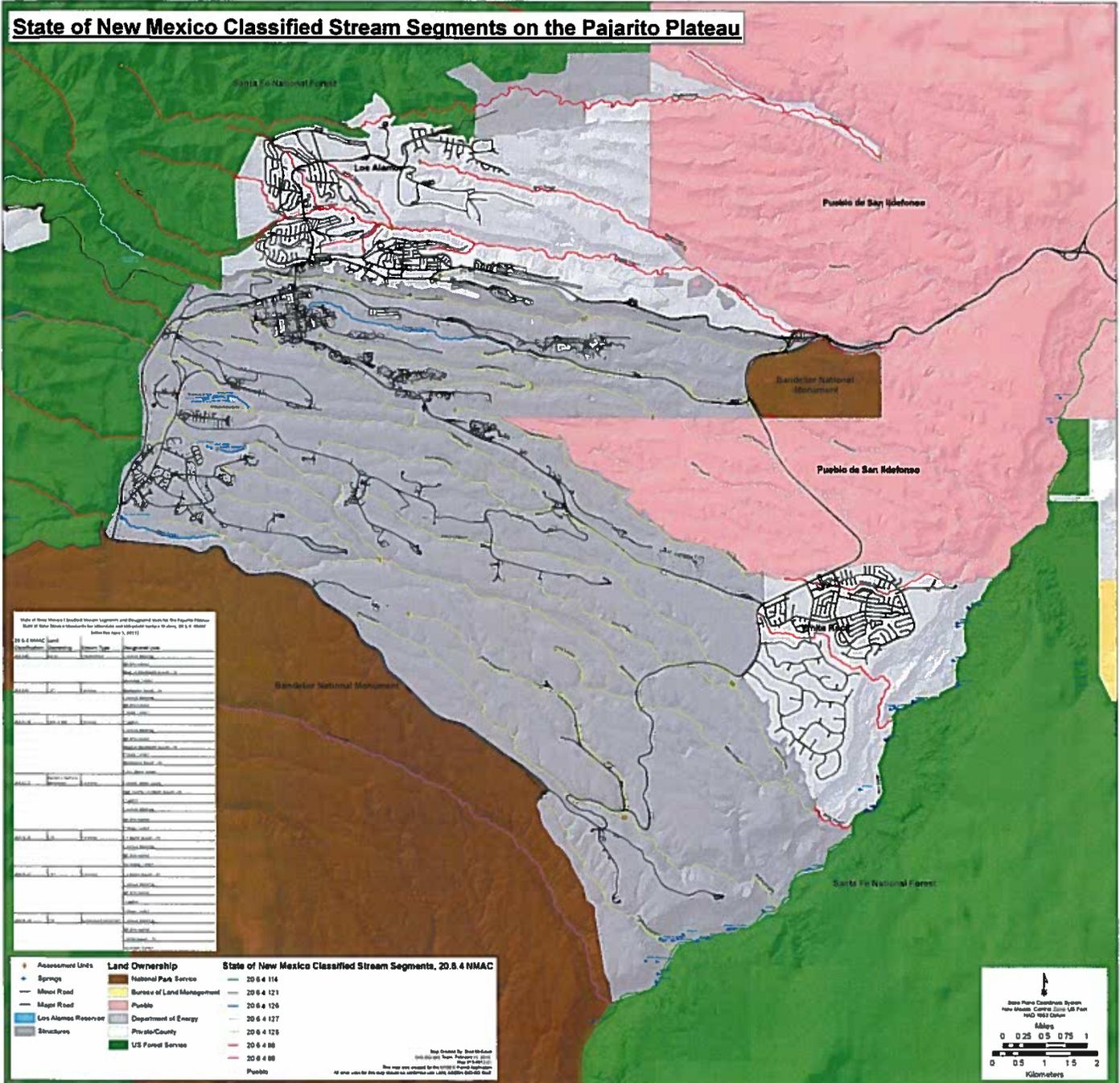
The Department's proposed changes to the domestic water supply use in most cases weaken the associated criteria because the proposed changes disregard the potential health effects to people who both drink the water and eat fish from the same water source. The EPA recommended criteria for consumption of water plus organism (these were the standards that the WQCC currently applies to the domestic water supply use) should continue to apply to the domestic water supply use. These criteria can be found in the November 2002 EPA Human Health Criteria Calculation Matrix. As a Clean Water Act trainer and through my work on New Mexico water policy issues, to my knowledge, all waters that have a domestic water supply use also has an aquatic life use and thus it is likely that some people both fish and drink from these waters. In fact, it is much more likely that both uses are conducted on the same waters than not. Many of the waters where people fish are also waters where people hike and camp and consume water. To protect these existing uses the more sensitive criteria for consumption of water and organism should apply. In addition, if protections are downgraded from consumption of water and organisms to only protecting for consuming water, a UAA is required. To my knowledge, UAAs for the multiple segments impacted have not been conducted.

## **11. 6T3 AND 4T3**

The Department's 7/6/09 proposal to include these new definitions and temperature criteria under the designated uses is of concern. Unfortunately the on the ground impacts of these additions appears to be a lowering of water quality standards. For example, the previous maximum standard for the marginal coldwater use was 25 degrees C but now the maximum temperature is 29 degrees C and the 6T3 temperature is 25 degrees C. I question whether the Department rarely, if ever, is out sampling the same location for 4 consecutive hours on four or more consecutive days. If these sampling conditions are rarely, if ever, met then the end result is basically increasing the maximum temperature criteria (since this will be the only criteria for which there will be monitoring data) for each designated aquatic use.

**Submitted by:  
Rachel Conn  
August 27, 2009**

**State of New Mexico Classified Stream Segments on the Pajarito Plateau**



# Exhibit 49

An official website of the United States government.



## Chemicals of Emerging Concern in the Columbia River



### What are chemicals of emerging concern?

Chemicals of emerging concern (also called "contaminants of emerging concern" or "CECs") can include nanoparticles, pharmaceuticals, personal care products, estrogen-like compounds, flame retardants, detergents, and some industrial chemicals with potential significant impact on human health and aquatic life.

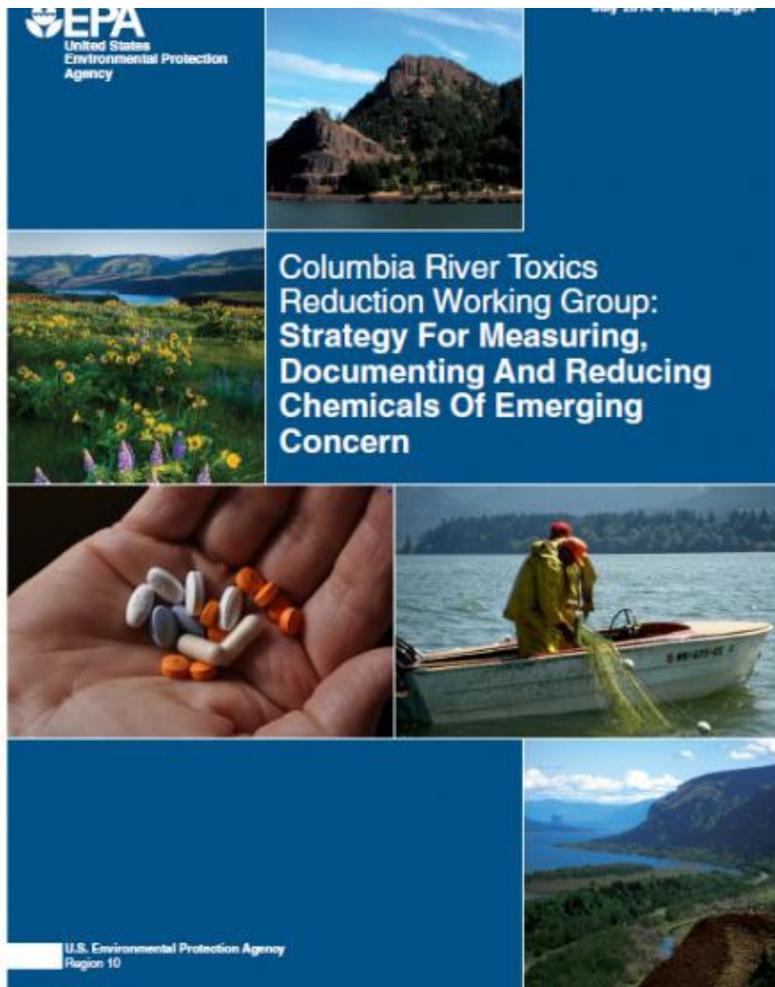
Some examples are:

- PAHs, PCBs, and PBDEs found throughout the lower Columbia River in water, sediment, and juvenile Chinook salmon. These contaminants are moving from river water and sediment into salmon prey and then into salmon tissue.
- 49 different chemicals of emerging concern were detected in sediments in the lower Columbia River main stem and several tributaries. Endocrine-disrupting compounds (contaminants that block or mimic hormones in the body and cause harm to fish and wildlife) were detected at 22 of 23 sites sampled.
- A myriad of pharmaceuticals and personal care products were detected in the effluent from wastewater treatment plants discharging to the Columbia River.

### Learn more about CECs:

- [Fish tissue contamination and CECs](#)
- [CECs including pharmaceuticals and personal care products](#)





## Research and Monitoring

The Columbia River Toxic Reduction Working Group's [Toxics Reduction Action Plan](#) identified the need for a Basin-wide research plan on contaminants of concern. Though some research on the effects of contaminants in the Basin ecosystem was being conducted by different agencies, there was no coordinated effort to identify the research priorities or gaps in our knowledge.

In 2014, the Working Group released its *Strategy for Measuring, Documenting and Reducing Chemicals of Emerging Concern* which provides an outline for a research and monitoring strategy, and a characterization of the biological impacts of CECs on aquatic and terrestrial wildlife.

### Read the report:

- [Columbia River: Strategy for Measuring, Documenting, and Reducing Chemicals of Emerging Concern](#)

LAST UPDATED ON FEBRUARY 5, 2019

# Exhibit 50

**WHITE PAPER**

**AQUATIC LIFE CRITERIA FOR CONTAMINANTS OF  
EMERGING CONCERN**

**PART I**

**GENERAL CHALLENGES AND RECOMMENDATIONS**

**Prepared by the  
OW/ORD Emerging Contaminants Workgroup**

**June 03, 2008**

**NOTICE**

**THIS DOCUMENT IS AN INTERNAL PLANNING DOCUMENT**

**It has been prepared for the purpose of Research & Development Planning.  
It has not been formally released by the U.S. Environmental Protection Agency and should  
not at this stage be construed to represent Agency guidance or policy.**

**DRAFT DOCUMENT**

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## **List of Acronyms:**

ACR	Acute to Chronic Ratio
ALC	Aquatic Life Criteria
Ah	Aryl Hydrocarbon (receptor)
AV	Acute (toxicity) value
AWQC	Ambient Water Quality Criteria
CCC	Criterion Continuous Concentration
CEC	Contaminants of Emerging Concern
CMC	Criterion Maximum Concentration
CV	Chronic (toxicity) value
CWA	Clean Water Act
CYP	Cytochrome enzymes (P450)
EDC	Endocrine Disrupting Chemicals
E2	Estradiol (natural estrogen)
EE2	Ethinylestradiol (synthetic pharmaceutical estrogen)
ELS	Early Life-Stage (toxicity test)
EPA	Environmental Protection Agency
FAV	Final Acute Value
FACR	Final Acute to Chronic Ratio
GMAV	Genus Mean Acute Value
GMCV	Genus Mean Chronic Value
HPG	Hypothalamic-Pituitary-Gonadal (axis)
HPT	Hypothalamic-Pituitary-Thyroid (axis)
LOEC	Lowest Observed Effect Concentration
MDR	Minimum Data Requirement
MOA	Mode of Action
NOEC	No Observed Effect Concentration
OECD	Organization for Economic Development and Cooperation
OWC	Organic Wastewater Contaminant
PBDE	Polybrominated Diphenyl Ether
PPCP	Pharmaceutical and Personal Care Product
POP	Persistent Organic Pollutant
SMAV	Species Mean Acute Value
TBT	Tributyltin
USGS	United States Geological Survey
VTG	Vitellogenin protein
<i>vtg</i>	Vitellogenin gene transcript
WWTP	Wastewater Treatment Plant

## 1.0 INTRODUCTION

Under the United States Clean Water Act (CWA) (33 U.S.C. Sections 1251-1387), EPA is required to take a number of actions to protect and restore the ecological integrity of the Nation's water bodies. Under Section 304(a) of the CWA, EPA must develop and publish ambient water quality criteria. Ambient water quality criteria (AWQC) are levels of individual pollutants, water quality characteristics, or descriptions of conditions of a water body that, if met, should protect the designated use(s) of the water. Examples of designated uses of a water body include swimming, drinking water, fishing, fish spawning, and navigation. States and authorized tribes establish designated uses for their water bodies. AWQC are recommended guidance that states and tribes may use as part of their water quality standards to protect water bodies for their designated use from chemical pollutants.

AWQC for aquatic life (aquatic life criteria, ALC) developed under Section 304(a) reflect the "latest scientific knowledge" concerning "all identifiable effects" of the pollutant in question. These criteria are based solely on data and scientific determinations on the relationship between environmental concentrations of the pollutant and its effects. Criteria do not consider social and economic impacts, or the technological feasibility of meeting the chemical concentration values in ambient water. Since the early 1980's, EPA has been developing ALC to protect aquatic organisms from chemical specific pollutants under Section 304(a) of the CWA. In 1985, EPA published *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (hereafter referred to as the "*Guidelines*"; Stephan et al. 1985). The *Guidelines* has provided uniformity and transparency in the derivation methodology of ALC for a large number of compounds among several classes of chemicals. The majority of EPA's currently recommended ALC have been derived using the methods outlined in the *Guidelines*.

While the *Guidelines* remain the primary instrument the Agency uses to meet its broad objectives for the development of ALC, there have been many advances in aquatic sciences, aquatic and wildlife toxicology, population modeling, and ecological risk assessment that are relevant to deriving ALC. Some of the advances have been addressed through supplemental guidance on the derivation or site-specific modification of criteria (Prothro 1993; U.S. EPA 1994a), while others have been incorporated directly into derivation of individual ALC for certain chemicals (e.g., saltwater chronic aquatic life criterion for tributyltin, U.S. EPA 2003). Recently, considerable attention has been generated by a widely ranging group of chemicals termed, in this document, contaminants of emerging concern (CECs). As is discussed in the body of this document, some of these CECs present challenges for the application of the *Guidelines* to ALC development.

### 1.1 What is a Contaminant of Emerging Concern?

The term "contaminant of emerging concern" is being used within the Office of Water to replace "emerging contaminant," a term that has been used loosely since the mid-1990s by EPA and others to identify chemicals and other substances that have no regulatory standard, have been recently "discovered" in natural streams (often because of improved analytical chemistry detection levels), and potentially cause deleterious effects in aquatic life at environmentally

relevant concentrations. They are pollutants not currently included in routine monitoring programs and may be candidates for future regulation depending on their (eco)toxicity, potential health effects, public perception, and frequency of occurrence in environmental media. CECs are not necessarily new chemicals. They include pollutants that have often been present in the environment, but whose presence and significance are only now being evaluated.

CECs include several types of chemicals:

- Persistent organic pollutants (POPs) such as polybrominated diphenyl ethers (PBDEs; used in flame retardants, furniture foam, plastics, etc.) and other global organic contaminants such as perfluorinated organic acids;
- Pharmaceuticals and personal care products (PPCPs), including a wide suite of human prescribed drugs (e.g., antidepressants, blood pressure), over-the-counter medications (e.g., ibuprofen), bactericides (e.g., triclosan), sunscreens, synthetic musks;
- Veterinary medicines such as antimicrobials, antibiotics, anti-fungals, growth promoters and hormones;
- Endocrine-disrupting chemicals (EDCs), including synthetic estrogens (e.g., 17 $\alpha$ -ethynylestradiol, which also is a PCPP) and androgens (e.g., trenbolone, a veterinary drug), naturally occurring estrogens (e.g., 17 $\beta$ -estradiol, testosterone), as well as many others (e.g., organochlorine pesticides, alkylphenols) capable of modulating normal hormonal functions and steroidal synthesis in aquatic organisms;
- Nanomaterials such as carbon nanotubes or nano-scale particulate titanium dioxide, of which little is known about either their environmental fate or effects.

## 1.2 Why is EPA Concerned with CECs?

The variety of chemicals labeled as CECs leads to a variety of concerns for EPA. Widespread uses, some indication of chemical persistence, effects found in natural systems, and public concerns have made clear the need for EPA to develop criteria that can be used to help assess and manage potential risk of some CECs in the aquatic environment. A recent U.S. Geological Survey (USGS) reconnaissance study (Kolpin et al. 2002) provides a good example of the prevalence of a wide range of CECs in U.S. streams. Improved analytical chemistry techniques were used to document the occurrence of what the authors called organic wastewater contaminants (OWCs) being released into surface waters from wastewater treatment plants (WWTPs). The targeted OWCs included PPCPs, veterinary medicines and other EDCs. The investigators found at least one of 95 different target OWCs in 80 percent of the 139 streams sampled. A median of seven, and as many as 38, OWCs were found in single samples.

The use and occurrence patterns associated with CECs are varied. Some CECs are similar to conventional toxic pollutants in that they are associated with industrial releases, whereas many others are used by the general public every day in homes, on farms, by businesses and industry (Daughton and Ternes 1999). PPCPs acting as EDCs can be released directly to the environment after passing through wastewater treatment processes, which are typically not designed to remove these pollutants from the effluent (Halling-Sorensen et al. 1998). Sludge from secondary treatment processes are land-applied as biosolids, supplying CECs which may leach or run off into nearby bodies of water. Pharmaceuticals used in animal feeding operations may be released

to the environment in animal wastes via direct discharge of aquaculture products (i.e., antibiotics), the excretion of substances in animal urine and feces of livestock animals, and the washoff of topical treatments from livestock animals (Boxall et al. 2003).

EDCs discharged at WWTPs are one group of CECs with potentially widespread environmental effects (Folmar et al. 1996; Folmar et al. 2001; Jobling et al. 1998; Woodling et al. 2006). Although particular concern has been expressed about the anthropogenic EDCs, there are also natural estrogens (estradiol and its metabolites estriol and estrone) entering the aquatic environment through wastewater discharge and excretion from domestic animals. Furthermore, little is known about the environmental occurrence, fate and, transport for any of these compounds after they enter aquatic ecosystems. Many of the man-made compounds have been in use for a long time, and there is concern about pharmacologically active ingredients and personal care products that are designed to stimulate a physiological response in humans, plants, and animals (Daughton and Ternes 1999).

Frequent detection of compounds by itself does not constitute a need for ALC. Rather, criteria development for CECs needs to focus efforts on chemicals that demonstrate a reasonable potential to adversely affect aquatic life. Of CECs now known to be found in some surface waters of the U.S., EDCs have received the most attention because field studies from around the world have demonstrated that very low concentrations of some of these compounds can significantly impact natural populations of aquatic vertebrates. For example, observational field studies (Jobling et al. 1998) have shown a high occurrence of intersex (the presence of both male and female characteristics) in wild populations of a fish known as roach (*Rutilus rutilus*) in rivers in the United Kingdom that are downstream from WWTPs. Similar results have recently been reported for white sucker (*Catostomus commersoni*) in northern Colorado, U.S.A (Woodling et al. 2006). In a multiyear study by Kidd et al. (2007), the authors showed that environmentally relevant concentrations of ethynylestradiol, EE2, caused reproductive failure and near collapse of a natural fathead minnow population in an experimental lake, and also had deleterious effects on the reproductive biology of the pearl dace. These direct effects resulting in loss of forage fish have led to cascading effects on the lake trout population due to lack of prey (Kidd, personal communication). Researchers from the U.S. Geological Survey (USGS) have observed intersex and testis-ova (the presence of eggs in the testis) in bass species collected from the Potomac River and its tributaries in West Virginia, Maryland, and Washington DC, and also quantified EDCs in their blood (Blazer et al. 2007; Chambers and Leiker 2006). The occurrence of intersex fish in the Potomac River, as well as documented occurrence of this and related effects in other waters of the US and internationally, prompted Congressional hearings that were held in October 2006 to inquire about the “State of the Science on EDCs in the Environment,” as well as EPA activities associated with EDCs.

### **1.3 Purpose and Organization of This White Paper**

The purpose of this white paper is to provide general guidance on how criteria development for CECs could be facilitated through a supplemental interpretation of the *Guidelines*, with particular attention to PPCPs with an EDC mode of action (MOA). Section 2 of this part (Part I) of the white paper describes the *Guidelines* procedures and identifies several areas in which these

procedures could be modified to address potential limitations for deriving criteria for CECs. Section 3 expands upon the areas of concern with respect to specific toxicological characteristics of some CECs. Section 4 summarizes these concerns and provides recommendations that could aid in the development of criteria for CECs in a resource efficient manner that takes best advantage of existing knowledge. Part II of this white paper further describes these concerns and recommendations using data for the synthetic pharmaceutical estrogen EE2.

## 2.0 CURRENT ALC METHODOLOGY

The *Guidelines* specify various data and procedural recommendations for criteria derivation, and also define general risk management goals for criteria, which are to provide a high level of protection for aquatic communities and for important species in these communities. ALC are defined to consist of two concentrations – the Criterion Maximum Concentration (CMC), intended to protect against severe acute effects, and the Criterion Continuous Concentration (CCC), intended to protect against longer term effects on survival, growth, and reproduction. The CMC is used in criteria to limit peak exposures by requiring that 1-hour averages of exposure concentrations not exceed the CMC more often than once in three years on average. The CCC is used in criteria to limit more prolonged exposures by requiring that 4-day averages of exposure concentrations not exceed the CCC more often than once in three years on average.

The CMC and CCC are usually derived from laboratory toxicity test results using specific standard procedures described in the *Guidelines*, but the *Guidelines* also have general provisions for deviating from these procedures as warranted by available information. The following text will first give an overview of the data requirements and calculations in the standard procedures, and then discuss how these procedures might vary under the umbrella of the *Guidelines*.

### 2.1 Standard ALC Derivation Procedures

The CMC is determined based on available Acute Values (AVs) – median lethal concentrations ( $LC_{50}$ s) or median effect (for a severe acute effect such as immobilization) concentrations ( $EC_{50}$ s) from aquatic animal acute toxicity tests (48- to 96-hours long) meeting certain data quality requirements. To compute a CMC, the *Guidelines* require that acceptable AVs be available for at least eight genera with a specified taxonomic diversity, in order to address a wide variety of the organisms constituting an aquatic animal community. These minimum data requirements include three vertebrates (a salmonid fish, a fish from a family other than salmonidae, and a species from a third chordate family) and five invertebrates (a planktonic crustacean, a benthic crustacean, an insect, a species from a phylum other than Chordata or Arthropoda, and a species from another order of insect or a fourth phylum).

For each genus, a Genus Mean Acute Value (GMAV) is calculated by first taking the geometric mean of the available AVs within each species (Species Mean Acute Value, SMAV) and then the geometric mean of the SMAVs within the genus. The fifth percentile of the set of GMAVs so obtained is calculated based on a specified estimation method, and designated the Final Acute Value (FAV). The FAV is then lowered to the SMAV for an important, sensitive species if appropriate. The CMC is set equal to half of the FAV to represent a low level of effect for the fifth percentile genus, rather than 50% effect.

The CCC is generally determined based on available Chronic Values (CVs), which are either (a) the geometric mean of the highest no-observed-effect concentration (NOEC) and lowest

observed effect concentration (LOEC) for effects on survival, growth, or reproduction in aquatic animal chronic tests or (b) in some recent criteria (e.g., ammonia), the  $EC_{20}$  in such tests based on concentration-effect regression analyses. Chronic tests for invertebrate species are required to include the entire life-cycle, but for fish partial life-cycle and early life-stage (ELS) testing protocols are accepted, the latter not including reproductive endpoints and not used if life-cycle or partial life-cycle tests are available and show more sensitive adverse effects.

If CVs are available for at least eight genera with the required taxonomic diversity, the CCC is set to the fifth percentile of Genus Mean Chronic Values (GMCVs), by the same procedure used to derive an FAV from GMAVs. Otherwise, the CCC is set to the FAV divided by a Final Acute Chronic Ratio” (FACR) that is based on acute to chronic ratios (ACRs – the ratio of the AV to the CV from parallel acute and chronic tests) for at least three species with a specified taxonomic diversity. The CCC can also be based on plant toxicity data if aquatic plants are more sensitive than aquatic animals, or on other data as deemed scientifically justified.

Further details on test requirements and calculation methods for the CMC and CCC are specified in the *Guidelines*, including deriving criteria that are a function of water quality characteristics.

## 2.2 Alternatives for ALC Derivation

The procedures described above enable broad application to toxic chemicals generally, and are only constrained by specific data requirements for quality and minimum taxonomic representation. Since they are not restricted with respect to specific types of chemicals, there is no reason to suppose that the standard data requirements and procedures specified by the *Guidelines* are any more or less applicable to CECs than to the chemicals for which criteria have already been developed. The *Guidelines* anticipated that rote application of the basic procedures may not yield the most appropriate criteria; consequently, the *Guidelines* provide flexibility when appropriate for deviation from the normal procedures regardless of the type of chemical, as indicated by the following provisions (hereafter referred to as the "Good Science" clauses:

*“These National Guidelines should be modified whenever sound scientific evidence indicates that a national criterion produced using these Guidelines would probably be substantially overprotective or underprotective of aquatic organisms and their uses on a national basis.”* (p. 18).

*"On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific evidence. If it is not, another criterion, either higher or lower, should be derived using appropriate modifications of these Guidelines."* (p. 57).

In addition, although the standard procedures in the *Guidelines* for deriving a CMC and CCC use only toxicity tests meeting certain requirements, the *Guidelines* also mandate the collation and examination of other data that might show effects that should be considered in criteria derivation:

*"Pertinent information that could not be used in earlier sections might be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on ... any other adverse effect that has been shown to be biologically important. Especially important are data for species for which no other data are available. ... Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important."* (p. 54).

While alternatives are allowed when a specific situation dictates, the *Guidelines* still require that any changes in the procedures are consistent with the level of protection represented by the standard procedures:

*"Derivation of numerical national water quality criteria for aquatic organisms and their uses is a complex process and requires knowledge in many areas of aquatic toxicology; any deviation from these Guidelines should be carefully considered to ensure that it is consistent with other parts of these Guidelines."* (p. iv).

Therefore, for the development of criteria for any chemical, the general strategy should be to start with the standard *Guidelines* procedures and then to adapt those procedures as warranted by available information on the effects of the chemical. This strategy applies to CECs as well, although certain considerations might more consistently be important for CECs. Specific attributes of CECs that might affect criteria derivations are considered in Section 3 of this paper, but several issues are introduced here that are of general concern.

#### ***Are data on acute toxicity needed for risk assessments?***

Some chemicals are not acutely toxic even at concentrations so high that they could not possibly occur in the environment (e.g., at the chemical solubility, or exceeding exposures possible based on known chemical production and discharges). The acute lethality of some classes of chemicals might be measurable, but would occur at environmental concentrations so much higher than those affecting reproduction, growth, or chronic survival that, in practice, environmental exposures will always be far below acutely lethal levels if those exposures are managed to limit chronic effects. Therefore, derivation of the CMC might be unnecessary or impossible. Thus, if the existing data indicate that it is reasonably certain that acute toxicity would not occur at environmentally relevant concentrations, conducting additional acute tests is likely to be unwarranted.

Even if a CMC is not needed, another use of acute toxicity data is for developing "acute to chronic ratios" (ACRs) that are used with the FAV to calculate the CCC (see pages 40-42 in the *Guidelines*), so that dropping acute testing requirements must consider this consequence as well. However, if acute effect concentrations are extremely high compared to chronic effect concentrations (large ACRs), whether the ACR approach should be even used warrants some consideration. Large ACRs are not, *per se*, less accurate than low ACRs, provided acute and chronic effect concentrations are well defined and the issue is simply extrapolating from acute to chronic toxicity within a species. However, for criteria calculations, the FACR needs to be a ratio that extrapolates from the fifth percentile of the acute effect concentration distribution to the fifth percentile of the chronic effect distribution. This requires appropriately combining ACR

information across species, the accuracy of which might be affected by large ACRs even if the accuracy of the individual ACRs is not. Therefore, in addition to not needing a CMC, extreme acute tolerance might also warrant direct calculation of the CCC rather than using the ACR approach, and thus eliminate the need for fulfilling all of the minimum acute toxicity test requirements as specified by the *Guidelines*.

***How should data requirements for tolerant taxa be addressed?***

The fifth percentile calculation methods for the CMC (as well as the CCC if the eight minimum data requirements noted above are met) require actual GMAV (or GMCV) values only for the four most sensitive genera. For more tolerant genera, it is only necessary to know that these toxicity values are greater than those of the four sensitive species. Therefore, toxicity test results that report "greater than" effect concentrations are acceptable for the tolerant taxa, and in fact are used in various criteria already.

If chronic tests have not already been done on some taxa needed for the minimum data requirements, but which are known to be tolerant, testing resources might be wasted by generating numbers that will not affect results. If methods such as inter-chemical or inter-species extrapolation methods, or assays (e.g., *in vitro* tests, biomarkers) that have been related to apical effects such as reductions in growth, survival, or reproduction can demonstrate these taxa to be insensitive compared to other taxa, actual chronic tests on these taxa may not be needed. In other words, can minimum data requirements for tolerant taxa be satisfied by some type of estimation rather than by an actual test result?

However, adding estimated data can become a rather open-ended process. Therefore, consideration must be given to how many estimated values should be allowed, relative to measured values, to produce an appropriate distribution of taxa in the data set used for criteria derivation.

***Should fish chronic tests be required to address reproduction?***

For chemicals (e.g., environmental estrogens) for which reproductive toxicity is of most concern, the allowance in the *Guidelines* for using ELS tests might need reconsideration. The *Guidelines* already give priority to life-cycle and partial life-cycle tests when they are available and show greater sensitivity than ELS tests. However, other information (from other species, similar chemicals, knowledge of the MOA) regarding latent or multigenerational reproductive effects may demonstrate the importance of sexual development and reproduction, so as to establish a basis for not considering ELS test results (or even partial life-cycle tests), but rather requiring life-cycle tests for fish.

***What endpoints can serve as surrogates for traditional chronic endpoints?***

Although chronic criteria are and will continue to be based on effects on reproduction, growth, and survival, another issue is whether only toxicity data directly addressing these endpoints is acceptable. Is there additional information (e.g., sub-organismal biomarkers, behavioral data) that can be used in criteria derivation because they are adequately correlated to reproduction, growth, and survival? Use of such data would be consistent with the *Guidelines* requirements to examine all pertinent data and make modifications to the criteria derivation procedures that are consistent with sound scientific evidence

### **2.3 Precedent for Deviating from Basic ALC Derivation Procedures**

The recent ALC document for tributyltin (TBT) provides a good example of some of the types of procedural criteria modifications discussed above. TBT is a highly toxic biocide that has been used extensively in anti-fouling paint to protect the hulls of large ocean-going ships. It is deemed a problem in the aquatic environment because it is extremely toxic to non-target organisms, and has been linked to imposex (the superimposition of male anatomical characteristics on females) and to immuno-suppression in snails and bivalves (U.S. EPA 2003). The concentrations reported to cause imposex in the laboratory are lower (range: 0.0093 to 0.0334  $\mu\text{g/L}$ ) than the FCV (0.0658  $\mu\text{g/L}$ ) calculated using the standard ALC derivation procedures (U.S. EPA 2003). The low effect concentrations established for female gastropods in the laboratory were subsequently corroborated in field studies. The CCC was lowered (to 0.0074  $\mu\text{g/L}$ ) based on the judgment that these effects were relevant for the risks of TBT to gastropod reproduction.

### 3.0 CHARACTERISTICS OF CECs AND THEIR INFLUENCE ON ALC DEVELOPMENT

As described in Section 1.0, chemicals become labeled as CECs for a variety of reasons, many of which have relatively little to do with their toxicological characteristics. Consequently, the Guidelines cannot be interpreted or modified in one particular way that would be universally appropriate for all CECs. However, some characteristics may be shared by various CECs, such that discussing the implications of these characteristics in the context of deriving water quality criteria is worthwhile. The expected outcome is additional guidance addressing key issues that may arise and how best to accommodate these issues in deriving criteria.

Much of the technical discussion that follows is centered on EDCs and, even more specifically, around chemicals that interact with the hypothalamic/pituitary/gonadal (HPG) axis. Endocrine function controlled via the HPG axis involves hormones broadly known as estrogens (“female” hormones such as estradiol) and androgens (“male” hormones such as testosterone), along with the body tissues and biochemical machinery with which they interact. Effects on this part of the endocrine system of various aquatic species have been documented in the literature, and publicized in the media, making toxicological disruption of this mechanism a good choice for discussing CECs in the context of the *Guidelines*. However, these types of substances are only a subset of EDCs, and an even smaller subset of CECs as a whole. While much of the discussion that follows uses HPG-active chemicals as a point of reference, the concepts presented may be useful in the derivation of ALC for many other chemicals as well. It is the principles more than the specifics that are important in considering the content of this report.

#### 3.1 Characteristics of HPG-Active EDCs

While estrogenic and androgenic hormones are a core component of the HPG axis, this system also includes a much larger group of tissues and biochemical machinery within the body which, in vertebrates, govern sexual development, maturation, and reproduction. Commensurate with this complexity, there are many places within the system that environmental chemicals may act to modify the normal function of the HPG-axis. Thinking simply of “estrogenicity” or “androgenicity” as toxicological modes of action is still too broad – these categorical classes are more the outward “symptoms” of disruption in the HPG axis than they are unique modes of toxicological action. For example, the synthetic steroids EE2 and trenbolone bind to (and act as agonists of) vertebrate estrogen and androgen receptors, respectively, with similar or greater affinity than the natural endogenous hormones, estradiol and testosterone. In contrast, a variety of other medicinal pharmaceuticals are specifically designed to do the opposite, to be antagonists of these same receptors. As examples, tamoxifen (breast cancer treatment) and flutamide (prostate cancer treatment) bind quite specifically to vertebrate estrogen and androgen receptors, respectively, thereby blocking the activity of endogenous steroid hormones. But disruptors of the HPG axis are not limited to chemicals that bind directly to estrogen or androgen receptors; they also include chemicals that interact elsewhere in the overall biochemical pathway. As an example, there are chemicals that exert their activity through interactions with CYP (cytochrome

P450) enzymes involved in steroid production. The pharmaceutical chemical fadrozole acts to inhibit CYP19 aromatase, the enzyme that converts estradiol to testosterone. A number of conazole fungicides act as competitive inhibitors of several CYPs further up the steroid synthesis pathway (Ankley et al. 2005).

Unlike many other chemicals that have either non-specific (e.g., narcotics) or more generalized reactive modes of action (e.g., electrophilic chemicals interacting with nucleic acids and proteins), HPG-active compounds tend to have very specific interactions with particular molecular targets within the biochemical pathway. There are a number of consequences arising from this specificity. One important consequence of target specificity is potency. Many pharmaceuticals are designed to be highly specific, and thus are extremely potent. For example, EE2 and trenbolone affect reproduction and development in fish at water concentrations in the very low ng/L (part-per-trillion) range (e.g., Ankley et al. 2003; Länge et al. 2001), well below effect concentrations for most chemicals for which current ALC have been derived. These very low biologically-active concentrations present substantial challenges for analytical determinations associated either with lab-based effects testing or field monitoring of *in situ* exposures. In the case of EE2 and/or trenbolone, effects observed in fish are at concentration levels below the methodological limit of detection for most laboratories even in laboratory test water, and even more so ambient water and effluents.

Such high potency can influence how one would approach criteria derivation when the chemicals exert minimal acute toxicity, but cause mostly long-term, sub-lethal effects. Trenbolone and EE2 illustrate this situation quite well. Like most pharmaceuticals (some exceptions being chemotherapy and anti-parasitical agents), these chemicals are designed to “adjust” the biochemistry of the body without causing acute toxicity or other significantly adverse side effects. As a consequence, these types of pharmaceuticals tend to have low toxicity in short-term lethality assays (Webb 2001). In the context of criteria development, this has implications for the use of ACRs. Most conventional toxic pollutants with EPA ALC have ACRs of 10 or less (Cunningham et al. 2006; Host et al. 1995). In contrast, ACRs for EE2 and 17 $\beta$ -trenbolone in fish have been shown to range from 1,000 to greater than 300,000 (Ankley et al. 2005). Again, this is a result of relatively low acute toxicity and high chronic potency. Importantly, limited data for other MOA classes of pharmaceuticals suggest that this phenomenon is not restricted to endocrine-active substances. For example, Huggett et al. (2002) reported an ACR in fish of about 50,000 for propranolol, a  $\beta$ -blocker. As discussed in Section 2, this large difference in acute and chronic potency may both make CMC values moot in the environmental management of these chemicals, and introduce uncertainty in the extrapolation between acute and chronic effects in the derivation of a CCC.

The specificity of the molecular target also can greatly affect those taxa likely to be sensitive to the chemical MOA of concern. While some biological pathways (e.g., energy metabolism) tend to be highly conserved across all organisms, others can be quite specific to certain phylogenetic groups. Although the control of reproductive function through the HPG axis is highly conserved across vertebrate classes, lower taxonomic groups such as invertebrates have different endocrine system structure that function differently. For example, Segner et al. (2003) tested several estrogenic chemicals, including EE2, in a variety of partial and full life-cycle tests with a model fish (zebrafish) and several aquatic invertebrate species. They found that the fish was by far most

sensitive to the effects of the estrogenic chemicals, and was the only species that responded to EE2 at environmentally-relevant concentrations. As a result, it is likely that chronic toxicity data for fish would be the most influential in setting the criterion for EE2, and correspondingly unlikely that toxicological data for invertebrate species would have much impact. Plants do not have comparable endocrine system structure or function, and would not be expected to be sensitive to these types of compounds, but there is research that indicates that algal species may be a uniquely sensitive taxa for the assessment of other types of CECs such as antibacterial products like triclosan (Orvos et al. 2002; Wilson et al. 2003).

Specificity in MOA can also affect how or if effects are expressed within a toxicity test, even in potentially sensitive species. In the case of chemicals that affect endocrine function, there are distinct “windows” when animals are likely to be sensitive and/or exhibit adverse outcomes. For example, a popular amphibian early developmental assay-FETAX (Frog Embryo Teratogenesis Assay-*Xenopus*) would be inadequate for detecting thyroid-active toxicants because the period of exposure and observation occurs early in development before the thyroid axis is functional in *Xenopus*. In the case of HPG-active toxicants, there are two windows of sensitivity during an animal’s life: during sexual differentiation in developing organisms (when “organizational” alterations occur), and during active reproduction in adults (when “activational” responses can be manifested; Ankley and Johnson 2004). As a result, it is critical that testing for HPG-active EDCs occur during periods when the system is vulnerable to disruption. It is equally critical that toxicity tests include observations during the periods when effects are expressed. Some of the changes caused during sensitive early developmental windows may not be expressed until later in life. For example, the ELS toxicity test protocol commonly used in criteria development to estimate the chronic sensitivity of fish contains the early life stages that could be sensitive to disruption of sexual development, but it does not extend through to maturation, and would therefore be insensitive for detecting disruption of sexual development.

### **3.2 Implications for ALC Development**

As is clear from the text above, some characteristics of HPG-active chemicals (and many other CECs) create the need to carefully interpret the intent of the *Guidelines*, not just the routine derivation process. As indicated in Section 2.0 there is nothing about CECs that invalidates the principles embodied in the *Guidelines*; however, the *Guidelines* were written before many of the issues discussed in Section 3.1 were known, so they do not necessarily contain prescriptive guidance for some of the nuances created by some CECs. The following paragraphs discuss the implications of these issues for criteria development, following the four general topic areas outlined in Section 2.0:

- The need for and relevance of acute toxicity data and a CMC;
- Defining minimum data requirements in terms of taxonomic coverage;
- Defining appropriate chronic toxicity data; and
- Selecting effect endpoints upon which to base criteria

### 3.2.1 The Need For and Relevance of Acute Toxicity Data and a CMC

As described in Section 2.2, there are two primary uses for acute toxicity data under the *Guidelines*: 1) the derivation of the CMC; and 2) establishment of the CCC when the FAV/FACR method is used. As a practical matter, if the CMC is more than 96-fold higher than the CCC, then it will always be the CCC that is more limiting. This is because in the standard formulation of criteria, the CMC has a one-hour averaging period and the CCC a 96-hour averaging period; thus, if the difference between them is more than 96-fold, it is mathematically impossible to exceed the CMC without also exceeding the CCC. A minor exception to this issue occurs when ALC are implemented in an NPDES permit such that the CMC is applied to whole effluent while the CCC is applied after mixing, and the available in-stream dilution is large. However, these exceptions are rare, and even the 96-fold difference discussed here pales in comparison to the factors of 1,000 to 300,000-fold discussed previously in regard to EE2 and trenbolone. In cases where such extreme differences between acute and chronic toxicity thresholds exist, establishing ALC as having only a CCC seems a reasonable approach.

While it is easy to see why a CMC would not be necessary when you have sufficient acute test data to show that the CMC would be dramatically higher than the CCC, this begs the question of how much data are needed to decide that this is the case. This decision should occur during the “problem formulation” step in the risk assessment for a specific chemical/class, and should be guided by the following types of information:

- the amount and phylogenetic spread of acute toxicity data that are available;
- toxicity data from short-term exposures that do not meet the strict definitions in the *Guidelines* of acute toxicity data acceptable for criteria derivation, but from which information on responses to acute exposures can be inferred;
- short-term effect data garnered from longer-term exposures;
- information from closely related chemicals that are thought to have the same MOA, and have more robust acute data sets; and
- knowledge of the degree of phylogenetic distribution of the toxicity pathway of concern.

A complicating issue resulting from a “moot” CMC is that data availability for acute effects will likely be limited. As such, having less than the required acute MDRs may preclude the ability to derive a CCC using the FAV/FACR approach typically used in the *Guidelines*. For chemicals with highly specific modes of action and large ACRs (such as many EDCs), it is very likely that the mechanisms for acute and chronic toxicity differ, since biological activity resulting in chronic effects is designed into the product and not a secondary consequence - such as many of the historical contaminants for which EPA has developed criteria. Also, sensitivity of different taxa classes to acute and/or chronic toxicity varies widely due to presence (or absence) and structure and function of phylogenetically-conserved systems. Both of these issues would introduce considerable uncertainty into the availability and interpretation of ACRs, and probably make it inadvisable to use the FAV/FACR approach anyway. The *Guidelines* discuss the inadvisability of using the ACR approach when ACRs vary by more than a factor of 10 without a clear relationship to taxonomy or acute sensitivity (page 41 of the *Guidelines*). A more advisable approach would generally be to develop a CCC directly from a sufficiently robust set of chronic data, using the procedures outlined in the *Guidelines* or an appropriate modification thereof.

### 3.2.2 Defining Minimum Data Requirements in Terms of Taxonomic Coverage

To develop a CCC directly from chronic toxicity data (rather than via FAV/FACR), the *Guidelines* require that acceptable chronic toxicity data be available from at least eight families with a taxonomic distribution fulfilling the requirements specified in the *Guidelines* (referred to as the “minimum data requirements” or “MDRs”). Having a blanket requirement for meeting the eight MDRs was included to insure a minimum level of “certainty” that the *Guidelines* will be protective of the broad phylogenetic distribution of organisms found in aquatic systems. Including this phylogenetic spread also enables criteria to be developed for chemicals for which the toxicological MOA is not known. Instead of “knowing” what organisms are most likely to be sensitive to a particular chemical, requiring a broad spread of empirical toxicity data makes it likely that whatever taxa may be sensitive to a chemical, they will be represented to some degree in the toxicity data set.

In the case of EDCs, PPCPs, and certain other chemical classes, we may have a reasonable understanding of the toxicological MOA for the chemical, and from that knowledge we may be able to infer what taxa are most likely to be sensitive to a particular chemical (Ankley et al. 2007; Williams 2005). As discussed in Section 2.2, the procedure used in the *Guidelines* for estimating the 5<sup>th</sup> percentile of a toxicity distribution is dependent on only the four lowest values; for this reason exact values for insensitive genera are not necessary, as long as there are sufficient data to infer that their sensitivity is lower than the four most sensitive genera.

So how does one determine that a particular taxon is insensitive? The general structure of the *Guidelines* presumes that sensitivity is determined by conducting an acceptable toxicity test with that taxon. However, one can infer that the actual need is only to have sufficient information to conclude that the taxon is insensitive; if that determination can be confidently made based on other information, the information need may be met even if an actual toxicity test with that particular organism and chemical has not been conducted. This does not change the intent of the *Guidelines*. It only acknowledges the possibility that there is more than one way to meet the information requirement.

Using the example of EE2, there is both physiological understanding and some empirical toxicity data to support the belief that vertebrates will be far more sensitive to EE2 toxicity than will invertebrates (see Part II of this white paper and Segner et al. 2003). As such, it would seem inappropriate to invest resources in testing a wide range of invertebrate taxa classes for sensitivity when all existing data indicate that the data would not affect the final criterion, which would be driven by sensitivity of vertebrates. In this case, it makes sense to argue that certain of the eight MDRs might be declared met not through direct testing, but through toxicological understanding of the chemical’s MOA and the physiology of those other taxa or existing toxicity data that establishes sensitivity differences among taxa.

While this logic is clear, one must be careful in presuming that the primary MOA demonstrated by organisms with the target physiology is the only toxic MOA for the chemical. Particularly given the phylogenetic diversity of organisms, it is always possible that a chemical that behaves

with one MOA in one class of organisms may exert toxicity through a different mechanism in a different phylogenetic group. There are precedents for this scenario (Ankley et al. 2007). For example, exposure to the non-steroidal anti-inflammatory drug diclofenac via consumption of dead livestock has greatly diminished some populations of vultures in several East Asian countries. Diclofenac kills the birds through renal failure, which is only a relatively minor side-effect of the drug in mammals. While the mechanism of renal toxicity in vultures is likely molecularly related to the mechanism of therapeutic action in man, i.e., both seem to occur due to inhibition of prostaglandin synthesis (Meteyer et al. 2005), the inhibition of similar molecular components appears to be manifested as dramatically different whole organism endpoints. The key is in achieving a reasonable balance between expending resources on collecting data most likely to influence the criterion, while maintaining some kind of backstop against initially unexpected toxicity in other organisms.

One possibility for enhancing confidence regarding phylogenetic sensitivity is in considering data for other, closely-related chemicals with the same MOA. While the *Guidelines* focus analysis on toxicity data for the specific chemical in question, an understanding of toxicological MOA can also lead to an understanding of how other chemicals might act to exploit the same biological system in the same way. For example, one might reasonably infer that the relative species sensitivity to EE2 is likely similar to 17- $\beta$  estradiol (E2), the natural hormone which EE2 mimics. If, for example, there were a taxon which had been tested and found insensitive to E2, but had not been tested with EE2, it seems a reasonable assumption that that taxon would also be insensitive to EE2.

The possibility of fulfilling certain information requirements using data other than from direct toxicity testing does raise some other interpretation challenges, in particular the definition of the sample size for determining the 5<sup>th</sup> percentile of the genera sensitivity distribution. For example, if one has reason to believe that all crustaceans would be insensitive to a chemical, how many genera does that assertion represent in the calculation of the genera sensitivity distribution? While this is a real issue that will have to be addressed, we believe the problem is tractable and the details of the resolution are left to later work.

Because of the risk that our mechanistic understanding of a chemical may be incomplete, it seems unlikely that one could justify completely bypassing several MDRs solely on theoretical arguments (e.g., developing a criterion for a testosterone mimic based only on chronic toxicity data for vertebrates, with no invertebrate data at all). At the same time, prudent application of other data types to fulfill certain information requirements for criteria derivation does seem appropriate. Given the tremendous variation in understanding and availability of data likely to exist for different CECs, it is presumed that at least initial application of this approach will have to be justified on a chemical-by-chemical basis using appropriate scientific judgment. However, lines of evidence that might be applicable to this determination include:

- an in-depth understanding of the toxicological (or, in the case of drugs, therapeutic) MOA;
- information on the basic physiology of other taxa in relation to the MOA;
- toxicity data from chronic exposures or other relevant experiments that do not meet the strict definitions of acceptable chronic data given in the *Guidelines*, but from which

- information on relative taxon sensitivity can be inferred; and
- information from closely related chemicals that are thought to have the same MOA and have more robust acute or chronic data sets.

