Recent advances in accumulation and effects of MOSH in rats – an overview

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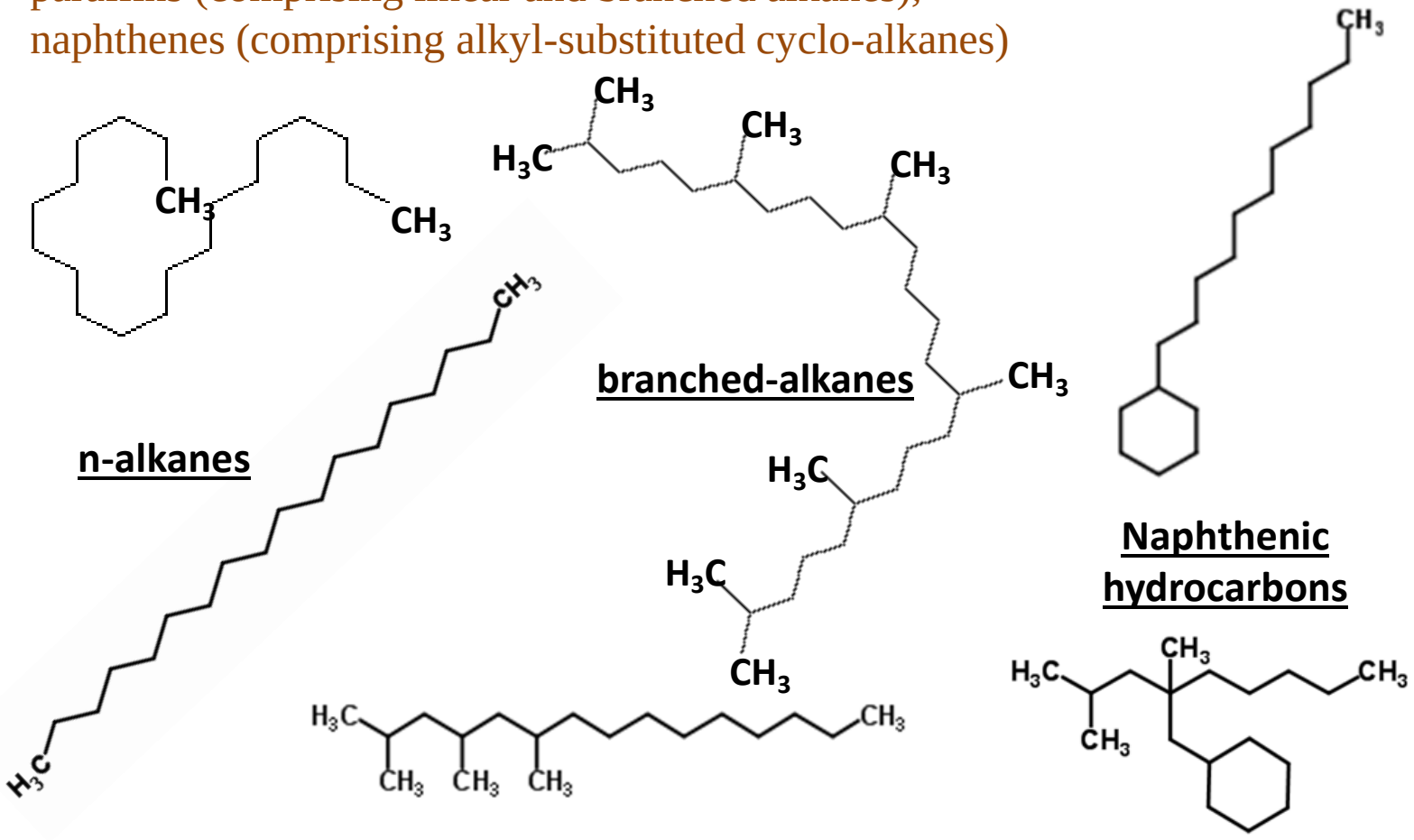
What are mineral oil hydrocarbons (MOHs) ?

- Mineral oils are a complex mixture of hydrocarbons (> 100 000 compounds) derived from petroleum.
- Technical grade mineral oil hydrocarbons typically contain 15-35 % aromatics (MOAH), the rest being almost exclusively constituted of mineral oil saturated hydrocarbons (MOSH).
- Food grade mineral oil hydrocarbon products are treated in such a way that the aromatic content is minimized.
- Little is known about the composition of commercially available products, as specifications are generally expressed in terms of physico-chemical properties (such as viscosity). Products with the same physico-chemical specification may vary considerably in their composition, depending on the source of the oil and its processing.

What are mineral oil saturated hydrocarbons (MOSH) ?

- MOSH consist of two major classes of compounds:
- paraffins (comprising linear and branched alkanes),
- naphthenes (comprising alkyl-substituted cyclo-alkanes)



- For MOSH found in food, the number of carbon atoms typically ranges from 12 to 40

Sources of MOSH in food

- **MOSH of natural origin**
  - e.g. n-alkanes from algae, plants and insects (with a predominance of odd number of carbon atoms

- **Environmental contamination**
  - e.g. oil spills in marine and freshwater ecosystems, pesticide formulations

- **Food processing**
  - e.g. release agents in bakery, dust suppressant for grain, protective coating for raw fruits

- **MOSH migrating from food contact materials**
  - Jute and sisal bags
  - Waxed packaging materials
  - Plastic materials
  - Lubricating oils for cans
  - Printing inks
  - Recycled board
  - Adhesives.

MOSH toxicological targets

- MOSH have low acute oral toxicity
- MOSH at high dose can cause moderate liver hypertrophy
- MOSH having carbon number between 16 and 35 may accumulate in different tissues including adipose tissue, lymph nodes, spleen and liver.
- In rats, bioaccumulation of MOSH can lead to formation of microgranulomas in liver and mesenteric lymph nodes (MLN). Microgranulomas in MLN are considered of low toxicological concern because they do not progress to adverse lesions. In contrast, liver microgranulomas are associated with inflammatory reactions.
- Food grade MOSH may differ in their bioaccumulation potential and in their ability to cause liver granulomas.
- There is evidence from studies in experimental animals that at least some saturated hydrocarbons are able to induce autoimmune responses when administered at high concentrations via a parenteral route.

