



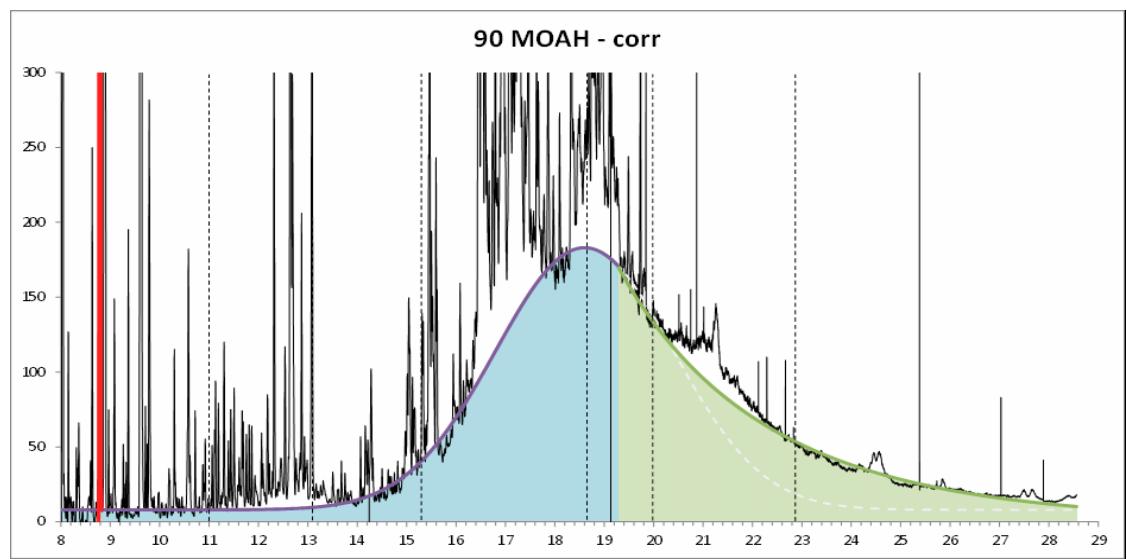
JRC TECHNICAL REPORT

Mineral oil in infant formulas - guidelines for integrating chromatograms

*JRC IF 2021-04: a virtual
inter-laboratory comparison*

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Beldi G., Senaldi C., Valzacchi S. and Hoekstra E.

2022



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Executive summary

Mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) consist of a mixture of various hydrocarbons that are difficult to resolve in a chromatographic column. They produce complex unresolved chromatographic signals (called humps). The quantification of total MOSH and total MOAH mass fractions requires proper integration of the respective humps, after background subtraction and the removal of the remaining interfering peaks. Such an operation is prone to subjective judgement that may increase the scatter of reported results.

The European Union Reference Laboratory for Food Contact Materials (EURL-FCM) organised the first virtual interlaboratory comparison (e-ILC) "JRC-IF-2021-04" to assess the capabilities of the participants in "**integrating chromatograms of mineral oils in infant formulas (IF)**".

A set of chromatograms in "cdf" (binary) or "csv" (ascii) formats - related to IF samples, hexane baselines, and retention time calibrations - were provided to the participants. They were requested to submit results for the mass fraction of total MOSH and total MOAH in seven IF samples.

Thirty participants registered to this round and submitted results. Most of the participants (26 out of 30) reported satisfactory peak areas for the two internal standards (CyCy and 2-MN) with a robust relative standard deviation (RSD) of 0.5 %. A significantly larger scatter of results was observed for total MOSH and total MOAH mass fractions, with RSD ranging from 6 to 18 %. This may be attributed to the various integration strategies used by the participants - keeping or removing interfering peaks and overriding humps - and the different baseline corrections applied.

Specific guidance is provided based on the good practices identified and problems encountered by the laboratories monitoring MOSH/MOAH in infant formulas. These guidelines aim to decrease the variability of results due to the integration of chromatograms.

Detailed information is provided in this report.

1. Introduction

The Foodwatch report (dated 24.10.2019) [1] and the RASFF notification (Rapid Alert System for Food and Feed, Notification Nr.408917, dated 25/10/2019) [2] mentioned the presence of mineral oil aromatic hydrocarbons (MOAH) in infant formula (IF) and follow-on formula. Consequently, the Directorate General for Health and Food Safety (DG SANTE) of the European Commission requested the Joint Research Centre (JRC) to provide a **harmonised and validated method for the determination of MOAH mass fractions in infant formula**, in order to ensure comparable and reliable results.

Bauwens et al. recognised [3] that, in addition to the sample preparation (*), the interpretation and the integration of collected chromatograms are major contributors to the variation of reported results. Since the quantification of the total MOSH and total MOAH mass fractions in the infant formula samples requires proper integration of the complex chromatograms (after background subtraction and the removal of the remaining interfering peaks), the European Union Reference Laboratory for Food Contact Materials (EURL-FCM) decided to organise a dedicated **virtual interlaboratory comparison** (e-ILC).

The outcome of this round (denoted **JRC IF 2022-04**) is presented in this report.

(*) Note: This measurement procedure includes several steps, such as saponification with KOH, hexane extraction, clean-up, epoxidation and the determination by LC-GC-FID (liquid chromatography hyphenated with gas-chromatography with flame ionisation detector). The detailed standard operating procedure was provided to the participants of the ongoing method validation ring trial.

2. Scope

JRC IF 2021-04 is designed to assess the capability of participants to **integrate complex LC-GC-FID chromatograms, obtained when analysing MOSH and MOAH in infant formulas**. Specific guidance is provided based on good practices identified and problems encountered by the participants.

3. Set-up of the exercise

Selection of the chromatograms

Several chromatograms (MOSH and MOAH in IF) were selected by the EURL-FCM, including simple and more challenging cases containing major chromatographic interferences. Seven different infant formulas were selected, and the following chromatograms were provided for each sample:

- MOSH and MOAH chromatograms of the test items, obtained on a single channel instrument;
- Retention time (RT) mix chromatogram, used to define the region of interest for integration (from C10 to C50);
- Hexane chromatograms for MOSH and MOAH baseline corrections.

[1] <https://www.foodwatch.org/en/news/2019/foodwatch-laboratory-tests-suspected-carcinogenic-mineral-oil-residues-in-baby-milk/>

[2] https://www.foodwatch.org/fileadmin/-DE/Themen/Mineraloel/Dokumente/Mineraloel_RASFF_BVL_30-03-2020.pdf

[3] Bauwens G., Pantó S., Purcaro G., Journal of Chromatography A, 1643 (2021) 462043; <https://doi.org/10.1016/j.chroma.2021.462044>

Time frame

The JRC IF 2021-04 round was announced by e-mail on December 8, 2021 (Annex 1) to laboratories having participated to the previous proficiency testing rounds (see reports of JRC IF-2020-1 [4], JRC IF 2020-2 [5]; and JRC IF-2021-3 [6]). The invitation letter, including instructions to participants (Annex 2), the reporting template (Annex 3) and the above-mentioned chromatograms were sent by email on December 26, 2021. The initial deadline for reporting of results was set to January 2, 2022. It was further extended until February 11, 2022, upon request from several laboratories. Finally, a preliminary report was sent by email (dated February 17, 2022) asking participants to check whether their results were correctly transcribed for further evaluation.

Confidentiality

The procedures used for the organisation of this e-ILC guarantee that the identity of the participants and the information they provided are treated as confidential. The participants in this ILC received a unique laboratory code used throughout this report.

Distribution

Each participant received a total of 26 chromatograms in “cdf” (binary) and/or “csv” (ascii) format(s):

- MOSH and MOAH in six infant formula samples (IF40, IF50, IF90, IF92, IF544 and IF669);
- MOSH and MOAH in hexane (baseline reference);
- RT calibration mixture (the same for MOSH and MOAH).
- MOAH in sample IF2A, the corresponding matrix blank baseline, and the respective RT calibration mix.

Instructions to participants

Participants were asked to import the “cdf” or “csv” files in their software to perform the quantification of total MOSH/MOAH. They were requested to report:

- The area of the peaks of the internal standards IS (baseline-corrected);
- The total area of the hump (C10-C50) with the riding peaks (baseline-corrected);
- The total area attributed to the MOSH/MOAH (C10-C50) (total hump, baseline corrected and riding peaks subtracted); and
- The mass fraction of total MOSH (C10-C50) and total MOAH (C10-C50) expressed in mg/kg IF, for the samples IF40, IF50, IF90, IF92, IF544, IF669 and IF2A (only MOAH).

[4] Bratinova S., Robouch P., Karasek L., Goncalves C., Beldi G., Senaldi C., Jakubowska N., Valzacchi S., Conneely P., Hoekstra E., and Emons H. *Determination of MOAH in Infant Formula, JRC IF 2020-01 - an exploratory interlaboratory comparison*, European Commission, Geel, 2020, JRC 121915 EN - <https://europa.eu/!qM3Hjk>

[5] Bratinova S., Robouch P., Beldi G., Senaldi C., Goncalves C., Karasek L., Valzacchi S., Conneely P., Hoekstra E., and Emons H. *Determination of MOAH in Infant Formula, JRC IF 2020-02 - The second interlaboratory comparison*, European Commission, Geel, 2021, JRC 125669 EN - <https://europa.eu/!M4wccG>

[6] Bratinova S., Robouch P., Goncalves C., Karasek L., Beldi G., Senaldi C., Valzacchi S., and Hoekstra E. *Determination of MOSH/MOAH in Shell SN500* mineral oil; JRC IF 2021-03 - The third interlaboratory comparison*, Publications Office (OP) of the European Union, Luxembourg, 2022, ISBN 978-92-76-47525-5, doi:10.2760/23771, JRC 127743 - <https://europa.eu/!hTTgR8>

4. Results and Discussions

At first 32 external laboratories were invited to this exercise; 30 of them reported results, together with JRC-Ispra and JRC-Geel, while laboratories L13 and L21 did not. Annexes 4 to 7 present the “Tables of results”, the corresponding “graphs” and the “chromatograms” that were integrated.

Integration software

Most of the participants (15) used the **Clarity Chromatography** Software (by DataApex.com). Since the earlier version of this software did not import correctly “cdf” (binary) files, participants either installed the latest release or imported “csv” (ascii) files. Seven participants used the **Thermo Chromeleon** software, while five others used the newly designed **Chrolibri** software to integrate the chromatograms and to calculate the total MOSH/MOAH contents. Laboratory L07 reported two sets of results obtained with different software. The remaining few laboratories integrated the chromatograms using the Agilent OpenLab Chemstation, the Shimadzu LabSolutions or the LECO ChromaTOF software. Annex 5.1 presents the list of software used.

