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June 8, 2018

Mr. Brian d'Amico
Branch Chief
Engineering and Analysis Division
Office of Science and Technology
Office of Water
United States Environmental Protection Agency
Mail Code 4303 T
1200 Pennsylvania Avenue Northwest
Washington, DC 20460

Dear Mr. D'Amico:

On behalf of our members, the American Petroleum Institute (API) and American Fuel and Petrochemical Manufacturers (AFPM) are providing the following update and comments concerning the Environmental Protection Agency's (EPA's) on-going Detailed Study of effluent limitation guidelines (ELGs) for the petroleum refining point source category. API is a nationwide, non-profit, trade association that represents over 625 members engaged in all aspects of the petroleum and natural gas industry, including exploration, production, refining, and distribution of petroleum products. AFPM is a national trade association representing nearly 400 companies that encompass virtually all U.S. refiners and petrochemical manufacturers. AFPM members operate 120 U.S. refineries comprising more than 95 percent of U.S. refining capacity. API and AFPM members are subject to effluent limitation guidelines, including those in the petroleum refining point source category, and so are directly affected by all aspects of the on-going Detailed Study.

We appreciate the cooperative and trusted relationship cultivated over the last several years we have worked together on the Detailed Study. As we have discussed on multiple occasions, API and AFPM members have invested heavily in wastewater treatment technologies where warranted for addressing local water quality concerns. API and AFPM believe EPA has sufficient data, including discharge monitoring reports, toxic release inventories, site visit reports, and the 308 Questionnaire responses, to determine that the existing effluent limitation guideline technology-based limits (TBELs), taken in combination with water-quality-based effluent limits (WQBELs), are protective of human health and the environment, and that revisions to existing petroleum refining TBELs are not warranted. We request EPA analyze the aforementioned discharge monitoring reports, toxic release inventories, site visit reports, and the 308 questionnaire responses, to inform whether it is necessary to proceed with the refinery self-

monitoring program. We believe EPA upon doing so will agree that the data support the conclusion that ELG revisions are not warranted.

If EPA determines the refinery self-monitoring program is justified, EPA should narrowly tailor the program to filling gaps in the available data. Also, EPA should remove naphthenic acids (NAs) and alkylated polynuclear aromatic hydrocarbons (alkylated-PAHs) from the scope of the sampling phase. While we have yet to receive EPA's preliminary analysis, we do appreciate the responsive nature by which EPA shared documentation for the analytical method(s) for alkylated-PAHs and NAs. That said, after thorough and critical review of the documentation by leading industry experts, our members' concerns (detailed in Attachment A) are not resolved. API and AFPM membership strongly oppose inclusion in the Detailed Study of the proprietary analytical method for naphthenic acids and the non-promulgated method for alkylated-PAHs. Data derived from these methods could result in the EPA facing substantial scientific and legal challenge.

Moreover, EPA's use of the proprietary method for naphthenic acids is in clear contradiction to EPA's recent proposed rule to strengthen transparency in regulatory science (83 Fed. Reg. 18768, April 30, 2018, "Strengthening Transparency in Regulatory Science"). The summary of EPA's proposed rule states, "The proposed regulation provides that when EPA develops regulations, including regulations for which the public is likely to bear the cost of compliance, with regard to those scientific studies that are pivotal to the action being taken, EPA should ensure that the data underlying those are publicly available in a manner sufficient for independent validation." Independent validation is clearly not possible when a proprietary analytical method is used to generate the data. In the interest of transparency, per its own proposed rule, EPA should abandon the use of this proprietary method in the Detailed Study.

API's and AFPM's remaining concerns are summarized as follows:

A. Analysis of collected data

EPA has yet to share preliminary analysis of existing data, including discharge monitoring reports, toxic release inventories, site visits, and the 308 Questionnaire responses. Sharing the analysis will clarify the necessity and scope of the sampling phase as well as attain early scientific concurrence with stakeholders. Analysis of existing data should be complete before EPA moves forward with additional data collection through the self-monitoring program.

B. Method not proved in analysis of refinery wastewaters

The method developed by Axys Laboratories, intended for use for analysis of samples in the Study, has never been tested on refinery wastewaters. The documentation provided by EPA suggests that interferences in complex matrices (e.g., refinery wastewaters and effluent), may impact data quality, giving rise to highly variable data, including false positive and/or negative results.

C. Proprietary method impairs validity of data

The proposed analytical method for naphthenic acids is neither an EPA-approved nor an industry-adopted method. In fact, it is Axys Laboratories' proprietary method which directly prevents our members from validating, evaluating or replicating any results. This is a deviation from past EPA procedures and provides neither sufficient transparency nor scientific validity to the Study.

D. Absence of documented environmental benefits

EPA has not identified the environmental concern for including NAs and alkylated-PAHs in the Study. As per the well-established procedures used in past effluent guideline studies, constituents should have an associated toxicity to determine the measurable environmental benefit that may result, if removed. The science and data for the toxicity of NAs and alkylated-PAHs are still a work in progress.

In this regard, we note that of the naphthenic acids and alkylated-PAHs that would be analyzed by the prescribed methods, the vast majority of specific compounds within these mixtures are of a size that could not cross biological membranes to cause toxicity. Typically, compounds with log octanol:water partition coefficients exceeding 6.4 are excluded from toxicity assessments by the target lipid model approach. Quantifying these analytes within "total NAs" or "total alkylated-PAHs" introduces error/bias.

EPA should make available API/AFPM for our review any petroleum refinery toxicity identification evaluation (TIE) data demonstrating naphthenic acid and/or alkylated-PAH toxicity constituting the basis for inclusion of these broad classes of analytes within the Detailed Study.

API and AFPM members believe in due diligence and support EPA in developing sound science. We therefore strongly recommend that EPA remove naphthenic acids and alkylated-PAHs from the Detailed Study. Rather, we recommend that these constituents and their analytical methods be addressed in a project outside of the Study, in which the industry will be a willing participant. A separate project would also allow EPA to follow the appropriate public notice and comment period required to gain method approval. API and AFPM will be happy to discuss the concerns and suggestions in a face-to-face meeting and come to an agreement that addresses the need for validated, reproducible science in support of environmental goals.

In summary, API/AFPM believe refining ELG revisions are not warranted. If EPA continues the Detailed Study, EPA should narrowly tailor the refinery self-monitoring program to filling gaps in the available data. And API/AFPM strongly recommend EPA remove naphthenic acids and alkylated PAHs from the Detailed Study. API/AFPM would participate with EPA in a project outside the Detailed Study to address analytical methods for naphthenic acids and alkylated PAHs.

If you have any questions about these concerns or would like to arrange a face-to-face meeting, please feel free to contact us.

Sincerely,

Roger E. Claff
Senior Scientific Advisor, API

Jeff Gunnulfsen
Director, Security and Risk Management Issues, AFPM

Attachment

cc: R. Wood, EPA
D. Ross, EPA
L. Forsgren, EPA

Attachment A - Report to API and AFPM on Issues with the EPA Proposed Analytical Methods for Groups of Naphthenic Acids and alkylated-PAHs, and the Potential Impact on an ELG Investigation

Introduction

The American Petroleum Institute and American Fuel and Petrochemical Manufacturers (API/AFPM) received a number of documents from the U.S. Environmental Protection Agency (EPA) concerning experimental methods used by AXYS Laboratories for the analysis of naphthenic acids (NAs) and alkylated polynuclear aromatic hydrocarbons (PAHs). Two documents were brief method summaries of the laboratory's analytical procedures. Also included in these documents were Inter-laboratory studies involving these two analytical methods. API/AFPM has examined these documents in considerable detail, and has a number of concerns about these methods, as described in the following report. Our overall conclusions are that these methods are currently highly experimental and should not be used to evaluate refinery wastewater or develop wastewater regulations for the refinery industry.

