Development Document for the Proposed Effluent Limitations Guidelines and Standards for the Meat and Poultry Products Industry Point Source Category (40 CFR 432)

EPA-821-B-01-007

January 2002

U.S. Environmental Protection Agency
Office of Water (4303T)
Washington, DC 20460

Complete proposed document available at:
http://www.epa.gov/ost/guide/mpp/
The Final Development Document is available as well.

APPENDIX A

ANALYTICAL METHODS AND BASELINE VALUES

The analytical methods described in this appendix were used to determine pollutant levels in wastewater samples collected by EPA and industry at a number of meat and poultry product facilities (sampling efforts are described in Section 3.) In developing the proposed rule, EPA sampled facilities to determine the levels of *Aeromonas*, ammonia as nitrogen, biochemical oxygen demand (BOD), carbonaceous biochemical oxygen demand, chemical oxygen demand (COD), chloride, *Cryptosporidium*, dissolved biochemical oxygen demand, dissolved total phosphorus, *E. coli*, fecal coliform, fecal streptococcus, 21 metals, oil and grease (measured as hexane extractable material (HEM)), nitrate/nitrite, six pesticides, *Salmonella*, total coliform, total dissolved solids (TDS), total kjeldahl nitrogen (TKN), total organic carbon (TOC), total orthophosphate, total phosphorus, total residual chlorine, total suspended solids (TSS), and volatile residue. As explained in Section 7, EPA is regulating a subset of these pollutants.

Sections A.1 and A.2 of this appendix provide explanations of nominal quantitation limits and baseline values. Section A.3 describes the reporting conventions used by laboratories in expressing the results of the analyses. Section A.4 describes each analytical method and the corresponding baseline values that EPA used in determining the pollutants of concern. Section A.5 defines total nitrogen. Table A-1 lists the analytical methods and baseline values used for each pollutant.

A.1 NOMINAL QUANTITATION LIMITS

The nominal quantitation limit is the smallest quantity of an analyte that can be reliably measured with a particular method. Protocols used for determination of nominal quantitation limits in a particular method depend on the definitions and conventions that EPA used at the time the method was developed. The nominal quantitation limits associated with the methods addressed in this section fall into five categories.

1) The first category pertains to EPA Methods 1660 and 1664, which define the minimum level (ML) as the lowest level at which the entire analytical system must give a recognizable signal and an acceptable calibration point for the analyte. These methods are described in Section A.4.1.

2) The second category pertains specifically to EPA Method 1620, and is explained in detail in Section A.4.2.

3) The third category pertains to the remainder of the chemical methods (classical wet chemistry and pesticides) in which a variety of terms are used to describe the lowest level at which measurement results are quantitated. In some cases (especially with the classical wet chemistry analytes) the methods date to the 1970s and 1980s when different concepts of quantitation were employed by EPA. These methods typically list a measurement range or lower limit of measurement. The terms differ by method and, as discussed in subsequent sections, the levels presented are not always representative of the lowest levels laboratories currently can achieve.

   For those methods associated with a calibration procedure, the laboratories demonstrated through a low-point calibration standard that they were capable of reliable quantitation at method-specified (or lower) levels. In such cases these nominal quantitation limits are operationally equivalent to the ML (though not specifically identified as such in the methods). In the case of titrimetric or gravimetric methods, the laboratory adhered to the established lower limit of the measurement range published in the methods. Details of the specific methods are presented in Section A.4.3 through A.4.17.

4) The fourth category pertains to *Cryptosporidium*. There is currently no detection limit associated with the method used to determine *Cryptosporidium* (EPA Method 1622 described in Section A.4.18); so when *Cryptosporidium* was not found in the sample, there was no number that was associated with the sample. Therefore, there is no nominal quantitation limit for *Cryptosporidium*.

5) The fifth category pertains to all microbiological methods except *Cryptosporidium*. The fifth category pertains specifically to the multiple-tube test procedure and is explained in detail in Section A.4.19.

A.2 BASELINE VALUES

As described further in Section 7, in determining the pollutants of concern, EPA compared the reported concentrations for each pollutant to a multiple of the baseline value. As described in Section A.3 and shown in Table A-1, for most pollutants, the baseline value was set equal to the nominal quantitation limit for the analytical method. EPA made two general types of exceptions which are briefly described below. Section A.4 provides additional details about these exceptions in the context of the analytical method.

The first type of exceptions were baseline values that were different than the nominal quantitation limits in the analytical methods. When the baseline values had lower values, EPA made these exceptions because the laboratory had submitted data that demonstrated reliable measurements could be obtained at lower levels for those pollutants. When the baseline values had higher values, EPA concluded that the nominal quantitation limit for a specified method was less than the level that laboratories could reliably achieve and adjusted the baseline value upward.

The second type of exceptions were baseline values set at a common value for multiple analytical methods for the same pollutant. For some analytes, EPA permitted the laboratories to choose between methods to accommodate sample characteristics. When these methods had different nominal quantitation limits, EPA generally used the one with the lowest value or the one associated with the method used for most samples.