Laboratory L35 applied an alternative approach using MS Excel, instead of the dedicated chromatography software provided by instrument manufacturers. The sample and the corresponding baseline “csv” files were first imported in XLS, to perform the baseline correction. The resulting chromatogram was then fitted by two “**modified gaussian equations**” (see blue and green areas in Figure 1) to simulate the envelop under the rising peaks and overriding humps. The resulting “netto hump” was corrected for any residual “pedestal” (cf. horizontal dotted line) and integrated (from C10 to C50) to derive the corresponding total MOSH (or MOAH) mass fractions.

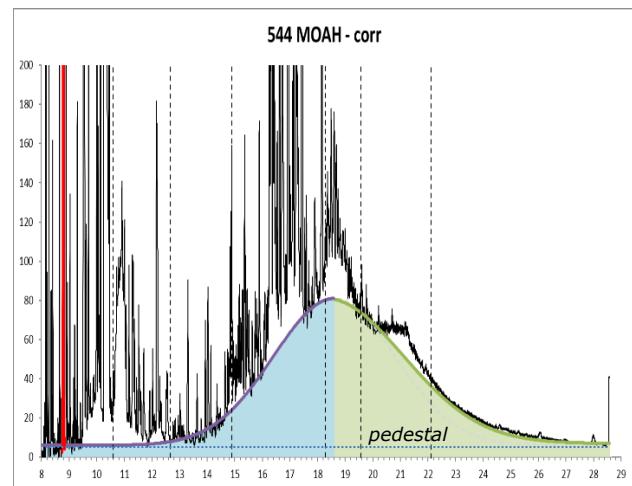


Figure 1: Fitted hump reported by L35 for **MOAH** in IF 544

Reporting units

All chromatograms were provided as a “Response” (expressed in mV) versus “time” (in minutes). It was somewhat surprising to see the broad variety of units used by the participants when reporting the “**peak areas**” of the internal standards and the different humps (as such, baseline corrected, or after removal of the riding peaks). Only few participants used the proper units, such as “mV * min”, “mV * s” or “V *s”. The vast majority did not correct the erroneous units suggested in the reporting template, and expressed their peak areas in “mV/s”, “mV/min”, “kV/s”, or “V/min”. Laboratories L31 and L35 reported the “total counts” collected in the regions of interest of the IS and of the hump (from C10 to C50). Disregarding the issue of the “units”, several conversion factors were used to express all the values in “mV * s”, thus allowing the comparability of the reported peak areas of the IS (see CyCy in Annex 4.2; and 2-MN in Annex 6.2).

Statistical evaluation

For the evaluation of the collected results, the EURL-FCM applied **robust statistics** (where no outlier test is required) using the “MS Excel Add-in for Robust Statistics” available from the Statistical Subcommittee of the Analytical Methods Committee of the UK Royal Society of Chemistry [7]. The following parameters were computed (see Annexes 4.2; 5.1; 6.2 and 7.1):

- i. A15, the estimator of the robust mean;
- ii. MADe, the estimator of the robust standard deviation; and
- iii. robust relative standard deviation, calculated as $RSD = 100 * MADe / A15$ (expressed in %).

Consequently, the percent difference ($D\%$), calculated as $D\% = 100 * (x - A15) / A15$, was used to identify some results (x) deviating from the corresponding robust mean (highlighted in Annexes 4.2, 5.1, 6.2 and 7.1).

Peak areas of internal standards

The internal standard **CyCy** (used for the “calibration” of the FID response to quantify the MOSH hump) presents a single peak that was easy to integrate, as shown by the **excellent agreement of results**: 189 values (out of 192; 98 %) lead to a **robust RSD ranging from 0.2 to 0.5 %** (see statistics in Annex 4.2, and graphs in Annex 4.3). Only two values seem to be significantly different from their respective A15 values: L16 (IF544), and L20 (IF92).

Two methylnaphthalene peaks (1-MN and 2-MN) can be used to “calibrate” the FID response to quantify MOAH hump. However, participants were requested to (i) integrate the **2-MN** peak and (ii) to quantify total MOAH using this internal standard. Since no information about the column used to generate the chromatograms and the 1-MN/2-MN order of elution was provided to participants, two laboratories (L04 and L11) “inverted” the internal standards, and reported slightly deviating results related to 1-MN. As soon as the preliminary report was sent to the participants, L11 spotted their “inversion” and provided a corrected set of results that was included in the current evaluation. Nevertheless, the vast majority of participants (95 %) reported satisfactory peak areas for 2-MN, with a **robust RSD ranging from 0.1 to 0.5 %** across the samples (see statistics in Annex 6.2, and graphs in Annex 6.3). If we exclude the “inverted” values reported by L04 (mentioned above), only three values deviate by 10 % to 20 % from their respective robust means values (see L09 (IF40); L16 (IF544); and L07b (IF50)). The value reported by L32 for 2-MN in IF544 seems unrealistic.

Integration of the corrected hump

The chromatograms of MOSH and MOAH in seven samples are presented in Annexes 5.3 and 7.3, together with the corresponding baselines.

All participants reported the mass fractions of **total MOSH and total MOAH** derived from the C10-C50 region of interest (Annex 5.1 and 7.2). Unlike the internal standards, **significantly larger scatters of results** were observed with a **robust RSD ranging from 6 to 18 %** (see Table 1, Figure 2 and Annexes 5 & 7). Since robust statistics was applied, no outlier test was performed and no outlying values were excluded from the calculation. However, the percent difference ($D\%$) was used to identify values deviating from the robust mean (A15) with a $|D\%| > 35\%$ (listed in Table 1).

[7] MS EXCEL Add-in for Robust Statistics available from: <https://www.rsc.org/membership-and-community/connect-with-others/through-interests/divisions/analytical/amc/software/>

Robust statistics – how not to reject outliers. Analytical Methods Committee, Analyst, 114 (1989) 1489
<https://doi.org/10.1039/AN9891401693>

Table 1: Summary overview of the ranges reported by the participants for the total mass fraction of MOSH and MOAH in seven infant formulas (IF). The robust relative standard deviations (RSD, expressed in %) and several deviating values reported by laboratories are presented, together with the corresponding percent difference, D% (between brackets).

MOSH (cf. Annex 5.1)			MOAH (cf. Annex 7.1)		
IF	RSD	Ranges & deviating values (*)	IF	RSD	Ranges & deviating values (*)
40	10 %	L06(-19 %) - L35(+26 %)	40	13 %	L16(-20 %) - L10(+27 %) & L27(+40 %), L28(+56 %)
50	6.0 %	L32(-21 %) - L30(+14 %)	50	14 %	L16(-24 %) - L28(+34 %)
90	6.0 %	L32(-15 %) - L10(+16 %)	90	10 %	L04(-22 %) - L28(+31 %) & L06(-34 %), L10(+45 %)
92	9.1 %	L12(-18 %) - L10(+30%) & L20(+119 %)	92	18 %	L03(-31 %) - L11(+29 %) & L10(+70 %)
544	9.5 %	L08(-19 %) - L11 (+17 %) & L16(-86 %), L33(-42 %), L32(-38 %) and L20(+111%)	544	17 %	L06(-29 %) - L15(+35 %) & L32(-53 %); L31(+71 %); L24(+92 %), L16(+580 %) See comments in the text
669	12 %	L26(-32 %) - L15(+16 %) & L10(+45 %)	669	16 %	L09(-27 %) - L04(+29 %)
(*) Lab(D%)			IF2A	7.2 %	L29(-13 %) - L10(+30 %)

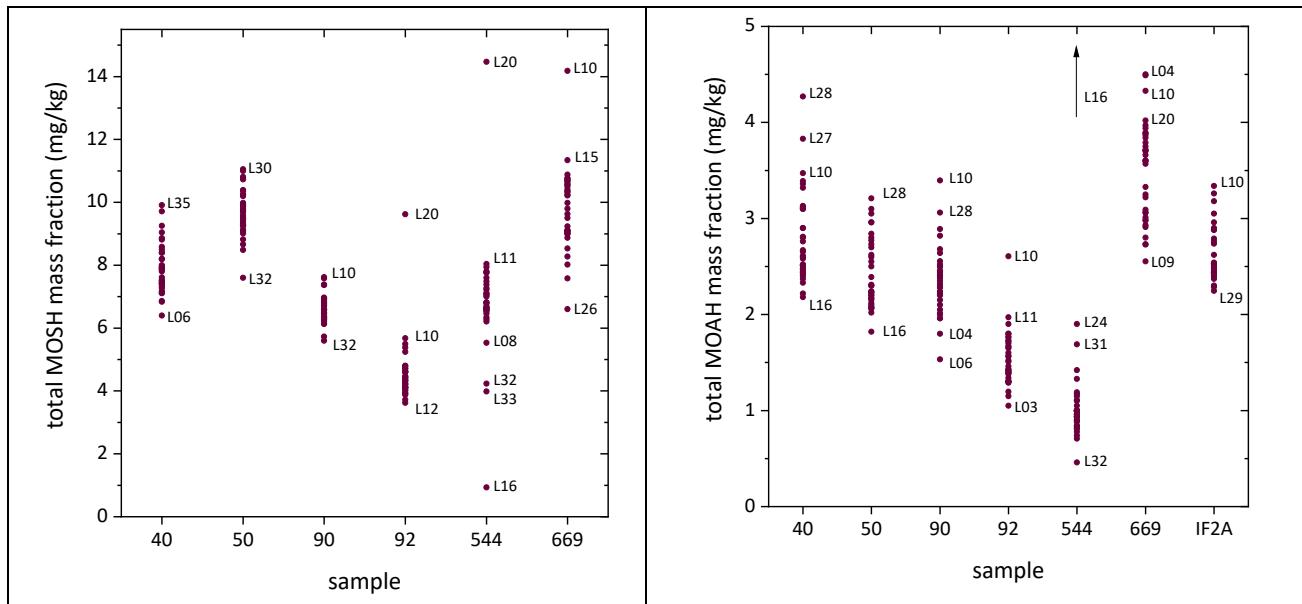


Figure 2. Overview of the **total MOSH & MOAH** mass fractions reported for seven IF samples.
The lowest, highest and some deviating values are labelled by the respective laboratory codes.