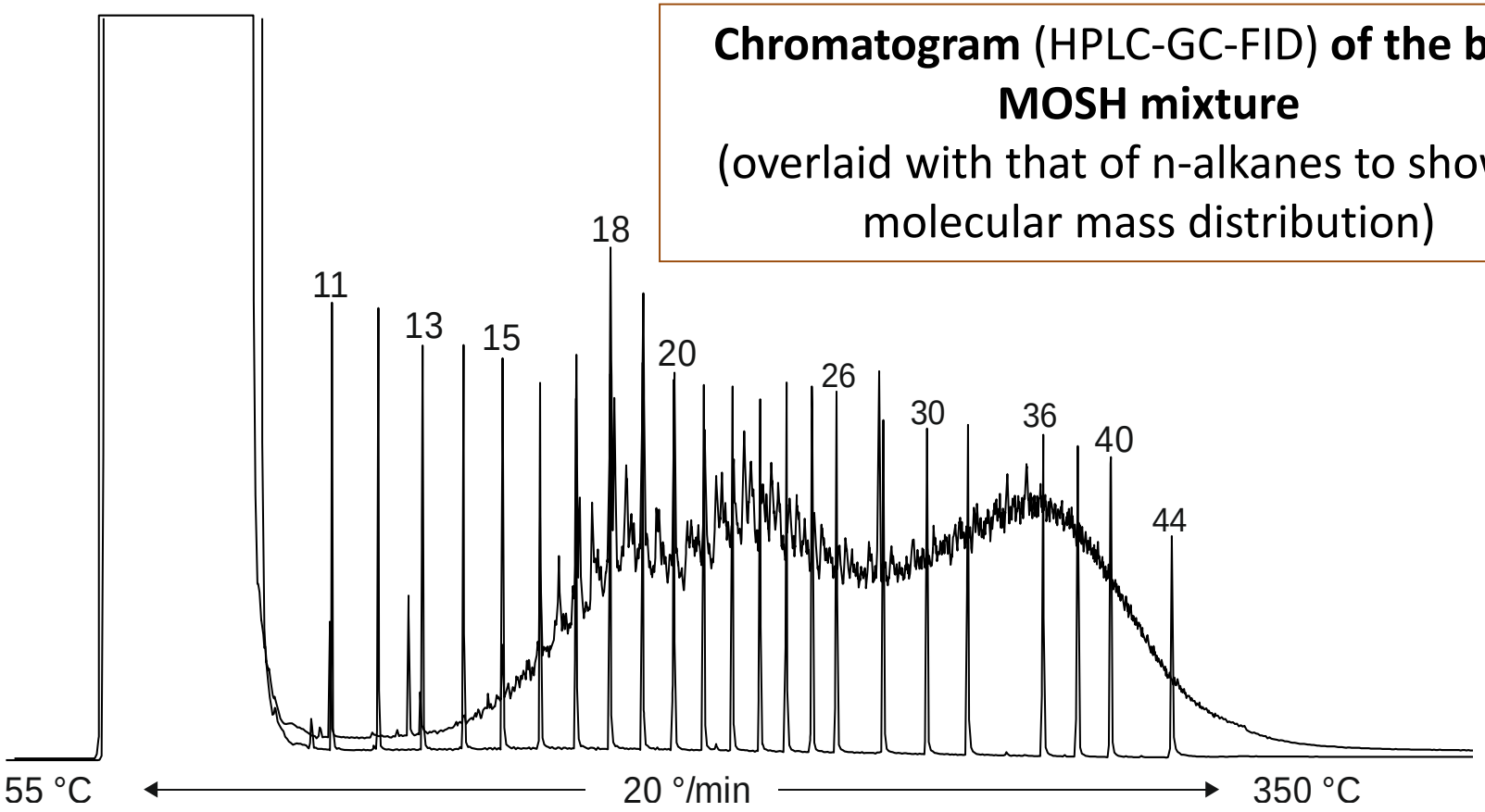
A series of studies supported by EFSA

- To investigate the accumulation and toxicity of a broad MOSH mixture representative of the whole MOSH range to which humans are exposed via the diet.
- To identify the MOSH fractions and sub-categories with higher bioaccumulation potentials.
- To analyse the correlation between accumulation and formation of hepatic microgranulomas.
- To study the potential immune perturbation of MOSH mixtures