A.3 ANALYTICAL RESULTS REPORTING CONVENTIONS

The laboratories expressed results of the analyses either numerically or as not quantitated[1] for a pollutant in a sample. If the result is expressed numerically, then the pollutant was quantitated[2] in the sample. All of the analytical chemistry data were reported as liquid

[1] Elsewhere in this document and in the preamble to the proposed rule, EPA refers to pollutants as "not detected" or "non-detected". This appendix uses the term "not quantitated" or "non-quantitated" rather than non-detected.

[2] Elsewhere in this document and in the preamble to the proposed rule, EPA refers to pollutants as "detected". This appendix uses the term "quantitated" rather than detected.

concentrations in weight/volume units, e.g., micrograms per liter (µg/L). *Cryptosporidium* results were reported in the calculated number of *Cryptosporidium* oocysts detected per liter. Bacteriological data generated using multiple-tube fermentation techniques were reported as most probable number (MPN)/100 mL.

For example, for a hypothetical pollutant X, the result would be reported as "15 µg/L" when the laboratory quantitated the amount of pollutant X in the sample as being 15 µg/L. For the non-quantitated results, for each sample, the laboratories reported a "sample-specific quantitation limit."[3] For example, for the hypothetical pollutant X, the result would be reported as "<10 µg/L" when the laboratory could not quantitate the amount of pollutant X in the sample. That is, the analytical result indicated a value less than the sample-specific quantitation limit of 10 µg/L. The actual amount of pollutant X in that sample is between zero (i.e., the pollutant is not present) and 10 µg/L. The sample-specific quantitation limit for a particular pollutant is generally the smallest quantity in the calibration range that can be measured reliably in any given sample. If a pollutant is reported as non-quantitated in a particular wastewater sample, this does not mean that the pollutant is not present in the wastewater, merely that analytical techniques (whether because of instrument limitations, pollutant interactions or other reasons) do not permit its measurement at levels below the sample-specific quantitation limit.

In its calculations, EPA generally substituted the reported value of the sample-specific quantitation limit for each non-quantitated result. In a few cases described in Section A.4.1, when the sample-specific quantitation limit was less than the baseline value, EPA substituted the baseline value for the non-quantitated result. And in a few instances also described in Section A.4.1, when the quantitated value was below the baseline value, EPA considered these values to be non-quantitated in the statistical analyses and substituted the baseline value for the measured value.

[3] Elsewhere in this document and in the preamble to the proposed rule, EPA refers to a "sample-specific quantitation limit" as a "sample-specific detection limit" or, more simply, as a "detection limit."

A.4 ANALYTICAL METHODS

EPA analyzed all of the meat product facility wastewater samples using methods identified in Table A-1. (As explained in Section 7, EPA is proposing to regulate only a subset of these analytes.) EPA generally used either EPA methods from "Methods for Chemical Analysis of Water and Wastes' (MCAWW) or the American Public Health Association's "Standard Methods for the Examination of Water and Wastewater." Table A-1 provides a summary of the analytical methods, the associated pollutants measured by the method, the nominal quantitation levels, and the baseline levels. The following sections provide additional information supporting the summary in Table A-1.

In analyzing samples, EPA generally used analytical methods approved at 40 CFR 136 for compliance monitoring or methods that had been in use by EPA for decades in support of effluent guidelines development. Exceptions for use of non-approved methods are explained in the method-specific subsections that follow Table A-1. Except for nitrate/nitrite, EPA proposed limitations or standards based only upon data generated by methods approved in 40 CFR Part 136. As explained in Section A.4.10, EPA used nitrate/nitrite data from Method 300.0 to develop the proposed limitations and standards for total nitrogen and is proposing the use of Method 300.0 for compliance.

Each of the following sections state whether the method is approved for compliance monitoring in 40 CFR Part 136 (even if the pollutant was not proposed to be regulated), provides a short description of the method, identifies the nominal quantitation limit, and explains EPA's choice for the baseline value. The sections are ordered alphabetically by analyte name within the five categories identified in Section A.1.