The case of **MOAH in IF544** was scrutinised to understand the broad range of results (RSD = 17 %) submitted by the participants when **integrating the same chromatogram**. Figure 3 compares the integrations applied by eight laboratories that reported total MOAH mass fractions ranging from 0.9 to 6.7 mg /kg (L32 and L16, respectively). Such scattered results may be due to the combination of several approaches, where laboratories integrated/ included, after baseline subtraction:

- ❶ the initial hump (RT: 9 – 14 min); and/or
 - ❷ the riding/interfering peaks (RT: 16 – 17.5 min); and/or
 - ❸ the riding/interfering peaks (RT: 18 – 20 min); and/or
 - ❹ the superimposed hump (RT: 20 – 22 min); and/or
 - ❺ the additional correction for the residual offset/"pedestal".
- (for numbering, refer to the last chromatogram in Figure 3)

As shown in the summary table included in Figure 3, L16 integrated all humps and most of the interfering peaks, and reported the highest result. On the contrary, L05 performed a conservative integration - excluding most peaks and other humps - and obtained a significantly smaller area.

- Note 1. In the case of MOAH in IF90, the area of the baseline-corrected & fitted hump was 894 mV*min. L35 observed a residual response after baseline correction (an offset) of 8 mV at RT ranging from 8 to 9 min, which corresponded to an offset/ pedestal of 117 mV*min (= 8 * (22.8 - 8.2)). Hence, the resulting *net hump* (of 777 mV*min) was reduced by 13%.
- Note 2: The visual inspection of the baseline corrected chromatogram is highly recommended, in order to check whether the baseline correction is sufficient (or to identify the presence of an offset/ pedestal to be further subtracted).

General remarks

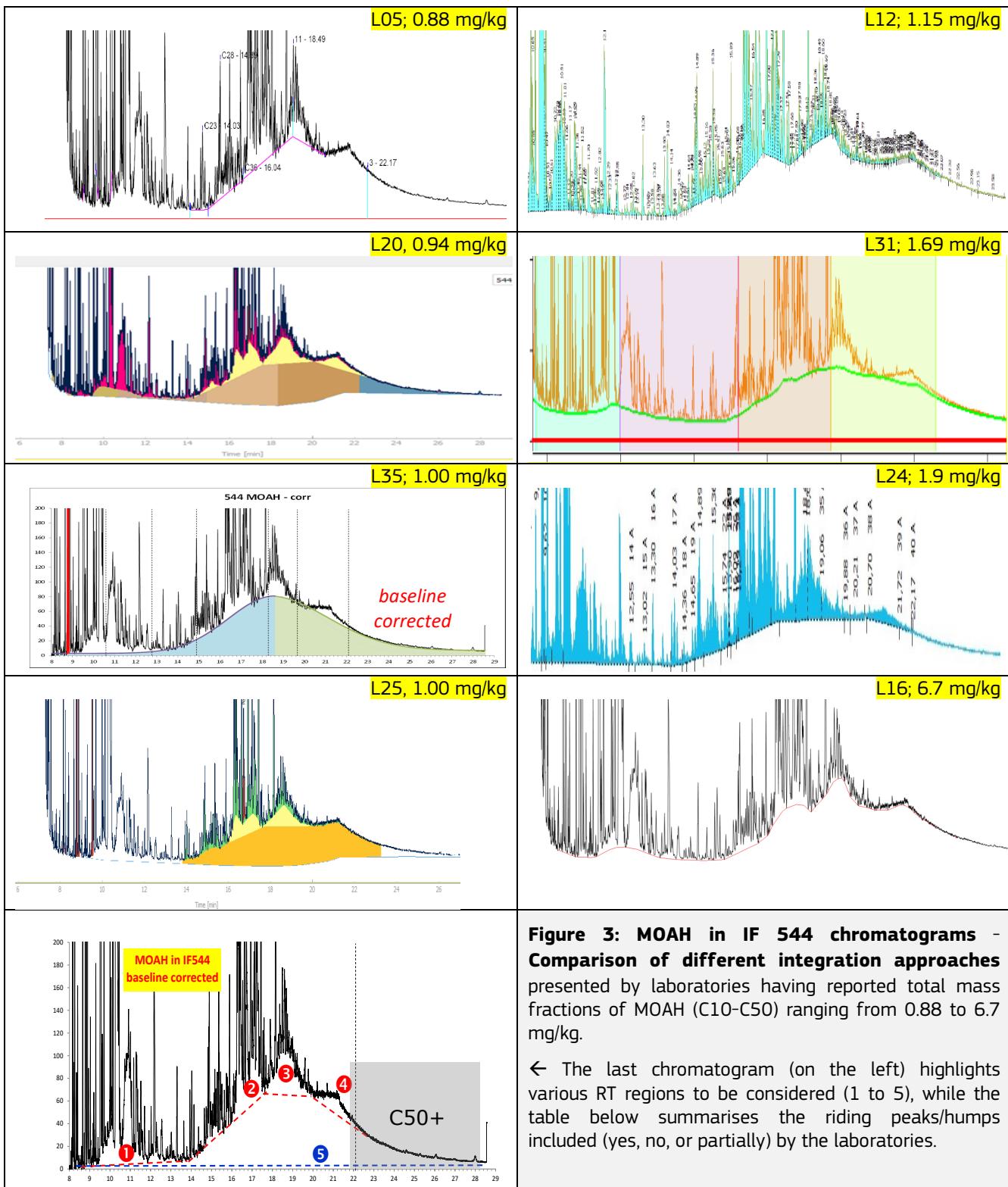
In general, no bias was observed depending on the software used. However, some participants stated that some software do not allow a straightforward baseline correction.

None of the laboratories described how they performed the baseline correction. It is highly probable that participants did not check the resulting offset, and may have overestimated the area of the trimmed hump.

Several participants regretted that the ILC organiser did not provide the chromatograms of the reagent blanks (instead of the hexane baselines). This would have allowed the removal/subtraction of interferences due to the reagents.

Several laboratories commented on the high **column bleeding** compared with the height of the hump in the MOAH chromatograms in IF90, IF92 and IF544. In normal conditions, they stated that they would have reported a "less than value".

Significant **C50+ fractions** were observed for MOAH in IF90 (ca. 15 %); IF544 (19 %); IF92 (20 %) and IF669 (35 %) – as shown in Annex 7.3.

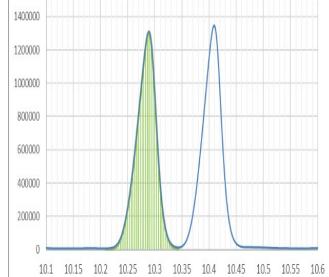
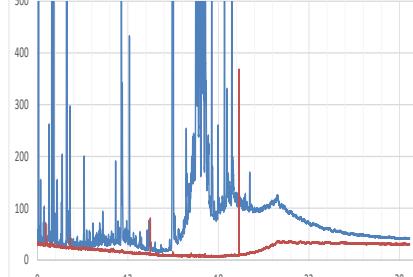
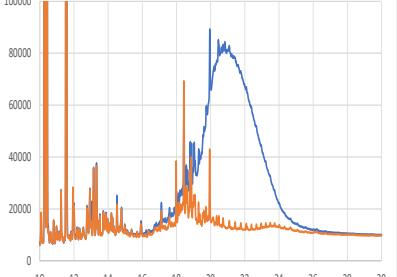
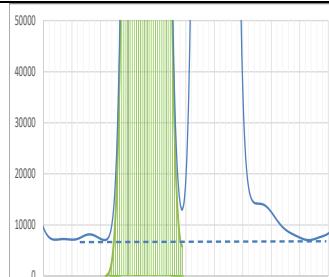
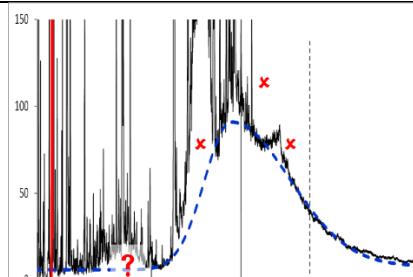
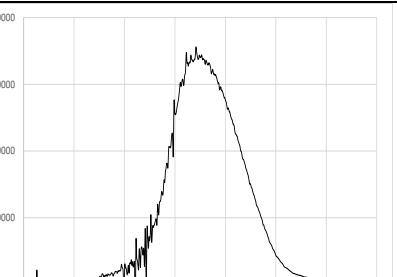


Summary table:

	L05	L20	L35	L25	L12	L31	L24	L16
①	No	Yes	No	No	No	part.	No	Yes
②	No	No	No	No	Yes	No	No	Yes
③	part.	No	No	No	Yes	No	No	Yes
④	Yes	No	No	Yes	Yes	No	No	Yes
(offset corr) ⑤	?	Yes	Yes	Yes	?	?	?	?
tot[MOAH] mg/kg	0.88	0.94	1.0	1.0	1.15	1.69	1.9	6.7

Recommendations for integration

The following recommendations are provided to improve the comparability of results reported when integrating the same chromatogram.

	MN in IF2A	MOAH in IF92	MOAH in IF2A
Sample & baseline		 Hexane baseline – in red	 baseline: matrix blank – in orange
Baseline corrected	 baseline corrected IS peak area - in green	 ? x x offset/pedestal	
<ul style="list-style-type: none"> Subtract the baseline from the IS signal/response; Integrate the IS peak: from baseline to baseline (after vertical split for 1- and 2-MNs) as shown in the 1st column above; Check visually that the offset in the range of the IS is negligible, or correct for it, if relevant; Calculate the IS peak area; Subtract the baseline from the sample chromatogram, using the hexane baseline (see 2nd column); or the procedural/reagent blank, or the matrix blank (when available, see 3rd column); Identify the interfering peaks and/or overriding humps to remove (or keep); Trim the hump accordingly; Check (visually) that the offset in the range of the IS is negligible, and correct for it, when relevant; Integrate the hump in the region of interest (C10-C50); Report the total MOSH or MOAH mass fraction calculated; and Specify the interfering peaks and overriding humps removed. 			

Conclusions

A total of 30 external laboratories and the EURL-FCM reported results to the first virtual interlaboratory comparison "JRC-IF-2021-04" organised to assess the capabilities of the participants in "integrating chromatograms of mineral oils in infant formulas (IF)".

