European Commission

JRC TECHNICAL REPORT

Determination of MOAH in Infant Formula

JRC IF 2020-02 - The second interlaboratory comparison

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Executive summary

The second interlaboratory comparison (ILC, JRC IF 2020-02) was organised by the Joint Research Centre (JRC) of the European Commission as a follow up of the first exploratory ILC (JRC IF 2020/01) to improve the harmonised standard operating procedure (SOP) for the determination of mineral oil aromatic hydrocarbons (MOAH) in infant formula (IF).

Participants were requested to apply the specific SOP, drafted by the JRC based on the outcome of the first ILC, to determine the MOAH content (total and several MOAH fractions) in the two IF test items provided. Satisfactory method performance characteristics were obtained for the rapeseed oil based sample (IF02A). However, several modifications were identified for a final SOP. They should improve the method performance characteristics for the determination of MOAH in challenging IF formulations, such as the palm oil based matrices (e.g., as used here for IF02B).

This report is presenting the outcome of the ILC attended by 28 participants from 8 European countries.

1 Introduction

Following the Rapid Alert System for Food and Feed (RASFF) notification message 2019.3734 (dated 25/10/2019) [1] and the Foodwatch report [2] related to mineral oil aromatic hydrocarbons (MOAH) in infant formula and follow-on formula (IF), the Directorate General for Health and Food Safety (DG SANTE) of the European Commission requested the Joint Research Centre (JRC) to organise a Roundtable Workshop on the determination of MOAH in IF [3]. The meeting held in Brussels on December 5, 2019, was attended by various stakeholders (e.g. official control laboratories, industry and NGOs), DG SANTE and EFSA. The comparability and reliability of the analytical procedures applied by laboratories to monitor the MOAH content in IF were thoroughly discussed. A broad variety of experimental procedures was reviewed. Participants identified the need for a harmonised analytical method to be validated and further standardised. DG SANTE requested the JRC to coordinate the work.

In 2020 the JRC organised an exploratory interlaboratory comparison [4] (ILC) to evaluate the analytical procedures applied to determine the MOAH mass fraction in an infant formula sample. On this basis aA draft SOP has been proposed and the second ILC has been organised by the JRC with two challenging test items. This report is presenting the outcome of this ILC, named **JRC IF 2020-02**.

2 Scope

ILC JRC IF 2020-02 has been designed to familiarise the participants of the MOAH in IF project with the new standard operating procedure (SOP) drafted by the JRC and to explore its applicability to challenging test items containing potential interfering substances. This ILC should allow (i) the identification of modifications to be implemented in the SOP and (ii) the selection of competent laboratories for the final ring-trial validation study.

3 Set-up of the exercise

Since the beginning of the project, the JRC has collaborated with Special Nutrition Europe (SNE) to produce tailored test materials (i) based on simple and/or challenging formulations (prone to chromatographic interferences), (ii) containing different MOAH contents (iii) in amounts suitable for running a method validation ring trial.

After several virtual meetings the following strategy was adopted:

I. In August 2020, the pilot plant identified by SNE produced three bulk IF materials of different compositions:

   a) **Rapeseed oil based IF** blank material (not containing MOAH), with little chromatographic interferences for the MOAH determination (BL1).

   b) **Palm oil based IF** blank material (not containing MOAH) - a "worst case" resulting in complex chromatograms with interferences (BL2).

[1] https://www.foodwatch.org/fileadmin/-DE/Themen/Mineraloel/Dokumente/Mineraloel_RASFF_BVL_30-03-2020.pdf
[2] https://www.foodwatch.org/en/news/2019/foodwatch-laboratory-tests-suspected-carcinogenic-mineral-oil-residues-in-baby-milk/
[3] Report from the Roundtable meeting: https://ec.europa.eu/jrc/en/eurl/food-contact-materials/technical-guidelines
[4] Bratinova S., Robouch P., Karasek L., Goncalves C., Beldi G., Senaldi C., Jakubowska N., Valzacchi S., Conneely P., Hoekstra E., Emons H. (2020) Determination of MOAH in Infant Formula, JRC IF 2020-01 - an exploratory interlaboratory comparison, European Commission, Geel, JRC 121915

c) **BL1 spiked** with 50 mg kg^{-1} SN500 mineral oil (BL1SP). The spiking was performed in the oil ingredient before mixing and spray drying of the IF, to ensure a homogeneous distribution of the mineral oil in the spiked material.

II. By mid-September the JRC Reference Material Unit blended BL1 and BL2 with the BL1SP to produce two test items (IF02A and IF02B) with little or many chromatographic interferences over the so-called MOAH hump. The JRC homogenised the newly produced materials and filled 100 ml brown glass bottles each with 45 g of powder. All necessary measures were taken to prevent cross-contamination:

- the bottles were baked before filling at 400 °C for at least 6 h;
- the crimp caps used for closure contained Teflon lining; and
- an aluminium (Al) foil was inserted between the caps and the bottle neck. In addition, the bottles were wrapped in Al foil to prevent any potential gas-phase contaminations during the shipment and storage.

III. Homogeneity was assessed by the JRC in October 2020, after the preparation of the test items and before distribution to the participants. Ten bottles were randomly selected and analysed by on-line LC-GC/FID in 2 replicates each. Results were evaluated according to ISO 13528:2015 [5] and proved that the two test items were adequately homogeneous (Annex 1).

Confidentiality

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

Time frame

The ILC JRC IF 2020-02 round was announced by e-mail on September 10, 2020 (Annex 2). The proposed SOP was sent to the interested participants on October 16, 2020 (Annex 3). All samples were dispatched on November 10, 2020 to the registered participants. At first, the deadline for the reporting of results was set to January 4, 2021. However, due to the ongoing pandemics, the deadline was extended until January 25, 2021.

Distribution

Each participant received:

- Two test items (IF02A and IF02B, one bottle each);
- The "Instruction to participants" (Annex 4); and
- The "Confirmation of receipt" form to be sent back to the PT coordinator after receipt of the test item (Annex 5).

Instructions to participants

[5] ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison https://www.iso.org/obp/ui/#iso:std:iso:13528:ed-2:v2:en

Detailed instructions were provided to the participants by e-mail (Annex 4). They were requested to apply the experimental protocol described in the SOP. In addition, they were allowed to provide a second set of results obtained with an alternative method.

The following measurands were defined in line with the JRC Guideline [6]:

- "the mass fraction of **total MOAH** in IF", expressed in mg kg^{-1}
- "the mass fraction of the MOAH in IF corresponding to the retention time of n-alkanes from n-C10 to n-C16 (**MOAH C10-C16**)", expressed in mg kg^{-1}
- "the mass fraction of the MOAH in IF corresponding to the retention time of n-alkanes from n-C16 to n-C25 (**MOAH C16-C25**)", expressed in mg kg^{-1}
- "the mass fraction of the MOAH in IF corresponding to the retention time of n-alkanes from n-C25 to n-C35 (**MOAH C25-C35**)", expressed in mg kg^{-1}
- "the mass fraction of the MOAH in IF corresponding to the retention time of n-alkanes from n-C35 to n-C50 (**MOAH C35-C50**)", expressed in mg kg^{-1}

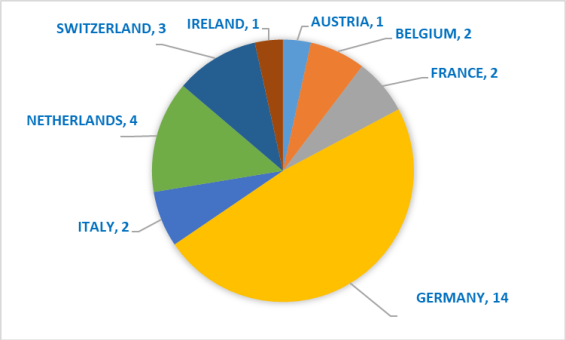
Participants were asked to check whether the test items were undamaged after transport and to report, if necessary, using the "Confirmation of receipt form" (Annex 5).

In addition, participants were requested to:

- Perform three independent measurements and report the three results in mg kg^{-1} (R1, R2, R3);
- Report the final (mean) value as they would report to their customer (in mg kg^{-1}), together with the associated expanded measurement uncertainty (in mg kg^{-1}), specifying the coverage factor;
- Provide the experimental details using the online questionnaire [7] (Annex 6) and the deviations from the SOP (if any); and
- Provide the recorded chromatograms for the two samples (IF02A and IF02B).

4 Results and Discussions

A total of 31 laboratories registered to the ILC JRC IF 2020-02 round. However, only 28 participants from 8 European countries reported results.



[6] JRC Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials https://publications.jrc.ec.europa.eu/repository/handle/JRC115694

[7] https://ec.europa.eu/eusurvey/runner/JRC_IF_2020_01A

4.1 Results

The ILC JRC IF 2020-02 aimed to familiarise the participants of the MOAH in IF project with JRC's proposed SOP and to explore its limitations with more potential interferences in the IF test items than in the first round. It was not meant as a proficiency test; hence, the reported results were not scored.

Results, as submitted by the participants, are presented in Annexes 7-11 for IF02A and in Annexes 12-16 for IF02B. Participants reported quantitative results (numerical values) for total MOAH and the C25-C35 and the C35-C50 fractions. Truncated "Less than" values were mainly reported for the C10-C16 and the C16-C25 fractions.

Seven laboratories (01A, 03A, 06A, 15A, 17A, 18A and 20A) submitted results using an alternative method, while laboratory 02A applied major modifications to the SOP investigated.

The performance characteristics of the JRC SOP presented in the upper part of Table 1, are compared to those derived from the reported results obtained by all the methods, including the alternative ones. The relative standard deviations for reproducibility (RSD_R), obtained by using the JRC SOP for the rapeseed oil samples (IF02A), ranged from 20 % to 25 %. They are much smaller than those achieved in the frame of the previous ILC JRC IF 2020-01 [4] due to the further harmonisation of several steps in the SOP. As expected from an interference prone matrix (cf. palm oil samples IF02B), larger RSD_R are observed for the analysis of the second test item, ranging from 26 to 32 %. Similarly, higher RSD_R values are obtained when pooling the results obtained with all the methods used (JRC SOP and the alternative methods).

The reported results together with their associated expanded measurement uncertainties are presented graphically in Figures 1-3.

Table 1. Statistical parameters for the results obtained (i) by the JRC SOP and (ii) all the methods, including the alternative ones. The relative standard deviations for reproducibility predicted by the Horwitz equation ($RSD_{Horwitz}$) are compared to those derived from N results reported in the frame of this ILC (observed RSD_R).

Methods	Samples	MOAH fraction	N	Kernel density mode	Robust mean	$RSD_{Horwitz}$	observed RSD_R
JRC SOP (only)	IF02A	total	26	2.46	2.42	14.0 %	20.7 %
		C25-C35	27	0.81	0.83	16.5 %	25.2 %
		C35-C50	27	1.70	1.49	14.8 %	21.6 %
	IF02B	total	27	2.92	3.00	13.6 %	31.9 %
		C25-C35	27	1.08	1.22	15.8 %	40.9 %
		C35-C50	27	1.65	1.64	14.8 %	35.5 %
All methods	IF02A	total	33	2.45	2.33	14.0 %	26.5 %
		C25-C35	34	0.80	0.80	16.5 %	34.6 %
		C35-C50	34	1.43	1.44	15.2 %	29.3 %
	IF02B	total	34	2.98	2.94	13.6 %	33.9 %
		C25-C35	34	1.14	1.19	15.7 %	43.7 %
		C35-C50	34	1.61	1.62	14.9 %	35.3 %

