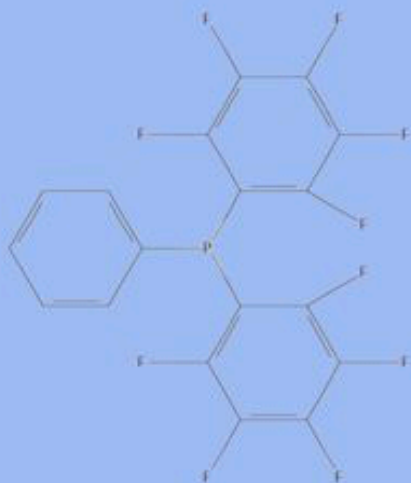


A Brief History of EPA's Criteria for GC/MS Tuning Compounds

Harry McCarty and Kevin Roberts

CSC



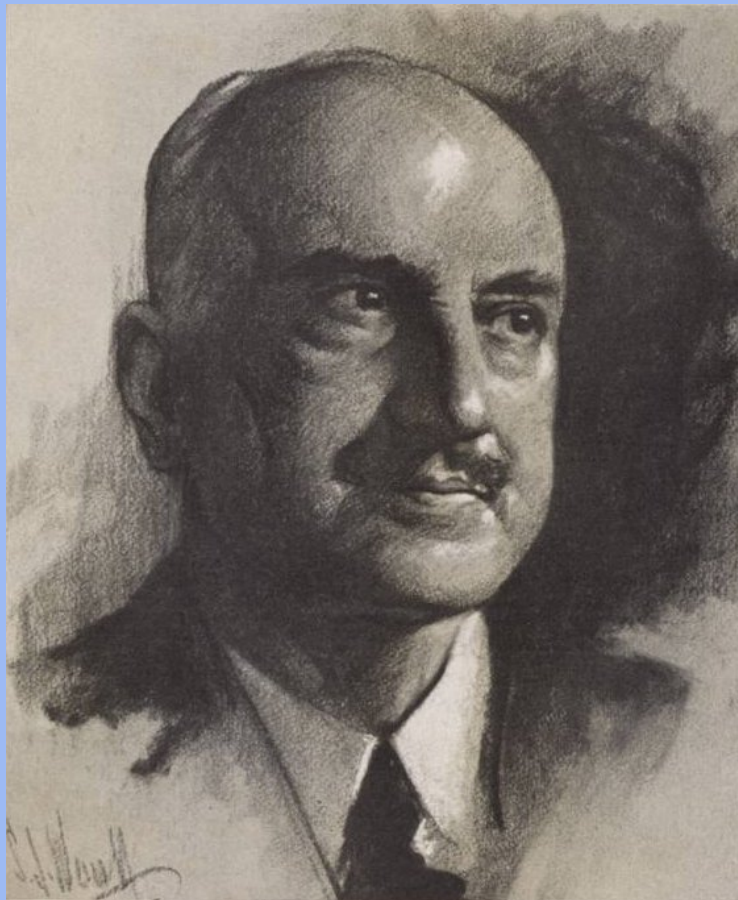
Disclaimer

- Although the authors are EPA contractors and much of this material is taken directly from EPA documents and studies, the contents of this presentation have not been subjected to EPA review, nor do the opinions of the authors reflect EPA policy.

Why Care Now?

- Disruptions in the supply of high quality helium has lead to wide-scale efforts to substitute hydrogen as the carrier gas in GC methods used to monitor compliance with various EPA regulations, including those under the Clean Water Act, Safe Drinking Water Act, and Resource Conservation and Recovery Act.
- Reports of laboratories switching to hydrogen, but having trouble meeting the existing acceptance criteria for the tuning compounds employed in many GC/MS methods

Jorge Agustín Nicolás Ruiz de Santayana y Borrás, known as George Santayana



"Those who cannot remember the past are condemned to repeat it." - *The Life of Reason*

So we start with some history ...

- Mass spectrometry (w/o a GC) came of age during WWII, as “calutrons” were used to separate uranium isotopes during the Manhattan Project.
- James and Martin are credited with developing MS as a detector for GC systems in 1952.
- In 1964, EAI tasked Bob Finnigan to develop a GC with a computerized quadrupole mass spectrometer as the detector.