Excellent results were collected for the peak areas of internal standards, as shown by the robust RSD ranging from 0.2 to 0.5 %. A much larger scatter was observed for total MOSH and total MOAH mass fractions, for which the robust RSD ranged from 5 to 18 %. This may be attributed to the various integration strategies used by the laboratories (keeping or removing interfering peaks and overriding humps), and the different baseline corrections applied.

When reporting results, laboratories should (1) specify which interfering peaks and overriding humps were subtracted (by providing chromatogram screenshots) and (2) assess the quality of the background correction applied, in line with the recommendations presented in the report. This will reduce the scatter of results due to the chromatogram's integration, improve the accuracy of the reported measurand values (e.g. mass fraction of total MOSH or MOAH in infant formula) and affect the associated measurement uncertainties. Nevertheless the interpretation and integration of the mineral oils' chromatograms, alongside with the sample preparation, remain amongst the main contributors to the scatter of the results.

Acknowledgements

The European Union Reference Laboratory for Food Contact Materials (EURL-FCM) acknowledges the contribution of the participants listed below.

Organisation	Country
Graz University of Technology	Austria
Primoris	Belgium
Liege University	Belgium
Sciencesano	Belgium
ITERG	France
NQAC NESTLE France Laboratory	France
Bavarian Health and Food Safety Authority	Germany
Bundesinstitut für Risikobewertung (BfR)	Germany
Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe (CVUA-MEL)	Germany
CVUA Stuttgart	Germany
Fraunhofer IVV	Germany
Eurofins WEJ Contaminants GmbH	Germany
GALAB Laboratories GmbH	Germany
Institut Kirchhoff Berlin GmbH	Germany
Landesbetrieb Hessisches Landeslabor	Germany
mas GmbH	Germany
SGS Institut Fresenius GmbH	Germany
IFP Institut für Produktqualität GmbH	Germany
Dublin Public Analyst's Laboratory	Ireland
NEOTRON SPA	Italy
University of Udine	Italy
NOFALAB	Netherland
Eurofins Lab Zeeuws-Vlaanderen (CNL027)	Netherlands
Wageningen Food Safety Research	Netherlands
Dr A Verwey	Netherlands
Centro Nacional Alimentación-AESAN	Spain
Nestlé Research	Switzerland
Official Food Control Authority of the Canton of Zurich	Switzerland
Swiss Quality Testing Services	Switzerland
Nestle RD	China

List of abbreviations

DG SANTE	Directorate General for Health and Food Safety
EURL-FCM	European Union Reference Laboratory for Food Contact Materials
e-ILC	virtual interlaboratory comparison
JRC	Joint Research Centre of the European Commission
LC-GC-FID	Liquid chromatography coupled with gas chromatography and flame ionisation detector
MOAH	Mineral oil aromatic hydrocarbons
MOSH	Mineral oil saturated hydrocarbons
RT	Retention time (expressed in min)

Annex 1 – Announcement e-mail

From: BRATINOVA Stefanka Petkova (JRC-GEEL)
Sent: Wednesday, December 8, 2021 8:48 AM
To: JRC EURL FCM <JRC-EURL-FCM@ec.europa.eu>
Subject: further steps - MOAH in IF

Dear all,

I hoped that with one mail I would have been able to cover all the pending topics of the MOAH in IF project. However the time flies and I feel necessary to inform you about the further developments.

1. Hope that very soon you will receive the final report on the ring trial for characterisation of the Shell SN500* mineral oil.
2. By the end of the year you will receive the revised SOP to be validated in a collaborative trial planned to start in February 2022. Our efforts to achieve full saponification led to additional challenges to be explored and solution proposed. Apparently the better efficiency of the saponification the more significant difference in the behaviour of MNs and TBB. One option to resolved it was by using 12 g Silica column that will retain the remaining fats and will compensate the non-full saponification. However to make the SOP more user and environmental friendly and to be as close as possible to the proposed SOP for improvement of the EN 16995:2017 we decided the following:
 - Proposing the conditions to achieve full SAPO, but introducing a second extraction step
 - Using 3 g silica column for clean-up, which is more time and environmental friendly
 - Compensating the different behaviour of MNs (two ring aromatics) and TBB (mono aromatics) by quantification of the MOAH hump based on averaging the 2MNs and TBB signals.
3. Next step even before the collaborative trial for the MVS will include a ring trial on integration. By the end of the year we are planning to send few files in *.cdf (AIA) format which will include: MOSH/MOAH chromatograms of blank; RT mixture and chromatograms of IF samples. We will ask you to import the files in the softwares that you use routinely (Clarity, Chromeleon, Chrolibri, Agilent Chenstation, LabSolution,...) and to quantify the MOSH/MOAH content. You will have all necessary data for that. The registration to report the results from the integration will be open simultaneously with the dissemination of the files for integration. There will be no deadline for registration beforehand. You will have 4 weeks to report.

Please let me know in case you are not interested in participation in the further steps.

Kind regards
Stefka

Stefanka BRATINOVA



European Commission
Directorate General Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
F.5.

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Joint Research Centre:
The European Commission's science and knowledge service
Discover JRC Science Hub: <https://ec.europa.eu/irc>
Connect with us on :



The views expressed are purely those of the writer and may not in any circumstances be regarded as stating an official position of the European Commission
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Annex 2 – Invitation letter



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel/Ispra)
Food & Feed Compliance



Geel, 21 December 2021

Attn.: «Title» «Firstname» «Surname»
«Organisation»
«Department»
«Zip» «Town»
«Country»

Subject: Participation in JRC IF 2021/04

Dear «Title» «Surname»,

Thank you for participating to the interlaboratory comparison “**JRC-IF-2021/04**” – “**Integration exercise on MOAH in IF**”. This round is organised to evaluate how you integrate the chromatograms provided.

You received total of 27 chromatograms in “cdf” format:

- MOSH and MOAH in six infant formula samples (IF40, IF50, IF90, IF92, IF544 and IF669);
- MOSH and MOAH in hexane (baseline reference);
- RT calibration mixture (the same for MOSH and MOAH).
- MOAH in sample IF2A, the corresponding matrix blank baseline, and the respective RT calibration mix.

All the chromatograms are presented in the attached word file.

In addition, another word file is attached for reporting.

Do not hesitate to contact us if you are not able to import the “*.cdf” files provided in the software you use for the MOSH/MOAH quantification.

You are expected to report:

- the area of the peaks of the internal standards IS (baseline corrected),
- the total area of the hump (C10-C50) with the riding peaks (baseline corrected);
- the total area attributed to the MOSH/MOAH (C10-C50) (total hump – baseline – riding peaks);
- The total content of MOSH (**C10-C50**) and MOAH (**C10-C50**) expressed in mg/kg IF, for the samples IF40, IF50, IF90, IF92, IF544, IF669 and IF2A (only MOAH).

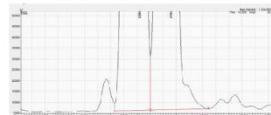
The following sample amounts and internal standard concentrations were used:

sample #	sample amount	IS*	Corresponding conc.**
	g	μ l	mg/kg
40	5.07	20	1.18
50	5.03	20	1.19
90	10.03	10	0.30
92	10.07	10	0.30
544	10.04	10	0.30
669	9.97	5	0.15
2A	5.05	10	0.60

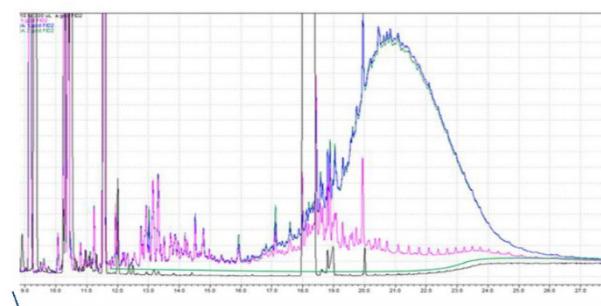
*IS solution: cycy/MN, each 300 mg/l
 ** concentration of cycy/MN related to sample

Take into consideration the following General Guidance on integration that should be applied for the MOAH quantification:

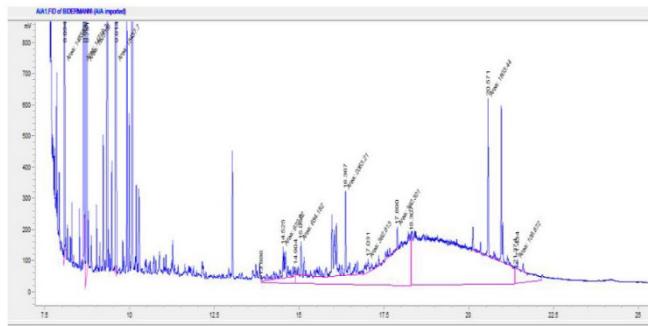
1. Integration of the IS (1MN and 2MN) – if the two peaks are not well resolved, they should be split until the baseline and then integrated. Integration valley-to-valley does not represent the entire area of the IS and compromise the results.
2. Subtract any superimposed humps and riding peaks over the main hump.
3. How to position the baseline?



An offset may be observed in the RT zone before & after the hump, between the "hexane" background chromatogram and the sample chromatogram (see offset between black and pink/green chromatograms for e.g. RT 16 min and RT 27 min). This offset should be reduced by positioning (moving up) the hexane baseline close to the sample chromatogram. Note: The area between the black and the green line is not due to MOAH.



You are requested to **provide a screen shot of the seven chromatogram integrations** (IF40, IF50, IF90, IF92, IF544, IF669 and IF2A) to show your background correction and the riding peaks subtraction (as shown below)



The deadline for submission of the results is set to **January 28, 2022**.

A draft report to participants will be circulated shortly after the end of the round to present the reported values from all participants with their laboratory codes. This code will be disclosed only to the concerned participant.

Your participation in this project is greatly appreciated.

Do not hesitate to contact me for further information.

