On the presence of Mineral Oil Hydrocarbons (MOSH and MOAH) in edible fats and oils: a report from the SISSG related Workshop*

Lanfranco CONTE

Società Italiana per lo Studio
delle Sostanze Grasse - SISSG
Via Giuseppe Colombo, 79
20133 Milano, Italia

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CORRESPONDING AUTHOR:
Lanfranco Conte
lanfrancoconte@gmail.com

La presenza di contaminanti organici tipici della composizione degli “oli minerali” è stata evidenziata in molti alimenti già dalla seconda metà degli anni '90, tuttavia è diventata un problema non indifferente per il commercio internazionale negli ultimi anni.

Il problema non è di poco conto, in quanto può rivestire anche aspetti legati alla salute del consumatore, il che ha spinto alcune compagnie della grande distribuzione internazionali ad applicare livelli molti bassi (2 mg/kg), tuttavia, non esiste ad oggi un metodo analitico standardizzato e validato per questa determinazione analitica e l'unica esperienza in tal senso ha dimostrato che è difficile scendere sotto i 10 mg/kg come riproducibilità.

La Società Italiana per lo Studio delle Sostanze Grasse ha ritenuto di fare cosa utile al comparto organizzando un workshop in dicembre 2019 chiamando a dibattere questo argomento i rappresentanti dei principali laboratori europei coinvolti nel problema,

I risultati dei lavori hanno dimostrato che non esiste un approccio analitico univoco, soprattutto per la purificazione del campione e per la integrazione dei cromatogrammi e come alcune tecniche di preparazione del campione (epossidazione) possano anche portare alla presenza di artefatti con la generazione di risultati analitici errati.

I singoli laboratori applicando protocolli validati internamente assicurano buone performances, ma i dati appaiono non paragonabili e la riproducibilità rimane non inferiore ai 10 mg/kg.

1. INTRODUCTION

The presence of Mineral Oil Hydrocarbons (MOH) was detected since the last '90th, then it was established that two or more classes of compounds were present, namely Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH).

The presence of these compounds in edible fats and oils and in foods moved some concerns for human health and a number of studies were published dealing with the measurement of their concentration.

Results were not always reliable, because of lack of an analytical method standardization and validation, beside the “in house” validation level, furthermore, poor separation of peaks strongly affected the measurements.

Just one validation work was carried out by ISO showing that reproducibility cannot be lower than 10 mg/kg, despite this, many retailers applied more severe limits (2 mg/kg) that, in lack of normative or legislative indication and method harmonization, leads to trade litigation with important consequences for companies.

The Italian Society for Fat Researches (SISSG) promoted in 2019, December, a workshop on this topic, inviting as lecturers chemists of main laboratories involved into this topic.

Results confirmed that even if within a single laboratory, very low level of LOQ (1-2 mg/kg) can be obtained, however a complete validation of the method is nowadays still far to be reached and that the LOQ level strongly depends on the nature of the matrix to be analysed and consequently on the sample preparation procedure that can produce artifacts leading to wrong results.

The contamination of foods with hydrocarbons of supposed petrogenical origin (distinguished in Mineral Oil Saturated Hydrocarbons – MOSH and Mineral Oil Aromatic Hydrocarbons – MOAH) is a hot topic of food chemistry and food safety control since many years, the earliest paper reporting the occurrence of these compounds being published in 1996 [1], later, several studies[2-6] were devoted to this issue, despite of this, only in a few cases it was possible to identify the origin of the contamination (technical oil used in the oil mill, batching oil from jute bags, diesel oil). In many instances the mineral paraffins were already in the seeds and had a composition resembling that in the atmospheric particulate matter, suggesting an environmental origin [4]

The main episode that moved the interest of control bodies was in 2008 with a strong contamination of mineral oil on sunflower oil for human consumption produced in Ukraine.

Even if some opinions by EFSA had been published, not an ultimate vision on toxicity of the two classes of compounds exists and nowadays no validated method is available, probably depending, too, on the fact that there is not a precise information on limits established on toxicological studies, even if a consensus exists about their being extraneous substances to foods.

Early papers dealing with this topic were published in 2001 [3] and later, other methods were published, with the purpose to improve the reliability of analytical results [7]

The presence of MOSH and MOAH was associated to toxicological concerns, despite EFSA published some reports [8] on this topic, no official limit had been established by UE or other normative sources, one of main drawbacks being the absence of a validated method.