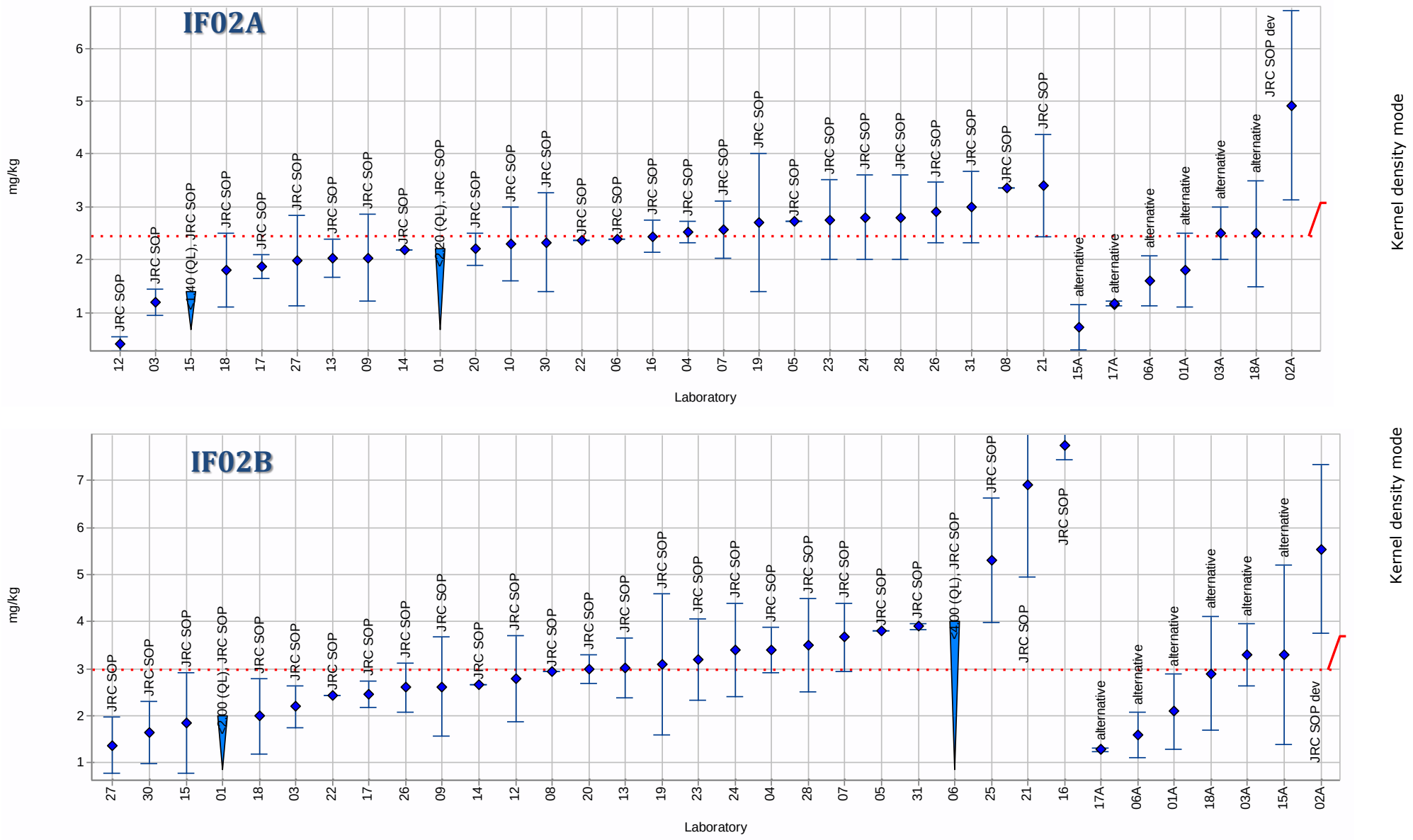


Figure 1: Content of total **MOAH (final)** in mg kg^{-1} with the associated measurement uncertainties as reported by the participants, according to (i) the requested JRC SOP; (ii) an alternative method; and (iii) a modified JRC SOP

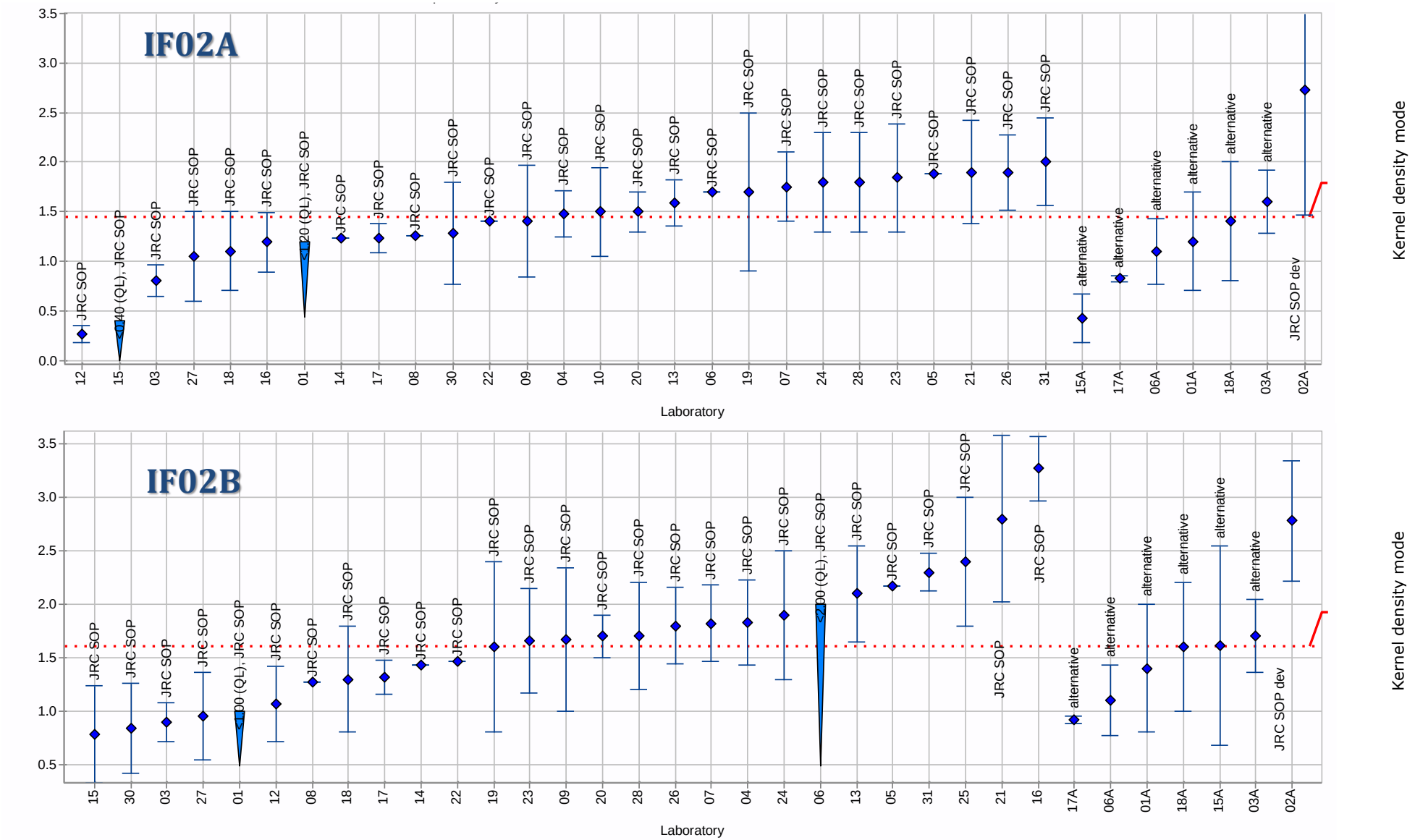


Figure 2: Content of **MOAH C35-C50** (according to Guideline [6]) in mg kg^{-1} with the associated measurement uncertainties as reported by the participants according to (i) the requested JRC SOP; (ii) an alternative method; and (iii) a modified JRC SOP

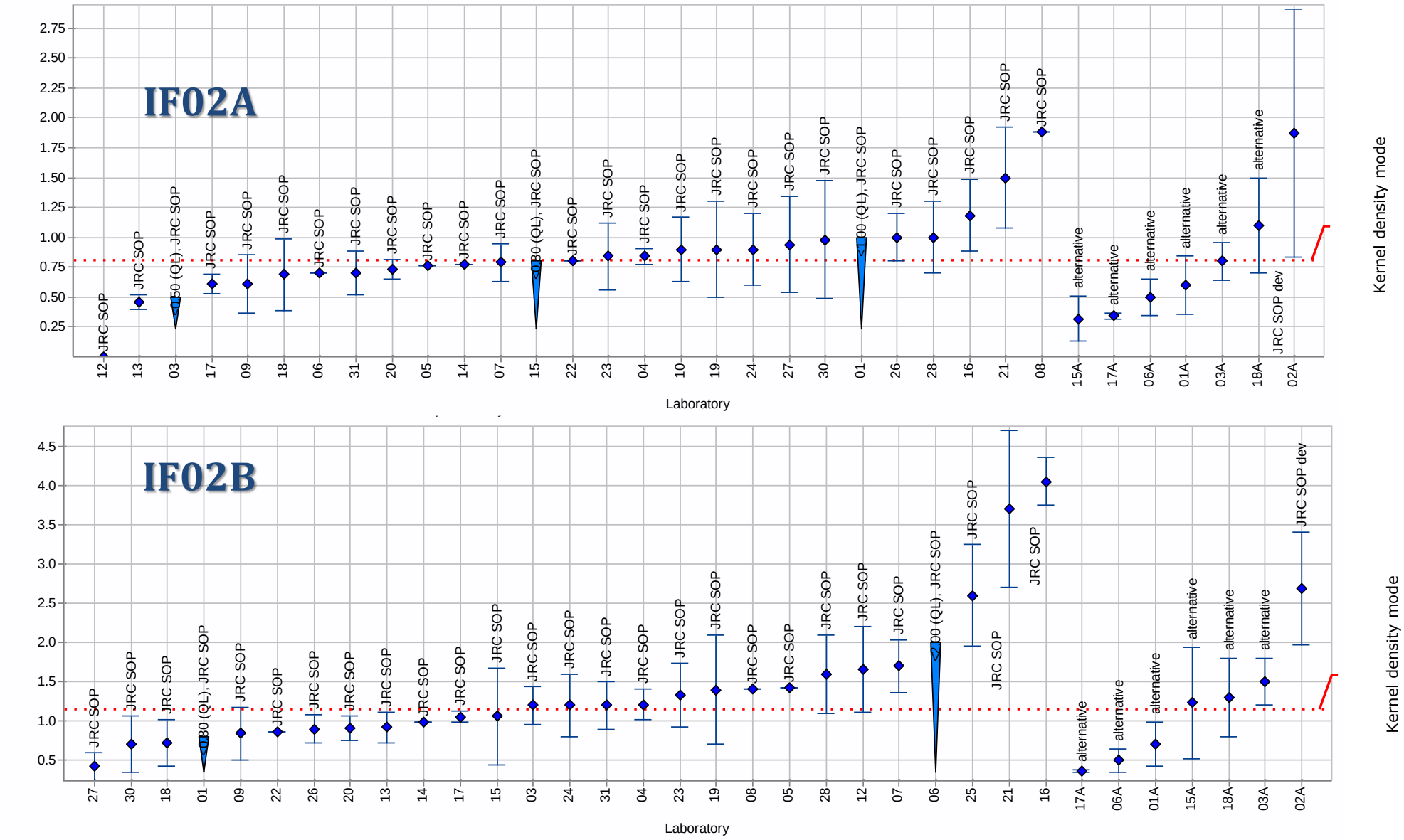


Figure 3: Content of **MOAH C25-C35** (according to Guideline [6]) in mg kg^{-1} with the associated measurement uncertainties as reported by the participants according to (i) the requested JRC SOP; (ii) an alternative method; and (iii) a modified JRC SOP

4.2 Questionnaire

The online questionnaire (Annex 6) has been designed to gather information related to (i) the implemented deviations from the proposed SOP, (ii) the experimental difficulties encountered, and to collect (iii) proposals for further improvements of the experimental procedure.

4.2.1 Main experimental difficulties reported

While satisfactory performance characteristics have been obtained for the interference-free samples (IF02A), many participants reported problems encountered during the sample preparation steps when applying the procedure.

It became clear that the procedure for saponification is not robust enough. The efficiency of the saponification should be improved in order to reduce the fat content in the extract before injection onto the silica clean-up column.

The following experimental challenges were reported by the laboratories:

- The reconstitution of 5 g IF powder in only 5 ml water.
- The concentration of the viscous extract obtained after incomplete saponification down to 1 ml and the successive separation in the HPLC column. The extract had to be diluted to a final volume larger than 1 ml.
- The formation of many riding peaks in the chromatogram after incomplete epoxidation.
- The precipitation of crystals after epoxidation.
- The loss of methyl naphthalene (MN) used as internal quantification standards. Therefore, quantification against tri-tert-butyl benzene (TBB) was required.

4.2.2 An overview of the proposals for improvement

Several modifications of the SOP were identified for a final SOP to be applied to all types of IF available on the market.

The following modifications are considered:

- **Reconstitution of 5 g IF in 10 ml of warm water** (instead of 5 ml) resulting in a homogeneous and clump-free solution to be further saponified.
- **Use of an excess of KOH for saponification** to reduce significantly the fat content in the extract. In addition, several participants mentioned the influence of vigorous shaking on the efficiency of saponification (*investigation ongoing*).
- Second saponification to complement the efficiency of the first one. This step could be avoided, if full saponification is reached by increasing the KOH amount.
- Second extraction to compensate for the low recovery of the internal standard (IS). This step does not seem to be relevant, since the majority of laboratories reported satisfactory recoveries of the IS after the first extraction. This was further confirmed by the ratios of the relevant verification standards measured by the participants and additional JRC experiments.
- **Washing of the extract after saponification with ethanol/water** as mandatory to ensure a better phase separation and to remove the polar substances from the extract.

- **Column clean-up with silica gel before and after epoxidation**. In cases when 100 % efficiency of the saponification can be guaranteed (i.e. no remaining fat in the extract), the column clean-up could be skipped, which would open the possibility for automation of the analysis. However, a column clean-up before epoxidation was preferred by a number of participants as a prevention to take potential variability of saponification rates between different labs into account. A clean-up after epoxidation could optionally remain, as it could potentially increase the lifetime of the HPLC column and could eliminate interferences from meta-chloroperoxybenzoic acid (mCPBA) impurities.
- Use of **12 g or 3 g silica gel for the column clean-up**. A 12 g column is more efficient in removing the fat residues remaining after the saponification step. Some participants have reduced the sample intake from 5 g to 2-3 g for matching the efficiency of the 3 g silica gel column. However, this could conflict with the goal to detect lower levels of MOAH in IF. In view of the aim to improve saponification with a new SOP, work is ongoing to evaluate the feasibility of continuing with a 3 g silica column used as a precaution against any potential fat residues from the saponification.
- Use more m-CPBA and increase the reaction time. Increasing the epoxidation time and temperature did not increase the removal of biogenic olefins. Therefore, the current conditions are deemed suitable.
- Other epoxidation step (stronger epoxidation, second epoxidation or epoxidation in dichloromethane (DCM)). An alternative epoxidation is not recommended in view of avoiding an additional loss of MOAH.