Table A-1. Analytical Methods and Baseline Values

Analyte	Method	CAS Number	Nominal Quantitation Value	Baseline Value	Unit
Aeromonas	9260L	C2101	2.0	2.0	/100mL
Ammonia as Nitrogen	350.2	7664417	0.20	0.20	mg/L
Antimony	1620	7440360	20.0	20.0	µg/L
Arsenic	1620	7440382	10.0	10.0	µg/L
Barium	1620	7440393	200.0	200.0	µg/L
Beryllium	1620	7440417	5.0	5.0	µg/L
BOD_5	405.1	C003	2.0	2.0	mg/L
Boron	1620	7440428	100.0	100.0	µg/L
Cadmium	1620	7440439	5.0	5.0	µg/L
Carbonaceous BOD_5	5210	C002	2.0	2.0	mg/L
	405.1	C002	2.0	2.0	mg/L
Carbaryl	632	63252	1.0	1.0	µg/L
COD	410.1	C004	50.0	5.0**	mg/L
	410.2	C004	5.0	5.0**	mg/L
	410.4 (automated)	C004	3.0	5.0**	mg/L
	410.4 (manual)	C004	20.0†		
	5220B	C004	5.0	5.0	mg/L
Chloride	300.0	16887006	0.05	1.0	mg/L
	325.3	16887006	1.0	1.0	mg/L
Chromium	1620	7440473	10.0	10.0	µg/L
cis-Permethrin	1660	61949766	5.0	5.0	µg/L
Cobalt	1620	7440484	50.0	50.0	µg/L
Copper	1620	7440508	25.0	25.0	µg/L
Cryptosporidium	1622	137259508			per_L
Dichlorvos	1657	62737	2.0	2.0	µg/L
Dissolved BOD_5	405.1	C003D	2.0	2.0	mg/L
Dissolved Total Phosphorus	365.2	14265442D	0.01	0.01	mg/L
	365.3	14265442D	0.01	0.01	mg/L
E. coli	9221F	C050	2.0	2.0	/100mL
Fecal Coliform	9221E	C2106	2.0	2.0	/100mL
Fecal Streptococcus	9230B	C2107	2.0	2.0	/100mL
HEM	1664	C036	5.0	5.0	mg/L
Lead	1620	7439921	50.0	50.0	µg/L
Malathion	1657	121755	2.0	2.0	µg/L
Manganese	1620	7439965	15	15	µg/L
Mercury	1620	7439976	0.20	0.20	µg/L
Molybdenum	1620	7439987	10.0	10.0	µg/L

Analyte	Method	CAS Number	Nominal Quantitation Value	Baseline Value	Unit
Nickel	1620	7440020	40.0	40.0	µg/L
Nitrate/Nitrite	300.0	C005	0.01	0.05	mg/L
	353.1	C005	0.01	0.05	mg/L
	353.2	C005	0.05	0.05	mg/L
Salmonella	FDA-BAM	68583357	2.0	2.0	/100mL
Selenium	1620	7782492	5.0	5.0	µg/L
Silver	1620	7440224	10.0	10.0	µg/L
Tetrachlorvinphos	1657	22248799	2.0	2.0	µg/L
Thallium	1620	7440280	10.0	10.0	µg/L
Tin	1620	7440315	30.0	30.0	µg/L
Titanium	1620	7440326	5.0	5.0	µg/L
Total Coliform	9221B	E10606	2.0	2.0	/100mL
Total Dissolved Solids	160.1	C010	10.0	10.0	mg/L
Total Kjeldahl Nitrogen	351.2	C021	0.10	0.5	mg/L
	351.3	C021	0.50	0.5	mg/L
Total Organic Carbon	415.1	C012	1.0	1.0	mg/L
Total Orthophosphate	300.0	C034	0.20	0.01	mg/L
	365.2	C034	0.01	0.01	mg/L
Total Phosphorus	365.2	14265442	0.01	0.01	mg/L
	365.3	14265442	0.01	0.01	mg/L
Total Residual Chlorine	HACH 8167	7782505	0.10	0.20	mg/L
	330.5	7782505	0.20	0.20	mg/L
Total Suspended Solids	160.2	C009	4.0	4.0	mg/L
trans-Permethrin	1660	61949777	5.0	5.0	µg/L
Vanadium	1620	7440622	50.0	50.0	µg/L
Volatile Residue	160.4	C030	10.0	10.0	mg/L
Yttrium	1620	7440655	5.0	5.0	µg/L
Zinc	1620	7440666	20.0	20.0	µg/L

**The baseline value was adjusted to reflect the lowest nominal quantitation limit of the titrimetric procedures (i.e., 410.1, 410.2, and 5220B). See Section A.4.6 for a detailed explanation.

†Method 410.4 lists two different quantitation limits that are dependent upon whether the automated or manual protocols were followed. The automated method limit =3 mg/L and the manual method limit =20 mg/L.

A.4.1 EPA Methods 1660 and 1664 (*cis*-Permethrin, *trans*-Permethrin, HEM)

Laboratories used EPA Method 1660 to measure *cis*-permethrin and *trans*-permethrin, and EPA Method 1664 to measure *n*-hexane extractable material (HEM). While 40 CFR Part 136 lists Method 1664 as an approved method for compliance monitoring of HEM, Part 136 does not list any methods for the pesticides *cis*-permethrin and *trans*-permethrin. However, Table 7 in 40 CFR 455 lists Method 1660 as approved for compliance monitoring of permethrin for the Pesticide Chemicals Point Source Category. (Permethrin is the common name given to any mixture of the two isomers, *cis*-permethrin and *trans*-permethrin.)

These methods use the minimum level (ML) concept for quantitation of the pollutant(s). The ML is defined as the lowest level at which the entire analytical system must give a recognizable signal and an acceptable calibration point for the analyte. When an ML is published in a method, the Agency has demonstrated that the ML can be achieved in at least one well-operated laboratory. When that laboratory or another laboratory uses that method, the laboratory is required to demonstrate, through calibration of the instrument or analytical system, that it can achieve pollutant measurements at the ML.