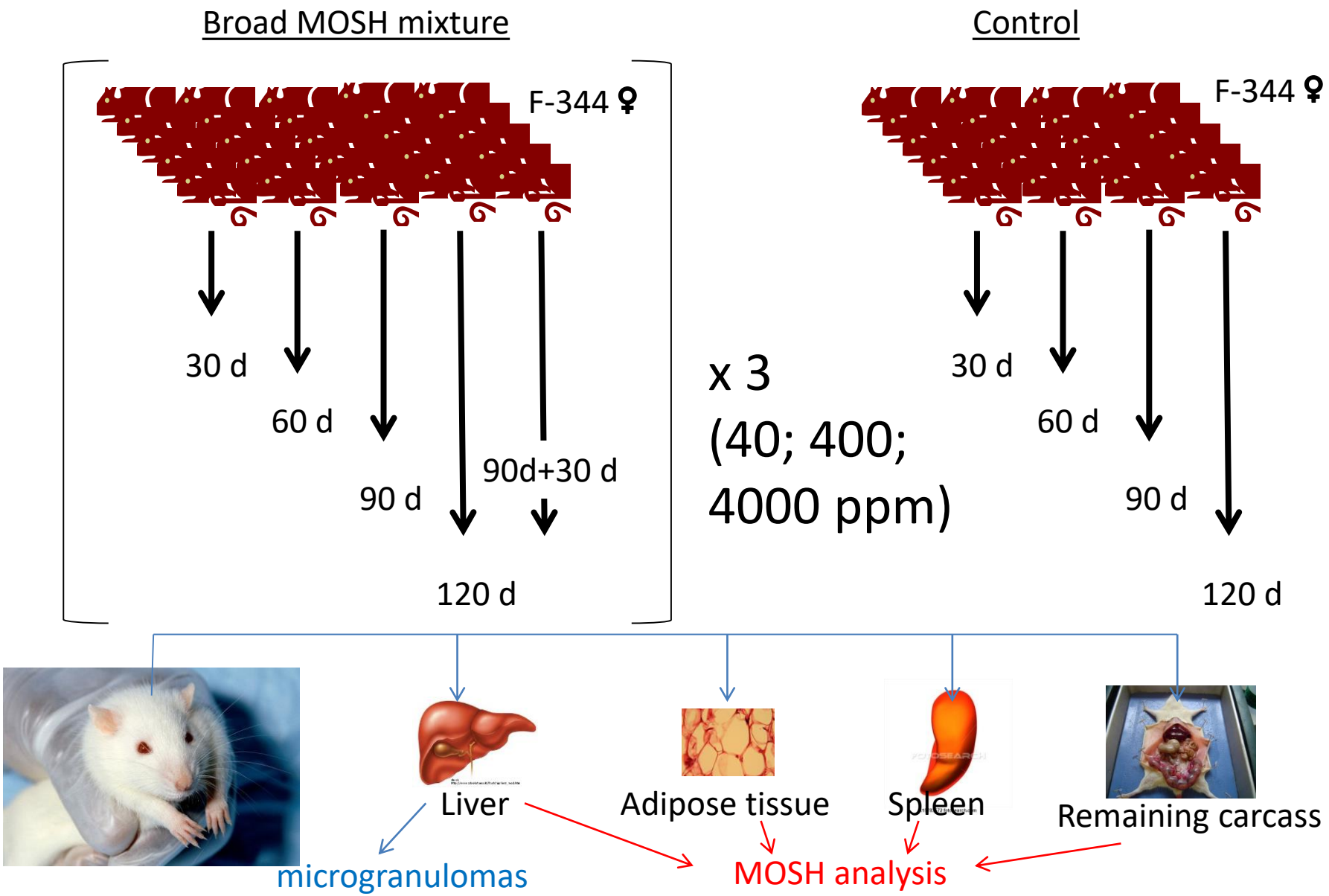
Preparation of a broad MOSH mixture representative of the whole MOSH range to which humans are exposed.

A MOSH mixture with an approximately constant concentration of hydrocarbons per carbon atom was prepared by combining several white oils.

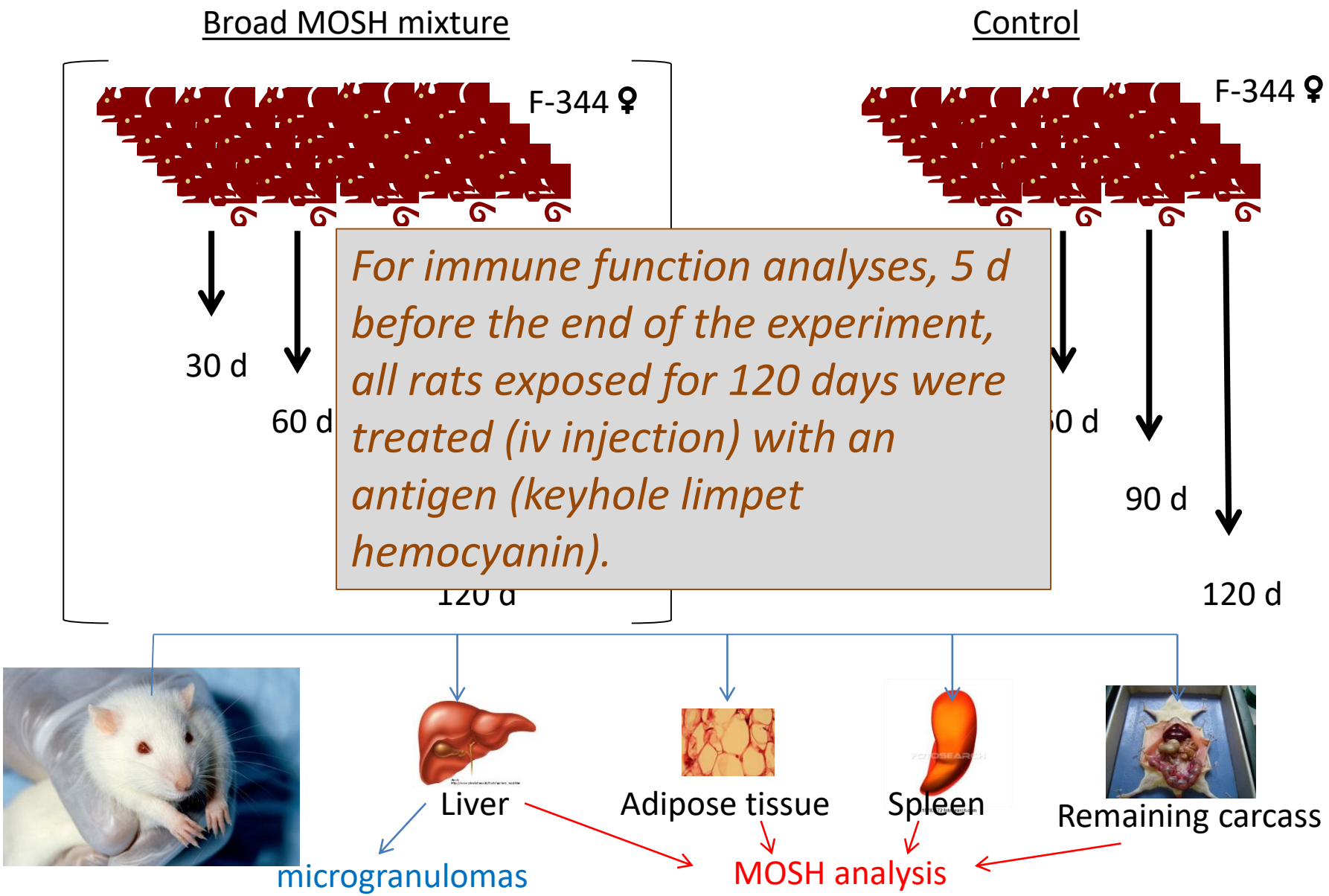
Chromatogram (HPLC-GC-FID) **of the broad MOSH mixture**
(overlaid with that of n-alkanes to show the molecular mass distribution)