I. Summary of Issues

1. The AXYS method for naphthenic acids is proprietary to AXYS. As such, EPA did not and could not provide the method procedures for review and comment. EPA intends to require use of the AXYS naphthenic acids method in the petroleum refining detailed study refinery self-monitoring program, notwithstanding the method is proprietary to AXYS. This intention is in clear contradiction to EPA's recent proposed rule to strengthen transparency in regulatory science (83 Fed. Reg. 18768, April 30, 2018, "Strengthening Transparency in Regulatory Science). The summary of EPA's proposed rule states, "The proposed regulation provides that when EPA develops regulations, including regulations for which the public is likely to bear the cost of compliance, with regard to those scientific studies that are pivotal to the action being taken, EPA should ensure that the data underlying those are publicly available in a manner sufficient for independent validation." Independent validation is clearly not possible when a proprietary analytical method is used to generate the data. If EPA seeks transparency, per its own proposed rule, EPA will abandon the use of this proprietary method in the petroleum refining detailed study.

2. The exact definitions of compounds to be included in both the naphthenic acid compound and alkylated PAH compound groups are still not decided, and the analytical lists for each vary widely. In the Environment Canada Inter-laboratory Study on Alkylated PAHs, part of the conclusion states: "This first assessment of the current state of the PAH and alkyl-PAH analysis of environmental samples was rather ambitious. Over 100 separate measurands were asked to be reported in 3 separate matrices. Future studies will focus on a target list more closely approximating the one found in ASTM D7363-11." They also stated they should focus on one matrix per study. This is a concession that the analytical method is unwieldly and matrix

effects are poorly understood, and the reported quantitative results for many of the PAH homologs were extremely poor.

3. For the NAs, Environment Canada is promoting the concept that aromatic naphthenic acids should be included in the "total naphthenic acids" analytical categories. The aromatic NAs are not currently included in the category, and API/AFPM strongly opposes their inclusion. If they were included with other NAs, this would imply that the toxicological and physical-chemical properties of aromatic NAs are basically the same as the properties for the NAs with no aromatic rings in their structure, and this comparability is not known or understood at this time. To determine this, a dependable and vetted method must be developed to analyze aromatic NAs as separate entities, so that their properties can be determined. There currently is no EPA peer reviewed and approved method for either the non-aromatic or aromatic NA categories.

4. The summary AXYS Analytical Method for NAs provided by EPA (the version was dated February 15, 2018) is an extremely complex and detailed method that attempts to separate the NAs in aqueous samples into 60 different categories of compounds. API/AFPM has concerns about several specific issues, some of which may have been overlooked in the necessarily abbreviated AXYS summary overview of the method. Some of our concerns and reservations are discussed below. All of these concerns and others are discussed in the full report.

   - The calibration curve for all sixty categories of naphthenic acid compounds is only provided by a single compound: 1-pyrenebutyric acid, which does not even qualify as a naphthenic acid due to the aromatic rings in its side chain. Further, 1-pyrenebutryic acid is used to generate response factors for the quantification of target compounds. Using a single compound to calibrate perhaps a hundred compounds, without evaluation of consideration of the various structural groups, will result in response factors orders of magnitude apart and will generate a highly biased data set.

   - The summary method states that several of the sixty categories either can or do contain some aromatic NAs, particularly in categories where the "z value" equals minus ten or minus twelve. It is unclear if the method can recognize which compounds are aromatic, but it appears the answer may be no, because otherwise they could be subtracted out from the total for each group. It is also unclear whether additional aromatic compounds may be present in some of the other analytical groups but cannot be detected as such by molecular weight.

   - The summary provides no discussion, for example, of the QC controls on the completeness of the derivatization reaction. We are concerned that di- or tri-carboxylic acids might get counted if only one carboxyl group is derivatized, while mono-carboxylic acids might be missed. Conversely, if two or three carboxylic acid groups per molecule do get derivatized, could molecular weight (MW) fragments of an original di- or tri-carboxylic acid be mistaken for some of the mono-carboxylic acids that are the intended analytical target?

- We note that for at least two of the chromatograms depicted on page six, there seems to be significant interfering overlap of some peaks within the same molecular weight. We are concerned that the interference could be many times greater for actual refinery wastewater, and that these interferences might be "double-counted" in any final total result, especially in highly complex wastewater matrices.

5. For naphthenic acids, the two Inter-laboratory Studies provided by EPA from Environment Canada did not provide any comparison of the analyses of different categories of naphthenic acids. The quantitative assessment was limited only to "total naphthenic acids" and included analyses by several different methods. For total NAs, the AXYS laboratory was evaluated with a somewhat high overall recovery for total NA (115-120%), which was typical of the labs using some form of liquid chromatography/mass spectroscopy (LC/MS) method in this study. (We are again concerned whether in more complex wastewater samples, this slight high bias might be much higher.) Given the dates of these studies (2012 and 2016), it is unclear whether the version of the AXYS Method (dated 2/15/18) described in the summary provided by EPA/AXYS was the same version as used for these two earlier studies.

6. Conclusion Number 8 for the 2016 Naphthenic Acid Inter-laboratory Study stated the following: "The complexity of the background matrix needs to be increased further. The synthetic toxicity testing matrix is suitable for method validation purposes but future inter-laboratory studies should use a natural water matrix for all samples." API/AFPM agrees that this is needed, and has stated that actual refinery samples, especially untreated wastewater samples, can greatly complicate the analytical process for many well established methods, let alone experimental procedures currently being developed.

7. EPA provided one Inter-laboratory Study for Alkylated PAHs. Most of the laboratories performed quite well on the traditional single-compound PAHs, with on average about a 22% Relative Target Standard Deviation (RTSD) per compound for aqueous samples. However, the story was entirely different for the alkyl-PAH homolog groups. For aqueous samples, the average RTSD was extremely large at 80%, with some PAH homolog groups being well over 100% RTSD. If the standard data acceptance criterion of plus or minus three standard deviations is applied to this data, it is difficult to describe the analysis of these PAH homologs as being even semi-quantitative. The literature documents errors associated with EPA 8270, resulting in overestimation of alkylated PAH concentrations (Wilton et al. *Analytica Chimica Acta* 977 (2017), pp. 20-27).

8. We are also concerned about how toxic weighting factors (TWF) might be developed and applied to analytical groups or subgroups (such as naphthenic acids or alkylated PAH compounds) that could include hundreds of different compounds. Typically, toxicity testing is performed using pure individual compounds; this assures that during toxicity testing, the

source of any toxicity can be attributed to that specific compound. We are concerned that for large groups of unidentified compounds, any perceived TWF observed during toxicity testing could be due to a very few compounds that are not representative of the overall group or are only present in that group of compounds when analyzed from a specific source. These few compounds may or may not be present in an analytical group from other sources or other types of wastewater. It should be noted that in Conclusion number 6 to the 2016 total Naphthenic Acid Inter-laboratory Study, Environment Canada expressed concern that the commercially available standard, Merichem Naphthenic Acid Solution (used to spike the samples, and presumably a similar mixture might be used for any toxicity testing), did not seem to match the contaminants in wastewater at the Athabasca oil sands region (sample OSPW in the study). By inference, this comment suggests that if the current naphthenic acid standard mixture solutions are not representative of oil sands process-affected water (OSPW), they are unlikely to be representative of other types of water matrices such as treated refinery wastewater either and therefore are inappropriate for determining what constituents might cause toxicity in refinery wastewater.

II. Issues Concerning an Exact and Appropriate Definition of the Compounds Being Analyzed for both Naphthenic Acids and alkyl-PAH Homologs

Based on published scientific literature discussing the analyses of both Alkylated PAHs and Naphthenic Acids, there are significant discrepancies as to exactly what types of compounds are considered appropriate to include into each of these groups. The grouping of compounds varies between different agencies (EPA, Canada, various US states), environmental papers, and also with the laboratories analyzing the samples (even in the inter-laboratory study by Environment Canada). There should be a clear and vetted definition of exactly what is intended to be measured and included within each of these broad analytical groups, and only peer-reviewed and approved methods should be used.