A separate, but related issue arises in respect to data from species not resident to North America. The *Guidelines* specify the use of toxicity data only from species resident to North America. However, particularly in regard to the study of EDCs, some fish species not resident to the U.S. have been advanced as experimental models for the evaluation of the chronic effects of EDCs to fish. Two clear examples at the time of this report are the zebrafish (*Brachydanio rerio*) and the Japanese medaka (*Oryzias latipes*), for which equivalency of EDC test data (with the fathead minnow) has been proposed through international groups such as the Organization for Economic Cooperation and Development (OECD; Ankley and Johnson 2004). These species have a rich toxicological database, and we know of no reason to believe that their sensitivities would be expected to be substantively different from sensitivities of at least some fish resident to the U.S. In keeping with international harmonization, we suggest that toxicity data from species with recognized international equivalency be included in criteria derivation with the full weight given to data from resident species.

### **3.2.3 Defining Appropriate Chronic Toxicity Data**

The *Guidelines* state that acceptable chronic tests for criteria derivation are full life-cycle exposures (F<sub>0</sub> egg to F<sub>1</sub> offspring) for both vertebrates and invertebrates, as well as partial life-cycle (adult to juvenile) and early life-stage (ELS; egg to juvenile) tests for fish. The acceptance of ELS tests in particular as acceptable chronic tests is predicated on the work of McKim et al. (1978) and other evidence that the toxicity thresholds obtained from ELS tests are generally within a factor of two of the thresholds from life-cycle chronic tests.

While this general approach has been applied with apparent success for many chemicals, the *Guidelines* intimate concerns with the approach, noting that for some chemicals, ELS tests might not be good predictors of chronic toxicity, which would violate the principle underlying the use of ELS tests as chronic data (page 39 in the *Guidelines*). As noted previously, toxicological data for chemicals like EE2 show that certain chemicals may have potent effects on life processes that lie outside the exposure period represented by ELS tests (e.g., pronounced effects on reproduction), or on life processes for which the expression of effects does not occur until after the ELS period (e.g., embryo or larval exposure resulting in effects on sexual development and maturation in adult fish; see Section 3.1). It is clear from these examples that there are chemicals for which ELS tests should not be used as surrogates for full life-cycle exposures. In fact, chemicals that affect sexual differentiation may not be adequately assessed even with partial life-cycle exposures, since these protocols do not generally include observation of sexual development/maturation in fish exposed during early development.

While the “Good Science” clause and other text in the *Guidelines* would not support reliance on ELS tests as chronic data for chemicals known to have specific effects on other life processes, such as sexual development or reproduction, the *Guidelines* would allow the development of a criterion using only ELS data for fish if there were not any specific data to indicate that this approach would be inappropriate. This is akin to an “innocent until proven guilty” approach.

However, we believe experiences with chemicals like EDCs make clear the need to move from the previous approach to one of “guilty until proven innocent.” In other words, it is probably wiser to require that the chronic toxicity data for fish include exposure and observation over a full life-cycle unless there is an affirmative reason to believe that it is not necessary (note: this issue is equally relevant to invertebrates species, but the ELS tests discussed in the *Guidelines* are focused explicitly on fish; invertebrate tests would already be required to be life-cycle). In keeping with this shift in emphasis, we believe the requirements for chronic toxicity data in the *Guidelines* should be tightened by adding the further requirements that either:

- 1) Full life-cycle data be available for at least one fish species; or
- 2) There is a body of experimental information indicating that life processes outside the ELS or partial life-cycle exposure/observation windows would not be important to capturing the important toxicological effects of the chemical.

At first glance, #2 may seem like requiring the proof of a negative, in that one would have to actually conduct the life-cycle test required by #1 in order to show that #2 is true. However, we believe there may be circumstances in which there may be data that speak to the sensitivity of different life stages that come from studies that, while scientifically valid, for some reason do not meet all the requirements of a valid life-cycle test as defined in the *Guidelines*. For example, there may be data for a life-cycle test with a non-resident species that includes the relevant life processes but does not qualify as an acceptable chronic test for the derivation of criteria because it is non-resident. Alternatively, there may be data from experiments that violate other requirements of acceptable toxicity data under the *Guidelines*, but still provide insight into sensitive exposure periods or life processes. Even though CVs from such data may not be used directly to calculate a chronic criterion, it seems reasonable to use such data to evaluate the question of where in the life-cycle there are important windows of exposure and/or effect and how that constrains the adequacy of ELS tests to represent chronic toxicity. In other cases, there may be sufficient information from other types of research to demonstrate to a reasonable level of certainty that a chemical’s toxicological mechanism(s) would not preclude the use of ELS tests as indicators of chronic toxicity.

Where life-cycle toxicity data are available, the results of those experiments should be carefully examined to determine the likelihood that important windows of exposure or effect lie outside ELS test protocols. Obviously, if there is meaningful potential for effects outside the ELS exposure period, ELS tests should not be used as surrogates for more involved chronic tests. It may also be that the knowledge of toxicological mechanism for a particular chemical may be sufficient that meaningful chronic toxicity data could be developed from exposures that have a structure different from the life-cycle, partial life-cycle, and ELS protocols defined explicitly in the *Guidelines*. While defining such alternate protocols is beyond the scope of this document, we recognize the potential for such a situation and leave it to appropriate implementation of the “Good Science” clause to allow for inclusion of such alternative exposure protocols as surrogates for chronic toxicity data, most likely in addition to, rather than instead of, data from life-cycle toxicity tests.

At the other end of the spectrum lie toxicity tests that extend beyond the definition of a full life-

cycle test, often referred to as multi-generational tests. Because they encompass the full range of life processes as a life-cycle test, we feel that they should be included as acceptable chronic tests, assuming they meet all other requirements for test acceptability. Some studies have reported effects from EDC or other chemicals in which exposure to one generation creates effects in a later generation that were not observed in prior generations even at the same life stage (Nash et al. 2004). If substantial, such effects could create a situation where even full life-cycle toxicity tests might underestimate the chronic toxicity of a chemical and therefore produce criteria that are potentially under-protective. While we recognize the potential for this situation, at present we believe there is not sufficient reason to make multi-generational testing a requirement for criteria development, unless there is specific, compelling information that a criterion would be substantially under-protective if multi-generational effects were not rigorously considered.

### **3.2.4 Selecting Effect Endpoints Upon Which to Base ALC**

The selection of endpoints appropriate to the derivation of ALC must be tied to the narrative intent of the overall *Guidelines*. The stated goal of ALC is to “protect aquatic organisms and their uses” (see *Water Quality Standards Handbook*; U.S. EPA 1994b). While the exact meaning of “protection” is not defined, there is considerable discussion in the *Guidelines* document that makes clear that protection does not mean the prevention of any measurable biological effect in any organism. Instead, there is discussion of endpoints that are “biologically important” and prevention of “unacceptable effects”; this implies that in the context of criteria there are effects that are “biologically unimportant” and/or levels of effect that are “acceptable.” Related concepts include the idea that natural populations can withstand some magnitude/frequency of disturbance and still meet the intent of the *Guidelines*.

With “protection of aquatic organisms and their uses” as the assessment endpoint, a decision must be reached as to which biological responses (measurement endpoints in risk assessment parlance) are appropriate to address this goal. Survival, growth, and reproduction are processes that are generally accepted as being directly related to this goal, as these are all demographic parameters that directly affect population dynamics (although, the exact quantitative relationship is not always fully determined). However, there are many more biological responses that have been observed in response to toxicant exposure, both at the whole organism level (e.g., behavior) and at lower levels of biological organization (e.g., biochemical or histological changes). For many of these endpoints, their relationship to the assessment goal, “protection of aquatic organisms and their uses,” is less clear. In this regard, we must consider an additional goal of the *Guidelines* – that criteria “provide a reasonable and adequate amount of protection, with only a small possibility of considerable overprotection or under-protection” (page 5 of the *Guidelines*). In keeping with this intent, it is important that criteria focus on endpoints that affect the assessment endpoint, but not create overprotection by preventing any measurable effect (or possibility of that effect). There must be a reasonable, affirmative connection between the measured response and the assessment endpoint.

The Agency’s *Framework for Ecological Risk Assessment* (U.S. EPA 1992) identifies this problem:

*In many cases, measurement endpoints at lower levels of biological organization may be more sensitive than those at higher levels. However, because of compensatory mechanisms and other factors, a change in a measurement endpoint at a lower organizational level (e.g., a biochemical alteration) may not necessarily be reflected in changes at a higher level (e.g., population effects). (p. 14)*

And later on:

*Ideally, the stressor-response evaluation quantifies the relationship between the stressor and the assessment endpoint. When the assessment endpoint can be measured, this analysis is straightforward. When it cannot be measured, the relationship between the stressor and measurement endpoint is established first then additional extrapolations, analyses, and assumptions are used to predict or infer changes in the assessment endpoint. (p. 23)*

*Measurement endpoints are related to assessment endpoints using the logical structure presented in the conceptual model. In some cases, quantitative methods and models are available, but often the relationship can be described only qualitatively. Because of the lack of standard methods for many of these analyses, professional judgment is an essential component of the evaluation. It is important to clearly explain the rationale for any analyses and assumptions. (p. 23)*

Existing criteria documents contain many types of data that were not used in the criteria derivation (the documents collate and review these data, but they are not used to actually define the criterion concentration) and it is useful to the discussion here to consider how such data have been interpreted. For example, the following text is derived from the most recent criteria document for ammonia (U.S. EPA 1999, see Appendix 5):

*Endpoint indices of abnormalities such as reduced growth, impaired reproduction, reduced survival, and gross anatomical deformities are clinical expressions of altered structure and function that originate at the cellular level. Any lesion observed in the test organism is cause for concern and such lesions often provide useful insight into the potential adverse clinical and subclinical effects of such toxicants as ammonia. For purposes of protecting human health or welfare these subclinical manifestations often serve useful in establishing ‘safe’ exposure conditions for certain sensitive individuals within a population.*

*With fish and other aquatic organisms the significance of the adverse effect can be used in the derivation of criteria only after demonstration of adverse effects at the population level, such as reduced survival, growth, or reproduction. Many of the data indicate that the concentrations of ammonia that have adverse effects on cells and tissues do not correspondingly cause adverse effects on survival, growth, or reproduction. No data are available that quantitatively and systematically link the effects that ammonia is reported to have on fish tissues with effects at the population level. This is not to say that the investigators who*

*reported both tissue effects and population effects within the same research did not correlate the observed tissue lesions and cellular changes with effects on survival, growth, or reproduction, and ammonia concentrations. Many did, but they did not attempt to relate their observations to ammonia concentrations that would be safe for populations of fish under field conditions nor did they attempt to quantify (e.g., increase in respiratory diffusion distance associated with gill hyperplasia) the tissue damage and cellular changes (Lloyd 1980; Malins 1982). Additionally, for the purpose of deriving ambient water quality criteria, ammonia-induced lesions and cellular changes must be quantified and positively correlated with increasing exposures to ammonia.*

*In summary, the following have been reported:*

- 1. Fish recover from some histopathological effects when placed in water that does not contain added ammonia.*
- 2. Some histopathological effects are temporary during continuous exposure of fish to ammonia.*
- 3. Some histopathological effects have occurred at concentrations of ammonia that did not adversely affect survival, growth, or reproduction during the same exposures.*

*Because of the lack of a clear connection between histopathological effects and effects on populations, histopathological endpoints are not used in the derivation of the new criterion, but the possibility of a connection should be the subject of further research.*

As discussed in greater detail below, chemicals such as EDCs have been shown to produce a wide variety of measurable changes at many different levels of biological organization. The challenge is to select from among those the endpoints that have sufficiently clear connection to expected effects on populations of aquatic organisms.

#### ***3.2.4.1 Specific Examples of Measurable Changes at Different Levels of Biological Organization***

The range of organismal endpoints that have been reported in the literature is vast, and varies to some degree on the organism and toxicant. With respect to only the HPG axis in vertebrates, this range of endpoints over and above direct measures of survival, growth, and reproduction includes:

- Biochemical measures (e.g., the female-specific yolk precursor protein vitellogenin; native hormones estradiol, testosterone, 11-ketotestosterone);
- Histopathological measures (e.g., proportion of spermatogonia, presence of testis-ova, oocyte atresia, Leydig cell hyperplasia/hypertrophy);

- Gross morphology (e.g., secondary sex characteristics: nuptial tubercles, coloration, ovipositors); and
- Behavioral measures (e.g., nest building, defense/aggression).

A comprehensive survey and evaluation of all such endpoints is far beyond the scope of this document. In lieu of that, this section presents in depth discussion of several individual measures relevant to the HPG axis, including their strengths and weaknesses as direct indicators of likely population level effects. The point of this discussion is simply to provide examples of the issues that must be considered in making a decision as to the biological importance and scientific defensibility for a specific endpoint, organism, or toxicant as it pertains to ALC derivation. These decisions will likely require case by case consideration; in certain circumstances, the suitability of a particular endpoint may vary across chemicals depending on how an individual chemical influences that endpoint.

One of the challenges that arises when incorporating alternative endpoints into criteria derivation is the need to not only conclude that the endpoint warrants consideration, but also establish some definition of what level of effect on that endpoint is unacceptable. While these links may not be completely quantitative, one would not want the definition of an unacceptable effect on one endpoint to be grossly disproportional to that considered unacceptable for another (i.e., if a 20% reduction in reproduction is considered unacceptable, what degree of estradiol (E2) suppression is equivalent to a 20% reduction in reproduction?).

In the text that follows, endpoints are categorized as being either “organizational” or “activational.” Organizational endpoints are those that are a result of changes to the normal growth and development of an organism, and are generally not reversible with cessation of exposure. Activational endpoints are those that occur in comparatively plastic tissues in response to exposure, but which may revert to their prior or normal condition with cessation of exposure.

#### *Organizational Endpoint: Sex Reversal*

Exposure of developing fish to endocrine-active materials during sensitive “windows” in early development can skew phenotypic sex dramatically toward either females (estrogenic chemicals) or males (androgenic chemicals). This response has been exploited by aquaculturists, who for many years have used potent natural or synthetic steroids to produce mono-sex stocks. The sensitivity of fish to this type of “sex reversal” is species-specific, and critical windows of exposure can vary markedly across species. The response can be manifested in several different ways, ranging from more or less completely sex-reversed animals (i.e., occurrence of gonads and secondary sex characteristics completely reflective of the opposite sex) to more subtle changes, such as the occurrence of intersex gonads (discussed further below). A significant challenge in assessing this condition—either in the lab or field—is knowledge of actual genetic sex of the fish. Since the molecular basis of sex determination in many fish is not known, reliable genetic markers of what sex an animal is programmed to be are not available for most test species (one notable exception here is the Japanese medaka, which is commonly used for endocrine testing in some parts of the world; Ankley and Johnson 2004). The net result of this is that the only way to practically monitor sex reversal in most fish species is indirectly, through analysis of sex ratios (generally based on phenotypic sex). This requires, of course, considerable background

knowledge concerning “normal” sex ratios for a species (or even strain) of fish. For some lab test species (e.g., fathead minnow), the normal sex ratio appears to be about 1:1, while for other commonly-tested small fish models (e.g., zebrafish), the ratio can be quite variable (Ankley and Johnson 2004). In field studies, collection of accurate sex ratio data also can be exceedingly difficult, depending on variables such as sampling gear and location and timing of collections.

Changes in the sex ratio of populations of fish, either in the lab or field, can be quite indicative of an endocrine-specific MOA, indicating exposure to estrogenic or androgenic chemicals (or even chemicals that block the actions of sex steroids). Significantly, from a risk assessment perspective, alterations in sex ratio could also have direct implications for spawning success and population viability. The degree to which sex ratios are critical in determining embryo production will vary based on reproductive strategies of the species of concern (e.g., broadcast versus paired spawners); however, from an evolutionary perspective, one would speculate that any departure from normal sex ratios for a species/population might be considered maladaptive.

#### *Organizational Endpoint: Intersex*

Exposure to certain classes of endocrine-active chemicals during critical windows in early development can produce intersex gonads (commonly termed testis-ova), a situation in which the gonads simultaneously contain both ovarian and testicular tissue. Different studies from around the world have shown an elevated occurrence of intersex fish downstream of municipal effluents containing natural and synthetic steroidal estrogens, including EE2 (WHO 2002). In fact, collection of intersex fish from the field has been one of the most visible manifestations of the effects of EDCs on fish/wildlife. For example, in a widely publicized USGS study, Blazer et al. (2007) recently reported that in the South Branch of the Potomac River and select nearby drainages, more than 80 percent of all the male smallmouth bass sampled had oocytes growing within their testicular tissue. Although histology is required to determine and quantify intersex, the techniques involved are relatively straight-forward. What is more challenging than measurement is interpretation of the results. For example, it appears that some degree of background intersex can occur, even in species held under carefully-controlled conditions (Grim et al. 2007). The degree of background intersex and sensitivity to chemically-induced intersex appear to be quite species-specific, requiring a thorough understanding of normal gonad differentiation and development in the species of concern.

Even in species for which background intersex is low, there is uncertainty as to the degree to which the condition could occur and not interfere with normal reproductive function. For example, in a field study in the UK, Jobling et al. (1998) noted a wide range of intersex in roach collected, even from the same site, with severity of the response ranging from occurrence of a few primary oocytes in otherwise normal testicular tissue to instances where there was a complete absence of sperm ducts in the males. Arguably, the former fish could produce viable sperm, while the latter certainly would not. So, although intersex is an intuitively reasonable endpoint upon which to base predictions of possible adverse effects of endocrine-active chemicals on reproductive success, determination of the relationship between severity of the condition and production of viable embryos is required to conduct this analysis.

### *Activational Endpoint: Behavior*

Although not usually considered a biomarker in the traditional sense, behavior is an endpoint that historically has been seldom used for ecological risk assessment, including the derivation of ALC. There are several reasons for this: (1) the types of assays used to assess behavior can be quite labor-intensive, (2) many methods for assessing toxicant-induced behavior have some degree of subjectivity, (3) many behavioral changes (e.g., gill ventilation in fish) are relatively non-specific in that they do not necessarily reflect exposure to chemicals with a specific MOA, and (4) translation of behavioral changes into adverse effects on endpoints such as survival, growth and reproduction can be difficult. Nonetheless, virtually all environmental toxicologists recognize the potential for chemically-induced alterations in behavior to influence the health of individual animals and populations.

There are some compelling reasons to consider behavior as a potentially useful/important endpoint in assessing the ecological risk of certain classes of endocrine-active chemicals. First, estrogens and androgens are known to play relatively specific roles in a variety of reproductive behaviors in fish, including competition for mates, courtship and nest-holding/guarding. Alterations in any of these behaviors theoretically could affect reproductive success and, hence, population status. In recognition of this there have been several recent papers describing straightforward, relatively quantitative assays for assessing the effects of endocrine-active substances on fish reproductive behavior. For example, Martinovic et al. (2007) conducted a study in which they showed that male fathead minnows exposed to a relatively low concentration of 17 $\beta$ -estradiol, and subsequently placed in a competitive spawning situation with non-exposed males, failed to compete successfully for nesting sites/females. Similar types of results have been reported for other fish species exposed to steroidal estrogens (e.g., EE2; see Part II of this white paper), suggesting that behavioral alterations could be important to consider, especially if they occur at exposure concentrations below those that cause effects on more traditional endpoints such as development and egg production.

### *Activational Endpoint: Secondary Sex Characteristics*

As described above, exposure of developing animals to endocrine-active chemicals can alter phenotypic sex, resulting in skewed sex ratios in populations. These organizational changes observed in secondary sex characteristics (in sexually dimorphic species) and/or gonads typically are not reversible. However, it also is possible to alter secondary sex characteristics, usually in a reversible manner, in sexually-mature fish through exposure to endocrine-active substances. For example, estrogens or anti-androgens can reduce expression of androgen-dependent secondary sex characteristics in males. Similarly, androgenic chemicals can cause female fish to develop male secondary sex characteristics, such as nuptial tubercles in the fathead minnow or elongated anal fins in the Japanese medaka (Seki et al. 2006). Alterations in secondary sex characteristics are much less useful indicators of endocrine-mediated responses in test species, such as zebrafish, with limited sexual dimorphism (Seki et al. 2006).

Alterations in secondary sex characteristics in adult fish can, in some instances, be subtle and somewhat subjective with respect to interpretation. For example, reductions in the status of

existing structures in fish (such as nuptial tubercles in male fathead minnows or anal fin length in male medaka) can be difficult to quantify. However, when there is a *de novo* synthesis of structural characteristics where none previously existed (such as tubercles in female fathead minnows), the response is not only quite specific (in this case to an androgenic MOA), but very easy to detect (i.e., the baseline, control condition is zero).

Although changes in secondary sex characteristics appear to be reasonable mechanistic biomarkers for some endocrine MOA, their utility as a predictor of adverse outcomes (e.g., egg production) is uncertain. Specifically, given our current understanding of fish reproductive physiology/endocrinology, causative links between secondary sex characteristics and gamete quality would be difficult to define. At best, a correlative association may be identified between the two parameters. For example, in studies with the synthetic androgenic steroid 17 $\beta$ -trenbolone, egg production appeared to be reduced at about the same test concentration that caused some degree of nuptial tubercle formation in females (Ankley et al. 2003).

#### *Activational Endpoint: Vitellogenin*

Vitellogenin status is probably the most commonly measured endpoint in studies with endocrine-active chemicals in fish. Measurement of the lipoprotein (or its mRNA) is relatively easy via a variety of methods (although most techniques have some degree of species specificity; Wheeler et al. 2005). Production of mRNA (*vtg*) and protein (VTG) in the liver of female oviparous (egg-laying) vertebrates is normally stimulated by activation of the estrogen receptor by endogenous estradiol. The protein is released to the plasma and subsequently deposited in the ovary where it forms a key constituent of developing oocytes. VTG levels in male oviparous animals typically are non-detectable due largely to very low circulating estradiol concentrations; however, males retain the molecular “machinery” in the liver necessary to produce VTG. Hence, exposure to even relatively low amounts of exogenous estrogen or estrogen mimics can stimulate a marked induction of VTG in males. The response not only is specific and sensitive (in part due to a baseline of essentially zero), but relatively sustained after exposure, as the males have no mechanism whereby to clear the protein from their blood.

Although *vtg* and/or VTG induction in male fish is an excellent biomarker of exposure to estrogens (Lattier et al. 2002), the response appears to have little direct (i.e., causative) value in terms of predicting adverse effects on reproduction (e.g., Wheeler et al. 2005). This perhaps should not be surprising given that VTG production in males is not part of any normal physiological pathway. It is possible, however, that correlative relationships between *vtg* and VTG induction in males by exogenous estrogens (such as EE2) and overall effects on fish population status could be derived (e.g., Kidd et al. 2007). This certainly merits additional study, but at present, it appears that the most technically-defensible use of VTG occurrence in male fish is as an indicator of exposure to estrogenic substances in the field and/or confirmation of chemical MOA in laboratory studies.

As opposed to males, VTG has a clear physiological role in females in that the protein is essential to egg production. Concentrations of VTG in females can be reduced by endocrine-active chemicals that directly or indirectly inhibit steroid (ultimately estradiol) production. For example, aromatase inhibitors such as some conazole fungicides decrease steroid production by

inhibiting enzymes involved in steroidogenesis, while other androgenic chemicals like trenbolone decrease steroid production through feedback inhibition in the HPG axis. As a consequence, these classes of endocrine-active chemicals reduce normal VTG production in female fish, thereby reducing fecundity and, ultimately, affecting population status (Miller et al. 2007). Therefore, in the case of females, VTG status may be effectively used as a biomarker both of exposure and effects. Kidd et al. (2007) found that VTG was elevated in female fathead minnows outside of their spawning season. Therefore, elevated VTG in females outside of the spawning season may also be an important measure of stress.

### **3.3 Pathways and Receptors Beyond the HPG-Axis**

As was explained at the outset, this section (Section 3) has a substantial focus on the HPG-axis not because it is the only MOA that is of concern in this document, but because it is currently prominent in both social and scientific arenas. However, it is important to re-emphasize that the use of HPG-active chemicals as a basis for discussion does not imply that this is the only group of CECs of concern with respect to the development of ALC, or the only group for which there may be need for supplementation of the explicit procedures outlined in the *Guidelines*.

As an example, the hypothalamic-pituitary-thyroid (HPT) axis is another endocrine system present in vertebrates that governs important biological pathways and is potentially subject to disruption. Similar to the role of steroid hormones in the HPG axis, actions of the HPT axis are mediated through thyroid hormone, which is involved in the regulation of metabolic activity, energy consumption and muscular activity in adult animals, and the regulation of postembryonic or perinatal growth and development in developing animals, especially in the central nervous system (Chatterjee and Tata 1992). Thyroid hormone is also responsible for the obligatory induction and maintenance of metamorphosis in amphibian and other poikilotherms, and may also play a role in male reproduction (Peterson et al. 1997). Since the actions of thyroid hormone are mediated via binding to highly-conserved nuclear thyroid hormone receptors and modulating transcription of specific genes, disruption of the HPT axis can be disrupted in many ways parallel to those discussed for the HPG axis (Farwell and Braverman 2006), and in doing so, create similar challenges for the development of ALC. Only a few of the developmental actions of thyroid hormones, however, are the result of the direct interaction of the hormone and receptor. Instead, most are indirect via the influence of thyroid hormone on other hormone or growth factors. For example, some of the growth-promoting effects of thyroid hormones on juveniles are indirectly mediated via growth hormone released from the pituitary gland (Chatterjee and Tata 1992).

The amphibian metamorphosis assay is one of the thyroid-relevant *in vivo* screening assays EPA has developed to detect chemicals that interfere with the thyroid hormone system. The assay represents a generalized vertebrate model to the extent that it is based on the conserved structure and functions of the thyroid systems, and thus mirrors some of the assays developed and discussed earlier for the HPG axis. This particular assay is important because amphibian metamorphosis provides a well-studied, thyroid-dependent process which responds to substances active within the HPT axis (Fort et al. 2007). The utility of this and other similar HPT-specific assays for development of ALC is predicated on the principle that the dramatic morphological

changes that occur during post-embryonic development of vertebrates are dependent on the normal function of the HPT axis, and that interference with this process leads to quantifiable effects (Zoeller and Tan 2007).

Other pathways relevant to this discussion could include any of a number of those regulated by different nuclear hormone-type transcription factors, such as the progesterone, glucocorticoid and aryl hydrocarbon (Ah) receptors. Of these the Ah receptor is of particular interest because it has been well studied and is key to the toxicity of several important environmental contaminants such as dioxins and PCBs. Ah receptor agonists are extremely toxic to early life stages of some vertebrates species (e.g., adult fish are at least 10 times less sensitive than early life stages), can induce delayed mortality not captured in short-term (e.g., 96-hour) toxicity tests, and are not very toxic to invertebrates, which lack the receptor (Cook et al. 1993; Mount et al. 2003; Tanguay et al. 2005). Hence, as is true for HPG-active chemicals, knowledge that a contaminant may be an Ah receptor agonist can help focus testing to determine ecological risk (Cook et al. 1993).

Although the previous systems are generally found in vertebrates but not invertebrates, parallel developmental, reproductive, and homeostatic systems exist in invertebrates (Lintelmann et al. 2003) and are most likely just as susceptible to disruption by xenobiotic chemicals. In fact, many pesticides are designed explicitly to disrupt biochemical pathways specific to invertebrates or sub-groups of invertebrates as a means to reduce effects on non-target (vertebrate) organisms. Some endocrine-mediated processes unique to certain taxa of invertebrates include molting, limb generation, diapause, pheromone production, pigmentation and coloration, and metamorphosis. For these processes, the most important endocrine regulators in arthropods are ecdysone and related compounds (ecdysteroids), which are involved in embryonic development, molting, metamorphosis, reproduction, and pigmentation (Lintelmann et al. 2003). Juvenile hormones in insects and methylfarnesoate in crustaceans (both belonging to the class of sexual hormones called terpenoids) are also deemed necessary to mediate the regulatory functions of ecdysteroids (DeFur et al. 1999). Research on the effects of CECs on these systems is still in its early stages, but the parallels with other systems that are susceptible to disruption are clear, and may therefore create similar issues for the development of ALC.

## 4.0 SUMMARY AND RECOMMENDATIONS

Through its deliberations, the workgroup concluded that the basic framework and conceptual underpinnings of the *Guidelines* apply to CECs as well as other chemicals. Further, the “Good Science” clause of the *Guidelines* provides the flexibility to adopt procedures that will produce a technically rigorous and protective criterion. The focus of this report has been the interpretation and adaptation of the principles set forth in the *Guidelines* with respect to common toxicological characteristics of CECs. In that regard, the workgroup identified a number of possible modifications or alternate interpretations that might aid those developing criteria for CECs to do so in a resource efficient manner that takes best advantage of existing knowledge.

Although some of the recommendations involve increasing flexibility in meeting certain data requirements, the intent is to guide the generation of ALC for CECs that have the same technical rigor as 304(a) criteria developed for other chemicals; these are not methods for “short-cut” criteria. This is a significant point, because an important feature of the *Guidelines* is defining a minimum technical rigor that criteria must have; if insufficient information exists to achieve a minimum level of confidence in the calculated criterion, then criteria should not be derived. The important consequence of this for risk assessors and managers is that when criteria are used to make regulatory decisions, one can have confidence that uncertainty regarding the criterion is not excessive. In other words, criteria derived using the *Guidelines* are often used as both “walk away values” (i.e., there is high confidence that there is little or no risk when exposures are below criteria) and as indicators of risk (implying that effects are likely when criteria are exceeded). If greater uncertainty were allowed in criteria, then the ability to use the values in this way would be compromised.

A negative aspect to establishing a minimum level of information for criteria is that there may be chemicals for which regulatory guidance is needed, but for which toxicological data are insufficient to meet the minimum standards of the *Guidelines*. In such cases, there may still be a need for alternate approaches to derive interim regulatory guidance values on which to base decisions that must be made before sufficient information for a complete water quality criterion can be gathered. While much of the discussion in this report might be useful to inform the development of such an approach, it must be emphasized that developing procedures to derive interim regulatory guidance values based on limited toxicity information is a separate matter and would require considerable additional analysis.

The subsequent sections summarize the issues and recommendations of the workgroup according to the areas of concern identified above.

### 4.1 Relevance of Acute Toxicity Effect Levels in Setting ALC for CECs

Some CECs may not be acutely toxic, or may only be acutely toxic at environmentally irrelevant concentrations. Thus, if the minimum data requirements for acute toxicity data are not already met by existing data, conducting additional acute tests might be unwarranted. Indication of lack

of acute toxicity in key aquatic species might also warrant direct calculation of the CCC rather than using the FAV/FACR approach, and thus eliminate the need for the full suite of acute toxicity tests normally required.

For a CEC of interest, available information should be reviewed to determine if the CMC would be sufficiently higher than the CCC such that developing the CMC is not needed. Exactly how much data is a risk management judgment, and probably does not have a unique answer. We recommend that the following information be considered when addressing this issue:

- the amount and phylogenetic spread of acute toxicity data available;
- toxicity data from short-term exposures that may not meet the strict definitions in the *Guidelines* of acute toxicity data acceptable for criteria derivation, but from which information on responses to acute exposures can be inferred;
- data on short-term exposures garnered from longer-term exposures;
- information from closely related chemicals thought to have the same MOA that have more robust acute data sets; and
- knowledge of the degree of phylogenetic distribution of the toxicity pathway of concern.

#### **4.2 Defining Minimum Data Requirements in Terms of Taxonomic Coverage**

One consequence of dropping acute testing requirements in criteria derivation is the inability to calculate a CCC using the ACR approach, i.e., as the quotient of the FAV and FACR. In addition, for chemicals with large ACRs, it is likely that the mechanisms for acute and chronic toxicity differ (Welshons et al. 2003) and that the sensitivity of different taxa to acute and/or chronic toxicity varies widely. Both of these issues introduce uncertainty into the interpretation of ACRs, and probably make it inadvisable to use the FAV/FACR approach. Under such a circumstance, a prudent approach would generally be to develop a CCC directly from a sufficiently robust set of chronic data, using the procedures outlined in the *Guidelines*. If there is insufficient data from actual toxicity tests to fulfill the MDRs to develop a CCC directly from chronic toxicity data, a reasonable understanding of the toxicological MOA for the chemical may allow inferences as to what taxa (and endpoints) are most likely to be insensitive, such that measured chronic values for those taxa might not be needed. One important consideration in this process is to avoid an excessive number of taxa estimated to be insensitive, relative to those for which actual test results are available, and thus to distort the phylogenetic distribution from that implicit in the MDRs and typical of ALC.

Accordingly, the workgroup recommends that, for chemicals without complete chronic toxicity data sets fulfilling all MDRs, there be an evaluation of whether sufficient information exists to conclude that certain taxa would not be sensitive to the chemical. Given the variation in understanding and availability of data likely to exist for different CECs, it is presumed that at least initial application of this approach would have to be justified on a chemical-by-chemical basis using appropriate scientific judgment. However, lines of evidence that might be applicable to this determination include:

- an in depth understanding of the toxicological (or, in the case of drugs, therapeutic)

MOA;

- information on the basic physiology of other taxa in relation to the MOA;
- toxicity data from chronic exposures or other relevant experiments that do not meet the strict definitions of acceptable chronic data given in the *Guidelines*, but from which information on relative taxon sensitivity can be inferred; and
- information from closely related chemicals thought to have the same MOA that have more robust acute or chronic data sets.

### 4.3 Use of Non-Resident Species in ALC Development

Historically, EPA has not used data derived from toxicity testing with non-resident species in the actual criteria derivation process. Excluding species simply because they are not resident may be unnecessarily restrictive for the purposes of deriving national criteria, and may actually increase rather than decrease uncertainty. Because ALC are intended to protect “most of the species, most of the time” and use distributions of test data for point estimation, increasing the species representation in the toxicological database should allow better estimation of species sensitivity distributions.

The workgroup recommends that some non-resident species be considered for use in criteria derivation calculations, focusing on those species with widely used and standardized test methods and for which there is no reason to believe would misrepresent the sensitivity of comparable resident species. Furthermore, we specifically suggest accepting data for zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*), to reflect international efforts toward data equivalency (Ankley and Johnson 2004). This recommendation pertains to the direct use of chronic toxicity data in the calculation of a CCC as is currently done for resident species. It is worth noting that even non-resident species that are not included in criteria calculations may still provide important information on MOA, sensitivity of endpoints, etc., as expanded on further below.

### 4.4 Defining Appropriate Chronic Toxicity Data

The *Guidelines* state that acceptable chronic tests for criteria derivation are full life-cycle exposures (egg/birth to egg/birth) for both vertebrates and invertebrates, as well as partial life-cycle (adult to juvenile) and early life-stage (ELS; egg to juvenile) tests for fish. For chemicals for which sexual development/maturation or reproductive effects are of most concern, the allowance in the *Guidelines* for using ELS or partial life-cycle fish tests might need reconsideration. The *Guidelines* already give priority to life-cycle tests when they are available and show greater sensitivity than other tests. However, other information indicating the importance of sexual development and reproduction (from other species, similar chemicals, knowledge of the MOA) might also establish a basis for not considering ELS data and for requiring life-cycle or partial life-cycle tests for fish.

At present, a CCC could be derived for a chemical for which chronic toxicity data for fish are limited to ELS exposures. Because of the importance of sexual maturation and reproduction for

determining the chronic toxicity of chemicals like EDCs, the workgroup recommends strengthening the *Guidelines* such that the chronic toxicity data requirements require that either:

- 1) Full life-cycle data be available for at least one fish species; or
- 2) There is a body of experimental information indicating that life processes outside the ELS or partial life-cycle exposure/observation windows would not be important to capturing the important toxicological effects of the chemical.

We note further that although this report is focused on CECs, this recommendation may be important to implement for all chemicals, not just CECs.

Regarding the latter, we recognize that there may be circumstances where the information that shows the sensitivity of different life stages comes from studies that, while scientifically valid, for some reason do not meet all the requirements of a valid life-cycle test as defined in the *Guidelines*. Alternatively, there may be data from experiments that violate other requirements of acceptable toxicity tests under the *Guidelines*, but still provide insight into sensitive exposure periods or life processes. Even though chronic values from such data may not be used directly to calculate a CCC, it seems a reasonable use of such data to evaluate the question of where in the life-cycle there are important windows of exposure and/or effect, and how that impinges on criteria derivation.

It may also be that meaningful chronic toxicity data could be developed from exposures that have a structure different from the life-cycle, partial life-cycle, and ELS protocols defined explicitly in the *Guidelines*; e.g., a short-term (21-day) reproduction assay with the fathead minnow (U.S. EPA 2001) or a multi-generational study – see example for EE2 reported in Nash et al. (2004). While defining such alternate protocols is beyond the scope of this document (see Ankley and Johnson 2004 for more detail), we recognize the potential for such a situation and leave it to appropriate implementation of the “Good Science” clause to allow for inclusion of such alternative test protocols as surrogates for chronic toxicity data, most likely in addition to, rather than instead of, data from life-cycle toxicity tests.

#### **4.5 Selection of Effect(s) Endpoints Upon Which to Base ALC**

Although chronic criteria typically are based on direct effects on reproduction, growth, and survival, there may be other endpoints indirectly related to these responses that could be useful for criteria derivation. The selection of endpoints appropriate to the derivation of ALC must be tied to the narrative intent of the overall *Guidelines*. The stated goal of ALC is to “protect aquatic organisms and their uses.” While the exact meaning of “protection” is not defined, there is considerable discussion in the *Guidelines* document that makes clear that protection does not mean the prevention of any measurable biological effect in any organism. Instead, there is discussion of endpoints that are “biologically important” and prevention of “unacceptable effects”; this implies that in the context of criteria there are effects that are “biologically unimportant” and/or levels of effect that are “acceptable.”

Chronic test data and other data should be examined to determine whether, for the specific chemical or MOA, endpoints beyond those traditionally used for criteria derivation may have intrinsic “biological importance” and therefore could be used as a basis for defining threshold of effect (e.g., sex ratio). Specifically, in the context of EDCs:

- Other “endocrine-sensitive endpoints” (e.g., VTG, testis-ova) should be examined to determine whether they can be relied upon as definitive indicators of other biologically important endpoints (e.g., reproduction), with the idea that they may be incorporated into calculation of the criterion. Important sources of this information would include full life-cycle tests in which these other endpoints were measured alongside traditional chronic endpoints, and may include tests with other chemicals with the same MOA (e.g., E2 for EE2).
- If endpoints, such as VTG or testis-ova, are used as direct or indirect indicators of effect, it is critically important that the baseline condition (e.g., variation during normal development) be understood sufficiently to define when changes are biologically meaningful.
- Selection of appropriate endpoints (and their associated effect thresholds) may, in some instances, transcend “biological importance” (the focus of the *Guidelines*) to reflect societal concerns (e.g., physical appearance of wild-caught fish).

#### **4.6 Involvement of an Expert Panel**

As becomes clear from the preceding issues, development of appropriate criteria for CECs may be unusually dependent on technical interpretations of a wide range of toxicological information pertinent to specific chemicals. One of the recommendations from a SETAC Pellston workshop (Mount et al. 2003), consistent with much of the above, was that expert panels be used to provide professional judgment during stages of the problem formulation and data interpretation associated with criteria development, particularly for chemicals with specific MOA. The involvement of the panel would “ensure consideration of other existing data for the chemical of concern, enable a significant degree of up-front technical input, and provide a level of peer review that should facilitate wider and more ready acceptance of the recommended criteria.” The workgroup agrees with this recommendation and suggests that it be incorporated into criteria development of CECs.

To maximize effectiveness, this panel should be convened very early in the criteria development process such that it will be able to assist in problem formulation, identification of important data, and scoping of particular issues that will be important. We envision these panels as being formed around specific chemicals, or groups of chemicals with a similar MOA, in order to access the most specialized expertise available.

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**WHITE PAPER**

**AQUATIC LIFE CRITERIA FOR CONTAMINANTS OF  
EMERGING CONCERN**

**PART II**

**ILLUSTRATION OF RECOMMENDATIONS USING DATA FOR  
17 $\alpha$ -ETHYNYLESTRADIOL (EE2)**

**Prepared by the  
OW/ORD Emerging Contaminants Workgroup**

**June 03, 2008**

**NOTICE**

**THIS DOCUMENT IS AN INTERNAL PLANNING DOCUMENT**

**It has been prepared for the purpose of Research & Development Planning.  
It has not been formally released by the U.S. Environmental Protection Agency and should  
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**DRAFT DOCUMENT**

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## 1.0 INTRODUCTION

In Part I of this white paper, toxicological characteristics of some contaminants of emerging concern (CECs) important to the derivation of ambient water quality criteria for aquatic life (aquatic life criteria, ALC) were described, and recommendations were made to facilitate ALC derivation for these chemicals. In Part II of this white paper, toxicity data for a model CEC, 17 $\alpha$ -ethynylestradiol (EE2), are used to further illustrate and explore those recommendations. Ethynylestradiol was chosen as a model compound for a several reasons. First, it possesses many of the toxicological characteristics described in Part I, and sufficient toxicity data exist to allow evaluation of the principles underlying the Part I recommendations. Second, toxicological effects of EE2 have been found both in the laboratory, the source of toxicological data for criteria development, and in the field, where criteria are used to enforce the regulatory authorities of the Clean Water Act. Finally, there is interest in deriving an EE2 ALC, and using EE2 as a basis for discussion should help advance that goal. While acknowledging that interest, it is important to note that the data and discussion presented are not intended to represent the formulation of an actual ALC, and potential ALC concentrations should not be inferred. The information from the ecotoxicological literature used here is for illustrative purposes and should not be considered as comprehensive, nor have all the data been fully examined for quality and applicability to ALC development.

The synthetic estrogenic steroid EE2 is the active pharmacological component of most oral contraceptives, and acts as a potent estrogen receptor agonist in vertebrates. After use and excretion of the contraceptive, domestic sewage treatment plant (STP) effluents become the primary source of EE2 entering the aquatic environment (Damstra 2002). Kolpin et al. (2002) found EE2 in 5.7% of 139 streams monitored in the U.S. While the concentrations of EE2 in Kolpin et al. (2002) have been debated (Ericson et al. 2002; Till 2003), other studies have noted concentrations ranging from 0.1 – 5.1 ng/L in surface waters (as reviewed by Campbell et al. 2006). Overall, it is somewhat uncertain at this time how high environmental concentrations of EE2 may be. Reliable analytical methods for the detection of EE2 have not been in existence very long, nor have they been widely validated in independent multi-laboratory studies. Some modeling efforts by the pharmaceutical industry indicate that based on the level of production and use of EE2 in the U.S., concentrations found in effluents should be less than 1 ng/L (Anderson, P.D. and D'Aco, V, personal communication, 2008). Complicating assessment of the possible risk of EE2 is the fact it co-occurs in STP effluents with the natural steroid hormones estradiol and estrone, though EE2 is generally found at lower concentrations. These three estrogens reportedly account for the majority of the estrogenic activity present in domestic wastewater effluents (Desbrow et al. 1998; Snyder et al. 2001), but EE2 is the most potent and resistant to degradation of the three (Nash et al. 2004; Gross-Sorokin et al. 2006). Data collected from fish and surface waters downstream of STPs over the past decade have implicated steroidal estrogens as the primary constituents in domestic effluents leading to the occurrence of intersex fish (Gross-Sorokin et al. 2006).

The remainder of this part of the white paper consists of a brief description of some relevant acute and chronic toxicity data available for EE2 (Section 2) and the evaluation of these data with respect to the recommendations made in Part I (Section 3).

## 2.0 DESCRIPTION OF THE ACUTE AND CHRONIC TOXICITY DATA SUMMARIZED FOR EE2

Acute and chronic toxicity data were identified via a literature search and review of relevant articles from EPA's ECOTOX database in April 2007. This list of potentially useful articles was supplemented with a few additional reports and articles as they became published or available. Only those studies with EE2 effect data on individual aquatic organisms or their populations were retained. For this particular effort, all endpoints expressing effects of EE2 at the whole animal and cellular levels were initially considered. Because the EE2 dataset is relatively large, and because many studies report more than one endpoint of possible consideration for ALC development, the data have been broadly summarized in an appendix (Appendix A). The tables comprising Appendix A are organized by endpoint, and include separate tables for endpoints typically used to derive ALC (survival, growth and reproduction) as well as for other endpoints relevant to the estrogenic mode of action of EE2. Table A.1 contains the data available on the acute (lethal) toxicity of EE2 to aquatic animals. This table is followed by others containing the chronic (long-term) effects of EE2 on survival (Table A.2) and growth of aquatic animals (Table A.3). Tables A.4 through A.9 present data directly (fecundity, fertility) or indirectly (sex reversal, intersex, sexual behavior, vitellogenin) related to the effects of EE2 on reproduction. Finally, Table A.10 presents a summary of the significant effects of EE2 on aquatic animals based on other potentially relevant endpoints. *In vitro* effects were not considered in the data analysis.

Within each table in Appendix A, data are first separated by studies where significant effects were observed, and then by studies where significant effects were not observed (i.e., where no effect was observed at the highest concentration tested). Each table in the appendix combines data for aquatic vertebrates and invertebrates for both freshwater and saltwater species, the latter designated by asterisks. All tables are organized by increasing effect concentrations, and all chronic effect endpoints are as reported by the authors.

Many studies of the effects of EE2 on aquatic organisms did not use standard toxicity test protocols, particularly those measuring sublethal responses. This is probably due in part to these studies having been designed for purposes other than ALC development, such as exploration of toxicity mechanisms, identification of sensitivity windows, bioassay development, etc. Adequate quantification of effect concentrations is also difficult for some of these studies because of the use of widely spaced treatment concentrations and by problems with analytical detection of exposure concentrations near the threshold for reproductive effects. While the results from such studies might limit their use in ALC development according to the definitions in the *Guidelines*, they were included in this document because they may inform other aspects of criteria derivation, as explained in general terms in Part I and in detail in the sections that follow.

### 3.0 EE2 DATA EVALUATION AND CONSIDERATIONS FOR ALC DEVELOPMENT

This section considers the application of the data in Appendix A toward criteria derivation in the context of the several areas of concern and general recommendations identified in Part I of this white paper.

#### 3.1 RELEVANCE OF ACUTE TOXICITY EFFECT LEVELS IN SETTING ALC FOR EDCS

One of the recommendations from Part I of this document was to determine whether the acute sensitivities of aquatic organisms to a chemical of interest are sufficient, relative to chronic sensitivity and expected exposures, to warrant derivation of a criterion maximum concentration (CMC) under the *Guidelines* procedures. This is especially important if there is not sufficient acute toxicity data to meet the minimum data requirements of the *Guidelines*, in order to avoid wasting resources on unnecessary additional testing. EE2 provides a good example of a chemical having insufficient acute toxicity data to derive a CMC according to *Guidelines* procedures, but enough data to demonstrate that deriving a CMC is not necessary.

Table 3.1 provides information on GMAVs that might be considered in CMC derivation. These values were derived from Table A.1 for any tests meeting *Guidelines* requirements, including "greater than" values indicative of the highest tested concentration eliciting less than 50% mortality. For genera without such acceptable tests, EC50/LC50s from Table A.1 for tests of 24 h duration and from Table A.2 for tests up to 30 days were also used. The EC50/LC50 values for these longer tests are designated as "greater than" values to indicate the expectation that acute EC50/LC50s would be higher. Values for medaka and zebrafish are included in accordance with the recommendation from Part I of this document that some latitude be adopted regarding species not resident to North America. Acute tests with embryos, not usually included in CMC calculations, are also included here because they suggest greater sensitivity of this life stage.