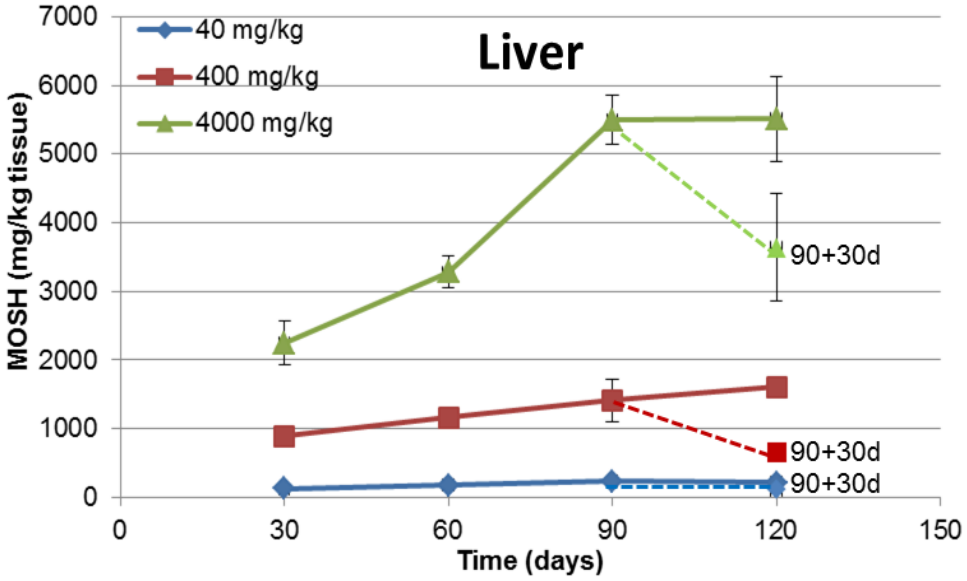
Experiment I : Broad MOSH mixture

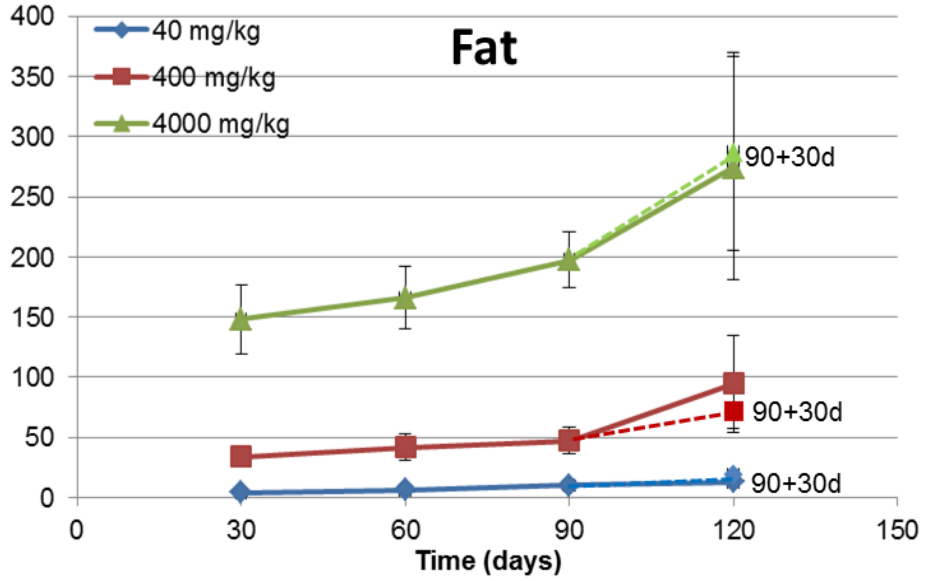


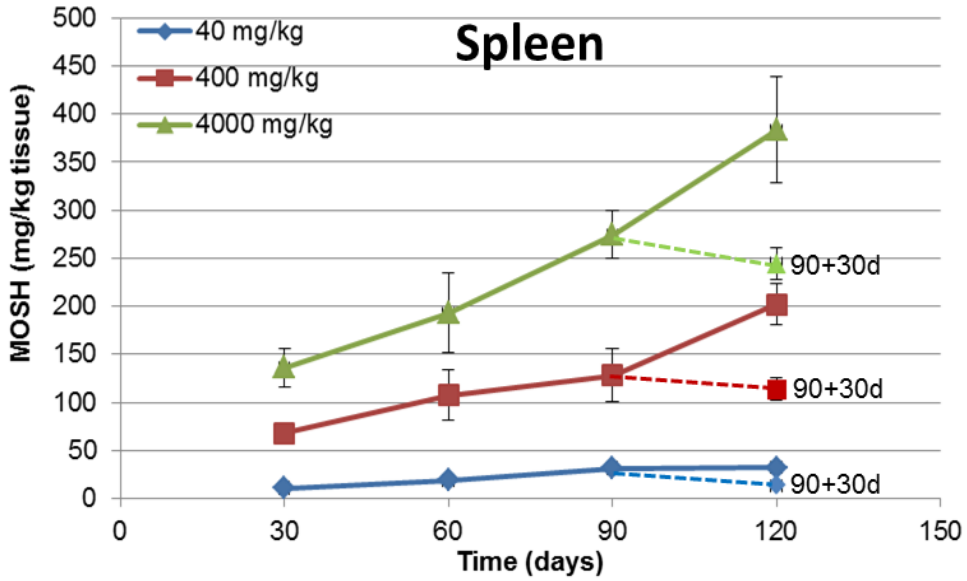
Experiment I : Broad MOSH mixture

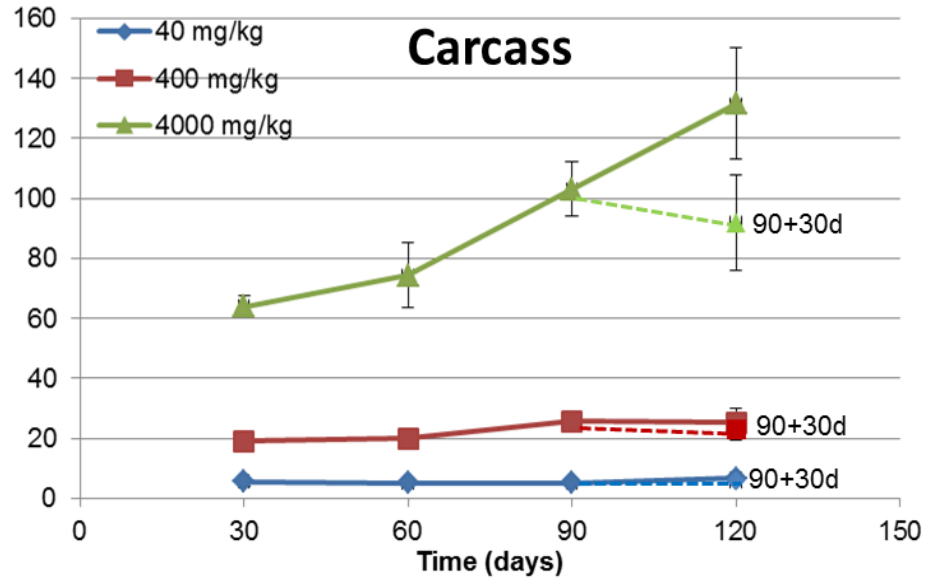


MOSH concentrations (mg/kg) in tissues versus time.