A. Naphthenic Acids: Strict Definition and Potential Issues

The AXYS Laboratory definition of a naphthenic acid is any configuration of fatty acid chain that 1) contains between twelve and twenty-one carbons, 2) that does not contain any aromatic carbon rings, 3) has only a single carboxylic acid group, and 4) is either saturated or has a degree of unsaturation defined by a negative "z" number that can equal the even numbers 0, -2, -4, -6, -8, -10, or -12, with each negative even number progressively corresponding to the loss of two more hydrogen atoms due to double bonds or alkyl carbon rings. The general formula is: $C_nH_{2n+z}O_2$. In common language, this definition and formula includes most naturally occurring fatty acids, and these can be saturated (maximum number of hydrogens: $z = 0$), monounsaturated (missing two hydrogen atoms due to a double-bond or cyclic non-aromatic ring: $z = -2$), or polyunsaturated (multiple double bonds, or more rarely, multiple cyclic, non-aromatic rings: z = higher even negative numbers up to -12). This definition of naphthenic acid (and, perhaps, any definition) is far from universally held, making data comparisons nearly impossible. There are some other

definitions in use (or that have been used) that utilize greater or lesser numbers of carbon atoms, a larger number of carboxylic acid groups, the presence (or absence) of some cyclo-alkane compounds, or different degrees of saturation. This particular definition used by AXYS might be due to the analytical method being used, or to the industrial wastewater being studied, or to certain common chemical properties these acids have in common. However, this definition of naphthenic acids is already very broad and can include hundreds or even thousands of compounds (including isomers).

Most of these fatty acids that meet this strict definition are essential components in vegetable oils, dairy products, animal fats, and also in processed foods such as dehydrogenated or polyunsaturated fats or fatty acids and are unlikely to be toxic. However, there evidently is a movement to broaden the definition of naphthenic acid to include carboxylic acids that contain aromatic rings, and Environment Canada has come out in favor of this. (Aromatic carbon rings are the primary constituents of benzene and PAH compounds.) API/AFPM would oppose such a move, because these compounds, if present in treated refinery wastewater, could possibly have significantly different characteristics from the normal aliphatic NAs that are presumably the main target for the analysis. API/AFPM opposes any such change on the grounds that any toxicity that might be measured could be due almost entirely to the inclusion of these aromatic compounds, which might then be transferred to other aliphatic NAs that have little or no toxicity to humans. (The human toxicity factor, or carcinogenicity, is nearly always the main driver when organic compounds are assigned a high TWF.) API/AFPM believes that the compounds that contain aromatic rings in their side-chains might have significantly different toxicological and physical-chemical properties than the standard defined naphthenic acids. Therefore, if they are found to be present in refinery wastewater, they should be evaluated separately from naphthenic acids. This is discussed in more detail in the portion of this report on the potential assignment of TWFs by EPA to analytical results that represent large groups of related compounds.

B. Alkylated PAHs: Definition has apparently been changed several times in recent years

In just the last few years, there have been numerous papers published discussing alkylated PAHs, and nearly all of the papers are different in assuming which types of compounds are to be included under that label. Many of the compounds discussed clearly do not fit the strict scientific definition of alkylated PAHs, i.e. a group of fused hydrocarbon aromatic rings (usually two to five) with substitutions of alkyl groups (methyl, ethyl, propyl, etc.) at some of the available locations around the fused rings. Some of these additional compounds have perhaps incorrectly been justified for inclusion in the group because they are frequently associated with PAH compounds, such as being common components of coal tar (which is to a large extent made up of PAH compounds). Others have even less justification for inclusion in the group. It appears that EPA is currently favoring the list of analytes that is provided with the AXYS Method (MSU 21C, provided by EPA).

Table 1 is a list of compound categories that are or have been suggested to be included in a list of alkylated PAH compounds that could be analyzed. The top three categories of compounds have been included in the AXYS analytical list, along with the traditional single compound PAHs. Compounds towards the bottom of Table 1 are not currently included in the AXYS list of analytical categories but are discussed in various other papers as possibly being identified as alkylated PAHs. It is unlikely that there is any single laboratory currently analyzing all of the compound/group categories in Table 1, and we believe it unlikely that any laboratory is using a method where all possible combinations within each compound group category are analyzed. Even AXYS and the other participants in the Environment Canada Inter-laboratory study (for alkylated PAHs) did not each perform the analysis on all of the over 100 "measurands" (combined individual compounds and homologous groups) requested by Environment Canada.

Table 1: Compounds/groups that do not meet the strict definitions of "PAH" or "alkylated-PAH"

Compound/Group	Comments
Biphenyl (plus alkyl-substituted Biphenyls)	Not really a PAH, as there are no fused rings. However, it is a common component of coal tar, and is therefore found with PAHs. They are on the AXYS analytical list.
Various alkyl substituted PAHs, also termed "alkyl-PAH Homologs"	While these type compounds do meet the "alkyl-PAH" definition, these are not analyzed as individual compounds, but as compound groupings. Each group can contain dozens of compounds, and there can be any number of different groupings possible. (No single laboratory analyzes for all possible alkyl-PAH groupings.) The AXYS Laboratory Analytical List does include an intermediate number of alkylated PAH groups, more than some laboratories, less than others. API/AFPM does not believe these groups should be included, because the quantitative analysis of the PAH homologs in aqueous samples in the 2015 Environment Canada Inter-laboratory Study was almost a complete failure (as described later in this report).
Dibenzothiophene, (plus alkyl-substituted DBTs)	This is a heterocycle (a sulfur atom in the middle ring), and therefore not a PAH. However, it is considered to be chemically similar to anthracene, and is frequently detected in heavy oil fractions. They are on the AXYS analytical list.
Dibenzofuran, other oxygen heterocycles	These are listed in the paper source below[1], and dibenzofuran is included in the alkyl-PAH listing for several laboratories, but these are not PAHs, since they contain oxygen in at least one of the fused rings. The AXYS list does not include dibenzofuran or any other oxygen heterocyclic compounds.
Nitro-pyrene, other nitro-substituted compounds	Some papers list these, and the Minnesota Pollution Control Board (MPCB) incorporates them into their "extended PAH" list. Nitro-substituted compounds have their own chemistry (explosives). These also can be groups of compounds. These are not included on the AXYS analytical list.

Nitrogen heterocycles such as Carbazole, dibenzocarbazole, dibenzoacridines (including groups of alkyl-substitutions)	Minnesota Pollution Control Board (MPCB) incorporates several of these nitrogen heterocyclic compounds into their "extended PAH" list. However, these all contain nitrogen in at least one of the aromatic rings, which greatly alters the chemistry of these compounds. They are polynuclear and aromatic but are not hydrocarbons. These are not included in the AXYS list.

[1]"Time to Say Goodbye to the 16 EPA PAHs? Toward an Up-to-Date Use of PACs for Environmental Purposes" Jan T. Andersson and Christine Achten (2015)

API/AFPM believes it is impractical to analyze samples for all of the possible combinations of compounds and compound groups in all of the above categories. The result would be hundreds of "measurands" (combined single compounds and homologous groups) where the compound groups could each further represent hundreds of additional compounds.

API/AFPM is also opposed to the analysis of alkyl-PAH homologs and any other groups of PAH-like compounds analyzed as a group, because they are not individual compounds, and the 2015 inter-laboratory study clearly indicates that currently they cannot be quantitatively analyzed. This would also apply to other compound groups that may not have been analyzed in the 2015 Inter-laboratory Study. Also, analogous to the argument for naphthenic acids, any toxicity assigned to a mixed group of alkyl-PAH isomers could be dominated by only one or a few compounds that may have unique features that are grouped with a larger number of compounds that have negligible toxicity. It should be noted that for the "traditional 16" PAH compounds, the assigned TWF ranges from 100 for benzo(a)pyrene to 0.008 for acenaphthylene. That is a TWF range of greater than four orders of magnitude. This problem with grouping alkyl-PAHs is discussed further in the portion of this report on the potential danger of assigning TWFs by EPA to analytical results that represent large groups of related compounds.

API/AFPM is not opposed to the analysis of individual non-PAH compounds if EPA can justify that such compounds can be or are often associated with other PAH compounds with similar physical-chemical and toxicological properties and an appropriate, recognized and vetted analytical method can be employed. We note that the AXYS analytical list already includes the analysis of biphenyl and dibenzothiophene as separate compounds. The individual compounds dibenzofuran and carbazole are already commonly included on many laboratory semi-volatile organic analytical lists and will likely be analyzed as independent compounds anyway. As to the other heterocycles, we think EPA should justify the investigation of those compounds, as some of them seem unlikely to be present and are rarely if ever analyzed by most laboratories.

