



EUROPEAN COMMISSION  
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers and Reference Materials (Geel)  
**Food and Feed Compliance**

## MINUTES

### of the Roundtable Workshop on the Determination of MOAH in Infant Formula

05 December 2019

Conference Centre Albert Borschette - Brussels

A Roundtable Workshop on the determination of mineral oil aromatic hydrocarbons (MOAH) in infant formula (IF) was organised by the Joint Research Centre (JRC) of the European Commission on short-term request of DG SANTE. A total of 77 participants representing all stakeholders (official control laboratories, industry and NGOs), DG SANTE, EFSA (via videoconference) and DG JRC attended the workshop. The meeting was opened and chaired by Hendrik Emons, Head of Unit 'Food and Feed Compliance' at the JRC.

Frans Verstraete (SANTE) explained the circumstances leading to the request to organise the roundtable with all stakeholders. Hendrik Emons (JRC) was setting the frame for the scientific-technical discussions aiming at harmonising as much as possible of the whole analytical method for the determination of MOAH in IF.

The morning was mainly dedicated to all stakeholders willing to present their methods/concerns related to the topic. Presentations were given by Romi Fengler (Fraunhofer Institute IVV), Fernando Campos (FEDIOL), Sander Koster (FoodDrinkEurope), Eva Mavromichali (SNE), Alex Moler (GBA Group Food), Roberto Ronzoni (NEOTRON), Jan Kuhlmann (SGS) and Maurus Biedermann (KLZH).

**All the presenters agreed to the statement that the measurand targeted here is operationally defined and that a step-by-step harmonisation of the procedure is necessary for obtaining comparable results .**

The need for reference materials and proficiency tests (PTs) was raised by several presenters as a prerequisite to assess the comparability and performance characteristics of the analytical approaches and the proficiency of the laboratories.

The second part of the roundtable was dedicated to the harmonisation process itself. Eddo Hoekstra (JRC) was the moderator of the discussions covering all steps of the analytical process for the determination of MOAH in IF.

## **1. Extraction of MOAH from IF**

It was acknowledged that this is a critical step as MOAH could be encapsulated in IF particles (for some types of IF) and denaturing of the proteins is necessary. An agreement was reached that acidic digestion creates problems. The recommended approach by all participants is alkaline digestion and saponification with KOH at 60 °C for 30 min after reconstitution of the powdered IF with water. Then liquid-liquid extraction with hexane is removing interfering components from the sample.

## **2. Clean-up by epoxidation**

Two main approaches were presented with some modifications within each. It was stated that epoxidation is in general a very harsh procedure and losing part of the MOAH is unavoidable. Consequently there is a crucial need for harmonisation. Maurus Biedermann informed the participants that an upcoming publication will demonstrate the equivalence of both approaches – epoxidation with m-CPBA in DCM at sub-ambient temperatures and in ethanol at room temperature, respectively. Jan Kuhlman informed that an interlaboratory study had been organised in Germany to compare different epoxidation methods. According to him the outcome indicated a slight preference for epoxidation in ethanolic solution at 40 °C. There were no objections when the epoxidation with 20% (purified) m-CPBA in ethanol at 40 °C for 15 min has been proposed as a harmonised clean-up step for the determination of the MOAH content in IF. Matrix removal by silica gel column chromatography with dichloromethane as eluent should be performed before or after epoxidation.

## **3. MOSH/MOAH separation**

On-line LC-GC is the method of choice, however it was agreed that the accepted approach should not be limited to it as off-line manual column-chromatographic separation should provide equivalent results as indicated by Oliver Kappenstein (BfR).

## **4. Quantification**

It was stated that the quantification of MOAH should be done with FID. Some participants expressed the view that it should be clear that the so-called hump signal could not only be attributed to MOAH as other interfering compounds could remain. Another concern was voiced by BfR with respect to a signal integration by different laboratories in cases where the solvent peak tailing is disturbing the C<sub>10</sub> (and even the C<sub>11</sub>) peak or when the baseline towards the end of the chromatograms is raising due to the column bleeding. In such situations the column has to be changed as it will not fulfil the performance requirements anymore. It was agreed to follow the JRC Guidance for quantification.

## **5. Confirmation of identification**

It was acknowledged that a confirmation of the structural identity of the detected molecules is necessary to prove the aromatic character of the compounds under the signal hump from FID.

Several participants presented the approach using GCxGC-TOF, enabling the separation of the aromatics depending on the number of aromatic rings in their molecules. The method is semi-quantitative and could indicate the ratio between different aromatic groups. Susanne Kühn (Kirchhoff Institut) presented results of Luxembourg from the determination of MOAH in IF with a characterisation of the MOAH hump. She recognized the possibility of reporting false positive results for MOAH caused by compounds not belonging to epoxidized biogenic substances.