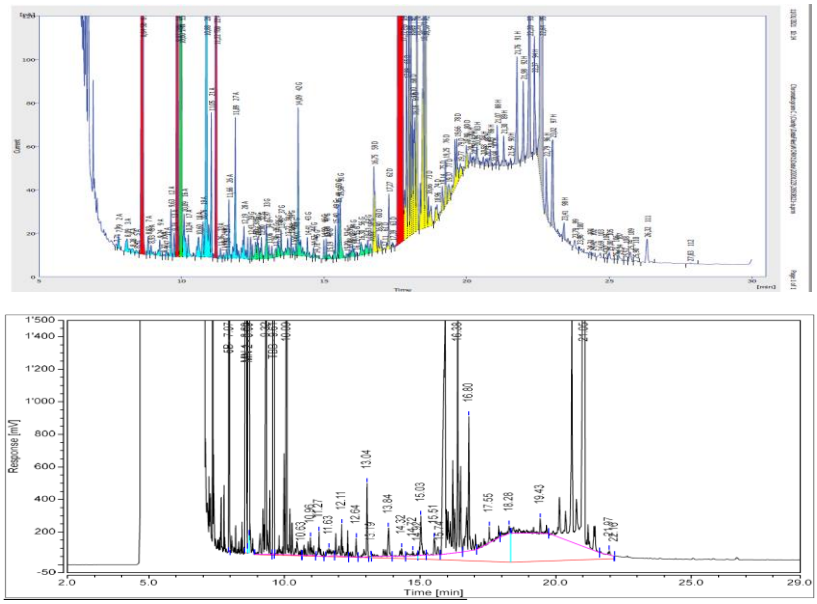


Figure 4 MOAH chromatograms from 2 participants with similar shape of the hump, but different approaches for signal integration

The **chromatographic signal integration is another critical step, which needs harmonisation**. Depending on the integration process applied on the very same chromatogram, significantly different

results could be obtained. This is even more critical in the case of complex chromatograms with unresolved signals (like for MOSH or MOAH), many riding peaks and/or superimposed humps (Figure 4).

While the JRC Guidance document [6] is recommending to integrate the entire chromatogram when determining the total MOAH content, more than half of the participants reported the total MOAH as a sum of the content of the different fractions quantified, applying a lower bound approach. Such an approach would provide underestimated results, when MOAH are detected but not quantified in some of the fractions.

Not many participants have replied to the question on the percentage of the very challenging IF samples in their routine work. The received answers were spread from 5 to 80 % of all analysed IF samples. Most of the participants observed MOAH signals extending beyond the C50 fraction in a high percentage of their analysed samples (60-100 %).

4.3 LOQ estimation

Despite the fact that all laboratories were requested to apply the same SOP, the reported limits of quantification (LOQ) broadly ranged from 0.05 to 0.8 mg kg^{-1} MOAH per fraction. This may be attributed to (i) the chromatographic interferences that were differently integrated in some of the fractions, and to (ii) different instrumental setups requiring different instrumental parameters or different injection volumes into the HPLC or GC columns of the on-line systems.

The estimation of the LOQ for the mass fraction of total MOAH (LOQ_{tot}(MOAH)) does not appear to be harmonised yet. Figure 5 shows that almost half of the participants reported an **underestimated** LOQ_{tot}(MOAH) equal to the LOQs of the individual fractions (LOQ_i(MOAH). Another third of the laboratories reported an **overestimated** and conservative LOQ_{tot}(MOAH) equal to the sum of the LOQ_i(MOAH) of the four fractions. The remaining laboratories reported a more **realistic** LOQ_{tot}(MOAH) equal to the sum of two LOQ_i(MOAH), based on the fact that the MOAH contamination in the samples investigated covers only the "C25-C35" and "C35-C50" fractions.

Obviously, further harmonisation is needed for the evaluation and reporting of the LOQ for total MOAH. This important step is under discussion and the JRC will provide further guidance.

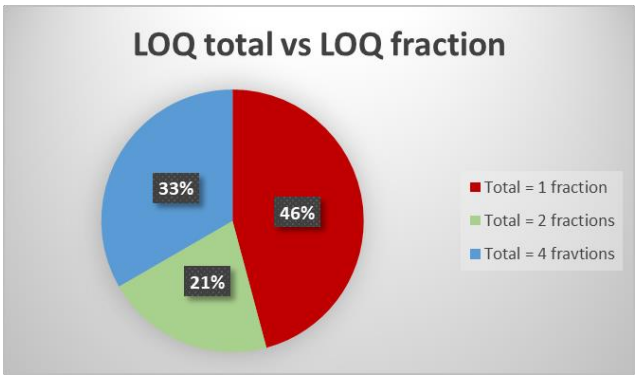


Figure 5. How participants calculated the LOQ of total MOAH

5 Conclusions

The second ILC was organised to identify relevant analytical aspects to be improved and implemented in the SOP used for determination of the MOAH in infant formula. Satisfactory performance was obtained for the analysis on the rapeseed-based sample having less interferences. Several modifications to the SOP have been identified - in particular related to the saponification step - to improve the quantification of the mass fraction of MOAH in IF, as also for palm oil based samples containing more interferences.

At first, the suggested modifications will be implemented, tested and validated in the JRC laboratories. Then a dedicated ring-trail validation study will be organised to determine the performance characteristics of the optimised SOP for the quantification of MOAH mass fraction levels in various IFs.

Acknowledgements

The European Reference Laboratories for Food Contact materials (EURL-FCM) would like to acknowledge Special Nutrition Europe (NESTLE pilot plant) for producing the different powder infant formulas and the Reference Material Unit of the JRC for processing the material and delivering high quality test items. Furthermore, the 28 laboratories listed hereafter are kindly acknowledged for their participation to this ILC round.

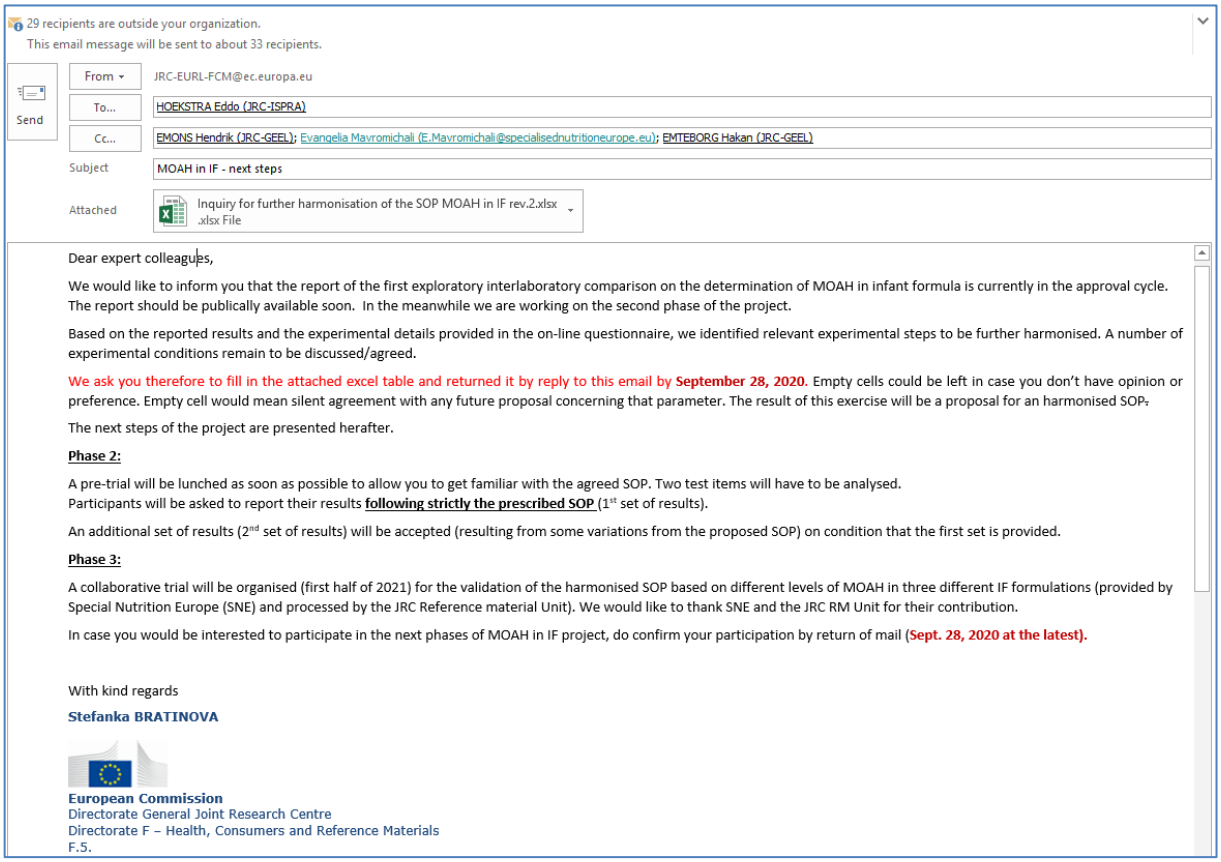
Organisation	Country
Graz University of Technology	Austria
Primoris	Belgium
Liege University	Belgium
ITERG	France
NQAC NESTLE France Laboratory	France
Bavarian Health and Food Safety Authority	Germany
bilacon GmbH	Germany
Bundesinstitut für Risikobewertung (BfR)	Germany
Chemisches und Veterinäruntersuchungsamt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

Annex 1: Homogeneity study (all values in mg kg^{-1})

Sample	IF02A		IF02B	
1	2.384	2.335	2.824	2.805
2	2.115	2.236	2.746	2.630
3	2.456	2.291	2.874	2.728
4	2.211	2.325	2.904	2.959
5	2.212	2.216	2.795	2.891
6	2.140	2.258	2.958	2.878
7	2.117	2.132	2.747	2.668
8	2.250	2.270	2.938	2.853
9	2.192	2.214	2.947	2.753
10	2.162	2.177	2.833	2.758
mean	2.235		2.825	
s_{bb}	0.081		0.082	
s_r	0.060		0.074	
u_{hom}	0.069		0.063	
σ_{pt} (20 %)	0.447		0.565	
0.3 σ_{pt}	0.134		0.169	
$u_{hom} < 0.3\ \sigma_{pt}$	passed		passed	

Where: s_{bb} is the between-bottle standard deviation,
s_r is the analytical standard deviation under repeatability conditions,
u_{hom} is the standard deviation due to inhomogeneity,
σ_{pt} is the standard deviation for performance assessment.

Annex 2. Invitation letter



29 recipients are outside your organization.
This email message will be sent to about 33 recipients.

Send

From: JRC-EURL-FCM@ec.europa.eu
To: HOEKSTRA Eddo (JRC-ISPRA)
Cc: EMONS Hendrik (JRC-GEEL); Evangelia Mavromichali (E.Mavromichali@specialisednutritioneurope.eu); EMTEBORG Hakan (JRC-GEEL)
Subject: MOAH in IF - next steps
Attached: Inquiry for further harmonisation of the SOP MOAH in IF rev.2.xlsx .xlsx File

Dear expert colleagues,

We would like to inform you that the report of the first exploratory interlaboratory comparison on the determination of MOAH in infant formula is currently in the approval cycle. The report should be publically available soon. In the meanwhile we are working on the second phase of the project.

Based on the reported results and the experimental details provided in the on-line questionnaire, we identified relevant experimental steps to be further harmonised. A number of experimental conditions remain to be discussed/agreed.

We ask you therefore to fill in the attached excel table and returned it by reply to this email by **September 28, 2020**. Empty cells could be left in case you don't have opinion or preference. Empty cell would mean silent agreement with any future proposal concerning that parameter. The result of this exercise will be a proposal for an harmonised SOP.

The next steps of the project are presented herafter.

Phase 2:

A pre-trial will be lunched as soon as possible to allow you to get familiar with the agreed SOP. Two test items will have to be analysed.
Participants will be asked to report their results **following strictly the prescribed SOP** (1st set of results).

An additional set of results (2nd set of results) will be accepted (resulting from some variations from the proposed SOP) on condition that the first set is provided.

