Toxicity of mineral oil hydrocarbons

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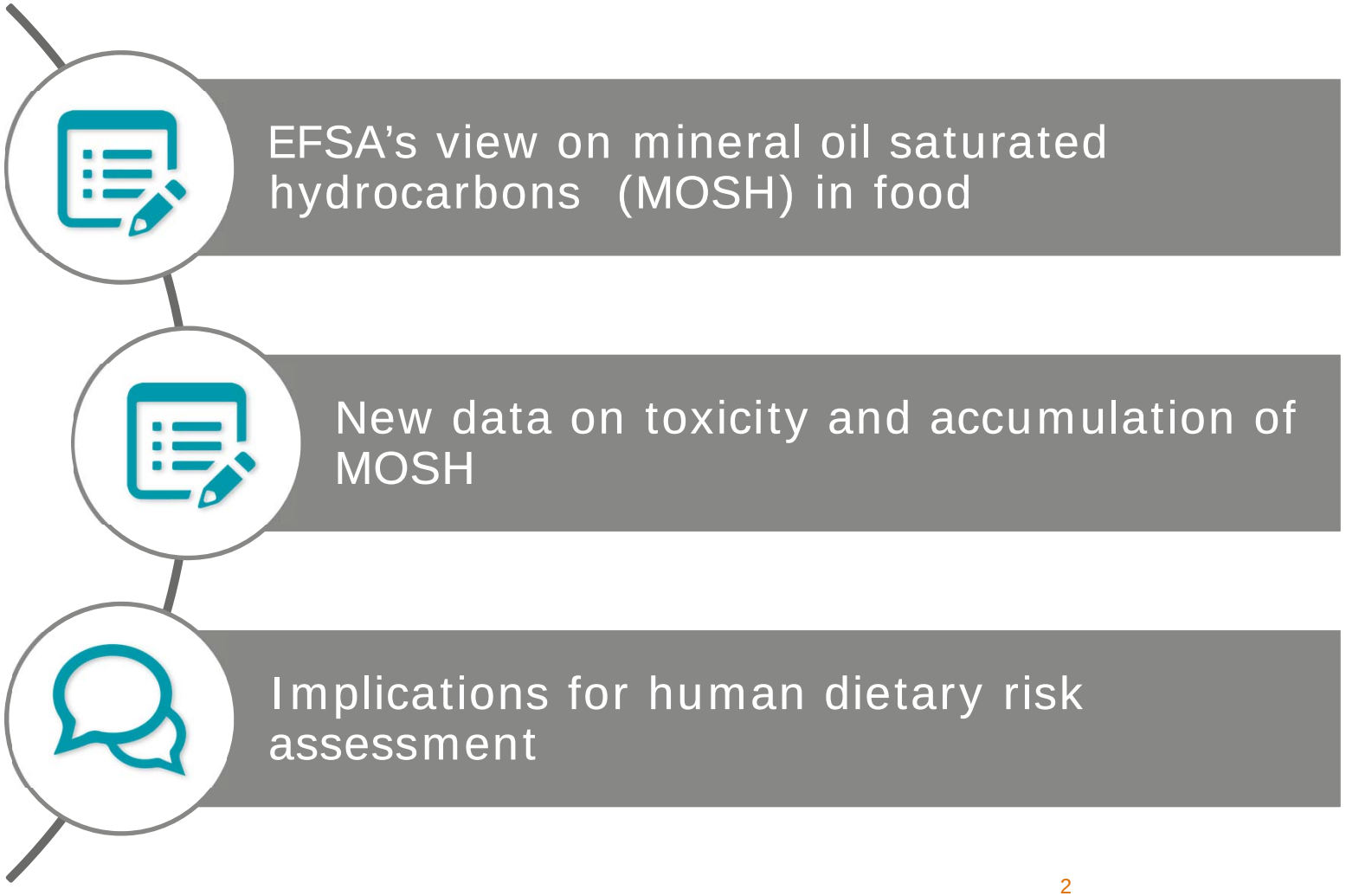
OUTLINE


EFSA's view on mineral oil saturated hydrocarbons (MOSH) in food
New data on toxicity and accumulation of MOSH
Implications for human dietary risk assessment

INTRODUCTION

- Mineral oil hydrocarbons (MOH) are present in food from many sources, following intended uses of food grade MOH or contamination via different routes
- Depending on the source, the composition of MOH mixtures varies
- Main sources of occurrence are
  - Contamination via the environment
  - Food processing
  - Migration from food contact materials
  - Food additives
  - Pesticides
- The complexity of MOH preclude the possibility to identify toxicological hazards based on single substance properties.

EFSA ASSESSMENTS OF MOH

- Food grades MOH have been evaluated by EFSA for several applications. The two most recent opinions were adopted by the ANS Panel for food additive applications:

  - ✓ High viscosity mineral oils (EFSA , 2009)
  - ✓ Medium viscosity (class I) mineral oils (EFSA, 2013)

- These evaluations are relative to specific products and applications, without considering the cumulative exposures from different sources.

- In 2012 the CONTAM Panel issued a Scientific Opinion assessing the risks to human health related to the the range of MOH that have been detected in food.