Despite this, some retailers imposed a limit of no more than 2 mg/kg for olive oils and other edible oils and this became an important problem for the suppliers.

The limit depends on the applied method, but without a suitable validation, reliability of methods is a source of litigation in trade.

The Italian Society for Fats Researches (SISSG) decided to promote a meeting between main laboratories involved in this subject and the workshop took place in Bologna in 2019, December 12th; this paper reports what was discussed in that occasion, based on the lecturers' presentation, with the aim to improve the knowledge about different analytical approach and to facilitate a shared vision of the problem that could also lead to an accepted hypothesis of limit in edible fats and oils.

The works were introduced by a plenary lecture by Konrad Grob (Kantonales Labor Zürich), entitled “MOSH and MOAH: occurrence and toxicological evaluation”. K. Grob provoked, opening by the often-heard statement “measurements are wrong!” This may be an excuse to reject inconvenient results, but, indeed, the analysis is demanding: it needs skilled laboratories, the interpretation of chromatograms (the main source of error) cannot be standardized and the cost of analysis increases when additional separation steps are required. Methods were developed to reliably determine MOSH and MOAH in edible fats and oils below 1 mg/kg, but this presupposes auxiliary techniques.

The basic method involves preseparation of MOSH and MOAH in liquid phase, preferably, but not necessarily by HPLC, and GC-FID for quantitation and a first characterization of the MOSH and MOAH. FID is preferred to MS because of a virtually equal response of all hydrocarbons. Without enrichment, but with possible reconcentration of extracts to inject a maximum of 20 mg triglycerides into the commonly used 25 cm × 2 mm i.d. silicagel HPLC column, the limits of quantification (LOQ) are the following:

- Low fat (≤ 4%) samples: LOQ ~ 0,1 mg/kg, with a concentration of extracts by a factor of 10 (10 g food to 1 mL n-hexane);
- Medium fat (~ 20%): no reconcentration (1 g of food to 1 mL n-hexane); LOQ ~ 0,5 mg/kg
- High fat (~ 40%): only half amount /concentration (0,5 g to 1 mL); LOQ ~ 1 mg/kg
- Vegetable oils (20% solution) LOQ ~ 2,5 mg/kg.

Then, Grob reviewed sample pre-treatment methods. Off-line or on-line chromatography on activated aluminium oxide enables the removal of long-chain n-alkanes, including disturbing natural n-alkanes, above about n-C_{24} [9]. The mechanism of this separation is poorly understood: it presupposes absence of humidity and somewhat polar solvents, and n-hexane causes the long-chain n-alkanes to be retained, while isooctane elutes them.

Enrichment for analysis in fats and oils is conveniently combined with the removal of the interfering n-alkanes, using a column packed with activated silica gel above a mixture of activated aluminium oxide and silica gel with 0.3% silver nitrate [10, 11]

So far, epoxidation [12] (proposed by Carlo Mariani) is the best, though imperfect method for the elimination of interfering olefins of natural origin or resulting from raffination of edible oils and fat (e.g. squalene and its isomerization products, sterenes and carotenoids). Partial loss of MOAH as well as incomplete removal of interferences in some cases seem unavoidable. The method recently proposed by Nestola and Schmidt [13], performing the reaction in ethanol, is an improvement: (i) it avoids cooling and facilitates automation by autosamplers, (ii) as the reaction is stopped kinetically, no stopping oil is required, and (iii) no evaporation step is required for exchanging the solvent. However, residual interferences, encountered primarily in refined oils, must still be recognized to avoid overestimates of MOAH concentrations.

For the verification and characterization of MOSH and MOAH, MS is of limited usefulness owing to little specific fragments and little selectivity obtained by high resolution MS. However, comprehensive two-dimensional GC (GC × GC) with FID provides rich information with characteristic bands for the various types of hydrocarbons as well as showing the mineral oil markers steranes, hopanes and marker for recycled paper and board, the diisopropyl naphthalenes. GC × GC is also the most effective way of checking for residual interferences after epoxidation.

For the determination of the sources of contaminations by mineral oil hydrocarbons, it is important to distinguish between environmental contributions: the producers cannot be made responsible for and those introduced during harvesting, processing and packaging. The dominant part of the environmental contribution is of a molecular mass suggesting that it is from particulate matter, such as soot, lubricating oils from diesel engines or debris from road tar. The contamination was shown to be in the order or 0.1-2 mg/kg in oil extracted from sunflower seeds collected around Zurich. It is unclear whether these hydrocarbons are deposited from the air onto the plants or picked up from the contaminated soil. Characteristically they are virtually free of MOAH, but there is no analytical method to selectively determine environmental contamination.