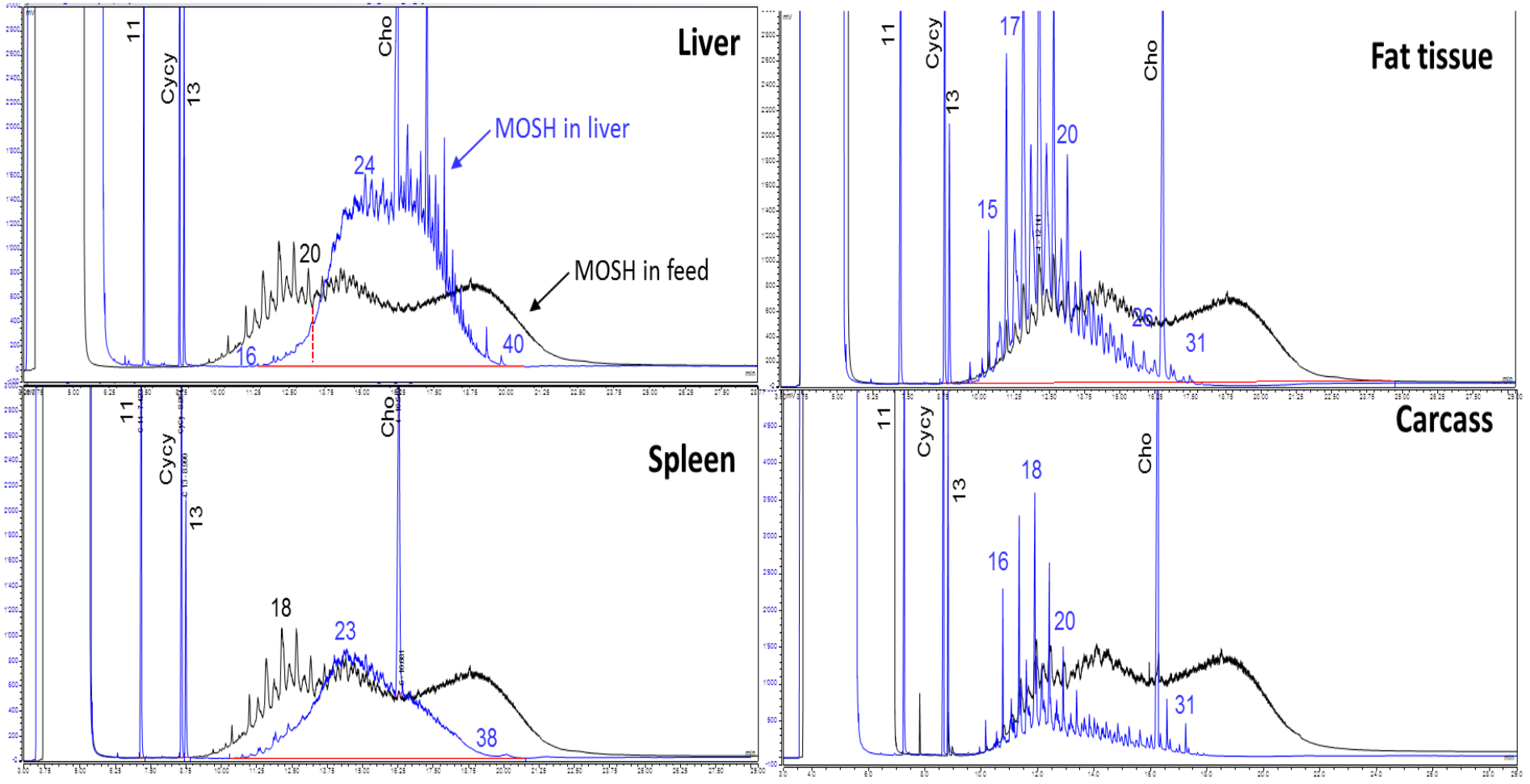






When the dose increased from 40 to 4000 mg/kg, the concentrations in the tissues increased by factors of 2.6-11.5 rather than by a factor of 100

HPLC-GC-FID chromatograms of MOSH in liver, adipose tissue, spleen and carcass.