III. Analytical Methods Used for Naphthenic Acids: Analytical Problems and Inter-laboratory Studies

Currently, all environmental laboratories only analyze naphthenic acids either as total naphthenic acids, or as groups of compounds with the general formula $C_nH_{2n+z}O_2$. There are no calibrations

performed that are utilized to quantitate individual compounds, and the type and number of calibration standards prepared for different compound groups varies by the method and laboratory using them. Naphthenic acids (NA) can be analyzed as a single result reported as "total naphthenic acids" using Fourier-transform Infrared Spectroscopy (FTIR, a type of infrared spectrophotometry). Using LC/MS methods, it may be possible to calibrate and analyze for some individual NA compounds, however each group of NA compounds can contain dozens or even hundreds of specific compounds and isomers, making this a daunting task. Laboratories utilizing an LC/MS method often simply report "total naphthenic acids" as the sum of the NA concentrations measured within each NA subgroup that is analyzed by their method.

A. A Brief Description of the AXYS method for analyzing NAs

The AXYS Method is a very complex and ambitious proprietary method for the measurement of naphthenic acids. EPA provided API/AFPM a short summary of this complicated method suitable for public review (MSU-077C, R01, dated February 15, 2018) that describes in general terms the various steps involved. Due to the very recent date assigned, it is not clear whether this exact version of the method was used in either of the inter-laboratory studies (performed in 2012 and 2016) provided by EPA and discussed later in this report. The general procedure is presented in the following.

Aqueous samples can be extracted in the laboratory, or samples can be collected in the field using up to three Polar Organic Chemical Integrative Sampler (POCIS) sampling disks, (which can be used to concentrate samples if desired). Each extract is derivatized with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), to form the corresponding naphthenic acid-EDC derivatives. This means that there is a reaction with the carboxylic group, so that an acid-EDC complex is generated. This step is presumably performed to enhance the solubility, chromatography, and/or mass spectral pattern of the naphthenic acids. Analysis of the extracts is performed by high performance liquid chromatography (HPLC) with triple quadrupole mass spectrometer detection (LC-MS/MS). A fully detailed analysis report using this method would contain values for 60 different analytical groups of naphthenic acids (an amazing amount).

These 60 groups fit the generic formula $C_nH_{2n+z}O_2$, but are restricted as listed in Table 1 of the provided MSU-077C, R01 document (and reproduced later in this report):

- The number of carbon atoms allowed for this NA analysis are only in the range of C12 through C21.
- The carbon chain should not contain aromatic rings.

- The unsaturation factor "z" for the number of hydrogens can only be zero (saturated fatty acid), or negative even integers -2 (unsaturated), -4, -6, -8, -10, or -12 (these last are polyunsaturated). Not every carbon number includes this complete list of "z" values; this serves to limit the number of NA groups to 60 categories. Each category is capable of containing dozens or sometimes hundreds of compounds meeting the same generic formula for the group.

- The AXYS method analysis is supposed to be limited only to parent ions that originally had a single carboxylic acid group (that is the CO_2H element prior to derivatization).

B. Possible issues with the AXYS method for naphthenic acids

We are concerned about several potential problems when this method is applied to actual refinery wastewater.[1] Some of these problems may be left out of the short summary provided, but others might have a major effect on the interpretation of these results, and how they might be used for development of an effluent limitations guideline (ELG). The following bullets identify these issues. They are arranged roughly in order of concern.

1. The method only uses a single calibration curve to quantitate all 60 of the different analytical categories of naphthenic acids, and the calibration uses only a single compound, 1-pyrenebutyric acid (injected at three concentration levels). This particular compound does not even qualify as a naphthenic acid by the scientific definition of that class of compounds, due to the presence of an aromatic PAH group in the side-chain. This type of representative calibration is to our knowledge never employed when the compound itself is not included among the targeted analytes. The inter-laboratory studies discussed below provide little comfort in this area, since those studies are only evaluated on the total naphthenic acid concentration, and not on the 60 different sub-categories included in this method. For the total NA analysis, the AXYS laboratory performed reasonably well (an overall moderately high bias, as did most of the laboratories using some kind of LC/MS method), but for individual categories, the results might be very high or very low. We do not know how much importance EPA might place on individual naphthenic acid categories that have been measured, but if there are great differences in toxicity for these categories, this could be problematic. We realize there are other QC controls, including a Merichem Refined NA Mix that may give reproducible results, however, it appears that the individual compounds contained in this commercial mix are unknown.

[1] Please do not assume that any of the identified problems are a reflection on AXYS Laboratories, which we know is recognized as one of the premier environmental research laboratories in North America. Our concerns are about an experimental method still under development, its possible weaknesses, and how some of the results of this method might potentially be used in the development of a new refinery ELG by EPA.

Table 2. Reproduction of Table 1 in AXYS Method MLA-077: Molecular weights of NA groups that are analyzed with this method

n (C #)	Z # (hydrogen deficiency)						
	0	-2	-4	-6	-8	-10	-12
12	200	198	196	194		--	--
13	214	212	210	208			--
14	228	226	224	222	220		--
15	242	240	238	236	234	232 *	230 *
16	256	254	252	250	248	246	244 *
17	270	268	266	264	262	260	258 *
18	284	282	280	278	276	274	272
19	298	296	294	292	290	288	286
20		310	308	306	304	302	300
21		324	322	320	318	316	314

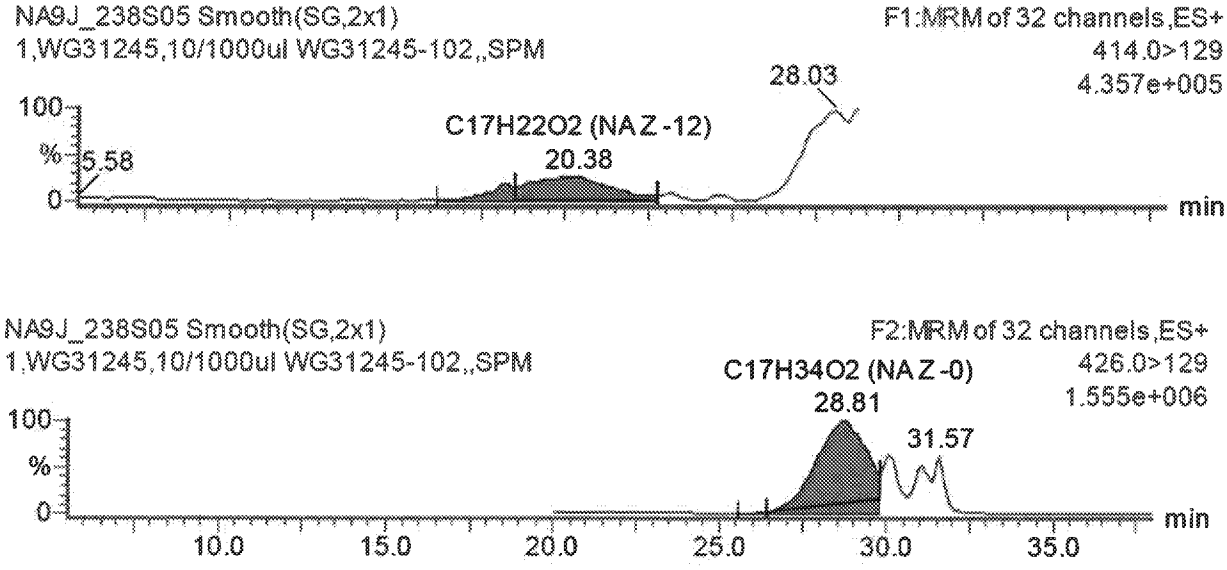
* Compounds that don't fit the strict definition of NA as they contain at least one aromatic ring may be included.

2. Table 2 is a copy of Table 1 from the AXYS Method (page 1 of the MSU-077C summary document. The table shows each of the sixty separate analytical categories of naphthenic acids reported to be analyzed using the AXYS method. Note that four of the 60 NA categories are asterisked, stating that it is possible that some of the compounds within those analytical groups might contain one or more aromatic rings, which do not fit the "strict definition" of a naphthenic acid. This also seems to suggest that the commercial mix "Merichem NA" that the method uses for control samples may also contain some aromatic acid species and possibly some di- or tricarboxylic acids.[2] Because the laboratory states that these aromatic compounds would be included within these categories, this logically seems to mean that the AXYS method cannot recognize whether the observed unsaturation in a particular parent mass spectral ion is caused by double bonds or by an aromatic ring (at least not by the molecular weight of the ion alone). A six-carbon aromatic ring is unsaturated by the equivalent of six hydrogens, so it would have a "z" number of "-6", before it is attached in some manner to the rest of the fatty acid chain, but this could be masked by the "z" factor present in the rest of the carbon chain. If the presence of aromatic rings could be determined by the method, then presumably such compounds could have been subtracted from the results for these analytical groups. This could have significant implications if the toxicological properties of NA's with aromatic rings are significantly different than those of the

[2] Environment Canada has concerns about the representativeness of the Merichem NA mixes compared to oil-sands process-affected water as described later in this report.

aliphatic NA compounds. Furthermore, if the presence of an aromatic ring in the carbon chain of an NA cannot be recognized, how does AXYS know whether there could be other aromatic NAs included within some of the other categories?