With kind regards,

Dr. Stefanka Bratinova
JRC IF 2021/04 Coordinator

Annex 3 – Reporting template



Directorate F - Health, Consumers & Reference Materials (Geel/Ispra)
Food & Feed Compliance



Geel, 21 December 2021

Attn.: «Title» «Firstname» «Surname»
«Organisation», «Department»
«Zip» «Town», «Country»

Subject: Participation in JRC IF 2021/04 – "Integration exercise on MOAH in IF"

Lab. Code: «**LabCode**»

		Infant formula sample code			
	Units*	40	50	90	92
	MOAH				
area 2-MN	mV/s				
area of total hump with the riding peaks	mV/s				
area of the hump attributed to MOAH C10-C50 (trimmed hump)	mV/s				
MOAH C10-C50 content	mg/kg				
	MOSH				
area CyCy	mV/s				
area of total hump with the riding peaks	mV/s				
area of the hump attributed to MOSH C10-C50 (trimmed hump)	mV/s				
MOSH C10-C50 content	mg/kg				

	Units*	2A
	MOAH	
area 2-MN	mV/s	
area of the hump attributed to MOAH C10-C50 (trimmed hump)	mV/s	
MOAH content	mV/s	

Annex 4 – CyCy peak areas – Table of results and graphs

4.1 Reported results, expressed in different units

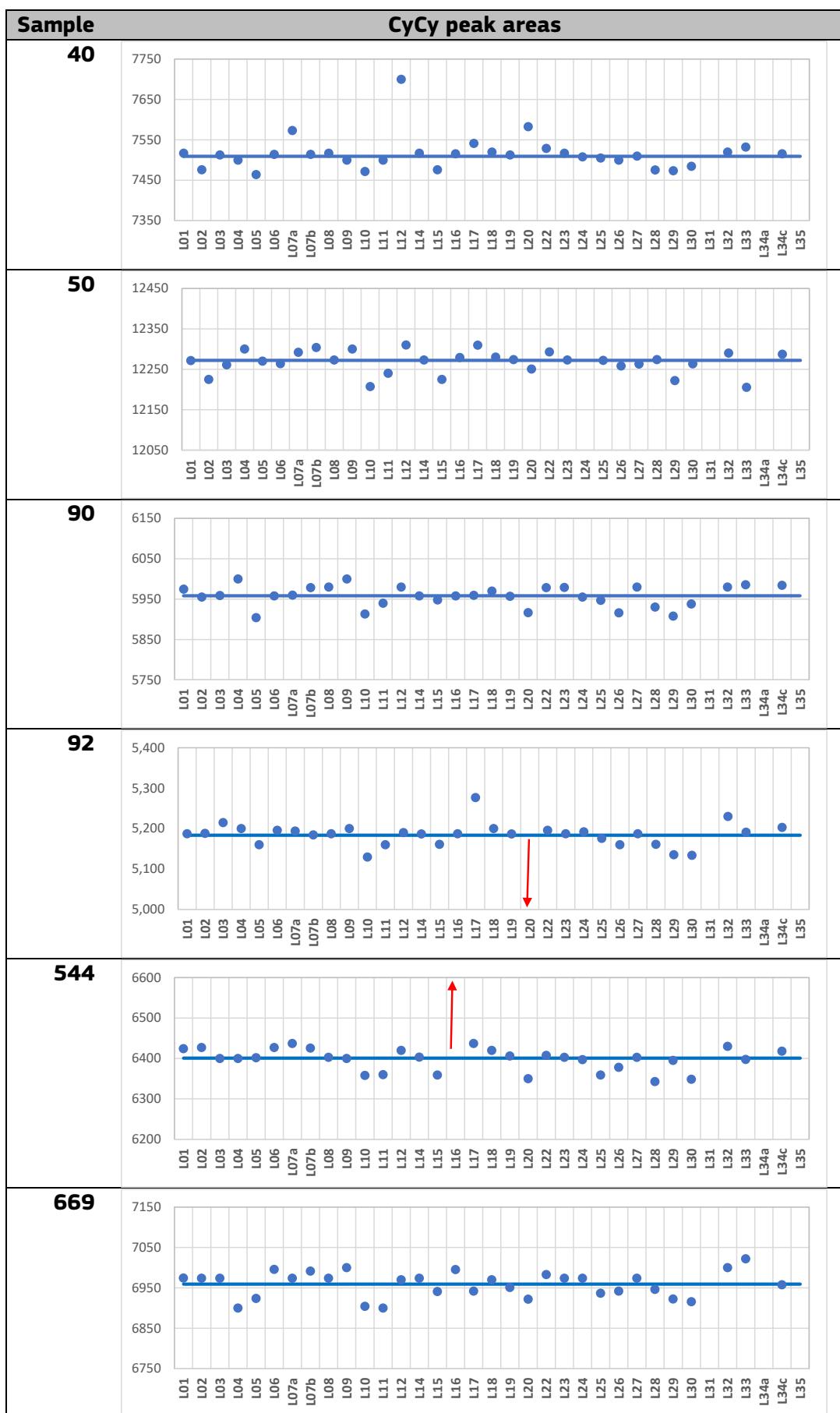
		sample code					
Lab ↑↓	Unit ↓↑	40 ↓↑	50 ↓↑	90 ↓↑	92 ↓↑	544 ↓↑	669 ↓↑
L01	mV/s	7517	12272	5975	5187	6424	6974
L02	mV/s	7476	12225	5955	5188	6427	6974
L03	kV/s	7.513	12.261	5.959	5.215	6.4	6.974
L04	V	7.5	12.3	6	5.2	6.4	6.9
L05	mV*min	124.4	204.5	98.4	86	106.7	115.4
L06	mV*s	7.514	12.264	5.958	5.196	6.427	6.996
L07a	pA*s	7573.15	12291.89	5959.76	5193.75	6436.94	6974.11
L07b	pA*s	7514.03	12303.98	5978.34	5184.37	6425.67	6991.69
L08	mV/s	7517.045	12272.7	5979.779	5187.303	6402.805	6974.023
L09	mV/s	7.5	12.3	6	5.2	6.4	7
L10	mV/s	124527	203450	98555	85490	105966	115077
L11	mV*min	125	204	99	86	106	115
L12	mV/s	7.70	12.31	5.98	5.19	6.42	6.97
L14	mV/s	7517	12273	5958	5187	6403	6974
L15	mV/min	7476	12225	5948	5161	6359	6941
L16	mV*min	125.262	204.652	99.302	86.453	121.004	116.587
L17	mV/s	7541.4	12309.7	5959.2	5276.6	6437.2	6942
L18	V/min	7.52	12.28	5.97	5.2	6.42	6.97
L19	kV/s	7.513	12.274	5.957	5.187	6.406	6.951
L20	mV/s	7583	12250.4	5916.7	2619	6350	6922
L22	V*s	7528.8	12292.9	5978.3	5195.8	6407.3	6982.8
L23	mV/s	7517.045	12272.7	5978.971	5187.303	6402.805	6974.023
L24	mV/s	7.508	12.5	5.955	5.192	6.397	6.974
L25	mV/s	7505	12272	5947	5176	6359	6937
L26	mV/s	1250	2043	986	860	1063	1157
L27	mV/s	7509.862	12263.063	5979.779	5187.252	6402.805	6974.023
L28	mV/s	7475.85	12273.64	5930.27	5161.0619	6342.57	6946.23
L29	mV/min	124.5594	203.7002	98.4639	85.5869	106.5864	115.3728
L30	mV/s	7484.86	12263.17	5937.85	5133.69	6348.51	6915.75
L31	mV/s	187732	306602	148937	129616	160089	174360
L32	kV	7.52	12.29	5.98	5.23	6.43	7
L33	mV/s	75321	122054	59856	51913	63973	70221
L34a	mV/s						
L34c	mV/s	7515770	12287274	5984115	5202807	6418133	6957972
L35	counts	187492	306796	148908	129423	159355	173339

Counts?

4.2 Converted results (for comparison) and corresponding robust statistics

Lab	40	50	90	92	544	669
L01	7517	12272	5975	5187	6424	6974
L02	7476	12225	5955	5188	6427	6974
L03	7513	12261	5959	5215	6400	6974
L04	7500	12300	6000	5200	6400	6900
L05	7464	12270	5904	5160	6402	6924
L06	7514	12264	5958	5196	6427	6996
L07a	7573	12292	5960	5194	6437	6974
L07b	7514	12304	5978	5184	6426	6992
L08	7517	12273	5980	5187	6403	6974
L09	7500	12300	6000	5200	6400	7000
L10	7472	12207	5913	5129	6358	6905
L11	7500	12240	5940	5160	6360	6900
L12	7700	12310	5980	5190	6420	6970
L14	7517	12273	5958	5187	6403	6974
L15	7476	12225	5948	5161	6359	6941
L16	7516	12279	5958	5187	7260	6995
L17	7541	12310	5959	5277	6437	6942
L18	7520	12280	5970	5200	6420	6970
L19	7513	12274	5957	5187	6406	6951
L20	7583	12250	5917	2619	6350	6922
L22	7529	12293	5978	5196	6407	6983
L23	7517	12273	5979	5187	6403	6974
L24	7508	12500	5955	5192	6397	6974
L25	7505	12272	5947	5176	6359	6937
L26	7500	12258	5916	5160	6378	6942
L27	7510	12263	5980	5187	6403	6974
L28	7476	12274	5930	5161	6343	6946
L29	7474	12222	5908	5135	6395	6922
L30	7485	12263	5938	5134	6349	6916
L31						
L32	7520	12290	5980	5230	6430	7000
L33	7532	12205	5986	5191	6397	7022
L34a						
L34c	7516	12287	5984	5203	6418	6958
L35						
count	32	32	32	32	32	32
A15 mean	7509	12272	5958	5184	6400	6960
MADe	20	24	30	18	29	35
RSD	0.3%	0.2%	0.5%	0.3%	0.4%	0.5%