TOXICITY OF MOSH – TOXICOKINETICS

- **Oral absorption decreases with increasing carbon number. Virtually no absorption at > C_{50}.** Branched-alkanes are slightly less absorbed than linear and cyclo-alkanes of comparable molecular weights.
- Alkanes are metabolised to the fatty alcohols and generally to fatty acids. **Linear alkanes more easily metabolised than cyclic- and branched-alkanes**.
  - Species (and strain) differences:
    - Biotransformation of heptadecane (*in vitro* microsome incubation)
      - Human >> Rat
      - Wistar rat > Sprague Dawley rat > Fischer rat[1]
- MOSH with carbon number between **C_{16} and C_{45} can accumulate in the organism**. High accumulation potential observed in Fischer 344 rats. **Accumulation in different tissues, such as adipose tissue, lymph nodes, liver and spleen, is observed in humans**[2].

[1]Cravedi and Perdu, 2012.
[2]Concin et al., 2008; Barp et al., 2014, 2015.

TOXICITY OF MOSH

- Laboratory toxicity data are available for a series of refined MOSH grades used/proposed as food additives.

- The main limitation of these data is that food grades mineral oils and waxes are classified mainly by physico-chemical properties, and not by chemical composition:

Name	Viscosity at 100 °C (mm^2/s)	Average relative molecular mass	Carbon number at 5% distillation point
Microcrystalline wax High melting point wax (HMPW)	≥ 11	≥ 500	≥ 25
Low melting point wax	3.3	380	22
Mineral oil (high viscosity) P100(H)	>11	≥ 500	≥ 28
Mineral oil (medium and low viscosity) class I P70(H)	8.5 – 11	480-500	≥ 25
Mineral oil (medium and low viscosity) class II N70(H)	7.0 – 8.5	400-480	≥ 22
Mineral oil (medium and low viscosity) class III P15(H), N15(H)	3.0 – 7.0	300-400	≥ 17

TOXICITY OF MOSH

- **Main findings in food grade oils and waxes**
  - ✓ **Negative results in genotoxicity tests**
  - ✓ Following 13-week exposure in female Fischer rats, formation of **microgranulomas are observed in the liver and mesenteric lymph node (MLN)**.
    - ❑ MLN microgranulomas are considered adaptive changes to high exposure to high MW substances with poor absorption, not progressing to adverse effects following longer exposure.
    - ❑ **Hepatic microgranulomas are associated to adverse effects** (inflammatory response, cell death and fibrosis).
    - ❑ Other species and other rats strains show lower sensitivity to these effects
    - ❑ Different grades shows different potency, **LMPW > Class II and III low and medium viscosity oils > others**
  - ✓ Chronic study with P70(H) and P100(H) showed no progression of liver and MLN microgranulomas into a prolonged inflammatory response or other severe pathological changes.

ACCEPTABLE DAILY INTAKES

	JECFA (2002, 2012)				EFSA (2009, 2013)			
	ADI (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Uncertainty factor	Comments	ADI (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Uncertainty factor	Comments
High viscosity P100(H)	0-20	1 951	100	90-day NOAEL	12	1 200	100	2-year NOAEL
Medium and low viscosity, class I P70(H)	0-10	1 200	100	2-year NOAEL	12	1 200	100	2-year NOAEL
Medium and low viscosity, class II N70(H)	0-0.01*	(2)	(200)	90-day NOAEL	–	–	–	
Medium and low viscosity, class III P15(H), N15(H)	0-0.01*	(2)	(200)	90-day NOAEL	–	–	–	
Microcrystalline wax (HMPW)	0-20	1 951	100	90-day NOAEL	–	–	–	Quantum satis

*temporary ADI, withdrawn in 2012

The CONTAM Panel did not consider any of the existing ADIs as relevant for the risk assessment of MOSH in food

OUTCOME OF CONTAM PANEL OPINION

- Considering the formation of liver microgranulomas as the critical endpoint, The CONTAM Panel concluded that there is potential concern associated to the current background exposure to MOSH.

- A number of recommendations for generation of further data were also issued:
  - Future monitoring should distinguish between MOSH and MOAH, and between subclasses of MOSH based on carbon numbers and chemical structures.
  - Human relevance of liver microgranulomas observed in rat should be investigated.
  - The potential of MOSH to induce altered immune functions following oral exposure should be investigated.
  - Toxicological evaluation of MOH should focus on the molecular mass range and structural sub-classes, rather than chemico-physical properties such as viscosity.

EFSA FUNDED STUDY ON TOXICITY AND BIOACCUMULATION OF MOSH

Objectives of the EFSA call

- ✓ Study of accumulation and toxicity of a broad MOSH mixture representative of the range to which humans are exposed via the diet
- ✓ Identification of the fraction(s) based on carbon number and chemical structure with higher bioaccumulation potentials
- ✓ Analysis of the correlation between accumulation and formation of hepatic microgranulomas
- ✓ Analysis of the correlation between the changes in immune functions and the formation of microgranulomas
- ✓ Study of the autoimmune response to oral exposure to MOSH

The EFSA grant was awarded by a consortium formed by INRA (coordinator) and NIPH (with KLZH as subcontractor)

Two year project finalised in September 2016, with final report published:
http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2017.EN-1090/epdf (Cravedi et al., 2017)

OUTLINE OF THE STUDY

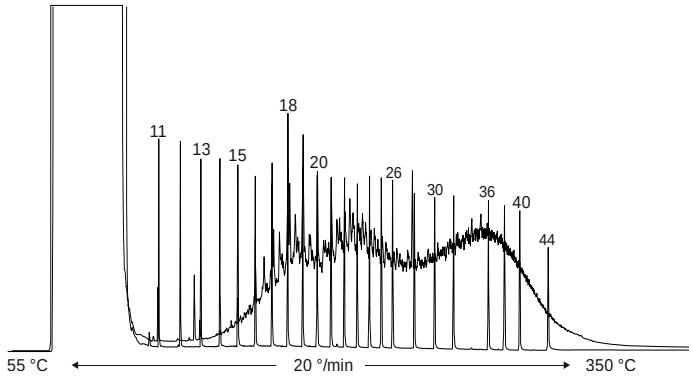
1. Subchronic oral toxicity/bioaccumulation study with broad MOSH mixture in female Fischer 344 rats.