Phase 3:

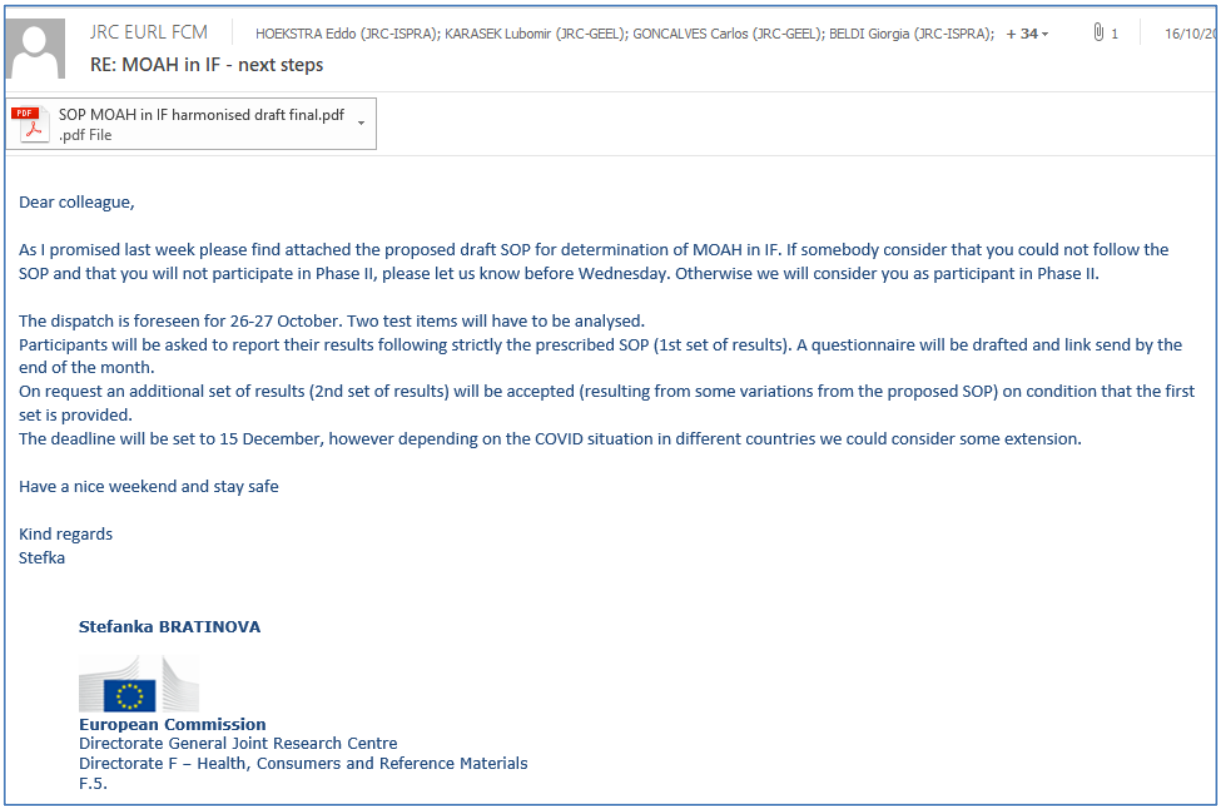
A collaborative trial will be organised (first half of 2021) for the validation of the harmonised SOP based on different levels of MOAH in three different IF formulations (provided by Special Nutrition Europe (SNE) and processed by the JRC Reference material Unit). We would like to thank SNE and the JRC RM Unit for their contribution.

In case you would be interested to participate in the next phases of MOAH in IF project, do confirm your participation by return of mail (**Sept. 28, 2020 at the latest).**

With kind regards

Stefanka BRATINOVA

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



JRC EURL FCM | HOEKSTRA Eddo (JRC-ISPRA); KARASEK Lubomir (JRC-GEEL); GONCALVES Carlos (JRC-GEEL); BELDI Giorgia (JRC-ISPRA); + 34 | 1 | 16/10/2

RE: MOAH in IF - next steps

SOP MOAH in IF harmonised draft final.pdf .pdf File

Dear colleague,

As I promised last week please find attached the proposed draft SOP for determination of MOAH in IF. If somebody consider that you could not follow the SOP and that you will not participate in Phase II, please let us know before Wednesday. Otherwise we will consider you as participant in Phase II.

The dispatch is foreseen for 26-27 October. Two test items will have to be analysed.
Participants will be asked to report their results following strictly the prescribed SOP (1st set of results). A questionnaire will be drafted and link send by the end of the month.
On request an additional set of results (2nd set of results) will be accepted (resulting from some variations from the proposed SOP) on condition that the first set is provided.
The deadline will be set to 15 December, however depending on the COVID situation in different countries we could consider some extension.

Have a nice weekend and stay safe

Kind regards
Stefka

Stefanka BRATINOVA

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

Annex 3: JRC SOP

Standard Operation Procedure

Official method for control of the mineral oil aromatic hydrocarbons (MOAH) content in infant formula powder (IF)

2020

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Aim

This document specifies a method for the determination of mineral oil aromatic hydrocarbons (MOAH) in infant formula powder (IF).

The EURL-FCM was requested by DG SANTE to coordinate the harmonisation of a method for the determination of MOAH in IF with the aim of improving the comparability of the results reported by different laboratories. Therefore, an exploratory ring-trial had been arranged during February – June 2020, in which the participants were asked to analyse a commercial test item following the requirements of the JRC Guidance[1] and the decisions of the 05 December roundtable[2], to fill in a detailed questionnaire for describing every step of the applied procedure and to send corresponding chromatograms. The outcome has been summarised in a report[3] distributed to the participants. It served as a basis for the procedure described in the following.

Scope

This Standard Operating Procedure specifies the official control method for the determination of total MOAH (from C10 to C50), including the quantification of four MOAH fractions in this carbon number range, in IF.

The description is based on the following analytical steps:

An aliquot of powder IF is reconstituted in hot water and saponified with KOH. MOAH are extracted with hexane. The extract is cleaned over a column filled with activated silica gel, eluted with 30 % DCM in hexane and re-concentrated. Interfering substances (e.g. olefins like squalene) are epoxidised with mCPBA and the reaction is stopped with a sodium thiosulfate solution. After removing the aqueous phase, the extract is washed out with a sodium carbonate solution. The upper organic phase is transferred to a vial and additionally re-concentrated, if needed, before its injection into the LC-GC on-line system.

Remark: The instrumental setup for MOSH/MOAH separation is not subject of this SOP version. It will be tackled in the next version established after the pre-trial. The labs should apply their own procedure following the requirements of the JRC Guidance[1].

For the manual MOSH/MOAH separation an additional clean-up through a silica gel column is necessary. After re-concentration of the eluate (30 % DCM in hexane), MOSH are separated from MOAH over a column filled with silica gel covered with silver nitrate. MOAH are eluted from the column with a hexane/DCM/toluene mixture, re-concentrated to 0.4 ml and transferred to a vial for injection into the GC/FID.

The total MOAH content and the MOAH fractions are expressed as mass fractions (mg/kg IF). The method is appropriate for the quantitative determination of MOAH for MOAH fractions above 0.5 mg/kg IF.

Materials and reagents

All reagents must be of analytical grade.

[1] *S. Bratinova, E. Hoekstra (Editors) Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials, Luxembourg: Publications Office of the European Union, 2019 ISBN 978-92-76-00172-0, doi:10.2760/208879, JRC115694*

[2] *https://ec.europa.eu/jrc/sites/jrcsh/files/summary_roundtable_moah_in_if_20191205.pdf*

[3] Bratinova S., Robouch P., Karasek L., Goncalves C., Beldi G., Senaldi C., Jakubowska N., Valzacchi S., Conneely P., Hoekstra E., Emons H. *Determination of MOAH in Infant Formula, JRC IF 2020-01 - an exploratory interlaboratory comparison*, European Commission, Geel, 2020, JRC 121915 EN

Chemicals and materials

MOSH/MOAH internal standard solution (IS):
Toluene solution containing n-undecane (C11), cyclohexylcyclohexane (CyCy), 1-methylnaphthalene (1-MN), 2-methylnaphthalene (2-MN), n-pentylbenzene (5B) and 1,3,5-tri-tert-butylbenzene (TBB) at 300 µg/ml, cholestane (Cho) and perylene (Per) at 600 µg/ml and n-tridecane (C13) at 150 µg/ml, from Restek Corporation (Bellefonte, PA, USA)

Alkane standard solution C10-C50

n-Hexane for gas chromatography > 99 %

Dichloromethane for gas chromatography >99.8 %

Ethanol, puriss. p.a., >99.9 %

Toluene

Potassium hydroxide pellets for analysis EMSURE®, w > 85 %

Water (Millipore)

Sodium sulfate, ≥ 99 %

3-chloroperbenzoic acid, ≤ 77 %

Sodium thiosulfate, ≥ 99 %

Sodium carbonate, ≥ 99.5 %

Silica gel 60, size range: 0.063-0.200 mm (70-230 mesh ASTM), activated at 400 °C for 48 h

60 ml or 40 ml centrifuge glass vials

250-ml round bottom flask

Glass columns with glass frits, different sizes

Note 1: *Materials and chemicals listed above and used for this analysis must be checked for the presence of mineral oils by preparing a reagent blank. Glassware could be rinsed before the analysis with acetone followed by hexane to avoid contaminations.*

Solutions and preparations

Saponification Solution: Saturated potassium hydroxide (KOH) in ethanol/water 1:1

Dissolve approximately 27 g KOH pellets in 100 ml of an ethanol/water (1:1) solution. The solution should be freshly prepared each day.

Note 2: ***Warning*** *- Mixing KOH with water leads to an exothermic reaction.*

Epoxidation reagent solution: 0.2 g/ml 3 % 3-chloroperbenzoic acid in ethanol

2 g mCPBA is purified (if needed) three times with 10 ml hexane and dissolved in 10 ml EtOH .

Note 3: *A clouding of the solution does not disturb the reaction. The solution could be used up to one week, if stored at +4 °C. However, it is recommended to prepare daily a fresh solution. Do not dry at elevated temperatures, otherwise the perbenzoic acid may become unstable and there is a* ***danger of explosion****!*

Eluent solution 1: hexane/dichloromethane (7:3) - 30 % DCM in hexane

Add 70 ml of hexane into a 100-ml flask and fill to the mark with dichloromethane.

Eluent solution 2: hexane/dichloromethane/toluene (75:25:5, v:v:v) (only for the manual MOSH/MOAH separation)

Add 25 ml of dichloromethane and 5 ml of toluene into a 100-ml volumetric flask and fill to the mark with n-hexane.

Ethanol/water solution (1:1) - Mix equal volumes of ethanol and water in a flask.

Sodium thiosulfate solution in water – 100 g/l

Sodium carbonate solution in water – 100 g/l

Silver nitrate - silica gel mix: Silica gel 60 activated containing 0.3 % of silver nitrate (only for the manual MOSH/MOAH separation)

Weigh 29 g ± 0.1 g of silica gel 60 activated into a 250-ml round bottom flask. Add 1.0 g ± 0.1 g of silver nitrate on silica gel 10 % pre-purchased. Mix thoroughly the mixture by repeated shaking and inverting the round bottom flask for at least one minute to ensure a good homogenisation.

Note 4: *This mixture has to be freshly prepared before filling SPE columns. Therefore, prepare only the needed quantity knowing that 3 g of this silica gel mixture is necessary to prepare one SPE column.*

Apparatus

General

Milli-Q Water purifier

N_2 flow evaporator or Rotavap

Analytical Balance

Centrifuge

Muffle furnace

On-line coupled LC-GC-FID system or GC/FID

GC performance

Since the MOH analysis includes hydrocarbons of up to n-C50, laboratories should use a temperature programme and a GC column that allow determining mineral oil of up to n-C50 without significant column bleeding. The response ratio for the signals of n-C50 to n-C20, measured in the alkane standard solution C10-C50, should be between 0.8 and 1.2. The same n-alkane standard solution is used to identify the retention times of the n-alkanes.

The instrumental parameter settings for the GC should target obtaining a well-shaped chromatogram with a hump that facilitates the integration. A faster ramp for the oven temperature is recommended.

Procedure

Sample preparation

Around 5.0 g of an IF powder sample is used for this analysis.

Note 5: *The personnel performing sampling, extraction and analysis should take all necessary precautions to avoid contamination of the sample. For example, the use of cosmetics such as hand cream should be avoided. Each new batch of sample containers and reagents should be checked for mineral oil contamination.*

Before analysis, the samples should be stored in the laboratory at room temperature.

Reconstitution, saponification and extraction of the powder IF

Weigh 5 g of IF powder in a centrifuge glass, add 20 µl ISTD working solution and add 5 ml of water (pre-heated to approximately 35 °C), heat for 5 min to 60 °C and shake at 120 rpm in a water bath.

Note 6: *Preliminary reconstitution of the IF powder in hot water helps to melt entirely the fatty acids.*

Add 10 ml of KOH solution (0) and heat at 60 °C for 30 min under shaking (120 rpm) in a water bath.

Note 7: *The solubility of KOH in ethanol is limited. The KOH solution should be prepared daily. Under routine conditions, it is faster to dissolve KOH in water and add ethanol separately, e.g 5 ml of 50 % KOH solution in water and 5 ml ethanol. Both approaches should result in a comparable outcome.*

Cool the solution for about one minute and extract the solution with 15 ml of hexane while shaking vigorously for 2 min. Centrifuge, if needed for better phase separation.