**Table 3.1. Potential GMAVs for Application to EE2 CMC.**

Genus	GMAV (ng/L)	Comments
<b>Freshwater</b>		
<i>Rana</i>	>760,000	14-d test
<i>Gammarus</i>	>840,000	10-d test
<i>Medaka</i>	>1,000,000	
<i>Danio</i>	1,700,000	
<i>Ceriodaphnia</i>	1,800,000	
<i>Hydra</i>	3,800,000	
<i>Sida</i>	>4,100,000	24-h test

<b>Genus</b>	<b>GMAV (ng/L)</b>	<b>Comments</b>
<i>Daphnia</i>	>5,000,000	
<i>Chironomus</i>	9,100,000	24-h test
<b>Saltwater</b>		
<i>Lytechinus</i>	30,000	Embryo
<i>Strongylocentrotus</i>	30,000	Embryo
<i>Acartia</i>	88,000	Embryo
<i>Tisbe</i>	>100,000	21-d test
<i>Acartia</i>	1,100,000	
<i>Neomysis</i>	1,200,000	

The data summarized in Table 3.1 show several deficiencies in meeting the minimum data requirements for deriving a CMC under the Guidelines. For freshwater application, only four genera, rather than the minimum of eight, meet the acute test requirements, even if the prohibition for non-resident species is ignored. If the shorter and longer tests are included, the requirement of at least eight genera is met, but the requirement for a salmonid fish is not. Even if this requirement is also ignored, two of the lowest four genera are "greater than" values, whereas CMC calculations can only be made if the four most sensitive genera have definite values ("greater than" values are permitted only for more tolerant genera.) For saltwater, there are even greater deficiencies in meeting the minimum data requirements.

Although these data are insufficient for deriving CMCs, they do provide ample evidence that a CMC is not needed and that it is unnecessary to conduct further tests to meet the minimum data requirements. For freshwater, there is still a rather broad taxonomic representation, including three vertebrates from two different classes, four crustaceans from two orders, and a third phylum. The acute LC50s/EC50s are consistently near and above 1 mg/L, several orders of magnitude above both the most sensitive chronic endpoints (Tables A.4 – A.9) and the highest environmental concentrations that organisms might be exposed to. The saltwater data do show greater sensitivity for the embryonic stages of some genera, but whether this reflects a lifestage or taxa sensitivity issue, these LC50s/EC50s are still four orders of magnitude above the most sensitive chronic endpoints and environmentally-relevant exposures.

### **3.2 USE OF NON-RESIDENT SPECIES IN ALC DEVELOPMENT**

Under the *Guidelines*, toxicity values from aquatic species not resident to the contiguous 48 United States, Alaska, or Canada are excluded from ALC derivation. One of the recommendations in Part I of this white paper is that this prohibition be relaxed and that data for non-resident species be allowed where deemed suitable, especially for species such as medaka and zebrafish which have become standard test organisms commonly used worldwide. Any tested species, whether resident or not, serves as a surrogate for estimating a sensitivity

distribution relevant to assessing risks in a variety of aquatic communities with a multitude of untested species. Therefore, the issue here is whether a non-resident species can serve as a reasonable surrogate for assessing the sensitivity of untested resident species. The use of such species would still be contraindicated if there is reason to believe they are significantly more or less sensitive than resident species.

The data in Appendix A support the use of medaka and zebrafish data in criteria calculations. Although there are no resident fish species with which to compare the acute sensitivities of medaka and zebrafish (see Table 3.1 and Table A.1), their lack of acute sensitivity is consistent with that of resident amphibians and invertebrates in the available data. The sensitivities of these fish species for long-term survival (Table A.2), growth (Table A.3), and reproduction (Table A.4) are interspersed with those of resident fish species, so there is no indication of either substantially higher tolerance or sensitivity to contraindicate their use. This is also generally true of the other endpoints summarized in Appendix A. The similarity among fish species of different geographic origins is not surprising, since the MOA of EE2 involves receptors and pathways that are highly conserved among vertebrates. If similar trends are seen in the data once they are thoroughly examined for quality and applicability to ALC development, data from these non-resident species should be included in criteria development.

The data in Appendix A also underscore pragmatic advantages of including non-resident species in criteria development. Medaka and zebrafish provided a large fraction of the available data regarding EE2 effects on fish. Removing them from the dataset simply because they are not resident would limit information on the distribution of species sensitivity and may actually increase rather than decrease uncertainty regarding resident species. Another use of data from non-resident species could be to assist in extrapolations of information across species, chemicals, and endpoints. For example, life-cycle tests with medaka could be used to evaluate whether early life-stage or partial life-cycle tests with resident species should or should not be accepted in criteria calculations for specific classes of chemicals with a defined MOA. The relationship of reproductive effects in non-resident fish (Table A.4) to other endpoints (Tables A.5-A.9) could also be used to determine how to apply information on these other endpoints for resident species lacking direct toxicological information on reproduction.

### **3.3 MINIMUM DATA REQUIREMENTS REGARDING TAXONOMIC COVERAGE**

As discussed in section 3.1, deriving a CMC for EE2 is not useful because acutely-toxic concentrations are so much higher than both chronic effects concentrations and expected environmental concentrations. In addition, developing a CMC would require additional acute toxicity tests to meet the minimum data requirements (MDRs) specified in the *Guidelines*. Without a CMC, the criterion continuous concentration (CCC) must be calculated directly from the available data, rather than through extrapolation using an acute to chronic ratio (ACR); this is probably not advisable anyway for such large ACRs. Since the ACR method is moot, the *Guidelines* calculation procedures for the CCC require that there be sufficient chronic toxicity tests to satisfy the MDRs for estimating the fifth percentile of the chronic database. For

freshwater criteria, these MDRs include a species from: the family Salmonidae; a species from a second family in the class Osteichthyes; a species from a third family in the phylum Chordata; a planktonic species from the Class Crustacea; a benthic species from the Class Crustacea; a species from the Class Insecta; a species from a phylum other than Chordata or Arthropoda; and a species from an order of insects or a phylum not otherwise represented.

Few existing ALC have chronic data that meet the MDRs, and this will likely be true of CECs as well. Significant expense would be incurred conducting new chronic tests to satisfy all the requirements. As recommended in Part I of this white paper, because only the four most sensitive genus mean chronic values (GMCVs) are used in the criteria calculations, chronic testing requirements for a taxon needed to meet an MDR should be waived if there is sufficient information to conclude that this taxon is more tolerant than the four most sensitive genera. A value (or values) for this taxon would still be included in the data set, but its GMCV would simply be specified to be greater than the fourth lowest GMCV.

Table 3.2 lists chronic values for the toxicity of EE2 to various freshwater genera to illustrate data that might be included in freshwater criteria calculations. These chronic values were obtained from Tables A.2-A.4, using *Guidelines* data selection procedures where possible, but also included some additional data to support discussion of how certain data deficiencies might be addressed. For invertebrates, the *Guidelines* require life-cycle tests that include reproductive endpoints, but if that type of test was not available, then other tests are reported here, with their limitations noted. For fish, the *Guidelines* preference order of life-cycle, partial life-cycle, and early life stage (ELS) was followed, but other tests were also reported as needed for illustrative purposes, with their limitations also noted. For all genera, the most sensitive endpoint among chronic survival, growth, and reproduction was selected, which was from the reproduction data of Table A.4, except for *Chironomus* (for which development from egg to pre-emergence was tested and the effects concentration was from Table A.3). Each chronic value (CV) was calculated as the geometric mean of the reported no observed and lowest observed effect concentrations (NOEC and LOEC) for an adverse effect. When the LOEC was the lowest exposure concentration, a "less-than" concentration is reported for the CV and, when the NOEC was the higher exposure concentration for insensitive species, a "greater-than" concentration is reported. As explained in Sections 1 and 2, these data are still under review and subject to modification. The specific values here should not be misconstrued as final, but rather as examples to illustrate trends and indicate needs that support the recommendations being addressed here.

**Table 3.2. Potential Chronic Values for Application to EE2 CCC.**

Genus	CV(s) (ng/L)	Notes
<i>Danio</i>	0.6, 1.5, <1.1	Life-cycle tests; for 1.5 ng/L CV, there was a 9-fold difference between LOEC and NOEC and the LOEC was a 100% effect
<i>Pimephales</i>	<0.32, 1.5	Life-cycle tests; for <0.32 ng/L CV, LOEC showed reduced fertilization but increased egg production so total reproduction not adversely affected; for 1.5 CV, 4-fold difference between NOEC and LOEC
<i>Oryzias</i>	3.2	F <sub>0</sub> from 1 d through spawning; 10-fold difference between NOEC and LOEC
<i>Oncorhynchus</i>	<16	Adult exposure only; fertilization success only endpoint examined
<i>Potamopyrgus</i>	50	Adult exposure only; embryo production over 9 wk test
<i>Gammarus</i>	>7,600	Population size over 100 d test; increased population size at 760 and 7,600 ng/L
<i>Daphnia</i>	45,000	5-fold difference between NOEC and LOEC
<i>Tisbe</i>	>100,000	Saltwater copepod included to further indicate arthropod insensitivity
<i>Chironomus</i>	320,000	Larval growth and molting schedule only; did not include emergence and reproduction
<i>Brachionus</i>	800,000	Intrinsic rate of population increase over 72 hr test

The data in Table 3.2 indicate high sensitivity of vertebrates to EE2. Significant reproductive effects in the life-cycle tests for zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*) occur at concentrations near and perhaps below 1 ng/L. Although the chronic test for medaka (*Oryzias latipes*) did not cover the entire life cycle, it included life stages likely important for reproduction and indicated a sensitivity similar to zebrafish and fathead minnow. For rainbow trout (*Oncorhynchus mykiss*), a more limited exposure addressing only effects on fertilization suggests that reproductive effects on this species should also be present in the low ng/L range. However, the absence of a definite toxicity value for rainbow trout will be an important impediment to criteria calculations, both for leaving an MDR unsatisfied and for being one of the four most sensitive genera in this set. Actual criteria development will require a decision whether to (a) require more information for this species, (b) use other information to help estimate rainbow trout sensitivity or (c) justify setting the MDR aside (see Section 3.5).

The invertebrate data in Table 3.2 indicate lower sensitivity, especially for arthropods (*Gammarus*, *Daphnia*, *Tisbe*, *Chironomus*) and rotifers (*Brachionus*). Some data, like the *Chironomus* test, fail to satisfy the *Guidelines* requirement for a life-cycle test and the copepod *Tisbe* is a saltwater species included here only to reinforce conclusions about arthropod sensitivity. Also, the tests for *Gammarus* and *Brachionus* are not standard life-cycle tests, but could be considered to satisfy *Guidelines* requirements because exposures span a life cycle and

include reproductive effects. The snail (*Potamopyrgus*) toxicity test showed moderate sensitivity, although still about an order of magnitude less than the fish, and also does not involve a full life-cycle test.

These data demonstrate the potential for a situation in which the GMCVs for taxa reasonably expected to be insensitive do not need to be quantified. For example, although the *Chironomus* test was not a full life-cycle test and thus could not fully define the GMCV under *Guidelines* requirements, it indicates such a degree of insensitivity for growth and development, such that it can be reasonably presumed that a full life-cycle test would still show much less sensitivity than the vertebrates, especially because other arthropods are observed to be similarly insensitive. Likewise, the snail test, although not for a full life-cycle, involved the effects of long exposures on reproduction, and can be argued to be sufficiently less sensitive than fish reproduction so that it would not reasonably be expected to be among the four most sensitive genera if a life cycle test was conducted. These inclusions, along with the data for *Daphnia*, *Gammarus*, and *Brachionus*, satisfy the *Guidelines* MDRs for invertebrates, and would allow an ALC to be calculated from the four sensitive vertebrate genera, provided the value for the rainbow trout was resolved.

Assessing that taxa are likely to be insensitive could involve other lines of evidence, especially for CECs with more limited chronic toxicity data than EE2. Tests involving endpoints such as those in Tables A.5-A.10 could be used to establish that certain taxa are sufficiently less sensitive than others to preclude the need for tests on their chronic survival, growth, and reproduction (Tables A.2-A.4), the endpoints typically used in ALC development. Information from other chemicals might also be used, such as using the insensitivity of arthropods to EE2 to preclude testing this taxonomic class with chemicals with the same MOA. Such a strategy could be used to help the evaluation of EE2, particularly regarding the snail sensitivity. For example, the sensitivity of this or similar species relative to that of vertebrates for other chemicals could be used to strengthen a conclusion that they are less sensitive to EE2 than are fish.

### **3.4 DEFINING APPROPRIATE CHRONIC TOXICITY DATA**

As discussed in Part I of this white paper, characteristics of some CECs require that careful consideration be given to the selection of chronic toxicity data appropriate for ALC development. Specifically, the use of data from early-life stage (ELS) or partial-life cycle (PLC) exposures as estimates of life-cycle chronic effect thresholds is inadvisable for chemicals whose MOA would result in biological effects for which critical periods of induction and/or expression would lie outside the exposure/observation window provided by the test procedure.

An examination of data specifically for EE2 provides evidence to support emphasis on full life-cycle exposures for determining the chronic toxicity of EE2. Länge et al. (2001) conducted a full life-cycle chronic exposure with fathead minnows which were exposed from fertilized eggs ( $F_0$ ) through maturation, spawning, and early-life stage development of the  $F_1$  generation. Nominal exposure concentrations of EE2 were 0.2, 1, 4, 16, and 64 ng/L (for convenience, nominal concentrations are used in this discussion as the important point is relative endpoint sensitivity

rather than absolute concentrations inducing effects). As part of this exposure, measurements of growth (as length) and survival were made at 28 days post hatch (dph) which would correspond to the end of a standard ELS exposure with fathead minnows. At 28 dph, there were no effects on survival. The length endpoint showed a 16% reduction at 64 ng/L, a smaller but significant reduction of 6% at 16 ng/L, and no effect at 4 ng/L. Accordingly, the NOEC and LOEC for an ELS test with EE2 would have been 4 and 16 ng/L, respectively, and an EC20 based on length would be >64 ng/L. However, as exposure continued throughout the life cycle, pronounced effects were observed for other endpoints at lower exposures. There was no reproduction at all in fish exposed to 4 ng/L, and a trend, though not significant, toward lower reproduction at 0.2 and 1 ng/L. Other significant effects observed included a 16% reduction in weight of adult female fish at 1 ng/L after 301 d exposure, and 5 to 10 percent reductions in weight of F<sub>1</sub> offspring at 28 dph, though the authors questioned the biological significance of the F<sub>1</sub> growth effects. Regardless, the clear indication is that life-cycle exposure showed substantially greater sensitivity to EE2 than was evidenced from ELS endpoints alone. This was much larger than the factor of 2 difference generally found for other chemicals by McKim et al. (1978).

A similar conclusion can be drawn from the study of Parrott and Blunt (2005). This involved exposure from fertilized egg through reproduction, including measures of fertilization success (but not ELS development) in the F<sub>1</sub> generation. Exposure was to nominal concentrations of 0.32, 0.96, 3.2, 9.6, and 32 ng/L EE2. Measurements of survival and growth at 30 dph showed no effects (NOEC  $\geq$  32 ng/L). However, continuation of exposure through adulthood showed no reproduction in the 3.2, 9.6 and 32 ng EE2/L treatments, and all fish in these treatments were phenotypic females. There was also suggestion of effects on fertilization success at 0.32 and 0.96 ng/L, although interpretation of these effects is complicated by an increase in number of eggs produced in these same treatments, such that the total number of fertilized eggs was not as dramatically affected. Regardless, the message relative to definition of chronic sensitivity is the same in that effects were apparent after life-cycle exposure at concentrations well below those that would be expected to show effects in an ELS test.

Additional comparisons can be extracted from the work of Wenzel et al. (2001), who conducted a multi-generational study of zebrafish exposed to EE2 concentrations from 0.05 to 10 ng EE2/L. Observations of survival and length of exposed fish showed no effects at 21 and 42 dph (NOEC  $\geq$  10 ng/L). However, with continued exposure, a variety of effects were observed around an EE2 test concentration of 1 ng/L, including effects on adult length, time to spawning, egg production and fertilization. As for the fathead minnow studies, survival and growth measured during the period comparable to an ELS study were far less sensitive to EE2 exposure than were endpoints measured in full life-cycle studies (Tables A.2, A.3, A.4).

The reason for these differences between ELS and full life-cycle tests is obvious when one considers the MOA for EE2, which interferes with sexual differentiation, development, maturation, and spawning. Because the endpoints measured in ELS tests are limited to survival and growth, and because the effects of EE2 on sexual differentiation are not apparent (at least not at a gross morphological level) in the tested species by 28-30 dph, the ELS test is comparatively insensitive to toxicity mediated through an estrogen receptor signaling pathway. It is interesting

to note that even though a standard ELS test is relatively insensitive to detecting the effects of EE2 exposure, other work has shown that key windows of exposure do in fact occur during the ELS exposure window. Van Aerle et al (2002) demonstrated that larval fathead minnows exposed to EE2 only during brief windows during early development (e.g., 10-15 dph) showed altered sexual development of male fish at 100 dph, including the development of an ovarian-like cavity and changes in the distribution of testicular cell types (Table A.6). The issue for interpreting chronic toxicity data is that, even though effects may be induced during ELS exposure, they are not expressed unless exposed fish are observed later in sexual development.

This latter observation also has implications for the suitability of PLC tests for detecting the effects of EE2 or other chemicals acting through a similar pathway. As discussed in the *Guidelines*, PLC tests are acceptable chronic tests for fish species that require more than one year to reach sexual maturity, such as the common species of trout. PLC tests are to begin exposure “with immature juveniles at least 2 months prior to active gonad development, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation.” If salmonids (or other species for which PLC tests might be conducted) were to express effects from larval exposure to EE2 as observed by Van Aerle et al. (2002) for fathead minnows, one would expect that a PLC exposure would not be as sensitive as a full life-cycle exposure. That is, even though a PLC test includes the observation periods shown to be sensitive in full life-cycle exposures, it might not include the exposure windows important to inducing chronic effects on sexual differentiation and development.

While the rationale for emphasizing full life-cycle chronic tests has clear grounding in the MOA for EE2, it has practical implications in terms of the fish species likely to be tested. Most species for which life-cycle chronic tests are most commonly conducted (primarily fathead minnow, zebrafish, medaka, flagfish, and sheepshead minnow), are small fish that develop rapidly and are continuous spawners (as opposed to annual spawners like rainbow trout or bluegill sunfish). Whether or not these life history traits influence sensitivity to EE2 is unknown, but because of the investment necessary to conduct true life-cycle exposures with annually spawning fish that take much longer to develop, it may be unlikely that comparative data will be developed. Better understanding these implications is a worthy subject for future research.

Finally, there are some scattered indications in the literature for trans-generational effects of EE2 exposure. As mentioned above, Lange et al. (2001) found small effects on growth in the F<sub>1</sub> generation that were not observed after comparable exposure of the F<sub>0</sub>. Wenzel et al. (2002) also report some suggestions of growth inhibition in subsequent generations at exposure below those causing such effects in the first exposed generation (Table A.3). The mechanisms by which such effects might occur are not clear, nor are their implications (in fact, Lange et al. actively dismiss them as being biologically unimportant). At this point, it does not seem that the evidence for trans-generational effects is sufficient for requiring their inclusion in the definition of an acceptable chronic test, but the potential for the existence and importance of trans-generational effects should be re-evaluated in the future as additional data become available.

### 3.5 SELECTION OF EFFECT(S) ENDPOINTS UPON WHICH TO BASE ALC

Aquatic studies with EE2, particularly those using fish, have measured a variety of endpoints not traditionally used for criteria derivation, including reproductive behavior, abnormal sex ratios, changes in secondary sexual characteristics, altered histopathology (typically gonadal), changes in steroid hormones, and modifications in the expression (or activity) of a variety of proteins/enzymes. Many of these endpoints were evaluated because they are known (or hypothesized) to be responsive to estrogenic MOA, and not because the intended result was the quantitative assessment of risk. Among the challenges in using data from these types of mechanism-based endpoints is that such measurements are seldom standardized or straightforward in their interpretation. For example, alterations in behavior are difficult to objectively quantify, it is challenging to accurately measure steroid hormone concentrations in small fish, and the capability of measuring gene expression or enzyme activity can be quite lab/method-specific. A second source of uncertainty in using most of the mechanism-specific endpoints evaluated in EE2 studies is a lack of knowledge concerning the functional relationship between changes in endpoints and responses of primary concern for risk assessment, such as survival, growth and reproduction. Even in considering these challenges related to measurement and interpretation, however, there are a handful of mechanistic endpoints/responses that exhibit utility for supporting ALC derivation for EE2 (or other xenobiotic compounds with estrogenic activity). Three of these are discussed in greater detail below.

A frequently measured mechanism-specific endpoint in fish exposed to EE2, is induction of vitellogenin mRNA (*vtg*) or expression of circulating vitellogenin protein (VTG) in males (Table A.9). The most attractive attribute of this endpoint is its specificity for an estrogenic MOA, since there are no other chemically linked biological phenomena known to consistently activate the vitellogenin gene or elevate vitellogenin protein in male fish. Further, since the vitellogenin gene is quiescent in male fish, which implies a zero baseline of vitellogenin, the response is unambiguous with regard to exposure. Additionally, this exposure mediated induction of *vtg* is sensitive to low levels of exogenous estrogen. Because vitellogenin protein has been frequently evaluated in fish studies, accurate methods of measurement (including several commercial kits) are available for many fish species, including the small fish models for which much of the EE2 chronic toxicity data exist. Given these attributes, male VTG has and should continue to be a very useful endpoint for monitoring the occurrence of estrogenic chemicals (including EE2) in the environment. A major drawback to using male-specific circulating protein to assess exposure and risk of EE2 (or other estrogens), including the derivation of ALC, is the lack of an established functional linkage between expression of the protein and adverse endpoints related to early development or reproduction (Wheeler et al. 2005). This is in large part due to the fact that VTG plays no physiological role in male reproductive processes. As such, any associations that might exist between VTG induction in males and reproductive success is likely more correlative than causal.

Despite the fact that the appearance of VTG in males appears not to be a robust predictor of adverse effects on reproduction, the response could nonetheless play an important role in reducing the uncertainty of ALC for EE2, or the development of ALC for other chemicals which

might be estrogenic. From Table 3.2 above, it is apparent that data from life-cycle tests with fish would be appropriate (and critical) to setting the final ALC for EE2 and, by extension, other estrogens. Hence, knowledge that a less well-studied chemical than EE2 induces VTG in males could be used to help identify those instances when one (or more) life-cycle fish assay(s) would be recommended for generating robust data for ALC derivation. Another possible use of protein data, that could have more direct applicability to developing an ALC for EE2, involves use of the endpoint as a basis for evaluating relative species sensitivity. Specifically, reproductive data suitable for an EE2 ALC are largely from three species: fathead minnow, medaka and zebrafish (Table 3.2); however, there are studies with numerous species that have evaluated the ability of EE2 to induce vitellogenin mRNA and protein in males. Provided that a common dose metric could be established across these studies, a dataset could be developed to provide an indication of the relative sensitivity distribution of fish species to EE2, in addition to other estrogens. This would enable a direct comparison of values along the continuum of estrogen sensitivity for those fish species wherein chronic data exist (based on VTG induction) and, as such, could provide a quantitative indication of uncertainty for a proposed EE2 criterion.

There are two mechanism-specific endpoints that have been measured in a number of EE2 studies that might, with additional research and analysis, have a direct bearing on criteria derivation: alterations in sex ratio (i.e., generation of genotypic males with a female phenotype) and the occurrence of intersex/testis-ova (Tables A.5, A.6). As opposed to VTG induction in males, the functional linkage between skewed sex ratios or abnormal gonad development and reproductive success in fish, at both the individual and population levels, is readily apparent. Specific endpoints, however, can be difficult to measure. For example, detailed histological analyses are needed to identify and, especially, quantify testis-ova. To detect an alteration in sex ratio, a genotypic marker of gender (available in medaka but not fathead minnow or zebrafish) or a relatively large representative sample is required to reliably detect chemically-induced changes within a proportion of males and females in a population. Probably more difficult than measurement of the endpoints is definition of the quantitative linkage between changes in sex ratio or occurrence of testis-ova and effects on reproductive success for individuals and populations. For example, unless one assumes that any deviation in sex ratio from the norm (e.g., 1:1) is adverse, it is necessary to know (in the case of estrogenic effects) the magnitude of shift in respective gender numbers that is likely to result in cases where fewer young are produced. Similarly, it is probable that some degree of testis-ova would not be considered adverse in terms of reducing reproductive success, especially considering that the condition can exist at some degree, even in control animals (Grim et al. 2007). It is certain that at some level of manifestation, the condition will impair gonad function sufficiently such that acceptable levels of normal sperm cannot be produced. The frequency of this phenomenon, however, is currently unknown for any fish species. Definition of this relationship would support use of testis-ova occurrence in fish not only for prospective assessments (like criterion derivation), but in environmental monitoring studies focused on chemicals with an estrogenic MOA.

At present, uncertainties regarding measurement and interpretation hamper use of data from any of the mechanism-specific endpoints mentioned above as a basis for derivation of an ALC for EE2. Eith appropriate research, however, induction of vitellogenin in males, changes in sex

ratios and occurrence of testis-ova, all have the potential to contribute insights to different facets of quantitative risk assessment for estrogenic chemicals, including derivation of ALC. There is one noteworthy additional observation relative to use of non-traditional endpoints for an EE2 ALC. Several fish life-cycle studies using EE2 have been conducted in which typical measures of reproductive success (e.g., fecundity, fertility) have been made in conjunction with induction of VTG, sex ratio and/or testis-ova data (e.g., Länge et al 2001; Wenzel et al. 2001; Nash et al. 2004; Parrott and Blunt 2005). Although experimental design variables make some of the endpoint sensitivity comparisons challenging, it does not appear that there are substantial differences in EE2 test concentrations that produce adverse effects on egg production/fertility, versus those that alter the mechanism-specific endpoints. Hence from a pragmatic perspective, at least for the near-term, it seems reasonable to base an EE2 ALC on traditional measures of long-term reproductive success in fish.

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**APPENDIX A**

**Table A.1. Effects of EE2 on Aquatic Animals (Short-term survival).**

Species	Life stage	Method	Duration	EC50 or LC50 (ng/L)	Reference	Remarks
<b>Traditional Acute (1-7 day timeframe)</b>						
*Sea urchin, <i>Strongylocentrotus purpuratus</i>	embryo	S,U	96 h	30,000	Roepke 2005	EC50 - abnormal development
*Sea urchin, <i>Lytechinus anamesus</i>	embryo	S,U	96 h	30,000	Roepke 2005	EC50 - abnormal development
*Copepod, <i>Acartia tonsa</i>	egg	R,U	5 d	88,000	Andersen et al. 2001	EC50-Inhibition of naupliar development
Medaka, <i>Oryzias latipes</i>	adult	-	96 h	>1,000,000	Thompson 2000	
*Copepod, <i>Acartia tonsa</i>	10-12 d adult	S,U	48 h	1,100,000	Andersen et al. 2001	
*Opossum shrimp, <i>Neomysis integer</i>	Juv, 2-4 mm	R, U	96 h	1,200,000	Verslycke et al. 2004	
Zebrafish, <i>Danio rerio</i>	adult	-	96 h	1,700,000	Wenzel et al. 2001	
Cladoceran, <i>Ceriodaphnia reticulata</i>		S,U	24 h	1,800,000	Jaser et al. 2003	EC50 mobility
Cnidarian, <i>Hydra vulgaris</i>	Adult male	R, U	96 h	3,800,000	Pascoe et al. 2002	
Cladoceran, <i>Sida crystallina</i>		S,U	24 h	>4,100,000	Jaser et al. 2003	EC50 mobility
Cladoceran, <i>Daphnia magna</i>	<24 h	S,U	48 h	>5,000,000	Goto and Hiromi 2003	
Midge, <i>Chironomus riparius</i>	4 <sup>th</sup> instar	S,M	24 h	9,100,000	Lee and Choi 2006	

\*Indicates saltwater species.

**Table A.2. Effects of EE2 on Aquatic Animals (Long-term survival).**

Species	Life Stage	Method	Duration	NOEC- survival (ng/L)	LOEC - survival (ng/L)	Reference	Remarks
<b>Significant Effect Observed</b>							
Zebrafish, <i>Danio rerio</i>	1 d old	R,U	38 d	10	100	Orm et al 2006	100% mortality at LOEC
Zebrafish, <i>Danio rerio</i>	2 dph	R,U	58 d	10	100	Hill and Janz 2003	90% mortality at LOEC (45% control mortality); excess solvent
Medaka, <i>Oryzias latipes</i>	1 d	R,M	LC: 85 to 110 dph	29	290	Metcalfe et al. 2001	83% mortality at LOEC
*Sheepshead minnow, <i>Cyprinodon variegatus</i>	juv	F,M	PLC:59-7 dph F1	120	330	Zilliox et al. 2001	50% mortality at LOEC (42 days)
Medaka, <i>Oryzias latipes</i>	6 mo.	F,M	21 d	260	490	Seki et al. 2002	42% mortality at LOEC (4 of 5 dead males)
Rainbow trout, <i>Oncorhynchus mykiss</i>	1+ year	F,M	62 d pre-spawning	130	750	Schultz et al. 2003	100% mortality at LOEC (57 days)
Medaka, <i>Oryzias latipes</i>	4 mo.	R,U	14 d	500	2,000	Thompson 2000; Tilton et al. 2005	Significant mortality at LOEC
Zebrafish, <i>Danio rerio</i>	fert. eggs	R,U	5 wk	-	5,000	Ortiz-Zarragoitia et al. 2006	50% mortality of exposed animals
*Copepod, <i>Tisbe battagliai</i>	<24 h	R,U	10 d	-	>100,000	Hutchinson et al. 1999	Value is an LC50
Wood frog, <i>Rana sylvatica</i>	Gosner 26	R,U	14 d	-	560,000	Hogan et al. 2006	Value is an LC50
Amphipod, <i>Gammarus pulex</i>	3-5 mm	R,M	10 d	-	840,000	Watts et al. 2001	Value is an LC50
Leopard frog, <i>Rana pipiens</i>	Gosner 26	R,U	14 d	-	890,000	Hogan et al. 2006	Value is an LC50
Leopard frog, <i>Rana pipiens</i>	Gosner 36	R,U	14 d	-	1,200,000	Hogan et al. 2006	Value is an LC50
<b>No Significant Effects Observed (NOEC Equals Highest Test Concentration)</b>							
*Sand goby, <i>Pomatoschistus minutus</i>	juv	F,U	7 mo	6	-	Robinson et al. 2003	
Fathead minnow, <i>Pimephales promelas</i>	fert eggs	F,U	125 d	10	-	Parrot et al. 2003	
Fathead minnow, <i>Pimephales promelas</i>	juvenile	F,M	21 d	20	-	Panter et al. 2002	Conc. only 40% of nominal

<b>Species</b>	<b>Life Stage</b>	<b>Method</b>	<b>Duration</b>	<b>NOEC- survival (ng/L)</b>	<b>LOEC - survival (ng/L)</b>	<b>Reference</b>	<b>Remarks</b>
Chinese rare minnow, <i>Gobiocypris rarus</i>	7 mo	F,U	28 d	25	-	Zha et al. 2007	10% mortality
Zebrafish, <i>Danio rerio</i>	fert. eggs	F,U	3 mo	25	-	Van den Belt et al. 2003	40% mortality after 5 mo. recovery
Fathead minnow, <i>Pimephales promelas</i>	mature male	F,U	35 d	50	-	Schmid et al. 2002	
Snail, <i>Potamopyrgus antipodarum</i>	adult	R,U	9 wk	100	-	Jobling et al. 2004	
Medaka, <i>Oryzias latipes</i>	1 d	R,U	2 mo	100	-	Scholz and Gutzeit 2000	8% mortality
Zebrafish, <i>Danio rerio</i>	4 wk	R,U	33 d	100	-	Versommen and Janssen 2004	6.6% mortality; excessive carrier solvent
Guppy, <i>Poecilia reticulata</i> (male)	< 7 d	F,M	108 d	110	-	Nielsen and Baatrup 2006	
Sturgeon, <i>Acipenser fulvescens</i>	1 yr	F,M	25 d	120	-	Palace et al. 2001	
Rainbow trout male, <i>Oncorhynchus mykiss</i>		F,M	3 wk	130	-	Hook et al. 2007	
African clawed frog, <i>Xenopus laevis</i>	adult	R,U	4 wk	2,960	-	Urbatzka et al. 2007	
Wood frog, <i>Rana sylvatica</i>	Gosner stage 25	R,M	76 d	4,100	-	Mackenzie et al. 2003	
Amphipod <i>Gammarus pulex</i>	mixed age	F,M	100 d	7,600	-	Watts et al. 2002	
Amphipod adult, <i>Hyalella azteca</i>	pre-copulatory	R,M	10 wk - 2 x gen	10,000	-	Vandenbergh et al. 2003	
*Copepod, <i>Tisbe battagliai</i>	<24 hr	R,U	21 d	100,000	-	Hutchinson et al. 1999	
*Copepod, <i>Tisbe battagliai</i>	<24 hr	R,U	21 d	100,000	-	Pounds et al. 2002	
Cladoceran, <i>Sida crystallina</i>		R,U	34 d	500,000	-	Jaser et al. 2003	
Cladoceran, <i>Daphnia magna</i>		R,U	25 d	500,000	-	Goto and Hiromi 2003	

\*Indicates saltwater species.

**Table A.3. Effects of EE2 on Aquatic Animals (Growth).**

Species Significant Effects Observed	Life Stage	Method	Duration	NOEC- growth (ng/L)	LOEC- growth (ng/L)	Reference	Remarks
Zebrafish , <i>Danio rerio</i>	fert egg	F,M	LC – F1	0.10	0.3	Wenzel et al. 2001	7% reduction at LOEC (75 dph)
Zebrafish , <i>Danio rerio</i>	fert egg	F,M	PLC	0.30	1.1	Wenzel et al. 2001	2% reduction at LOEC (78 dph)
Zebrafish, <i>Danio rerio</i>	20 dph	R,M	40 d	0.60	1.5	Orm et al. 2003	<b>Increased</b> juvenile wet weight
Fathead minnow, <i>Pimephales promelas</i>	<24 hr	F,M	LC	0.76	2.8	Länge et al. 2001	
Zebrafish , <i>Danio rerio</i>	fert egg	F,U	3 mo	1	10	Van den Belt et al. 2003	
Zebrafish, <i>Danio rerio</i>	2 dph	R,U	58 d	1	10	Lin and Janz 2006	
Fathead minnow, <i>Pimephales promelas</i>	embryo	F,M	114 d	-	12	Bogers et al. 2006b	
Chinese rare minnow, <i>Gobiocypris rarus</i>	7 mo	F,U	28 days	5	25	Zha et al. 2007	
Fathead minnow, <i>Pimephales promelas</i>	fert eggs	F,U	60 dph	10	32	Parrot and Wood 2002	
Zebrafish , <i>Danio rerio</i>	4 wk	R,U	33 d	10	100	Versonnen and Janssen 2004	Excessive carrier solvent
Guppy, <i>Poecilia reticulata</i>	<7d	F,M	108 d	44	112	Nielsen and Baatrup 2006	<b>Increased</b> adult wet weight.
Medaka, <i>Oryzias latipes</i>	1 day old	R,M	LC	29	290	Metcalfe et al. 2001	
Midge, <i>Chironomus riparius</i>	4th instar	S,U	48 hr	50	500	Lee et al. 2006	<b>Increased</b> larval dry weight
Midge, <i>Chironomus riparius</i>	1st instar	R,M	egg - pupa	100,000	1,000,000	Watts et al. 2003	

**No Significant Effects Observed (NOEC Equals Highest Test Concentration)**

Zebrafish, <i>Danio rerio</i>	fert egg	F,M	2xGen	4.5	-	Nash et al. 2004
*Three-spined stickleback, <i>Gasterosteus aculeatus</i>	fry	R,U	14 days	7.3	-	Hahlbeck et al. 2004b

Species	Life Stage	Method	Duration	NOEC-growth (ng/L)	LOEC-growth (ng/L)	Reference	Remarks
Fathead minnow, <i>Pimephales promelas</i>	fert eggs	F,U	60 dph	10	-	Parrot et al. 2003	
Fathead minnow, <i>Pimephales promelas</i>	juv	F,M	21 days	20	-	Panter et al. 2002	
Prosobranch mollusc, <i>Potamopyrgus antipodarum</i>	adult	R,U	9 wk	100	-	Jobling et al. 2004	
*Sheepshead minnow, <i>Cyprinodon variegatus</i>	juv	F,M	PLC	330	-	Zillioux et al. 2001	
Wood frog, <i>Rana sylvatica</i>	Gosner stage 25	R,M	76 d	4,100	-	Mackenzie et al. 2003	

\*Indicates saltwater species.

**Table A.4. Chronic Reproductive Effects of EE2 on Aquatic Animals (Fecundity, Fertility, and Population Growth).**

Species	Life Stage	Method	Duration	Endpoint	NOEC (ng/L)	LOEC (ng/L)	Reference	Remarks
<b>Significant Effects Observed</b>								
Fathead minnow, <i>Pimephales promelas</i>	40-60 h	F,M	LC	Percent fertilized of eggs laid	-	0.32	Parrott and Blunt 2005	
Zebrafish, <i>Danio rerio</i>	fert egg	F,M	LC	Number of fertilized eggs per female	0.30	1.1	Wenzel et al. 2001	
Fathead minnow, <i>Pimephales promelas</i>	<24 hr	F,M	LC	Mean no. eggs laid per breeding day	0.76	2.8	Länge et al. 2001	
Zebrafish, <i>Danio rerio</i>	fert egg	F,M	118 d	No. eggs spawned and prop fertilized	-	3	Fenske et al. 2005	
Fathead minnows, <i>Pimephales promelas</i>	-	Field	3 yrs	Population crash	-	3.2 – 8.9	Kidd et al. 2007	
Green frog, <i>Rana clamitans</i>	fert egg	Field	2 yr	Hatching success	-	3.2 – 8.9	Park and Kidd 2005	
Zebrafish, <i>Danio rerio</i>	fert egg	F,M	2 x gen	Proportion of non-viable eggs	0.50	4.5	Nash et al. 2004	Complete Rep. failure at LOEC
*Sand goby, <i>Pomatoschistus minutus</i>	juv	F,U	7 months	Fertile eggs and hatching success	-	6	Robinson et al. 2003	
Fathead minnow, <i>Pimephales promelas</i>	6-11 mo.	F,M	3 wk	Fert rate and no. eggs spawned	0.75	7.5	Pawlowski et al. 2004	<b>Increased</b> No. eggs spawned up to 0.75 ng/L
Zebrafish, <i>Danio rerio</i>	2 dph	R,U	60 d	% viable eggs, % hatch, % swim-up	1	10	Hill and Janz 2003	Excessive carrier solvent
Medaka, <i>Oryzias latipes</i>	1 d	R,U	2 mo	Female egg production	1	10	Scholz and Gutzeit 2000	No effect on male fert at 10 ng/L
Zebrafish, <i>Danio rerio</i>	fert egg	F,U	3 mo	No. spawning females & egg prod	1	10	Van den Belt et al. 2003	
Zebrafish, <i>Danio rerio</i>	8 mo	R,U	14 d	Absence of intact eggs in ovaries	1	10	Versommen and Janssen 2004	
Rainbow trout, <i>Oncorhynchus mykiss</i>	1+ year	F,M	PLC	Fertilization success	-	16	Schultz et al. 2003	EC50; same response@131 ng/L
Snail, <i>Potamopyrgus antipodarum</i>	adult	R,U	9 wk	Embryo production	25	100	Jobling et al. 2004	EE2 at 25 ng/L stimulatory
*Sheepshead minnow, <i>Cyprinodon variegatus</i>	juv	F,M	PLC	Hatching success	18	120	Zillioux et al. 2001	
Medaka, <i>Oryzias latipes</i>	6 mo.	F,M	21 d	Fecundity	260	490	Seki et al. 2002	

Species	Life Stage	Method	Duration	Endpoint	NOEC (ng/L)	LOEC (ng/L)	Reference	Remarks
Medaka, <i>Oryzias latipes</i>	4 mo.	R,U	14 d	Spawning frequency, % fertilized and % hatch.	5	500	Thompson 2000; Tilton et al. 2005	
Cladoceran, <i>Daphnia magna</i>	-	R,U	25 d	Embryo production	20,000	100,000	Goto and Hiromi 2003	
Rotifer, <i>Brachionus calyciflorus</i>		S,U	72 hr	Ratio of ovigerous/non-ovigerous females	202,000	510,000	Radix et al. 2002	
Rotifer, <i>Brachionus calyciflorus</i>		S,U	72 hr	Intrinsic rate population increase r	510,000	1,300,000	Radix et al. 2002	
<b>No Significant Effects Observed (NOEC Equals Highest Test Concentration)</b>								
Fathead minnow, <i>Pimephales promelas</i>	>6 mo.	F,M	3 wk	No. spawnings and eggs per spawn	1.5	-	Brian et al. 2007	
Mink frog, <i>Rana septentrionalis</i>	fert egg	Field	2 yr	Hatching success	3.2 – 8.9	-	Park and Kidd 2005	
Zebrafish, <i>Danio rerio</i> (females)	5-6 mo	R,U	15 d	Sterility in females	5,000	-	Ortiz-Zarragoitia et al. 2006	
Amphipod, <i>Gammarus pulex</i>	mixed ages	F, M	100 d	Population growth (total pop. size)	7,600	-	Watts et al. 2002	<b>Increase</b> in population size
*Copepod, <i>Tisbe battagliai</i>	<24 hr	R,U	21 days	Fecundity	100,000	-	Hutchinson et al. 1999	
*Copepod, <i>Tisbe battagliai</i>	<24 hr	R,U	21 days	Reproduction	100,000	-	Pounds et al. 2002	

\* Indicates saltwater species.

**Table A.5. Chronic Reproductive Effects of EE2 on Aquatic Animals (Sex Reversal).**

Species	Life Stage	Method	Duration	Effect	NOEC (ng/L)	LOEC (ng/L)	Reference	Remarks
<b>Significant Effects Observed</b>								
Zebrafish, <i>Danio rerio</i>	fert egg	F,U	3 mo	Delayed sexual differentiation	-	0.10	Van den Belt et al. 2003	No males at LOEC
Zebrafish, <i>Danio rerio</i>	20 dph	R,M	40 d	Male:female sex ratios	-	0.60	Orm et al. 2003	Complete sex reversal at 1.5 ng/L
Fathead minnow, <i>Pimephales promelas</i>	40-60 h old	F,M	LC	Male:femal sex ratio	0.32	0.96	Parrott and Blunt 2005	Complete ex. femin. at 3.5 ng/L
Fathead minnow, <i>Pimephales promelas</i>	fert. eggs	F,U	60 d	Male:female sex ratio	0.32	1.0	Parrot and Wood 2002	Complete ex. femin. at 3.2 ng/L
Zebrafish, <i>Danio rerio</i>	2 dph	R,U	58 d	Male:female sex ratio	-	1.0	Lin and Janz 2006	
Fathead minnow, <i>Pimephales promelas</i>	<24 hr	F,M	LC	Sex reversal - all female	0.76	2.8	Länge et al. 2001	Sex ratio at 0.76 ng/L 54:46
Zebrafish, <i>Danio rerio</i>	fert egg	F,M	42 d	Male feminization	-	3.0	Fenske et al. 2005	
Fathead minnow, <i>Pimephales promelas</i>	fert eggs	F,U	125 d	Sex reversal - all female	-	10	Parrot et al. 2003	
Zebrafish, <i>Danio rerio</i>	1 dph	R,U	60 d	Complete feminization	-	10	Orm et al 2006	
Zebrafish, <i>Danio rerio</i>	1 dph	R,U	60 d	Complete feminization	-	10	Yamani 2004	
Fathead minnow, <i>Pimephales promelas</i>	embryo	F,M	114 d	75% female gonads; 15% undeveloped	-	12	Bogers et al. 2006b	
Zebrafish, <i>Danio rerio</i>	fert egg	R,M	60 d	Complete feminization	-	15	Andersen et al. 2003b	
Medaka, <i>Oryzias latipes</i>	1 d	R,M	LC	Male:female sex ratio	2.9	29	Metcalfe et al. 2001	
*Three-spined stickleback, <i>Gasterosteus aculeatus</i>	Larvae	R,U	42 d	Sex reversal and intersex	-	50	Hahlbeck et al. 2004a	
Medaka, <i>Oryzias latipes</i>	1 d	R,U	2 mo	Sex reversal with ovary	10	100	Scholz and Gutzzeit 2000	
Medaka, <i>Oryzias latipes</i>	1 d	R,U	60 d	88% female, 2% male, 10% intersex	10	100	Yamani 2004 and Orm et al 2006	
Amphipod, <i>Gammarus pulex</i>	mixed ages	F, M	100 d	Male:femal ratio	-	104	Watts et al. 2002	No dose-response >104 ng/L

Species	Life Stage	Method	Duration	Effect	NOEC (ng/L)	LOEC (ng/L)	Reference	Remarks
Guppy, <i>Poecilia reticulata</i>	< 7 d	F,M	108 d	Male:female sex ratio	44	110	Nielsen and Baatrup 2006	
<b>No Significant Effects Observed (NOEC Equals Highest Test Concentration)</b>								
Green frog, <i>Rana clamitans</i>	fert egg	Field	2 yr	Male:female sex ratio	3.2 – 8.9	-	Park and Kidd 2005	
Mink frog, <i>Rana septentrionalis</i>	fert egg	Field	2 yr	Male:female sex ratio	3.2 – 8.9	-	Park and Kidd 2005	
Amphipod, <i>Hyalella azteca</i>	adult	R,M	10 wk; 2 gen	Male:femal sex ratio	10,000	-	Vandenbergh et al. 2003	
*Copepod, <i>Tisbe battagliai</i>	<24 hr	R,U	21 d	Male:female sex ratio	100,000	-	Hutchinson et al. 1999	
Cladoceran, <i>Daphnia magna</i>	-	R,U	25 d	Male:female ratio	500,000	-	Goto and Hiromi 2003	

\*Indicates saltwater species.

**Table A.6. Chronic Reproductive Effects of EE2 on Aquatic Animals (Intersex).**

Species	Life Stage	Method	Duration	Effect	NOEC (ng/L)	LOEC (ng/L)	Reference	Comments
<b>Significant Effects Observed</b>								
Pearl dace, <i>Margariscus margarita</i>	mature	Field	3 yr	Presence of testis-ova	-	3.2 – 8.9	Palace et al. 2006	Edema in ovaries
Fathead minnows, <i>Pimephales promelas</i>		Field	3 yr	Presence of testis-ova	-	3.2 – 8.9	Kidd et al. 2007	Testicular malformations
Mink frog, <i>Rana septentrionalis</i>	fert eggs	Field	2 yr	Intersex gonads (5 – 12 %)	-	3.2 – 8.9	Park and Kidd 2005	
Chinese rare minnow, <i>Gobiocypris rarus</i>	7 mo	F,U	28 d	Testis-ova in males	1.0	5.0	Zha et al. 2007	No sperm detectable
Fathead minnow, <i>Pimephales promelas</i>	egg	F,U	5 d	Ovarian cavities in males (8%)	-	10	Van Aerle et al. 2002	
Fathead minnow, <i>Pimephales promelas</i>	5-10 dph	F,U	5 d	Ovarian cavities in males (38%)	-	10	Van Aerle et al. 2002	
Fathead minnow, <i>Pimephales promelas</i>	10-15 dph	F,U	5 d	Ovarian cavities in males (64%)	-	10	Van Aerle et al. 2002	
Fathead minnow, <i>Pimephales promelas</i>	15-20 dph	F,U	5 d	Ovarian cavities in males (43%)	-	10	Van Aerle et al. 2002	
Fathead minnow, <i>Pimephales promelas</i>	egg	F,U	20 d	Ovarian cavities in males (22%)	-	10	Van Aerle et al. 2002	
Medaka, <i>Oryzias latipes</i>	1 d	R,M	LC	Sex inversion and testis-ova	2.9	29	Metcalfe et al. 2001	4 of 4 males with TO
*Three-spined stickleback, <i>Gasterosteus aculeatus</i>	Larvae	R,U	42 d	Intersexed gonads	-	50	Hahlbeck et al. 2004a	
Medaka, <i>Oryzias latipes</i>	6 mo	F,M	21 d	Testis-ova in males (33%)	33	64	Seki et al. 2002	No histological abnormalities in females
Medaka, <i>Oryzias latipes</i>	1 d	R,U	2 mo	All males developed an ovary	10	100	Scholz and Gutzeit 2000	No effect on male fertility
Medaka, <i>Oryzias latipes</i>	1 d	R,U	60 d	Intersexed gonads (10%)	10	100	Yamani 2004 and Orm et al 2006	
Cuppy, <i>Poecilia reticulata</i>	< 7 d	F,M	108 d	Feminization of male reproductive ducts	44	110	Nielsen and Baatrup 2006	
Amphipod, <i>Hyalella azteca</i>	fert eggs	R,M	2 x gen	Oocyte-like structures in males	23	320	Vandenbergh et al. 2003	
Leopard frog, <i>Rana pipiens</i>	G-osner 25	R,M	162 d	Intersex and altered testicular development	41.4	4,140	Mackenzie et al. 2003	

Species	Life Stage	Method	Duration	Effect	NOEC (ng/L)	LOEC (ng/L)	Reference	Comments
Wood frog, <i>Rana sylvatica</i>	Gosner 25-28	R,M	76 d	Intersex and altered testicular development	-	4,140	Mackenzie et al. 2003	
<b>No Significant Effects Observed (NOEC Equals Highest Test Concentration)</b>								
Green frog, <i>Rana clamitans</i>	fert eggs	Field	2 yr	Intersexed gonads	3.2 – 8.9	-	Park and Kidd 2005	
Zebrafish, <i>Danio rerio</i>	2 dph	R,U	58 d	Testis-ova	10	-	Lin and Janz 2006	
Zebrafish, <i>Danio rerio</i>	2 dph	R,U	58 d	Testis-ova	10	-	Hill and Janz 2003	Excessive carrier solvent; high control mortality
Zebrafish, <i>Danio rerio</i>	adult	F,U	21 d	Feminization of testes	25	-	Islinger et al. 2003	

\*Indicates saltwater species.