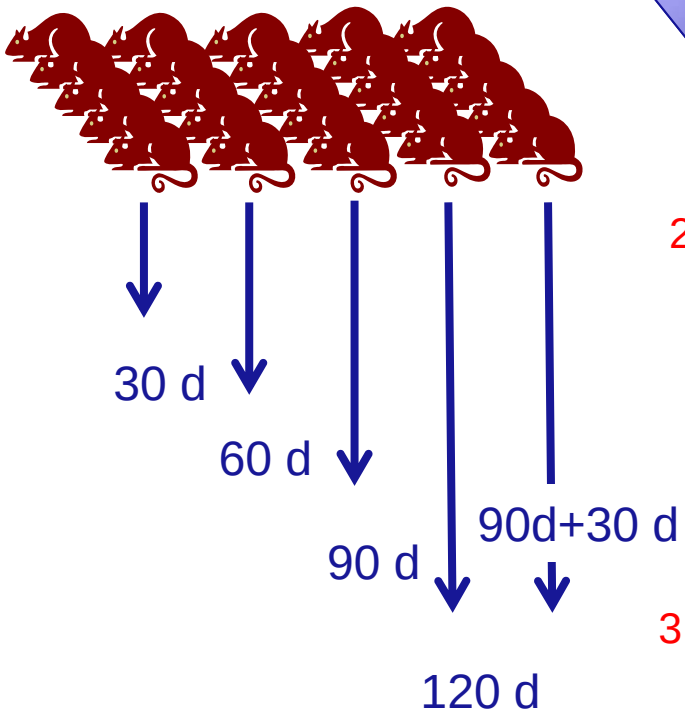
2. Subchronic study of autoimmune response following oral exposure to broad MOSH mixture in dark Agouti rats.