3. Ionization efficiency of NAs change with the structure of the compound and the matrix of the sample. This variation in ionization efficiency renders HPLC MS with electrospray ionization problematic for such complex mixtures.
4. On page six of the AXYS method summary, there are a series of seven chromatograms of groups of NAs containing 17 carbons, showing (presumably derivatized) mass values with parent MWs of 414 through 426. Presumably because these peaks are generated by a number of different isomers, the peaks have very broad retention times. Most are greater than five minutes, and all have undulations within each peak. In particular, in the mass 414 chromatogram the peak that crests at 20.38 minutes seems to have its low end retention time (RT) window clipped short due to another peak of the same mass appearing within the original RT window. Also, for mass 426, the peak at 28.81 minutes is clearly significantly influenced by some later peaks of the same mass, and apparently a manual integration was necessary. EPA requires all manual integration to be well documented. A highly experienced analyst can exercise his or her professional judgement on these integration issues (provided there is appropriate documentation), but this has its limits, and may become impossible if the chromatograms become too complex. Below are the chromatograms in question, for MW 414 and MW 426.



5. We do not know whether the chromatograms from page 6 (depicted above) are of a quality control (QC) sample or a real oil sands sample. Nor do we know if a smoothing

function has been used, as suggested by the label, "smooth," and if so, if that practice altered the analytical results. Particularly for untreated refinery wastewater which can be generated from many types of raw crude and be products of differing refinery processes, it is likely that these chromatograms could become far more complex, with substantially more likelihood of uncertainty entering into the analysis. Environment Canada mentioned this as one of their conclusions to the 2016 Inter-laboratory Study they conducted. They stated: "The complexity of the background matrix needs to be increased further. The synthetic toxicity testing matrix is suitable for method validation purposes but future inter-laboratory studies should use a natural water matrix for all samples." Presumably this would also include refinery wastewater matrices for studying refineries. The 2016 Inter-laboratory was focused on oil-sands process-affected water and is not representative of refinery wastewater, either untreated or treated.

6. We note that this AXYS summary does not discuss any QC analytical check on the verification of the completeness of the derivatization efficiency, or address how the derivatization might perform on actual refinery samples, which presumably may contain di- or tri-carboxylic acids. Does the instrument recognize di and tri-carboxylic acids, even if they form fragments that contain only one carboxyl group? Does a fresh reagent fully derivatize all carboxyl groups in any compound? What if only one of the carboxylic groups is successfully derivatized in a di- or tri-carboxylic acid? Could the parent compound, or a potential mass ion fragment of the parent compound, be mistakenly identified as a monocarboxylic acid, and counted as a naphthenic acid? How is it determined whether stored derivatization reagent has become less effective over time? Finally, even if di- and tri-carboxylic acids are not included in the NA quantification when using the AXYS method, they possibly still could be present in acid extractions from samples containing naphthenic acids, which may have implications when performing toxicity studies on these extractions.

C. Inter-laboratory studies of the analysis of naphthenic acids

There were two inter-laboratory studies performed for the naphthenic acids analyses, one in 2012, and a second in 2016. However, the primary focus of both of these studies was the analysis of "total naphthenic acids" and only the total NA values were evaluated as to accuracy and precision among all of the participating laboratories. Triplicate samples were typically provided, and the laboratories reported their individual results as well as the mean of their triplicate analyses. (The mean value reported was the value that was evaluated in most cases.) The samples included reagent water blanks, spikes generated from Merichem naphthenic acid reference material, and other samples were of oil sands process-affected waters (OSPW). There were two main categories of analyses for total NA. An FTIR Method that can only give results as total naphthenic acids was used by many of the laboratories. There were a variety of LC/MS and LC/MS-MS methods also used by several laboratories. While these methods can achieve varying degrees of speciation

depending on the method, they also can be used to obtain a total NA value by summing up the values from all of the measured subcategories of NAs. Environment Canada evaluated the score for these laboratories only using the total naphthenic acid results since the degree and type of speciation varied greatly among the different laboratories and was evidently not comparable.

The 2012 Environment Canada Naphthenic Acids Inter-laboratory (ECNAIL) study found that some of the laboratories using both FTIR and some of the LC/MS methods could reasonably reproduce total naphthenic acid results. There was some speciation information displayed in Appendix A of the 2012 study from the various GC/MS, LC/MS, and LC/MS-MS methods, however the speciation was limited to different degrees of saturation (the "z" factor, even numbers zero through twelve, forming seven speciation categories). These categories did not differentiate based on the number of carbon atoms. The 2012 report concludes regarding speciation of the NA compounds: "The data demonstrated the capability of certain methodologies to characterize NA by carbon number as a percentage of the Total $C_nH_{2n+z}O_2$ species, however, complexity of the speciation data made comparative evaluation impractical."

The 2016 ECNAIL study report was smaller, involving only nine laboratories, but it did not address potential speciation of the NAs. Four of the nine laboratories used an FTIR method. Five of the nine laboratories used some variant of LC/MS or LC/MS-MS methods, but it is unknown whether any of these methods were identical to one-another. On average, the FTIR methods were biased low at 78% of the target values on average, with every FTIR laboratory having a negative bias. The LC/MS labs were biased somewhat high, on average 108% recovery, but the range of biases by laboratory was -19% on up to +40% (that is, the average percent recovery by laboratories performing an LC/MS method ranged from 81% to 140%). The OSPW samples had on average lower recovery by all methods, averaging 67% recovery, while the Merichem NA standard reference material had on average 113% recovery by all methods. These values demonstrated that for "total naphthenic acids" these analyses in general were reasonably quantitative among the different laboratories, but there were some significant differences depending on the sources of the reference materials.

The AXYS laboratory participated in both the 2012 and 2016 study. In both studies, they tended to be biased somewhat high for total NA (approximately +20% of the target values on samples with NA values greater than 1 mg/L), and they were approximately in the middle of the ranges for laboratories using one of the LC/MS or LC/MS-MS methods. Their in-lab precision was good, and they had no outlier results from either study.

The conclusions from the 2016 study (pages 18 and 19) contain some interesting comments that are reported below, roughly in order of importance:

- Environment Canada states in conclusion number 7: "The current definition of Total Naphthenic Acids ($C_nH_{2n+z}O_2$) as used in this study needs to be broadened to include aromatic

O2 species." API/AFPM does not agree with this conclusion, as described in Section VI of this report.

- Conclusion number 3 states: "The correlation coefficient for all laboratories is >0.96 for all laboratories indicating that main factor in any laboratory imprecision is a bias of some kind as opposed to some random errors or blunders in the laboratory." API/AFPM agree with this conclusion. Among the items that likely creates an inherent bias is trying to use a single calibration material to quantitate mixtures of compounds that can differ significantly in their overall makeup from site to site. It should be noted the calibration ranges were different across all of the methods in the interlaboratory study, with some being outside of the measured analyte range. This practice results in an inherent bias in the study.

- Conclusion number 6: "There is a need to establish a traceable quantification standard to achieve consistent analytical results. Merichem® is a commercially available mixture of naphthenic acids that allowed for an inter-laboratory comparison of laboratories' abilities to measure Total NA. It is currently the best available representation of the Total Naphthenic Acids ($C_nH_{2n+z}O_2$) which are reported in this study. However, it needs to be replaced with a commercially available, traceable material (single component or mixture) that better represents the NA components found in relevant matrices of the Athabasca oil sands region (e.g. OSPW)." This is also an important issue for API/AFPM. The assay information on these Merichem NA mixtures (from Appendix A of the 2016 study) indicates only that they are 95-99% naphthenic acids, and 1-5% petroleum distillates. It has a total acid number of 191 (with an acceptance range of 170-210). There is no information whatsoever as to specific quantities of which categories of naphthenic acids are included in this material, and it is not a traceable standard.