Grob provided a historical perspective on the toxicological evaluation of mineral oil hydrocarbons. It was characterized by a number of pitfalls [14]. In the years 1950-1990, numerous publications reported frequent occurrence of granulomas in human tissues that were related to mineral oil hydrocarbons. They remained unexplained up to today. There are no reliable data on their present occurrence, but it is believed that the reduced exposure let them largely disappear.

Granuloma formation and related inflammation were also considered the pivotal end points in animal tests, but these were misleading. The “old” toxicological evaluations, almost exclusively on MOSH, were based on experiments with entire mineral oil product and little information about their composition beyond viscosity. In Fischer 344 rats, granuloma formation was found for mineral oil products of relatively low molecular mass, which prompted the risk assessors (SCF and JECFA, 2002) to specify a low TDI (0.01 mg/kg body weight) for mineral oil products with more than 5% hydrocarbons below C25, but ADIs of 10-20 mg/kg body weight for the higher mass oils with less than 5%. Recent research with test materials of known composition revealed, however, that this granuloma formation in Fischer 344 rats is a particularity of this rat strain, as it seems to be unable to metabolize n-alkanes: n-alkanes above about C25 are believed to crystallize in the tissues and trigger granuloma formation [13]. They are not accumulated in humans and, therefore, they cannot form granulomas [15] This was the first pitfall: as the mineral oil products of relatively low molecular mass tested contained n-alkanes, they triggered granuloma formation in the Fischer 344 rats and, consequently, were considered to be of concern, whereas oils of higher mass were more effectively deparaffinated, contained hardly any n-alkanes and did not cause granuloma formation in these rats.

The second pitfall was due to lacking analysis of MOSH in human tissue: it turned out that exactly these higher molecular mass mineral oil products that contain hydrocarbons which are strongly accumulated in human tissues, possibly over lifetime. This accumulation results in concentrations in human tissues that are far higher than expected from animal experiments – for a quarter of the subjects investigated, the body burden exceeded 5 g mineral oil hydrocarbons [16]. At the elevated concentrations found in human spleens and livers, test animals showed strongly increased organ weights, which are signs of reactions to compensate for damaged organ functioning. If Fischer rats are adequate models for humans in this respect, also humans may affected in this respect. Hence, the MOSH with high ADIs (i.e. authorized with high tolerance) are exactly those of main concern. As a result of these confusions, there are still no legal limits or health-related reference values to determine tolerable levels of food contamination with MOSH.

Often the MOAH are considered as the main risk owing to genotoxic constituents. However, it is a subject of debate to what extent these genotoxic components (polyaromatic MOAH) are eliminated by the raffination process. In fact, the commonly applied hydrogenation is most effective for the polyaromatic MOAH, leaving a high proportion of alkylated benzenes. The MOAH composition of mineral oil products varies widely; most mineral oils found in food do not contain more than 2 aromatic rings with the exception of jute and sisal bags. For this reason, the analytical method should provide separate determinations of MOAH with more than two aromatic rings (which should be feasible, e.g. using on-line HPLC-GC × GC). MOAH are not accumulated by humans.

A conclusive toxicological evaluation of MOSH and MOAH is badly needed, also to avoid overreaction when these are discovered in food, but the data presently available might not allow for this. Owing to the misleading interpretation of the data from Fischer

344 rats, the evaluation needs complete revision.

Grob highlighted that one of the problem is the lack of a harmonized method to assess the presence and the concentration of mineral oils in fats and oils or, at least, of a method with characteristics accepted and applied by anyone who is involved into this topic, the work on standardization of methods had been the topic of the lecture by Florence Lacoste by ITERG.

After giving the definition of Mineral oil composition according to Concawe (division of the European Petroleum Refiners Association), Lacoste reported some examples of edible fats and oil contamination, then she elucidated the principles of methods and the critical points that are: a) Integration of the hump subtraction of the "natural hydrocarbons"; b) quantification of hydrocarbons without losing the volatile ones; c) Removal of interfering compounds (olefins in the MOAH fraction); d) Limit of quantification as low as possible; e) Blank level to be under control.