3. Subchronic toxicity/bioaccumulation study with three MOSH fractions in female Fischer 344 rats.

1. SUBCHRONIC STUDY WITH BROAD MOSH MIXTURE

1. A broad MOSH mixture with an approximately constant concentration of hydrocarbons per carbon atom was prepared

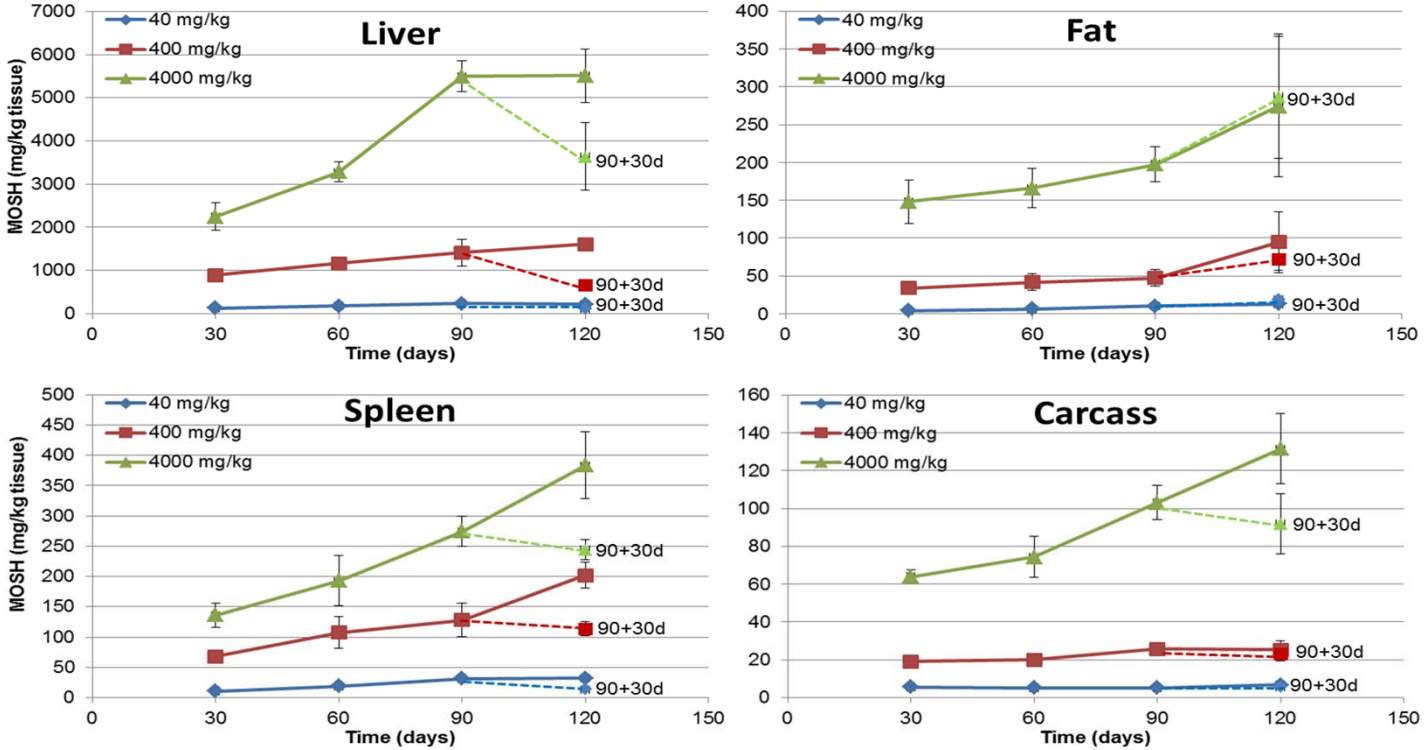




2. Five rats/timepoint/dose exposed up to 120 days to 0, 40, 400 or 4000 mg MOSH/kg feed (approximately 0, 2, 22 or 222 mg/kg bw per day)

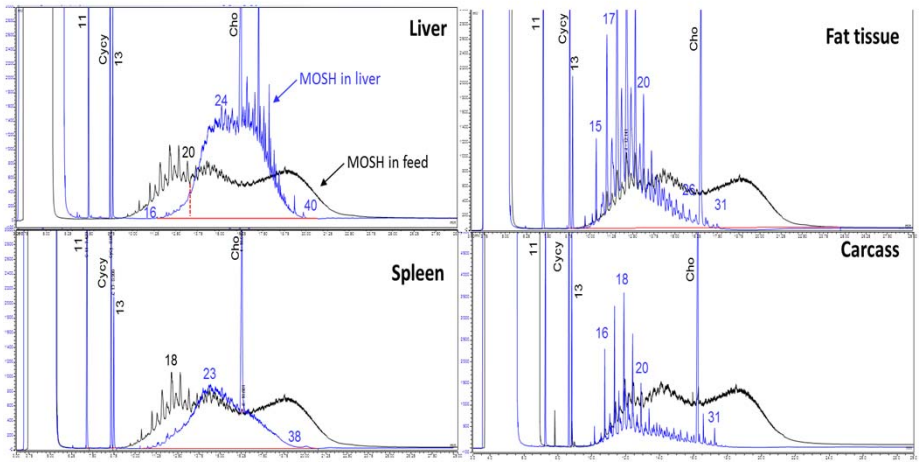
3. - MOSH levels in liver, spleen, adipose tissue and remaining carcass were measured.
- Liver histopathology analysis (incidence of microgranulomas) and immune functions were studied

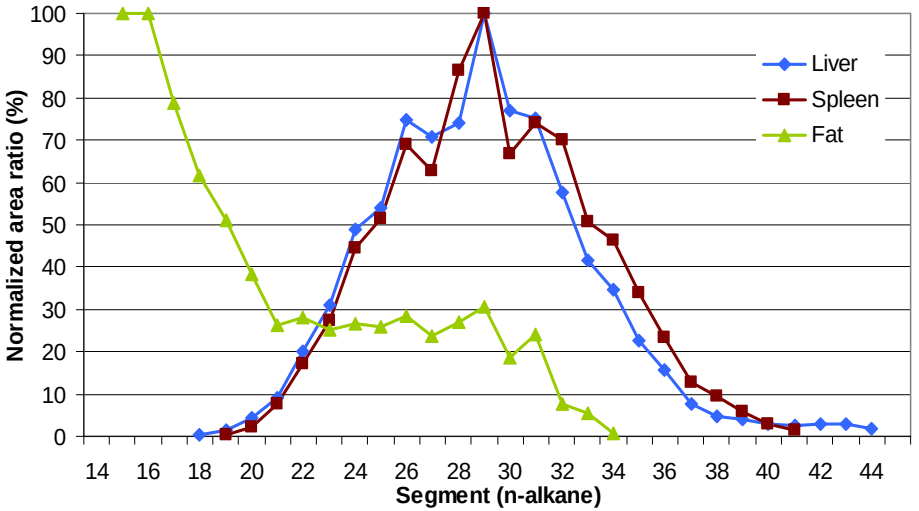
BIOACCUMULATION OF MOSH BROAD MIXTURE



- ✓ Accumulation occurred mainly in the liver
- ✓ Clearance was observed in all tissues but in the adipose tissue following a 30 day recovery period
- ✓ No steady state for tissue bioaccumulation was reached after 120 days
- ✓ Accumulation rate was relatively higher at lower doses (concentrations in the tissues increased by factors 2.6-11.5 in different tissues when going from 400 to 4000 mg/kg)

BIOACCUMULATION OF MOSH BROAD MIXTURE





Accumulation varied both in term of carbon numbers and chemical classes in different tissues

- GCxGC analysis revealed a preferential accumulation of n-alkanes/little branched paraffins with low carbon number in the adipose tissue.
- In the liver, a slightly higher retention was observed for strongly branched (cyclo)alkanes, likely due to the more difficult biotransformation and/or elimination.