For *cis*-Permethrin, *trans*-Permethrin, and HEM, EPA used the method-specified MLs as the baseline values. In determining the pollutants of concern and in calculating the HEM standards, if a quantitated value or sample-specific quantitation limit was reported with a value less than the ML specified in the method, EPA substituted the value of the ML and assumed that the measurement was not quantitated. For example, for *cis*-permethrin with an ML of 5 µg/L, if the laboratory reported a quantitated value of 3 µg/L, EPA would have assumed that the concentration was not quantitated[4] with a sample-specific quantitation limit of 5 µg/L. The objective of this comparison was to identify any results for the three pollutants reported below the method-defined ML. Results reported below the ML were changed to the ML to ensure that all results used by EPA were reliable. In most cases, the quantitated values and sample-specific quantitation limits were equal to or greater than the baseline values.

[4] As explained in Appendix C, EPA applied different statistical assumptions to quantitated and non-quantitated results.

A.4.2 EPA Method 1620 (Metals)

Laboratories used EPA Method 1620 to measure the concentrations of 21 metals. While EPA Method 1620 is not listed in 40 CFR Part 136 as an approved method for compliance monitoring, it represents a consolidation of the analytical techniques in several 40 CFR 136-approved methods such as EPA Method 200.7 (inductively coupled plasma atomic emission (ICP) spectroscopy of trace elements) and Method 245.1 (mercury cold vapor atomic absorption technique). This method was developed specifically for the effluent guidelines program. EPA Method 1620 includes more metal analytes than are listed in the approved methods and contains quality control requirements at least as stringent as the 40 CFR Part 136-approved methods.

EPA Method 1620 employs the concept of an instrument detection limit (IDL). The IDL is defined as "the smallest signal above background noise that an instrument can detect reliably."[5] Data reporting practices for EPA Method 1620 analyses follow conventional metals reporting practices used in other EPA programs, in which values are required to be reported at or above the IDL. In applying EPA Method 1620, IDLs are determined on a quarterly basis by each analytical laboratory and are, therefore, laboratory-specific and time-specific. Although EPA Method 1620 contains MLs, these MLs pre-date EPA's recent refinements of the ML concept described earlier. The ML values associated with EPA Method 1620 are based on a consensus opinion reached between EPA and laboratories during the 1980s regarding levels that could be considered reliable quantitation limits when using EPA Method 1620. These limits do not reflect advances in technology and instrumentation since the 1980s. Consequently, the IDLs were used as the lowest values for reporting purposes, with the general understanding that reliable results can be produced at or above the IDL. Though the baseline values were derived from the MLs (or adjusted MLs) in EPA Method 1620, EPA used the laboratory-reported quantitated values and sample-specific quantitation limits, which captured concentrations down to the IDLs, in its data analyses.

[5] Keith, L.H., W. Crummett, J. Deegan, R.A. Libby, J.K. Taylor, G. Wentler (1983). "Principles of Environmental Analysis," Analytical Chemistry, Volume 55, Page 2217.

In general, EPA used the MLs specified in Method 1620 as the baseline values. However, EPA adjusted the baseline value for lead to 50 µg/L and boron to 100 µg/L. In EPA Method 1620, lead has an ML of 5 µg/L for graphite furnace atomic absorption (GFAA) spectroscopy analysis; EPA determined, however, that it was not necessary for the laboratories to measure down to such low levels, and that lead could be analyzed by inductively couple plasma atomic emission (ICP) spectroscopy.[6] Consequently, the ML requirement was adjusted to 50 µg/L, the ML for the ICP method. In EPA Method 1620, boron has an ML of 10 µg/L, but laboratory feedback years ago indicated that laboratories could not reliably achieve this low level. As a result, EPA only required laboratories to measure values at 100 µg/L and above. Thus, EPA adjusted the baseline value to 100 µg/L.

A.4.3 Method 350.2 (Ammonia as Nitrogen)

Ammonia as nitrogen was measured using Method 350.2, which is listed as approved for compliance monitoring in 40 CFR Part 136. Method 350.2 utilizes either colorimetric, titrimetric, or electrode procedures to measure ammonia.

Method 350.2 has a lower measurement range limit of 0.20 mg/L for the colorimetric and electrode procedures, and a lower measurement range limit of 1.0 mg/L for the titrimetric procedure. Rather than use different baseline values for the same pollutant, EPA used the 0.20 mg/L because it represented a value at which ammonia as nitrogen can be measured reliably by several determinative techniques in Method 350.2, as well as in other methods approved at 40 CFR 136.

A.4.4 Methods 405.1 and SM5210B (BOD_5, Carbonaceous BOD_5, and Dissolved BOD_5)

Biochemical Oxygen Demand (BOD_5), Carbonaceous BOD_5 ($cBOD_5$), and Dissolved BOD_5 were measured using Method 405.1 and Standard Method (SM) 5210B, both of which are approved for compliance monitoring in 40 CFR Part 136. BOD_5 and $cBOD_5$ are essentially the

[6] Also antimony, arsenic, selenium, and thallium were analyzed by ICP instead of GFAA. The method MLs were used because the laboratories demonstrated that their IDLs were able to quantitate below the ML for these four analytes.

same method, except an organic compound is added to the $cBOD_5$ test to inhibit nitrogenous oxygen deemand. If the sample does not include any nitrogenous demand to inhibit, the results should be comparable for BOD_5 and $cBOD_5$. BOD_5 and dissolved BOD_5 are the same method, except that the dissolved BOD_5 sample is filtered prior to analysis (either in the field or immediately upon receipt by the laboratory).