The work program for method standardization was developed by ISO (ISO/TC34/SC11), with the aim to meet the EU requirements (CEN/TC275/WG13), however, ISO only considered MOSH in crude and refined vegetable oils, while CEN worked on a method suitable to distinguish MOSH and MOAH in crude and refined vegetable oils and in foodstuffs on basis of vegetable oils.

Results were the method ISO 17780:2015 for MOSH with a limit of application of 50 mg/kg and the method EN 16995:2017 for MOSH&MAOH, with limit of application 10 mg/kg (without prior clean-up).

The reproducibility of the ISO 17780 was problematic according to the value of the HORRAT and for this reason, the limit of application was established at 50 mg/kg for MOSH.

The on line HPLC-GC-FID method used by the EN 16995:2017 method is intended for application to fats and oils and foodstuffs on basis on vegetable oils only and is not intended for other matrices.

The method was based on fractionation of MOSH&MOAH by means of HPLC with UV detection and on line large volume injection of each fraction, while quantification was obtained by the use of two i.s.

The clean up by epoxidation is mandatory, according to the results of the collaborative study, the method has been proven suitable for mass concentration above 10 mg/kg both for MOSH and for MOAH.

Lacoste also report, as a frame of the current situation of the analysis of MOSH and MOAH the results published by Koster et al. (2019) [17] that reported that the acceptable deviation from the estimated "true value" is quite high below 10 mg/kg for both MOSH and MOAH and that amounts of MOAH below 2 mg/kg are so difficult to quantify that statistical analysis cannot be performed.

In 2019/2020, 19 laboratories will participate to a collaborative study and proposed several improvements to the method, mainly dealing with sample clean up or fraction enrichment; the evaluation of hopane as marker of petrogenic origin was considered, too, by means of $SiO_2/AgNO_3$ purification, followed by ISO 17780 and GC/MS-SIM, by using m/z 191 fragment for identification.

Lacoste concluded that despite the presence of MOH in foods is nowadays elucidated, as well as some production chain steps as responsible for contamination, till now no EU legislation had been published, even if a recommendation on the monitoring of MOH in foods had been published; in the meantime, standardized methods for MOSH&MAOH published in 2017 had a LOQ at 10 mg/kg, even if it may be improved by sample enrichment prior the on line LC-GC/FID analysis, however, at present, that is the LOQ.

After these introducing lectures, floor was given to some laboratories to speak about their experiences on MOSH&MAOH analysis.

S. Moret from Udine University (Italy) reported experiences developed on LC-LC-GC analysis in 1995 when a method was developed in cooperation with Kantonales Laboratory of Zürich with the aim to determine alkylated polycyclic aromatic hydrocarbons (MOAH): the first SiO_2 column retained TAGs while MOSH and MOAH fraction was eluted and transferred to a second column, amino phase, suitable to fraction aromatics according to their ring number.

The first method for routine analysis applying LC-GC was developed by Biedermann et Al in 2009 [7] , then Barp et al. modify it [18] by using a rapid gradient to oven and shortened the backflush reconditioning time; this permitted to carry out up to 62 runs per day, to a lower solvent consumption, in the meantime increasing method sensitivity.

Moret reported that when high amount of interfering olefins are presents, as is the case of vegetable oils, epoxidation is mandatory. At present two different epoxidation protocols are reported in the literature [9-11], and probably further modified protocols are used in different laboratories. Nevertheless, no published data on method performances comparison are available. When applied to different pomace olive oils, epoxidation showed a sample dependent behaviour.

When followed by on-line HPLC-GC, SPE on 1 g of $SiO_2/AgNO_3$ [19] may be an alternative to epoxidation and it was observed that in the case of EVOO, interfering olefins were removed completely, while removal was negligible in the case of palm oil.

To improve the sensitivity of the method and remove interfering olefins, reaching LOQ around 0.5-1 mg/kg, automated SPE (speed extractor, Büchi) equipped with 10 mL cells loaded with 5 g of activated $SiO_2/AgNO_3$ and 0.25 g of sample, was used. Automated SPE was also used to remove *n*-alkanes experimenting the use of alumina or alumina/activated SiO_2. Higher enrichment factors with minimal solvent consumption can be reached by applying fast microwave assisted saponification with simultaneous extraction of unsaponifiable.

The on line coupled HPLC-GC-FID is applied by Jan Kuhlmann who reported data for validation of MOSH/MOAH determination at SGS of Hamburg (Germany) by applying both ISO 17780 and DIN EN 16995:2017-08, despite the declared LOQ which are respectively 50 mg/kg for MOSH and 10 mg/kg both for MOSH and MOAH, SGS was able to reach LOQ values for MOSH, as well as for MOAH is 1 mg/Kg, but in some interlaboratory comparisons, reproducibility is sometimes poor at the low level of ppm.