- Conclusion number 10 also discusses reference materials: "An OSPW derived reference material is required that can be used to compare without bias the various methods being used for NA analysis." API/AFPM is very concerned about this. Does this mean that each site or each refinery might need its own reference material for calibrations?

- Conclusion number 1 from the 2016 study discusses how the results from this study are significantly improved over much poorer results that were obtained from a 2014 inter-laboratory study for naphthenic acids, where the overall RSD values for the samples varied from 64% to 168%, with only the three highest samples having RSDs below 100%. (API/AFPM believes that if these RSD results are correct, this constitutes unacceptable method performance.) **This 2014 naphthenic acid study was not included in the information given to API/AFPM.**

- Conclusion number 8: "The complexity of the background matrix needs to be increased further. The synthetic toxicity testing matrix is suitable for method validation purposes but future inter-laboratory studies should use a natural water matrix for all samples." API/AFPM agrees that this is needed, and has stated that actual refinery samples, especially untreated wastewater samples, can greatly complicate the analytical process for many well-established methods let alone these AXYS experimental procedures currently being developed.

IV. Discussion of Analytical Methods for Alkylated PAH Compounds and the 2015 Environment Canada Inter-laboratory Study

A. Overview of methodology

The analytical list for "alkylated PAHs usually includes the 16 standard EPA priority pollutant PAHs, "extended PAHs" (meaning additional single-compound PAHs or PAH-associated compounds), and alkylated PAHs, which are analyzed as individual groups of alkyl-substituted PAH homologs. Most laboratories use a GC/MS instrument as is used in EPA SW-846 Method 8270D.[3] Many labs operate the MS in a selective ion monitoring (SIM) mode to obtain greater sensitivity, with the possible drawback being they do not obtain a full mass spectrum of each compound. The SGS-AXYS Laboratory Method MSU-21C uses their MS operating in an Electron-Impact Ionization (EI) mode using Multiple Ion Detection (MID). We are not currently familiar with the advantages/disadvantages inherent to this type of MS setting. The main point here is that the methods used by the participating laboratories in the 2015 study discussed in Section B below, though similar in instrumentation, may not be exactly the same. In Section I of this report, we have also discussed that there is ongoing debate within the analytical community as to which extended PAH compounds and alkylated PAH homologs should routinely be included in the parameter list for this determination.

B. 2015 environment Canada inter-laboratory study shows major problems in quantifying the groups of PAH homologs

Environment Canada performed an Inter-laboratory Study for Alkylated PAH compounds, the report of which is dated April, 2015. API/AFPM received a copy of this report from EPA. Three sample matrices were tested (with four samples provided for each matrix): extract samples consisting of three different diluted oils, one National Institute of Standards and Technology (NIST) standard in methylene chloride, and synthetic soils samples spiked with three different oil sources. Four samples were provided for each matrix. Our primary concern here is on the four aqueous samples, but we also include a comparative discussion on the analyses of the extract that is spiked with the NIST certified mixture.

The results for the aqueous samples in this inter-laboratory study paint a completely different picture of two types of PAH analyses (see Table 3 below, which is a compilation of the aqueous results from Tables 3 and 4 on pages 10 and 11 from the 2015 Environment Canada Inter-laboratory study on Alkylated PAH analyses). As expected, all of the laboratories analyzed the parent PAHs (all single compounds, each with their own calibration curves) and achieved

[3] EPA, *Test Method for Evaluating Solid Waste: Physical-Chemical Methods Compendium (SW-846)*, Office of Land and Emergency Management, Washington, D.C.

acceptable Relative Target Standard Deviations (RTSD), with the average values being between 20 and 25% RTSD.[4] The parent PAH data for water and the other matrices is presented in Table 3 on page 9 of the Environment Canada Report.

However, for the PAH homolog analyses (found in Table 4 on page 11 of the Environment Canada report), the results of the RTSDs are shockingly different, and API/AFPM considers them unacceptable. (It is important to remember that the alkylated PAH homologs are actually groups of related PAH compounds, where the calibration is based only on a single compound intended to represent the entire group.) The average RTSD for the four water samples is almost 80%, an extremely high value, and some of the RTSDs for some homolog compound groups were over 100%. Typically, in these type studies, results outside of two standard deviations are given a warning, but are still considered acceptable, and results outside of three standard deviations are considered as unacceptable. To illustrate how terrible an RTSD of 80% is (which represents only a single standard deviation around the target value), consider a spiked sample with a value of 1,000 µg/L for a particular PAH homolog group. If a result within +/- 3 std. deviations is acceptable, then in this case (using an 80% RTSD for one standard deviation, multiplied by 3 SDs), any result between the values of 0 (or non-detected) up to 3,400 µg/L would be considered an acceptable result. It is difficult to rate such results as even "semi-quantitative", because many "acceptable" results would not even be within the same order of magnitude of the true value (1,000 µg/L). It is clear that the analytical method proposed for the PAH homolog groups does not "quantitate" these compounds within any acceptable definition of quantitation. Therefore, this analytical method is unacceptable for evaluating the concentrations of such compounds in refinery wastewater.

In the Table 3 below, API/AFPM compares the average percent RTSD for the parent PAHs in the four aqueous samples with the average RTSD for the PAH homologs in these same four samples. We find that for the water samples alone, the RTSD average for the PAH homologs is actually 3.41 times higher than for the parent PAH compounds. This is significantly worse than the discussions within the Environment Canada report, which estimated that overall, the RTSD for the homologs was 2.5 to 3 times higher than the RTSD for the parent compounds. This seems to suggest that the problems analyzing aqueous samples for these parameters is significantly greater than for soils or extracts. Again, API/AFPM asserts that this performance cannot be considered as quantification of these compound/compound groups in water samples.

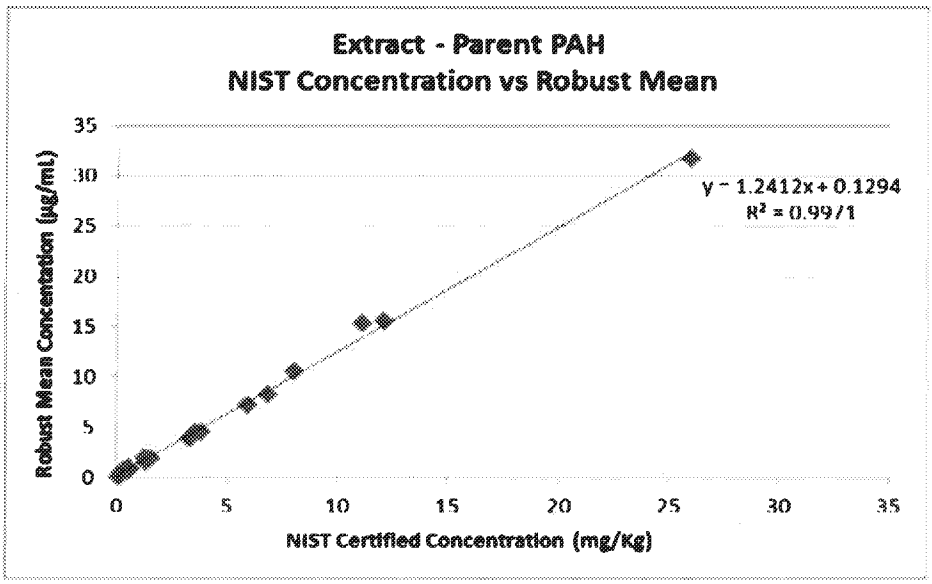
[4] An RTSD is the RSD around a known target value, instead of the mean of the reported results.