4.3 Graph of converted results



Annex 5 – Total MOSH mass fractions (C10-C50) – Table of results and graphs

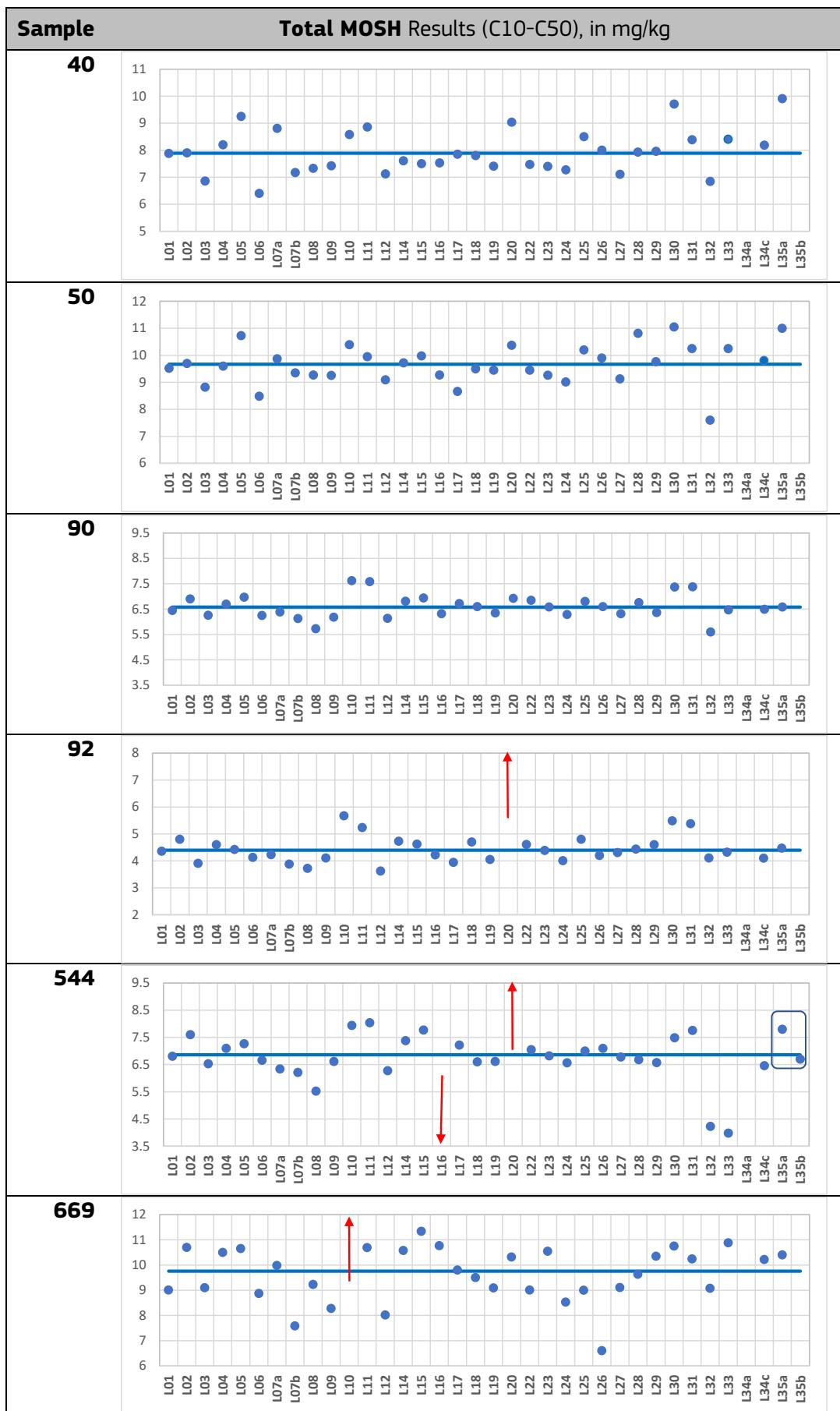
5.1 Reported results (expressed in different units) and corresponding robust statistics

Lab	40	50	90	92	544	669	Sotware
L01	7.88	9.52	6.45	4.36	6.81	9.01	Clarity
L02	7.90	9.70	6.90	4.80	7.60	10.70	Clarity
L03	6.86	8.82	6.26	3.91	6.53	9.10	Clarity
L04	8.20	9.60	6.70	4.60	7.10	10.50	Chameleon
L05	9.25	10.73	6.97	4.42	7.27	10.65	Chameleon
L06	6.397	8.485	6.253	4.132	6.659	8.871	Clarity
L07a	8.81	9.87	6.39	4.23	6.34	9.98	Chrolibri
L07b	7.17	9.35	6.13	3.88	6.21	7.58	Clarity
L08	7.33	9.27	5.73	3.72	5.53	9.23	Clarity
L09	7.43	9.25	6.19	4.11	6.62	8.28	Clarity
L10	8.58	10.39	7.62	5.68	7.94	14.18	Chameleon
L11	8.86	9.95	7.58	5.24	8.04	10.69	Chameleon
L12	7.12	9.09	6.14	3.62	6.28	8.02	Clarity
L14	7.61	9.72	6.81	4.73	7.38	10.58	--
L15	7.50	9.98	6.94	4.62	7.77	11.34	Clarity
L16	7.53	9.27	6.32	4.22	0.93	10.77	Chameleon
L17	7.85	8.66	6.72	3.94	7.22	9.80	Clarity
L18	7.80	9.50	6.60	4.70	6.60	9.50	Chemstation
L19	7.41	9.45	6.35	4.05	6.62	9.09	Clarity
L20	9.04	10.37	6.92	9.62	14.47	10.32	Chrolibry
L22	7.47	9.45	6.85	4.61	7.05	9.01	Clarity
L23	7.40	9.26	6.58	4.39	6.82	10.55	Clarity
L24	7.27	9.01	6.29	4.01	6.57	8.53	Clarity
L25	8.50	10.20	6.80	4.80	7.00	9.00	Chrolibry
L26	8.00	9.90	6.60	4.20	7.10	6.60	Chameleon
L27	7.11	9.13	6.32	4.31	6.78	9.11	Clarity
L28	7.93	10.81	6.76	4.44	6.68	9.63	Chrolibry
L29	7.96	9.77	6.36	4.60	6.57	10.35	Chameleon
L30	9.71	11.05	7.37	5.49	7.49	10.75	Chrolibry
L31	8.39	10.25	7.38	5.38	7.76	10.24	LECO ChromaTOF
L32	6.84	7.60	5.60	4.11	4.23	9.08	--
L33	8.41	10.25	6.47	4.32	3.99	10.88	Agilent MS
L34a							Lab.Solution
L34b	8.19	9.8	6.49	4.10	6.46	10.22	Lab.Solution
L35a	9.91	11.0	6.58	4.47	7.8	10.4	MS Excel
L35b					6.7		MS Excel

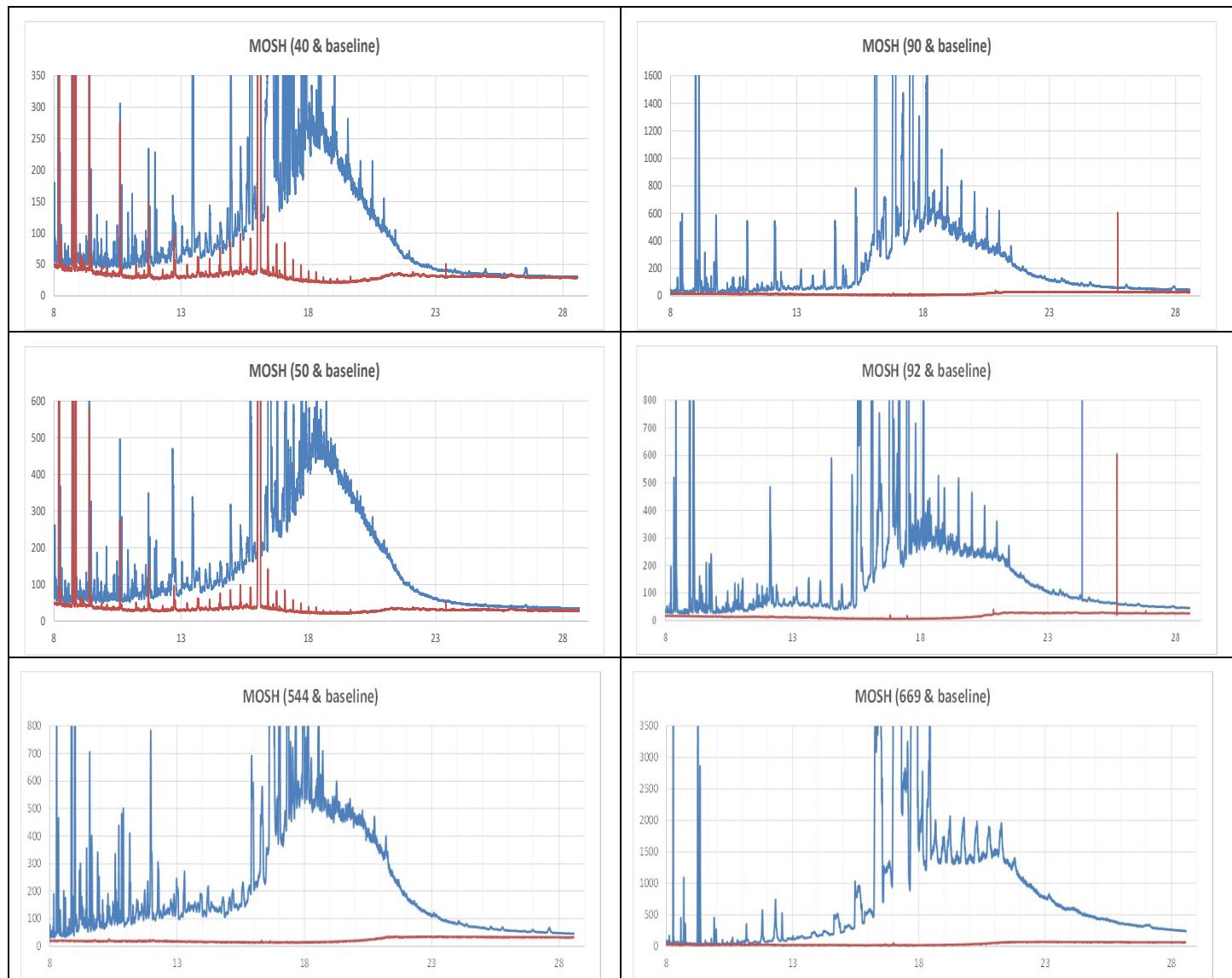
bimodal

count	34	34	34	34	35	34
A15 mean	7.89	9.66	6.58	4.40	6.86	9.75
MADe	0.79	0.58	0.39	0.40	0.65	1.19
RSD	10%	6.0%	6.0%	9.1%	9.5%	12%