Kuhlmann stressed that as several sample procedures might be applied, every laboratory goes its own way, dealing with different fat or analyte extraction, use or not of saponification, epoxidation (strong or mild), aluminium oxide column chromatography, manually or on line, silica gel column chromatography, before or after epoxidation and finally different strategies for raw data processing.

SGS applies saponification or silica gel clean up as early step, then aluminium oxide clean-up is applied for MOSH and epoxidation for MOAH, then separate measurement by LC-GC-FID.

Saponification has a significant impact on the matrix load of samples and might support efficiency of subsequently applied clean up steps, especially epoxidation, but it must bear in mind that every sample preparation step might result in losses of analyte and or carry-over contamination with a risk of overestimation or false positive results.

The use of a silica –gel as well as of a silica-gel/aluminium oxide column chromatography (SPE), it is a standard procedure that can be carried out in a reproducible manner, but elution solvent, elution speed, silica amount and its particle size and deactivation degree as well as column geometry and final solvent evaporation can greatly influence results, mainly recoveries, so that they must be strictly controlled, as a conclusion, SGS suggest that an agreement on the exact silica or silica/aluminium oxide SPE procedure is needed.

Epoxidation is a harsh chemical reaction which is difficult to be quantitatively controlled: it can cause losses of MOAH, with underestimation as a result, however, not applying epoxidation might result in overestimation due to the contribute of non-aromatic components to the MOAH hump: method harmonization should take care of these aspects by suggesting when epoxidation must be applied and by approving a devoted exact and harmonized protocol.

Advantages of automated LC-GC-FID are excellent linearity and signal/dose correlation independent upon nature of the analytes, drawbacks are that probably it is not the first choice for trace analysis and that it does not give information on analyte structure.

However, LC-GC-FID seems to be the best choice for reproducible quantification, but demand for LOQ must be realistic, otherwise official method validation might fail.

Any improvement in analytical method performances or validation makes no sense if no agreement is found in data processing: weak aspects are non-standardized integration protocols and changes in the instrumental or personal that might cause increased measurement uncertainty, mainly at low concentration level. An agreement on the exact procedure of data processing is mandatory in view of method harmonization.

Results verification, too, is an open question: not considering mosh results for verification of MOAH findings might be a wasted opportunity, GC × GC – TOF-MS could be used, however is a rather expensive apparatus and needs for high qualified and trained personnel.

Results should be reported after background subtraction and LOQ is evaluated at 1 mg/kg per fraction and in sum.

SGS stressed for care in evaluating sources of error as well and on need for a fully harmonized analytical protocols; furthermore, SGS also estimates that official validation for MOH determination in the very low ppm range might be not feasible with the current methodology, if the impact of the single sample preparation steps on reproducibility has not checked systematically.

Nadja Liebmann on behalf of Eurofins (Germany) reported that their laboratory for MOSH, POSH, PAO and MOAH uses LC-GC-MS and LC-GC × GC - MS for qualitative evaluation, while for quantitative measurement uses LC-GC-FID.

With the aim to lower the LOQ, Eurofins approach involves extraction that is usually carried out by n-hexane, then a clean-up step is applied by silica gel to remove simple fats in case of MOSH/MOAH, while aluminium oxide is used in case of MOSH is used to remove natural long chain (> C20) and epoxidation for MOAH to remove other natural compounds such as squalene, vitamin E and carotenoids; furthermore, saponification is under evaluation, as a tool to use when lecithine is present.

Three internal standards (namely, cholestane, TBB and perylene) were introduced, suitable to assess the beginning and the end of different fraction; the Eurofins validated method for oils and fats declares the following LOQs values: 1mg/kg for MOSH/POSH and 1 mg/kg for MOAH, even if it is aware that increasing of LOQ are possible, depending on matrixes interferences that depends on the king of vegetable matter that undergoes analysis.

Eurofins also presented data of a project carried out with Greek that monitored MOSH and MOAH content of a production of that country, even if results had been presented, however they are property of custom, and here we can just report that some hypothesis on the source of contamination (lubricating oil of chainsaws) had been reported that are in agreement with those of other research groups.

Further data by Eurofins highlighted the occurrence and similar level of MOSH & MOAH contamination in other oils, beside olive oil, like avocado and coconut (many thousands of samples each one).