Method 405.1 and SM5210B are identical and the nominal quantitation limit, which is expressed in the methods as the lower limit of the measurement range at 2 mg/L, is the same for all three forms of BOD_5. EPA used this nominal quantitation limit of 2 mg/L as the baseline value in determining the pollutants of concern.

A.4.5 EPA Method 632 (Carbaryl)

Carbaryl was determined by EPA Method 632. There are no methods approved in 40 CFR Part 136 for carbaryl. However, Method 632 is approved for compliance monitoring of carbaryl for the Pesticide Chemicals Point Source Category (see Table 7 in 40 CFR Part 455).

In this method, samples are prepared by liquid-liquid extraction with methylene chloride in a separatory funnel. The extract is analyzed by a high-pressure liquid chromatograph with a UV detector. The nominal quantitation limit was determined by a low-point calibration standard. The nominal quantitation limit for carbaryl is 1 μg/L and was used as the baseline value.

A.4.6 Methods 410.1, 410.2, 410.4, and SM5220B (Chemical Oxygen Demand)

Chemical Oxygen Demand (COD) was measured using Methods 410.1, 410.2, 410.4, and SM5220B, of which Methods 410.1, 410.2, and 410.4 are approved for compliance monitoring in 40 CFR Part 136. Methods 410.1 and 410.2 are titrimetric procedures that follow identical analytical protocols, but differ only in the range of COD concentration that they are designed to measure. Reagent concentrations and sample volumes are adjusted to accommodate a wide range of sample concentrations, since the dynamic range of the chemistry used to detect COD is somewhat limited. Standard Method 5220B is a titrimetric method that incorporates the different reagent concentrations and sample volumes listed in Methods 410.1 and 410.2 into one method.

Data from all three of these methods are directly comparable. Method 410.4 is a colorimetric procedure.

Method 410.1 is designed to measure mid-level concentrations (greater than 50 mg/L) of COD and is associated with a nominal quantitation limit of 50 mg/L. Method 410.2 is designed to measure low-level concentrations of these parameters in the range of 5-50 mg/L. Method 410.4 has a measurement range of 3-900 mg/L for automated procedures and measurement range of 20-900 mg/L for manual procedures. EPA contracts required that laboratories measure down to the lowest quantitation limit possible for whatever method is used. Therefore, if the laboratory analyzes a sample using Method 410.1 and obtains a non-quantitated result, it must reanalyze the sample using Method 410.2. Thus, the quantitation limit reported for non-quantitated results was equal to 5 mg/L, unless sample dilutions were required for matrix complexities.

For all COD data, EPA used the baseline value of 5 mg/L that is associated with the lower quantitation limit for the titrimetric procedures because most of the data used to determine the pollutants of concern had been obtained by the titrimetric procedures (i.e., Methods 410.1, 410.2, or SM5220B).

A.4.7 Methods 325.3 and 300.0 (Chloride)

Chloride was measured using Methods 325.3, which is approved for compliance monitoring in 40 CFR Part 136, and 300.0, which is not listed in Part 136. Method 325.3 is a colorimetric (actually titrimetric) procedure and measures concentrations greater than 1 mg/L. Method 300.0 uses ion chromatography and can measure down to 0.05 mg/L. EPA allowed laboratories to use Method 300.0 even though it is not approved at 40 CFR Part 136 because the analytical methods normally used for chloride are subject to interferences sometimes present in samples containing blood, animal tissue, and/or other particulates. With Method 300.0, the complex matrices are not a factor and this method has a lower nominal quantitation limit than Method 325.1. (Section A.4.10 provides a more detailed description of Method 300.0.)

For all chloride data, EPA used the baseline value of 1 mg/L that is associated with the higher quantitation limit for the colorimetric procedure because most of the data used in the

pollutants of concern analysis had been obtained by the colorimetric procedure (i.e., Method 325.3).

A.4.8 EPA Method 1657 (Dichlorvos, Malathion, Tetrachlorvinphos)

Laboratories used Method 1657 to measure dichlorvos, malathion, and tetrachlorvinphos concentrations in the samples. There is one approved method for malathion at 40 CFR Part 136: Standard Method 6630C; however, the other two pesticides are not listed in 40 CFR Part 136. EPA Method 1657 was selected for analysis of all three pesticides for several reasons, including:

- Method 1657 is approved for compliance monitoring of all three pesticides for the Pesticide Chemicals Point Source Category (see Table 7[7] in 40 CFR 455).
- EPA 1600-series methods were developed specifically for the effluent guidelines program; therefore, they have more stringent quality control requirements than Standard Methods; and
- It was more economical to use one method for the three pesticides, rather than analyzing malathion separately by SM6630C.