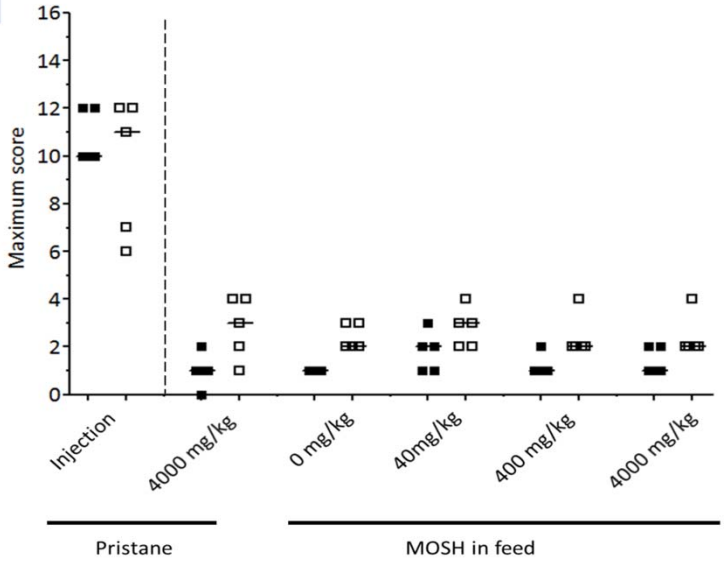
TOXICITY OF MOSH BROAD MIXTURE

- Slight increase in liver weight, mainly at 4000 mg/kg
- Liver microgranulomas observed only at 4000 mg/kg after 90 or 120 days of exposure
- No indication of pro-inflammatory changes or other signs of hepatotoxicity
- No significant changes in immune response were observed, but a decreased antibody production was observed at the highest dose.

2. STUDY OF AUTOIMMUNE RESPONSE IN AGOUTI RATS

Exposure	Route	n (females + males)	Dietary mineral oil exposure mg/kg/b.w./day (median; females + males)		Duration (days)
			Week 2	Week 10	
200 µl Pristane day 0	i.d.	5+5	0+0	0+0	40*
4000 mg Pristane/kg feed	p.o.	5+5	283.3+355.1	260.0+244.5	90
0 mg MOSH/kg feed	p.o.	5+5	0+0	0+0	90
40 mg MOSH/kg feed	p.o.	5+5	3.8+3.3	2.9+2.3	90
400 mg MOSH/kg feed	p.o.	5+5	30.3+30.8	29.4+25.7	90
4000 mg MOSH/kg feed	p.o.	5+5	317.6+309.3	280.1+238.8	90

- Oral exposure to pristane or to the broad MOSH did not result in an increase either in arthritis score, or in a series of arthritis markers in serum
- Intra-dermal injection of pristane induced arthritis symptoms (positive control)[3]



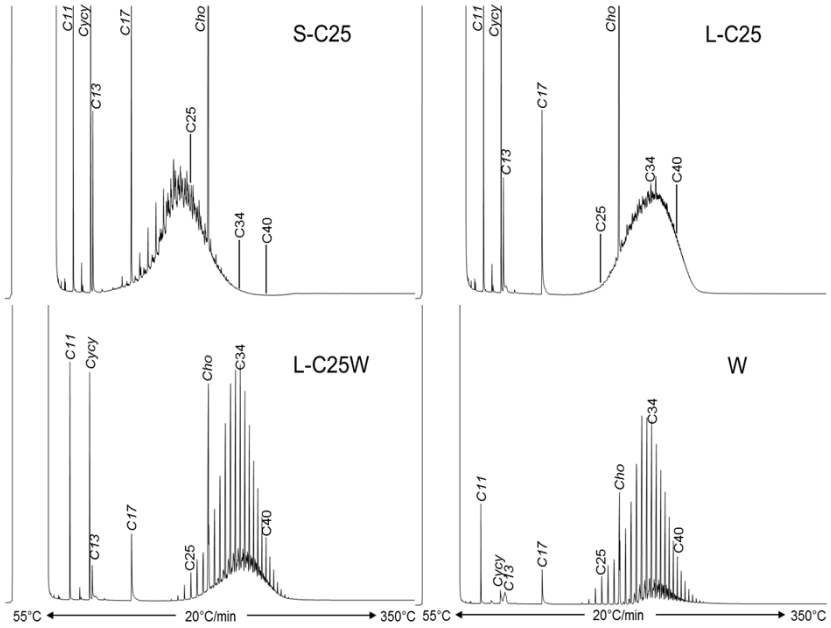
[3]Andreassen et al., 2017

3. SUBCHRONIC STUDY WITH 3 MOSH FRACTIONS

1. Three mixtures were selected to investigate the different accumulation and toxicity at different carbon number distributions and the possible role of n-alkanes:
   - ✓ S-C25: MOSH mainly below C25, lower viscosity
   - ✓ L-C25: MOSH mainly above C25, higher viscosity
   - ✓ L-C25W: 1:1 mixture of L-C25 and a wax (W)