**Table A.7. Chronic Reproductive Effects of EE2 on Aquatic Animals (Sexual Behavior).**

Species	Life Stage	Method	Duration	Effect	NOEC (ng/L)	LOEC (ng/L)	Reference	Remarks
<b>Significant Effects Observed</b>								
*Sand goby, <i>Pomatoschistus minutus</i>	juv	F,U	7 mo	Male nesting behavior	-	6	Robinson et al. 2003	High mortality in solvent controls in first month of exposure
Fathead minnow, <i>Pimephales promelas</i>	mature	F,M	27 d	Impaired ability to compete and acquire territory	2.0	8.9	Majewski et al. 2002	
*Three-spined stickleback, <i>Gasterosteus aculeatus</i>	mature	R,U	12 d	Time spent near nest and gluing frequency, but effect short-lived	-	10	Brian et al. 2006	
<b>No Significant Effects Observed (NOEC Equals Highest Test Concentration)</b>								
Zebrafish, <i>Danio rerio</i>	fert egg	F,M	2 x gen	Natural spawning behavior of adult male fish	4.5	-	Nash et al. 2004	Sexually compromised males still actively participated in the spawning act, i.e., chasing females and competing with healthy males
Amphipod, <i>Gammarus pulex</i>	3-5 mm	R,M	10 d	Pre-copulatory guarding behavior	3,700,000	-	Watts et al. 2001	Reproductive behavior was only disrupted at high concentrations where it would be unrealistic to attribute effects to and endocrine-mediated process.

**Table A.8. Data on Effects of EE2 on Aquatic Animals (Secondary Sexual Characteristics).**

Species	Life Stage	Method	Duration	Effect	Event Association	NOEC (ng/L)	LOEC (ng/L)	Reference	Comments
<b>Significant Effects Observed</b>									
Fathead minnow, <i>Pimephales promelas</i>	6-11 mo.	F,M	3 wk	Number of male nuptial tubercles	Activational	-	0.80	Pawlowski et al. 2004	
Fathead minnow, <i>Pimephales promelas</i>	40-60 h	F,M	LC	Ovipositor size, nuptial tubercles, banding strength	Organizational	0.32	0.96	Parrott and Blunt 2005	
Fathead minnow, <i>Pimephales promelas</i>	156 dph	F,M	LC	Nuptial tubercles, banding strength, dorsal fin dot, dorsal fat pad	Activational	0.32	0.96	Parrott and Blunt 2005	
Fathead minnow, <i>Pimephales promelas</i>	fert eggs	F,U	60 dph	Male sex index: nuptial tubercles, dorsal fat pad, dorsal fin dot, banding strength	Organizational	0.32	1.0	Parrot and Wood 2002	Complete femin. at 3.2 ng/L
Fathead minnow, <i>Pimephales promelas</i>	<24 hr	F,M	LC	Secondary sex characteristics – not specified	Organizational	0.76	2.8	Länge et al. 2001	50% sex ratio at 0.76 ng/L
Fathead minnow, <i>Pimephales promelas</i>	fert eggs	F,U	60 dph	Development and length of ovipositors	Organizational	1.0	3.2	Parrot and Wood 2002	Complete femin. at 3.5 ng/L
Zebrafish, <i>Danio rerio</i>	fert egg	F,M	2 x gen	Coloration and bright anal fin markings	Organizational	-	4.5	Nash et al. 2004	
*Sand goby, <i>Pomatoschistus minutus</i>	juv	F,U	7 mo	Delayed and inhibited nuptial coloration in males	Activational	-	6.0	Robinson et al. 2003	
Fathead minnow, <i>Pimephales promelas</i>	fert eggs	F,U	125 d	Ovipositor size, nuptial tubercles, banding strength	Organizational	-	10	Parrot et al. 2003	
Fathead minnow, <i>Pimephales promelas</i>	maturing	F,M	21 d	Number and prominence of nuptial tubercles and dorsal fat pad	Activational	-	11	Filby et al. 2007	
Fathead minnow, <i>Pimephales promelas</i>	embryo	F,M	114 d	Number and prominence of nuptial tubercles	Organizational	-	12	Bogers et al. 2006b	
Amphipod, <i>Hyalella azteca</i>	fert eggs	R,M	2 x gen	Male second gnathopods	Organizational	-	23	Vandenbergh et al. 2003	No effect >1,000 ng/L
<b>No Significant Effects Observed (NOEC Equals Highest Test Concentration)</b>									
Fathead minnow, <i>Pimephales promelas</i>	>6 mo.	F,M	3 wk	Relative fat pad weight, number of nuptial tubercles, nuptial tubercle prominence	Activational	1.5	-	Brian et al. 2007	
Medaka, <i>Oryzias latipes</i>	4 mo	R,U	14 d	Anal and dorsal fin shape	Organizational	500	-	Thompson 2000; Tilton et al. 2005	

\*Indicates saltwater species.

**Table A.9. Chronic Reproductive Effects of EE2 on Aquatic Animals (Vitellogenin).**

Species	Life Stage	Method	Duration	Effect	NOEC (ng/L)	LOEC (ng/L)	Reference	Comments
<b>Significant Effects Observed</b>								
Zebrafish, <i>Danio rerio</i>	adult males	F,M	40 d	Increased whole-blood VTG level	-	0.50	Nash et al. 2004	
Fathead minnow, <i>Pimephales promelas</i>	6-11 mo	F,M	3 wk	Increased plasma VTG levels	-	0.80	Pawlowski et al. 2004	
Rainbow trout, <i>Oncorhynchus mykiss</i>	immature female	F,M	14 d	Increased liver <i>vtg</i> mRNA expression and plasma VTG	0.21	1.0	Thomas-Jones et al. 2003	
Zebrafish, <i>Danio rerio</i>	20 dph	R,M	40 d	Increased whole body VTG levels	-	1.5	Orn et al. 2003	
Zebrafish, <i>Danio rerio</i>	adult male	R,U	21 d	Increased plasma VTG level	-	1.6	Fenske et al. 2001	
Rainbow trout, <i>Oncorhynchus mykiss</i>	Adult male	F,M	3 wk	Increased plasma VTG level	-	1.8	Jobling et al. 1996	
Zebrafish, <i>Danio rerio</i>	fert egg	F,M	42 d	Increased vitellogenin level	-	3.0	Fenske et al. 2005	
Fathead minnow, <i>Pimephales promelas</i>	-	Field	5 mo	Increased plasma VTG levels	-	3.2 – 8.9	Palace et al. 2002 (also see Kidd et al. 2007)	
Pearl dace, <i>Margariscus margarita</i>	-	Field	3 yrs	Increased whole body VTG level	-	3.2 – 8.9	Palace et al. 2006	
Zebrafish, <i>Danio rerio</i>	adult male	F,M	8 d	Increased whole-body VTG level	2.2	3.6	Rose et al. 2002	EC10 = 0.92 ng/L
Zebrafish, <i>Danio rerio</i>	adult females	F,M	40 d	Increased whole-blood VTG level	0.50	4.5	Nash et al. 2004	
Fathead minnow, <i>Pimephales promelas</i>	juv	F,M	21 d	Increased whole-body VTG level	2.0	5.0	Panter et al. 2002	
Fathead minnow, <i>Pimephales promelas</i>	8 mo	R,M	48 hrs	Increased liver <i>vtg</i> levels	2.5	5.0	Biales et al. 2007	
Ide, <i>Leuciscus idus</i>	juv	F,M	7 d	Increased plasma VTG levels	-	6.0	Allmer et al. 1999	
Zebrafish, <i>Danio rerio</i>	fert egg	R,M	4 d	Increased whole body VTG level	2.6	7.8	Bogers et al. 2006a	
Zebrafish, <i>Danio rerio</i>	adult females	R,M	21 d	Increased plasma VTG level	4.1	8.5	Van den Belt et al. 2004	
Zebrafish, <i>Danio rerio</i>	adult males	F,M	24 d	Increased plasma VTG level	-	9.0	Van den Belt et al. 2002	

Rainbow trout, <i>Oncorhynchus mykiss</i>	11 mo	F,M	2 wk	Increased plasma VTG level	0.87	10	Samuelsson et al. 2006
*Eelpout, <i>Zoarces viviparus</i>	adult female	F,M	3 wk	Increased plasma VTG level	5.0	10	Kotsgaard et al. 2002
Fathead minnow, <i>Pimephales promelas</i>	<24 hr	F,M	LC	Increased whole body VTG level	2.8	12	Länge et al. 2001
Zebrafish, <i>Danio rerio</i>	adults	F,M	168 hrs	Increased plasma VTG level	-	14	Hoffmann et al. 2006
Sturgeon, <i>Acipenser fulvescens</i>	1 yr	F,M	25 d	Increased plasma VTG levels	-	14	Palace et al. 2001
Lake trout, <i>Salvelinus namaycush</i>	immature	F,M	21 d	Increased plasma VTG level	-	15	Werner et al. 2003
*Baltic flounder, <i>Platichthys flesus</i>	adult	F,M	21 d	Increased plasma VTG level in male and female fish	-	15	Allen et al. 1999b
Medaka, <i>Oryzias latipes</i>	6 mo.	F,M	21 d	Increased liver Vtg levels	33	64	Seki et al. 2002
Rainbow trout, <i>Oncorhynchus mykiss</i>	juvenile	R,M	14 d	Increased plasma VTG level	10	100	Verslycke et al. 2002
*Sheepshead minnow, <i>Cyprinodon variegatus</i>	male	F,M	16 d	Increased liver vtg mRNA expression	24	110	Folmar et al. 2000
Rainbow trout, <i>Oncorhynchus mykiss</i>	mature male	F,M	61 d	Increased plasma VTG levels	-	140	Skillman et al. 2006
<b>No Significant Effects Observed (NOEC Equals Highest Test Concentration)</b>							
Zebrafish, <i>Danio rerio</i>	adult males	F,M	310 dpf F1	No effect on whole-blood VTG level	4.5	-	Nash et al. 2004
Zebrafish, <i>Danio rerio</i>	adult females	F,M	310 dpf F1	No effect on whole-blood VTG level	4.5	-	Nash et al. 2004

\*Indicates saltwater species.

**Table A.10. Chronic Effects of EE2 on Aquatic Animals (Other Relevant Endpoints).**

Species	Life Stage	Method	Duration	Effect	Concentration (ng/L)	Reference	Remarks
<b>Significant Effects Observed</b>							
Zebrafish, <i>Danio rerio</i>	17-20 dpf	R,U	3 d	Enhanced effect on CYP19A2 gene expression	0.30	Kazeto et al. 2004	Excessive carrier solvent
Zebrafish, <i>Danio rerio</i>	2 dph	R,U	58 d	Suppression of gametogenesis for males (no testes discernible) and females	1.0	Weber et al. 2003	Excessive carrier solvent
Chinese rare minnow, <i>Gobiocypris rarus</i>	7 mo	F,U	28 d	Increased GSI and renal somatic index (RSI) in males	1.0	Zha et al. 2007	
Fathead minnow, <i>Pimephales promelas</i>	40-60 h	F,M	LC	Reduced GSI in females	3.5	Parrott and Blunt 2005	
Atlantic salmon, <i>Salmo salar</i>	immature	S,U	7 d	Increased AchE and GST activities and lactate content after 3 days, but no effect at 7 days	5.0	Greco et al. 2007	
Chinese rare minnow, <i>Gobiocypris rarus</i>	7 mo	F,U	28 d	Reduced GSI in females	5.0	Zha et al. 2007	
Chinese rare minnow, <i>Gobiocypris rarus</i>	7 mo	F,U	28 d	Increased RSI in females	5.0	Zha et al. 2007	
Medaka, <i>Oryzias latipes</i>	4 mo	R,U	14 d	Increased male and female plasma E2 levels	5.0	Thomposn 2000; Tilton et al. 2005	
Fathead minnow, <i>Pimephales promelas</i>	6-11 mo	F,M	3 wk	Reduction on male GSI	7.5	Pawlowski et al. 2004	
Zebrafish, <i>Danio rerio</i>	Adult female	R,M	21 d	Reduced female ovarian somatic index	8.5	Van den Belt et al. 2004	
Rainbow trout, <i>Oncorhynchus mykiss</i>	11 mo	F,M	2 wk	Higher hepatosomatic index (HSI)	10	Samuelsson et al. 2006	
Fathead minnow, <i>Pimephales promelas</i>	fert eggs	F,U	125 d	Increased liver somatic index	10	Parrot et al. 2003	
Medaka, <i>Oryzias latipes</i>	1 d	R,U	4 mo	In both males and females, significantly increased number of necrotic hepatocytes and kidney tubule cells	10	Weber et al. 2004	
*Baltic flounder, <i>Platichthys flesus</i>	adult	F,M	21 d	Increased HSI in males	15	Allen et al. 1999	
Zebrafish, <i>Danio rerio</i>	adult male	S,M	21 d	Increased levels of cyp19a2 mRNA (aromatase)	21	Kallivretaki et al. 2006	
Chinese rare minnow, <i>Gobiocypris rarus</i>	7 mo	F,U	28 d	Increased HSI in males	25	Zha et al. 2007	

Species	Life Stage	Method	Duration	Effect	Concentration (ng/L)	Reference	Remarks
Chinese rare minnow, <i>Gobiocypris rarus</i>	7 mo	F,U	28 d	Ovary degeneration in females	25	Zha et al. 2007	
Zebrafish, <i>Danio rerio</i>	adult male	F,M	7 d	Decreased testosterone and 11-ketotestosterone levels	26	Andersen et al. 2006	
Atlantic salmon, <i>Salmo salar</i>	immature	S,U	72 hr	Induced expression of brain P450 Aromatase	50	Lyssimachou et al. 2006	
Sturgeon, <i>Acipenser fulvescens</i>	1 yr	F,M	25 d	Increased plasma Vit E, A1 and A2; Decreased Vit E and A in kidney	60	Palace et al. 2001	
*Sheepshead minnow, <i>Cyprinodon variegatus</i>	juv	F,M	PLC	Increased pathological condition of kidneys	120	Zillioux et al. 2001	Fish survived to reproduction
Rainbow trout, <i>Oncorhynchus mykiss</i>	mature male	F,M	3 wk	Changed gene expression profile	130	Hook et al. 2007	
Zebrafish, <i>Danio rerio</i>	18-21 d	R,U	72 hr	Stimulated expression of Cytochrome P450 aromatase (Aro-B)	300	Le Page et al. 2006	
Medaka, <i>Oryzias latipes</i>	mature	R,U	14 d	Induced ER protein and aromatase activity	500	Contractor et al. 2004	
Medaka, <i>Oryzias latipes</i>	adult	R,U	14 d	Increased hepatic estrogen receptor (ER)	500	Thompson 2000	
Medaka, <i>Oryzias latipes</i>	4 mo.	R,U	14 d	Decreased female and male GSI	500	Thompson 2000; Tilton et al. 2005	
African clawed frog, <i>Xenopus laevis</i>	adult	R,U	4 wk	Reduced Leutinizing hormone B mRNA expression	3,000	Urbatzka et al. 2006	
African clawed frog, <i>Xenopus laevis</i>	adult	R,U	4 wk	Reduced testosterone levels in both sexes	3,000	Urbatzka et al. 2007	
African clawed frog, <i>Xenopus laevis</i>	adult	R,U	4 wk	Reduced E2 level in females	3,000	Urbatzka et al. 2007	
Midge, <i>Chironomus riparius</i>	4th instar	S,U	24 h	Increased expression of heat shock proteins	8,000	Lee et al. 2006	
Medaka, <i>Oryzias latipes</i>	mature male	R,U	6 d	Increased mRNA expression of liver choriogenin L	10,000	Lee et al. 2002b	
Medaka, <i>Oryzias latipes</i>	mature male	R,U	6 d	Increased mRNA expression of liver choriogenin H levels	20,000	Lee et al. 2002b	
Medaka, <i>Oryzias latipes</i>	mature male	R,U	6 d	Increased mRNA expression of liver choriogenin H levels	20,000	Lee et al. 2002a	
Medaka, <i>Oryzias latipes</i>	juv	R,U	6 d	Increased mRNA expression of whole body Choriogenin H	50,000	Lee et al. 2002a	

No Significant Effects Observed (NOEC Equals Highest Test Concentration)

<b>Species</b>	<b>Life Stage</b>	<b>Method</b>	<b>Duration</b>	<b>Effect</b>	<b>Concentration (ng/L)</b>	<b>Reference</b>	<b>Remarks</b>
*Sand goby, <i>Pomatoschistus minutus</i>	juv	F,U	7 mo	No effect on GSI in males or females	6.0	Robinson et al. 2003	
Rainbow trout, <i>Oncorhynchus mykiss</i>	juvenile	R,M	14 d	No effect on GSI or HSI	100	Verslycke et al. 2002	
African clawed frog, <i>Xenopus laevis</i>	adult	R,U	4 wk	No effect on Gonadotropin Releasing Hormone mRNA expression	3,000	Urbatzka et al. 2006	

\*Indicates saltwater species.

# Exhibit 51

**Guidelines for Deriving  
Numerical National Water Quality Criteria  
for the Protection Of Aquatic Organisms  
and Their Uses**

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## **Notices**

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This document is available the public to through the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161.

### **Special Note**

This December 2010 electronic version of the 1985 Guidelines serves to meet the requirements of Section 508 of the Rehabilitation Act. While converting the 1985 Guidelines to a 508-compliant version, EPA updated the taxonomic nomenclature in the tables of Appendix 1 to reflect changes that occurred since the table were originally produced in 1985. The numbers included for Phylum, Class and Family represent those currently in use from the Integrated Taxonomic Information System, or ITIS, and reflect what is referred to in ITIS as Taxonomic Serial Numbers. ITIS replaced the National Oceanographic Data Center (NODC) taxonomic coding system which was used to create the original taxonomic tables included in the 1985 Guidelines document (NODC, Third Addition - see Introduction). For more information on the NODC taxonomic codes, see <http://www.nodc.noaa.gov/General/CDR-detdesc/taxonomic-v8.html>.

The code numbers included in the reference column of the tables have not been updated from the 1985 version. These code numbers are associated with the old NODC taxonomic referencing system and are simply replicated here for historical purposes. Footnotes may or may not still apply.

EPA is working on a more comprehensive update to the 1985 Guidelines, including new taxonomic tables which better reflect the large number of aquatic animal species known to be propagating in U.S. waters.

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## ***Executive Summary***

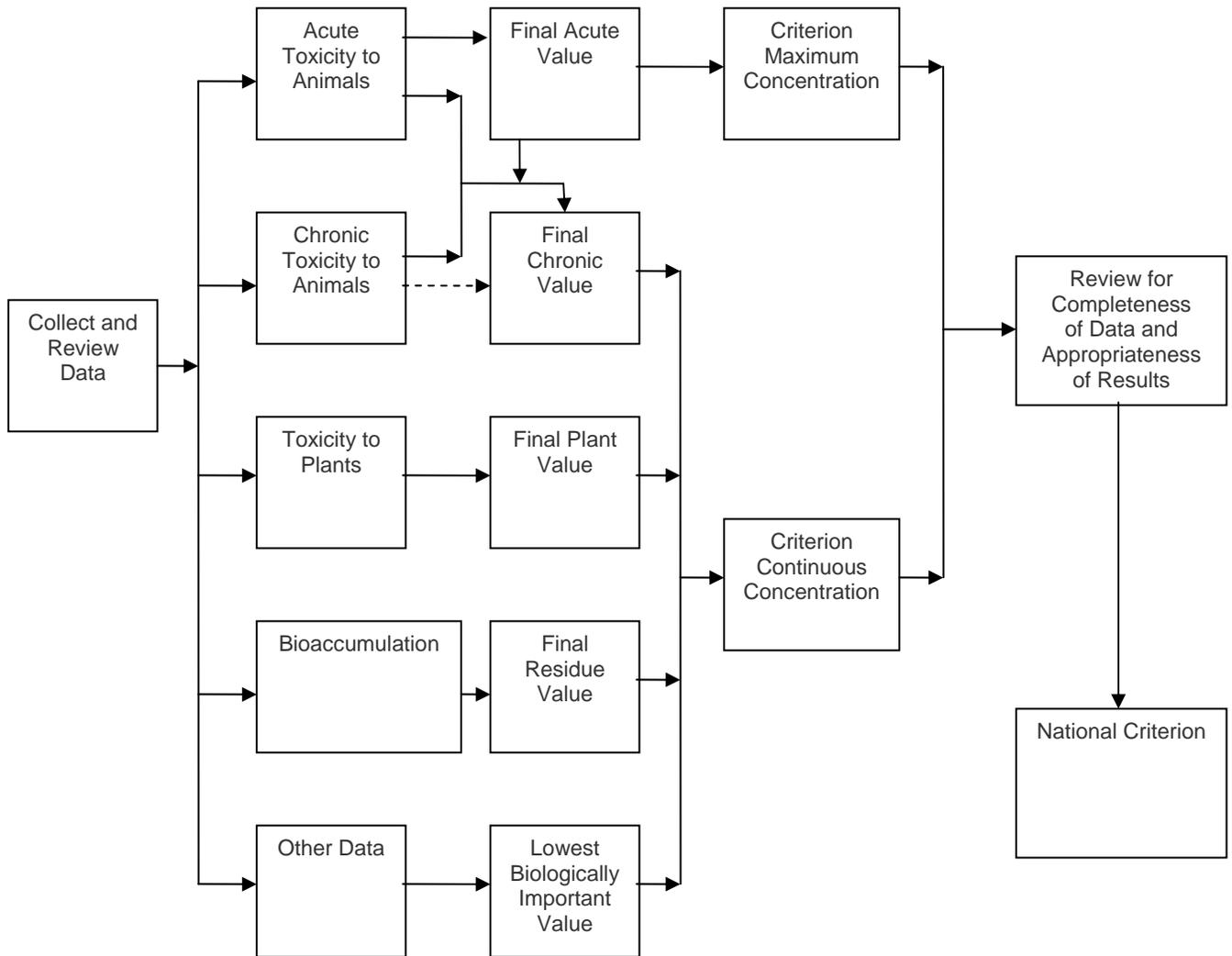
Derivation of numerical national water quality criteria for the protection of aquatic organism and their uses is a complex process (Figure 1) that uses information from many areas of aquatic toxicology. After a decision is made that a national criterion is needed for a particular material, all available information concerning toxicity to, and bioaccumulation by, aquatic organisms is collected, reviewed for acceptability, and sorted. If enough acceptable data on acute toxicity to aquatic animals are available, they are used to estimate the highest one-hour average concentration that should not result in unacceptable effects on aquatic organisms and their uses. If justified, this concentration is made a function of a water quality characteristic such as pH, salinity, or hardness. Similarly, data on the chronic toxicity of the material to aquatic animals are used to estimate the highest four-daily average concentration that should not cause unacceptable toxicity during a long-term exposure. If appropriate, this concentration is also related to a water quality characteristic.

Data on toxicity to aquatic plants are examined to determine whether plants are likely to be unacceptably affected by concentrations that should not cause unacceptable effects on animals. Data on bioaccumulation by aquatic organisms are used to determine if residues might subject edible species to restrictions by the U.S. Food and Drug Administration or if such residues might harm some wildlife consumers of aquatic life. All other available data are examined for adverse effects that might be biologically important.

If a thorough review of the pertinent information indicates that enough acceptable data are available, numerical national water quality criteria are derived for fresh water or salt water or both to protect aquatic organisms and their uses from unacceptable effects due to exposures to high concentrations for short periods of time, lower concentrations for longer periods of time, and combinations of the two.

**Figure 1**

Derivation of Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses



## ***Introduction***

Of the several possible forms of criteria, the numerical form is the most common, but the narrative (e.g., pollutants must not be present in harmful concentrations) and operational (e.g., concentrations of pollutants must not exceed one-tenth of the 96-hr LC50) forms can be used if numerical criteria are not possible or desirable. If it were feasible, a freshwater (or saltwater) numerical aquatic life national criterion\* for a material should be determined by conducting field tests on a wide variety of unpolluted bodies of fresh (or salt) water. It would be necessary to add various amounts of the material to each body of water in order to determine the highest concentration that would not cause any unacceptable long-term or short-term effect on the aquatic organisms or their uses. The lowest of these highest concentrations would become the freshwater (or saltwater) national aquatic life water quality criterion for that material, unless one or more of the lowest concentrations were judged to be outliers. Because it is not feasible to determine national criteria by conducting such field tests, these Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (hereafter referred to as the National Guidelines) describe an objective, internally consistent, appropriate, and feasible way of deriving national criteria, which are intended to provide the same level of protection as the infeasible field testing approach described above.

Because aquatic ecosystems can tolerate some stress and occasional adverse effects, protection of all species at all times and places is not deemed necessary. If acceptable data are available for a large number of appropriate taxa from an appropriate variety of taxonomic and functional groups, a reasonable level of protection will probably be provided if all except a small fraction of the taxa are protected, unless a commercially or recreationally important species is very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is too high or too low or just right.

Determining the validity of a criterion derived for a particular body of water, possibly by modification of a national criterion to reflect local conditions<sup>1, 2, 3</sup>, should be based on an operation definition of "protection of aquatic organisms and their uses" that takes into account the practicalities of field monitoring programs and the concerns of the public. Monitoring programs should contain sampling points at enough times and places that all unacceptable changes, whether caused directly or indirectly, will be detected. The programs should adequately monitor the kinds of species of concern to the public, i.e., fish in fresh water and fish and macroinvertebrates in salt water. If the kinds of species of concern cannot be adequately monitored at a reasonable cost, appropriate surrogate species should be monitored. The kinds of species most likely to be good surrogates are those that either (a) are a major food of the desired kinds of species or (b) utilize the same food as the desired species or (c) both. Even if a major adverse effect on appropriate surrogate species does not directly result in an unacceptable effect on the kinds of species of concern to the public, it indicates a high probability that such an effect will occur.

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\* The term "national criteria" is used herein because it is more descriptive than the synonymous term "section 304(a) criteria", which is used in the Water Quality Standards Regulation [1].

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term short-term effects on (1) commercially, recreationally, and other important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams, and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. Monitoring programs intended to be able to detect unacceptable effects should be tailored to the body of water of concern so that necessary samples are obtained at enough times and places to provide adequate data on the populations of the important species, as well as data directly related to the reasons for their being considered important. For example, for substances that are residue limited, species that are consumed should be monitored for contaminants to ensure that wildlife predators are protected, FDA action levels are not exceeded, and flavor is not impaired. Monitoring programs should also provide data on the number of taxa and number of individuals in the above-named assemblages that can be sampled at reasonable cost. The amount of decrease in the number of taxa or number of individuals in an assemblage that should be considered unacceptable should take into account appropriate features of the body of water and its aquatic community. Because most monitoring programs can only detect decreases of more than 20 percent, any statistically significant decrease should usually be considered unacceptable. The insensitivity of most monitoring programs greatly limits their usefulness for studying the validity of criteria because unacceptable changes can occur and not be detected. Therefore, although limited field studies can sometimes demonstrate that criteria are underprotective, only high quality field studies can reliably demonstrate that criteria are not underprotective.

If the purpose of water quality criteria were to protect only commercially and recreationally important species, criteria specifically derived to protect such species and their uses from the direct adverse effects of a material would probably, in most situations, also protect those species from indirect adverse effects due to effects of the material on other species in the ecosystem. For example, in most situations either the food chain would be more resistant than the important species and their uses or the important species and their food chains would be adaptable enough to overcome effects of the material on portions of the food chains.

These National Guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations. All North American bodies of water and resident aquatic species and their uses are meant to be taken into account, except for a few that may be too atypical, such as the Great Salt Lake, brine shrimp, and the siscowet subspecies of lake trout, which occurs in Lake Superior and contains up to 67% fat in the fillets<sup>4</sup>. Derivation of criteria specifically for the Great Salt Lake or Lake Superior might have to take brine shrimp and siscowet, respectively, into account.

Numerical aquatic life criteria derived using these National Guidelines are expressed as two numbers, rather than the traditional one number, so that the criteria more accurately reflect toxicological and practical realities. If properly derived and used, the combination of a maximum concentration and a continuous concentration should provide an appropriate degree of protection of aquatic organisms and their uses from acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms, without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these Guidelines are intended to be useful for developing water quality standards, mixing zone standards, effluent limitations, etc. The development of such standards

and limitations, however, might have to take into account such additional factors as social, legal, economic, and hydrological considerations, the environmental and analytical chemistry of the material, the extrapolation from laboratory data to field situations, and relationships between species for which data are available and species in the body of water of concern. As an intermediate step in the development of standards, it might be desirable to derive site-specific criteria by modification of national criteria to reflect such local conditions as water quality, temperature, or ecologically important species<sup>1,2,3</sup>. In addition, with appropriate modifications these National Guidelines can be used to derive criteria for any specific geographical area, body of water (such as the Great Salt Lake), or group of similar bodies of water, if adequate information is available concerning the effects of the material of concern on appropriate species and their uses.

Criteria should attempt to provide a reasonable and adequate amount of protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a national criterion be the best estimate that can be obtained using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Therefore, these National Guidelines specify certain data that should be available if a numerical criterion is to be derived. If all the required data are not available, usually a criterion should not be derived. On the other hand, the availability of all required data does not ensure that a criterion can be derived.

A common belief is that national criteria are based on "worst case" assumptions and that local considerations will raise, but not lower, criteria. For example, it will usually be assumed that if the concentration of a material in a body of water is lower than the national criterion, no unacceptable effects will occur and no site-specific criterion needs to be derived. If, however, the concentration of a material in a body of water is higher than the national criterion, it will usually be assumed that a site-specific criterion should be derived. In order to prevent the assumption of the "worst case" nature of national criteria from resulting in the underprotection of too many bodies of water, national criteria must be intended to protect all or almost all bodies of water. Thus, if bodies of water and the aquatic communities in them do differ substantially in their sensitivities to a material, national criteria should be at least somewhat overprotective for a majority of the bodies of water. To do otherwise would either (a) require derivation of site-specific criteria even if the site-specific concentration were substantially below the national criterion or (b) cause the "worst case" assumption to result in the underprotection of numerous bodies of water. On the other hand, national criteria are probably underprotective of some bodies of water.

The two factors that will probably cause the most difference between national and site-specific criteria are the species that will be exposed and the characteristics of the water. In order to ensure that national criteria are appropriately protective, the required data for national criteria include some species that are sensitive to many materials and national criteria are specifically based on tests conducted in water relatively low in particulate matter and organic matter. Thus, the two factors that will usually be considered in the derivation of site-specific criteria from national criteria are used to help ensure that national criteria are appropriately protective.

On the other hand, some local conditions might require that site-specific criteria be lower than national criteria. Some untested locally important species might be very sensitive to the material of concern, and local water quality might not reduce the toxicity of the material. In addition,

aquatic organisms in field situations might be stressed by diseases, parasites, predators, other pollutants, contaminated or insufficient food, and fluctuating and extreme conditions of flow, water quality, and temperature. Further, some materials might degrade to more toxic materials, or some important community functions or species interactions might be adversely affected by concentrations lower than those that affect individual species.

Criteria must be used in a manner that is consistent with the way in which they were derived if the intended level of protection is to be provided in the real world. Although derivation of water quality criteria for aquatic life is constrained by the ways toxicity and bioconcentration tests are usually conducted, there are still many different ways that criteria can be derived, expressed, and used. The means used to derive and state criteria should relate, in the best possible way, the kinds of data that are available concerning toxicity and bioconcentration and the ways criteria can be used to protect aquatic organisms and their uses.

The major problem is to determine the best way that the statement of a criterion can bridge the gap between the nearly constant concentrations used in most toxicity and bioconcentration tests and the fluctuating concentrations that usually exist in the real world. A statement of a criterion as a number that is not to be exceeded any time or place is not acceptable because few, if any, people who use criteria would take it literally and few, if any, toxicologists would defend a literal interpretation. Rather than try to reinterpret a criterion that is neither useful nor valid, it is better to develop a more appropriate way of stating criteria.

Although some materials might not exhibit thresholds, many materials probably do. For any threshold material, continuous exposure to any combination of concentrations below the threshold will not cause an unacceptable effect (as defined on pages 1 and 2) on aquatic organisms and their uses, except that the concentration of a required trace nutrient might be too low. However, it is important to note that this is a threshold of unacceptable effect, not a threshold of adverse effect. Some adverse effect, possibly even a small reduction in the survival, growth, or reproduction of a commercially or recreationally important species, will probably occur at, and possibly even below, the threshold. The Criterion Continuous Concentration (CCC) is intended to be a good estimate of this threshold of unacceptable effect. If maintained continuously, any concentration above the CCC is expected to cause an unacceptable effect. On the other hand, the concentration of a pollutant in a body of water can be above the CCC without causing an unacceptable effect if (a) the magnitudes and durations of the excursions above the CCC are appropriately limited and (b) there are compensating periods of time during which the concentration is below the CCC. The higher the concentration is above the CCC, the shorter the period of time it can be tolerated. But it is unimportant whether there is any upper limit on concentrations that can be tolerated instantaneously or even for one minute because concentrations outside mixing zones rarely change substantially in such short periods of time.

An elegant, general approach to the problem of defining conditions (a) and (b) would be to integrate the concentration over time, taking into account uptake and depuration rates, transport within the organism to a critical site, etc. Because such an approach is not currently feasible, an approximate approach is to require that the average concentration not exceed the CCC. The average concentration should probably be calculated as the arithmetic average rather than the geometric mean<sup>5</sup>. If a suitable averaging period is selected, the magnitudes and durations of concentrations above the CCC will be appropriately limited, and suitable compensating periods below the CCC will be required.

In the elegant approach mentioned above, the uptake and depuration rates would determine the effective averaging period, but these rates are likely to vary from species to species for any particular material. Thus the elegant approach might not provide a definitive answer to the problem of selecting an appropriate averaging period. An alternative is to consider that the purpose of the averaging period is to allow the concentration to be above the CCC only if the allowed fluctuating concentrations do not cause more adverse effect than would be caused by a continuous exposure to the CCC. For example, if the CCC caused a 10% reduction in growth of rainbow trout, or a 13% reduction in survival of oysters, or a 7% reduction in reproduction of smallmouth bass, it is the purpose of the averaging period to allow concentrations above the CCC only if the total exposure will not cause any more adverse effect than continuous exposure to the CCC would cause.

Even though only a few tests have compared the effects of a constant concentration with the effects of the same average concentration resulting from a fluctuating concentration, nearly all the available comparisons have shown that substantial fluctuations result in increased adverse effects<sup>5, 6</sup>. Thus if the averaging period is not to allow increased adverse effects, it must not allow substantial fluctuations. Life-cycle tests with species such as mysids and daphnids and early life-stage tests with warmwater fishes usually last for 20 to 30 days. An averaging period that is equal to the length of the test will obviously allow the worst possible fluctuations and would very likely allow increased adverse effects.

An averaging period of four days seems appropriate for use with the CCC for two reasons. First, it is substantially shorter than the 20 to 30 days that is obviously unacceptable. Second, for some species it appears that the results of chronic tests are due to the existence of a sensitive life stage at some time during the test<sup>7</sup>, rather than being caused by either long-term stress or long-term accumulation of the test material in the organism. The existence of a sensitive life stage is probably the cause of acute-chronic ratios that are not much greater than 1, and is also possible when the ratio is substantially greater than 1. In addition, some experimentally determined acute-chronic ratios are somewhat less than 1, possibly because prior exposure during the chronic test increased the resistance of the sensitive life stage<sup>8</sup>. A four-day averaging period will probably prevent increased adverse effects on sensitive life stages by limiting the durations and magnitudes of exceedences\* of the CCC.

The considerations applied to interpretation of the CCC also apply to the CMC. For the CMC the averaging period should again be substantially less than the lengths of the tests it is based on, i.e., substantially less than

48 to 96 hours. One hour is probably an appropriate averaging period because high concentrations of some materials can cause death in one to three hours. Even when organisms do not die within the first hour or so, it is not known how many might have died due to delayed effects of this short of an exposure. Thus it is not appropriate to allow concentrations above the CMC to exist for as long as one hour.

The durations of the averaging periods in national criteria have been made short enough to restrict allowable fluctuations in the concentration of the pollutant in the receiving water and to restrict the length of time that the concentration in the receiving water can be continuously above

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\* Although "exceedence" has not been found in any dictionary, it is used here because it is not appropriate to use "violation" in conjunction with criteria, no other word seems appropriate, and all appropriate phrases are awkward.

a criterion concentrations. The statement of a criterion could specify that the four-day average should never exceed the CCC and that the one-hour average should never exceed the CMC. However, one of the most important uses of criteria is for designing waste treatment facilities. Such facilities are designed based on probabilities and it is not possible to design for a zero probability. Thus, one of the important design parameters is the probability that the four-day average or the one-hour-average will be exceeded, or, in other words, the frequency with which exceedences will be allowed.

The frequency of allowed exceedences should be based on the ability of aquatic ecosystems to recover from the exceedences, which will depend in part on the magnitudes and durations of the exceedences. It is important to realize that high concentrations caused by spills and similar major events are not what is meant by an "exceedence", because spills and other accidents are not part of the design of the normal operation of waste treatment facilities. Rather, exceedences are extreme values in the distribution of ambient concentrations and this distribution is the result of the usual variations in the flows of both the effluent and the receiving water and the usual variations in the concentrations of the material of concern in both the effluent and in the upstream receiving water. Because exceedences are the result of usual variation, most of the exceedences will be small and exceedences as large as a factor of two will be rare. In addition, because these exceedences are due to random variation, they will not be evenly spaced. In fact, because many receiving waters have both one-year and multi-year cycles and many treatment facilities have daily, weekly, and yearly cycles, exceedences will often be grouped, rather than being evenly spaced or randomly distributed. If the flow of the receiving water is usually much greater than the flow of the effluent, normal variation and the flow cycles will result in the ambient concentration usually being below the CCC, occasionally being near the CCC, and rarely being above the CCC. In addition, exceedences that do occur will be grouped. On the other hand, if the flow of the effluent is much greater than the flow of the receiving water, the concentration might be close to the CCC much of the time and rarely above the CCC, with exceedences being randomly distributed.

The abilities of ecosystems to recover differ greatly, and depend on the pollutant, the magnitude and duration of the exceedence, and the physical and biological features of the ecosystem. Documented studies of recoveries are few, but some systems recover from small stresses in six weeks whereas other systems take more than ten years to recover from severe stress<sup>3</sup>. Although most exceedences are expected to be very small, larger exceedences will occur occasionally. Most aquatic ecosystems can probably recover from most exceedences in about three years. Therefore, it does not seem reasonable to purposely design for stress above that caused by the CCC to occur more than once every three years on the average, just as it does not seem reasonable to require that these kinds of stresses only occur once every five or ten years on the average.

If the body of water is not subject to anthropogenic stress other than the exceedences of concern and if exceedences as large as a factor of two are rare, it seems reasonable that most bodies of water could tolerate exceedences once every three years on the average. In situations in which exceedences are grouped, several exceedences might occur in one or two years, but then there will be, for example, 10 to 20 years during which no exceedences will occur and the concentration will be substantially below the CCC most of the time. In situations in which the concentration is often close to the CCC and exceedences are randomly distributed, some adverse effect will occur regularly, and small additional, unacceptable effects will occur about every

third year. The relative long-term ecological consequences of evenly spaced and grouped exceedences are unknown, but because most exceedences will probably be small, the long-term consequences should be about equal over long periods of time.

The above considerations lead to a statement of a criterion in the frequency-intensity-duration format that is often used to describe rain and snow fall and stream flow, e.g., how often, on the average, does more than ten inches of rain fall in a week? The numerical values chosen for frequency (or average recurrence interval), intensity (i.e., concentration), and duration (of averaging period) are those appropriate for national criteria. Whenever adequately justified, a national criterion may be replaced by a site-specific criterion<sup>1</sup>, which may include not only site-specific criterion concentrations<sup>2</sup>, but also site-specific durations of averaging periods and site-specific frequencies of allowed exceedences<sup>3</sup>.

The concentrations, durations, and frequencies specified in criteria are based on biological, ecological, and toxicological data, and are designed to protect aquatic organisms and their uses from unacceptable effects. Use of criteria for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of water quality criteria, but a steady-state model might have to be used instead of a dynamic model in some situations. Regardless of the model that is used, the durations of the averaging periods and the frequencies of allowed exceedences must be applied correctly if the intended level of protection is to be provided. For example, in the criterion statement frequency refers to the average frequency, over a long period of time, of rare events (i.e., exceedences). However, in some disciplines, frequency is often thought of in terms of the average frequency, over a long period of time, of the years in which rare events occur, without any consideration of how many rare events occur within each of those eventful years. The distinction between the frequency of events and the frequency of years is important for all those situations in which the rare events, e.g., exceedences, tend to occur in groups within the eventful years. The two ways of calculating frequency produce the same results in situations in which each rare event occurs in a different year because then the frequency of events is the same as the frequency of eventful years.

Because fresh water and salt water have basically different chemical compositions and because freshwater and saltwater (i.e., estuarine and true marine) species rarely inhabit the same water simultaneously, these National Guidelines provide for the derivation of separate criteria for these two kinds of water. For some materials sufficient data might not be available to allow derivation of criteria for one or both kinds of water. Even though absolute toxicities might be different in fresh and salt waters, such relative data as acute-chronic ratios and bioconcentration factors often appear to be similar in the two waters. When data are available to indicate that these ratios and factors are probably similar, they are used interchangeably.

The material for which a criterion is desired is usually defined in terms of a particular chemical compound or ion, or a group of closely related compounds or ions, but it might possibly be defined in terms of an effluent. These National Guidelines might also be useful for deriving criteria for temperature, dissolved oxygen, suspended solids, pH, etc., if the kinds of data on which the Guidelines are based are available.

Because they are meant to be applied only after a decision has been made that a national water quality criterion for aquatic organisms is needed for a material, these National Guidelines do not

address the rationale for making that decision. If the potential for adverse effects on aquatic organisms and their uses is part of the basis for deciding whether an aquatic life criterion is needed for a material, these Guidelines will probably be helpful in the collection and interpretation of relevant data. Such properties as volatility might affect the fate of a material in the aquatic environment and might be important when determining whether a criterion is needed for a material; for example, aquatic life criteria might not be needed for materials that are highly volatile or highly degradable in water. Although such properties can affect how much of the material will get from the point of discharge through any allowed mixing zone to some portion of the ambient water and can also affect the size of the zone of influence in the ambient water, such properties do not affect how much of the material aquatic organisms can tolerate in the zone of influence.

This version of the National Guidelines provides clarifications, additional details, and technical and editorial changes from the previous version<sup>9</sup>. These modifications are the result of comments on the previous version and subsequent drafts<sup>10</sup>, experience gained during the U.S. EPA's use of previous versions and drafts, and advances in aquatic toxicology and related fields. Future versions will incorporate new concepts and data as their usefulness is demonstrated. The major technical changes incorporated into this version of the National Guidelines are:

1. The requirement for acute data for freshwater animals has been changed to include more tests with invertebrate species. The taxonomic, functional, and probably the toxicological, diversities among invertebrate species are greater than those among vertebrate species and this should be reflected in the required data.
2. When available, 96-hr EC50s based on the percentage of fish immobilized plus the percentage of fish killed are used instead of 96-hr LC50s for fish; comparable EC50s are used instead of LC50s for other species. Such appropriately defined EC50s better reflect the total severe acute adverse impact of the test material on the test species than do LC50s or narrowly defined EC50s. Acute EC50s that are based on effects that are not severe, such as reduction in shell deposition and reduction in growth, are not used in calculating the Final Acute Value.
3. The Final Acute Value is now defined in terms of Genus Mean Acute Values rather than Species Mean Acute Values. A Genus Mean Acute Value is the geometric mean of all the Species Mean Acute Values available for species in the genus. On the average, species within a genus are toxicologically much more similar than species in different genera, and so the use of Genus Mean Acute Values will prevent data sets from being biased by an overabundance of species in one or a few genera.
4. The Final Acute Value is now calculated using a method<sup>11</sup> that is not subject to the bias and anomalous behavior that the previous method was. The new method is also less influenced by one very low value because it always gives equal weight to the four values that provide the most information about the cumulative probability of 0.05. Although the four values receive the most weight, the other values do have a substantial effect on the Final Acute Value (see examples in Appendix 2).
5. The requirements for using the results of tests with aquatic plants have been made more stringent.

6. Instead of being equal to the Final Acute Value, the Criterion Maximum Concentration is now equal to one-half the Final Acute Value. The Criterion Maximum Concentration is intended to protect 95 percent of a group of diverse genera, unless a commercially or recreationally important species is very sensitive. However, a concentration that would severely harm 50 percent of the fifth percentile or 50 percent of a sensitive important species cannot be considered to be protective of that percentile or that species. Dividing the Final Acute Value by 2 is intended to result in a concentration that will not severely adversely affect too many of the organisms.
7. The lower of the two numbers in the criterion is now called the Criterion Continuous Concentration, rather than the Criterion Average Concentration, to more accurately reflect the nature of the toxicological data on which it is based.
8. The statement of a criterion has been changed (a) to include durations of averaging periods and frequencies of allowed exceedences that are based on what aquatic organisms and their uses can tolerate, and (b) to identify a specific situation in which site-specific criteria<sup>1,2,3</sup> are probably desirable.

In addition, Appendix 1 was added to aid in determining whether a species should be considered resident in North America and its taxonomic classification. Appendix 2 explains the calculation of the Final Acute Value.

The amount of guidance in these National Guidelines has been increased, but much of the guidance is necessarily qualitative rather than quantitative; much judgment will usually be required to derive a water quality criterion for aquatic organisms and their uses. In addition, although this version of the National Guidelines attempts to cover all major questions that have arisen during use of previous versions and drafts, it undoubtedly does not cover all situations that might occur in the future. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these Guidelines and should be consistent with the spirit of these Guidelines, i.e., to make best use of the available data to derive the most appropriate criteria. These National Guidelines should be modified whenever sound scientific evidence indicates that a national criterion produced using these Guidelines would probably be substantially overprotective or underprotective of the aquatic organisms and their uses on a national basis. Derivation of numerical national water quality criteria for aquatic organisms and their uses is a complex process and requires knowledge in many areas of aquatic toxicology; any deviation from these Guidelines should be carefully considered to ensure that it is consistent with other parts of these Guidelines.

## **I. Definition of Material of Concern**

- A. Each separate chemical that does not ionize substantially in most natural bodies of water should usually be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of various compounds and apparently have similar biological, chemical, physical, and toxicological properties.
- B. For chemicals that do ionize substantially in most natural bodies of water (e.g., some phenols and organic acids, some salts of phenols and organic acids, and most

inorganic salts and coordination complexes of metals), all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

- C. The definition of the material should include an operational analytical component. Identification of a material simply, for example, as "sodium" obviously implies "total sodium", but leaves room for doubt. If "total" is meant, it should be explicitly stated. Even "total" has different operational definitions, some of which do not necessarily measure "all that is there" in all samples. Thus, it is also necessary to reference or describe the analytical method that is intended. The operational analytical component should take into account the analytical and environmental chemistry of the material, the desirability of using the same analytical method on samples from laboratory tests, ambient water, and aqueous effluents, and various practical considerations, such as labor and equipment requirements and whether the method would require measurement in the field or would allow measurement after samples are transported to a laboratory.