Note 8: *Simultaneous extraction and saponification should give comparable results for MOAH in most of the cases, however the amount and the composition of the lipids in the extracts may be different.*

Note 9*: Washing of the organic phase with ethanol/water is optional but not necessary.*

Silica gel column purification and re-concentration

This step removes part of the remaining lipids after the saponification and ensure epoxidation in more controllable way.

Fill 3 g of activated silica gel into a glass column. Add 1 g Na_2SO_4 on the top. Rinse the column with 15 ml of the DCM solution (0). Add 14 ml of the extract (upper organic phase) after saponification to the column. Elute with adding 15 ml of the DCM solution (0) onto the column and collect the extract and the eluate (14+15 ml) into a 40 ml vial. Evaporate the extract to ca. 1 ml.

Note 10: *Losses of internal standards during reconcentration of the silica gel eluate may be an issue: the rotavapor is most robust, potentially a keeper is needed (e.g. MOH free vegetable oil, any higher boiling plasticizer such as diethylhexyl terephthalate). Reconcentration by an evaporation system such as TurboVap (Biotage) or Syncore (Büchi) has to be carefully optimized, there could be easily a loss of 10-20 % of the volatile standards including cycy and MNs, resulting in correspondingly elevated MOSH/MOAH contents.*

To evaporate less, depending on the dead volume of the column, less extract could be collected (e.g 7+15 ml) but it should be ensured that there is no break though of the extract (extract reaching the bottom of the column before the collection started)

Note 11: *In the JRC, the procedure is tested with a column filled with 12 g activated silica gel and elution with 40 ml DCM solution (0). According to other experts and additional tests, 3 g silica gel could be sufficient.*

Epoxidation

Add 0.5 ml m-CPBA solution (0) to the extract (1 ml) from 0 and shake briefly. Carry-out the epoxidation for 15 min at 40 °C in a water bath while shaking (120 rpm). Stop the reaction by adding 2 ml of $Na_2S_2O_3$ solution

(0) and shake the sample intensively for approximately 15 s. Remove the aqueous phase (bottom) with a Pasteur pipette and discard it. Wash out the organic phase with 2 ml of Na_2CO_3 solution (0) while shaking intensively for 15 s. Transfer the organic phase into a vial add a spatula tip of sodium sulfate for drying of the organic phase and inject onto the on-line LC-GC system or proceed to 0.

Note 12. *If needed, the 2 ml of Na_2CO_3 solution (0) could be added together with the thiosulfate.*

Silica gel column purification

This step is mandatory for the manual method for robust MOSH/MOAH separation. It is optional for the on-line LC-GC, for the preservation of the HPLC column from the polar products of the epoxidation.

Fill 3 g of activated silica gel into a glass column. Add 1 g Na_2SO_4 on the top. Rinse the column with 15 ml of the DCM solution (0). Load the extract from 0 (upper organic phase) to the column. Elute with 15 ml of the DCM solution (0) and collect the extract and the eluate (14+15 ml) into a 40 ml vial. Evaporate the extract to ca. 0.4-1.0 ml (see Note 10). Inject 1/10 onto the online HPLC-GC/FID system or proceed to the manual MOSH/MOAH separation.

Manual MOSH and MOAH separation (if necessary)

Prepare a silver nitrate-silica column by filling the glass column with 3 g of the silver nitrate-silica mixture (0). Rinse the column with 10 ml hexane before sample loading. Load the hexane extract (0) on to the SPE column and allow this volume to pass through. Wash with 2 ml of hexane. Discard the eluate.

Elute with 5 ml of hexane. Collect the eluate into a 40 ml vial containing 300 µl of isooctane. Add 1 ml of the hexane/dichloromethane/toluene (75:20:5, v:v:v) mix (0). Collect the eluate in the same vial. This fraction contains MOSH.

Replace the 40 ml vial by the new one. Add 10 ml of the hexane/dichloromethane/toluene (75:20:5, v:v:v) mix (0). Wait until complete elution. The eluate contains the MOAH fraction. Evaporate the MOAH eluate under a N_2 flow to 0.3 ml (see Note 10). Inject 50 µl into the GC-FID.

Quantification of total MOAH and the MOAH fractions

The parameter "total MOAH content" should be determined by integration of the whole signal interval in the chromatogram, starting at the retention time of the peak start of n-C10 and ending at the retention time of the peak end of n-C50, after the elimination of the identified sharp peaks above the hump and taking the baseline of the blank into account. Sharp peaks above the hump are assumed to come from non-MOAH interferences.

The total MOAH content is quantified according to the equation listed in Section 4.4 of the JRC Guidance[1].

$$w_{MOAH} = \frac{A_i \times m_{IS} \times 1000}{A_{IS} \times m}$$

Where:

A_i is the signal area attributed to MOAH (total or C-fraction) after the elimination of the identified sharp peaks above the hump and if possible, elimination of POH and/or POA signals;

A_{IS} is the peak area of the internal standard (1-MN or an equivalent IS);

m_{IS} is the mass of the internal standard added to the sample in [mg];

m is the mass of the test portion, in [g].

The following MOAH sub-fractions should be analysed according to the JRC Guidance:

- the mass fraction of total MOAH in IF", expressed in mg/kg

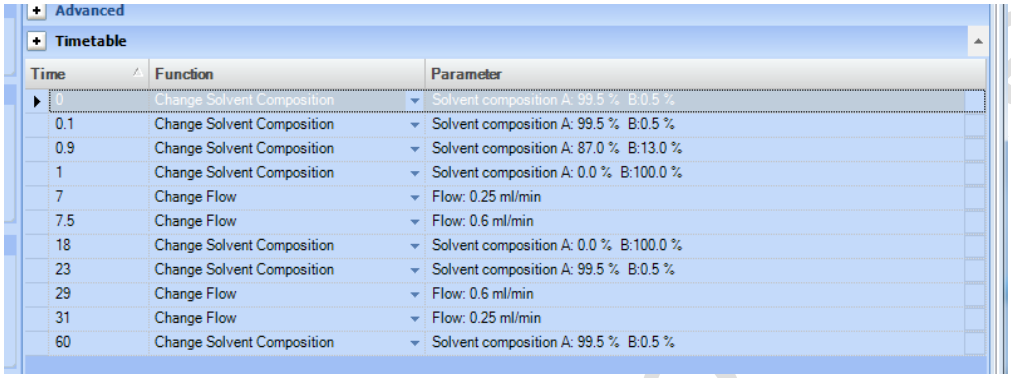
- the mass fraction of the MOAH in IF corresponding to the retention time of n-alkanes from n-C35 to n-C50 (MOAH C35-C50)", expressed in mg/kg
- the mass fraction of the MOAH in IF corresponding to the retention time of n-alkanes from n-C25 to n-C35 (MOAH C25-C35)", expressed in mg/kg
- the mass fraction of the MOAH in IF corresponding to the retention time of n-alkanes from n-C16 to n-C25 (MOAH C16-C25)", expressed in mg/kg
- the mass fraction of the MOAH in IF corresponding to the retention time of n-alkanes from n-C10 to n-C16 (MOAH C10-C16)", expressed in mg/kg

Annex – Example for instrumental conditions

Semi on-line HPLC-GC/FID (JRC-Ispra)

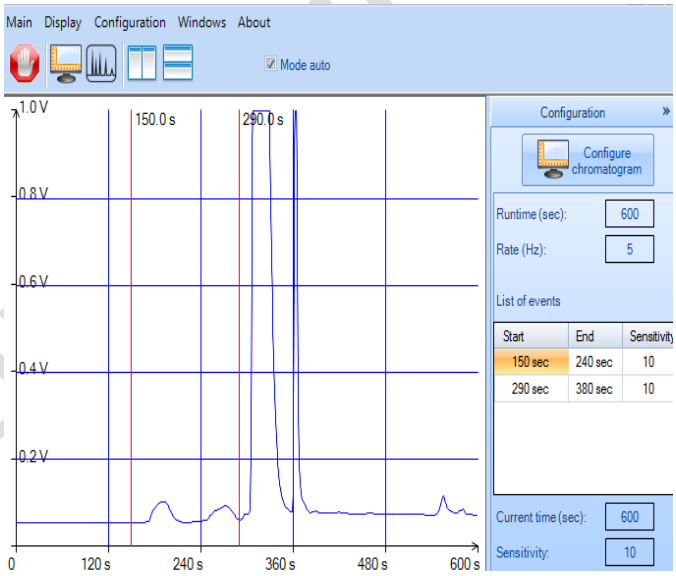
HPLC operating conditions

Mobile phase A: Hexane
Mobile phase B: Dichloromethane
Injection volume: 100 µl
Wavelenght: 220 nm
Elution programme: end time 60 min



SRA interface

Windows to collect MOSH and MOAH fractions from the flow cell should be optimised using the MOSH internal standard solution (4.1.1) and the response ratio among analyte signals should be verified according to the actual concentration.



GC-FID operating conditions

Injection port:	On column
Temperature:	55 °C
Pressure:	22 psi
Septum purge flow:	15 ml/min
Volume collected from flow cell:	250 µl
Collection speed of MOSH fraction:	4.0 µl
Collection speed of MOAH fraction:	2.5 µl

GC Column

Flow:	12 ml/min
Pressure:	21.85 psi
Average velocity:	146.57 cm/s
Hold up time:	1.17056 min

Temperature programme:

Rate [°C/min]	Temperatura [°C]	Time [min]
	50	3.2
25	250	0
20	350	6

Pressure programme:

Rate [psi/min]	Pressure [psi]	Hold time [min]	Run time [min]
	21.86	3	3
97.09	13.16	7	10.09
47.47	32.15	1	22.2

Flow programme:

Flow rate [ml/min*min]	Flow [ml/min]	Hold time [min]	Run time [min]
	12	3	3
67	6	7	10.09
10	10	1	22.2

Note: *The operator should perform a check, if all the instrument parameters are properly set after switching on the instrument. All the operations are conducted following the HPLC-GC-FID manual.*

GC/FID operational parameters for the manual method (JRC-Geel)

The GC instrumental parameters listed below were found suitable for obtaining well-shaped chromatograms with a sharper hump that facilitates the integration. Changes from these conditions may be used for the purpose of better chromatographic separation or sensitivity or increased sample throughput, but any changes have to be verified.

Injection port:	PTV (Gerstel CIS, MPS) with on-column adapter insert
Injection volume:	50 µl
Injector temperature programme (PTV):	Fast ramp mode, 55 °C (6 min) – 15 °C/s to 150 °C – 5 °C/s to 350 ° (10 min)
GC Column Uncoated precolumn:	 7 m x 0.53 mm ID, press-fit connector
Separation column:	DB-1HT (15 m x 0.32 mm ID × 0.10 µm film thickness)
Carrier gas:	helium (ramped pressure/flow mode)
Pressure ramp programme:	150 kPa (3 min) – @100 kPa/min to 80 kPa - @ 1 kPa/min to 90 kPa (held till the end of run)
Oven temperature programme:	50 °C (4.5 min) – 20 °C/min to 280 °C – 30 °C/min to 350 °C (10 min), total time 28.33 min
Detector: FID Temperature:	 350 °C
H_2 flow:	35 ml/min
Air flow:	350 ml/min
Make up flow (Nitrogen):	30 ml/min (make-up + carrier gas constant flow)

Annex 4. Instructions to participants

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

EURL European Union Reference Laboratory for Food Contact Materials

Geel, 21 October 2020
Ref. Ares (2020)xxxx - 21/10/2020

Attn.: «Title» «Firstname» «Surname»
«Organisation»
«Department»
«Address2»
«Zip» «Town»
«Country»

Subject: Participation in JRC IF 2020/02 – "Determination of MOAH in IF"

Dear «Title» «Surname»,

Thank you for participating in the pre-trial **JRC-IF-2020/02 – "Determination of MOAH in IF".** This round is organised to familiarise and test the proposed analytical procedure for the analysis of MOAH in infant formula (IF).

The measurands are mass fractions (mg kg^{-1}) of total MOAH (C10-C50) and the corresponding **fraction cuts**, as defined in the "Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials on mineral oil hydrocarbons" in the frame of Recommendation (EU) 2017/84.

Please integrate the hump only until RT corresponding to n-C50!

The parcels contain two 100 ml brown glass bottles filled with approximately 40 g powder IF, crimp capped and wrapped in Al foil each.

Upon arrival of this parcel, please check whether the bottles are undamaged after transport.

You are requested to send the "**Confirmation of receipt**" form within 3 days after receipt of the samples to Stefanka-Petkova.BRATINOVA@ec.europa.eu.

The procedure used for the analyses should **follow exactly the enclosed draft SOP**

Please report the following:

- the results from the **three replicate** measurements (in mg kg^{-1})
- the **final value** you would report to customers (may be different from the mean of the 3 replicates);
- the associated expanded **uncertainty** of the final value (in mg kg^{-1});
- the **coverage factor;** and
- the procedure used – JRC SOP or in-house SOP.

The results should be reported in the same format (e.g. number of significant figures) as you normally report to customers.

European Commission, JRC-Geel, Belgium. Telephone: (32) 14571800.
e-mail: jrc-eurl-fcm@ec.europa.eu URL: https://ec.europa.eu/jrc/en/eurl/food-contact-materials

The reporting website **https://web.jrc.ec.europa.eu/ilcReportingWeb/** will be open on **30/10/20.**

Then you will receive the link to the questionnaire (via EUSurvey platform) and **the personal password key that you need for the reporting website**.