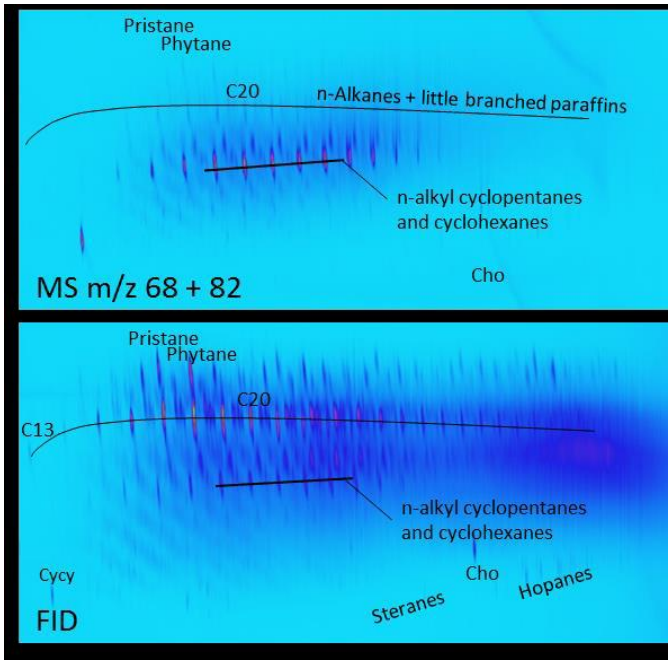


- Maximum retention in liver and spleen at C29
- Maximum retention in adipose tissue: ≤C16

MOSH composition in broad classes

- The broad MOSH mixture consisted of :
- 31% n-alkanes + little branched paraffins,
- 9.9% multibranched paraffins,
- 59% naphthenes.

- There was no significant change in this composition in liver and spleen, but a significant shift to the open chain hydrocarbons in the adipose tissue.



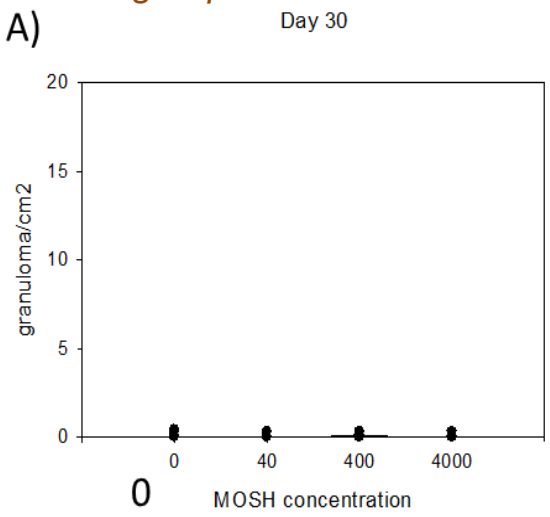
GCxGC plots of the broad MOSH mixture added to the feed

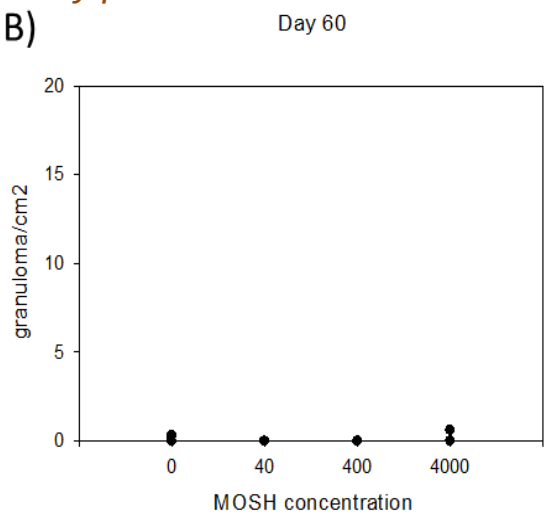
Effect of the broad MOSH mixture on bw, spleen and liver weights

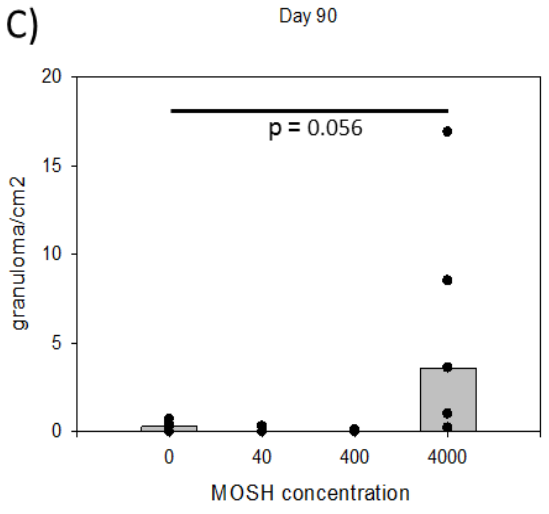
Exposure Period (d)	Dose (mg/kg)	Body weight	Liver	Spleen	HSI
	0	137.0 ± 5.34	5.69 ± 0.28	0.44 ± 0.013	0.0415 ± 0.0011
30	40	137.2 ± 8.81	5.73 ± 0.53	0.45 ± 0.029	0.0417 ± 0.0018
	400	141.4 ± 8.26	6.27 ± 0.34	0.48 ± 0.019	0.0444 ± 0.0023
	4000	142.8 ± 3.96	6.71 ± 0.25**	0.48 ± 0.014	0.0470 ± 0.0026**
	0	177.6 ± 5.03	6.57 ± 0.34	0.54 ± 0.042	0.0370 ± 0.0011
60	40	177.2 ± 6.50	6.23 ± 0.52	0.55 ± 0.019	0.0352 ± 0.0029
	400	173.0 ± 11.62	6.20 ± 0.46	0.54 ± 0.036	0.0358 ± 0.0017
	4000	174.8 ± 5.63	6.75 ± 0.35	0.59 ± 0.041	0.0386 ± 0.0013
	0	178.0 ± 23.56	5.79 ± 0.79	0.55 ± 0.053	0.0325 ± 0.0010
90	40	196.4 ± 9.56	6.55 ± 0.25	0.58 ± 0.040	0.0334 ± 0.0010
	400	193.0 ± 11.60	6.78 ± 0.47*	0.61 ± 0.054	0.0351 ± 0.0014*
	4000	190.6 ± 9.45	6.86 ± 0.11**	0.62 ± 0.044	0.0361 ± 0.0022**
	0	194.0 ± 13.73	6.39 ± 0.81	0.57 ± 0.043	0.0328 ± 0.0018
120	40	199.6 ± 10.99	6.36 ± 0.42	0.55 ± 0.042	0.0319 ± 0.0008
	400	196.0 ± 15.35	6.42 ± 0.65	0.65 ± 0.111	0.0328 ± 0.0016
	4000	206.6 ± 10.62	7.48 ± 0.46*	0.69 ± 0.060	0.0362 ± 0.0009**
	0	203.4 ± 20.71	6.92 ± 0.77	0.59 ± 0.072	0.0340 ± 0.0012
90+30	40	192.6 ± 5.13	6.40 ± 0.43	0.56 ± 0.036	0.0332 ± 0.0014
	400	192.6 ± 13.54	6.78 ± 0.79	0.59 ± 0.052	0.0351 ± 0.0018
	4000	188.6 ± 5.98	6.50 ± 0.32	0.64 ± 0.056	0.0345 ± 0.0012