The primary requirements of the operational analytical component are that it be appropriate for use on samples of receiving water, that it be compatible with the available toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection or overprotection of aquatic organisms and their uses. Because an ideal analytical measurement will rarely be available, a compromise measurement will usually have to be used. This compromise measurement must fit with the general approach that if an ambient concentration is lower than the national criterion, unacceptable effects will probably not occur, i.e., the compromise measurement must not err on the side of underprotection when measurements are made on a surface water. Because the chemical and physical properties of an effluent are usually quite different from those of the receiving water, an analytical method that is acceptable for analyzing an effluent might not be appropriate for analyzing a receiving water, and vice versa. If the ambient concentration *calculated* from a measured concentration in an effluent is higher than the national criterion, an additional option is to *measure* the concentration after dilution of the effluent with receiving water to determine if the measured concentration is lowered by such phenomena as complexation or sorption. A further option, of course, is to derive a site-specific criterion<sup>1,2,3</sup>. Thus, the criterion should be based on an appropriate analytical measurement, but the criterion is not rendered useless if an ideal measurement either is not available or is not feasible.

**NOTE:** The analytical chemistry of the material might have to be taken into account when defining the material or when judging the acceptability of some toxicity tests, but a criterion should not be based on the sensitivity of an analytical method. When aquatic organisms are more sensitive than routine analytical methods, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

## II. Collection of Data

- A. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants, (b) FDA action levels <sup>12</sup>, and (c) chronic feeding studies and long-term field studies with wildlife species that regularly consume aquatic organisms.
- B. All data that are used should be available in typed, dated, and signed hard copy (publication, manuscript, letter, memorandum, etc.) with enough supporting information to indicate that acceptable test procedures were used and that the results are probably reliable. In some cases it may be appropriate to obtain additional written information from the investigator, if possible. Information that is confidential or privileged or otherwise not available for distribution should not be used.
- C. Questionable data, whether published or unpublished, should not be used. For example, data should usually be rejected if they are from tests that did not contain a control treatment, tests in which too many organisms in the control treatment died or showed signs of stress or disease, and tests in which distilled or deionized water was used as the dilution water without addition of appropriate salts.
- D. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the material of concern should not be used.
- E. For some highly volatile, hydrolyzable, or degradable materials it is probably appropriate to use only results of flow-through tests in which the concentrations of test material in the test solutions were measured often enough using acceptable analytical methods.
- F. Data should be rejected if they were obtained using:
  - 1. Brine shrimp, because they usually only occur naturally in water with salinity greater than 35 g/kg.
  - 2. Species that do not have reproducing wild populations in North America (see Appendix 1).
  - 3. Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.
- G. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with non-resident species in North America or previously exposed organisms may be used to provide auxiliary information but should not be used in the derivation of criteria.

## III. Required data

- A. Certain data should be available to help ensure that each of the four major kinds of possible adverse effects receives adequate consideration. Results of acute and chronic toxicity tests with representative species of aquatic animals are necessary so that data available for tested species can be considered a useful indication of the sensitivities of

appropriate untested species. Fewer data concerning toxicity to aquatic plants are required because procedures for conducting tests with plants and interpreting the results of such tests are not as well developed. Data concerning bioaccumulation by aquatic organisms are only required if relevant data are available concerning the significance of residues in aquatic organisms.

- B. To derive a criterion for freshwater aquatic organisms and their uses, the following should be available:
1. Results of acceptable acute tests (see Section IV) with at least one species of freshwater animal in at least eight different families such that all of the following are included:
    - a. the family Salmonidae in the class Osteichthyes
    - b. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
    - c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
    - d. a planktonic crustacean (e.g., cladoceran, copepod, etc.)
    - e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
    - f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
    - g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
    - h. a family in any order of insect or any phylum not already represented.
  2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that one of the three species:
    - at least one is a fish
    - at least one is an invertebrate
    - at least one is an acutely sensitive freshwater species (the other two may be saltwater species).
  3. Results of at least one acceptable test with a freshwater alga or vascular plant (see Section VIII). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available (see Section IX).
- C. To derive a criterion for saltwater aquatic organisms and their uses, the following should be available:
1. Results of acceptable acute tests (see Section IV) with at least one species of saltwater animal in at least eight different families such that all of the following are included:
    - a. two families in the phylum Chordata
    - b. a family in a phylum other than Arthropoda or Chordata
    - c. either the Mysidae or Penaeidae family
    - d. three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used above)
    - e. any other family.
  2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that of the three species:
    - at least one is a fish
    - at least one is an invertebrate
    - at least one is an acutely sensitive saltwater species (the other two may be freshwater species).
  3. Results of at least one acceptable test with a saltwater alga or vascular plant (see Section VIII). If plants are among the aquatic organisms most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.
  4. At least one acceptable bioconcentration factor determined with an appropriate saltwater species, if a maximum permissible tissue concentration is available (see Section IX).
- D. If all the required data are available, a numerical criterion can usually be derived, except in special cases. For example, derivation of a criterion might not be possible if the available acute-chronic ratios vary by more than a factor of ten with no apparent pattern. Also, if a criterion is to be related to a water quality characteristic (see Sections V and VII), more data will be necessary.

Similarly, if all required data are not available, a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it might be possible to derive a criterion if the available data

clearly indicate that the Final Residue Value should be much lower than either the Final Chronic Value or Final Plant Value.

- E. Confidence in a criterion usually increases as the amount of available pertinent data increases. Thus, additional data are usually desirable.

#### **IV. Final Acute Value**

- A. Appropriate measures of the acute (short-term) toxicity of the material to a variety of species of aquatic animals are used to calculate the Final Acute Value. The Final Acute Value is an estimate of the concentration of the material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acceptable acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value of a commercially or recreationally important species is lower than the calculated Final Acute Value, then that Species Mean Acute Value replaces the calculated Final Acute Value in order to provide protection for that important species.
- B. Acute toxicity tests should have been conducted using acceptable procedures<sup>13</sup>.
- C. Except for test with saltwater annelids and mysids, results of acute tests during which the test organisms were fed should not be used, unless data indicate that the food did not affect the toxicity of the test material.
- D. Results of acute tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between acute toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.
- E. Acute values should be based on endpoints which reflect the total severe acute adverse impact of the test material on the organisms used in the test. Therefore, only the following kinds of data on acute toxicity to aquatic animals should be used:
  - 1. Tests with daphnids and other cladocerans should be started with organisms less than 24 hours old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC50 based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC50 is not available from a test, the 48-hr LC50 should be used in place of the desired 48-hr EC50. An EC50 or LC50 of longer than 48 hr can be used as long as the animals were not fed and the control animals were acceptable at the end of the test.
  - 2. The result of a test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones, should be the 96-hr EC50 based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an EC50 is not available from a test, the lower of the 96-hr EC50 based on the percentage of organisms with incompletely developed shells and the 96-hr LC50

should be used in place of the desired 96-hr EC50. If the duration of the test was between 48 and 96 hr, the EC50 or LC50 at the end of the test should be used.

3. The acute values from tests with all other freshwater and saltwater animal species and older life stages of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimps, and abalones should be the 96-hr EC50 based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed. If such an EC50 is not available from a test, the 96-hr LC50 should be used in place of the desired 96-hr EC50.
  4. Tests with single-celled organisms are not considered acute tests, even if the duration was 96 hours or less.
  5. If the tests were conducted properly, acute values reported as "greater than" values and those which are above the solubility of the test material should be used, because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species.
- F. If the acute toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Acute Equation should be derived based on that water quality characteristic. Go to Section V.
- G. If the available data indicate that one or more life stages are at least a factor of two more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the Species Mean Acute Value because a species can only be considered protected from acute toxicity if all life stages are protected.
- H. The agreement of the data within and between species should be considered. Acute values that appear to be questionable in comparison with other acute and chronic data for the same species and for other species in the same genus probably should not be used in calculation of a Species Mean Acute Value. For example, if the acute values available for a species or genus differ by more than a factor of 10, some or all of the values probably should not be used in calculations.
- I. For each species for which at least one acute value is available, the Species Mean Acute Value (SMAV) should be calculated as the geometric mean of the results of all flow-through tests in which the concentrations of test material were measured. For a species for which no such result is available, the SMAV should be calculated as the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial concentrations (nominal concentrations are acceptable for most test materials if measured concentrations are not available) of test material.

**NOTE:** Data reported by original investigators should not be rounded off. Results of all intermediate calculations should be rounded <sup>14</sup> to four significant digits.

**NOTE:** The geometric mean of N numbers is the Nth root of the product of the N numbers. Alternatively, the geometric mean can be calculated by adding the logarithms of the N numbers, dividing the sum by N, and taking the antilog of the quotient. The geometric mean of two numbers is the square root of the product of the two numbers, and the geometric mean of one number is that number. Either natural (base e) or common (base 10) logarithms can be used to calculate geometric means as long as they are used consistently within each set of data, i.e., the antilog used must match the logarithm used.

**NOTE:** Geometric means, rather than arithmetic means, are used here because the distributions of sensitivities of individual organisms in toxicity tests on most materials and the distributions of sensitivities of species within a genus are more likely to be lognormal than normal. Similarly, geometric means are used for acute-chronic ratios and bioconcentration factors because quotients are likely to be closer to lognormal than normal distributions. In addition, division of the geometric mean of a set of numerators by the geometric mean of the set of corresponding denominators will result in the geometric mean of the set of corresponding quotients.

- J. For each genus for which one or more SMAVs are available, the Genus Mean Acute Value (GMAV) should be calculated as the geometric mean of the SMAVs available for the genus.
- K. Order the GMAVs from high to low.
- L. Assign ranks, R, to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.
- M. Calculate the cumulative probability, P, for each GMAV as R/(N+1).
- N. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are less than 59 GMAVs, these will always be the four lowest GMAVs).
- O. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\sum ((\ln GMAV)^2) - ((\sum \ln GMAV))^2 / 4}{\sum (F) - ((\sum (\sqrt{P}))^2 / 4)}$$

$$L = (\sum (\ln GMAV) - S(\sum (\sqrt{P}))) / 4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

(See <sup>11</sup> for development of the calculation procedure and Appendix 2 for an example calculation and computer program.)

**NOTE:** Natural logarithms (logarithms to base e, denoted as ln) are used herein merely because they are easier to use on some hand calculators and computers than common (base 10) logarithms. Consistent use of either will produce the same result.

- P. If for a commercially or recreationally important species the geometric mean of the acute values from ~~the~~ flow-through tests in which the concentrations of test material were measured is lower than the calculated Final Acute Value, then that geometric mean should be used as the Final Acute Value instead of the calculated Final Acute Value.
- Q. Go to Section VI.

## V. Final Acute Equation

- A. When enough data are available to show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be taken into account as described in Sections B-G below or using analysis of covariance<sup>15, 16</sup>. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two or more factors affect toxicity, multiple regression analysis should be used.
- B. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the acute toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95% confidence limits for each species.

**NOTE:** Because the best documented relationship is that between hardness and acute toxicity of metals in fresh water and a log-log relationship fits these data, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics, such as pH, temperature, or salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section.

- C. Decide whether the data for each species is useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, acute values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic, the acute values available for a species or genus differ by more than a factor of 10, rejection of some or all of the values is probably appropriate. If useful slopes are not available for at least one fish and one invertebrate or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between acute toxicity and the water quality characteristic, return to Section IV.G., using the results of tests

conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

- D. Individually for each species calculate the geometric mean of the available acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.
- E. Similarly normalize the values of the water quality characteristic for each species individually.
- F. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95% confidence limits will be identical to those obtained in Section B above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.
- G. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope,  $V$ , and its 95% confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.
- H. For each species calculate the geometric mean,  $W$ , of the acute toxicity values and the geometric mean,  $X$ , of the values of the water quality characteristic. (These were calculated in steps D and E above.)
- I. For each species calculate the logarithm,  $Y$ , of the SMAV at a selected value,  $Z$ , of the water quality characteristic using the equation:  
$$Y = \ln W - V(\ln X - \ln Z).$$
- J. For each species calculate the SMAV at  $Z$  using the equation:  $\text{SMAV} = e^Y$ .  
**NOTE:** Alternatively, the SMAVs at  $Z$  can be obtained by skipping step H above, using the equations in steps I and J to adjust each acute value individually to  $Z$ , and then calculating the geometric mean of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted acute values for each species.
- K. Obtain the Final Acute Value at  $Z$  by using the procedure described in Section IV.J-O.
- L. If the SMAV at  $Z$  of a commercially or recreationally important species is lower than the calculated Final Acute Value at  $Z$ , then that SMAV should be used as the Final Acute Value at  $Z$  instead of the calculated Final Acute Value.
- M. The Final Acute Equation is written as:  $\text{Final Acute Value} = e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])}$ , where  $V$  = pooled acute slope and  $A$  = Final Acute Value at  $Z$ . Because

V, A, and Z are known, the Final Acute Value can be calculated for any selected value of the water quality characteristic.

## VI. Final Chronic Value

- A. Depending on the data that are available concerning chronic toxicity to aquatic animals, the Final Chronic Value might be calculated in the same manner as the Final Acute Value or by dividing the Final Acute Value by the Final Acute-Chronic Ratio. In some cases it may not be possible to calculate a Final Chronic Value.

**NOTE:** As the name implies, the acute-chronic ration (ARC) is a way of relating acute and chronic toxicities. The acute-chronic ratio is basically the inverse of the application factor, but this new name is better because it is more descriptive and should help prevent confusion between "application factors" and "safety factors". Acute-chronic ratios and application factors are ways of relating the acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond the known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it will usually be greater than one; this should avoid the confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

- B. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which the concentrations of test material in the test solutions were properly measured at appropriate times during the test.
- C. Results of chronic tests in which survival, growth, or reproduction in the control treatment was unacceptably low should not be used. The limits of acceptability will depend on the species.
- D. Results of chronic tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between chronic toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.
- E. Chronic values should be based on endpoints and lengths of exposure appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:
1. Life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species to a different concentration of the test material throughout a life cycle. To ensure that all life stages and life processes are exposed, tests with fish should begin with embryos or newly hatched young less than 48 hours old, continue through maturation and reproduction, and should end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young less than 24 hours old and last for not less than 21 days. Tests with mysids should begin with young less than 24 hours old and continue until 7 days past the median time of first brood release in the

controls. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female. For mysids, data should be obtained and analyzed on survival, growth, and young per female.

2. Partial life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species of fish to a different concentration of the test material through most portions of a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with immature juveniles at least 2 months prior to active gonad development, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.
3. Early life-stage toxicity tests consisting of 28- to 32-day (60 days post hatch for salmonids) exposures of the early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained and analyzed on survival and growth.

**NOTE:** Results of an early life-stage test are used as predictions of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests are possibly not good predictions of the results of comparable life-cycle or partial life-cycle tests.

- F. A chronic value may be obtained by calculating the geometric mean of the lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of adverse effect on any of the specified biological measurements, and (c) below which no tested concentration caused an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of adverse effect on one or more of the specified biological measurements, and (c) above which all tested concentrations also caused such an effect.

**NOTE:** Because various authors have used a variety of terms and definitions to interpret and report results of chronic tests, reported results should be reviewed carefully. The amount of effect that is considered unacceptable is often based on a statistical hypothesis test, but might also be defined in terms of a specified percent reduction from the controls. A small percent reduction (e.g., 3%) might be

considered acceptable even if it is statistically significantly different from the control, whereas a large percent reduction (e.g., 30%) might be considered unacceptable even if it is not statistically significant.

- G. If the chronic toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section VII.
- H. If chronic values are available for species in eight families as described in Sections III.B.1 or III.C.1, a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values available for the species, and appropriate Genus Mean Chronic Values should be calculated. The Final Chronic Value should then be obtained using the procedure described in Section IV.J-O. Then go to Section VI.M.
- I. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of the results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which the concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. The acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water, but in a different study, may be used. If no such acute tests are available, results of acute tests conducted in the same dilution water in a different laboratory may be used. If no such acute tests are available, an acute-chronic ratio should not be calculated.
- J. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios available for that species.
- K. For some materials the acute-chronic ratio seems to be the same for all species, but for other materials the ratio seems to increase or decrease as the Species Mean Acute Value (SMAV) increases. Thus the Final Acute-Chronic Ratio can be obtained in four ways, depending on the data available:
  - 1. If the species mean acute-chronic ratios seems to increase or decrease as the SMAV increases, the Final Acute-Chronic Ratio should be calculated as the geometric mean of the acute-chronic ratios for species whose SMAVs are close to the Final Acute Value.
  - 2. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all the species mean acute-chronic ratios available for both freshwater and saltwater species.
  - 3. For acute tests conducted on metals and possibly other substances with embryos and larvae of barnacles, bivalve molluscs, sea urchins, lobsters,

crabs, shrimp, and abalones (see Section IV.E.2), it is probably appropriate to assume that the acute-chronic ratio is 2. Chronic tests are very difficult to conduct with most such species, but it is likely that the sensitivities of embryos and larvae would determine the results of life-cycle tests. Thus, if the lowest available SMAVs were determined with embryos and larvae of such species, the Final Acute-Chronic Ratio should probably be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B).

4. If the most appropriate species mean acute-chronic ratios are less than 2.0, and especially if they are less than 1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B).

If the available species mean acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a Final Chronic Value probably cannot be calculated.

- L. Calculate the Final Chronic Value by dividing the Final Acute Value by the Final Acute-Chronic Ratio. If there was a Final Acute Equation rather than a Final Acute Value, see also Section VII.A.
- M. If the Species Mean Chronic Value of a commercially or recreationally important species is lower than the calculated Final Chronic Value, then that Species Mean Chronic Value should be used as the Final Chronic Value instead of the calculated Final Chronic Value.
- N. Go to Section VIII.

## **VII. Final Chronic Equation**

- A. A Final Chronic Equation can be derived in two ways. The procedure described here in Section A will result in the chronic slope being the same as the acute slope. The procedure described in Sections B-N will usually result in the chronic slope being different from the actual slope.
  1. If acute-chronic ratios are available for enough species at enough values of the water quality characteristic to indicate that the acute-chronic ratio is probably the same for all species and is probably independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the available species mean acute-chronic ratios.
  2. Calculate the Final Chronic Value at the selected value Z of the water quality characteristic by dividing the Final Acute Value at Z (see Section V.M.) by the Final Acute-Chronic Ratio.

3. Use  $V =$  pooled acute slope (see section V.M.) as  $L =$  pooled chronic slope.
  4. Go to Section VII.M.
- B. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be taken into account as described in Sections B-G below or using analysis of covariance<sup>15, 16</sup>. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two more factors affect toxicity, multiple regression analysis should be used.
- C. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the chronic toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95% confidence limits for each species.

**NOTE:** Because the best documented relationship is that between hardness and acute toxicity of metals in fresh water and a log-log relationship fits these data, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics, such as pH, temperature, or salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section. It is probably preferable, but not necessary, to use the same transformation that was used with the acute values in Section V.

- D. Decide whether the data for each species is useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, chronic values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic, the chronic values available for a species or genus differ by more than a factor of 10, rejection of some or all of the values is probably appropriate. If a useful chronic slope is not available for at least one species or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between chronic toxicity and the water quality characteristic, it might be appropriate to assume that the chronic slope is the same as the acute slope, which is equivalent to assuming that the acute-chronic ratio is independent of the water quality characteristic. Alternatively, return to Section VI.H, using the results of tests

conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

- E. Individually for each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.
- F. Similarly normalize the values of the water quality characteristic for each species individually.
- G. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and the 95% confidence limits will be identical to those obtained in Section B above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.
- H. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope, L, and its 95% confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.
- I. For each species calculate the geometric mean, M, of the toxicity values and the geometric mean, P, of the values of the water quality characteristic. (These were calculated in steps E and F above.)
- J. For each species calculate the logarithm, Q, of the Species Mean Chronic Value at a selected value, Z, of the water quality characteristic using the equation:  $Q = \ln M - L(\ln P - \ln Z)$ .

**NOTE:** Although it is not necessary, it will usually be best to use the same value of the water quality characteristic here as was used in Section V.I.

- K. For each species calculate a Species Mean Chronic Value at Z using the equation:  $SMCV = e^Q$ .

**NOTE:** Alternatively, the Species Mean Chronic Values at Z can be obtained by skipping step J above, using the equations in steps J and K to adjust each acute value individually to Z and then calculating the geometric means of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted chronic values for each species.

- L. Obtain the Final Chronic Value at Z by using the procedure described in Section IV.J-O.
- M. If the Species Mean Chronic Value at Z of a commercially or recreationally important species is lower than the calculated Final Chronic Value at Z, then that Species Mean

- N. The Final Chronic Equation is written as: Final Chronic Value =  $e^{(L[\ln(\text{water quality characteristic}) + \ln S - L \ln Z])}$ , where L = pooled chronic slope and S = Final Chronic Value at Z. Because L, S and Z are known, the Final Chronic Value can be calculated for any selected value of the water quality characteristic.

### VIII. Final Plant Value

- A. Appropriate measures of the toxicity of the material to aquatic plants are used to compare the relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting the results of toxicity tests with plants are not well developed, results of tests with plants usually indicate that criteria which adequately protect aquatic animals and their uses will probably also protect aquatic plants and their uses.
- B. A plant value is the result of a 96-hr test conducted with an alga or a chronic test conducted with an aquatic vascular plant.

**NOTE:** A test of the toxicity of a metal to a plant usually should not be used if the medium contained an excessive amount of a complexing agent, such as EDTA, that might affect the toxicity of the metal. Concentrations of EDTA above about 200 µg/L should probably be considered excessive.

- C. The Final Plant Value should be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured and the endpoint was biologically important.

### IX. Final Residue Value

- A. The Final Residue Value is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from affecting marketability because of exceedance of applicable FDA action levels and (b) protect wildlife, including fishes and birds, that consume aquatic organisms from demonstrated unacceptable effects. The Final Residue Value is the lowest of the residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level <sup>12</sup> for fish oil or for the edible portion of fish or shellfish, or (b) a maximum acceptable dietary intake based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section X because no Final Residue Value can be derived.
- B. Bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution in which the organism had been living. A BCF is intended to account only for net uptake directly from water, and thus almost

has to be measured in a laboratory test. Some uptake during the bioconcentration test might not be directly from water if the food sorbs some of the test material before it is eaten by the test organisms. A BAF is intended to account for the net uptake from both food and water in a real-world situation. A BAF almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey that itself could have accumulated the material from both food and water. The BCF and BAF are probably similar for a material with a low BCF, but the BAF is probably higher than the BCF for materials with high BCFs. Although BCFs are not too difficult to determine, very few BAFs have been measured acceptably because it is necessary to make enough measurements of the concentration of the material in water to show that it was reasonably constant for a long enough period of time over the range of territory inhabited by the organisms. Because so few acceptable BAFs are available, only BCFs will be discussed further. However, if an acceptable BAF is available for a material, it should be used instead of any available BCFs.

- C. If a maximum permissible tissue concentration is available for a substance (e.g., parent material, parent material plus metabolites, etc.), the tissue concentration used in the calculation of the BCF should be for the same substance. Otherwise, the tissue concentration used in the calculation of the BCF should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.
- D.
  - 1. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of the test material in tissue and in the test solution, and the exposure continued at least until either apparent steady-state or 28 days was reached. Steady-state is reached when the BCF does not change significantly over a period of time, such as two days or 16 percent of the length of the exposure, whichever is longer. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached, (b) the highest BCF obtained, if apparent steady-state was not reached, and (c) the projected steady-state BCF, if calculated.
  - 2. Whenever a BCF is determined for a lipophilic material, the percent lipids should also be determined in the tissue(s) for which the BCF was calculated.
  - 3. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to a BCF obtained with unaffected organisms of the same species at lower concentrations that did not cause adverse effects.
  - 4. Because maximum permissible tissue concentrations are almost never based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight BCF by 0.1 for plankton and by 0.2 for individual species of fishes and invertebrates <sup>17</sup>.

5. If more than one acceptable BCF is available for a species, the geometric mean of the available values should be used, except that if the BCFs are from different lengths of exposure and the BCF increases with length of exposure, the BCF for the longest exposure should be used.
- E. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:
1. For each available maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of the tested wildlife species.
  2. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion (muscle for decapods, muscle with or without skin for fishes, adductor muscle for scallops, and total soft tissue for other bivalve molluscs) of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.
- F. For lipophilic materials, it might be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percent lipids from one tissue to another and from one species to another<sup>18, 19, 20</sup>, extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percent lipids.
1. For each BCF for which the percent lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by the percent lipids. This adjustment to a one percent lipid basis is intended to make all the measured BCFs for a material comparable regardless of the species or tissue with which the BCF was measured.
  2. Calculate the geometric mean normalized BCF. Data for both saltwater and freshwater species should be used to determine the mean normalized BCF, unless the data show that the normalized BCFs are probably not similar.
  3. Calculate all possible residue values by dividing the available maximum permissible tissue concentrations by the mean normalized BCF and by the percent lipids values appropriate to the maximum permissible tissue concentrations, i.e.,
- $$\text{Residue Value} = \frac{(\text{maximum permissible tissue concentration})}{(\text{mean normalized BCF})(\text{appropriate percent lipids})}$$
- a. For an FDA action level for fish oil, the appropriate percent lipids value is 100.

- b. For an FDA action level for fish, the appropriate percent lipids value is 11 for freshwater criteria and 10 for saltwater criteria because FDA action levels are applied on a species-by-species basis to commonly consumed species. The highest lipid contents in the edible portions of important consumed species are about 11 percent for both the freshwater chinook salmon and lake trout and about 10 percent for the saltwater Atlantic herring <sup>21</sup>.
  - c. For a maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, the appropriate percent lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.
- G. The Final Residue Value is obtained by selecting the lowest of the available residue values.

**NOTE:** In some cases the Final Residue Value will not be low enough. For example, a residue value calculated from an FDA action level will probably result in an average concentration in the edible portion of a fatty species that is at the action level. Some individual organisms, and possibly some species, will have residue concentrations higher than the mean value but no mechanism has been devised to provide appropriate additional protection. Also, some chronic feeding studies and long-term field studies with wildlife identify concentrations that cause adverse effects but do not identify concentrations which do not cause adverse effects; again no mechanism has been devised to provide appropriate additional protection. These are some of the species and uses that are not protected at all times in all places.

## **X. Other Data**

Pertinent information that could not be used in earlier sections might be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other adverse effect that has been shown to be biologically important. Especially important are data for species for which no other data are available. Data from behavioral, biochemical, physiological, microcosm, and field studies might also be available. Data might be available from tests conducted in unusual dilution water (see IV.D and VI.D), from chronic tests in which the concentrations were not measured (see VI.B), from tests with previously exposed organisms (see II.F), and from tests on formulated mixtures or emulsifiable concentrates (see II.D). Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important.

## **XI. Criterion**

- A. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

- B. The Criterion Maximum Concentration (CMC) is equal to one-half the Final Acute Value.
- C. The Criterion Continuous Concentration (CCC) is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value, unless other data (see Section X) show that a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the Final Residue Value by selecting the one, or the combination, that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section X) show that a lower value should be used.
- D. Round <sup>14</sup> both the CMC and the CCC to two significant digits.
- E. The criterion is stated as:

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where a locally important species is very sensitive, (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

where (1) = insert "freshwater" or "saltwater"

(2) = insert name of material

(3) = insert the Criterion Continuous Concentration

(4) = insert the Criterion Maximum Concentration.

## **XII. Final Review**

- A. The derivation of the criterion should be carefully reviewed by rechecking each step of the Guidelines. Items that should be especially checked are:
  1. If unpublished data are used, are they well documented?
  2. Are all required data available?
  3. Is the range of acute values for any species greater than a factor of 10?
  4. Is the range of Species Mean Acute Values for any genus greater than a factor of 10?
  5. Is there more than a factor of ten difference between the four lowest Genus Mean Acute Values?
  6. Are any of the four lowest Genus Mean Acute Values questionable?

7. Is the Final Acute Value reasonable in comparison with the Species Mean Acute Values and Genus Mean Acute Values?
  8. For any commercially or recreationally important species, is the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured lower than the Final Acute Value?
  9. Are any of the chronic values questionable?
  10. Are chronic values available for acutely sensitive species?
  11. Is the range of acute-chronic ratios greater than a factor of 10?
  12. Is the Final Chronic Value reasonable in comparison with the available acute and chronic data?
  13. Is the measured or predicted chronic value for any commercially or recreationally important species below the Final Chronic Value?
  14. Are any of the other data important?
  15. Do any data look like they might be outliers?
  16. Are there any deviations from the Guidelines? Are they acceptable?
- B. On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific evidence. If it is not, another criterion, either higher or lower, should be derived using appropriate modifications of these Guidelines.

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## **Appendix 1. Resident North American Species of Aquatic Animals Used in Toxicity and Bioconcentration Tests**

### **Introduction**

These lists identify species of aquatic animals which have reproducing wild populations in North America and have been used in toxicity or bioconcentration tests. "North America" includes only the 48 contiguous states, Canada, and Alaska; Hawaii and Puerto Rico are not included. Saltwater (i.e., estuarine and true marine) species are considered resident in North America if they inhabit or regularly enter shore waters on or above the continental shelf to a depth of 200 meters. Species do not have to be native to be resident. Unlisted species should be considered resident North American species if they can be similarly confirmed or if the test organisms were obtained from a wild population in North America.

The sequence for fishes is taken from A List of Common and Scientific Names of Fishes from the United States and Canada. For other species, the sequence of phyla, classes, and families is taken from the NODC Taxonomic Code, Third Edition, National Oceanographic Data Center, NOAA, Washington, DC 20235, July, 1981, and the numbers given are from that source to facilitate verification. Within a family, genera are in alphabetical order, as are species in a genus.

The references given are those used to confirm that the species is a resident North American species. (The NODC Taxonomic Code contains foreign as well as North American species.) If no such reference could be found, the species was judged to be nonresident. No reference is given for organisms not identified to species; these are considered resident only if obtained from wild North American populations. A few nonresident species are listed in brackets and noted as "nonresident" because they were mistakenly identified as resident in the past or to save other investigators from doing literature searches on the same species.

### **Special Note**

This December 2010 electronic version of the 1985 Guidelines serves to meet the requirements of Section 508 of the Rehabilitation Act. While converting the 1985 Guidelines to a 508-compliant version, EPA updated the taxonomic nomenclature to reflect changes that occurred since the tables were originally produced in 1985. The numbers included for Phylum, Class and Family represent those currently in use from the Integrated Taxonomic Information System, or ITIS, and reflect what is referred to in ITIS as Taxonomic Serial Numbers. ITIS replaced the National Oceanographic Data Center (NODC) taxonomic coding system which was used to create the original taxonomic tables included in the 1985 Guidelines document (NODC, Third Addition - see Introduction). For more information on the NODC taxonomic codes, see <http://www.nodc.noaa.gov/General/CDR-detdesc/taxonomic-v8.html>.

The code numbers included in the reference column of the tables have not been updated from the 1985 version. These code numbers are associated with the old NODC taxonomic referencing system and are simply replicated here for historical purposes. Footnotes may or may not still apply.

EPA is working on a more comprehensive update to the 1985 Guidelines, including new taxonomic tables which better reflect the large number of aquatic animal species known to be propagating in U.S. waters.

## Freshwater Species Table

Synonyms appear after the official Scientific Name and are marked with an asterisk (\*).  
 Non-resident species are noted in the Reference column and are marked with a dagger (†).

Class	Family	Species		Reference
		Common Name	Scientific Name	
<b>Phylum: Porifera (46861)</b>				
Demospongiae 47528	Spongillidae 47691	Sponge	<i>Ephydatia fluviatilis</i>	P93
<b>Phylum: Cnidaria (48738)</b>				
Hydrozoa 48739	Hydridae 50844	Hydra	<i>Hydra oligactis</i>	E318, P112
		Hydra	<i>Hydra littoralis</i>	E321, P112
<b>Phylum: Platyhelminthes (53963)</b>				
Turbellaria 53964	Planariidae 54502	Planarian	<i>Dugesia dorotocephala</i>	D22
		Planarian	<i>Dugesia lugubris</i> <i>Dugesia polychroa</i> <sup>†</sup>	D24
		Planarian	<i>Planaria gonocephala</i>	<sup>1</sup>
		Planarian	<i>Polycelis felina</i> <sup>§</sup>	nonresident
	Dendrocoelidae 54469	Planarian	<i>Procotyla fluviatilis</i> <i>Dendrocoelum lacteum</i> <sup>*</sup>	E334, P132, D63
<b>Phylum: Gastrotricha (57597)</b>				
Chaetonotida 57822	Chaetonotidae 57823	Gastrotrich	<i>Lepidodermella squamata</i> <i>Lepidodermella squamatum</i> <sup>*</sup>	E413
<b>Phylum: Rotifera (58239)</b>				
Eurotatoria (Formerly Bdelloidea) 654070	Philodinidae 58266	Rotifer	<i>Philodina acuticornis</i>	Y
		Rotifer	<i>Philodina roseola</i>	E487
Eurotatoria (Formerly Monogononta) 654070	Brachionidae 58344	Rotifer	<i>Keratella cochlearis</i>	E442, P188
		Rotifer	<i>Keratella sp.</i>	<sup>2</sup>
<b>Phylum: Annelida (64357)</b>				
Polychaeta (Formerly Archiannelida) 64358	Aeolosomatidae 68423	Worm	<i>Aeolosoma headleyi</i>	E528, P284
Clitellata (Formerly Oligochaeta) 568832	Lumbriculidae 68440	Worm	<i>Lumbriculus variegatus</i>	E533, P290
	Tubificidae 68585	Tubificid worm	<i>Branchiura sowerbyi</i>	E534, P289, GG
		Tubificid worm	<i>Limnodrilus hoffmeisteri</i>	E536, GG
		Tubificid worm	<i>Quistadrilus multisetosus</i> <i>Peloscolex multisetosus</i> <sup>*</sup>	E535, GG
		Tubificid worm	<i>Rhyacodrilus montanus</i>	GG
		Tubificid worm	<i>Spirosperma ferox</i> <i>Peloscolex ferox</i> <sup>*</sup>	GG
		Tubificid worm	<i>Spirosperma nikolskyi</i> <i>Peloscolex variegatus</i> <sup>*</sup>	E534, GG
		Tubificid worm	<i>Stylodrilus heringianus</i>	GG

<sup>‡</sup> Synonym

<sup>§</sup> Non-resident species

Class	Family	Species		Reference	
		Common Name	Scientific Name		
		Tubificid worm	<i>Tubifex tubifex</i>		E536, P289, GG
		Tubificid worm	<i>Varichaeta pacifica</i>	GG	
	Naididae 68854	Worm	<i>Nais sp.</i>	2	
		Worm	<i>Paranais sp.</i>	2	
		Worm	<i>Pristina sp.</i>	2	
Clitellata (Formerly Hirudinea) 568832	Erpobdellidea 69438	Leech	<i>Erpobdella octoculata</i>	Formerly nonresident (BB16)	
<b>Phylum: Mollusca (69458)</b>					
Gastropoda 69459	Viviparidae 70304	Snail	<i>Campeloma decisum</i>	P731, M216	
	Bithyniidae (Amnicolidae) (Bulimidae) (Hydrobiidae) 70745	Snail	<i>Amnicola sp.</i>	2	
		Pleuroceridae 71541	Snail	<i>Goniobasis livescens</i>	P732
			Snail	<i>Elimia virginica</i> <i>Goniobasis virginica</i>	E1137
			Snail	<i>Leptoxis carinata</i> <i>Nitocris carinata</i> <i>Mudalia carinata</i>	X, E1137
	Lymnaeidae 76483	Snail	<i>Nitocris sp.</i>	2	
		Snail	<i>Lymnaea acuminata</i> <sup>?</sup>	nonresident	
		Snail	<i>Lymnaea catascopium</i> <i>Lymnaea emerginata</i> <i>Stagnicola emerginata</i>	M328	
		Snail	<i>Lymnaea elodes</i> <i>Lymnaea palustris</i> <sup>*</sup>	E1127, M351	
		Snail	<i>Lymnaea luteola</i> <sup>?</sup>	nonresident M266	
		Snail	<i>Lymnaea stagnalis</i>	E1127, P728, M296	
	Planorbidae 76591	Snail	<i>Lymnaea sp.</i>	2	
		Snail	<i>Biomphalaria glabrata</i>	Formerly nonresident (M390)	
		Snail	<i>Gyraulus circumstriatus</i>	P729, M397	
		Snail	<i>Helisoma campanulatum</i>	M445	
	Physidae 76676	Snail	<i>Helisoma trivolvis</i>	P729, M452	
		Snail	<i>Aplexa hypnorum</i>	E1126, P727, M373	
		Snail	<i>Physa fontinalis</i> <sup>?</sup>	nonresident M373	
		Snail	<i>Physa gyrina</i>	E1126, P727, M373	
		Snail	<i>Physa heterostropha</i>	M378	
		Snail	<i>Physa integra</i>	P727	
	Bivalvia (Pelecypoda) 79118	Margaritiferidae 79914	Mussel	<i>Physa sp.</i>	2
		Unionidae (Formerly Amblemidae) 79913	Mussel	<i>Margaritifera margaritifera</i>	E1138, P748, J11
		Unionidae	Mussel	<i>Amblema plicata</i>	AA122
			Mussel	<i>Anodonta imbecillis</i>	J72, AA122

Class	Family	Species		Reference
		Common Name	Scientific Name	
	79913	Mussel	<i>Carunculina parva</i> <i>Toxolasma texasensis</i> *	J19, AA122
		Mussel	<i>Cyrtonaias tampicoensis</i>	P759, AA122
		Mussel	<i>Elliptio complanata</i>	J13
	Corbiculidae 81381	Asiatic clam	<i>Corbicula fluminea</i>	E1159
		Asiatic clam	<i>Corbicula manilensis</i>	P749
	Pisidiidae Sphaeriidae 81388	Fingernail clam	<i>Eupera cubensis</i> <i>Eupera singleyi</i> †	E1158, P763, G9
		Fingernail clam	<i>Musculium transversum</i> , <i>Sphaerium transversum</i>	M160, G11
		Fingernail clam	<i>Sphaerium corneum</i>	G12
	<b>Phylum: Arthropoda (82696)</b>			
Branchiopoda (Formerly Crustacea) 83687	Lynceidae 83769	Conchostracan	<i>Lynceus brachyurus</i>	E580, P344
	Sididae 83834	Cladoceran	<i>Diaphanosoma sp.</i>	2
	Daphniidae 83872	Cladoceran	<i>Ceriodaphnia acanthina</i>	E618
		Cladoceran	<i>Ceriodaphnia reticulata</i>	E618, P368
		Cladoceran	<i>Daphnia ambigua</i>	E607, P369
		Cladoceran	<i>Daphnia carinata</i>	3
		Cladoceran	<i>Daphnia cucullata</i> †	nonresident
		Cladoceran	<i>Daphnia galeata mendotae</i>	E610, P370
		Cladoceran	<i>Daphnia hyalina</i>	4
		Cladoceran	<i>Daphnia longispina</i>	5
		Cladoceran	<i>Daphnia magna</i>	E605, P367
		Cladoceran	<i>Daphnia parvula</i>	E611
		Cladoceran	<i>Daphnia pulex</i>	E613, P367
		Cladoceran	<i>Daphnia pulicaria</i>	A
		Cladoceran	<i>Daphnia similis</i>	E606, P367
		Cladoceran	<i>Simocephalus serrulatus</i>	E617, P370
	Cladoceran	<i>Simocephalus vetulus</i>	E617, P370	
	Moinidae (Formerly Daphniidae) 84162	Cladoceran	<i>Moina macrocopa</i>	E622, P372
		Cladoceran	<i>Moina rectirostris</i>	E623
Bosminidae 83935	Cladoceran	<i>Bosmina longirostris</i>	E624, P373	
Polyphemidae 83959	Cladoceran	<i>Polyphemus pediculus</i>	E599, P385	
Ostracoda (Formerly Crustacea) 84195	Cyprididae Cypridae 84462	Ostracod	<i>Cyprretta kawatai</i> †	nonresident U
		Ostracod	<i>Cypridopsis vidua</i>	E770, P430
Maxillopoda (Formerly Crustacea) 621145	Diaptomidae 85779	Copepod	<i>Eudiaptomus padanus</i> †	nonresident
	Temoridae 85855	Copepod	<i>Epischura lacustris</i>	E751, P407
	Cyclopidae 88634	Copepod	<i>Cyclops abyssorum</i> †	nonresident
		Copepod	<i>Cyclops bicuspidatus</i>	E807, P405
		Copepod	<i>Cyclops vernalis</i>	E804, P405
		Copepod	<i>Cyclops viridis</i> <i>Acanthocyclops viridis</i> *	E803, P397

Class	Family	Species		Reference	
		Common Name	Scientific Name		
		Copepod	<i>Acanthocyclops sp.</i>	2	
		Copepod	<i>Diacyclops sp.</i>	2	
		Copepod	<i>Eucyclops agilis</i>	P403	
		Copepod	<i>Mesocyclops leuckarti</i>	E812, P403	
Malacostraca (Formerly Crustacea) 89787	Asellidae 92657	Isopod	<i>Asellus aquaticus</i> <sup>†</sup>	nonresident (I2)	
		Isopod	<i>Caecidotea bicrenata</i> (Formerly <i>Asellus bicrenata</i> )	HH (I1,2)	
		Isopod	<i>Asellus brevicaudus</i>	E875, P447, I	
		Isopod	<i>Asellus communis</i>	E875, P448, I	
		Isopod	<i>Asellus intermedius</i>	E875, P448, I	
		Isopod	<i>Asellus meridionalis</i> <sup>†</sup> <i>Asellus meridianus</i> <sup>†</sup>	nonresident	
		Isopod	<i>Asellus racovitzai</i>	P449, I	
		Isopod	<i>Lirceus alabamae</i>	P875, I	
		Crangonyctidae (Formerly Gammaridae) 95080	Amphipod	<i>Crangonyx pseudogracilis</i>	P459, T68, FF23
		Gammaridae 93745	Amphipod	<i>Gammarus fasciatus</i>	E877, P458, T53
			Amphipod	<i>Gammarus lacustris</i>	E877, P458, FF23
			Amphipod	<i>Gammarus pseudolimnaeus</i>	E877, P458, T48
			Amphipod	<i>Gammarus pulex</i> <sup>†</sup>	nonresident
			Amphipod	<i>Gammarus tigrinus</i>	L51, FF17
			Amphipod	<i>Gammarus sp.</i>	2
		Hyalellidae (Talitridae) 94022	Amphipod	<i>Hyalella azteca</i> <i>Hyalella knickerbockeri</i> <sup>†</sup>	E876, P457, T154
		Palaemonidae 96213	Prawn	<i>Macrobrachium lamarrei</i> <sup>†</sup>	nonresident
			Prawn	<i>Macrobrachium rosenbergii</i>	<sup>6</sup>
			Prawn	<i>Palaemonetes kadiakensis</i>	E881, P484
		Cambaridae (Formerly Astacidae) 97336	Crayfish	<i>Cambarus latimanus</i>	E897
			Crayfish	<i>Faxonella clypeata</i>	E890
			Crayfish	<i>Orconectes immunis</i>	E894, P482
			Crayfish	<i>Orconectes limosus</i>	E893, P482
			Crayfish	<i>Orconectes propinquus</i>	E894, P482
			Crayfish	<i>Orconectes nais</i>	E894
			Crayfish	<i>Orconectes rusticus</i>	E893, P482
			Crayfish	<i>Orconectes virilis</i>	E894, P483
			Crayfish	<i>Pacifastacus trowbridgii</i>	E883
			Crayfish	<i>Procambarus acutus</i>	P482
			Crayfish	<i>Procambarus clarki</i> <i>Procambarus clarkii</i> <sup>†</sup>	E885, P482
			Crayfish	<i>Procambarus simulans</i>	E888, P482
			Crayfish	<i>Procambarus sp.</i>	2
Insecta 99208	Heptageniidae 100504	Mayfly	<i>Maccaffertium ithaca</i> <i>Stenonema ithaca</i>	S173, O205	
		Mayfly	<i>Maccaffertium modestum</i> <i>Stenonema rubrum</i> <sup>†</sup>	S178, O205	
		Baetidea	Mayfly	<i>Callibaetis skokianus</i>	S116, N9

Class	Family	Species		Reference
		Common Name	Scientific Name	
100755		Mayfly	<i>Callibaetis</i> sp.	2
		Mayfly	<i>Cloeon dipterum</i>	O173
Leptophlebiidae 101095		Mayfly	<i>Paraleptophlebia praepedita</i>	S89, O233
Ephemerellidae 101232		Mayfly	<i>Drunella doddsii</i> <i>Ephemerella doddsii</i>	O245
		Mayfly	<i>Drunella grandis</i> <i>Ephemerella grandis</i>	O245
		Mayfly	<i>Ephemerella subvaria</i>	N9, O248, S71
		Mayfly	<i>Ephemerella</i> sp.	2
Caenidea 101467		Mayfly	<i>Caenis diminuta</i>	S51, O268
Ephemeridae 101525		Mayfly	<i>Ephemera simulans</i>	S36, N9, O283
		Mayfly	<i>Hexagenia bilineata</i>	N9, S39, O290
		Mayfly	<i>Hexagenia rigida</i>	O290, S41, N9
		Mayfly	<i>Hexagenia</i> sp.	2
Libellulidae 101797		Dragonfly	<i>Pantala hymenaea</i> <i>Pantala hymenea</i>	N15, V603
Coenagrionidae Agrionidae Coenagriidae 102077		Damselfly	<i>Enallagma aspersum</i>	DD
		Damselfly	<i>Ischnura elegans</i>	nonresident
		Damselfly	<i>Ischnura verticalis</i>	N15, E918
		Damselfly	<i>Ischnura</i> sp.	2
Pteronarcyidae (Formerly Pteronarcidae) Pteronarcyidae 102470		Stonefly	<i>Pteronarcella badia</i>	L172
		Stonefly	<i>Pteronarcys californica</i>	L173
		Stonefly	<i>Pteronarcys dorsata</i>	E947
		Stonefly	<i>Pteronarcys</i> sp.	2
Nemouridae 102517		Stonefly	<i>Nemoura cinerea</i>	nonresident
Perlidae 102914		Stonefly	<i>Acroneuria lycorias</i>	N4, E953
		Stonefly	<i>Acroneuria pacifica</i>	E953, L180
		Stonefly	<i>Claassenia sabulosa</i>	E953
		Stonefly	<i>Agnatina capitata</i> <i>Neophasganophora capitata</i> <i>Phasganophora capitata</i>	E953, CC407
Perlodidae 102994		Stonefly	<i>Skwala americana</i> <i>Arcynopteryx parallela</i>	E954
Nepidae 103747		Water Scorpion	<i>Ranatra elongate</i> (Species cannot be confirmed in ITIS)	nonresident
Dytiscidae 111963		Beetle	-	2
Elmidae Elminthidae 114093		Beetle	<i>Stenelmis sexlineata</i>	W21
Hydropsychidae 115398		Caddisfly	<i>Arctopsyche grandis</i>	L251, II98
		Caddisfly	<i>Hydropsyche betteni</i>	N24
		Caddisfly	<i>Hydropsyche californica</i>	L253
		Caddisfly	<i>Hydropsyche</i> sp.	2
Limnephilidae 115933		Caddisfly	<i>Clistoronia magnifica</i>	II206
		Caddisfly	<i>Philarctus quaeris</i>	II272