For those participants that will report two sets of results, they will receive two password keys.

The system will guide you through the reporting procedure. Do not forget to submit and confirm when required. Please fill in the questionnaire as well. It will be much shorter then in Phase 1. You will be asked to **upload chromatograms** of the two test items in a scale that the **hump is clearly visible as well as the fraction cuts and integration lines**.

At present, the deadline for submission of results is set to **December 14, 2020**.

A report to participants will be circulated shortly after the end of the round to present the reported values from all participants with their lab codes. The laboratory code will be disclosed only to the respective participant together with the password key for reporting, to preserve the confidentiality of the data reported.

Your participation in this project is greatly appreciated.

Do not hesitate to contact me for further information.

With kind regards,

/signed electronically in Ares/

Dr. Stefanka Bratinova
JRC IF 2020/02 Coordinator

Cc: H. Emons (Head of Unit, Food & Feed Compliance, F.5),
E. Hoekstra (Operating Manager EURL-FCM)
P. Robouch (Standardisation group team leader)

European Commission, JRC-Geel, Belgium. Telephone: (32) 14571800.
e-mail: jrc-eurl-fcm@ec.europa.eu. URL: https://ec.europa.eu/jrc/en/eurl/food-contact-materials

Annex 5: Confirmation of receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials
European Union Reference Laboratory for Food Contact Materials

Attn.: «Title» «Firstname» «Surname»
«Organisation»
«Country»

Subject: Participation in JRC IF 2020/01 – "Determination of MOAH in IF"

Please return this form within 3 days of reception, to confirm that the package arrived well to your laboratory. If samples are damaged, please mention it below and contact us as soon as possible.

Date of package arrival: _____/_____/2020

Was the sample damaged? ☐ YES ☐ NO

Remarks

……..

……..

……..

……..

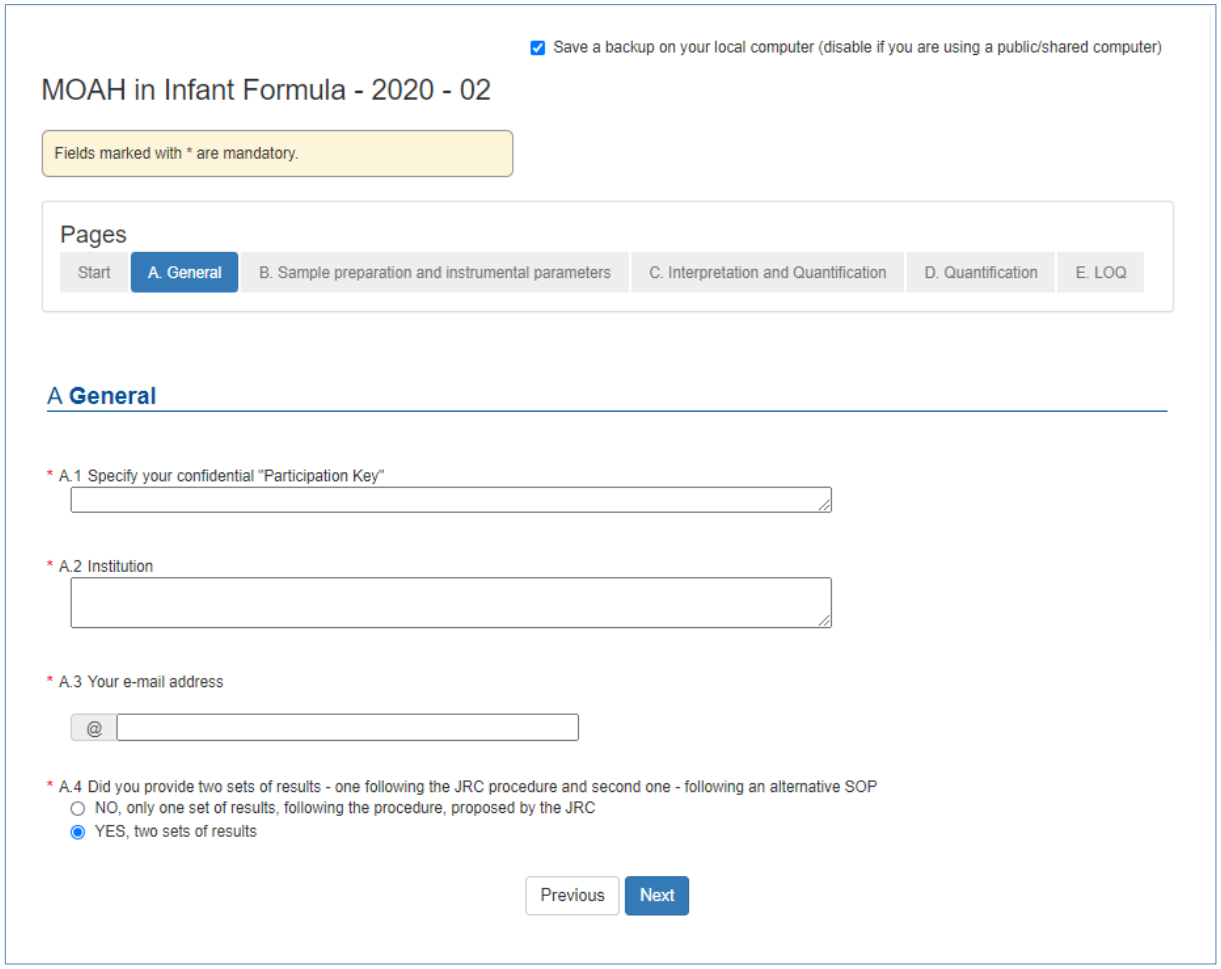
Signature

Thank you for returning this form by email to:
Stefanka-Petkova.BRATINOVA@ec.europa.eu
CC: jrc-eurl-fcm@ec.europa.eu

European Commission, JRC-Geel, Belgium. Telephone: (32) 14571800.
e-mail: jrc-eurl-fcm@ec.europa.eu. URL: https://ec.europa.eu/jrc/en/eurl/food-contact-materials

Annex 6. EuSurvey – online questionnaire

to collect experimental details used by the participants to analyse MOAH in IF (https://ec.europa.eu/eusurvey/runner/JRC_IF_2020_01A)



Pages

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B Sample preparation and instrumental parameters

* B.1 Did you follow the first step of the sample preparation as in § 5.1.1 of the procedure, proposed by the JRC?
For those reporting two sets of results the question relates to the set of results produced by applying the proposed by the JRC SOP.

- [ ] Yes, using 10 ml of saturated KOH in ethanol
- [ ] Yes, using 5 ml 50% KOH
- [ ] Partly, as per Note 8
- [ ] Additional washing of the organic phase
- [x] No, others

* B.2 Please provide details for the deviations

B.3 Do you suppose or have experience that reconstitution of 5 g IF in 5 ml water would be problematic for some of the IF formulations on the market?

- ○ No
- ○ Yes
- ○ no opinion

B.4 Do you find reconstitution of 5 g IF in 10 ml water better option?

- ○ No
- ○ Yes
- ○ no opinion

* B.5 Did you follow strictly the second step of the sample preparation procedure (§ 5.1.2)?
For those reporting two sets of results the question relates to the set of results produced by applying the proposed by the JRC SOP.

- ○ Yes
- ◉ No

* B.6 Please provide details for the deviations

* B.7 Did you use keeper during the pre-concentration?

- ○ Yes
- ○ No

* B.8 Please describe the equipment used for pre-concentration of the extract

* B.9 Did you follow the third step of the sample preparation procedure (§ 5.1.3)?
For those reporting two sets of results the question relates to the set of results produced by applying the proposed by the JRC SOP.

- ○ Yes, washing out the organic phase with carbonate solution;
- ○ Yes, by adding the carbonate to the stop reagent (thiosulphate) and washing with ethanol/water;
- ○ Yes, by adding the carbonate to the stop reagent (thiosulphate) and proceeding without washing
- ◉ No, others

* B.10 Please provide details for the deviations from the abovementioned epoxidation.

B.11 What would you do to eliminate the interferences, remained after epoxidation, especially for sample 2B, without uncontrollably compromise the MOAH

* B.12 Did you apply silica gel column clean-up after the epoxidation (§ 5.1.4)?
For those reporting two sets of results the question relates to the set of results produced by applying the proposed by the JRC SOP.

- (●) No
- ( ) Yes

B.14 Any comments on the sample preparation procedure?

* B.15 Did you follow the procedure for manual MOSH/MOAH separation (§ 5.1.5)?
For those reporting two sets of results the question relates to the set of results produced by applying the proposed by the JRC SOP.

- ( ) No, on-line LC-GC/FID used
- ( ) Yes

* B.16 Did your alternative procedure (for the second set of results) follow exactly the sample preparation steps used in the first pre-trial ?

- ( ) Yes, it is described in the answers from the questionnaire, submitted via EUSurvey during the first phase
- (●) No, there are deviations

B.17 If there are deviations, please describe the sample preparation or upload a file below with the steps of the respective sample preparation procedure

B.18 Please upload your file, with the sample preparation descriptions in any EU language (mandatory if the used SOP for the second set of results deviate from the one applied during the first pre-trail and not described above

Select file to upload

* B.19 Did you follow exactly the instrumental LC-GC conditions, described in the answers of the questionnaire from the first pre-trail phase?

- ( ) YES
- (●) NO

B.20 If there are deviations, please describe the instrumental parameters (LC and GC) or attach a file with the described instrumental parameter procedure

B.21 Please upload your file, describing instrumental conditions in any language (mandatory if not described above)

Select file to upload

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Start | A. General | B. Sample preparation and instrumental parameters | **C. Interpretation and Quantification** | D. Quantification | E. LOQ

C Interpretation and Quantification of the results, obtained by the JRC SOP

* C.1 Did you have problems with
- [ ] the baseline
- [ ] the peak tailing/broadening of the IS and/or markers?
- [ ] the loss of the IS (1-MN and 2-MN)
- [ ] the solvent peak
- [ ] the blank
- [ ] interferences on the IS
- [ ] interferences on the hump of IF2A that would compromise reliable subtraction
- [ ] interferences on the hump of IF2B that would compromise reliable subtraction
- [ ] other problems
- [x] no problems

* C.3 Have you set in your routine pre-defined acceptance criteria for some of the ratios between the area of the IS and verification standards?
- (●) Yes
- ( ) No

* C.4 Please describe the criteria

* C.5 How do you proceed when, for a given sample, these criteria are not met?

* C.6 Do you run C10-C50 mixture with every batch of samples to monitor the RT shift for proper fraction windows cuts?
- ( ) Yes
- ( ) No

* C.7 Please upload a chromatogram of IF02A and a blank in a scale that the height of the hump is more than 1/2 of the axe Y; the integration of the different fractions should be clearly distinguished

Select file to upload

* C.8 Please upload a chromatogram of IF02B and a blank in a scale that the height of the hump is more than 1/2 of the axe Y; the integration of the different fractions should be clearly distinguished

Select file to upload

* C.9 Did you quantify MOAH **as required in the proposed SOP**?
- ( ) Yes, against 1-MN;
- ( ) Yes, against 2 MN;
- ( ) Yes, against an average of both;
- ( ) No, we deviated from the SOP and quantified MOAH against TBB
- ( ) Other

* C.11 Were the riding interfering peaks well separated until baseline?

C.12 **For the results, obtained following the JRC procedure**, please report area of the peaks (in eV) and the initial content (in mg) of the following substances in the internal/verification standard mix, added to the sample aliquot in the beginning (You can choose the data from the best replicate)

	blank	IF02A	IF02B	content in mg, added to the sample
* 1-MN				
* 2-MN				
* 5B				
* TBB or DEHB				
* perylene				

C.13 **For the results, obtained following the JRC procedure,** please report the area of the fractions and total MOAH (in eV). (You can choose the data from the best replicate)

	blank	IF02A	IF02B
* C10-C16			
* C16-C25			
* C25-C35			
* C35-C50			
* total MOAH			

* C.14 How did you integrate the last fraction >C35 ?

- ○ integrated the hump until the RT of n-C50 alkane as required in the JRC Guidance and in the proposed SOP;
- ○ integrated the entire hump until reaching a baseline;

* C.15 How do you report the total MOAH content?

- ○ based on the integration of the entire chromatogram, following the requirement of the EURL Guideline and the proposed SOP
- ○ as the sum of different fractions applying the lower bound approach (if < LOQ then set to zero)

C.16 Any comments or difficulties you want to report?