In Method 1657, samples are prepared by liquid-liquid extraction. The extract is dried and concentrated and a 1-µL aliquot of the extract is injected into the gas chromatography. The nominal quantitation limit of 2 µg/L was used as the baseline value for all three pesticides. This nominal quantitation limit was determined from the results of low-point calibration standards.

A.4.9 Methods 365.2 and 365.3 (Dissolved Total Phosphorus and Total Phosphorus)

Dissolved total phosphorus and total phosphorus were measured using Method 365.2 and 365.3, respectively. Both methods are approved for compliance monitoring of total phosphorus in 40 CFR Part 136. Total phosphorus represents all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure.

[7] Table 7 lists tetrachlorvinphos as stirofos.

The two methods differ only in the preparation of one of the reagents. Method 365.2 specifies the separation of the ammonium molybdate and the antimony potassium tartrate from the ascorbic acid reagent. Method 365.3 allows combining these reagents into a single solution. Because the chemistry is unaffected, the data are directly comparable.

These methods have the same nominal quantitation limit of 0.01 mg/L for both analytes. EPA used this value as the baseline value for both dissolved total phosphorus and total phosphorus.

A.4.10 Methods 300.0, 353.1, and 353.2 (Nitrate/Nitrite)

Nitrate/nitrite was measured using Methods 300.0, 353.1, and 353.2. Methods 353.1 and 353.2 are approved for compliance monitoring in 40 CFR Part 136, while Method 300.0 is not listed in Part 136. However, because nitrate/nitrite is a component of total nitrogen (see Section A.5), EPA is proposing to approve EPA Method 300.0 at 40 CFR Part 432 for compliance monitoring of nitrate/nitrite. Alternatively, EPA may amend 40 CFR Part 136 to include Method 300.0 for determination of nitrate/nitrite from wastewaters in the meat and poultry products point source category. In the preamble to the proposed rule, EPA has requested comment on the use of this method for the meat and poultry point source category and whether the method should be approved at 40 CFR Part 432 or at 40 CFR Part 136 or both.

Many of the analytical methods for nitrite/nitrate that are currently approved at 40 CFR Part 136, including Methods 353.1 and 353.2, are based on colorimetric techniques (i.e., adding reagents to a sample that form a colored product when they react with the nitrate/nitrite and measuring the intensity of the colored product). Such methods can be subject to interferences in the difficult matrices associated with this industry where samples may contain blood, animal tissue, and/or other particulates which affect both the color development and ability to pass light through the sample to measure the intensity of the colored product. In contrast, Method 300.0 employs the technique known as ion chromatography to measure 10 inorganic anions, including nitrate and nitrite. Ion chromatography permits the various inorganic anions to be separated from one another, as well as from other materials and contaminants present in the sample. Each anion can be identified on the basis of its characteristic retention time (the time required to pass

through the instrumentation). After separation, the anions are measured by a conductivity detector that responds to changes in the effluent from the ion chromatograph that occur when the negatively charged anions (analytes) elute at characteristic retention times, thereby changing the conductivity of the solution. Thus, Method 300.0 offers better specificity for nitrate and nitrite in the presence of interferences compared to the approved colorimetric methods. Method 300.0 is located in the rulemaking record (Docket No. W-01-06, Record No.10036).

Methods 353.1 and 353.2 are essentially the same method, with variations in the technique used to reduce the nitrite (NO_2) present in the sample to nitrate (NO_3). Method 353.1 uses hydrazine to accomplish the reduction, while 353.2 uses cadmium granules. Method 353.2 is generally preferred simply because the cadmium granules are far easier to handle and less toxic than hydrazine. The chemistry of the colorimetric determination is the same, as are the interferences.

Each of the three methods lists slightly different nominal quantitation limits that are expressed in the methods as the lower limit of the measurement range. The nominal quantitation limit for Method 353.1 is 0.01 mg/L and the nominal quantitation limit for Method 353.2 is 0.05 mg/L. Rather than use different baseline values for the same pollutant, EPA used the nominal quantitation limit of 0.05 mg/L from Method 353.1 as the baseline value for nitrate/nitrite. EPA chose this value because Method 353.1 was used to obtain most of the data used in the pollutants of concern analysis. It is also the maximum of the nominal quantitation limits from the three methods.

A.4.11 Method 160.1 (Total Dissolved Solids)

Total Dissolved Solids (TDS) was measured by Method 160.1, which is approved for compliance monitoring in 40 CFR Part 136 (see 'residue – filterable'). Method 160.1 is a gravimetric method with a lower limit measurement range of 10 mg/L. EPA used this nominal quantitation limit of 10 mg/L as the baseline value.

A.4.12 Methods 351.2 and 351.3 (Total Kjeldahl Nitrogen (TKN))

Total Kjeldahl nitrogen (TKN) was measured by Methods 351.2 and 351.3, both of which are approved for compliance monitoring in 40 CFR Part 136. Method 351.2 is designed to be used with a flow colorimetry apparatus with a lower measurement range limit of 0.1 mg/L. Method 351.3 is a manual colorimetric analysis that has a lower measurement range limit of 0.5 mg/L. Rather than use different baseline values for the same pollutant, EPA used the nominal quantitation limit of 0.05 mg/L from Method 351.3 as the baseline value for TKN. EPA chose this value because Method 351.3 was used to obtain most of the data used in the pollutants of concern analysis. It is also the maximum of the nominal quantitation limits from the two methods.