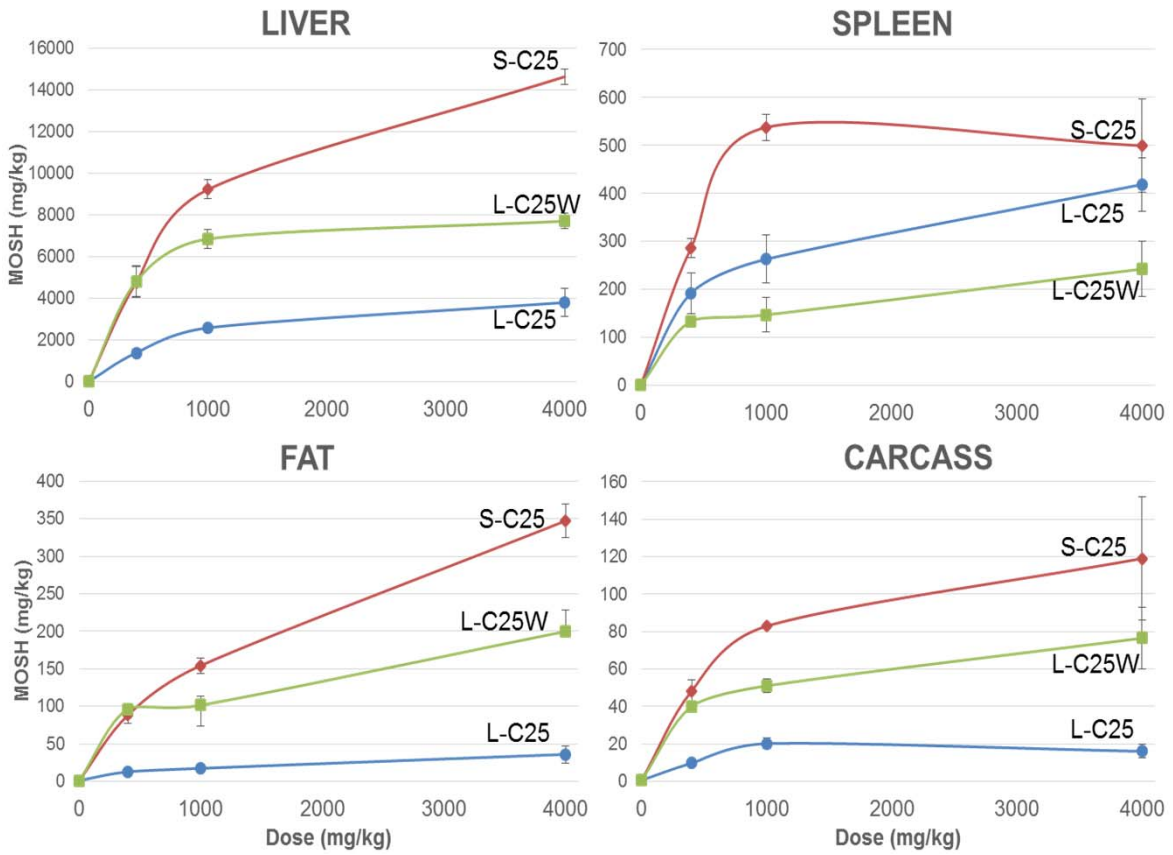
2. Eight rats/group were exposed for 120 days either to 0 or to 40, 1000 or 4000 mg mixture/kg feed

3. - MOSH levels in liver, spleen, adipose tissue and remaining carcass were measured.
   - Liver histopathology analysis and immune functions were studied