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D Interpretation and Quantification of the results, obtained by the alternative SOP

* D.1 When you analyzed the samples with the alternative SOP, did you have problems with
- [ ] the baseline
- [ ] the peak tailing/broadening of the IS and/or markers?
- [ ] the loss of the IS (1-MN and 2-MN)
- [ ] the solvent peak
- [ ] the blank
- [ ] interferences on the IS
- [ ] interferences on the hump of IF2A that would compromise reliable subtraction
- [ ] interferences on the hump of IF2B that would compromise reliable subtraction
- [ ] other problems
- [ ] no problems

* D.3 Please upload a chromatogram of IF02A and a blank, obtained by applying the **alternative** SOP in a scale that the height of the hump is more than 1/2 of the axe Y; the integration of the different fraction should be clearly distinguished

Select file to upload

* D.4 Please upload a chromatogram of IF02B and a blank, obtained by applying the **alternative** SOP in a scale that the height of the hump is more than 1/2 of the axe Y; the integration of the different fraction should be clearly distinguished

Select file to upload

* D.5 How did you quantify MOAH in the results set, complying with the **alternative** procedure
- ○ Against 1-MN;
- ○ Against 2 MN;
- ◉ Against an average of both;
- ○ Against TBB
- ○ Other

* D.7 Were the riding interfering peaks well separated until baseline?

D.8 For the results, obtained following the **alternative** procedure, please report area of the peaks (in eV) and the initial content (in mg) of the following substances in the internal/verification standard mix, added to the sample aliquot in the beginning
(You can choose the data from the best replicate)

	blank	IF02A	IF02B	content in mg, added to the sample
* 1-MN				
* 2-MN				
* 5B				
* TBB				
* perylene				

D.9 Please report area of the fractions and total MOAH (in eV) corresponding to the results set, complying with the **alternative** procedure.
(You can choose the data from the best replicate)

	blank	IF02A	IF02B
* C10-C16			
* C16-C25			
* C25-C35			
* C35-C50			
* total MOAH			

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Start | A. General | B. Sample preparation and instrumental parameters | C. Interpretation and Quantification | D. Quantification | E. LOQ

E Limit of Quantification (LOQ) and others

E.1 Do you find the two samples in this round as representative enough for the IFs on the market with low and high analytical challenge?
(For those with experience in analyses of many different IF formulations)

E.2 What is the % of the very challenging IF samples that you have to analyze in your routine practice? What is your approach to those samples?
(For those with experience in analyses of many different IF formulations)

E.3 Do you always compare the profile of MOAH with MOSH chromatogram before deciding if a hump in MOAH chromatogram belongs to MOAH?

E.4 Do you have criteria for taking decision when one IF sample should be subjected to confirmatory methods? Please describe

* E.5 Are you aware of IF formulations with interferences in the last fraction (C35-C50), after epoxidation?
(For those with experience in analyses of many different IF formulations)

E.6 What is the % of IF test samples that you analyse routinely, where the hump extends beyond the RT of nC50?
(For those with experience in analyses of many different IF formulations)

E.7 How **would you estimate** (in mg/kg) the LOQs of the different fractions and the total MOAH for each of the IF test items, when applying the JRC procedure?

	LOQ IF2A	LOQ IF2B
* 1. MOAH ≥ n-C10 to ≤ n-C16		
* 2. MOAH > n-C16 to ≤ n-C25		
* 3. MOAH > n-C25 to ≤ n-C35		
* 4. MOAH > n-C35 to ≤ n-C50		
* 5. Total MOAH		

E.8 Are the LOQs of **the alternative procedure**, used for the second set of results, much better for those two samples?

- ○ Yes
- ○ No
- ○ Similar

E.9 Any other comment from your side

Thank you for your contribution.
Rest assured that this information will be treated with due confidentiality

MOAH in Infant Formula

Previous | Submit

Annex 7. Results as reported by the participants for the mass fraction of total MOAH in IF02A (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<2.2		<2.5	<2	<2	JRC SOP
01A	1.8	0.7	1.7	1.8	1.8	alternative
02A	4.92	1.79	3.63	4.55	6.59	JRC SOP dev
03	1.2	0.24	1.1	1.2	1.3	JRC SOP
03A	2.5	0.5	2.6	2.4	2.4	alternative
04	2.52	0.21	2.40	2.56	2.60	JRC SOP
05	2.73		2.73	2.73		JRC SOP
06	1.6	30	1.6	1.7	1.6	JRC SOP
06A	2.4		3	2.9	2.4	alternative
07	2.58	0.54	2.69	2.47	2.57	JRC SOP
08	3.358		4.002	3.332	2.741	JRC SOP
09	2.04	0.816	2.04	2	2.07	JRC SOP
10	2.3		2.2	2.4	2.4	JRC SOP
12	0.412	0.136	0.445	0.379	0	JRC SOP
13	2.02	0.36	2.02	1.97	2.07	JRC SOP
14	2.19		2.17	2.4	2.27	JRC SOP
15	<1.4		<1.4	<1.4	<1.4	JRC SOP
15A	0.73	58	0.64	<0.6	0.94	alternative
16	2.44	0.3	2.49	2.43	2.46	JRC SOP
17	1.866	0.22	1.993	1.813	1.791	JRC SOP
17A	1.156	0.05	1.164	1.15	1.185	alternative
18	1.8	0.7	2	1.7	1.8	JRC SOP
18A	2.5	1	2.5	2.5	2.6	alternative
19	2.7	1.3	1.3	1.2	2.7	JRC SOP
20A	2.2	0.3	2.2	2.4	2.1	alternative
21	3.4	0.96	3.5	3.5	3.3	JRC SOP
22	2.37		2.4	2.3	2.4	JRC SOP
23	2.76	0.76	2.66	2.62	2.99	JRC SOP
24	2.8	0.8	2.8	2.9	2.7	JRC SOP
26	2.9	20	3	3.1	2.9	JRC SOP
27	1.99	43	1.99	2.11	1.29	JRC SOP
28	2.8	0.8	2.6	2.8	3.1	JRC SOP
30	2.33	40	2.3	2.38	2.3	JRC SOP
31	3	0.68	3.3	2.8	-	JRC SOP

Annex 8. Results as reported by the participants for the mass fraction of MOAH C10-C16 in IF02A (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<0.5		<0.5	<0.5	<0.5	JRC SOP
01A	<0.5		<0.5	<0.5	<0.5	alternative
02A	<0.143		<0.133	<0.100	<0.195	JRC SOP dev
03	<0.5		<0.5	<0.5	<0.5	JRC SOP
03A	<0.5		<0.5	<0.5	<0.5	alternative
04	0.04	0.01	0.05	0.04	0.05	JRC SOP
05	0		0	0		JRC SOP
06	<0.5		<0.5	<0.5	<0.5	JRC SOP
06A	<0.5		<0.5	<0.5	<0.5	alternative
07	<0.1		<0.1	<0.1	<0.1	JRC SOP
08	0.003		0.007	0.001	0	JRC SOP
09	0	0	0	0	0	JRC SOP
10	<0.2		<0.2	<0.2	<0..2	JRC SOP
12	0	0	0	0		JRC SOP
13						JRC SOP
14	<0.5		<0.5	<0.5	<0.5	JRC SOP
15	<1.0		<1.0	<1.0	<1.0	JRC SOP
15A	<0.5		<0.5	<0.5	<0.5	alternative
16	<0.15		<0.15	<0.15	<0.15	JRC SOP
17	<0.2		<0.2	<0.2	<0.2	JRC SOP
17A	<0.2		<0.2	<0.2	<0.2	alternative
18	<0.5		<0.5	<0.5	<0.5	JRC SOP
18A	<0.8		<0.8	<0.8	<0.8	alternative
19	<0.5		<0.5	<0.5	<0.5	JRC SOP
20A	<0.5		<0.5	<0.5	<0.5	alternative
21	<0.3		0.3	0.3	0.3	JRC SOP
22	<1		<1	<1	<1	JRC SOP
23	<0.5		<0.5	<0.5	<0.5	JRC SOP
24	0	0	0	0	0	JRC SOP
26	<0.05		<0.05	<0.05	<0.05	JRC SOP
27	0	43	0	0.01	0	JRC SOP
28	<0.5		<0.5	<0.5	<0.5	JRC SOP
30	<0.05		<0.05	<0.05	<0.05	JRC SOP
31	0	0	0	0	-	JRC SOP

Annex 9. Results as reported by the participants for the mass fraction of MOAH C16-C25 in IF02A (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<0.5		<0.5	<0.5	<0.5	JRC SOP
01A	<0.5		<0.5	<0.5	<0.5	alternative
02A	<0.069		<0.064	<0.049	<0.094	JRC SOP dev
03	<0.5		<0.5	<0.5	<0.5	JRC SOP
03A	<0.5		<0.5	<0.5	<0.5	alternative
04	0.16	0.09	0.21	0.12	0.15	JRC SOP
05	0.02		0.03	0.04		JRC SOP
06	<0.5		<0.5	<0.5	<0.5	JRC SOP
06A	<0.5		<0.5	<0.5	<0.5	alternative
07	<0.1		<0.1	<0.1	<0.1	JRC SOP
08	0.22		0.408	0.159	0.093	JRC SOP
09	<0.5		<0.5	<0.5	<0.5	JRC SOP
10	<0.2		<0.2	<0.2	<0.2	JRC SOP
12	0	0	0	0		JRC SOP
13						JRC SOP
14	<0.5		<0.5	<0.5	<0.5	JRC SOP
15	<0.9		<0.9	<0.9	<0.9	JRC SOP
15A	<0.4		<0.4	<0.4	<0.4	alternative
16	<0.15		<0.15	<0.15	<0.15	JRC SOP
17	<0.2		<0.2	<0.2	<0.2	JRC SOP
17A	<0.2		<0.2	<0.2	<0.2	alternative
18	<0.5		<0.5	<0.5	<0.5	JRC SOP
18A	<0.8		<0.8	<0.8	<0.8	alternative
19	<0.5		<0.5	<0.5	<0.5	JRC SOP
20A	<0.5		<0.5	<0.5	<0.5	alternative
21	<0.3		0.3	0.3	0.3	JRC SOP
22	<1		<1	<1	<1	JRC SOP
23	<0.5		<0.5	<0.5	<0.5	JRC SOP
24	0	0	0.1	0	0	JRC SOP
26	<0.1		<0.1	<0.1	<0.1	JRC SOP
27	0	43	0	0	0	JRC SOP
28	<0.5		<0.5	<0.5	<0.5	JRC SOP
30	0.07	50	0.05	0.07	0.1	JRC SOP
31	0.3	0.04	0.3	0.2	-	JRC SOP

Annex 10. Results as reported by the participants for the mass fraction of MOAH C25-C35 in IF02A (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<1		<1	<1	<1	JRC SOP
01A	0.6	0.24	0.58	0.6	0.62	alternative
02A	1.87	1.04	1.43	1.72	2.44	JRC SOP dev
03	<0.5		<0.5	<0.5	<0.5	JRC SOP
03A	0.8	0.16	0.9	0.8	0.8	alternative
04	0.84	0.07	0.81	0.85	0.87	JRC SOP
05	0.76		0.74	0.78		JRC SOP
06	0.5	30	0.5	0.5	0.5	JRC SOP
06A	0.7		1	0.9	0.7	alternative
07	0.79	0.16	0.84	0.78	0.76	JRC SOP
08	1.884		2.104	1.963	1.585	JRC SOP
09	0.61	0.244	0.63	0.59	0.62	JRC SOP
10	0.9		0.9	0.7	1.0	JRC SOP
12	0	0	0	0	0	JRC SOP
13	0		0	0	0	JRC SOP
14	0.77		0.76	0.76	0.8	JRC SOP
15	<0.8		<0.8	<0.8	<0.8	JRC SOP
15A	0.32	58	<0.3	<0.3	0.37	alternative
16	1.18	0.3	1.17	1.17	1.19	JRC SOP
17	0.607	0.08	0.652	0.575	0.595	JRC SOP
17A	0.343	0.025	0.344	0.338	0.349	alternative
18	0.69	0.3	0.77	0.62	0.66	JRC SOP
18A	1.1	0.4	1.1	1	1.2	alternative
19	0.9	0.4	<0.5	<0.5	0.9	JRC SOP
20A	0.73	0.08	0.75	0.76	0.67	alternative
21	1.5	0.42	1.5	1.5	1.5	JRC SOP
22	0.8		0.8	0.8	0.8	JRC SOP
23	0.84	0.28	0.83	0.78	0.91	JRC SOP
24	0.9	0.3	0.9	1	0.9	JRC SOP
26	1	20	1.01	1.02	0.98	JRC SOP
27	0.94	43	0.94	1.05	0.52	JRC SOP
28	1	0.3	1	1	1	JRC SOP
30	0.98	50	0.92	0.99	1.02	JRC SOP
31	0.7	0.18	0.8	0.7	-	JRC SOP