5.2 Graphs of reported results



5.3 MOSH chromatograms provided (sample and baseline)



Annex 6 – 2-MN peak areas – Table of results and graphs

6.1 Reported results, expressed in different units

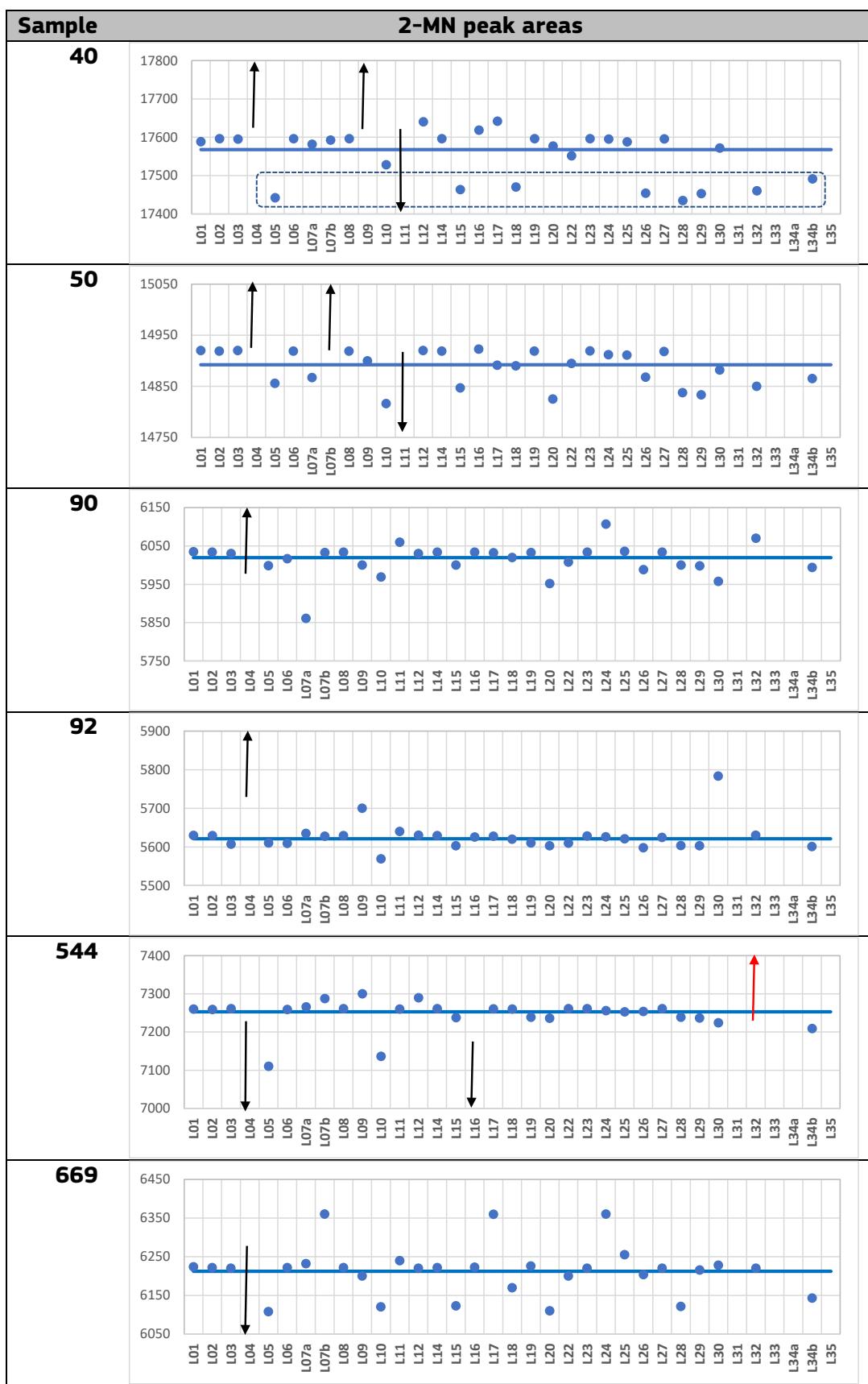
		sample code					
Lab ↑↓	Un its ↓	40 ↓	50 ↓	90 ↓	92 ↓	544 ↓	669 ↓
L01	mV/s	17588.061	14920.12	6034.959	5629.577	7260.129	6224.043
L02	mV/s	17596	14919	6034	5629	7259	6222
L03	kV/s	17.595	14.92	6.03	5.607	7.261	6.22
L04	V	19	16.2	7.3	6.4	6.3	5.1
L05	mV*min	290.7	247.6	99.98	93.5	118.5	101.8
L06	mV*s	17.596	14.919	6.017	5.609	7.259	6.222
L07a	pA*s	17581.5	14867.1	5860.83	5635.02	7266.05	6231.86
L07b	pA*s	17592.21	17562.62	6032.63	5627.49	7287.811	6360.21
L08	mV/s	17596	14919	6034	5629	7261	6222
L09	mV/s	19.1	14.9	6	5.7	7.3	6.2
L10	mV/s	292135	246935	99487	92815	118940	102006
L11	mV*min	288	244	101	94	121	104
L12	mV/s	17.64	14.92	6.03	5.63	7.29	6.22
L14	mV/s	17596	14919	6034	5629	7261	6222
L15	mV/min	17463	14847	6000	5603	7238	6123
L16	mV*min	293.642	248.714	100.562	93.763	106.752	103.71
L17	mV/s	17642	14891.1	6032.1	5627.8	7260.9	6359.5
L18	V/min	17.47	14.89	6.02	5.62	7.26	6.17
L19	kV/s	17.596	14.919	6.033	5.61	7.239	6.226
L20	mV/s	17577	14825	5952	5603	7236	6110
L22	V*s	17551.3	14894.7	6007.9	5609.7	7261.3	6200.4
L23	mV/s	17596.254	14919.481	6033.811	5628.382	7260.923	6220.151
L24	mV/s	17.595	14.912	6.107	5.626	7.256	6.36
L25	mV/s	17588	14911	6036	5621	7253	6255
L26	mV/s	2909	2478	998	933	1209	1034
L27	mV/s	17595.688	14918.09	6033.811	5624.708	7260.923	6220.151
L28	mV/s	17434.47	14837.61	6000.39	5603.7	7238.55	6121.21
L29	mV/min	290.879	247.2177	99.97	93.381	120.612	103.5913
L30	mV/s	17571.57	14881.96	5957.4	5783.39	7224.13	6228
L31	mV/s	439921.67	373015.08	150816.86	140650.28	181533.51	155522.22
L32	kV/s	17.46	14.85	6.07	5.63	14.92	6.22
L33							
L34a							
L34b	mV/s	17491	14865	5994	5601	7209	6143
L35	counts	438748	371732	150363	140041	181604	153588

Note: L11 submitted a corrected set of results (dated 21/02/2022)

6.2 Converted results (for comparison) and corresponding robust statistics

Lab	40	50	90	92	544	669
L01	17588	14920	6035	5630	7260	6224
L02	17596	14919	6034	5629	7259	6222
L03	17595	14920	6030	5607	7261	6220
L04	19000	16200	7300	6400	6300	5100
L05	17442	14856	5999	5610	7110	6108
L06	17596	14919	6017	5609	7259	6222
L07a	17582	14867	5861	5635	7266	6232
L07b	17592	17563	6033	5627	7288	6360
L08	17596	14919	6034	5629	7261	6222
L09	19100	14900	6000	5700	7300	6200
L10	17528	14816	5969	5569	7136	6120
L11	17280	14640	6060	5640	7260	6240
L12	17640	14920	6030	5630	7290	6220
L14	17596	14919	6034	5629	7261	6222
L15	17463	14847	6000	5603	7238	6123
L16	17619	14923	6034	5626	6405	6223
L17	17642	14891	6032	5628	7261	6360
L18	17470	14890	6020	5620	7260	6170
L19	17596	14919	6033	5610	7239	6226
L20	17577	14825	5952	5603	7236	6110
L22	17551	14895	6008	5610	7261	6200
L23	17596	14919	6034	5628	7261	6220
L24	17595	14912	6107	5626	7256	6360
L25	17588	14911	6036	5621	7253	6255
L26	17454	14868	5988	5598	7254	6204
L27	17596	14918	6034	5625	7261	6220
L28	17434	14838	6000	5604	7239	6121
L29	17453	14833	5998	5603	7237	6215
L30	17572	14882	5957	5783	7224	6228
L31						
L32	17460	14850	6070	5630	14920	6220
L33						
L34a						
L34b	17491	14865	5994	5601	7209	6143
L35						
count	31	31	31	31	31	31
A15 median	17568	14892	6019	5621	7253	6212
MADe	45	30	33	21	10	24
RSD	0.3%	0.2%	0.5%	0.4%	0.1%	0.4%