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Brachycentridae 116905	Caddisfly	<i>Brachycentrus sp.</i>	2
	Tipulidae 118840	Crane fly	<i>Tipula sp.</i>	2
	Ceratopogonidae 127076	Biting midge	-	2
	Culicidae 125930	Mosquito	<i>Aedes aegypti</i>	EE3
		Mosquito	<i>Culex pipiens</i>	EE3
	Chironomidae 127917	Midge	<i>Chironomus plumosus</i> <i>Tendipas plumosus</i>	L423
		Midge	<i>Chironomus tentans</i>	Q
		Midge	<i>Chironomus thummi</i> <sup>†</sup>	nonresident
		Midge	<i>Chironomus sp.</i>	2
		Midge	<i>Paratanytarsus parthenogeneticus</i>	7
		Midge	<i>Paratanytarsus dissimilis</i> <i>Tanytarsus dissimilis</i>	R11
	Athericidae (Formerly Rhagionidae) Leptidae 130928	Snipe fly	<i>Atherix sp.</i>	2
<b>Phylum: Ectoprocta (155470)</b>				
Phylactolaemata 156688	Pectinatellidae (Formerly Pectinatelcidae) 156729	Bryozoan	<i>Pectinatella magnifica</i>	E502, P269
	Lophopodidae 156714	Bryozoan	<i>Lophopodella carteri</i>	E502, P2671
	Plumatellidae 156690	Bryozoan	<i>Plumatella emarginata</i>	E505, P272
<b>Phylum: Chordata (158852)</b>				
Agnatha 159693	Petromyzontidae 159697	Sea lamprey	<i>Petromyzon marinus</i>	F11
Actinopterygii (Formerly Osteichthyes) 161061	Anguillidae 161125	American eel	<i>Anguilla rostrata</i>	F15
	Salmonidae 161931	Pink salmon	<i>Oncorhynchus gorboscha</i>	F18
		Coho salmon	<i>Oncorhynchus kisutch</i>	F18
		Sockeye salmon	<i>Oncorhynchus nerka</i>	F19
		Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	F19
		Mountain whitefish	<i>Prosopium williamsoni</i>	F19
		Golden Trout	<i>Oncorhynchus aguabonita</i> (Formerly <i>Salmo aguabonita</i> )	F19
		Cutthroat trout	<i>Oncorhynchus clarki</i> (Formerly <i>Salmo clarki</i> )	F19
		Rainbow trout Steelhead trout <sup>*</sup>	<i>Oncorhynchus mykiss</i> (Formerly <i>Salmo gairdneri</i> )	F19
		Atlantic salmon	<i>Salmo salar</i>	F19
		Brown trout	<i>Salmo trutta</i>	F19
		Brook trout	<i>Salvelinus fontinalis</i>	F19
Lake trout	<i>Salvelinus namaycush</i>	F19		

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Esocidae 162137	Northern pike	<i>Esox lucius</i>	F20
	Cyprinidae 163342	Chiselmouth	<i>Acrocheilus alutaceus</i>	F21
		Longfin dace	<i>Agosia chrysogaster</i>	F21
		Central stoneroller	<i>Campostoma anomalum</i>	F21
		Goldfish	<i>Carassius auratus</i>	F21
		Common carp	<i>Cyprinus carpio</i>	F21
		Zebra danio Zebrafish	<i>Danio rerio</i> <sup>*</sup> <i>Brachydanio rerio</i> <sup>*</sup>	nonresident F96
		Silverjaw minnow	<i>Notropis buccatus</i> <i>Ericymba buccata</i>	F21
		Golden shiner	<i>Notemigonus crysoleucas</i>	F23
		Pugnose shiner	<i>Notropis anogenus</i>	F23
		Emerald shiner	<i>Notropis atherinoides</i>	F23
		Striped shiner	<i>Luxilus chrysocephalus</i> <i>Notropis chrysocephalus</i> <sup>*</sup>	F23
		Common shiner	<i>Luxilus cornutus</i> <i>Notropis cornutus</i> <sup>*</sup>	F23
		Pugnose minnow	<i>Opsopoeodus emiliae</i> <i>Notropis emiliae</i>	F24
		Spottail shiner	<i>Notropis hudsonius</i>	F24
		Red shiner	<i>Cyprinella lutrensis</i> <i>Notropis lutrensis</i>	F24
		Spotfin shiner	<i>Cyprinella spiloptera</i> <i>Notropis spilopterus</i>	F25
		Sand shiner	<i>Notropis stramineus</i>	F25
		Steelcolor shiner	<i>Cyprinella whipplei</i> <i>Notropis whipplei</i>	F25
		Northern redbelly dace	<i>Phoxinus eos</i>	F25
		Bluntnose minnow	<i>Pimephales notatus</i>	F25
		Fathead minnow	<i>Pimephales promelas</i>	F25
		Northern squawfish	<i>Ptychocheilus oregonensis</i>	F25
		Blacknose dace	<i>Rhinichthys atratulus</i>	F25
		Speckled dace	<i>Rhinichthys osculus</i>	F25
		Bitterling	<i>Rhodeus sericeus</i>	F26
		Rudd	<i>Scardinius erythrophthalmus</i>	F26
		Creek chub	<i>Semotilus atromaculatus</i>	F26
		Pearl dace	<i>Margariscus margarita</i> <i>Semotilus margarita</i>	F26
		Tench	<i>Tinca tinca</i>	F26
		Catostomidae 163892	White sucker	<i>Catostomus commersoni</i>
	Mountain sucker		<i>Catostomus platyrhynchus</i>	F26
	Ictaluridae 163995	Black bullhead	<i>Ameiurus melas</i> <i>Ictalurus melas</i> <sup>*</sup>	F27
		Yellow bullhead	<i>Ameiurus natalis</i> <i>Ictalurus natalis</i> <sup>*</sup>	F27
		Brown bullhead	<i>Ameiurus nebulosus</i> <i>Ictalurus nebulosus</i> <sup>*</sup>	F27
		Channel catfish	<i>Ictalurus punctatus</i>	F27

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Clariidae 164118	Walking catfish	<i>Clarias batrachus</i>	F28
	Adrianichthyidae (Formerly Oryziidae) 165623	Medaka	<i>Oryzias latipes</i>	nonresident F96
	Cyprinodontidae 165629	Banded killifish	<i>Fundulus diaphanus</i>	F33
		Flagfish	<i>Jordanella floridae</i>	F33
	Poeciliidae 165876	Mosquitofish	<i>Gambusia affinis</i>	F33
		Amazon molly	<i>Poecilia formosa</i>	F34
		Sailfin molly	<i>Poecilia latipinna</i>	F34
		Molly	<i>Poecilia sp.</i>	
		Guppy	<i>Poecilia reticulata</i> ( <i>Lebistes reticulatus</i> , Obs.)	F34
		Southern platyfish	<i>Xiphophorus maculatus</i>	F34
	Gasterosteidae 166363	Brook stickleback	<i>Culaea inconstans</i>	F35
		Threespine stickleback	<i>Gasterosteus aculeatus</i>	F35
		Ninespine stickleback	<i>Pungitius pungitius</i>	F35
	Percichthyidae 170315	White perch	<i>Morone americana</i> ( <i>Roccus americanus</i> , Obs.)	F36
		Striped bass	<i>Morone saxatilis</i> ( <i>Roccus saxatilis</i> , Obs.)	F36
	Centrarchidae 168093	Rock bass	<i>Ambloplites rupestris</i>	F38
		Green sunfish	<i>Lepomis cyanellus</i>	F38
		Pumpkinseed	<i>Lepomis gibbosus</i>	F38
		Orangespotted sunfish	<i>Lepomis humilis</i>	F38
		Bluegill	<i>Lepomis macrochirus</i>	F38
		Longear sunfish	<i>Lepomis megalotis</i>	F38
		Redear sunfish	<i>Lepomis microlophus</i>	F38
		Smallmouth bass	<i>Micropterus dolomieu</i>	F39
		Largemouth bass	<i>Micropterus salmoides</i>	F39
		White crappie	<i>Pomoxis annularis</i>	F39
		Black crappie	<i>Pomoxis nigromaculatus</i>	F39
		Percidae 168356	Rainbow darter	<i>Etheostoma caeruleum</i>
	Johnny darter		<i>Etheostoma nigrum</i>	F40
	Orangethroat darter		<i>Etheostoma spectabile</i>	F40
	Yellow perch		<i>Perca flavescens</i>	F41
	Walleye		<i>Sander vitreus</i> <i>Stizostedion vitreum vitreum</i> *	F41
	Sciaenidae 169237	Freshwater drum	<i>Aplodinotus grunniens</i>	F45
	Cichlidae 169770	Oscar	<i>Astronotus ocellatus</i>	F47
		Blue tilapia	<i>Tilapia aurea</i>	F47
		Mozambique tilapia	<i>Oreochromis mossambicus</i> <i>Tilapia mossambica</i>	F47
	Cottidae 167196	Mottled sculpin	<i>Cottus bairdi</i>	F60
Amphibia 173420	Ranidae 173433	Bullfrog	<i>Rana catesbeiana</i>	B206
		Green frog	<i>Rana clamitans</i>	B206

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Pig frog	<i>Lithobates grylio</i> <i>Rana grylio</i>	B206
		River frog	<i>Rana heckscheri</i>	B206
		Leopard frog	<i>Rana pipiens</i>	B205
		Wood frog	<i>Rana sylvatica</i>	B206
		Frog	<i>Rana temporaria</i> <sup>1</sup>	nonresident
		Leopard frog	<i>Lithobates sphenoccephalus sphenoccephalus</i> (Formerly <i>Rana spenocephala</i> )	JJ
	Microhylidae 173465	Eastern narrow-mouthed toad	<i>Gastrophryne carolinensis</i>	B192
	Bufonidae 173471	American toad	<i>Anaxyrus americanus americanus</i> <i>Bufo americanus</i>	B196
		Toad	<i>Bufo bufo</i> <sup>2</sup>	nonresident
		Green toad	<i>Anaxyrus debilis debilis</i> <i>Bufo debilis</i>	B197
		Fowler's toad	<i>Anaxyrus fowleri</i> <i>Bufo fowleri</i> <sup>1</sup>	B196
		Red-spotted toad	<i>Anaxyrus punctatus</i> <i>Bufo punctatus</i>	B198
		Woodhouse's toad	<i>Anaxyrus woodhousii woodhousii</i> <i>Bufo woodhousii</i>	B196
	Hylidae 173497	Northern cricket frog	<i>Acris crepitans</i>	B203
		Southern gray treefrog	<i>Hyla chrysoscelis</i>	B201
		Spring creeper	<i>Pseudacris crucifer</i> <i>Hyla crucifer</i> <sup>1</sup>	B202
		Barking treefrog	<i>Hyla gratiosa</i>	B201
		Squirrel treefrog	<i>Hyla squirella</i>	B201
		Gray treefrog	<i>Hyla versicolor</i>	B200
		Northern chorus frog	<i>Pseudacris triseriata</i>	B202
	Pipidae 173547	African clawed frog	<i>Xenopus laevis</i>	Z16
	Ambystomatidae 173588	Spotted salamander	<i>Ambystoma maculatum</i>	B176
		Mexican axolotl	<i>Ambystoma mexicanum</i> <sup>1</sup>	nonresident
		Marbled salamander	<i>Ambystoma opacum</i>	B176
	Salamandridae 173613	Newt	<i>Notophthalmus viridescens</i> <i>Triturus viridescens</i> <sup>1</sup>	B179

## Footnotes for Freshwater Species

- <sup>1</sup> Apparently this is an outdated name (D19, 20). Organisms identified as such should only be used if they were obtained from North America.
- <sup>2</sup> Apparently this is an outdated name (D19, 20). Organisms identified as such should only be used if they were obtained from North America.
- <sup>3</sup> If from North America, it is resident and should be called *D. similis* (C). If not from North America, it should be considered nonresident.
- <sup>4</sup> If from North America, it is resident and may be any one of a number of species such as *D. laevis*, *D. dubia*, or *D. galeate mendoca* (C). If not from North America, it should be considered nonresident.

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- <sup>5</sup> If from North America, it is resident and may be any one of a number of species such as *D. ambigua*, *D. longiremis*, or *D. rosea* (C). If not from North America, it should be considered nonresident.
- <sup>6</sup> This species might be established in portions of the southern United States.
- <sup>7</sup> The taxonomy of this species and this and similar genera has not been clarified, but this species should be considered resident.

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## Saltwater Species Table

Synonyms appear after the official Scientific Name and are marked with an asterisk (\*).

Non-resident species are noted in the Reference column and are marked with a dagger (†).

Class	Family	Species		Reference
		Common Name	Scientific Name	
<b>Phylum: Cnidaria (Coelenterata) (48738)</b>				
Hydrozoa 48739	Campanulariidae 49470	Hydroid	<i>Campanularia flexiosa</i> ** <i>Campanularia flexuosa</i> **	B122, E81
		Hydroid	<i>Laomedea loveni</i> ††	nonresident
		Hydromedusa	<i>Phialidium</i> sp.	† (E81)
	Campanulinidae 49756	Hydroid	<i>Eirene viridula</i> †	nonresident
<b>Phylum: Ctenophora (53856)</b>				
Tentaculata 53858	Pleurobrachiidae 53860	Ctenophore	<i>Pleurobrachia pileus</i>	B218, E162
	Mnemiidae 53915	Ctenophore	<i>Mnemiopsis mccradyi</i>	C39, I94
<b>Phylum: Nemertea (Rhynchozoela) (57411)</b>				
Heteronemertea 57438	Lineidae 57443	Nemertine worm	<i>Cerebratulus fuscus</i>	B252
<b>Phylum: Rotifera (Rotatoria) (58239)</b>				
Monogononta 58342	Brachionidae 58344	Rotifer	<i>Brachionus plicatilis</i>	B272
<b>Phylum: Annelida (64357)</b>				
Polychaeta 64358	Phyllodoceidae 65228	Polychaete worm	<i>Phyllodoce maculata</i> <i>Anaitides maculata</i> <i>Nereiphylla maculata</i> *	E334
			Nereididae (Nereidae) 65870	Polychaete worm
	Polychaete worm	<i>Neanthes vaali</i> †		nonresident
	Polychaete worm	<i>Nereis diversicolor</i> <i>Neanthes diversicolor</i> *		E337, F527
	Sand worm	<i>Nereis virens</i> <i>Neanthes virens</i> *		B317, E337, C58
	Polychaete worm	<i>Nereis</i> sp.		
	Dorvilleidae 66478	Polychaete worm	<i>Ophryotrocha diadema</i>	P23
		Polychaete worm	<i>Ophryotrocha labronica</i> † <i>Ophryotrocha labronica</i> †	nonresident
	Spionidae 66781	Polychaete worm	<i>Polydora websteri</i>	E338
	Cirratulidae 67116	Polychaete worm	<i>Cirriformia spirabanchia</i>	G253
	Ctenodrilidae 67217	Polychaete worm	<i>Ctenodrilus serratus</i>	G275
	Capitellidae 67413	Polychaete worm	<i>Capitella capitata</i>	B358, E337
	Arenicolidae 67500	Polychaete worm	<i>Arenicola marina</i>	B369, E337

\*\* Synonym

†† Non-resident species

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Sabellidae 68076	Polycheate worm	<i>Eudistylia vancouveri</i>	DD
Oligochaeta 68422	Tubificidae 68585	Oligochaete worm	<i>Limnodriloides verrucosus</i>	Z
		Oligochaete worm	<i>Monopylephorus cuticulatus</i>	Z
		Oligochaete worm	<i>Peloscolex gabriellae</i> <i>Tubificoides gabriellae</i>	Z
<b>Phylum: Mollusca (69458)</b>				
Gastropoda 69459	Haliotididae 566897	Black abalone	<i>Haliotis cracherodii</i>	C88, D17
		Red abalone	<i>Haliotis rufescens</i>	D18
	Calyptraeidae 72611	Common Atlantic slippershell	<i>Crepidula fornicata</i>	C90, D141
	Muricidae 73236	Oyster drill	<i>Urosalpinx cinerea</i> <i>Urosalpinx cinereus</i>	B646, D179, E264
	Melongenidae (Neptuneidae) 74069	Channeled whelk	<i>Busycotypus canaliculatus</i> (Formerly <i>Busycon canaliculatum</i> )	B655, D223, E264
	Nassariidae (Nassidae) 74102	Mud snail	<i>Nassarius obsoletus</i> <i>Nassa obsoleta</i> <i>Icyanassa obsoleta</i>	B649, D226, E264
Bivalvia (Pelecypoda) 79118	Mytilidae 79451	Northern horse mussel	<i>Modiolus modiolus</i>	D434
		Blue mussel	<i>Mytilus edulis</i>	B566, C101, D428, E299
		Mediterranean mussel	<i>Mytilus galloprovincialis</i> <sup>†</sup>	nonresident
	Pectinidae 79611	Bay scallop	<i>Argopecten irradians</i>	D447
	Ostreidae 79866	Pacific oyster	<i>Crassostrea gigas</i>	C102, D456, E300
		Eastern oyster	<i>Crassostrea virginica</i>	D456, E300
		Oyster	<i>Crassostrea sp.</i>	1
		Oyster	<i>Ostrea edulis</i>	E300
	Cardiidae 80865	Cockle	<i>Cerastoderma edule</i> <sup>†</sup> <i>Cardium edule</i> <sup>†</sup>	nonresident
	Mactridae 80942	Clam	<i>Mulinia lateralis</i>	D491
		Common rangia	<i>Rangia cuneata</i>	D491, E301
		Surf clam	<i>Spisula solidissima</i>	B599, D489, E301
	Tellinidae 81032	Clam	<i>Macoma inquinata</i>	D507
		Bivalve	<i>Tellina tenuis</i> <sup>†</sup>	nonresident
	Veneridae 81439	Quahog clam	<i>Mercenaria mercenaria</i>	D523, E301
		Common Pacific littleneck	<i>Protothaca staminea</i>	D526
		Japanese littleneck clam	<i>Tapes philippinarum</i>	D527
Myidae 81688	Soft-shell clam	<i>Mya arenaria</i>	B602, D536, E302	
<b>Phylum: Arthropoda (82696)</b>				
Merostomata 82698	Limulidae 82701	Horseshoe crab	<i>Limulus polyphemus</i>	B533, E403, H30
Branchiopoda (Formerly Crustacea) 83687	Artemiidae 83689	Brine shrimp	<i>Artemia salina</i> <sup>†</sup>	<sup>2</sup> nonresident
Maxillopoda (Formerly Crustacea) 621145	Calanidae 85259	Copepod	<i>Calanus helgolandicus</i>	Q25
		Copepod	<i>Undinula vulgaris</i>	Q29
	Eucalanidae 85299	Copepod	<i>Eucalanus elongatus</i>	AA
		Copepod	<i>Subeucalanus pileatus</i> <i>Eucalanus pileatus</i>	AA
	Pseudocalanidae 85351	Copepod	<i>Pseudocalanus minutus</i>	E447, I155, Q43

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Euchaetidae 85524	Copepod	<i>Euchaeta marina</i>	Q63
	Metridinidae (Formerly Metridiidae) 593501	Copepod	<i>Metridia pacifica</i>	X179, Y
	Pseudodiaptomidae 85847	Copepod	<i>Pseudodiaptomus coronatus</i>	E447, I154, Q101
	Temoridae 85855	Copepod	<i>Eurytemora affinis</i>	E450, I155, Q111
	Pontellidae 86038	Copepod	<i>Labidocera scotti</i>	R157
	Acartiidae 86083	Copepod	<i>Acartia clausi</i>	E447
		Copepod	<i>Acartia tonsa</i>	E447, I154
	Harpacticidae 86329	Copepod	<i>Tigriopus californicus</i>	J78
		Copepod	<i>Tigriopus japonicus</i> <sup>†</sup>	nonresident
	Tisbidae 86444	Copepod	<i>Tisbe holothuriae</i>	BB
	Ameiridae (Formerly Canthocamptidae) 86999	Copepod	<i>Nitokra spinipes</i> <i>Nitocra spinipe</i>	Q240
	Archaeobalanidae (Formerly Balanidae) 89681	Barnacle	<i>Semibalanus balanoides</i> <i>Balanus balanoides</i> <sup>†</sup>	B424, E457
	Balanidae 89599	Barnacle	<i>Balanus crenatus</i>	B426, E457
		Barnacle	<i>Balanus eburneus</i>	B424, E457
Barnacle		<i>Balanus improvisus</i>	B426, E457	
Malacostraca (Formerly Crustacea) 89787	Mysidae 89856	Mysid	<i>Heteromysis formosa</i>	E513, K720
		Mysid	<i>Americamysis bahia</i> <i>Mysidopsis bahia</i> <sup>†</sup>	U173
		Mysid	<i>Americamysis bigelowi</i> <i>Mysidopsis bigelowi</i> <sup>†</sup>	E513, K720
		Mysid	<i>Neomysis sp.</i>	1
	Idoteidae 92564	Isopod	<i>Idotea balthica</i> <i>Idothea baltica</i> <sup>†</sup>	B446, E483
		Isopod	<i>Idotea emarginata</i> <sup>†</sup>	nonresident
		Isopod	<i>Idotea neglecta</i> <sup>†</sup>	nonresident
	Janiridae 92810	Isopod	<i>Jaera albifrons</i> <sup>†</sup>	nonresident
		Isopod	<i>Jaera albifrons sensu</i> <sup>†</sup>	nonresident
		Isopod	<i>Jaera nordmanni</i> <sup>†</sup>	nonresident
	Ampeliscidae 93320	Amphipod	<i>Ampelisca abdita</i>	E488, L136
	Eusiridae (Pontogeneiidae) 93681	Amphipod	<i>Pontogeneia sp.</i>	1
	Gammaridae 93745	Amphipod	<i>Gammarus duebeni</i>	L56
		Amphipod	<i>Gammarus oceanicus</i>	E489, L50
		Amphipod	<i>Gammarus tigrinus</i>	L51
		Amphipod	<i>Gammarus zaddachi</i> <sup>†</sup>	nonresident
		Amphipod	<i>Marinogammarus obtusatus</i>	L58
	Uristadae (Formerly Lysianassidae) 621432	Amphipod	<i>Anonyx sp.</i>	1
	Euphausiidae (Thysanopodidae) 95500	Euphausiid	<i>Euphausia pacifica</i>	M15
	Penaeidae	Brown shrimp	<i>Penaeus aztecus</i>	E518, N17

Class	Family	Species		Reference
		Common Name	Scientific Name	
	95602	Pink shrimp	<i>Penaeus duorarum</i>	E518, N17
		White shrimp	<i>Penaeus setiferus</i>	E518, N17
		Blue Shrimp	<i>Penaeus stylirostris</i> <sup>†</sup>	nonresident
	Palaemonidae 96213	Shrimp	<i>Leander paucidens</i> <sup>†</sup>	nonresident
		Prawn	<i>Leander squilla</i> <sup>†</sup> <i>Palaemon elegans</i> <sup>†</sup>	nonresident
		Prawn	<i>Macrobrachium rosenbergii</i>	<sup>3</sup>
		Korean shrimp	<i>Palaemon macrodactylus</i>	T380
		Grass shrimp	<i>Palaemonetes pugio</i>	E521, N59
		Grass shrimp	<i>Palaemonetes vulgaris</i>	B500, E521, N56
	Hippolytidae 96746	Sargassum shrimp	<i>Latreutes fucorum</i>	N78
	Pandalidae 96965	Coon stripe shrimp	<i>Pandalus danae</i>	T306, W163
		Shrimp	<i>Pandalus goniurus</i>	W163
		Pink shrimp	<i>Pandalus montagui</i>	B494, E522, W163
	Crangonidae 97106	Sand shrimp	<i>Crangon crangon</i> <sup>†</sup>	nonresident
		Bay shrimp	<i>Crangon franciscorum</i> <i>Crango franciscorum</i> <sup>†</sup>	V176, W164
		Shrimp	<i>Crangon nigricauda</i>	V176, W164
		Sand shrimp	<i>Crangon septemspinosa</i>	B500, E522
	Nephropidae (Homaridae) 97307	American lobster	<i>Homarus americanus</i>	B502, E532
		European lobster	<i>Homarus gammarus</i> <sup>†</sup>	nonresident
	Paguridae 97774	Hermit crab	<i>Pagurus longicarpus</i>	B514, E537, N125
	Cancridae 98670	Rock crab	<i>Cancer irroratus</i>	B518, E543, N175
		Dungeness crab	<i>Cancer magister</i>	T166, V185, W177
	Portunidae 98689	Blue crab	<i>Callinectes sapidus</i>	B521, C80, E543, N168
		Green crab	<i>Carcinus maenas</i>	C80, E543
	Xanthidae (Pilumnidae) 98748	Mud crab	<i>Eurypanopeus depressus</i>	B522, E543, N195
		Crab	<i>Leptodius floridanus</i>	S80
		Mud crab	<i>Rhithropanopeus harrisi</i>	E543, N187
	Varunidae (formerly Grapsidae) 621521	Shore crab	<i>Hemigrapsus nudus</i>	CC
		Shore crab	<i>Hemigrapsus oregonensis</i>	CC
Sesamidae (formerly Grapsidae) 621520	Drift line crab	<i>Armases cinereum</i> ( <i>Sesarma cinereum</i> )	B526, E544, N222	
	Crab	<i>Sesarma haematocheir</i> <sup>†</sup>	nonresident	
Ocypodidae 99080	Fiddler crab	<i>Uca pugnator</i>	B526, E544, N232	
<b>Phylum: Echinodermata (156857)</b>				
Asteroidea 156862	Asteriidae 157212	Starfish	<i>Asterias forbesi</i>	B728, E578, O392
Ophiuroidea 157325	Ophiothricidae 157792	Brittle star	<i>Ophiothrix spiculata</i>	O672, T526
Echinoidea 157821	Arbaciidae 157904	Sea urchin	<i>Arbacia lixula</i> <sup>†</sup>	nonresident
		Sea urchin	<i>Arbacia punctulata</i>	B762, E572
	Toxopneustidae 157919	Sea urchin	<i>Lytechinus pictus</i>	T253
		Sea urchin	<i>Pseudocentrotus depressus</i> <sup>†</sup>	nonresident
	Echinidae 157940	[chinoderm]	<i>Paracentrotus lividus</i> <sup>†</sup>	nonresident
Echinometridae 157955	Coral reef echinoid	<i>Echinometra mathaei</i> <sup>†</sup>	nonresident [Hawaii only]	

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Strongylocentrotidae 157965	Sea urchin	<i>Strongylocentrotus purpuratus</i>	O574, T202
	Dendrasteridae 158008	Sand dollar	<i>Dendraster excentricus</i>	O537, V363
<b>Phylum: Chaetognatha (158650)</b>				
Sagittoidea 158655	Sagittidae 158726	Arrow worm	<i>Ferosagitta hispida</i> <i>Sagitta hispida</i>	E218
<b>Phylum: Chordata (158852)</b>				
Chondrichthyes 159785	Rajidae 160845	Thornback ray	<i>Raja clavata</i> <sup>†</sup>	nonresident
Actinopterygii (Formerly Osteichthyes) 161061	Anguillidae 161125	American eel	<i>Anguilla rostrata</i>	A15
	Clupeidae 161700	Atlantic menhaden	<i>Brevoortia tyrannus</i>	A17
		Gulf menhaden	<i>Brevoortia patronus</i>	A17
		Atlantic herring	<i>Clupea harengus</i> <i>Clupea harengus harengus</i> <sup>*</sup>	A17
		Pacific herring	<i>Clupea pallasii</i> <i>Clupea harengus pallasii</i> <sup>*</sup>	A17
		Herring	<i>Clupea harengus</i>	A17
	Engraulidae 553173	Northern anchovy	<i>Engraulis mordax</i>	A18
		Nehu	<i>Encrasicholina purpurea</i> <sup>†</sup> <i>tolephorus purpureus</i> <sup>†*</sup>	nonresident [Hawaii only]
	Salmonidae 161931	Pink salmon	<i>Oncorhynchus gorbuscha</i>	A18
		Chum salmon	<i>Oncorhynchus keta</i>	A18
		Coho salmon	<i>Oncorhynchus kisutch</i>	A18
		Sockeye salmon	<i>Oncorhynchus nerka</i>	A19
		Chinook salmon	<i>Oncorhynchus tshawytscha</i>	A19
		Rainbow trout (Steelhead trout)	<i>Oncorhynchus mykiss</i> (Formerly <i>Salmo gairdneri</i> )	A19
		Atlantic salmon	<i>Salmo salar</i>	A19
	Gadidae 164701	Atlantic cod	<i>Gadus morhua</i>	A30
		Haddock	<i>Melanogrammus aeglefinus</i>	A30
	Cyprinodontidae 165629	Sheepshead minnow	<i>Cyprinodon variegatus</i>	A33
		Mummichog	<i>Fundulus heteroclitus</i>	A33
		Striped killifish	<i>Fundulus majalis</i>	A33
		Longnose killifish	<i>Fundulus similis</i>	A33
	Poeciliidae 165876	Mosquitofish	<i>Gambusia affinis</i>	A33
		Sailfin molly	<i>Poecilia latipinna</i>	A34
	Atherinidae 165984	Inland silverside	<i>Menidia beryllina</i>	A34
		Atlantic silverside	<i>Menidia menidia</i>	A34
		Tidewater silverside	<i>Menidia peninsulae</i>	A34
	Gasterosteidae 166363	Threespine stickleback	<i>Gasterosteus aculeatus</i>	A35
Fourspine stickleback		<i>Apeltes quadracus</i>	A35	
Syngnathidae 166443	Northern pipefish	<i>Syngnathus fuscus</i>	A36	
Percichthyidae 170315	Striped bass	<i>Morone saxatilis</i> ( <i>Roccus saxatilis</i> , Obs.)	A36	
Kuhliidae 168083	Mountain bass	<i>Kuhlia sandvicensis</i> <sup>†</sup>	nonresident [Hawaii only]	
Carangidae 168584	Florida Pompano	<i>Trachinotus carolinus</i>	A43	

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Sparidae 169180	Pinfish	<i>Lagodon rhomboides</i>	A45
	Sciaenidae 169237	Spot	<i>Leiostomus xanthurus</i>	A46
		Atlantic croaker	<i>Micropogonias undulatus</i>	A46
		Red drum	<i>Sciaenops ocellatus</i>	A46
	Embiotocidae 169735	Shiner perch	<i>Cymatogaster aggregata</i>	A47
		Dwarf perch	<i>Micrometrus minimus</i>	A48
	Pomacentridae 170044	Blacksmith	<i>Chromis punctipinnis</i>	A48
	Labridae 170477	Cunner	<i>Tautoglabrus adspersus</i>	A49
		Bluehead	<i>Thalassoma bifasciatum</i>	A49
	Mugilidae 170333	Mullet	<i>Aldrichetta forsteri</i> <sup>†</sup>	nonresident
		Striped mullet	<i>Mugil cephalus</i>	A49
		White mullet	<i>Mugil curema</i>	A49
	Ammodytidae 171670	Pacific sand lance	<i>Ammodytes hexapterus</i>	A53
	Gobiidae 171746	Longjaw mudsucker	<i>Gillichthys mirabilis</i>	A54
		Naked goby	<i>Gobiosoma bosci</i>	A54
	Cottidae 167196	Tidepool sculpin	<i>Oligocottus maculosus</i>	A61
	Bothidae 172714	Speckled sanddab	<i>Citharichthys stigmatæus</i>	A64
		Summer Flounder	<i>Paralichthys dentatus</i>	A64
	Pleuronectidae 172859	Dab	<i>Limanda limanda</i> <sup>†</sup>	nonresident
		Plaice	<i>Pleuronectes platessa</i> <sup>†</sup>	nonresident
		English sole	<i>Parophrys vetulus</i>	A65
		Winter flounder	<i>Pseudopleuronectes americanus</i>	A65
	Balistidae 173128	Planehead filefish	<i>Monacanthus hispidus</i>	A66
	Tetraodontidae 173283	Northern puffer	<i>Sphoeroides maculatus</i>	A66

## Footnotes for Saltwater Species

- <sup>1</sup> Organisms not identified to species are considered resident only if obtained from wild populations in North America.
- <sup>2</sup> This species should not be used because it might be too atypical.
- <sup>3</sup> This species might be established in portions of the southern United States.

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## Appendix 2. Example Calculation of Final Acute Value, Computer Program, and Printouts

### A. Example Calculation

N = total number of MAVs in data set = 8

Rank	MAV	ln(MAV)	ln(MAV) <sup>2</sup>	P = R / (N+1)	√P
4	6.4	1.8563	3.4458	0.44444	0.66667
3	6.2	1.8245	3.3290	0.33333	0.57735
2	4.8	1.5686	2.4606	0.22222	0.47140
1	0.4	-0.9163	0.8396	0.11111	0.33333
Sum		4.3331	10.0750	1.11110	2.04875

$$S^2 = \frac{10.0750 - (4.3331)^2 / 4}{1.11110 - (2.04875)^2 / 4} = 87.134$$

$$S = 9.3346$$

$$L = [4.3331 - (9.3346)(2.04875)] / 4 = -3.6978$$

$$A = (9.3346) (\sqrt{0.05}) - 3.6978 = -1.6105$$

$$FAV = e^{-1.6105} = 0.1998$$

### B. Example Computer Program in BASIC Language for Calculating the FAV

```

10   REM This program calculates the FAV when there are less than
20   REM 59 MAVs in the data set
30   X = 0
40   X2 = 0
50   Y = 0
60   Y2 = 0
70   PRINT "How many MAVs are in the data set?"
80   INPUT N
90   PRINT "What are the four lowest MAVs?"
100  FOR R = 1 TO 4
110      INPUT V

```

```

120          X = X + LOG(V)
130          X2 = X2 + (LOG(V)) * (LOG(V))
140          P = R / (N + 1)
150          Y2 = Y2 + P
160          Y = Y + SQR((X2 - X * X / 4))
170 NEXT R
180 S = SQR((X2 - X * X / 4) / (Y2 - Y * Y / 4))
190 L = (X - S * Y) / 4
200 A = S * SQR(0.05) + L
210 F = EXP(A)
220 PRINT "FAV = " F
230 END

```

### C. Example Printouts from Program

```

How many MAVs are in the data set?
? 8
What are the four lowest MAVs?
? 6.4
? 6.2
? 4.8
? .4
FAV = 0.1998

```

```

How many MAVs are in the data set?
? 16
What are the four lowest MAVs?
? 6.4
? 6.2
? 4.8
? .4
FAV = 0.4365

```

# Exhibit 52

An official website of the United States government.

## How to Get Methods Approved



### Background

The Clean Water Act requires EPA to establish testing procedures for analysis of pollutants through a formal notice and comment rulemaking process. These testing methods must be used for measuring pollutants for NPDES (National Pollutant Discharge Elimination System) permit applications and any NPDES reporting requirements. The Office of Science and Technology (OST) within EPA's Office of Water (OW) is responsible for developing, reviewing and promulgating these methods as well as developing alternatives to existing methods.

### Methods Update Rules

Because promulgating methods through individual rulemakings would be very resource-intensive, EPA periodically combines new methods and modifications to existing methods into a single package – a proposed “Methods Update Rule” (MUR). Once EPA promulgates final rules, it codifies the approved methods at 40 CFR Part 136. These approved methods must be used for determining compliance with pollutant discharge limitations.

- Regulatory history - Methods Update Rules

For its MURs, EPA considers new or revised methods from two major sources. One is the Agency's Alternate Test Procedure (ATP) program. Under this program, method developers submit an application for a proposed new method or modification to an approved Part 136 method – an “alternative method” – directly to OST for evaluation for nationwide use. There are established, formal protocols for the ATP program that lay out specific requirements for submitting methods/modifications for consideration.

## VCSB Methods

The second major source for new or revised methods is those methods that are adopted by a **voluntary consensus standards body** (VCSB) such as ASTM International and *Standard Methods*, or another government agency such as the United States Geological Survey. VCSBs may submit methods and modifications to OST under the provisions of the National Technology Transfer and Advancement Act (NTTAA). The NTTAA requires EPA to adopt methods approved by VCSBs, unless doing so would be inconsistent with applicable laws or is otherwise impractical. When VCSBs or other government agencies submit adopted methods for consideration, they must include the method in its final form, documentation that it has been approved/published by that VCSB or agency, the validation study plan, and the validation study report, including data and analysis that supported the method's development and adoption. The VCSB or agency must comply with its own internal method testing criteria (e.g., ASTM D2777).

LAST UPDATED ON JUNE 12, 2019

# Exhibit 53

\* \* \* \* \*

[FR Doc. 2014-19557 Filed 8-18-14; 8:45 am]

BILLING CODE 6560-50-P

**ENVIRONMENTAL PROTECTION AGENCY**

**40 CFR Parts 122 and 136**

[EPA-HQ-OW-2009-1019; FRL-9915-18-OW]

RIN 2040-AC84

**National Pollutant Discharge Elimination System (NPDES): Use of Sufficiently Sensitive Test Methods for Permit Applications and Reporting**

**AGENCY:** Environmental Protection Agency.

**ACTION:** Final rule.

**SUMMARY:** The Environmental Protection Agency (EPA) is finalizing minor amendments to its Clean Water Act (CWA) regulations to codify that under the National Pollutant Discharge Elimination System (NPDES) program, permit applicants must use “sufficiently sensitive” analytical test methods when completing an NPDES permit application and the Director must prescribe that only “sufficiently sensitive” methods be used for analyses of pollutants or pollutant parameters under an NPDES permit.

The final rule is based on requirements in the CWA and clarifies existing EPA regulations. It also codifies existing EPA guidance on the use of “sufficiently sensitive” analytical methods with respect to measurement of mercury and extends the approach outlined in that guidance to the NPDES program more generally. Specifically, EPA is modifying existing NPDES application, compliance monitoring, and analytical methods regulations. The amendments in this rulemaking affect only chemical-specific methods; they do not apply to the Whole Effluent Toxicity (WET) methods or their use.

**DATES:** These final regulations are effective September 18, 2014. For judicial review purposes, this final rule is promulgated as of 1:00 p.m. Eastern Time, on September 2, 2014, as provided in 40 CFR 23.2.

**ADDRESSES:** The record for this rulemaking is available for inspection and copying at the Water Docket, located at the EPA Docket Center (EPA/DC), EPA West 1301 Constitution Ave. NW., Washington, DC 20004. The record is also available via EPA Dockets at <http://www.regulations.gov> under docket number EPA-HQ-OW-2009-1019. The rule and key supporting

documents are also available electronically on the Internet at <http://cfpub.epa.gov/npdes/ssmethods.cfm>.

**Docket:** All documents in the docket are listed in the [www.regulations.gov](http://www.regulations.gov) index. Some information, however, is not publicly available, e.g., confidential business information (“CBI”) or other information whose disclosure is restricted by statute. Certain other material, such as copyrighted material, is publicly available only in hard copy. Publicly available docket materials are available electronically in [www.regulations.gov](http://www.regulations.gov) or in hard copy at the Water Docket, EPA Docket Center, EPA West, Room 3334, 1301 Constitution Avenue NW., Washington, DC. The Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566-1744, and the telephone number for the Water Docket is (202) 566-2426.

**FOR FURTHER INFORMATION CONTACT:** For additional information, contact Kathryn Kelley, Water Permits Division, Office of Wastewater Management (4203M), Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460; telephone number: (202) 564-7004, email address: [kelly.kathryn@epa.gov](mailto:kelly.kathryn@epa.gov).

**SUPPLEMENTARY INFORMATION:**

- I. General Information
  - A. Potentially Affected Parties
  - B. Legal Authority
- II. Background
- III. Summary of Public Comments and EPA’s Response
- IV. The Final Rule
- V. Impacts
- VI. Compliance Dates
- VII. Statutory and Executive Order Reviews
  - A. Executive Order 12866: Regulatory Planning and Review
  - B. Paperwork Reduction Act
  - C. Regulatory Flexibility Act
  - D. Unfunded Mandates Reform Act
  - E. Executive Order 13132: Federalism
  - F. Executive Order 13175: Consultation and Coordination With Indian Tribal Governments
  - G. Executive Order 13045: Protection of Children From Environmental Health and Safety Risks
  - H. Executive Order 13211: Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use
  - I. National Technology Transfer and Advancement Act
  - J. Executive Order 12898: Federal Actions To Address Environmental Justice in Minority Populations and Low-Income Populations

**I. General Information**

**A. Potentially Affected Parties**

In the NPDES program, point source dischargers obtain permits that are issued by EPA regions and authorized NPDES States, Territories, and Indian tribes (collectively referred to as “permitting authorities”). These point source dischargers include publicly owned treatment works (POTWs) and various industrial and commercial facilities (collectively referred to as “NPDES applicants or permittees”). Permitting authorities issue NPDES individual permits after analyzing the information contained in the application and making a determination that the application is “complete” under 40 CFR 122.21(e). In the case of a general permit, authorization to be covered by the permit is given if the information submitted demonstrates eligibility for coverage under 40 CFR 122.28. The NPDES permit prescribes the conditions under which the facility is allowed to discharge pollutants into waters of the United States and the conditions that will ensure the facility’s compliance with the CWA’s technology-based and water quality-based requirements. NPDES permits typically include restrictions on the mass and/or concentration of pollutants<sup>1</sup> that a permittee may discharge as well as requirements that the permittee conduct routine sampling and reporting of various parameters measured in the permitted discharge. In general, NPDES applicants and permittees are required to use EPA-approved methods<sup>2</sup> when measuring the pollutants in their discharges.

The purpose of today’s final rule is to codify that where EPA-approved methods exist, NPDES applicants must use sufficiently sensitive EPA-approved analytical methods when quantifying the presence of pollutants in a

<sup>1</sup> Where the term “pollutant” is used, it refers to both pollutants and pollutant parameters.

<sup>2</sup> For purposes of this rule, the term “EPA-approved methods” refers to methods that have been approved under 40 CFR part 136 or are required under 40 CFR chapter I, subchapter N or O. This includes analytical methods for CWA pollutants developed by EPA, voluntary consensus standards bodies (VCSBs), and other government agencies (such as the U.S. Geological Survey), as well as Alternate Test Procedures (ATPs) developed by commercial method developers for nation-wide use. These methods have been reviewed by EPA and approved for use in compliance monitoring under the CWA. EPA publishes lists of the EPA, VCSB, and other agency methods as well as ATPs that it has found to be acceptable for such use at 40 CFR Part 136, and at 40 CFR Chapter I, subchapters N and O. As a point of clarification, this includes approved ATPs as described in 40 CFR 136.4 and 136.5.

discharge, and the Director<sup>3</sup> must prescribe that only sufficiently sensitive EPA-approved methods be used for analyses of pollutants or pollutant parameters under the permit. The broad universe of entities<sup>4</sup> that would be affected by this final action includes

NPDES permitting authorities and municipal and industrial applicants and permittees (Table I–1). This rule does not apply to *indirect dischargers* as defined in 40 CFR 122.2. The impact of this action, however, would only affect those entities that use or allow the use

of any EPA-approved analytical methods (for one or more parameters) that are not “sufficiently sensitive” to detect pollutants being measured in the discharge.

TABLE I–1—ENTITIES POTENTIALLY REGULATED BY THIS RULE

Category	Examples of potentially affected entities
State, Territorial, and Indian Tribal Governments.	States, Territories, and Indian tribes authorized to administer the NPDES permitting program; States, Territories, and Indian tribes that provide certification under section 401 of the CWA.
Municipalities .....	POTWs required to apply for or seek coverage under an NPDES individual or general permit and to perform routine monitoring as a condition of any issued NPDES permit.
Industry .....	Facilities required to apply for or seek coverage under an NPDES individual or general permit and to perform routine monitoring as a condition of any issued NPDES permit.

If you have any questions regarding the applicability of this action to a particular entity, consult the person listed under **FOR FURTHER INFORMATION CONTACT**.

*B. Legal Authority*

EPA is issuing today’s final rule pursuant to the authority of sections 301, 304(h), 308, 402(a), and 501(a) of the CWA [33 U.S.C. 1311, 1314(h), 1316, 1318, 1342(a), 1343, and 1361(a)]. Section 301(a) of the CWA prohibits the discharge of any pollutant except in compliance with an NPDES permit issued under section 402 of the act. Section 402(a) of the CWA authorizes the Administrator to issue permits that require a discharger to meet all the applicable requirements under sections 301, 302, 306, 307, 308, and 403. Section 301(b) of the CWA further requires that NPDES permits include effluent limitations that implement technology-based standards and, where necessary, water quality-based effluent limitations (WQBELs) that are as stringent as necessary to meet water quality standards. With respect to the protection of water quality, NPDES permits must include limitations to control all pollutants that the NPDES permitting authority determines are or might be discharged at a level that “will cause, or contribute to an excursion above any state water quality standard,” including both narrative and numeric criteria [40 CFR 122.44(d)(1)(i)]. If the Director determines that a discharge causes, has the reasonable potential to cause, or contributes to such an excursion, the permit must contain WQBELs for the pollutant [40 CFR

122.44(d)(1)(iii)]. Section 402(a)(2) of the CWA requires EPA to prescribe permit conditions to ensure compliance with requirements, “. . . including conditions on data and information collection, reporting and such other requirements as [the Administrator] deems appropriate.” Thus, a prospective permittee might need to measure various pollutants in its effluent at two stages: First, at the permit application stage so that the Director can determine what pollutants are present in the applicant’s discharge and the amount of each pollutant present and, second, to quantify the levels of each pollutant limited in the permit to determine whether the discharge is in compliance with the applicable limits and conditions.

Section 304(h) of the CWA requires the Administrator of EPA to “. . . promulgate guidelines establishing test procedures for the analysis of pollutants that shall include the factors which must be provided in any certification pursuant to [section 401 of this Act] or permit application pursuant to [section 402 of this Act].” Section 501(a) of the act authorizes the Administrator to “. . . prescribe such regulations as are necessary to carry out this function under [the act].” EPA generally has codified its test procedure regulations (including analysis and sampling requirements) for CWA programs at 40 CFR part 136, although some requirements are codified in other parts (e.g., 40 CFR chapter I, subchapters N and O).

The Director is required under 40 CFR 122.21(e) to determine when an NPDES permit application is complete. Moreover, the Director shall not begin

processing an application for an individual permit until the applicant has fully complied with the application requirements for that permit [40 CFR 124.3(a)(2)]. Under 40 CFR 122.21(g)(13), applicants are required to provide to the Director, upon request, such other information as the Director may reasonably require to assess the discharge. Finally, 40 CFR 122.41(j)(1) requires NPDES permits to include a standard condition specifying that “samples and measurements taken for the purpose of monitoring shall be representative of the monitored activity.”

Among other things, section 308 of the CWA authorizes EPA to require owners or operators of point sources to establish records, conduct monitoring activities, and make reports to enable the permitting authority to determine whether there is a violation of any prohibition or any requirement established under provisions including section 402 of the CWA. Under sections 308(c) and 402(b)(2)(A), a state’s authorized NPDES program must have authorities to inspect, monitor, enter, and require reports to at least the same extent as required in section 308.

As summarized above, the legal requirements and authorities exist for EPA to require NPDES applicants and permittees to use sufficiently sensitive EPA-approved analytical methods when quantifying the presence of pollutants in a discharge and to require the Director to require and accept only such data.

**II. Background**

Multiple analytical test methods exist for many pollutants regulated under the CWA. Therefore, EPA has generally

<sup>3</sup> The term “Director” refers to the permitting authority. See definition at 40 CFR 122.2.

<sup>4</sup> Although terms such as “authorities,” “applicants,” and “permittees” imply individuals,

EPA uses these terms to refer to entities. For example, EPA uses the term “NPDES permitting authorities” to mean the EPA Regions, States, Territories, and Indian tribes granted authority to implement and manage the NPDES program. EPA

uses the term “NPDES applicants” or “NPDES permittees” to mean facilities that have applied for, sought coverage under, or been issued an NPDES individual or general permit.

approved multiple methods for CWA pollutants under 40 CFR part 136 and 40 CFR chapter I, subchapters N and O. Some of the approved analytical test methods have greater sensitivities and lower minimum levels<sup>5</sup> or method detection limits (MDLs)<sup>7</sup> than other approved methods for the same pollutant. This situation often occurs because of advances made in instrumentation and in the analytical protocols themselves. Many metals and toxic compounds (for example, mercury) have an array of EPA-approved methods, including some methods that have greater sensitivities and lower minimum levels than the others.

Although EPA has approved multiple analytical methods for individual pollutants, the Agency has historically expected that applicants would select from the array of available methods a specific analytical method that is sufficiently sensitive to quantify the presence of a pollutant in a given discharge. EPA has not expected that NPDES permit applicants would select a method with insufficient sensitivity, thereby masking the presence of a pollutant in their discharge, when an EPA-approved sufficiently sensitive method is available. Further, EPA anticipated that NPDES permitting authorities would specify an EPA-approved method in an NPDES permit where the Director determined that a particular analytical method was needed to provide meaningful results relative to the permit limit. EPA believes that the authority to prescribe a specific analytical method in an NPDES permit exists under the current

<sup>5</sup> The term “minimum level” refers to either the sample concentration equivalent to the lowest calibration point in a method or a multiple of the method detection limit (MDL). Minimum levels may be obtained in several ways: They may be published in a method; they may be sample concentrations equivalent to the lowest acceptable calibration point used by a laboratory; or they may be calculated by multiplying the MDL in a method, or the MDL determined by a lab, by a factor. [See: (A) 40 CFR 136, appendix A, footnotes to table 2 of EPA Method 1624 and table 3 of EPA Method 1625 (49 FR 43234, October 26, 1984); (B) 40 CFR 136, section 17.12 of EPA Method 1631E (67 FR 65876–65888, October 29, 2002); (C) 61 FR 21, January 31, 1996; and (D) “Analytical Method Guidance for the Pharmaceutical Manufacturing Point Source Category,” EPA 821–B–99–003, August 1999].