Mass Calibrants

- Early mass calibrants for mass spectrometers began with such simple approaches as an air leak or background levels of hydrocarbons that allowed the analyst to establish a reference mass-to-charge ratio (m/z) in the instrument.
- By the mid-1940s, the National Bureau of Standards (NBS, now NIST) provided hydrocarbon standards as mass calibrants for m/z s well above those found in air.
- In cooperation with NBS, the American Petroleum Institute developed fluorinated organic calibrants such as perfluorokerosene (PFK) and perfluorotributylamine (PFTBA) that are still in use today.
- These perfluorinated compounds produce distinct m/z s over a relatively wide range of masses.
- They are also stable and relatively volatile and can be used in GC/MS systems.

EPA GC/MS Methods

- Development of affordable quadrupole GC/MS systems supported early efforts by EPA's Office of Research and Development (ORD) laboratory in Cincinnati to develop reliable GC/MS procedures for volatile and semivolatile organic compounds in drinking water and wastewater samples.
- ORD staff published the results of a study of decafluorotriphenylphosphine (DFTPP) conducted in 1973 and proposed its use as a mass calibrant for semivolatile organics (Eichelberger *et al.*, 1975), with the familiar abundances.

Eichelberger *et al.* Criteria

Mass	Ion Abundance Criteria
51	30 - 60 % of mass 198
68	< 2 % of mass 69
70	< 2 % of mass 69
127	40 - 60 % of mass 198
197	< 1 % of mass 198
198	Base peak, 100 % relative abundance
199	5 - 9 % of mass 198
275	10 - 30 % of mass 198
365	1 % of mass 198
441	< mass 443
442	> 40 % of mass 198
443	17 - 23 % of mass 442

Study Details

- 15 GC/MS systems, including 11 quadrupole instruments from Finnigan and four magnetic sector instruments were used to generate data.
- Pooled ion abundances for DFTPP, covering a mass range from 51 to 443.
- *But, the Devil is in the details!*

Careful Reading of the Original Paper Indicates That:

- The data that were pooled were only those from the 6 quadrupole instruments that yielded a base peak of 198,
- The results for the other 5 quadrupole instruments and all 4 magnetic sector instruments were not used to develop the criteria.

Incorporation into EPA Methods

- The DFTPP criteria were included in EPA Methods 525 and 625.
- In 1984, when Method 625 was promulgated under 40 CFR Part 136, the criteria took on the force of law.
- OSW (now ORCR) included the criteria in SW-846 when they used Method 625 as the basis for Method 8250.
- OSWER (Superfund) included the criteria into the Contract Laboratory Program (CLP) SOWs in the early 1980s.

Other Tuning Criteria

- In developing the isotope dilution methods 1624 and 1625 (as variants of 624 and 625), the Office of Water developed its own tuning criteria from the results of their own multi-lab validation studies.
- Their DFTPP criteria are different from the 1975 Eichelberger *et al.* criteria.

Mass	Method 625	Method 1625
51	30 - 60 % of mass 198	8 - 82 % of mass 198
68	< 2 % of mass 69	< 2 % of mass 69
69	no criterion	11 - 91 % of mass 198
70	< 2 % of mass 69	< 2 % of mass 69
127	40 - 60 % of mass 198	32 - 59 % of mass 198
197	< 1 % of mass 198	< 1 % of mass 198
198	Base peak	Base peak
199	5 - 9 % of mass 198	4 - 9 % of mass 198
275	10 - 30 % of mass 198	11 - 30 % of mass 198
365	> 1 % of mass 198	no criterion
441	Present, but < mass 443	44 - 110 % of mass 443
442	Greater than 40 % of mass 198	30 - 86% of mass 198
443	17 - 23 % of mass 442	14 -24 % of mass 443

Method 1625 Criteria

- However, in 1984, when Method 1625B was promulgated for NPDES compliance monitoring, the powers that be replaced the new DFTPP tuning criteria with the Method 625 criteria.
- The Office of Water restored the criteria in Method 1625C, but that version of the method has not been promulgated at 40 CFR Part 136 to date. (These criteria also appear in Method 1653 for chlorophenols.)

Other than that, everything was fine ...

- Until 1987 or so, when one or more instrument vendors started to look into data system algorithms that could adjust the DFTPP spectrum and all of the associated sample results to conditions that met the tuning criteria.
- They called the process “post-acquisition data manipulation.”

EPA's Reaction

- While other EPA Offices may not have taken notice, the CLP did and were very concerned with the term “*post-acquisition data manipulation*” since they were defending CLP results in court cases against parties who were responsible for disposal of hazardous wastes.
- CLP responded by forming a work group and designing a study to look into the importance of tuning on sample results.

CLP Tuning Work Group

- Two-day meeting in May 1987
- Members included:
 - Staff from OERR,
 - OERR contractors,
 - Staff from ORD and other Program Offices,
 - Representatives of several other Federal Agencies and organizations involved in environmental monitoring, and
 - GC/MS instrument manufacturers.