6.3 Graph of converted results



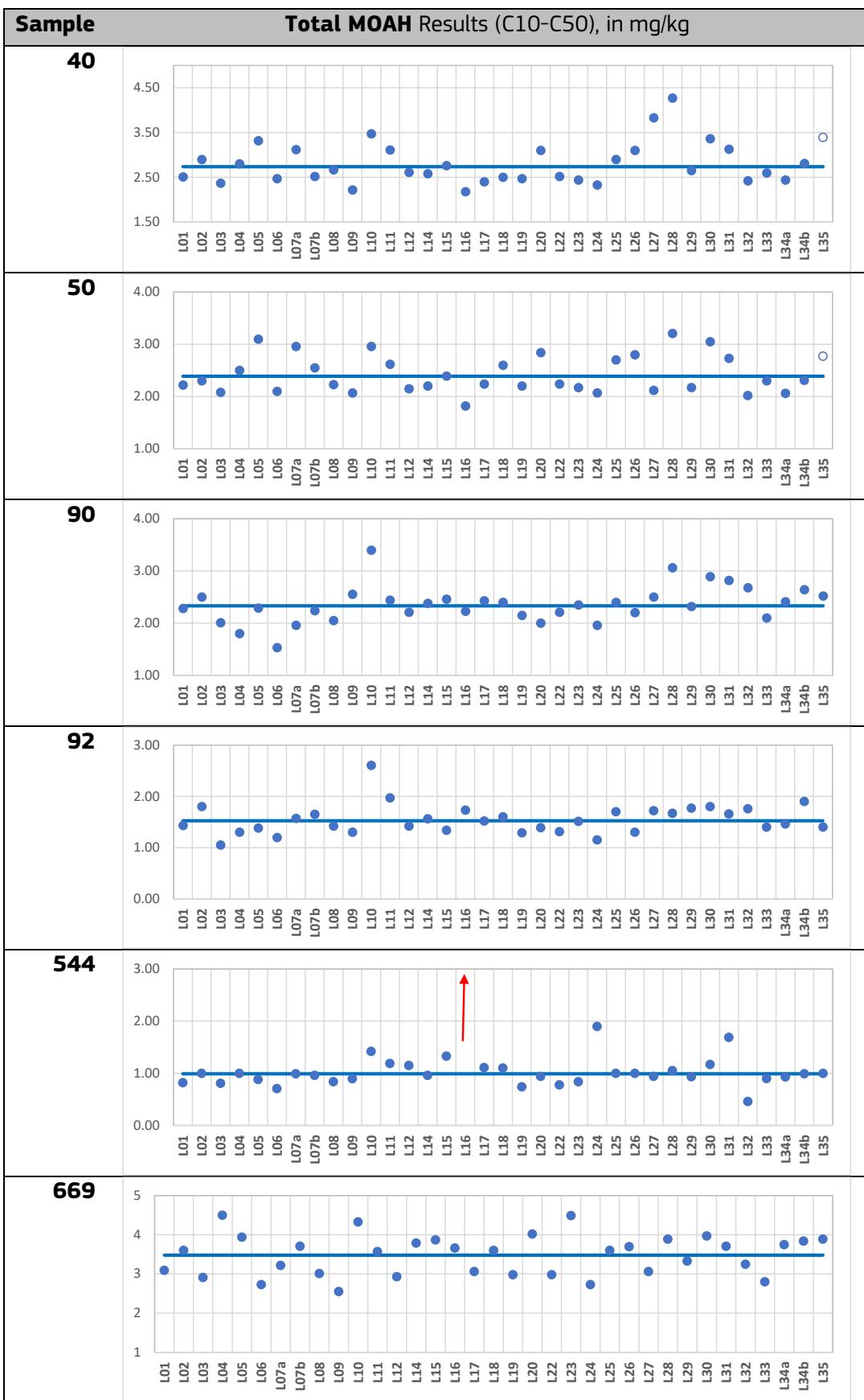
Annex 7 – Total MOAH mass fractions (C10-C50) - Table of results and graphs.

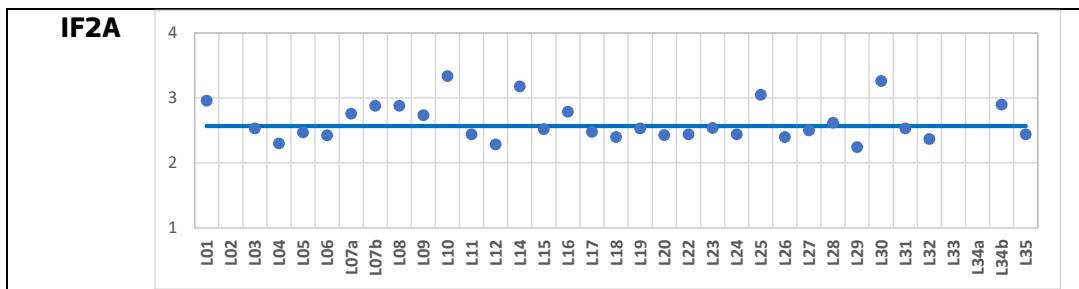
7.1 Reported results (expressed in mg/kg) and corresponding robust statistics

Lab	sample code						
	40	50	90	92	544	669	IF2A
L01	2.51	2.22	2.28	1.43	0.82	3.09	2.96
L02	2.90	2.30	2.50	1.80	1.00	3.60	nd
L03	2.37	2.08	2.01	1.05	0.81	2.91	2.53
L04	2.80	2.50	1.80	1.30	1.00	4.50	2.30
L05	3.32	3.10	2.29	1.38	0.88	3.94	2.47
L06	2.47	2.10	1.53	1.20	0.71	2.73	2.42
L07a	3.12	2.96	1.96	1.57	0.99	3.22	2.76
L07b	2.52	2.55	2.24	1.65	0.96	3.71	2.88
L08	2.67	2.23	2.05	1.42	0.84	3.01	2.88
L09	2.22	2.07	2.56	1.30	0.90	2.55	2.74
L10	3.47	2.96	3.40	2.61	1.42	4.33	3.34
L11	3.11	2.62	2.44	1.97	1.19	3.57	2.44
L12	2.61	2.15	2.21	1.42	1.15	2.93	2.29
L14	2.58	2.20	2.38	1.56	0.96	3.79	3.18
L15	2.76	2.39	2.46	1.34	1.33	3.87	2.52
L16	2.18	1.82	2.23	1.73	6.70	3.66	2.79
L17	2.40	2.24	2.43	1.52	1.11	3.06	2.48
L18	2.50	2.60	2.40	1.60	1.10	3.60	2.40
L19	2.47	2.20	2.15	1.29	0.74	2.98	2.53
L20	3.10	2.84	2.00	1.39	0.94	4.02	2.43
L22	2.52	2.24	2.21	1.31	0.78	2.98	2.44
L23	2.44	2.17	2.35	1.51	0.84	4.49	2.54
L24	2.33	2.07	1.96	1.15	1.90	2.73	2.44
L25	2.90	2.70	2.40	1.70	1.00	3.60	3.05
L26	3.10	2.80	2.20	1.30	1.00	3.70	2.40
L27	3.83	2.12	2.50	1.72	0.94	3.06	2.50
L28	4.27	3.21	3.06	1.67	1.05	3.89	2.62
L29	2.65	2.17	2.32	1.77	0.93	3.33	2.25
L30	3.36	3.05	2.89	1.80	1.17	3.97	3.26
L31	3.13	2.73	2.82	1.66	1.69	3.71	2.53
L32	2.42	2.02	2.68	1.76	0.46	3.25	2.37
L33	2.60	2.30	2.10	1.40	0.90	2.80	nd
L34a	2.44	2.06	2.41	1.46	0.93	3.75	nd
L34b	2.81	2.31	2.64	1.90	0.99	3.84	2.90
L35	3.39	2.77	2.52	1.40	1.00	3.89	2.44
Count	34	34	34	34	34	34	31
A15 mean	2.74	2.39	2.33	1.52	0.99	3.48	2.57
MADe	0.37	0.34	0.22	0.28	0.16	0.55	0.19
RSD	13%	14%	10%	18%	17%	16%	7%

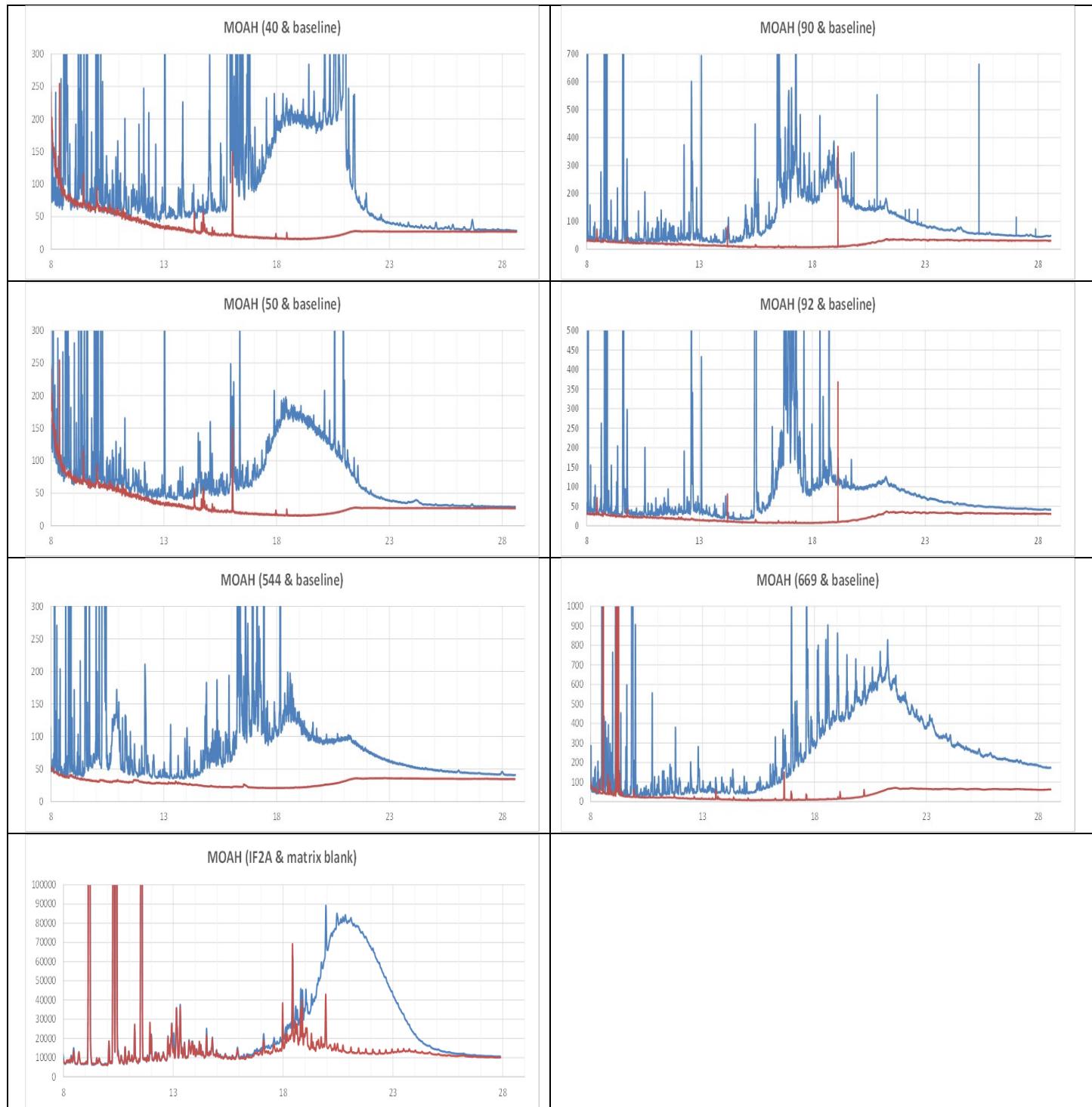
Note: L11 submitted a corrected set of results (dated 21/02/2022)

7.2 Graph of reported results





7.3 MOAH chromatograms provided



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