A.4.13 Method 415.1 (Total Organic Carbon (TOC))

Total organic carbon (TOC) was determined by Method 415.1, which is approved for compliance monitoring in 40 CFR Part 136. Method 415.1 is a combustion (or oxidation) method with a lower measurement range limit of 1 mg/L. EPA used this nominal quantitation limit of 1 mg/L as the baseline value.

A.4.14 Methods 365.2 and 300.0 (Total Orthophosphate)

Methods 365.2 and 300.0 were used to measure orthophosphate concentrations. Total orthophosphate is the inorganic phosphorus (PO_4) in the sample. Method 365.2 is approved for compliance monitoring of total orthophosphate in 40 CFR Part 136, while Method 300.0 is not. As explained previously (see Sections A.4.7 and A.4.10), EPA allowed laboratories to use Method 300.0 because interferences, sometimes present in samples containing blood, animal tissue, and/or other particulates, are not a factor in the analysis.

Method 365.2 is a colorimetric method for determining orthophosphate and measures concentrations greater than 0.01 mg/L. Method 300.0 uses ion chromatography and can measure down to 0.20 mg/L. For all orthophosphate data, EPA used the baseline value of 0.01 mg/L, that is associated with the lower quantitation limit for the colorimetric procedure because the

laboratories used Method 365.2 to produce the majority of the data used in the pollutants of concern analysis.

A.4.15 Methods HACH 8167 and 330.5 (Total Residual Chlorine)

Total residual chlorine was determined by Methods 330.5 and HACH 8167. Method 330.5 is approved for compliance monitoring in 40 CFR Part 136. Methods 330.5 and HACH 8167 use the same colorimetric reagent, N,N-diethyl-p-phenylene diamine (DPD), and are essentially the same procedure; thus, the data are directly comparable.

The nominal quantitation limit in Method 330.5 is 0.2 mg/L; the nominal quantitation limit for method HACH 8167 is 0.1 mg/L. Rather than use two different baseline values for the same pollutant, EPA used the value associated with Method 330.5 (i.e., 0.2 mg/L) as the baseline value because Method 330.5 produced the majority of the data used in the pollutants of concern analysis. It also is the higher of the two values.

A.4.16 Method 160.2 (Total Suspended Solids)

Total suspended solids (TSS) was determined by Method 160.2, which is approved for compliance monitoring in 40 CFR Part 136. Method 160.2 is a gravimetric method with a lower limit measurement range of 4 mg/L. The nominal quantitation limit of 4 mg/L was used as the baseline value.

A.4.17 Method 160.4 (Volatile Residue)

Volatile residue was determined by Method 160.4, which is approved for compliance monitoring in 40 CFR Part 136. Method 160.4 is a gravimetric and ignition method with a lower limit measurement range of 10 mg/L. The nominal quantitation limit of 10 mg/L was used as the baseline value.

A.4.18 EPA Method 1622 (*Cryptosporidium*)

Cryptosporidium was determined by EPA Method 1622, which, as explained in Section A.1, has not been approved for compliance monitoring. There are no 40 CFR Part 136-approved

methods for *Cryptosporidium*; however, EPA proposed Method 1622 for ambient water monitoring on August 30, 2001 (66 FR 169, pages 45811-45829). In Method 1622, the laboratory filters a 10-L sample through an absolute-porosity filter to capture any target organisms that may be present, elutes the filter, concentrates the eluate, purifies the concentrate using immunomagnetic separation, and applies the purified sample to a microscope slide. The purified sample is stained with an antibody stain and a vital dye stain, and target organisms are identified and counted based on immunofluorescence assay, differential interference microscopy, and vital dye staining characteristics.

Due to the high turbidity of the sample matrices for these episodes, it was necessary for the analytical laboratory to modify the sample processing steps of the method, depending on the nature of the particulates in the sample. For samples that contained a high concentration of biological particles, a small volume of the sample (100 - 250 mL) was concentrated using centrifugation and then processed according to EPA Method 1622. For samples with lower concentrations of biological particulates that could be filtered, a 10-L sample was filtered through a compressed foam filter, the filter was eluted, and the eluate was concentrated by centrifugation and then processed according to EPA Method 1622.

As explained earlier, there is no detection limit or baseline value associated with EPA Method 1622; however, EPA used the baseline value of zero in the pollutant of concern analysis. Further, if *Cryptosporidium* was not quantitated, the sample was reported as zero.