Table 3: Extracts of the Aqueous Analyses RTSDs data for alkyl-PAH Homologs (originally from Table 4 in the 2015 alkyl-PAH Inter-laboratory Study) and a summary of the average RTSDs from the aqueous analyses for the parent PAH compounds (calculated from Table 3 of 2015 report)

Aqueous samples Relative Target Standard Deviation% for PAH Homologs analyzed in Environment Canada 2015 Inter-lab Study				
Aqueous Sample Number	AAP-01	AAP-02	AAP-03	AAP-04
C1-Naphthalene	71	46	30	40
C2- Naphthalene	123	59	57	64
C3- Naphthalene	120	77	68	60
C4- Naphthalene	106	83	77	68
C1-Fluorene	91	76	66	60
C2-Fluorene	66	65	63	40
C3-Fluorene	100	95	86	91
C4-Fluorene	105	215	217	126
C1-Phenanthrene	55	45	44	29
C2- Phenanthrene	45	52	49	41
C3- Phenanthrene	80	77	79	81
C4- Phenanthrene	108	129	109	108
C1-Fluoranthene	91	76	66	60
C2- Fluoranthene	93	84	74	100
C3- Fluoranthene	68	50	57	68
C4- Fluoranthene	128	132	121	103
C1-Chrysene	27	29	31	34
C2- Chrysene	102	76	94	88
C3- Chrysene	96	96	98	81
C4- Chrysene	178	184	187	129
C1-Benzopyrene	73	78	78	78
C2-Benzopyrene	63	78	100	62
C1-Dibenzothiophene	54	42	42	42
C2-Dibenzothiophene	51	52	40	45
C3-Dibenzothiophene	83	55	57	66
C4-Dibenzothiophene	53	44	62	69
Average RTSD per sample for PAH homologs	85.77	80.58	78.92	70.50
Average RTSD per Aqueous sample for 18 parent PAH compounds	22.5	23.9	21.6	25.11
Overall RTSD Ratio Homolog over parent PAHs per sample	3.81	3.37	3.65	2.81
Average of all four ratios				3.41

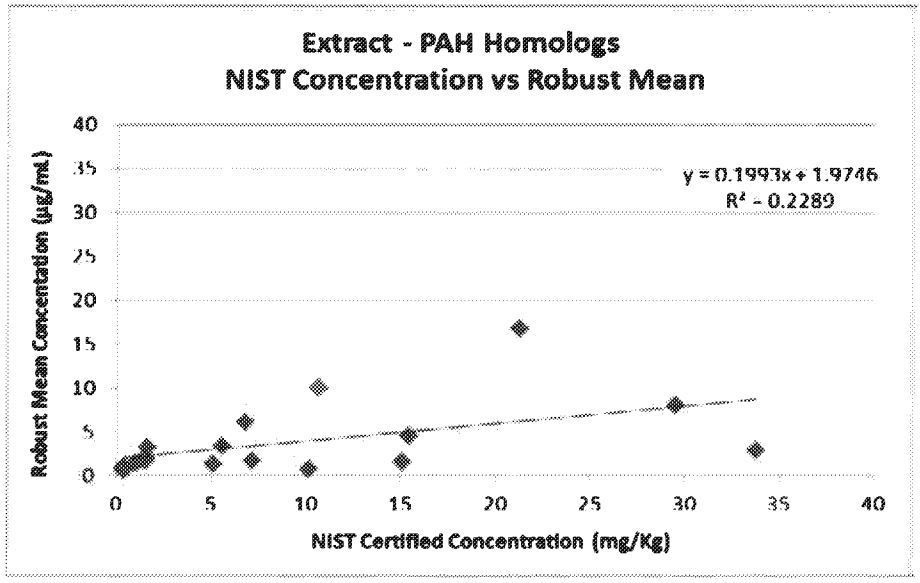
Another indication of problems related to the analysis of the PAH homologs can be seen in the extract sample that was spiked with the NIST standard. Here, any errors or biases due to sample extraction have been eliminated, and all of the values for the parent PAHs and their PAH homologs are certified. There are graphs of the analytical results of this sample on page 13 of the Environment Canada 2015 report, and two of these are shown below. It should be noted that these graphs are based on the "robust mean" and "robust standard deviation" of the data for this sample. "Robust" is defined as a statistical program that reduces the influence of any outlier results on the calculation of the "robust mean" and "robust SD" (without totally eliminating the outlying data points), so that these calculations are not unduly influenced by such outliers. Therefore, these graphs already contain a degree of correction for the worst outlier results.

The first graph (below) is for the results of the parent PAH compounds in the NIST sample extract:



As can be seen, the correlation coefficient of the parent PAH compounds versus the robust mean of the NIST extract sample is satisfactory ($R^2 = 1.0000$ is perfect correlation).

This second graph is for the PAH homologs:



The correlation coefficient of the PAH homolog compounds vs. the robust mean is only 0.2289. This is extremely poor, especially for a sample that is a simple dilution of an NIST standard that did not have to be extracted. The evidence is clear that there are severe problems with the calibrations being used for the PAH homologs.

C. Summary of Conclusions Discussed in the 2015 Environment Canada Inter-laboratory Study for PAH and PAH homolog analysis

The Environment Canada conclusions show they are aware of the issues with the quantification of the PAH homologs. They first state that the results of the analyses of the parent PAH compounds were not unexpected. They stated that most of these compounds have been routinely analyzed by most environmental labs since the 1980's, and that percent RSD's of 20 to 25% are typical for these compounds.

The following is the Environment Canada assessment of the PAH homolog analysis in the conclusion to the 2015 report:

"The results for the analysis of the alkyl-PAH homologs are consistent with an analytical method that relies on only a few select compounds to represent an entire class. The quantitation of the homologs is generally done using a single compound to represent the entire class of alkyl-PAH being quantitated instead of individual compounds and this could be responsible for the increase in relative target standard deviations observed. This would be especially true if all of the compounds in a class do not exhibit the same response factors. A number of homologs in the solid samples were also too low in concentration to be accurately quantitated or even detected in some cases. This included the NIST SRM (1941b). A lack of traceable individual calibration standards for homologs may also play a part in the apparent low recoveries of the homologs as could some unfamiliarity with the practical application of some elements of the recently promulgated ASTM

D7363-11, *Standard Test Method for Determination of Parent and Alkyl Polycyclic Aromatics in Sediment Pore Water Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry in Selected Ion Monitoring Mode*."

API/AFPM believes that based on the results of this study, Environment Canada has greatly understated the problems observed in the aqueous analyses, especially when they state: "The quantitation of the homologs is generally done using a single compound to represent the entire class of alkyl-PAH being quantitated instead of individual compounds and this could be responsible for the increase in relative target standard deviations observed. This would be especially true if all of the compounds in a class do not exhibit the same response factors." We also note that the problems with the aqueous samples were for all four samples, not simply the low concentration results.

Environment Canada also states that this first study may have been too ambitious and possibly included too many compounds and homologs for analysis:

"This first assessment of the current state of the PAH and alkyl-PAH analysis of environmental samples was rather ambitious. Over 100 separate measurands were asked to be reported in 3 separate matrices. Future studies will focus on a target list more closely approximating the one found in ASTM D7363-11."

API/AFPM believes that the analyses of so many types of alkylated PAHs is far too complex and that methods for measuring groups of alkylated PAHs are nowhere near sufficiently developed for any EPA study of refinery wastewaters, or any follow-up rulemaking effort.

V. Concerns About Blanket Toxicity Assessments of Groups and Categories of Compounds

A. Brief Background

In the EPA ELG process, the pollutants estimated to be removed by a proposed rule have been given a toxic weighting factor (TWF) based on toxicological tests having been performed in the past on that specific pollutant. The calculated TWF for each pollutant is actually the sum of an aquatic life toxicity value, and a human health toxicity value that are both normalized to the TWF of copper.[5] The TWF formula for pollutants in water is:

$$TWF = (5.6/AQ_{value}) + (5.6/HH_{value})$$

Where:

[5] Copper as a reference toxicant was selected by EPA years ago because its toxicity was about in the middle of pollutants being tested at the time.

5.6 (μg/L) = acute aquatic toxicity of copper at a specified hardness that is used as the scaling factor to normalize the TWF in relation to copper

AQ = Aquatic Life Value (μg/L). This is determined experimentally through toxicity testing on aquatic organisms.

HH = Human Health Value (μg/L). A few pollutants have acute human toxicity, but most times the HH factor is based on potential carcinogenic properties of the compound.

Except in rare cases, the TWF is dominated by either the AQ value, indicating toxicity to aquatic life is the predominant effect, or the HH value if there is a significant human health risk. While there are rare exceptions due to acutely toxic properties of specific compounds or potential unusual human exposure pathways—for trace organic compound contamination in water, the HH value is typically not going to be significant to the TWF calculation unless that compound is demonstrated to have potential or confirmed carcinogenic properties.