Work Group Discussions

- Neither DFTPP nor BFB are used to actually tune the MS. They are used as “tuning check compounds.”
- Original goal of the criteria was to ensure that mass spectra generated across instruments were as similar as practical.
- CLP goals were somewhat different, as are those for most other EPA monitoring programs.

Work Group Consensus

- Some check of instrument tuning is needed.
- Changes to the existing tuning criteria should be considered.
- The monoisotopic peaks in the spectra must be considered accurate, but acceptance windows may be candidates for changes from the 1975 criteria.
- The rearrangement ions in the spectra are potential candidates for changes that reflect modern instrumentation.

Consensus – cont.

- Criteria should allow for the possibility that either m/z 198 or 442 may appear as the most abundant peak in the DFTPP spectra, and thus be called the “base peak.”
- An alternative method of introducing the tuning check compound should be considered.
- The use of software to assist in meeting the tuning criteria must be resolved (e.g., either explicitly allow it or outlaw it).
- The effects on forward library searches must be evaluated.

CLP Tuning Study Design

- Single-laboratory study by ORD in Las Vegas to identify practical limits of GC/MS operating conditions and data acquisition parameters that would still enable analysis of the CLP list of target analytes.
- The question became *“how much can you distort the DFTPP results and still successfully run the analysis?”*
- 2 studies, 1 by 4 instrument manufacturers, and 1 by 8 EPA Regional laboratories, designed to gauge the variability of DFTPP ion abundances in modern GC/MS instruments, especially for those m/zs in DFTPP with no “correct” abundances (e.g., 51 and 127).

CLP Tuning Study Design – cont.

- 7 CLP labs analyzed 60 CLP semivolatile organic target analytes under 5 sets of DFTPP conditions.
- The 5 conditions ranged from “tunes” grossly distorted to the low-mass end ions to those grossly distorted to the high-mass end, with the original DFTPP conditions and two intermediate “tunes” in between.
- Using standards of known concentrations, labs evaluated both analyte identification (using the EPA/NBS spectra library as the reference) and analyte quantification based on response factors generated under each set of DFTPP conditions.

CLP Tuning Study Results 1

Single-Lab Study Results (ORD-LV)

Tune Characteristics	Total Analytes	# Matched by Library Search	% Matched
1975 Criteria	86	71	83
Base peak = 51, 442 suppressed	86	69	80
Base peak = 51	86	71	83
Base peak = 442	86	73	85
Base peak = 442, 51 suppressed	86	76	88

CLP Tuning Study Results 2

Multi-Lab Study Results (7 CLP labs, 60 analytes)

Tune Characteristics	Total Analytes	# Matched by Library Search	% Matched
1975 Criteria	420	389	92.6
Base peak = 51, 442 suppressed	420	376	89.5
Base peak = 51	420	387	92.1
Base peak = 442	420	390	92.9
Base peak = 442, 51 suppressed	420	385	91.7

CLP Tuning Study Conclusions

- The effects of the tuning criteria on analyte identification are only evident at the two extremes of the tested conditions, where either m/z 51 or 442 was suppressed. No existing tuning criteria of that period approach either of those conditions.
- Studies by the manufacturers and the Regional labs (not summarized here) indicated that the manner in which the tuning data are acquired is more important than the actual ion abundances achieved or the acceptance criteria used.

Revised CLP DFTPP Criteria - 1991

Mass	1975 Criteria	1991 Revised CLP Criteria
51	30 - 60 % of mass 198	10 - 80 % of mass 198
68	< 2 % of mass 69	< 2 % of mass 69
69	--	Present
70	< 2 % of mass 69	< 2 % of mass 69
127	40 - 60 % of mass 198	10 - 80 % of mass 198
197	< 1 % of mass 198	< 2 % of mass 198
198	Base peak	Base peak

Revised CLP DFTPP Criteria – cont.