<sup>6</sup> For the purposes of this rulemaking, EPA is considering the following terms related to analytical method sensitivity to be synonymous: “quantitation limit,” “reporting limit,” “level of quantitation,” and “minimum level.”

<sup>7</sup> The MDL is determined using the procedure at 40 CFR Part 136, appendix B. It is defined as the minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

regulations. However, some state permitting authorities expressed concern that this authority was not explicit in current regulations, thus limiting states’ ability to prescribe an appropriate analytical method where needed to assess compliance with permit limits. This rule requires that, where EPA-approved methods exist, NPDES applicants must use sufficiently sensitive EPA-approved analytical methods when quantifying the presence of pollutants in a discharge and that the Director must prescribe that only sufficiently sensitive EPA-approved methods be used for analyses of pollutants or pollutant parameters under the permit.

EPA and state permitting authorities use data from the permit application to determine whether pollutants are present in an applicant’s discharge and to quantify the levels of all detected pollutants. These pollutant data are then used to determine whether technology- or water quality-based effluent limits are needed in the facility’s NPDES permit. It is critical, therefore, that applicants provide data that have been measured at levels that will be meaningful to the decision-making process. Among other things, data must be provided that will enable the Director to make a sound “reasonable potential” determination and, if necessary, establish appropriate water quality-based permit limits. The same holds true for monitoring and reporting relative to permit limits established for regulated parameters. The intent is for applicants and permittees to use analytical methods that are capable of detecting and measuring the pollutants at, or below, the respective water quality criteria or permit limits.<sup>8</sup>

For example, in 2002 and 2007 EPA published two new analytical methods for mercury that were several orders of magnitude more sensitive than previously available methods. In addition, a number of states have set water quality criteria for mercury that are below the detection levels of the older methods for mercury that EPA approved prior to 2002. Unlike the previous methods, the new methods are capable of measuring whether effluent samples are above or below the current water quality criteria. In 2007 EPA addressed this issue with respect to mercury in a memorandum titled “Analytical Methods for Mercury in NPDES Permits,” from James A. Hanlon, Director of EPA’s Office of Wastewater

<sup>8</sup> To address this situation some state permitting authorities have developed a list of monitored parameters and prescribed a required minimum level that must be achieved for each parameter as a part of their state regulations or policy.

Management, to the Regional Water Division Directors. This memorandum is available at [http://www.epa.gov/npdes/pubs/mercurymemo\\_analyticalmethods.pdf](http://www.epa.gov/npdes/pubs/mercurymemo_analyticalmethods.pdf). The memorandum explains EPA’s expectation that “All facilities with the potential to discharge mercury will provide with their NPDES permit applications monitoring data for mercury using Method 1631E or another sufficiently sensitive EPA-approved method. Accordingly, EPA strongly recommends that the permitting authority determine that a permit application that lacks effluent data analyzed with a sufficiently sensitive EPA-approved method such as Method 1631E, is incomplete unless and until the facility supplements the original application with data analyzed with such a method.”

Following issuance of the 2007 memorandum, EPA determined that the NPDES permit application regulations at 40 CFR 122.21 and the NPDES permit monitoring requirements at 40 CFR 122.44 should be revised to ensure that, where EPA-approved methods exist, applicants use sufficiently sensitive EPA-approved analytical methods when quantifying the presence of pollutants in a discharge and that Directors prescribe that only sufficiently sensitive EPA-approved methods be used to perform sampling and analysis for all pollutants, not just mercury. Therefore, in this rulemaking, EPA is revising the regulations to extend the requirement to use sufficiently sensitive EPA-approved analytical test methods, where they exist, to all pollutants and establish criteria for what qualifies as a “sufficiently sensitive” method.

This final rule requires that NPDES applicants must use sufficiently sensitive EPA-approved analytical methods, where they exist, when submitting information required by a permit application quantifying the presence of pollutants in a discharge. If the applicant does not provide data using a sufficiently sensitive EPA-approved analytical method, the Director may determine that the application is “incomplete” per 40 CFR 122.21(e). The Director may require that the applicant provide new screening data obtained using a sufficiently sensitive EPA-approved analytical method before making a completeness determination and moving forward with permit development. The final rule also requires that, as a condition of permit development, to assure compliance with permit limitations the permit shall include requirements to monitor according to sufficiently sensitive EPA-approved methods, where they exist.

Specifically, where an EPA-approved analytical method exists that would provide quantifiable results necessary to assess compliance with a permit limit and the permit allows monitoring to be conducted using different analytical methods that, although approved, would fail to produce data necessary to assess compliance, the permit would be inconsistent with the NPDES permitting requirements of 40 CFR 122.44(i).

EPA is defining the term “sufficiently sensitive” in two sections of the NPDES regulations: At 40 CFR 122.21(e) (Completeness), as a new subsection (3), and at 40 CFR 122.44(i)(1)(iv) (Monitoring Requirements). EPA is also modifying 40 CFR 136.1 (Applicability) by adding a new paragraph (c), which is simply a cross-reference to the changes being promulgated in 40 CFR 122.21(e)(3) and 40 CFR 122.44(i)(1)(iv). The new and revised sections indicate that an EPA-approved method is sufficiently sensitive where:

A. The method minimum level is at or below the level of the applicable water quality criterion or permit limitation for the measured pollutant or pollutant parameter; or

B. In the case of permit applications, the method minimum level is above the applicable water quality criterion, but the amount of the pollutant or pollutant parameter in a facility’s discharge is high enough that the method detects and quantifies the level of the pollutant or pollutant parameter in the discharge; or

C. The method has the lowest minimum level of the EPA-approved analytical methods.

The requirement to use a “sufficiently sensitive” EPA-approved method does not apply where no EPA-approved method exists. When no analytical method is approved under 40 CFR part 136 or required under subchapter N or O, and a specific method is not otherwise required by the Director, an NPDES applicant may use any suitable method; however, the applicant shall provide a description of the method.

The first two criteria, A and B, in the sufficiently sensitive definition address situations in which EPA has approved multiple methods for a pollutant and some of those approved methods have greater sensitivities and lower minimum levels than others. In this situation, the applicant or permitting authority may select a method based on the minimum level published in the EPA-approved method, where available, or using a derived minimum level. As noted in footnote 4, the minimum level may be explicitly listed in some EPA-approved methods. Where this is the case, the

applicant may reference the published minimum level when determining whether a method selected to provide data for their permit application is sufficiently sensitive. Where EPA has included a minimum level for a pollutant in a specific method, it reflects the minimum level obtained in a multi-laboratory study of the new method in a wide variety of matrices, many of which EPA selects due to their complex nature. EPA acknowledges that complex matrices exist and provides flexibility and suggestions for ways to mitigate interferences in such instances, often within the published method for a specific pollutant. EPA’s experience is that many laboratories find solutions to address difficult matrices and are able to achieve the published minimum level within the required quality assurance specifications. However, applicants have always had the option of calculating a matrix-specific method detection limit (MDL). Extreme matrices may necessitate the use of an elevated sample specific minimum level, in which case the laboratory should be able to show that a reasonable effort (e.g., published cleanup procedures) was attempted to achieve as low a minimum level as possible for those samples. The use of sample or matrix specific minimum levels rather than the published levels has always been an available option, and consistent with that flexibility, use of a matrix-specific minimum level may sometimes be necessary when determining which methods are sufficiently sensitive.

For EPA-approved methods that do not explicitly list minimum levels, the applicant can derive the minimum level from either the concentration of the lowest calibration standard in methods that dictate the concentrations of such standards, or as a multiple of the MDL or similar statistically derived detection limit concept. When the method dictates, or recommends, the concentration of the lowest calibration standard, that concentration can be converted to a minimum level by considering the weights and/or volumes of the sample and all of the intermediate preparation and analysis steps in the method. If a method provides a literature MDL for the matrix of interest, that MDL value can be used to estimate the minimum level as 10 times the standard deviation of the replicate measurements used to determine the MDL according to 40 CFR part 136, appendix B. However, MDLs are inherently method- and laboratory-specific, so whenever a permittee is contracting a laboratory for NPDES work, it is prudent to obtain that

laboratory’s MDL and compare it to the published MDL to ensure that both their MDL and their minimum level are appropriate for the intended application.

The third criterion, C, of the definition addresses situations in which none of the EPA-approved methods for a pollutant can achieve the minimum levels necessary to assess reasonable potential or to monitor compliance with a permit limit. In these situations, applicants or permittees must use the method with the lowest minimum level among the EPA-approved methods for the pollutant, and this method would meet the definition of sufficiently sensitive.

As explained above, the requirement to use a “sufficiently sensitive” EPA-approved method does not apply where no EPA-approved methods exist. The final rule addresses these situations, for permit applicants, where no approved analytical method exists under 40 CFR part 136 or is required under subchapter N or O, and one is not otherwise required by the Director. In such situations, an applicant may use any suitable method but shall provide a description of the method. With respect to pollutant limits in permits, where an EPA-approved analytical method does not exist, monitoring shall be conducted in accordance with a test procedure specified in the permit.

EPA recognizes that other factors beyond the minimum level or MDL can also be important in determining method performance, including a method’s resolution, accuracy, and precision. Where there are no EPA-approved methods, this rule does not affect how those other factors are considered in selecting a method. Rather, the rule notes that permit applicants may consider these other factors when selecting a suitable method where no EPA-approved method exists.

For EPA-approved methods, however, these factors have already been considered during the method validation and approval process. As explained above, EPA evaluates method performance in a wide variety of wastewater matrices and approves those methods that have sensitivity, precision and accuracy that are appropriate for wastewater compliance monitoring. 40 CFR 136.6 also allows flexibility to tailor approved methods to more challenging wastewater matrices or overcome methodological problems. Based on data and information provided to EPA by analytical laboratories, EPA finds that experienced laboratories are often capable of achieving minimum levels below those published with a

method while maintaining the precision and accuracy specified in the method.

EPA acknowledges that while rare, methodological problems may exist that could affect the determination of a “sufficiently sensitive” method. In such rare situations, the Director may consider additional technical factors when determining whether the method is still “sufficiently sensitive.” Specifically, where the permit applicant or permittees can demonstrate to the Director that despite a good faith effort to overcome these methodological problems due to challenging wastewater matrices, either (1) the method’s minimum level is higher than originally anticipated, or (2) the method results no longer meet the methods quality assurance/quality control (“QA/QC”) specification, the Director may take these factors into account when determining whether the permit applicant has met the requirements to use a “sufficiently sensitive” method or in prescribing a “sufficiently sensitive” method in the permit. In the first situation, the matrix or sample-specific minimum level should be used to evaluate which of the EPA-approved methods is “sufficiently sensitive.” In the second situation, if the method’s results are no longer consistent with the QA/QC specifications, then the method is not performing adequately and a “sufficiently sensitive” method should be selected from the remaining EPA-approved methods. In either case, the permit applicant or permittee is responsible for demonstrating that a published minimum level is unachievable or a reasonable effort was applied to bring the original sufficiently sensitive method within the QA/QC specifications in the given matrix before selecting another EPA-approved method (e.g., cleanup procedures, dilution when appropriate, etc.).

Additionally, where a technology-based requirement is specified as “zero discharge” or “no detect,” the permitting authority may take into account the sensitivity of the method used to establish the requirement when determining if a method is “sufficiently sensitive.” EPA recognizes that if a more sensitive method is approved after such a requirement has been established, its use may be inconsistent with the technological basis of the original requirement. In situations where a technology-based requirement reflects a technology that eliminates the discharge of the subject pollutant altogether, the newer sensitive method is appropriate. However, where a technology-based limit reflects a technology that may not achieve the minimum level of the newer more sensitive method, the Director may

determine that the method on which the requirement was originally based is “sufficiently sensitive” to determine compliance, as understood at the time the requirement was established.

For both EPA-approved methods and non-EPA-approved methods, EPA’s understanding of standard practice is that if an applicant/permittee or laboratory has questions regarding the suitability of a specific method in a given situation, or has technical questions on its use, it will consult with its permitting authority. EPA has the same expectations in connection with today’s rulemaking for questions specifically about which methods are sufficiently sensitive. The permitting authority continues to have the ultimate responsibility for determining whether an NPDES application is complete (40 CFR 122.21(e)) and establishing permit conditions, including monitoring and reporting requirements (40 CFR 122.44(i)).

The amendments in this rulemaking affect only chemical-specific methods; they do not apply to the Whole Effluent Toxicity (WET) methods or their use. Note that existing EPA regulations (40 CFR 122.44(d)(1)(ii)) and policy require permit writers to take into account the sensitivity of the species to toxicity testing when evaluating whole effluent toxicity. EPA has interpreted this provision as directing the permitting authority to develop criteria and limits based upon the most sensitive test species to ensure that the most sensitive species and all less sensitive species will be protected.

### III. Summary of Public Comments and EPA’s Response

On June 23, 2010, EPA proposed changes to the existing NPDES regulations (75 FR 35712) and requested comments from the public. EPA received 25 comment letters. The majority of the comments came from publicly owned treatment works and industry organizations, but EPA also received comments from laboratories, and state and federal agencies. The majority of comments covered the following categories: Implementation and technology; administration and timing; and burden. The complete list of comments and responses is available in the record of this rulemaking.

#### A. Implementation

##### 1. Effect of the Rule on Current Practices

EPA received several comments that indicated the approach outlined in the proposed rule would force applicants and permittees to make decisions regarding the selection of an appropriate

method without adequate information upon which to base a decision. Specifically, commenters indicated that issues related to the definition of the method minimum level would make this rule difficult to implement and that method sensitivity should not be the sole factor in deciding which method should be used in the permitting process. They indicated that there are other factors including accuracy, precision, selectivity, and whether the method has been validated that should be considered.

In response, EPA notes that applicants for NPDES permits have always needed to make decisions regarding which EPA-approved methods are the most appropriate for use when performing the screening analyses required under the various permit application regulations at 40 CFR 122.21. Similarly, NPDES permitting authorities, even before today’s rulemaking, have had to consider which of the EPA-approved methods are the most appropriate for permittees to use to meet their monitoring and reporting requirements under an NPDES permit. Today’s rule does not change the basic NPDES permit application or permit issuance process. Under 40 CFR 122.21, permittees seeking permit renewal or new applicants must provide the Director with adequate information to determine whether an NPDES application is complete. Once the Director makes this determination, the Director determines the applicable permit requirements, including any sampling or monitoring that must be taken that is “representative of the monitored activity.” See 40 CFR 122.41(j)(1). The effect of today’s final rulemaking is to codify that where EPA-approved methods exist, only “sufficiently sensitive” EPA-approved methods may be used in connection with permit applications and to conduct monitoring and reporting under a permit.

To determine whether an EPA-approved analytical method is “sufficiently sensitive” in any particular case, NPDES applicants/permittees and permit authorities should use the best information available on what the minimum level is for the method, and EPA believes that in general a method’s accurate minimum level will be readily ascertainable. Where the minimum level is explicitly listed in the EPA-approved method, applicants may reference the published minimum level when determining whether a method selected to provide data for their permit application is sufficiently sensitive. Alternatively, applicants have always had the option of providing matrix-specific method detection limits and

minimum levels rather than the published minimum levels, and nothing in today's rule changes that flexibility, including with respect to selecting a sufficiently sensitive EPA-approved method. For these cases the laboratory should be able to show that a reasonable effort (e.g., published cleanup procedures) was attempted to achieve as low a minimum level as possible for those samples. For EPA-approved methods that do *not* explicitly list minimum levels, the minimum level can be obtained or derived by the applicant or permitting authority. Indeed, many permitting authorities have developed guidance, policies or regulations that establish minimum levels for various methods, or specify specific methods to be used by applicants and permittees. Where applicable, these policies and regulations will continue to affect method selection, although at the same time, states must ensure that such policies and regulations conform with the criteria established in today's rulemaking that, where they exist, only "sufficiently sensitive" EPA-approved methods are being used when completing an NPDES permit application and when performing sampling and analysis pursuant to monitoring requirements in an NPDES permit. If the applicant does not provide data using a sufficiently sensitive EPA-approved analytical method where one exists, the Director may determine that the application is "incomplete" per 40 CFR 122.21(e). The Director may require that the applicant provide new screening data obtained using a sufficiently sensitive EPA-approved analytical method before making a completeness determination and moving forward with permit development. Thus, to avoid having the permitting authority reject data provided in an application because the data were not collected by means of a "sufficiently sensitive" method, the NPDES applicant should work closely with the permitting authority prior to conducting the required analyses. In addition, the permitting authority must ensure the permit includes a requirement to use a sufficiently sensitive EPA-approved analytical test method, where one exists, where necessary to perform sampling and analysis, consistent with 40 CFR 122.41(j) and 122.44(i).

## 2. Development of New or Alternate Test Procedures

EPA received several comments that indicated the proposed rule would require the development of new analytical methods where no EPA-

approved methods exist or where existing EPA-approved methods would not quantify the pollutant concentration at or below the level of the criterion or permit limit. Other commenters indicated that the rule would alter the existing requirements for developing Alternate Test Procedures under 40 CFR part 136. EPA has modified the proposal to address these comments, as explained below.

EPA has modified the proposed language for this final rule so that it does not change existing regulatory requirements with respect to unapproved methods. Where no EPA-approved analytical methods exist, an applicant will need to select a method from another source of available analytical methods (e.g., Standard Methods for the Examination of Water and Wastewater) to measure that pollutant or pollutant parameter. Today's final rule does not require the applicant to develop new methods. The situation in which there are no EPA-approved methods is uncommon because there are EPA-approved methods for most pollutants or pollutant parameters screened and regulated under the NPDES program. Under the existing regulations at 40 CFR 122.21(g)(7), the NPDES applicant has the flexibility to use any suitable analytical method when no EPA-approved analytical method exists for that pollutant or pollutant parameter. Additionally, under the existing regulations at 40 CFR 122.44(i)(1)(iv), the NPDES permitting authority specifies a method in the permit when there is no EPA-approved method.

Where EPA-approved methods exist, but none of the available methods will quantify the pollutant concentration at or below the level of the criterion or permit limit, today's rulemaking does not require the development of any new analytical methods. However, in this situation, the rule will now require the use of the most sensitive of the EPA-approved methods.

Finally, today's rulemaking does not alter any of the existing requirements related to the development or approval of alternative test procedures under 40 CFR 136.4 and 136.5.

## 3. Consideration of Matrix Effects in Selecting a Sufficiently Sensitive Method

EPA received several comments that indicated the approach outlined in the proposed rule would force applicants and permittees to make decisions regarding the selection of an appropriate method without adequate information upon which to base a decision. Specifically, commenters indicated that

issues related to the definition of the method minimum level would make this rule difficult to implement and that method sensitivity should not be the sole factor in deciding which method should be used in the permit process. They believe there are other critical factors including accuracy, precision, selectivity, and whether the method has been validated.

In response, as noted above, EPA has clarified that the requirement to use a "sufficiently sensitive" EPA-approved method does not apply where no EPA-approved method exists. EPA agrees that other factors beyond the minimum level can also be important in determining method performance, including a method's selectivity, resolution, accuracy, and precision. EPA has added language in the rule text that clarifies where no EPA-approved methods exist, permit applicants may consider these other factors, in conjunction with sensitivity, when selecting an appropriate method.

For EPA-approved methods, however, these factors have already been considered during the method validation and approval process. As explained above, EPA evaluates method performance in a wide variety of wastewater matrices and approves those methods that have selectivity, sensitivity, precision and accuracy that are appropriate for wastewater compliance monitoring. 40 CFR 136.6 also allows flexibility to tailor approved methods to more challenging wastewater matrices. EPA notes that applicants have always had the option of providing matrix or sample-specific minimum levels rather than the published levels and nothing in today's rule changes that flexibility, including with respect to selecting a sufficiently sensitive EPA-approved method. For these cases the laboratory should be able to show that a reasonable effort (e.g., published cleanup procedures) was attempted to achieve as low a minimum level as possible for those samples.

If the most sensitive method listed in 40 CFR Part 136 is not performing adequately in a given wastewater matrix (e.g., with regard to sensitivity, accuracy, and precision), several options are available and should be pursued. Dilution is often a good option if it does not drive the sample specific minimum level above the permit requirements. Cleanup procedures included in the method can also be utilized. If those cleanups do not prove adequate for a particular matrix, the analyst should consult "Solutions to Analytical Chemistry Problems with Clean Water Act Methods," EPA 821-R-07-002 (or more recent revisions) to

determine if another cleanup procedure may be appropriate. If a solution is still not apparent, the permittee should consult EPA or the permitting authority.

Based on data and information provided to EPA by analytical laboratories, EPA finds that experienced laboratories are often capable of achieving minimum levels below those published with a method while maintaining the precision and accuracy specified in the method. However, EPA acknowledges that while rare, situations may exist where a method cannot perform adequately in a specific matrix. In such rare situations, the Director may consider additional technical factors when determining whether the method is still "sufficiently sensitive." Specifically, where the permit applicant or permittees can demonstrate to the Director that despite a good faith effort to overcome these methodological problems due to challenging wastewater matrices, either (1) the method's minimum level is higher than originally anticipated, or (2) the method results no longer meet the methods QA/QC specification, the Director may take these factors into account when determining whether the permit applicant has met the requirements to use a "sufficiently sensitive" method or in prescribing a "sufficiently sensitive" method in the permit. In the first situation, the matrix or sample-specific minimum level should be used to evaluate which EPA-approved method is "sufficiently sensitive." In the second situation, if the method's results are no longer consistent with the QA/QC specifications, then the method is not performing adequately and a "sufficiently sensitive" method should be selected from the remaining EPA-approved methods. In either case, the permit applicant or permittee is responsible for demonstrating that a published minimum level is unachievable or a reasonable effort was applied to bring the original sufficiently sensitive method within the QA/QC specifications in the given matrix before selecting another EPA-approved method (e.g., cleanup procedures, dilution when appropriate, etc.). To illustrate the type of situations where this provision would be appropriate, EPA provides two examples below.

EPA received comments about the situation where there are multiple EPA-approved methods for an organic pollutant and the methods employ different technologies (i.e., gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS)). These commenters raised concern that, in some instances, while the GC method may provide a lower

detection limit, the GC/MS method provides a greater degree of confidence in the correct identification of the regulated parameter. As explained above, this is not an issue if the laboratory has demonstrated that it can achieve a minimum level for GC/MS that is lower than the NPDES permit limit for the regulated parameter, in which case GC/MS would be considered "sufficiently sensitive." EPA agrees that GC/MS is more selective than GC, but several options are available to remove the interferences from difficult matrices before using a dual-column GC method (e.g., solid-phase extraction as a cleanup procedure, Florisil cleanup, alumina cleanup, sulfur removal with copper or TBA sulfite, gel permeation chromatography, etc.). Generally, a result from a dual-column GC method would only be questioned if the chromatograms from the two columns did not yield similar numerical results or if the chromatograms contained many extraneous peaks that suggest interferences are present. If the permit applicant or permittee is still concerned that the peaks may be caused by a different contaminant, and the GC method provides a false positive result, the permit applicant or permittee could use a GC/MS to confirm the presence of the contaminant. However, since the GC/MS is less sensitive, it may not be able to confirm low-level dual column GC results. The more sensitive GC/MS method options (e.g., larger sample volume, smaller final extract volume, selected ion monitoring techniques, or high resolution GC/MS) may be necessary to prove whether the dual column GC result is a false positive. The permittee should also consult with EPA and/or its permitting authority for potential solutions. In this case, if the permittee has exhausted all practical options (e.g., solid-phase extraction as a cleanup procedure, Florisil cleanup, alumina cleanup, sulfur removal with copper or TBA sulfite, gel permeation chromatography, etc.) and has documentation to demonstrate that the dual-column GC creates false positive results for that specific matrix, then the Director would appropriately approve the selection of a different EPA-approved method that would then be considered a sufficiently sensitive method (e.g., GC/MS).

As another example, EPA also received comments specific to Method 1631 for mercury. These commenters noted that use of the "clean" sampling methods associated with this method to minimize potential contamination from the sampling technique itself is not possible in many industrial settings.

They noted that EPA's documentation of the sampling technique acknowledges it is not intended for treated and untreated discharges from industrial uses. EPA notes that since approval of this method and the associated clean sampling techniques, these techniques have been successfully used in some industrial settings. For example, sewage treatment plants accepting industrial wastewater have successfully eliminated permit exceedances for mercury as measured by Method 1631 by employing the clean sampling procedures. Where the permittee has documentation that clean sampling techniques cannot be adopted for the site-specific application, the Director would appropriately approve the selection of a different EPA-approved method that meets the definition of a sufficiently sensitive method (e.g., the one with the lowest minimum level of the remaining EPA-approved methods). If the ambient level of mercury contamination at the site is too high to use clean sampling methods, then using a less sensitive EPA-approved method can meet the definition of a sufficiently sensitive method.

Another commenter raised concerns specific to Method 1631. They questioned the method's suggestion to minimize laboratory contamination by soaking laboratory air filters in gold chloride solution so that mercury in incoming air will amalgamize with the filter's gold. This commenter questioned whether or not it was EPA's expectation that laboratories go to such lengths to employ such a sufficiently sensitive method where required under this rule. EPA notes the procedure described by the commenter is only a suggestion if laboratories are having problems with laboratory contamination. There are now many laboratories that perform Method 1631 without undue difficulty. In this case, where necessary to meet the definition of "sufficiently sensitive" in today's final rule, EPA would expect that the permittee use Method 1631, since the permittee should send their sample to a laboratory that can demonstrate it has control over sources of mercury within its own environment.

Finally, where a technology-based requirement is specified as "zero discharge" or "no detect," the permitting authority may take into account the sensitivity of the method used to establish the requirement when determining if a method is "sufficiently sensitive." EPA recognizes that if a more sensitive method is approved after such a requirement has been established, its use may be inconsistent with the technological basis of the original requirement. In situations where a

technology-based requirement reflects a technology that eliminates the discharge of the subject pollutant altogether, the newer sensitive method is appropriate. However, where a technology-based limit reflects a technology that may not achieve the minimum level of the newer more sensitive method, the Director may determine that the method on which the requirement was originally based is "sufficiently sensitive" to determine compliance, as understood at the time the requirement was established.

#### 4. Report of the Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs

EPA received a number of comments that identified concerns that the proposed rule uses terms, such as minimum level, that are not defined in new or existing regulations. Commenters also indicated that the proposed rule fails to address a variety of issues regarding detection and quantitation that were raised in the Report of the Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs. EPA agrees that there are a variety of related issues raised in the aforementioned report, yet notes that the members of the Federal Advisory Committee (FAC) were unable to reach consensus over several key issues in the report. While several of these issues, such as the definition of minimum level, are discussed in today's rulemaking, applicants and permitting authorities must still, on a regular and ongoing basis, choose which of the available analytical methods are most appropriate for use when screening effluent for permit applications and as part of permit conditions. This has always been the case, regardless of today's rulemaking.

EPA believes that the requirements of the rule are adequately described and can be implemented without having to address the myriad of issues considered by the FAC. For today's rulemaking, EPA is not redefining or establishing new method detection limits (MDLs) or minimum levels, developing new procedures for determining detection or quantitation, or maintaining a clearinghouse on detection and quantitation issues. EPA considers such issues to be outside the scope of today's rulemaking.

#### 5. Other Factors Affecting Selection of Analytical Methods

EPA received several comments that expressed concern that the rule would require the use of only the most sensitive available method, and that

other factors such as geographical isolation or unique sample collection constraints might preclude the use of certain available methods. Some comments also expressed concerns regarding the availability of laboratories qualified to conduct some of the more sensitive analytical methods, particularly where the state requires applicants and permittees to use laboratories certified by the state to conduct analyses.

EPA is not requiring the use of any specific analytical technology or practice over others; only that the selected EPA-approved method is sufficiently sensitive. EPA expects that, in general, factors such as geographical isolation, or unique sampling collection constraints would not preclude the selection of a sufficiently sensitive method. The definition does not require the use of the most sensitive EPA-approved method available, so long as a less sensitive approved method still meets the criteria for being "sufficiently sensitive." In cases where factors beyond a facility's control render the use of a particular method infeasible, such as extreme geographical isolation, the permitting authority could consider such factors in deciding which method best meets the definition of "sufficiently sensitive." EPA expects such situations would be rare.

Issues related to sampling procedures, such as holding times, are frequently prescribed by the test procedures in 40 CFR Part 136, and may be contingent on the unique physical, chemical, and biological characteristics of the discharge. Standard practice has been and continues to be that if an applicant/permittee or laboratory has questions regarding the appropriateness of using a specific method in a given situation, or has technical questions on its use, it should consult with its permitting authority prior to conducting monitoring.

#### B. Administration and Timing

EPA received a few comments regarding the effect of the rule on recordkeeping and reporting requirements. The rule does not change existing recordkeeping and reporting requirements at 40 CFR 122.21(p), 122.41(j) and 122.48. The permitting authority, however, has discretionary authority to require its applicants or permittees to provide information under the latter two provisions. In addition, a few comments asked whether the rule alters the terms or conditions of existing permits. The rule itself does not modify the terms or conditions of existing NPDES permits. If, under the requirements of today's rulemaking, a

change needs to occur in the analytical methods specified in an existing permit, that change would occur at the time of permit renewal, or it could occur through a permit modification under the procedures of 40 CFR Part 124, if the permitting authority determined that such a modification was appropriate.

EPA received a few comments regarding whether existing data, if collected using insufficiently sensitive methods, will be acceptable for submission with an application for permit renewal. NPDES application monitoring data that is collected after the effective date of the rule, or, if applicable, after an authorized state has revised its regulations to adopt the provisions of the rule,<sup>9</sup> must be based on the use of sufficiently sensitive test methods. However, the rule does not negate the existing requirement for applicants to submit data from previous years, even where these data may have been collected using methods that did not conform to the sufficiently sensitive criteria established in this rule. Based on all of the data submitted with the permit application, the permitting authority will determine whether it has information adequate to develop an NPDES permit. Where the permitting authority determines that data was collected using insufficiently sensitive methods, it may choose to disregard this information and accept only data collected employing sufficiently sensitive EPA-approved methods. In addition, even prior to the effective date of today's rulemaking, the permitting authority has the authority under the existing NPDES regulations to request additional data from applicants where insufficient data is provided with the application before considering an application complete.

EPA received a few comments pertaining to the rule's impact on indirect dischargers. The rule affects only direct dischargers (those applying for an individual NPDES permit) and state/EPA NPDES permitting authorities. The rule does not apply to indirect dischargers. POTWs with approved pretreatment programs may at their discretion (as authorized by their local ordinances and regulations) require their indirect dischargers to achieve specific minimum levels when performing analyses or may require the use of specific methods to enable them to better characterize contributions into their system. Where a state or EPA is the

<sup>9</sup> Authorized NPDES states have up to one year following rule issuance to revise their own regulations to conform to the requirements of this rule. Authorized NPDES states have up to two years to conform to the rule's requirements if they must make statutory changes.

pretreatment Control Authority, the specific requirements for analytical methods can be specified in the control mechanism issued to the indirect discharger.

EPA received several comments that indicated that while the commenters supported the concept established in the proposed rule, they believed additional flexibility should be provided to account for instream dilution. Specifically, the commenters requested that the criteria defining sufficiently sensitive be revised such that the minimum level would be compared to either “the applicable water quality criterion, wasteload allocation, permit limit, or other critical regulatory value.” EPA believes that the final rule need only require comparison of a method’s minimum level with the applicable water quality criterion, as proposed, and that this language is sufficiently flexible to address the commenters’ concern. Under this language, the permitting authority has adequate discretion to determine whether the data provided with a permit application were collected with methods that are sufficiently sensitive to measure at the relevant regulatory value. For example, where a permitting authority has conducted a timely and relevant dilution analysis (including an evaluation of ambient pollutant concentrations) and documented this analysis in the permit record, the permitting authority could provide this information to the applicant prior to the applicant sampling for the permit application. The applicant would then only need to show that the method it has selected has a minimum level that is at least as sensitive as necessary to determine compliance with the water quality criterion, after accounting for allowable dilution. The water quality criterion as adjusted for allowable dilution would be the “applicable water quality criterion” in this case, and the method would be “sufficiently sensitive” if it measures at this level. EPA considers this approach consistent with the requirements established in today’s rule. For these reasons, EPA is not revising the regulatory text to incorporate the language suggested by the commenters.

#### C. Burden

EPA received a few comments indicating that site-specific situations might increase the implementation costs of the rule beyond those costs outlined in the proposed rule. Some of these commenters provided examples of when site-specific conditions might result in increased costs. EPA recognizes that the burden estimated is a national average and that the cost for an individual

facility could be higher or lower than that average. However, EPA does not believe that the information provided by the commenters is representative of the impact for a typical facility affected by this rule, nor does it alter the Agency’s original burden estimates.

EPA also recognizes that in some cases, use of a more sensitive method could have the practical effect of requiring a facility to adopt additional pollution control measures, even if the permit limit remained unchanged. This is because a more sensitive method may detect the presence of a pollutant that was previously undetected. EPA emphasizes that this rule would not be responsible for any change in stringency of the permit requirements in such a case, but acknowledges that a facility may incur additional pollution control costs if a previously undetected pollutant is later detected by the use of a sufficiently sensitive method, and additional treatment is required to meet the existing permit limit. In general, when EPA develops a cost analysis for a new regulation, there is an assumption made of full compliance with existing requirements. EPA does not have data that would allow it to predict in advance where or how often this situation might occur, or what a facility would be required to do to address it. Therefore, EPA has not attempted to quantify any such costs, as they are outside the scope of this rulemaking.

As noted above, where a technology-based requirement is specified as “zero discharge” or “no detect,” the permitting authority may take into account the sensitivity of the method used to establish the requirement when determining if a method is “sufficiently sensitive.” EPA recognizes that if a more sensitive method is approved after such a requirement has been established, its use may be inconsistent with the technological basis of the original requirement. In situations where a technology-based requirement reflects a technology that eliminates the discharge of the subject pollutant altogether, the Agency included costs that reflect that technology, the newer sensitive method is appropriate, and the permittee would not incur additional costs. However, where a technology-based limit reflects a technology that may not achieve the minimum level of the newer more sensitive method, the Director may determine that the method on which the requirement was originally based is “sufficiently sensitive” to determine compliance, as understood at the time the requirement was established, and there would thus be no additional control costs incurred by the facility.

EPA received a few comments regarding compliance with requirements under the statutory and Executive Order reviews contained in the proposed rule. EPA believes that there was a misunderstanding on the part of the commenters regarding the intent of the rule that led the commenters to believe that the rule would result in a higher cost of implementation than that estimated by EPA. EPA believes that the Agency has met its responsibilities under the applicable statutory and Executive Orders.

#### IV. The Final Rule

The final rule adds a new 40 CFR 122.21(e)(3) and revises 122.44(i)(1)(iv) to require that where EPA-approved methods exist, NPDES applicants use sufficiently sensitive EPA-approved analytical methods when submitting information quantifying the presence of pollutants in a discharge and that the Director must prescribe that only sufficiently sensitive EPA-approved analytical test methods be used for analyses of pollutants or pollutant parameters under the permit. EPA is also providing a cross-reference to these changes in a new 40 CFR 136.1(c). For the purposes of this rulemaking, if monitoring requirements are included as a condition of a general permit, those requirements are subject to the provisions established in 122.44(i)(1)(iv). Only these specific parts of the regulations undergoing revision are subject to challenge under section 509(b) of the Clean Water Act.

In addition, based on public comments, EPA made certain minor modifications to the final rule from the original proposal. Specifically, EPA amended 122.21(e)(3)(i)(B) and 122.44(i)(1)(iv)(A)(1) to add the word “or” when defining the term “sufficiently sensitive,” which was unintentionally omitted in the proposed rule. In addition, EPA added “pollutant or pollutant parameter” to 122.21(e)(3)(i)(C) and 122.44(i)(1)(iv)(A) to clarify the applicability of the criteria established under the sufficiently sensitive method definition. EPA also removed the second “in accordance with” in the introductory paragraphs for 122.21(e)(3) and 122.44(i)(1)(iv) to clarify that the method selected must be approved under 40 CFR part 136 or required under 40 CFR chapter I, subchapter N or O.

EPA removed language in 122.44(i)(1)(iv)(A)(2) of the proposed rule because it was not applicable to requirements established in this section and created confusion about the implementation of the rule. In this instance, even if the permittee believes

they are discharging above the permit limit and could potentially use a less sensitive method, the permitting authority is responsible for prescribing an EPA-approved method, where available, that is sensitive enough to detect at or below the permit limit in order to properly assess compliance with the permit.

EPA revised the proposed regulatory text at 122.21(e)(3)(ii) and 122.41(i)(1)(iv)(B) for instances where there are no EPA-approved methods. The proposed language included additional requirements for situations where there are no EPA-approved methods. Specifically, the proposed rule would have required that applicants and permitting authorities select a “sufficiently sensitive” non EPA-approved method and that applicants provide a description of the method, including the minimum level. The situation in which there are no EPA-approved methods is uncommon because there are EPA-approved methods for most pollutants or pollutant parameters screened and regulated under the NPDES program. In addition, the existing regulations already require that applicants select a suitable method and provide a description of the method. Based on public comments, EPA determined that this additional requirement was unnecessary and has revised the regulatory text to revert the existing language in 40 CFR 122.21 and 122.41. As a result, today’s rule does not specify that non-EPA-approved methods

must be sufficiently sensitive. To clarify this point, EPA also added language to the introduction of 122.21(e)(3) to specify that the requirement to use a sufficiently sensitive method applies “except as specified in 122.21(e)(3)(ii).”

EPA amended 122.21(e)(3)(ii) by adding regulatory text to clarify that in the case where there are no EPA-approved methods, applicants may consider other relevant factors when selecting an appropriate method. In addition, EPA revised the proposed regulatory text to change “or otherwise required by the Director” to “and not otherwise required by the Director” to clarify that this provision applies to a situation where no EPA-approved methods exist *and* the Director has not required the use of a specific non-EPA-approved method. In this situation, the permit applicant may select a suitable non-EPA-approved method and provide a description of the method.

Finally, in both places where the new definition of “sufficiently sensitive” appears, EPA added a note to clarify that, consistent with 40 CFR part 136, permittees have the option of providing matrix or sample-specific minimum levels rather than the published levels. In addition, the note clarifies that where a permittee can demonstrate that, despite a good faith effort to use a method that would otherwise meet the definition of “sufficiently sensitive,” the analytical results are not consistent with the QA/QC specifications for that method, then the Director may determine that the method is not

performing adequately and a different method should be selected from the remaining EPA-approved methods consistent with 40 CFR 122.21(e)(3)(i) and 40 CFR 122.44(i)(1)(iv)(A). Where no other EPA-approved methods exist, a method should be selected consistent with 40 CFR 122.21(e)(3)(ii) and 40 CFR 122.44(i)(1)(iv)(B).

**V. Impacts**

Entities that discharge to waters of the United States vary in terms of the quantity of their discharges, the potential constituents contained in their discharges, and their operation and maintenance practices. Consequently, the Director’s NPDES application requirements vary depending on applicant type. For example, Form 2A for municipalities requires minimal screening for POTWs with design flows under 100,000 gallons per day; however, for POTWs with design flows above 1 million gallons per day, multiple priority pollutant scans are required. Similarly, existing industrial and commercial facilities that complete Form 2C are required to test for toxic pollutants based on the nature of their manufacturing operation. To assist permitting authorities (EPA regions, States, and Tribes), EPA developed several NPDES permit application forms. Table IV–1 provides a list of these forms and the discharger type(s) for which they are intended. Permitting authorities may use EPA’s forms or comparable forms of their own.

TABLE IV–1—EPA NPDES PERMIT APPLICATION FORMS BY APPLICANT TYPE

	Form or request	Applicant type
1 .....	Form 1 .....	New and existing applicants, except POTWs and treatment works treating domestic sewage.
2 .....	Form 2A .....	New and existing POTWs (i.e., municipal facilities).
3 .....	Form 2B .....	New and existing concentrated animal feeding operations (CAFOs) and aquatic animal production facilities.
4 .....	Form 2C .....	Existing industries discharging process wastewater.
5 .....	Form 2D .....	New industries discharging process wastewater.
6 .....	Form 2E .....	New and existing industries discharging non-process wastewater only.
7 .....	Form 2F .....	New and existing industries discharging stormwater.
8 .....	40 CFR 122.21(r) and 122.22(d)	New and existing industries with cooling water intake structures.
9 .....	Form 2S .....	New and existing POTWs and other treatment works treating domestic sewage (covers sludge).

As noted earlier, permitting authorities issue and develop effluent limitations for individual NPDES permits after analyzing the data contained in each permittee’s application. The NPDES permit prescribes the conditions under which the facility is allowed to discharge to ensure the facility’s compliance with the CWA’s technology-based and water quality-based requirements. NPDES permits typically include restrictions on

the quantity of pollutants that a permittee may discharge and require the permittee to conduct routine measurements of, and report on, a number of parameters using EPA-approved, pollutant-specific test procedures (or approved alternative test procedures).

In 2012 EPA submitted an Information Collection Request (ICR) to the Office of Management and Budget (OMB) that, in part, updated the Agency’s burden estimates for

applicants to complete Forms 1, 2A, 2C–2F, and 2S and for permitting authorities to review and process such forms.<sup>10</sup> The renewal ICR did not include updated estimates for Form 2B or for forms associated with cooling water intake structures (Item 8 in Table IV–1). Updated estimates to complete

<sup>10</sup> USEPA. “Information Collection Request (ICR) for National Pollutant Discharge Elimination System (NPDES) Program (Renewal),” OMB Control No. 2040–0004, EPA ICR No. 0229.20, March 2012.

those forms were contained in separate ICRs.<sup>11</sup> The existing ICRs include annual burden estimates for completing NPDES permit applications and for conducting ongoing compliance monitoring for both new and existing NPDES permittees. EPA's expectation is that permit applicants and permittees will use a range of methods based on a need to appropriately quantify pollutants in their discharge. To calculate cost and burden, the ICRs use an average cost for analytical methods, which is then translated into burden hours.

To assess the impact of this final rule, EPA also assessed the cost information for 40 CFR Part 136 methods found in the National Environmental Methods Index (NEMI) at <http://www.nemi.gov>. The NEMI site describes the "relative cost" as the cost per procedure of a typical analytical measurement using the specified methods (i.e., the cost of analyzing a single sample). Additional considerations affect total project costs (e.g., labor and equipment/supplies for a typical sample preparation, quality assurance/quality control requirements to validate results reported, number of samples being analyzed). EPA's review of the cost ranges provided in NEMI indicated that there was generally little difference in the cost ranges across the EPA-approved analytical methods for a particular pollutant. A table with the NEMI cost ranges is included in the record. While EPA acknowledges that there are cost differentials for some facilities based on case-specific situations, on the basis of the analytical cost ranges provided in NEMI, and the assumptions used in the current ICRs (i.e., that applicants and permittees will use a range of available approved methods), the final rule is expected to result in little or no new or increased analytical burden to applicants or permittees.

The existing ICRs also account for the ongoing burden to permitting authorities to review applications and to issue NPDES permits annually. They

also account for the ongoing burden associated with reviewing discharge monitoring and other reports for compliance assessment purposes. Finally, the existing ICRs account for program revisions where they are necessary because the controlling Federal statutes or regulations were modified.

As noted above, EPA also recognizes that in some cases, use of a more sensitive method could have the practical effect of requiring a facility to adopt additional pollution control measures, even if the permit limit remained unchanged. EPA does not have data that would allow it to predict in advance where or how often this situation might occur, or what a facility would be required to do to address it. EPA has not attempted to quantify the costs of any such new control measures that might be adopted, as they are outside the scope of this rulemaking.

#### VI. Compliance Dates

Following issuance of this rule, authorized states have up to one year to revise, as necessary, their NPDES regulations to adopt the requirements of this rule, or two years if statutory changes are needed, as provided at 40 CFR 123.62.

#### VII. Statutory and Executive Order Reviews

##### A. Executive Order 12866: Regulatory Planning and Review and Executive Order 13563: Improving Regulation and Regulatory Review

Under Executive Order 12866 (58 FR 51735, October 4, 1993), this action is a "significant regulatory action." Accordingly, EPA submitted this action to the Office of Management and Budget (OMB) for review under Executive Orders 12866 and 13563 (76 FR 3821, January 21, 2011) and any changes made in response to OMB recommendations have been documented in the docket for this action.

##### B. Paperwork Reduction Act

This action does not impose any new information collection burden. The final rulemaking requires the use of sufficiently sensitive EPA-approved analytical test methods, where they exist, when applying for an NPDES permit and when performing sampling and analysis pursuant to monitoring requirements in an NPDES permit. However, it does not change the recordkeeping or reporting requirements associated with the use of analytical methods. The Office of Management and Budget (OMB) has previously approved the information collection requirements

contained in the existing regulations (which cover all potential NPDES applicants) under the provisions of the Paperwork Reduction Act, 44 U.S.C. 3501 et seq. and has assigned OMB control numbers, as summarized in section V (Impacts) of this preamble. The OMB control numbers for EPA's regulations in 40 CFR are listed in 40 CFR part 9.

##### C. Regulatory Flexibility Act

The Regulatory Flexibility Act generally requires an agency to prepare a regulatory flexibility analysis of any rule subject to notice and comment rulemaking requirements under the Administrative Procedure Act or any other statute unless the agency certifies that the rule will not have a significant economic impact on a substantial number of small entities. Small entities include small businesses, small organizations, and small governmental jurisdictions.

For purposes of assessing the impacts of this final rule on small entities, "small entity" is defined as (1) a small business based on the Small Business Administration regulations at 13 CFR 121.201; (2) a small governmental jurisdiction that is a government of a city, county, town, school district, or special district with a population of less than 50,000; or (3) a small organization that is any not-for-profit enterprise which is independently owned and operated and is not dominant in its field.

After considering the economic impacts of this final rule on small entities, I certify that this action will not have a significant economic impact on a substantial number of small entities. EPA has determined that the incremental analytical costs that NPDES permit applicants and permittees may bear as a result of this rule are minimal and would not rise to the level of a significant economic impact on a substantial number of small entities.

##### D. Unfunded Mandates Reform Act

This rule does not contain a Federal mandate that might result in expenditures of \$100 million or more for state, local, and tribal governments, in the aggregate, or the private sector in any one year. Thus, this final rule is not subject to the requirements of sections 202 and 205 of the UMRA. EPA has further determined that this rule contains no regulatory requirements that might significantly or uniquely affect small governments. Thus, this final rule is not subject to the requirements of section 203 of UMRA.

<sup>11</sup> USEPA. "Supporting Statement for the Information Collection Request for the NPDES Regulation and Effluent Limitation Guidelines and Standards for Concentrated Animal Feeding Operations." OMB Control No. 2040-0250, EPA ICR No. 1989.09, January 2014.

USEPA. "Information Collection Request (ICR) for Cooling Water Intake Structures at Phase III Facilities (Final Rule)." OMB Control No. 2040-0268, EPA ICR No. 2169.05, January 2014.

USEPA. "Information Collection Request (ICR) for Cooling Water Intake Structures Phase II Existing Facilities (Renewal)." OMB Control No. 2040-0257, EPA ICR No. 2060.06, January 2014.

USEPA. "Information Collection Request (ICR) for Cooling Water Intake Structures New Facility Rule (Renewal)." OMB Control No. 2040-0241, EPA ICR No. 1973.05, December 2011.

*E. Executive Order 13132: Federalism*

This final rule does not have federalism implications. When promulgated, it will not have substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of governments, as specified in Executive Order 13132 (64 FR 43255, August 10, 1999). This final rule does not change the relationship between the national government and the States or change their roles and responsibilities. Rather, this final rulemaking requires that sufficiently sensitive EPA-approved analytical test methods be used, where they exist, when applying for an NPDES permit and when performing sampling and analysis pursuant to monitoring requirements in an NPDES permit. EPA does not expect this final rule to have any impact on local governments.

Furthermore, the revised regulations would not alter the basic state-federal scheme established in the CWA, under which EPA authorizes states to carry out the NPDES permitting program. EPA expects the revised regulations to have little effect on the relationship between, or the distribution of power and responsibilities among, the Federal and State governments.