As example of this, consider the sixteen PAH compounds currently on the EPA priority pollutant list. Seven of these compounds have been identified as potentially carcinogenic through the aqueous-fish-shellfish exposure pathway, and these seven have by far the highest TWFs of the sixteen compounds. Benzo(a)pyrene is the highest of the seven with a TWF of 100, and the lowest two are benzo(b) and benzo(k) fluoranthene, both with a TWF of 30.66. Of the nine considered to be "non-carcinogenic" PAHs, the highest is fluoranthene, with a TWF of 1.27.[6] The lowest TWF of the nine "non-carcinogenic" PAHs is acenaphthylene, with a TWF of 0.0084. This compound was found to have "no observed effect" on mice, and has no HH value, so this TWF is totally based on aquatic life impacts. Note that the acenaphthylene TWF is more than 10,000 times lower than that of benzo(a)pyrene. It is an indication that if an individual compound is not carcinogenic, a TWF based entirely on aquatic life toxicity may be thousands of times lower.

B. Relating TWF factors to mixed groups of compounds, and testing for toxicity

Because the discussion above is applicable to assigning TWFs to categories of mixed compounds, it creates significant problems. Carcinogenic effects are applicable to only specific compounds because the carcinogenic interaction is produced at the molecular level, at specific sites of the molecules that mimic critical enzymes. The addition of a methyl group to a critical area of a molecule may create a stearic hindrance that may completely prevent this molecular interaction. This is why, even among the 16 PAH priority pollutant compounds that are very similar in structure some have been found to be carcinogenic and others show no carcinogenic effect whatsoever.

Each analytical group of naphthenic acids can be mixtures of dozens or hundreds of different compounds, and the total naphthenic acids can consist of thousands of compounds. The only

[6] Though fluoranthene is not classified as a class 3 carcinogen to humans as are the other seven, one study has found it to possess carcinogenic properties to newborn mice, so it still retains a HH value.

common denominator among these compounds is that they contain a single carboxylic acid group, and the attached carbon chains must be aliphatic, (but even this is being questioned by Environment Canada). As we have previously stated, most of *aliphatic* NAs (in the C12 to C21 carbon range), that meet the strict definition of NAs as used by the AXYS are naturally occurring aliphatic saturated or polyunsaturated fatty acids that are commonly found in foods and dairy products, and these compounds should not be toxic.

Some papers have discussed how oil-sands process-affected water contains numerous organic compounds, including naphthenic acids (NAs), and a few papers have asserted NAs as a source of acute toxicity in oil-sands process-affected water. Total NAs, however, defy generic characterization and the toxicity of "NAs" cannot be meaningfully expressed as though NAs constituted a single compound or a consistent, reproducible mixture of compounds. To quote one scientific review on naphthenic acids[7]: "The field continues to be challenged by the lack of a cost-effective, accurate analytical technique for NAs or an understanding of all the organic constituents in process-affected water that may be contributing to observed toxicity and thus requiring treatment."

As discussed in this report, even possibly the most specific analyses for NAs such as the method used by AXYS laboratories can still include other types of compounds that do not meet the definition of naphthenic acids. Just as in the example for PAH compounds discussed earlier, it is entirely possible for only a very few compounds to be the drivers for most or all of the apparent toxicity when addressing a situation of a mixture of hundreds or thousands of compounds. Also, it is unknown, and unlikely, that the naphthenic acids that remain in refinery wastewater after treatment contain the same toxic compounds/mixes that appear to be present in oil-sands process water.

The fact that the analytical method measures total NAs makes the toxicological testing of these naphthenic acid mixes (and also mixes of PAH homologs) a very difficult and inexact procedure. There must be some kind of reference chemical available commercially that is used to perform the toxicity testing. If the toxicity is due to only a few highly toxic compounds present in a mostly non-toxic mixture and one does not know which compounds they are, whether they are present in every mix, or whether they are present in some mixes from some sources and not others, how can a TWF for the mixture be estimated? Are they present in only some wastewaters that contain naphthenic acids and not others? Regulation of total NAs on this basis will invariably result in false positives prompting exceedance violations for dischargers presenting no significant increase to environmental toxicity. These issues are why toxicity testing has (mostly) been limited to testing one pure individual compound at a time, to increase the likelihood that consistent and reproducible results can be obtained when using the same standard reference material.

[7] Oil Sands Naphthenic Acids: A Review of Properties, Measurement, and Treatment, Brown and Ulrich, 2015

There are some very serious shortcomings to the current commercially available consensus reference material used by AXYS, which is the Merichem NA mixture. This mixture was used as a standard reference for the NA comparative studies, and AXYS Laboratory also uses Merichem mixtures as their quality assurance (QA) samples for their proprietary naphthenic acid test method. This Merichem reference material apparently contains relatively consistent proportions of the 60 naphthenic acid subcategories analyzed by AXYS, so it can be used as a QC sample to verify consistent results in their analyses over time. However, the exact makeup of the various specific compounds is unknown, and these samples only demonstrate that the unknown can be reproduced consistently. The summary API/AFPM received of the AXYS method indicates that the laboratory appears to believe some of the fractions found in the commercial Merichem NA mixture do contain some aromatic naphthenic acids. It is possible that some of these aromatic acids could have much higher toxicity than the normal aliphatic NAs. Our impression is that the AXYS method cannot quantify the aromatic NAs separately, otherwise they could be subtracted out of the total. Finally, Environment Canada, in their conclusion to the 2016 NA Inter-laboratory Study stated: "There is a need to establish a traceable quantification standard to achieve consistent analytical results. Merichem® is a commercially available mixture of naphthenic acids that allowed for an inter-laboratory comparison of laboratories' abilities to measure Total NA. It is currently the best available representation of the Total Naphthenic Acids ($C_nH_{2n+z}O_2$) which are reported in this study. However it needs to be replaced with a commercially available, traceable material (single component or mixture) that better represents the NA components found in relevant matrices of the Athabasca oil sands region (e.g. OSPW)." (Important to note: Environment Canada here appears to be asking for a reference material that is representative of a single site. Does this mean that each site and each refinery should obtain a mix that matches their site alone?)

C. Summary of the Main Issues for determining toxicity for Naphthenic Acids (also generally applicable to alkylated PAH homologs)

The following bullet items are just a few of the complex issues that must be dealt with, if one is to apply a single TWF to large groups of compounds such as naphthenic acids or alkylated PAH homologs:

- These NA or alkylated PAH homologs mixtures can contain hundreds of compounds, and if present, it is very likely that only a tiny fraction of these compounds may have a high TWF but this fraction might drive the overall toxicity of the entire group. These few toxic compounds have likely not yet been identified, but they may be present in samples from one source, and not present in another, with dramatic effect on the future evaluation of the TWF.

- Performing the tests to determine toxicity: As stated by analysts and Environment Canada, there is not yet available a commercial material that is traceable quantitatively, where all the components are identified. If individual lot numbers of this commercial material are used as **a standard to determine toxicity**, it appears they face the same problem—do certain lots of the mix contain fewer or more of the limited number of compounds that can drive the toxicity,

and is the mix representative of the types of naphthenic acids present at various facilities? How do you prepare a mix to certain toxicity specifications, if you do not know what compounds are present in the wastewater that can create the most toxicity?

- In the case of determining the toxic-weighted pound equivalents (TWPE)[8] for a refinery effluent, the standard mix used to determine a TWF for NAs needs to be toxicologically representative of the naphthenic acids present in the discharge from a refinery after biological and other treatment. This is likely to be very different than the mix of naphthenic acids present in untreated refinery wastewater, and even further different than oil sands process water used to mine the oil.

- Environment Canada believes that aromatic-naphthenic acids (this term is seemingly self-contradictory, since the word "naphthenic" is used to define mixtures of organic fluids that are low in aromatic content) should be included in the analysis of NAs. If, as might be the case, the aromatic NAs have significantly different toxicological/environmental properties than the currently defined aliphatic NAs, then what is the justification for including them in the same category? Perhaps a separate definition and scientifically defensible analytical procedure should be devised that can analyze for aromatic NA's only.

[8] The TWPE is used by EPA to estimate the total mass loadings of all toxic pollutants in a specific industrial effluent category for the purposes of comparing industrial point source categories for their relative contribution ot surface water discharges of toxic pollutants.