Mass	1975 Criteria	1991 Revised CLP Criteria
199	5 - 9 % of mass 198	5 - 9 % of mass 198
275	10 - 30 % of mass 198	10 - 60 % of mass 198
365	1 % of mass 198	> 1 % of mass 198
441	< mass 443	Present, but < mass 443
442	> 40 % of mass 198	50 - 100 % of mass 198
443	17 - 23 % of mass 442	15 - 24 % of mass 442

Revised CLP BFB Criteria - 1991

Mass	1975 Criteria	1991 Revised CLP Criteria
50	20 - 40 % of Mass 95	15 - 40 % of Mass 95
75	50 - 70 % of Mass 95	30 - 80 % of Mass 95
95	Base peak	Base peak
96	5 - 9 % of Mass 95	5 - 9 % of Mass 95
173	< 1 % of Mass 174	< 2 % of Mass 174
174	> 50 % of Mass 95	50 - 120 % of Mass 95
175	5 - 9 % of Mass 174	5 - 9 % of Mass 174
176	> 50 % of Mass 174	95 - 101 % of Mass 174
177	5 - 9 % of Mass 176	5 - 9 % of Mass 176

1991 CLP acquisition requirements

- The mass spectrum of DFTPP or BFB must be acquired in the following manner:
 - Three scans (the peak apex scan and the scans immediately preceding and following the apex) are acquired and averaged.
 - Background subtraction required, and must be accomplished using a single scan acquired no more than 20 scans prior to the elution of DFTPP or BFB.
 - Do not subtract part of the DFTPP or BFB peak.

So What's Up Now?

- Various EPA Programs have proposed different DFTPP and BFB tuning criteria over the years.
- There have been calls for “harmonization” among Programs.

Old vs. New Comparison 1

Mass	1975 Criteria	1991 CLP	Method 525.3/8270E
51	30 - 60 % of mass 198	10 - 80 % of mass 198	
68	< 2 % of mass 69	< 2 % of mass 69	< 2 % of mass 69
69	no criterion	Present	Present
70	< 2 % of mass 69	< 2 % of mass 69	< 2 % of mass 69
127	40 - 60 % of mass 198	10 - 80 % of mass 198	

Old vs. New Comparison 2

Mass	1975 Criteria	1991 CLP	Method 525.3/8270E
197	< 1 % of mass 198	< 2 % of mass 198	< 2 % of mass 198
198	Base peak, 100 % relative abundance	Base peak	Base peak or present
199	5 - 9 % of mass 198	5 - 9 % of mass 198	5 - 9 % of mass 198
275	10 - 30 % of mass 198	10 - 60 % of mass 198	

Old vs. New Comparison 3

Mass	1975 Criteria	1991 CLP	Method 525.3/8270E
365	> 1 % of mass 198	> 1 % of mass 198	> 1 % of base peak
441	< mass 443	Present, but < mass 443	< 150 % of mass 443
442	Greater than 40 % of mass 198	50 - 100 % of mass 198	Base peak or present
443	17 - 23 % of mass 442	15 - 24 % of mass 442	15 - 24 % of mass 442

Summary

- DFTPP and BFB are used as instrument performance checks, not to perform the actual tuning of the GC/MS system.
- CLP's revised BFB and DFTPP tuning acceptance criteria from 1991 may not cover all of the exceptions reported to EPA as a result of the use of hydrogen as the carrier gas.
- However, the lessons learned in those CLP studies should be reviewed by EPA program offices before issuing any guidance in this matter.

Summary – cont.

- Based on the CLP study, the specific tuning criteria do not adversely affect the ability of the instrument to correctly identify and quantify the target analytes when the method specifies comparing the sample spectra to the spectra from the most recent calibration.
- The way in which the spectra for all of the target compounds (as well as BFB and DFTPP) are acquired is more important than the criteria themselves.
- Most older EPA methods provide no guidance at all on the acquisition of spectra.

Summary – cont.

- Methods promulgated for NPDES or SDWA compliance monitoring *do not* rely on forward library search results.
- Forward library search results from SW-846 methods may not be useful for RCRA compliance monitoring either.
- CLP forward library search results are at best a starting point for further work.
(All forward searches assume a RRF = 1.0)

DFTPP Concerns

- Historical issues with DFTPP criteria were often instrument manufacturer specific.
- Low end m/zs 68, 69, and 70 were often the issue.
- GC/MS manufacturing landscape has changed greatly since 1975.
- Are we developing criteria to fit current instruments, or to ensure adequate performance?

BFB Concerns

- Specific concerns about m/z 96 in the BFB spectrum exceeding the current 5 - 9% criteria.
- Current criterion for m/z 96 is based on the natural isotopic abundances of ^{13}C and ^2H , and the chance that one of the six carbon atoms or four hydrogen atoms in the benzene ring may contain the heavier isotope.
- It is not surprising that the use of hydrogen as the carrier gas is leading to protonation of the mass fragment that comprises m/z 95.
- However, as long as m/zs 95 and 96 can be readily distinguished from one another, it is difficult to imagine a scenario where the exact abundance of m/z 96 becomes a critical factor in successful application of the volatiles methods.