A.4.19 SM9221B, SM9221E, SM9221F, SM9230B, SM9260L, FDA-BAM Chapter 5 (total coliform, fecal coliform, *E. coli*, fecal Streptococcus, *Aeromonas*, *Salmonella*)

Laboratories measured the densities of total coliform, fecal coliform, *E. coli*, fecal Streptococcus, *Aeromonas*, and *Salmonella* in 100-mL samples using the multiple-tube fermentation test specified in Standard Methods. EPA used methods approved for compliance monitoring in 40 CFR Part 136 for total coliform (SM9221B), fecal coliform (SM9221E), and fecal streptococcus (SM9230B). There are no 40 CFR Part 136-approved methods for *E.coli*,

Aeromonas, and *Salmonella*; however, EPA proposed ambient water monitoring methods for *E.coli* on August 30, 2001 (66 FR 169, pages 45811-45829).

In measuring total coliforms (SM 9221B), fecal coliforms (SM 9221E), and *E. coli* (SM 9221F), samples were inoculated into a presumptive medium (Lauryl tryptose broth) and incubated. Tubes positive for growth and gas production were transferred into confirmatory media: brilliant green bile broth (for total coliforms), EC (for fecal coliforms), or EC-MUG (for *E. coli*). Tubes with acidic growth and gas production in their respective media were recorded as positive.

In measuring fecal streptococcus (SM 9230B), samples were inoculated into a presumptive medium (azide dextrose broth) and incubated. Tubes positive for turbidity (growth) were confirmed by streaking onto bile esculin agar plates. All plates with typical growth were recorded as positive for fecal streptococcus.

Aeromonas densities were determined using SM9260L, followed by the confirmation steps in EPA Method 1605, to minimize false positive results. Samples were inoculated into a presumptive medium (TSB30) and incubated. Tubes with growth were streaked onto ADA. All yellow colonies were isolated on nutrient agar and confirmed as Aeromonas if they were oxidase positive and were able to ferment trehalose. In addition to the biochemical confirmation, colony morphologies from ADA and nutrient agar were recorded and used to differentiate between Aeromonas and Bacillus.

The Food and Drug Administration-Biological Analytical Manual (FDA-BAM) Chapter 5 method was used to determine *Salmonella* densities. Samples were inoculated into a presumptive medium (tetrathionate broth) and incubated. Tubes with growth were streaked onto Hektoen enteric agar plates. Typical colonies were confirmed on triple sugar iron agar slants. The FDA-BAM method was used instead of the approved EPA Kenner-Clark method because FDA-BAM method performance is better suited for samples that contain blood and particulates.

The nominal quantitation limit for these analytes was determined using the most probable number (MPN) approach specified in Standard Methods. The MPN of each target organism per

100 mL was calculated based on the positive and negative results from the analysis of multiple replicates at multiple dilutions for each sample (see Table 9221.IV of Standard Methods and Table 2 in Appendix 2 of FDA-BAM). Based on the tables in Standard Methods, the nominal quantitation limit for all analytes was 2 MPN per 100 mL. The nominal quantitation limit was used as the baseline value. No values were reported below the baseline value.

Table II in 40 CFR 136.3 specifies holding times of six hours for some pathogens. In its sampling for this proposed rule, EPA measured counts in samples that had been retained longer than the six hours specified in Table II. In its data review narratives (located in Section 6.1.4.2 of the administrative record for the proposal), EPA has identified those samples that were retained longer than eight hours at the laboratory (includes the six hours holding time allotted for delivery to the laboratory plus an additional two hours at the laboratory). Method 9221E, an approved method[8] for fecal coliform, states that "Water treatment and other adverse environmental conditions often place great stress on indicator bacteria, resulting in an extended lag phase before logarithmic growth takes place." EPA is currently conducting a holding time study to assess potential changes in pathogen concentrations in effluents over time (8, 24, 30, and 48 hours after sample collection). This study will evaluate total and fecal coliforms, *Escherichia coli*, Aeromonas species, and fecal streptococci for both the meat products and aquaculture industries effluents. Additionally, Salmonella will be analyzed in meat products effluents. EPA is conducting this holding time study for two purposes: to evaluate the use of data in developing the limitations and standards; and for possible revisions to Table II. EPA notes that if the holding time can be extended to longer periods, overnight shipping of samples would be possible for compliance monitoring. However, EPA has not proposed any new limitations and standards for these analytes. Rather, EPA plans to retain the current limitations and standards for fecal coliform. The study plan for the holding time study is located at DCN 15060 in Section 6.1.4 of the administrative record for the proposal. In the forthcoming NODA, EPA will provide the data collected during the study and its evaluation of the results.

[8] Per Table IA of 40 CFR 136.3.

A.5 TOTAL NITROGEN

EPA proposes to regulate total nitrogen to ensure that the relationship between organic nitrogen (estimated by TKN) and inorganic nitrogen (estimated by nitrate/nitrite) is maintained, thus EPA is defining for the purposes of this industry 'total nitrogen' to be the sum of nitrate/nitrite and TKN. This summation will include nitrogen in the trinegative oxidation state (the dominant oxidation state of nitrogen in organic compounds), ammonia-nitrogen, and nitrogen in nitrite (NO_2^-) and nitrate (NO_3^-). In developing the limitations (see Section 13), EPA used a baseline value of 0.1 mg/L which is the sum of the baseline values for nitrate/nitrite (0.05 mg/L) and TKN (0.05 mg/L).