After recall the situation by the point of view of normative and legislation (stressing that no legal limit, no EU Regulations for MOSH and NOAH in oils and fats exists), A. Luisi reported the experiences by Chemiservice (IT) where an internal off-line method was developed and validated using solid liquid separation on chromatographic column and injection into a gas chromatograph with on column injector ad FID detector.

The method was successfully used for olive oils, while for other oils, recently, further clean up steps had been introduced, with the aim to low LOQ values.

A very careful procedure was described, both for the use of quality control of materials and use of several I.S. and for what concerns the problem of integration of the UCM in the MOSH area (mainly C17-C25 and more) by compare the use or not of epoxidation (in the case of pomace oil, also a purification step through an alumina cartridge was applied).

Some examples dealing with drawbacks in the analysis of EVOO with high contamination level were presented; method performances were also evaluated against request by JRC.

The method presented a very good linearity that was evaluated, by using an EVOO spiked with MOSH ranging from C10 to C60, which concentration ranged between 1,4 mg/kg and 290 mg/kg.

LOQ was evaluated for MOSH and MOAH and results were respectively 1,50 mg/kg and 1,1 mg/kg as average of 8 replicated determination.

Precision, accuracy and uncertainty of measurement were evaluated, too, within internal validation process and results were also evaluated against the epoxidaton procedure with complaint results.

Data of a huge number of samples of different kind of edible oils and fats has been presented, too, highlighting the problem involves most of them, both virgin, crude and refines, not depending on the fact they are extracted from fruits or seeds.

E. Schulz from Merieux Nutrisciences – Laboratories of Institut Kirchhoff – IKB (Berlin, D), presented the activities carried out in cooperation with Max Rubner Institute, too. After examining the recent international rules (that for EU means Recommendations, indeed), E. Shulz stated that considering the growing relevance of MOH within Europe, USA, Canada and China, LC-GC-FID is the method globally established.

Details on JRC guidelines were then presented, then the method used is described, that, after extraction and clean up (if necessary), the MOSH and MOAH are separated by means of online HPLC on a silica gel phase. The target fraction is then transferred to the GC on column, separated by boiling point and quantified by FID.

LOQ in the case of fats and oils is reported to be 1 mg/kg, with intermediate precision of 7% both for MOSH and for MOAH (validation data IKB).

Experiences of characterization of MOSH and MOAH with GC × GC – TOF-MS are reported, too, in this case, the characterization is obtained according to substance classes, but separation in single compounds is not completely feasible. Special marker substances are also visible with GC × GC - TOF-MS, which can give hints of the contamination source.

Studies on contamination sources had been carried out, too, that highlighted that often multiple sources exist, leading to overlapping distribution of related peaks.

IKB analyzed more than 240 lubricating oils to compare the chromatograms of them with the profile of food in case of high contamination. Synthetic lubricating oils based on polyalphaolefines (PAO) are frequently used in the food industry; these lubricating oils are also included in mineral oil analysis.

Problems also arise from matrix components and in some cases, an “hump” remains also after epoxidation, depending not on MOAH, but on biogenic substances, however, this “hump” can be considered as a signal of MOAH by laboratories which are not so familiar with this analytics, so that false positive results are reported, with significant consequences for raw material suppliers and food companies.

This, too, moved interest to deeply investigate the nature and the toxicology of these substances; according to BfR, contamination with MOAH should be avoided, however EFSA stressed that carcinogenic potential correlates with increasing number of aromatic systems and that MOAH with three or more, non or simple alkylated aromatic rings may be mutagenic and carcinogenic, for this reason it seems important to distinguish between MOAH with 1-2 and more aromatic rings.

As a conclusion of the workshop organized by SISSG, a round table with lecturers and representative of companies and their association took place.

It is clear that, despite the huge number of samples analyzed by many laboratories around the world, an ultimate and well established frame does not exists and consequently, it seems still difficult to have a complete consensus about possible limits.

Every lecturer, while gave details on the method that was in house validated that makes the determination of low concentration reliable within the laboratory itself, also presented severe drawbacks of the analytical protocols and the only international validation trial carried out established LOQ at 10 mg/kg, so far from request of retailers.

In the meantime, improved toxicological acquirements made clear that even the present applied analytical approaches are not adequately updated and not suitable to guarantee on one hand the consumers health safe and on the other hand not to improve litigation on market.

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