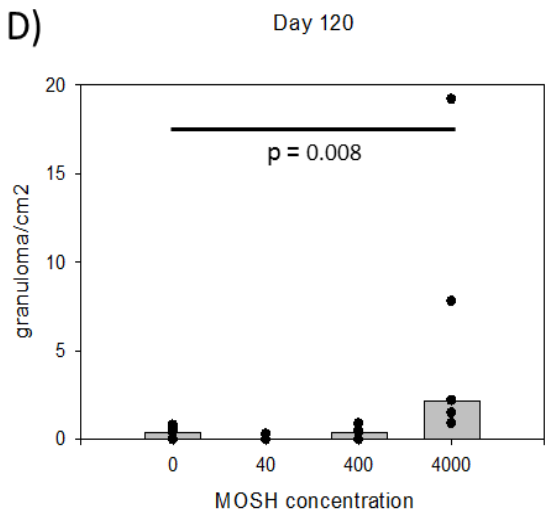
Granulomas/cm² in livers from rats fed for 30 (A), 60 (B), 90 (C), 120 (D) days with control feed or feed containing MOSH mixture 40, 400 and 4000 mg/kg, or for 90 d with the test feed + 30 d of control feed (E).

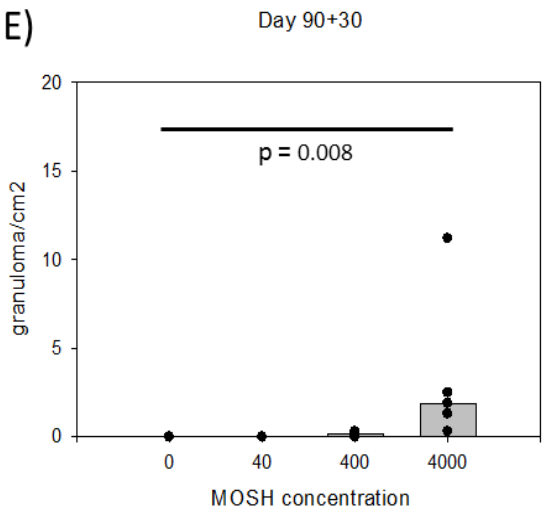
The dots represent the value for the individual animals while the bars represent the group median value. Significant group differences are denoted by p-values.