Hendrik Emons stressed the demand that the confirmation methods have to be “enforceable”, meaning not requiring too sophisticated instrumentation which would not be available in most of the OCLs. The JRC expressed a vision for confirming the presence of 3-7 PACs based on GC-MS monitoring of ions (SIM), characteristic for the respective 3-7 alkylated or hetero-PACs, as published for some environmental samples and in line with the recently published EFSA opinion on substances of potential concern.

Several participants voiced their scepticism to this proposed approach. According to them the outcome would not be conclusive as one would find always (even without a MOAH hump from FID) some patterns of the chosen characteristic ions in the extracts as they are not specific enough. The JRC admitted that the applicability of the proposed procedure has to be studied in detail. It is not the intention of the method for ‘MOAH in IF’ discussed today to quantify the PACs.

As it was not possible to agree on a harmonised fit-for-purpose approach for identity conformation, the JRC suggested to stop the discussion on this step in this roundtable workshop.

## **6. Limit of quantification**

The discussions were focussed on two issues:

- the convention procedure to estimate the LOQ;
- the target LOQ for MOAH in IF as performance parameter.

The JRC proposed to agree on a common procedure how to estimate the LOQ for the determination of MOAH in IF. Each laboratory should use the same spiking solution for the addition of MOAH to an extract of a blank IF. The LOQ would represent the content of MOAH in the IF that will produce a hump with a height at the maximum of the hump which is 10 times larger than the standard deviation of the blank signal there.

Several participants commented that the procedure is reasonable, however the problem for an LOQ estimation comes from interferences remaining after epoxidation in the MOAH fraction which could produce signals above 0.2 mg/kg. There seems to be huge differences from one IF to another and the LOQ would depend strongly on the type of the IF.

The target LOQ and the maximum acceptable LOQ for the determination of MOAH in IF should follow the requirement of the JRC Guideline.

Moreover it has been agreed that the analysis of two or more replicate samples should form the basis for the reported result.

A question was raised regarding the assessment in case that there is no indication for the presence of MOAH in IF from GC-FID measurements, but a GCxGC chromatogram would indicate the presence of 3-7 PACs. It has been agreed that the sample should be considered as negative (i.e. no MOAH present).

No more problems were raised by the participants. Consequently Hendrik Emons wrapped up the agreed conclusions:

1. Quantification of MOAH in IF is method dependent ('operationally defined measurand'), consequently sufficient harmonization of the whole analytical process is required.
2. The proposed method should be enforceable - the equipment for the proposed steps should be available not only in a very limited number of laboratories.
3. The following steps should be executed in a harmonised manner:
  - Reconstitution of powder IF shall be carried out with water followed by the addition of internal standards, alkaline denaturation of proteins and saponification with ethanolic KOH at 60 °C for 30 min;
  - Extraction (liquid/liquid) of total MOAH with *n*-hexane;
  - Matrix removal by silica gel column chromatography, elution with dichloromethane (could also be done after epoxidation);
  - Further clean-up of the extract by epoxidation with 20 % purified<sup>1</sup> *meta*-chloroperbenzoic acid (*m*-CPBA) in ethanol, vortex mixing at 40 °C for 15 min, reaction stopped with sodium thiosulfate;
  - MOSH/MOAH separation by on-line LC-GC or a manual column chromatography method;
  - Quantification by LC-GC-FID or GC-FID, application of internal standards;
  - Signal integration of the whole signal interval starting at  $t_R$  of the beginning of the *n*-C<sub>10</sub> peak and ending at  $t_R$  of the *n*-C<sub>50</sub> peak end after elimination of the sharp peaks above the hump for total MOAH; C-fraction shall be determined as described in the JRC Guideline<sup>2</sup>;
  - Identification/confirmation by mass spectrometric methods still needs to be further discussed;
  - Results should be reported based on the analysis of a minimum of 2 replicate samples;
  - A procedure for LOQ estimation in a conventional manner was agreed by the participants.

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<sup>1</sup> Purification with hexane extraction if needed. **Caution** – if you purify the CPBA too much there is risk of explosion!

It was agreed that laboratories analysing MOAH in IF samples shall follow the corresponding JRC "Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials"<sup>2</sup>. In addition, they shall apply the conclusions of this workshop to further harmonise their analytical procedures with the aim to obtain comparable data for risk assessment and risk management.

The Chair thanked all participants attending and contributing to the discussion on such a short notice. He acknowledged that the whole harmonisation process is iterative and indicated that the JRC will further work on it.

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<sup>2</sup> 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