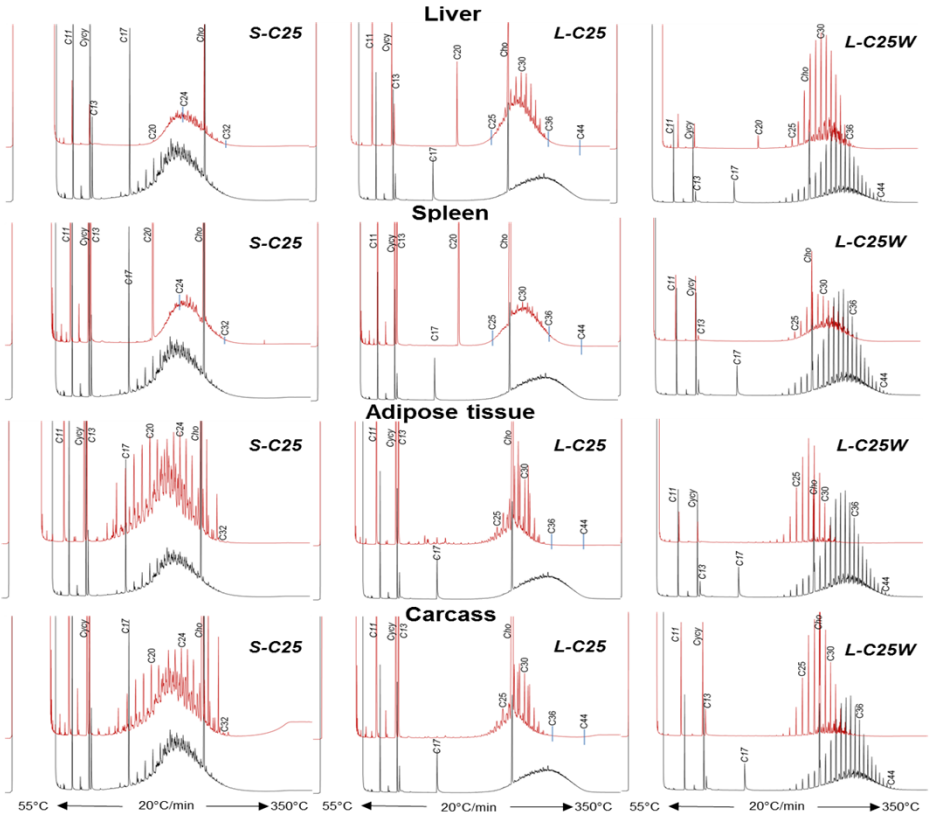
BIOACCUMULATION OF THE 3 MOSH FRACTIONS

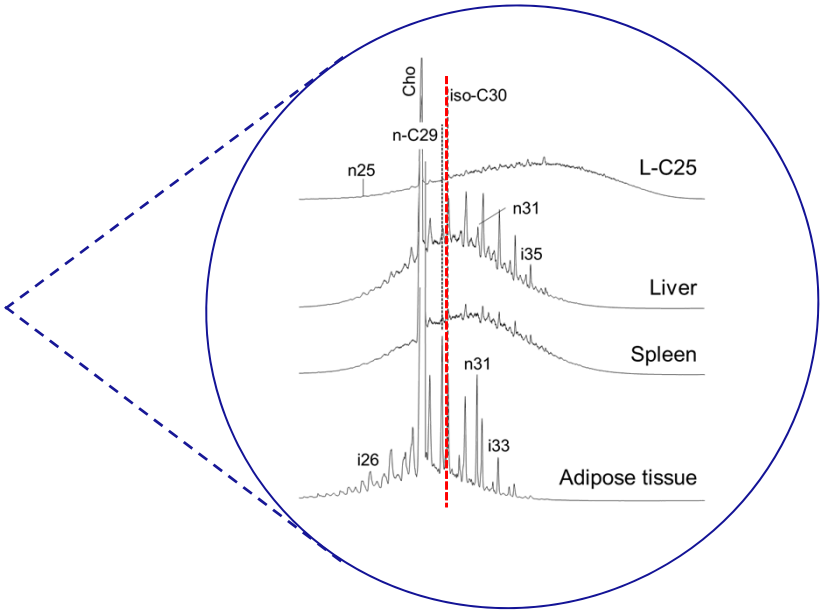
- Tissue accumulation was mixture specific, with higher retention for S-C25 and n-alkanes in the liver and adipose tissue.



SELECTIVE RETENTION IN DIFFERENT TISSUES

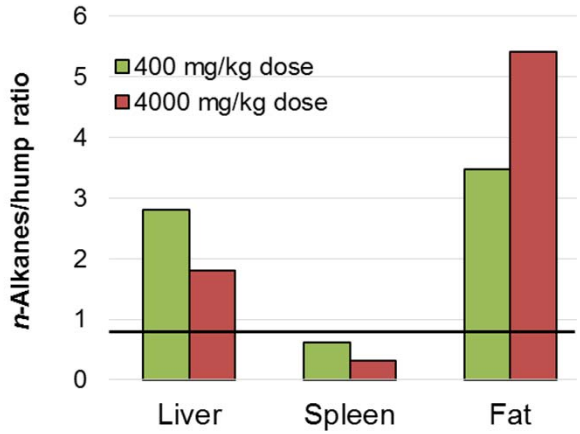
- High retention of n-alkanes in liver and adipose tissue
- Preferential retention of lower C numbers (S-C25) in adipose tissue in comparison to the liver (C-25(W))
- Accumulation in liver centered around C29-C30





BROAD MIXTURE FRACTIONS

n-alkane retention from L-C25W

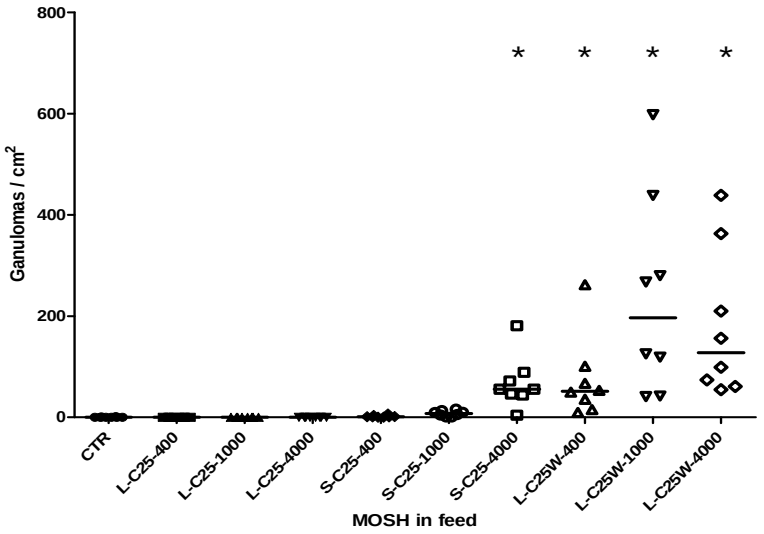


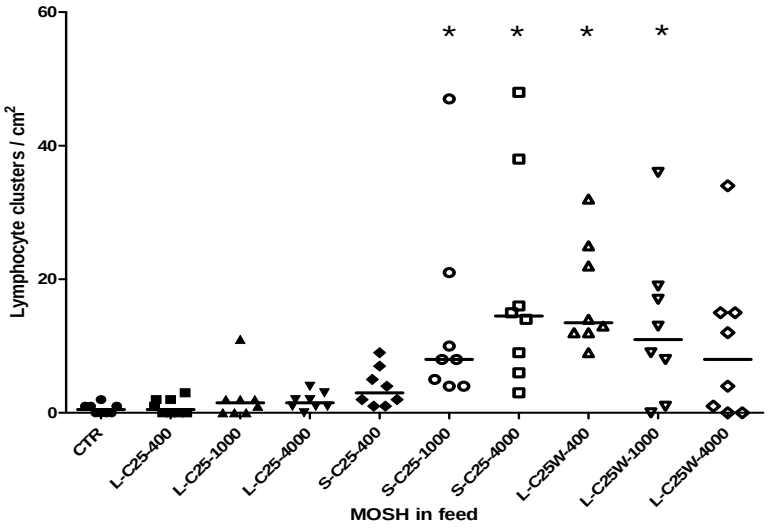
	Retention (%)		
	Liver	Spleen	Adipose tissue
n-C25	0.8	0.04	3.9
n-C27	3.1	0.11	6.8
n-C30	10.8	0.49	2.7
n-C33	7.5	0.89	0.1