Annex 11. Results as reported by the participants for the mass fraction of MOAH C35-C50 in IF02A (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<1.2		<1	<1	<1.5	JRC SOP
01A	1.2	0.5	1.1	1.2	1.2	alternative
02A	2.73	1.26	2.2	2.56	3.43	JRC SOP dev
03	0.8	0.16	0.7	0.8	0.8	JRC SOP
03A	1.6	0.32	1.7	1.6	1.6	alternative
04	1.48	0.24	1.34	1.56	1.53	JRC SOP
05	1.88		1.89	1.87		JRC SOP
06	1.1	30	1.1	1.2	1.1	JRC SOP
06A	1.7		1.9	1.8	1.7	alternative
07	1.75	0.35	1.81	1.66	1.78	JRC SOP
08	1.252		1.483	1.209	1.063	JRC SOP
09	1.41	0.564	1.4	1.39	1.43	JRC SOP
10	1.5		1.3	1.7	1.4	JRC SOP
12	0.262	0.087	0.288	0.237	0	JRC SOP
13	2.02	0.36	2.02	1.97	2.07	JRC SOP
14	1.23		1.21	1.11	1.38	JRC SOP
15	<0.4		<0.4	<0.4	0.42	JRC SOP
15A	0.42	58	0.34	0.34	0.57	alternative
16	1.19	0.3	1.19	1.18	1.19	JRC SOP
17	1.23	0.15	1.316	1.193	1.181	JRC SOP
17A	0.823	0.03	0.82	0.812	0.836	alternative
18	1.1	0.4	1.2	1.1	1.1	JRC SOP
18A	1.4	0.6	1.4	1.4	1.5	alternative
19	1.7	0.8	1.1	1	1.7	JRC SOP
20A	1.5	0.2	1.4	1.6	1.4	alternative
21	1.9	0.52	1.9	1.9	1.8	JRC SOP
22	1.4		1.4	1.4	1.4	JRC SOP
23	1.84	0.54	1.76	1.78	1.98	JRC SOP
24	1.8	0.5	1.8	1.9	1.8	JRC SOP
26	1.9	20	1.84	1.89	1.83	JRC SOP
27	1.05	43	1.05	1.04	0.77	JRC SOP
28	1.8	0.5	1.6	1.7	2	JRC SOP
30	1.28	40	1.33	1.32	1.18	JRC SOP
31	2	0.44	2.2	1.9	-	JRC SOP

Annex 12. Results as reported by the participants for mass fraction of total MOAH in IF02B (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<2		<2	<2	<2	JRC SOP
01A	2.1	0.8	2.1	2.1	2.1	alternative
02A	5.54	1.79	4.79	5.79	6.03	JRC SOP dev
03	2.2	0.44	2.3	1.9	2.3	JRC SOP
03A	3.3	0.66	3.4	3.2	3.4	alternative
04	3.40	0.48	3.53	3.12	3.55	JRC SOP
05	3.8		4.14	3.46		JRC SOP
06	1.6	30	1.7	1.6	1.7	JRC SOP
06A	<4		4.8	4.2	3.5	alternative
07	3.67	0.73	3.71	3.38	3.91	JRC SOP
08	2.933		3.389	2.397	3.014	JRC SOP
09	2.62	1.05	2.57	2.61	2.69	JRC SOP
12	2.783	0.918	2.581	2.985	0	JRC SOP
13	3.02	0.64	3.53	2.18	3.35	JRC SOP
14	2.67		2.68	2.54	2.79	JRC SOP
15	1.84	58	<1.4	2.39	1.74	JRC SOP
15A	3.3	58	2.79	2.4	4.71	alternative
16	7.73	0.3	9.55	6.8	6.83	JRC SOP
17	2.458	0.28	2.299	2.511	2.563	JRC SOP
17A	1.284	0.04	1.315	1.252	1.285	alternative
18	2	0.8	2	1.9	2	JRC SOP
18A	2.9	1.2	2.6	2.9	3.1	alternative
19	3.1	1.5	3.1	6.2	1.7	JRC SOP
20A	3	0.3	3.1	2.9	3.1	alternative
21	6.9	1.94	6.8	6.7	7.1	JRC SOP
22	2.43		2.3	2.3	2.7	JRC SOP
23	3.19	0.86	3.45	3.01	3.11	JRC SOP
24	3.4	1	2.9	3.7	3.5	JRC SOP
25	5.3	25	4.5	5.7	5.5	JRC SOP
26	2.6	20	2.7	2.8	2.8	JRC SOP
27	1.38	43	1.38	1.08	1.26	JRC SOP
28	3.5	1	3.5	3.5	3.6	JRC SOP
30	1.64	40	1.71	1.63	1.57	JRC SOP
31	3.9	0.06	3.9	4	-	JRC SOP

Annex 13. Results as reported by the participants-for the mass fraction of MOAH C10-C16 in IF02B (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<0.5		<0.5	<0.5	<0.5	JRC SOP
01A	<0.5		<0.5	<0.5	<0.5	alternative
02A	<0.0602		<0.0568	<0.0603	<0.0633	JRC SOP dev
03	<0.5		<0.5	<0.5	<0.5	JRC SOP
03A	<0.5		<0.5	<0.5	<0.5	alternative
04	0.05	0.00	0.05	0.05	0.05	JRC SOP
05	0		0	0		JRC SOP
06	<0.5		<0.5	<0.5	<0.5	JRC SOP
06A	<0.5		<0.5	<0.5	<0.5	alternative
07	<0.1		<0.1	<0.1	<0.1	JRC SOP
08	0.007		0.013	0.004	0.004	JRC SOP
09	<0.5		0	<0.5	<0.5	JRC SOP
12	0	0	0	0		JRC SOP
13						JRC SOP
14	<0.5		<0.5	<0.5	<0.5	JRC SOP
15	<1.0		<1.0	<1.0	<1.0	JRC SOP
15A	<0.5		<0.5	<0.5	<0.5	alternative
16	<0.15		<0.15	<0.15	<0.15	JRC SOP
17	<0.2		<0.2	<0.2	<0.2	JRC SOP
17A	<0.2		<0.2	<0.2	<0.2	alternative
18	<0.5		<0.5	<0.5	<0.5	JRC SOP
18A	<0.8		<0.8	<0.8	<0.8	alternative
19	<0.5		<0.5	<0.5	<0.5	JRC SOP
20A	<0.5		<0.5	<0.5	<0.5	alternative
21	<0.3		0.3	0.3	0.3	JRC SOP
22	<1		<1	<1	<1	JRC SOP
23	<0.5		<0.5	<0.5	<0.5	JRC SOP
24	0	0	0	0	0	JRC SOP
25	<1		<1	<1	<1	JRC SOP
26	<0.05		<0.05	<0.05	<0.05	JRC SOP
27	0	43	0	0	0	JRC SOP
28	<0.5		<0.5	<0.5	<0.5	JRC SOP
30	<0.05		<0.05	<0.05	<0.05	JRC SOP
31	0	0.02	0	0	-	JRC SOP

Annex 14. Results as reported by the participants for the mass fraction of MOAH C16-C25 in IF02B (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<0.5		<0.5	<0.5	<0.5	JRC SOP
01A	<0.5		<0.5	<0.5	<0.5	alternative
02A	<0.0292		<0.0276	<0.0293	<0.0307	JRC SOP dev
03	<0.5		<0.5	<0.5	<0.5	JRC SOP
03A	<0.5		<0.5	<0.5	<0.5	alternative
04	0.32	0.18	0.26	0.27	0.42	JRC SOP
05	0.11		0.13	0.09		JRC SOP
06	<0.5		<0.5	<0.5	<0.5	JRC SOP
06A	<0.5		<0.5	<0.5	<0.5	alternative
07	0.13	0.03	0.17	0.1	0.12	JRC SOP
08	0.25		0.385	0.063	0.302	JRC SOP
09	<0.5		<0.5	<0.5	<0.5	JRC SOP
12	0	0	0	0		JRC SOP
13						JRC SOP
14	<0.5		<0.5	<0.5	<0.5	JRC SOP
15	<0.9		<0.9	<0.9	<0.9	JRC SOP
15A	0.46	58	0.46	0.41	0.51	alternative
16	0.4	0.3	0.52	0.35	0.34	JRC SOP
17	<0.2		<0.2	<0.2	<0.2	JRC SOP
17A	<0.2		<0.2	<0.2	<0.2	alternative
18	<0.5		<0.5	<0.5	<0.5	JRC SOP
18A	<0.8		<0.8	<0.8	<0.8	alternative
19	<0.5		<0.5	<0.5	<0.5	JRC SOP
20A	<0.5		<0.5	<0.5	<0.5	alternative
21	<0.5		0.5	0.5	0.5	JRC SOP
22	<1		<1	<1	<1	JRC SOP
23	<0.5		<0.5	<0.5	<0.5	JRC SOP
24	0.1	0	0.1	0.1	0.1	JRC SOP
25	<1		<1	<1	<1	JRC SOP
26	<0.1		<0.1	<0.1	<0.1	JRC SOP
27	0	43	0	0	0	JRC SOP
28	<0.5		<0.5	<0.5	<0.5	JRC SOP
30	0.09	50	0.1	0.08	0.09	JRC SOP
31	0.3	0.06	0.4	0.3	-	JRC SOP

Annex 15. Results as reported by the participants for the mass fraction of MOAH C25-C35 IF02B (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<0.8		<0.5	<1	<1	JRC SOP
01A	0.71	0.28	0.7	0.72	0.7	alternative
02A	2.69	0.721	2.27	2.88	2.9	JRC SOP dev
03	1.2	0.24	1.4	1.1	1.1	JRC SOP
03A	1.5	0.3	1.5	1.4	1.6	alternative
04	1.21	0.20	1.17	1.13	1.32	JRC SOP
05	1.43		1.63	1.24		JRC SOP
06	0.5	30	0.5	0.5	0.5	JRC SOP
06A	<2		2.2	1.8	1.4	alternative
07	1.7	0.34	2	1.35	1.76	JRC SOP
08	1.405		1.542	1.242	1.432	JRC SOP
09	0.84	0.34	0.83	0.82	0.87	JRC SOP
12	1.662	0.548	1.598	1.726	0	JRC SOP
13	0.92	0.19	1.04	0.74	0.97	JRC SOP
14	0.99		1.04	0.9	1.03	JRC SOP
15	1.06	58	<0.8	1.55	0.84	JRC SOP
15A	1.23	58	1.22	0.75	1.71	alternative
16	4.05	0.3	5.16	3.46	3.53	JRC SOP
17	1.055	0.07	0.969	1.067	1.128	JRC SOP
17A	0.364	0.02	0.373	0.355	0.364	alternative
18	0.72	0.3	0.77	0.68	0.71	JRC SOP
18A	1.3	0.5	1.1	1.3	1.5	alternative
19	1.4	0.7	1.4	2.7	0.8	JRC SOP
20A	0.91	0.15	0.95	0.85	0.95	alternative
21	3.7	1	3.6	3.6	3.8	JRC SOP
22	0.87		0.8	0.8	1	JRC SOP
23	1.33	0.41	1.41	1.17	1.4	JRC SOP
24	1.2	0.4	1	1.5	1.4	JRC SOP
25	2.6	25	2.1	2.9	2.7	JRC SOP
26	0.9	20	0.88	0.93	0.87	JRC SOP
27	0.42	43	0.42	0.35	0.48	JRC SOP
28	1.6	0.5	1.8	1.5	1.5	JRC SOP
30	0.71	50	0.74	0.71	0.68	JRC SOP
31	1.2	0.3	1.1	1.4	-	JRC SOP

Annex 16. Results as reported by the participants for the mass fraction of MOAH C35-C50 in IF02B (in mg kg^{-1})

LabCode	Result	MU	Rep1	Rep2	Rep3	Method
01	<1		<1	<1	<1	JRC SOP
01A	1.4	0.6	1.4	1.4	1.3	alternative
02A	2.78	0.565	2.47	2.87	3.01	JRC SOP dev
03	0.9	0.18	0.8	0.7	1	JRC SOP
03A	1.7	0.34	1.8	1.6	1.7	alternative
04	1.83	0.40	2.05	1.68	1.77	JRC SOP
05	2.17		2.26	2.08		JRC SOP
06	1.1	30	1.1	1.1	1.1	JRC SOP
06A	<2		2.3	2.1	1.9	alternative
07	1.82	0.36	1.53	1.92	2.02	JRC SOP
08	1.271		1.449	1.088	1.276	JRC SOP
09	1.67	0.67	1.71	1.64	1.67	JRC SOP
12	1.071	0.353	0.94	0.201	0	JRC SOP
13	2.1	0.45	2.49	1.44	2.38	JRC SOP
14	1.43		1.38	1.33	1.58	JRC SOP
15	0.78	58	0.61	0.84	0.9	JRC SOP
15A	1.61	58	1.11	1.25	2.49	alternative
16	3.27	0.3	3.86	2.98	2.97	JRC SOP
17	1.316	0.16	1.278	1.341	1.327	JRC SOP
17A	0.92	0.03	0.942	0.897	0.922	alternative
18	1.3	0.5	1.3	1.2	1.3	JRC SOP
18A	1.6	0.6	1.5	1.6	1.7	alternative
19	1.6	0.8	1.6	3.5	0.9	JRC SOP
20A	1.7	0.2	1.7	1.6	1.8	alternative
21	2.8	0.78	2.7	2.8	2.9	JRC SOP
22	1.47		1.4	1.4	1.6	JRC SOP
23	1.66	0.49	1.8	1.68	1.51	JRC SOP
24	1.9	0.6	1.8	2	1.9	JRC SOP
25	2.4	25	2.2	2.6	2.5	JRC SOP
26	1.8	20	1.75	1.76	1.74	JRC SOP
27	0.95	43	0.95	0.73	0.77	JRC SOP
28	1.7	0.5	1.7	1.7	1.8	JRC SOP
30	0.84	50	0.87	0.84	0.8	JRC SOP
31	2.3	0.18	2.4	2.3	-	JRC SOP

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