*F. Executive Order 13175: Consultation and Coordination With Indian Tribal Governments*

This final rule does not have tribal implications, as specified in Executive Order 13175, "Consultation and Coordination with Indian Tribal Governments" (65 FR 67249, November 9, 2000). It will not have substantial direct effects on tribal governments, on the relationship between the Federal Government and Indian tribes, or on the distribution of power and responsibilities between the Federal Government and Indian tribes, as specified in Executive Order 13175. The final rule requires that sufficiently sensitive EPA-approved analytical test methods must be used, where they exist, when applying for an NPDES permit and when performing sampling and analysis pursuant to monitoring requirements in an NPDES permit. Nothing in this final rule would prevent an Indian tribe from exercising its own organic authority to deal with such matters.

*G. Executive Order 13045: Protection of Children From Environmental Health and Safety Risks*

The final rule is not subject to Executive Order 13045, "Protection of

Children from Environmental Health Risks and Safety Risks" (62 FR 19885, April 23, 1997), because it is not economically significant and the Agency does not believe that the environmental health and safety risks addressed by this action present a disproportionate risk to children.

*H. Executive Order 13211: Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use*

This rulemaking is not subject to Executive Order 13211, "Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use" (66 FR 28355, May 22, 2001), because it is not likely to have a significant adverse effect on the supply, distribution, or use of energy.

*I. National Technology Transfer and Advancement Act*

Section 12(d) of the National Technology Transfer and Advancement Act (NTTAA) of 1995 (Pub. L. 104-113, section 12(d), 15 U.S.C. 272 note) directs EPA to use voluntary consensus standards in its regulatory activities unless to do so would be inconsistent with applicable law or otherwise impractical. Voluntary consensus standards are technical standards (e.g., materials specifications, test methods, sampling procedures, and business practices) that are developed or adopted by voluntary consensus standard bodies. The NTTAA directs EPA to provide explanations to Congress, through OMB, when the Agency decides not to use available and applicable voluntary consensus standards. This final rulemaking does not change agency policy or requirements with respect to the use of voluntary consensus standards for the analysis of pollutants by NPDES permit applicants or permittees.

*J. Executive Order 12898 (Federal Actions To Address Environmental Justice in Minority Populations and Low-Income Populations)*

Executive Order 12898 (59 FR 7629, February 16, 1994) establishes federal executive policy on environmental justice. Its main provision directs federal agencies, to the greatest extent practicable and permitted by law, to make environmental justice part of their mission by identifying and addressing, as appropriate, disproportionately high and adverse human health or environmental effects of their programs, policies, and activities on minority populations and low-income populations in the United States.

EPA has determined that this final rule will not have disproportionately high and adverse human health or environmental effects on minority or low-income populations because it does not affect the level of protection provided to human health or the environment. As explained above, the Agency does not have reason to believe that the rule addresses environmental health and safety risks that present a disproportionate risk to minority populations and low-income populations.

*K. Congressional Review Act*

The Congressional Review Act, 5 U.S.C. 801 et seq., as added by the Small Business Regulatory Enforcement Fairness Act of 1996, generally provides that before a rule may take effect, the agency promulgating the rule must submit a rule report, which includes a copy of the rule, to each House of the Congress and to the Comptroller General of the United States. EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of the rule in the **Federal Register**. A major rule cannot take effect until 60 days after it is published in the **Federal Register**. This action is not a "major rule" as defined by 5 U.S.C. 804(2). This rule will be effective September 18, 2014.

**List of Subjects***40 CFR Part 122*

Administrative practice and procedure, Confidential business information, Environmental protection, Hazardous substances, Reporting and recordkeeping requirements, Water pollution control.

*40 CFR Part 136*

Environmental protection, Incorporation by reference, Reporting and recordkeeping requirements, Water pollution control.

Dated: August 6, 2014.

**Gina McCarthy**,  
Administrator.

For the reasons set out in the preamble, title 40, chapter I, of the Code of Federal Regulations is amended as follows:

**PART 122—EPA ADMINISTERED PERMIT PROGRAMS: THE NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM**

■ 1. The authority citation for part 122 continues to read as follows:

Authority: The Clean Water Act, 33 U.S.C. 1251 et seq.

2. Section 122.21, is amended by adding a new paragraph (e)(3), to read as follows:

122.21 Application for a permit (applicable to State programs, see § 123.25).

(e) (3) Except as specified in 122.21(e)(3)(ii), a permit application shall not be considered complete unless all required quantitative data are collected in accordance with sufficiently sensitive analytical methods approved under 40 CFR part 136 or required under 40 CFR chapter I, subchapter N or O.

(i) For the purposes of this requirement, a method approved under 40 CFR part 136 or required under 40 CFR chapter I, subchapter N or O is "sufficiently sensitive" when:

(A) The method minimum level (ML) is at or below the level of the applicable water quality criterion for the measured pollutant or pollutant parameter; or

(B) The method ML is above the applicable water quality criterion, but the amount of the pollutant or pollutant parameter in a facility's discharge is high enough that the method detects and quantifies the level of the pollutant or pollutant parameter in the discharge; or

(C) The method has the lowest ML of the analytical methods approved under 40 CFR part 136 or required under 40 CFR chapter I, subchapter N or O for the measured pollutant or pollutant parameter.

Note to paragraph (e)(3)(i)(C): Consistent with 40 CFR part 136, applicants have the option of providing matrix or sample specific minimum levels rather than the published levels. Further, where an applicant can demonstrate that, despite a good faith effort to use a method that would otherwise meet the definition of "sufficiently sensitive", the analytical results are not consistent with the QA/QC specifications for that method, then the Director may determine that the method is not performing adequately and the applicant should select a different method from the remaining EPA-approved methods that is sufficiently sensitive consistent with 40 CFR 122.21(e)(3)(i). Where no other EPA-approved methods exist, the applicant should select a method consistent with 40 CFR 122.21(e)(3)(ii).

(ii) When there is no analytical method that has been approved under 40 CFR part 136, required under 40 CFR chapter I, subchapter N or O, and is not otherwise required by the Director, the applicant may use any suitable method but shall provide a description of the method. When selecting a suitable method, other factors such as a

method's precision, accuracy, or resolution, may be considered when assessing the performance of the method.

\* \* \* \* \*

3. Section 122.44 is amended by revising paragraph (i) (1) (iv) to read as follows:

122.44 Establishing limitations, standards, and other permit conditions (applicable to State NPDES programs, see § 123.25).

\* \* \* \* \*

(i) (1) (iv)

(iv) According to sufficiently sensitive test procedures (i.e., methods) approved under 40 CFR part 136 for the analysis of pollutants or pollutant parameters or required under 40 CFR chapter I, subchapter N or O.

(A) For the purposes of this paragraph, a method is "sufficiently sensitive" when:

(1) The method minimum level (ML) is at or below the level of the effluent limit established in the permit for the measured pollutant or pollutant parameter; or

(2) The method has the lowest ML of the analytical methods approved under 40 CFR part 136 or required under 40 CFR chapter I, subchapter N or O for the measured pollutant or pollutant parameter.

Note to paragraph (i)(1)(iv)(A)(2): Consistent with 40 CFR part 136, applicants or permittees have the option of providing matrix or sample specific minimum levels rather than the published levels. Further, where an applicant or permittee can demonstrate that, despite a good faith effort to use a method that would otherwise meet the definition of "sufficiently sensitive", the analytical results are not consistent with the QA/QC specifications for that method, then the Director may determine that the method is not performing adequately and the Director should select a different method from the remaining EPA-approved methods that is sufficiently sensitive consistent with 40 CFR 122.44(i)(1)(iv)(A). Where no other EPA-approved methods exist, the Director should select a method consistent with 40 CFR 122.44(i)(1)(iv)(B).

(B) In the case of pollutants or pollutant parameters for which there are no approved methods under 40 CFR part 136 or methods are not otherwise required under 40 CFR chapter I, subchapter N or O, monitoring shall be conducted according to a test procedure specified in the permit for such pollutants or pollutant parameters.

\* \* \* \* \*

PART 136—GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

4. The authority citation for part 136 continues to read as follows:

Authority: Secs. 301, 304(h), 307, and 501(a) Pub. L. 95-217, 91 Stat. 1566, et seq. (33 U.S.C. 1251 et seq.) (The Federal Water Pollution Control Act Amendments of 1972 as amended by the Clean Water Act of 1977.)

5. Section 136.1 is amended by adding a new paragraph (c) to read as follows:

136.1 Applicability.

\* \* \* \* \*

(c) For the purposes of the NPDES program, when more than one test procedure is approved under this part for the analysis of a pollutant or pollutant parameter, the test procedure must be sufficiently sensitive as defined at 40 CFR 122.21(e)(3) and 122.44(i)(1)(iv).

[FR Doc. 2014-19265 Filed 8-18-14; 8:45 am]

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DEPARTMENT OF THE INTERIOR

Office of the Secretary

43 CFR Part 2

[145D0102DM DLSN00000.000000 DS62400000 DX62401]

RIN 1090-AA94

Privacy Act Regulations; Exemption for the Debarment and Suspension Program

AGENCY: Office of the Secretary, Interior. ACTION: Final rule.

SUMMARY: The Department of the Interior is issuing a final rule to amend its regulations to exempt certain records of the Debarment and Suspension Program system of records from particular provisions of the Privacy Act because these records contain investigatory material.

DATES: This final rule is effective September 18, 2014.

FOR FURTHER INFORMATION CONTACT: Teri Barnett, Departmental Privacy Officer, U.S. Department of the Interior, 1849 C Street NW., Mail Stop 5547 MIB, Washington, DC 20240. Email at privacy@ios.doi.gov.

SUPPLEMENTARY INFORMATION:

Background

The Department of the Interior (DOI) published a notice of proposed rulemaking in the Federal Register, 76 FR 52295, August 22, 2011, proposing to

# Exhibit 54



United States  
Environmental Protection  
Agency

Office of Water  
4304T

EPA-822-R-18-001  
December 2018

**FINAL  
AQUATIC LIFE AMBIENT WATER  
QUALITY CRITERIA FOR  
ALUMINUM  
2018**

FINAL  
AQUATIC LIFE  
AMBIENT WATER QUALITY CRITERIA FOR  
ALUMINUM - 2018

(CAS Registry Number 7429-90-05)

December 2018

U.S. ENVIRONMENTAL PROTECTION AGENCY  
OFFICE OF WATER  
OFFICE OF SCIENCE AND TECHNOLOGY  
HEALTH AND ECOLOGICAL CRITERIA DIVISION  
WASHINGTON, D.C.

## NOTICES

This document provides information to states and tribes authorized to establish water quality standards under the Clean Water Act (CWA), to protect aquatic life from toxic effects of aluminum. Under the CWA, states and tribes are to establish water quality criteria to protect designated uses. State and tribal decision makers retain the discretion to adopt approaches that are scientifically defensible that differ from these criteria to reflect site-specific conditions. While this document contains the Environmental Protection Agency's (EPA) scientific recommendations regarding ambient concentrations of aluminum that protect aquatic life, the Aluminum Criteria Document does not substitute for the CWA or the EPA's regulations; nor is it a regulation itself. Thus, the document does not impose legally binding requirements on the EPA, states, tribes, or the regulated community, and might not apply to a particular situation based upon the circumstances. The EPA may update this document in the future. This document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use. This document can be downloaded from:  
<https://www.epa.gov/wqc/aquatic-life-criteria-and-methods-toxics>.

## FOREWORD

The Clean Water Act (CWA) Section 304(a)(1) (P.L. 95-217) directs the Administrator of the Environmental Protection Agency (EPA) to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including groundwater. This document is a final ambient water quality criteria (AWQC) document for the protection of aquatic life based upon consideration of all available information relating to effects of aluminum on aquatic organisms.

The term Water Quality Criteria is used in two sections of the CWA, Section 304(a)(1) and Section 303(c)(2). The term has different meanings in each section. In Section 304, the term represents a non-regulatory, scientific assessment of ecological and human health effects. Criteria presented in this document are such a scientific assessment of ecological effects. In section 303, if water quality criteria associated with specific surface water uses are adopted by a state or the EPA as water quality standards, they become the CWA water quality standards applicable in ambient waters within that state or authorized tribe. Water quality criteria adopted in state water quality standards could have the same numerical values as recommended criteria developed under section 304. However, in some situations states might want to adjust water quality criteria developed under section 304 to reflect local water chemistry or ecological conditions. Alternatively, states and authorized tribes may develop numeric criteria based on other scientifically defensible methods, but the criteria must be protective of designated uses. It is not until their adoption as part of state water quality standards, and subsequent approval by the EPA under section 303(c), that criteria become CWA applicable water quality standards. Guidelines to assist the states and authorized tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 2014).

This document presents recommendations only. It does not establish or affect legal rights or obligations. It does not establish a binding requirement and cannot be finally determinative of the issues addressed. The EPA will make decisions in any particular situation by applying the CWA and the EPA regulations on the basis of specific facts presented and scientific information then available.

Deborah G. Nagle  
Director  
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## ACKNOWLEDGEMENTS

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## ACRONYMS

ACR	Acute to Chronic Ratio
AIC	Akaike Information Criterion
AVS	Acid Volatile Sulfide
AWQC	Ambient Water Quality Criteria
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BIC	Bayesian Information Criterion
CCC	Criterion Continuous Concentration
CMC	Criterion Maximum Concentration
CV	Chronic Value (expressed in this document as an EC <sub>20</sub> )
CWA	Clean Water Act
DOC	Dissolved Organic Carbon
ECOTOX	Ecotoxicology Database
EC <sub>x</sub>	Effect Concentration at X Percent Effect Level
ELS	Early-Life Stage
EPA	Environmental Protection Agency
EU	European Union
FACR	Final Acute-to-Chronic Ratio
FAV	Final Acute Value
FCV	Final Chronic Value
FDA	US Food and Drug Administration
GMAV	Genus Mean Acute Value
GMCV	Genus Mean Chronic Value
IC <sub>x</sub>	Inhibitory Concentration at X Percent Level
LC <sub>x</sub>	Lethal Concentration at X Percent Survival Level
LOEC	Lowest Observed Effect Concentration
MATC	Maximum Acceptable Toxicant Concentration (expressed mathematically as the geometric mean of the NOEC and LOEC)
MDR	Minimum Data Requirement
MLR	Multiple Linear Regression
NAWQA	USGS National Water Quality Assessment Program
NOAA	National Oceanic and Atmospheric Administration
NOEC	No Observed Effect Concentration
NPDES	National Pollutant Discharge Elimination System
QA/QC	Quality Assurance and Quality Control
SMAV	Species Mean Acute Value
SMCV	Species Mean Chronic Value
TMDL	Total Maximum Daily Load
TRAP	Toxicity Relationship Analysis Program
US	United States
USGS	United States Geological Survey
WQC	Water Quality Criteria
WQS	Water Quality Standards

## EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) is updating its aquatic life ambient water quality criteria (AWQC) recommendation for aluminum, in accordance with the provisions of section 304(a) directing the EPA to revise AWQC from time to time to reflect the latest scientific knowledge. The recommended aluminum aquatic life AWQC were developed using peer reviewed methods and data that are acceptable for the derivation of criteria, as described in the EPA's 1985 "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (Stephan et al. 1985, referred to herein as "1985 Guidelines"). The previous aquatic life AWQC for aluminum were developed in 1988 (EPA 440/5-86-008). These 2018 final recommended aquatic life AWQC for aluminum supersedes the 1988 recommended criteria.

The 2017 draft aquatic life AWQC for aluminum were posted to the Federal Register (Docket ID: EPA-HQ-OW-2017-0260) in late July 2017 for public comment. The public comment period was open for 90 days and closed in late October 2017. Public comments received were incorporated and addressed in these final AWQC, where applicable. The EPA responses to all of the public comments can be found on the website for the aluminum criteria (<https://www.epa.gov/wqc/aquatic-life-criteria-aluminum>).

Literature searches for laboratory tests published from 1988 to 2017 identified new studies describing the toxicity of aluminum to aquatic life. The EPA supplemented these studies with additional data made available by researchers in late-2017 and 2018. The EPA conducted a full evaluation of available data to determine test acceptability for criteria development. Appendix A of "*Quality Criteria for Water 1986*" (U.S. EPA 1986) provides an in-depth discussion of the minimum requirements for data quality needed to develop AWQC for aquatic life.

This update to the recommended aluminum aquatic life AWQC establishes freshwater criteria magnitude values resulting from the interactions of aluminum and three water chemistry parameters: pH, total hardness, and dissolved organic carbon (DOC). It also expands the toxicity database to include those studies conducted in waters with pH values below 6.5. There were insufficient data to establish an estuarine/marine aluminum criteria.

Multiple linear regression (MLR) models were developed to characterize the bioavailability of aluminum in aquatic systems, based on the effects of pH, total hardness and

DOC on aluminum toxicity (DeForest et al. 2018a,b). These authors used a dataset comprised of 22 chronic tests with the fathead minnow (*Pimephales promelas*), and 23 chronic tests with an invertebrate (*Ceriodaphnia dubia*) to evaluate the ability of MLR models to predict chronic toxicity of aluminum as a function of pH, total hardness and DOC water chemistry conditions. These three parameters are considered to be the most influential for aluminum bioavailability and can be used to explain the range of differences in the observed toxicity values. These datasets were supplemented in 2018 with an additional nine *C. dubia* toxicity tests and nine *P. promelas* toxicity tests to expand the range of water chemistry conditions for model development (OSU 2018a,b,d). All of the toxicity test data used in the model were subjected to independent external expert peer review.

Two models, one for invertebrates and one for vertebrates, were used to normalize freshwater aluminum toxicity values. These separate models correspond to effects on invertebrates and vertebrates due to differing effects of pH, total hardness and DOC on aluminum bioavailability and toxicity, and therefore enable the criteria magnitudes to be calculated as a function of the unique chemistry conditions at a given site. The EPA conducted both independent external expert peer review and internal reviews of these models, published by DeForest et al. (2018a,b), to verify the results. The updated aluminum criteria were derived using these MLR models to normalize the freshwater acute and chronic toxicity data. The MLR equations applied to the acute toxicity data were those developed using chronic tests, with the expectation that the effect of water chemistry on bioavailability remains consistent across exposure duration.

### **Freshwater Criteria Update**

The 1988 aluminum freshwater criteria (U.S. EPA 1988) are expressed as total recoverable aluminum. Acid soluble aluminum was considered but not used because the methods were not developed. These updated 2018 criteria are also based on total recoverable aluminum concentrations.

The 1988 criteria did not consider the variable effects of water chemistry on aluminum toxicity, but simply specified that the recommended criteria only applied to a pH range of 6.5 to 9.0. The 2018 final aluminum recommended AWQC take into account the effects of pH, total hardness and DOC on aluminum toxicity.

# Exhibit 55

# RECORD OF DECISION

## ADDENDUM

### New Mexico's Standards For Interstate and Intrastate Surface Waters 20.6.4 NMAC

The purpose of this addendum is to explain the Environmental Protection Agency (EPA's or the Agency's) decision on those provisions of New Mexico's *Standards for Interstate and Intrastate Surface Waters, 20.6.4. NMAC*, that EPA did not act on as part of its previous April 12, 2011 decision. EPA's decisions are based on a detailed review of supporting documentation for these provisions, discussions and correspondence with the State.

#### **20.6.4.10 D. Site-specific Criteria**

Federal regulations allow States the flexibility to modify EPA's 304(a) criteria to reflect site-specific conditions. Given this premise, EPA initially approved the majority of section **20.6.4.10(D) Site-specific Criteria** and took no action on subsection **20.6.4.10 (D)(1)(e)** because of specific concerns with that subsection of the provision. After additional analysis, EPA determined that section **20.6.4.10(D)** represents implementation procedures and does not constitute water quality standards that require the EPA's review or action under Section 303(c) of the Clean Water Act (CWA). Since the provisions in this section are not water quality standards, EPA has determined that it has no obligation to act on these provisions and as a result, rescinds that prior action. Section **20.6.4.10(D)** remains in effect for purposes of State law and may be used for the development of site-specific criteria; however, it is not a water quality standard that is effective for CWA purposes.

Although EPA is not approving the procedures in section **20.6.4.10 (D)** as water quality standards, we retain authority to act on site-specific criteria developed using these procedures. Given this authority, it is important that the State understand our concerns with subsection **20.6.4.10 (D)(1)(e)**. In a plain reading of this subsection, it is unclear what the reference to "...other factors or combinations of factors that...may warrant modifications of default criteria" means or how it will be applied or implemented. In an effort to determine the meaning and intent, EPA referred to the hearing record, the Commission's Statement of Reasons and the Hearing Officers Report. All referenced assurances from the New Mexico Environment Department (NMED) to 3<sup>rd</sup>-party petitioners that the Commission would consider "net ecological benefit" in establishing site-specific criteria. Given this, EPA believes it is important to reiterate the position outlined in comments provided to NMED that were included as Exhibit\_89 in the State's hearing record and subsequent submission. As explained in those comments, the "net ecological benefit" concept is not supportable from an ecological perspective and is not consistent with federal regulations. As such, EPA is unlikely to approve site-specific criteria based on a net ecological benefit concept.

#### 20.6.4.13 J. Turbidity

EPA believes that when this provision regarding criteria for turbidity was initially adopted, it was intended to address potential degradation from sources of turbidity expressed as numeric total dissolved solids values. Although the amendments were intended to provide some clarity, EPA's concern has been that if implemented as written, the provision could allow long-term or permanent degradation. However, EPA believes that if this provision is implemented consistent with the antidegradation policy and implementation contained in the State's standards and antidegradation implementation procedures in its Continuing Planning Process (CPP) and/or related documents, the amended provision is consistent with the CWA and the EPA's implementing regulations at 40 CFR 131. As a result, EPA approves the new and revised language in this provision with the understanding that – as with all of the State's water quality standards -- it will be implemented consistent with approved antidegradation policy and procedures in the State's standards and its CPP.

The State is currently addressing the effects of imbalances in suspended and bedded sediment on aquatic life uses through narrative or comparative standards found in section 20.6.4.13 NMAC, which include this turbidity provision. There is significant variability inherent to turbidity data and the degree that natural and anthropogenic sediment loads affect aquatic life are not specifically defined. As a result, Region 6 and NMED staff have been working towards developing benchmarks for bedded sediment by site class to better implement the existing narrative criterion. The analyses are to identify sediment characteristics that are expected under the range of environmental settings in New Mexico, especially in undisturbed reference streams. Through this characterization, it will be possible to identify situations where the expectations are not met, using sediment indicators that show responsiveness to disturbance. Associating biological measures with sediment indicators will further indicate situations where the disturbance causes biological imbalance and habitat degradation. EPA believes that the results of these analyses will aid in establishing quantitative sedimentation benchmarks on New Mexico perennial streams in future standards revisions.

#### 20.6.4.900 I. (1) Acute and (2) Chronic Hardness-based Metals Criteria

##### Aluminum:

New Mexico has adopted revised criteria for aluminum based on a proposal from a 3<sup>rd</sup>-party, Chevron Mining, Inc. The rationale and methods used to derive the proposed criteria were presented in a report prepared by GEI Consultants, Inc. The Commission adopted hardness-dependent equations for aluminum (based on analysis of total recoverable metal):

$$\begin{aligned} \text{Acute} &= e^{(1.3695[\ln(\text{hardness})]+1.8308)} \\ \text{Chronic} &= e^{(1.3695[\ln(\text{hardness})]+0.9161)} \end{aligned}$$

These hardness-dependent equations were derived through a recalculation of the toxicity database for EPA's 1988 Aluminum Criteria Document and newer studies published since the criteria document's publication. In the initial review, EPA identified concerns with the approach taken in the development of these recalculated criteria and conducted a detailed review to determine the appropriateness of applying these criteria statewide.

Based on our detailed review and correspondence with the State, EPA noted concerns with the selective exclusion and inclusion of specific studies that were used in the recalculation, including the use of non-native species. EPA learned that the recalculated criteria were derived by GEI as if they were an update to the national criteria. Although GEI generally followed methods outlined in EPA's criteria derivation and recalculation procedures (Stephan et al. 1985, USEPA 1994), since these updates are submitted by the State, EPA views them as State, not national criteria. As such, EPA recommends the use of indigenous species in the development of criteria intended to apply statewide.

Given that the implementation of metals criteria is complex due to the site-specific nature of their toxicity, the detailed review was also intended to determine if it would be appropriate to apply these recalculated values statewide. The studies GEI utilized were carried out over a pH range of 6.5 to 9.0. EPA previously established this pH range as an optimal in ambient freshwater (USEPA 1976), it is not reflective of the pH range that will be seen in all waters in New Mexico. Although GEI recognized the inverse toxicity and hardness relationship (within the pH range of 6.5 to 9.0) in the development of the acute equation, it does not appear that the significant effects that site-specific factors such as pH have on metals and particularly on aluminum toxicity were fully considered in applying these equations as statewide criteria. The pH significantly influences speciation and/or complexation of aluminum at low pH and should have been considered carefully in determining if these recalculated values would be appropriate when adopting these values as statewide criteria.

Given the significant variability in both pH and hardness in waters in New Mexico, EPA does not believe that these hardness-based equations are appropriate as a basis for statewide criteria and may not be protective of beneficial uses in all waters of the State. EPA has determined that the hardness-based equations would be protective for waters within the pH range of 6.5 to 9.0, particularly at low hardness levels, but would not be protective for waters below that pH range. Therefore, EPA is approving the hardness-based equation for aluminum for only those waters of the State where pH is equal to or greater than 6.5, but is disapproving these equations in waters where the pH is less than 6.5. To resolve this disapproval, EPA recommends that the State adopt a footnote for these equations specifying the following:

“Where pH is equal to or greater than 6.5 in the receiving water after mixing, the chronic hardness-dependent equation will apply. Where pH is 6.5 or less in the receiving water after mixing, either the 87 µg/l chronic total recoverable aluminum criterion or the criterion resulting from the chronic hardness-dependent equation will apply, whichever is more stringent.”

In the interim, for waters of the State where pH is 6.5 or less, in the receiving water after mixing, EPA will apply the 304(a) recommended 87 µg/L chronic total recoverable aluminum criterion.

Cadmium:

New Mexico has adopted revised criteria for dissolved cadmium based on a proposal from a 3<sup>rd</sup>-party, Chevron Mining, Inc. The rationale and methods used to derive the proposed criteria were presented in a report prepared by GEI Consultants, Inc. The Commission adopted hardness-dependent equations for cadmium (based on analysis of dissolved metal):

$$\begin{array}{ll} \text{Acute} = e^{(0.8968[\ln(\text{hardness})]-3.5699)} & \text{CF: } 1.136672-[(\ln \text{hardness})(0.041838)] \\ \text{Chronic} = e^{(0.7647[\ln(\text{hardness})]-4.2180)} & \text{CF: } 1.101672-[(\ln \text{hardness})(0.041838)] \end{array}$$

EPA identified concerns with the approach taken in the development of these recalculated criteria during its detailed review in an effort to determine the appropriateness of applying these recalculated criteria statewide. In this review, EPA concluded that there were concerns with the supporting documentation for the hardness-based cadmium criterion, specifically the use of a non-native species arctic grayling (*T. arcticus*) and juvenile rainbow trout (*O. mykiss*) as representative of the most sensitive life stage. In correspondence with the State, GEI indicated that it considers the fact that non indigenous species were used to be irrelevant because this update was to the national criteria. Since these updates are submitted by the State, EPA views these updates as State, not national criteria. As such the use of non indigenous species is not recommended in the development of criteria intended to apply statewide. However, EPA believes that overall, the new hardness-based equation will be adequately protective of the applicable designated use for all waters of the State. Therefore in today’s action, EPA is approving the new hardness-based equation for cadmium.

Zinc:

New Mexico has adopted revised criteria for zinc based on a proposal from a 3<sup>rd</sup>-party, Chevron Mining, Inc. The rationale and methods used to derive the proposed criteria were presented in a report prepared by GEI Consultants, Inc. The Commission adopted hardness-dependent equations for zinc (based on analysis of dissolved metal):

$$\begin{array}{ll} \text{Acute} = 0.978e^{(0.9094[\ln(\text{hardness})]+0.9095)} & \text{CF: } 0.978 \\ \text{Chronic} = 0.986e^{(0.90947[\ln(\text{hardness})]+0.6235)} & \text{CF: } 0.986 \end{array}$$

In our detailed review of the supporting documentation for the hardness-based zinc criterion, EPA noted the lack of a clear explanation on patterns between final acute/chronic ratio (FACR) values and acute values as consistent with EPA’s 1985 Guidelines, as well as the confusing presentation of data on the acute/chronic ratio (ACR) values. GEI provided an adequate response concerning the FACR values and confusing data presentation. As a result, EPA believes the new hardness-based equation is adequately protective of the applicable

designated use for all waters of the State. Therefore in today's action, EPA is approving the new hardness-based equation for zinc.

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GEI Consultants, Inc. Ambient Water Quality Standards for Zinc – Review and Update, August 2009.

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# Exhibit 56

# U.S. Department of Energy and TRIAD National Security

July 13, 2021

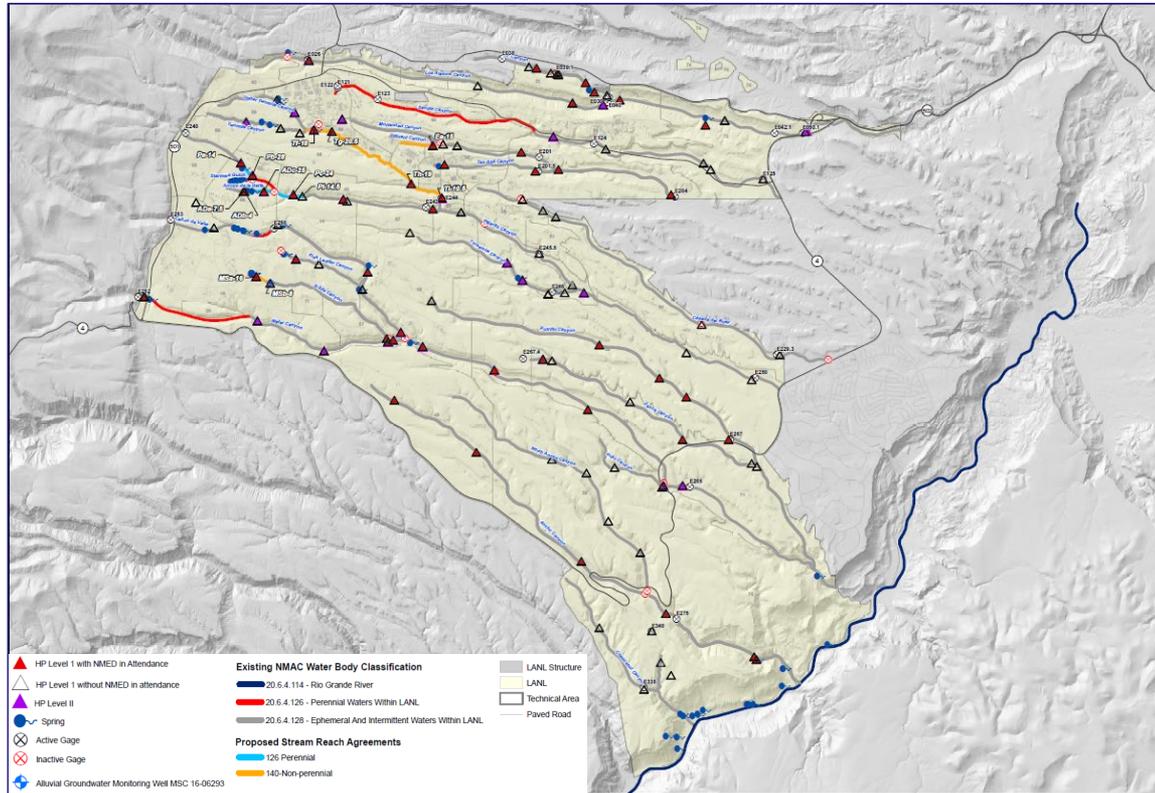


  
National Nuclear Security Administration  
Managed by Triad National Security, LLC for the U.S. Department of Energy's NNSA

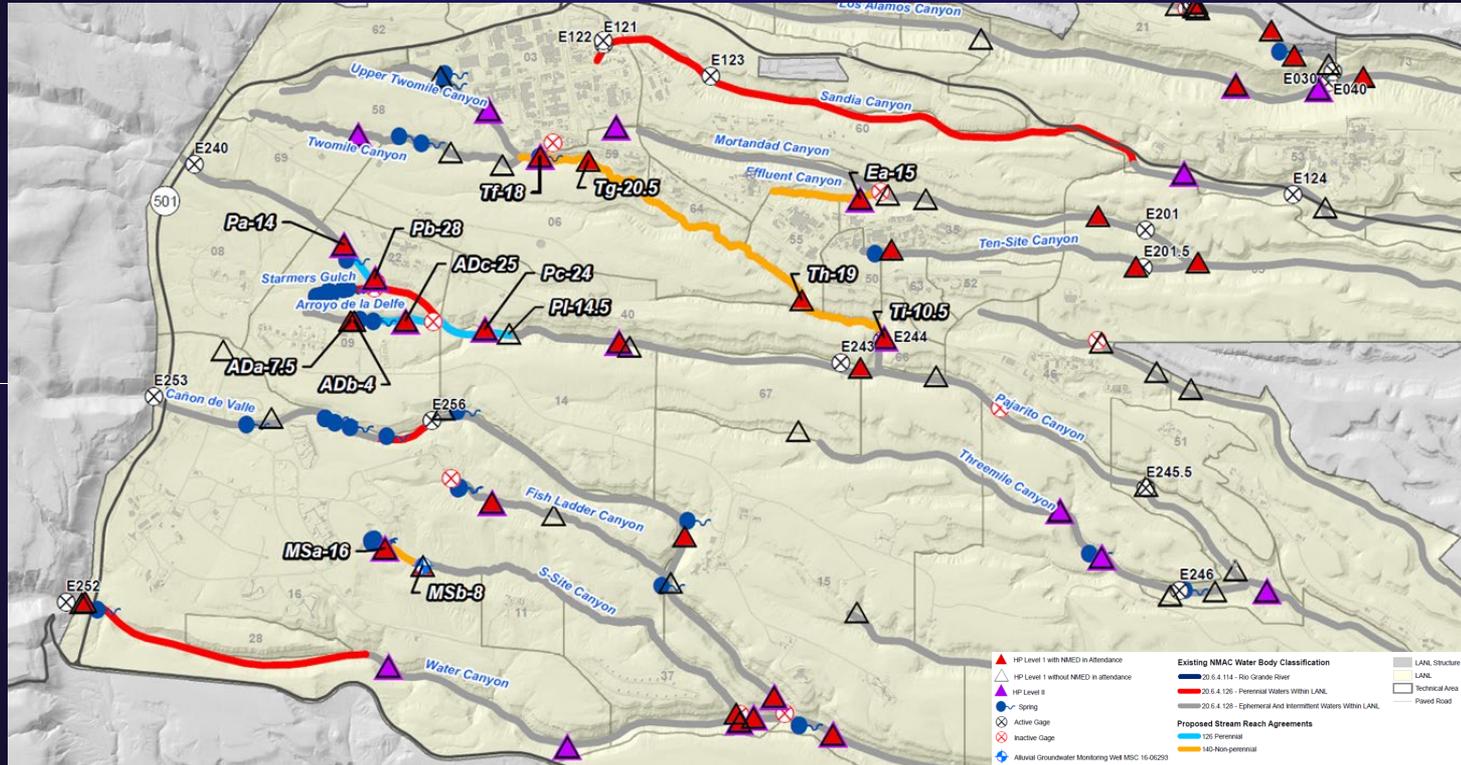
LA-UR-21-24127

2020 TR LANL-01041

# Hydrology Protocol Sites and Study Areas



# Proposed Section 126 and 140 Reaches



# Stream Hydrology/Use Classification Summary

## Current Stream Classification Summary

Perennial (20.6.4.126 NMAC)	4.8 Miles
Intermittent /Ephemeral (20.6.4.128 NMAC)	75.2 Miles

## New Proposed Stream Classification Summary

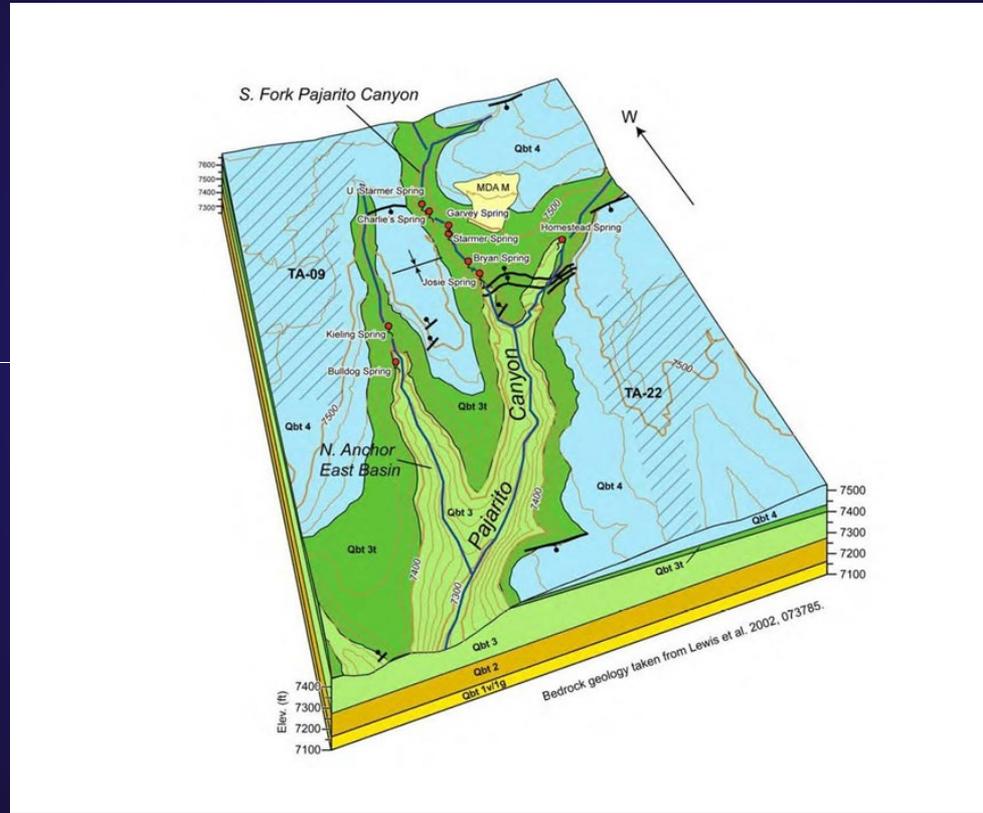
Perennial (20.6.4.126 NMAC)	5.8 Miles Total 4.8 Miles (Existing) + 1.0 Miles (New)
Intermittent 20.6.4.140 (NMAC)	2.5 Miles
Intermittent/Ephemeral 20.6.4.128 (NMAC)	71.7 Miles

# Joint Stipulated Agreement – October 2015

## Parties: NMED, Amigos Bravos and LANL

- Process created to evaluate water quality protections for LANL Segment 20.6.4.128 waters.
- Parties agree to meet, confer and share information.
- Strive to reach agreement on increased protections for LANL Segment 20.6.4.128 waters
- 117 Level I Hydrology Protocols Completed and Evaluated  
– Collaboration with NMED, Amigos Bravos, DOE and LANL
- 32 Level II Hydrology Protocols completed to collect additional information and support stream classification and use determination.
- Data Sources incorporated: precipitation, stream gage, level I and level II HP assessments, surface water monitoring and macroinvertebrate sampling and identification.

# Hydrogeologic Diagram of Pajarito Spring-Fed Reaches



## Proposed 20.6.4.126 (NMAC) - Pajarito Canyon below Homestead Spring



# Proposed 126 - Pajarito Canyon below Arroyo de la Delfe



# Gage Flow Statistics

Summary of Gage Flow Statistics

Station #	Station Name	Analysis Period	Average Percent Days of Flow	Flow Classification <sup>1</sup> Based on Percent Days with Flow	Current NMWQCC	Proposed NMWQCC
E240	Pajarito (below SR-501)	2000 - 2019	9.6%	Ephemeral / Intermittent	20.6.4.128	No change
E241	Pajarito (above Starmer's)	2000 - 2009	76.8%	Intermittent / Perennial	20.6.4.128	20.6.4.126
E242	Pajarito (Starmer's Gulch)	2000 - 2009	97.5%	Perennial	20.6.4.126	No change
E242.5	Arroyo de la Delfe (above Pajarito)	2000 - 2009	81.8%	Perennial	20.6.4.128	20.6.4.126
E244	Twomile (above Pajarito)	2003 - 2011; 2015 - 2019	34.0%	Intermittent	20.6.4.128	20.6.4.140

	Perennial flow
	Borderline Intermittent / Perennial flow

<sup>1</sup>Flow Classification based on criteria from Hedman & Osterkamp, 1982 (USGS Water Supply Paper #2193).

**Ephemeral:** measurable discharge generally occurring less than 10% of the time

**Intermittent:** measurable surface discharge between 10 and 80% of the time

**Perennial:** measurable surface discharge > 80% of the time

# Proposed 20.6.4140 (NMAC) - S-Site Canyon below Martin Spring



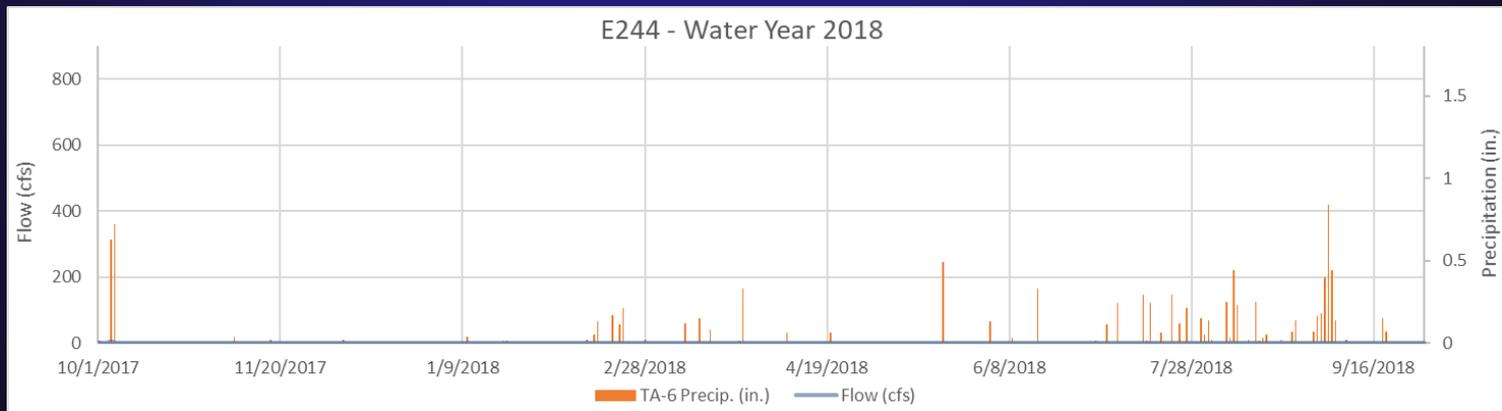
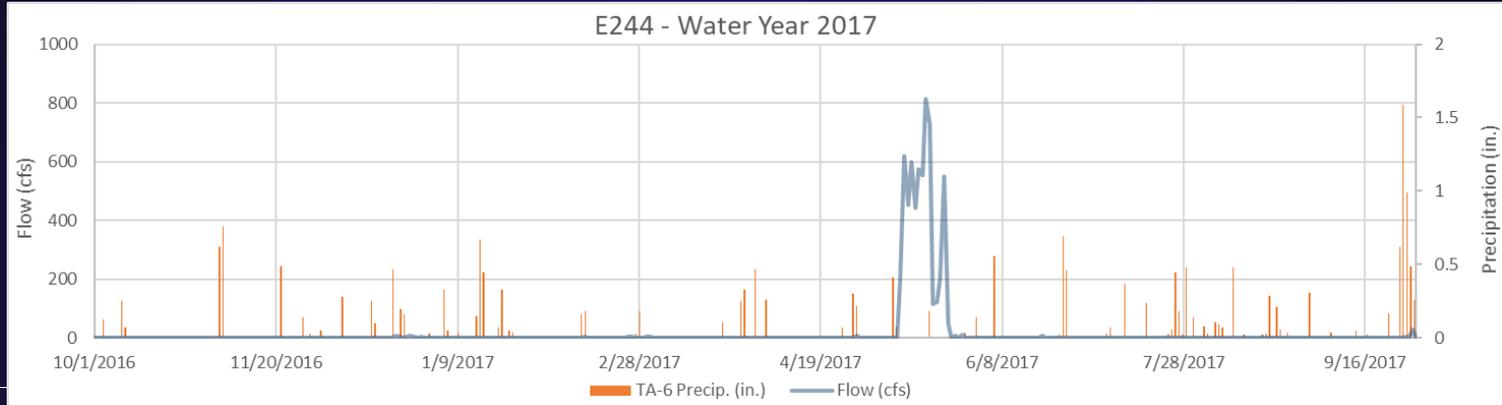
# Proposed 140 – Two Mile Canyon



## Retain 20.6.4.128 (NMAC) Two Mile Canyon above E244



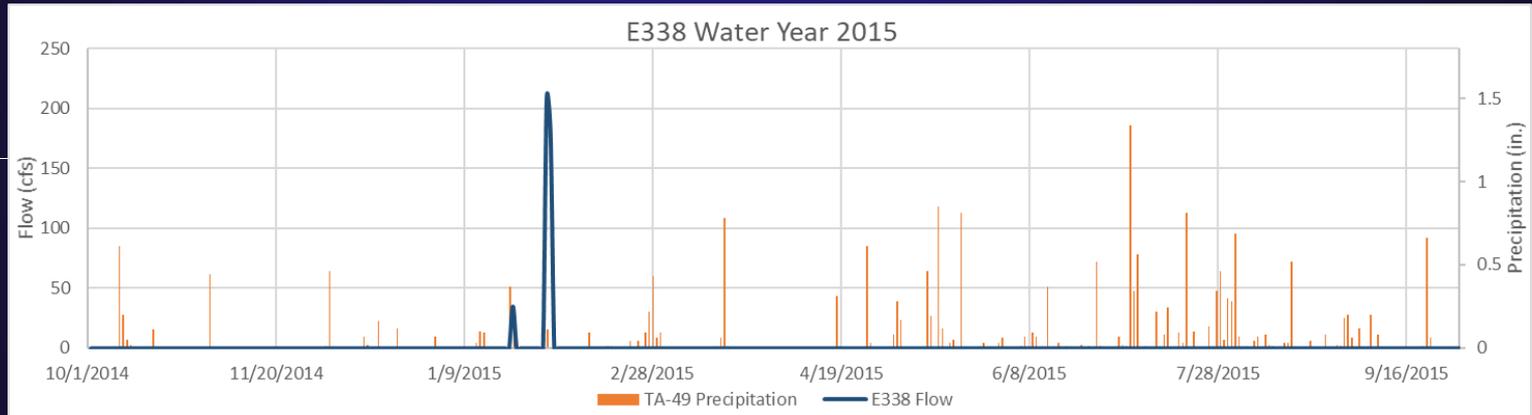
# Gage E244



## 20.6.4.128 (NMAC) Ephemeral/Intermittent – Chaquehui Canyon below E338



# Gage E338



# Benthic Summary

Summary of Benthic Data for Proposed Waters 1			
Segment	Level 1-2 Locations and Scores	Benthic Macroinvertebrate Narrative Score	EPT Taxa (Present/Absence)
Pajarito above Starmers Site 1	Pa-14	-	-
Pajarito canyon from Starmers Gulch to Homestead Spring	Pb-28	Moderate	Present
Pajarito canyon 0.5 miles below Arroyo de La Delfe	Pc-24	Moderate	Present
Arroyo de la Delfe from Pajarito canyon upstream to Kieling Spring	Ac-25	Strong <sup>1</sup>	Present
S-Site canyon from alluvial groundwater well MSC 16-06293 upstream to Martin Spring	MSa-16	Strong <sup>1</sup>	Present
Effluent canyon from Mortandad canyon confluence upstream its headwaters	Ea-15	Weak	Present
Two Mile Canyon below Confluence	Tf-18	Moderate	Present
Two Mile Canyon TA-59	Tg-20.5	Moderate	Present
Two Mile Canyon at TA-55 Confluence	Th-19	-	-
Two Mile above E244	Ti-10.5	-	-
1. bivalves present			

# Stream and Spring Temperature Data