- ✓ High retention of linear alkanes
- ✓ Different distributions in liver and adipose tissue
- ✓ Beside n-alkanes:
  - ✓ For S-C25 observed preferential accumulation of multibranched alkanes (and polycyclic MOSH) in liver, n-alkyl monocyclic alkanes in adipose tissue
  - ✓ For L-C25 preferential accumulation of n-alkyl monocyclic alkanes, mainly in liver.

TOXICITY OF BROAD MIXTURE FRACTIONS

- ✓ Increased spleen and liver weights were observed in rats exposed to L-C25 and L-C25W.
- ✓ Hepatic microgranulomas and inflammatory changes were observed at all L-C25W doses and mid/high S-C25 doses.
- ✓ No effects on immune functions were observed.





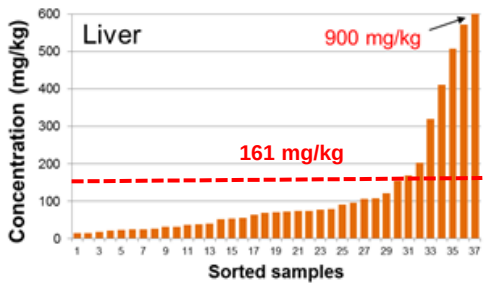
OVERALL CONCLUSIONS

- In female Fischer 344 rats accumulation of MOSH occurs mainly in the liver and to a lesser extent in the adipose tissue.
- Accumulation depends on the composition of the MOSH mixture.
  - ✓ In the liver, the accumulation is higher at C>25, whereas lower C numbers accumulate in the adipose tissue.
  - ✓ n-Alkanes (and unidentified isoalkanes) accumulate preferentially in the adipose tissue, but also in liver.
- MOSH exposure resulted in a significant increase in absolute and relative liver weights.
- Very strong granuloma formation was observed after ingestion of the wax-containing L-C25W mixture, suggesting that n-alkanes have an impact on this toxicological endpoint in Fischer 344 rats. This pattern appeared to be similar for pro-inflammatory changes in the liver (increased lymphoid cell clusters).

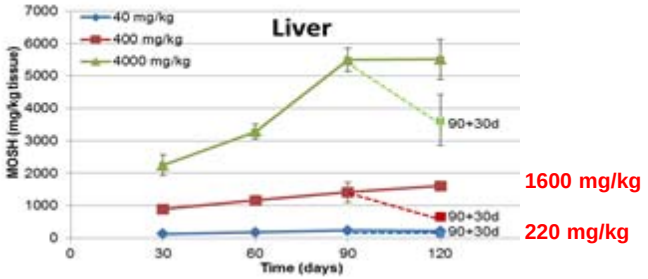
HUMAN RELEVANCE OF MOSH ACCUMULATION?

- MOSH are detected in human liver, MLN, spleen and adipose tissue.
- A recent study in 37 (25-91 years old) subjects showed substantially high levels[4]

Human liver



Rat liver



Estimated human dietary exposure: 0.03-0.3 mg/kg bw per day

Exposure to broad MOSH mixture: 2-22 mg/kg bw per day

Longer retention? Underestimated exposure?

- Different sites of accumulation:

  95% MOSH accumulate in human adipose tissue ⟺ 50% accumulation in Fischer rat liver

- Different retention profiles

  n-Alkanes do not appear to accumulate substantially in human liver and adipose tissue.

[4]Barp et al., 2014, 2015, 2017(a,b).

HUMAN RELEVANCE OF LIVER MICROGRANULOMAS?

- Lipogranulomas containing MOH have been observed in liver and spleen in a series of epidemiological studies, at higher incidence between the ’70s and the end of the ’80s[5].
- In most cases the correlation with dietary exposure to MOH was not clear or not confirmed.
- Lipogranulomas were generally not associated with signs of inflammation or other pathological effects or clinical abnormalities[6].
- Severe liver lesions were observed in cases of intoxication in which the extension of lipogranulomas affected the liver architecture[7].
- The current incidence of hepatic granulomas in human is likely <<4% [8].

[5]Boinott and Margolis, 1970; Dincsoy et al., 1982; Cruickshank et al., 1984.
[6]Carlton et al., 2001.
[7]Trivalle et al., 1991.
[8]Lagana et al., 2010.

OVERALL POINTS OF DISCUSSION FOR MOSH IN FOOD

Scientific debate should take place on the following aspects:

- Relevance of classification of MOSH based on viscosity and carbon numbers.
- Relevance of Fischer rats as a laboratory model to test MOSH.
- Relevance of human bioaccumulation as a toxicological endpoint.
- Scientific basis of the currently established ADIs.

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