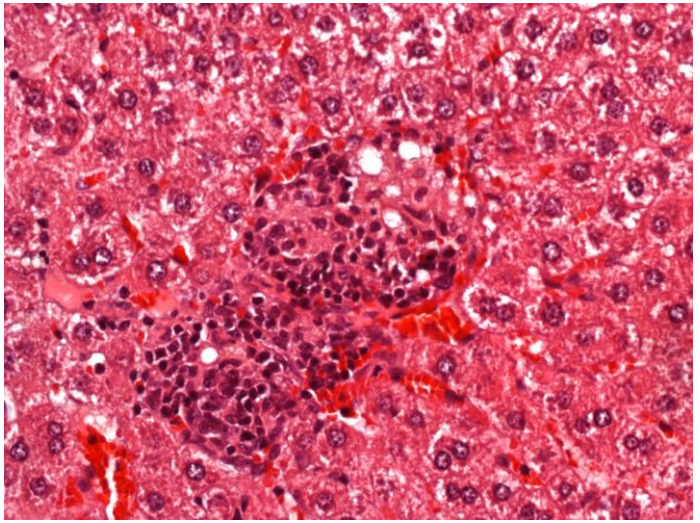










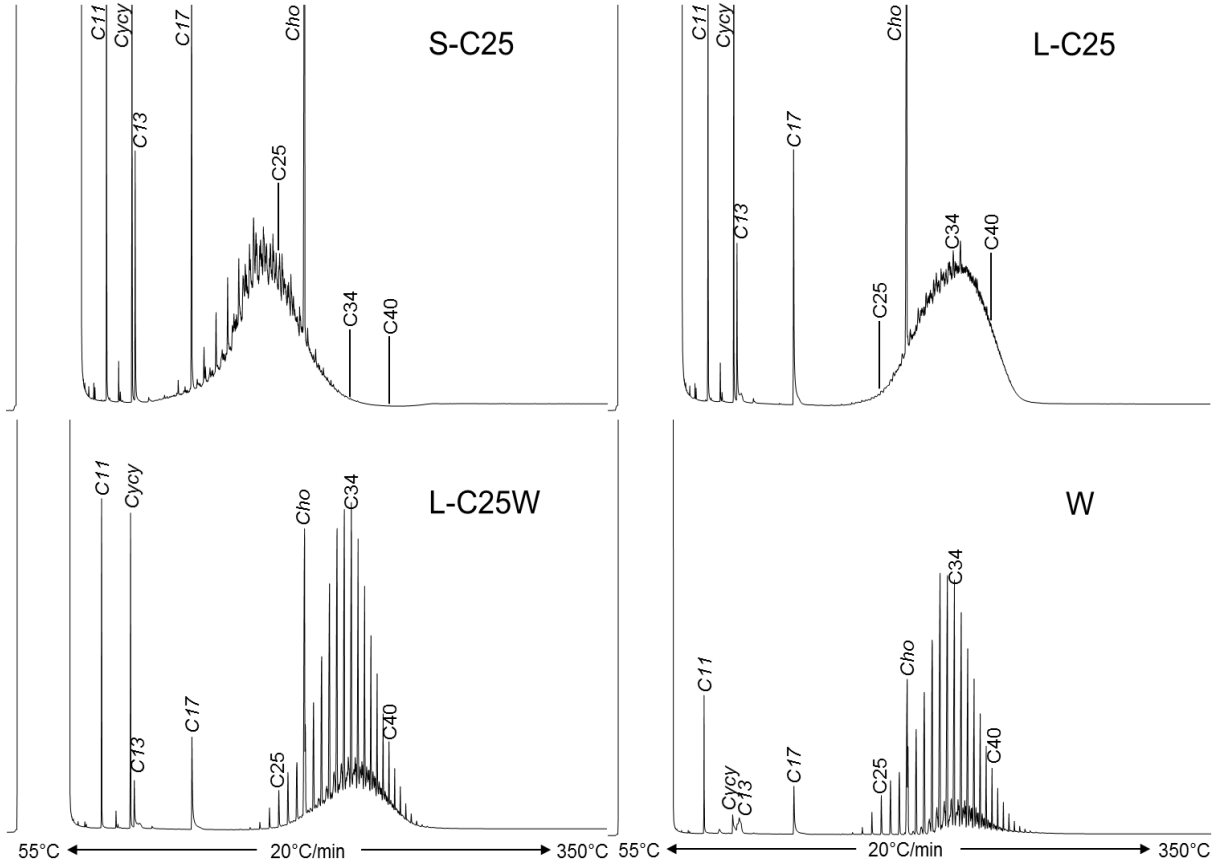


Effects of the exposure to MOSH broad mixture on other targets (liver, immune system)

- The increase in granuloma formation at the highest dose appeared to be accompanied by increased number of lymphoid clusters in the liver parenchyma,
- No significant changes due to MOSH exposure were observed for the KLH-specific IgM concentrations in serum.

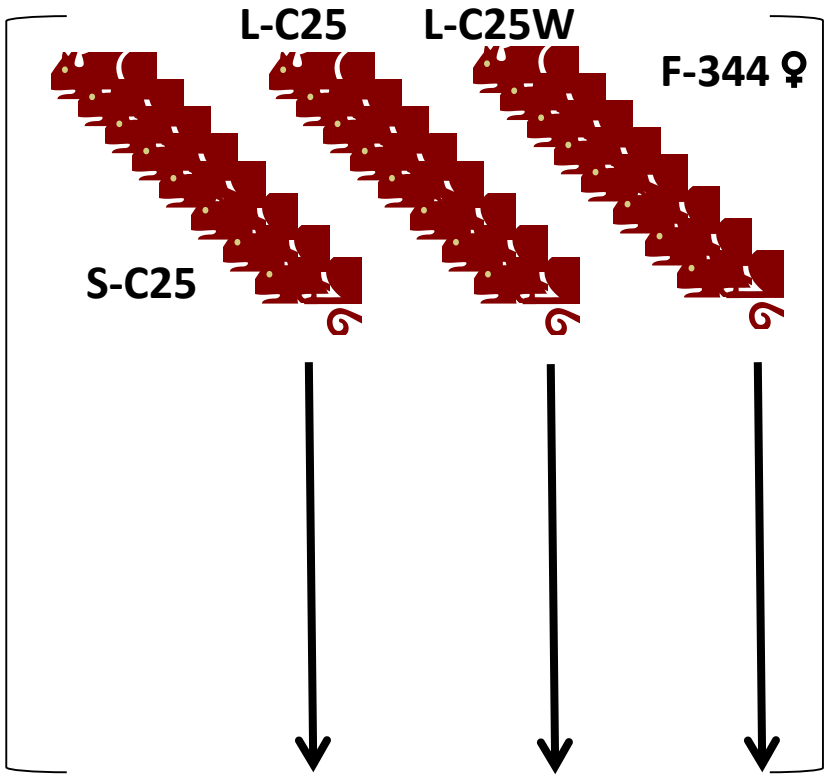
Preparation of 3 MOSH mixtures

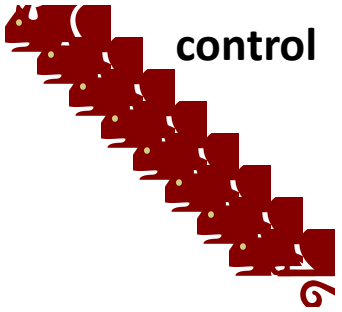
Rationale : to distinguish MOSH below (S-C25) and above C25 (L-C25) and to check whether not accumulated hydrocarbons (in particular n-alkanes) have an impact on granuloma formation, using mixtures having the same composition in terms of branching and cyclic constituents as in the previous experiment.



- S-C25: Fluka Paraffin wax Ph Eur, low viscosity, 76233
- L-C25: Fluka Paraffin wax Ph Eur, high viscosity, 76234
- L-C25W: 1:1 (*w*:*w*) mixture of L-C25 and wax,411663 (Sigma-Aldrich) (mp ≥65°C)

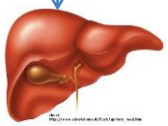
Experiment II: « narrow » MOSH mixture



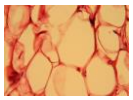


x 3
(400; 1000;
4000 ppm)

120 d 120 d 120 d 120 d



Liver



Adipose tissue

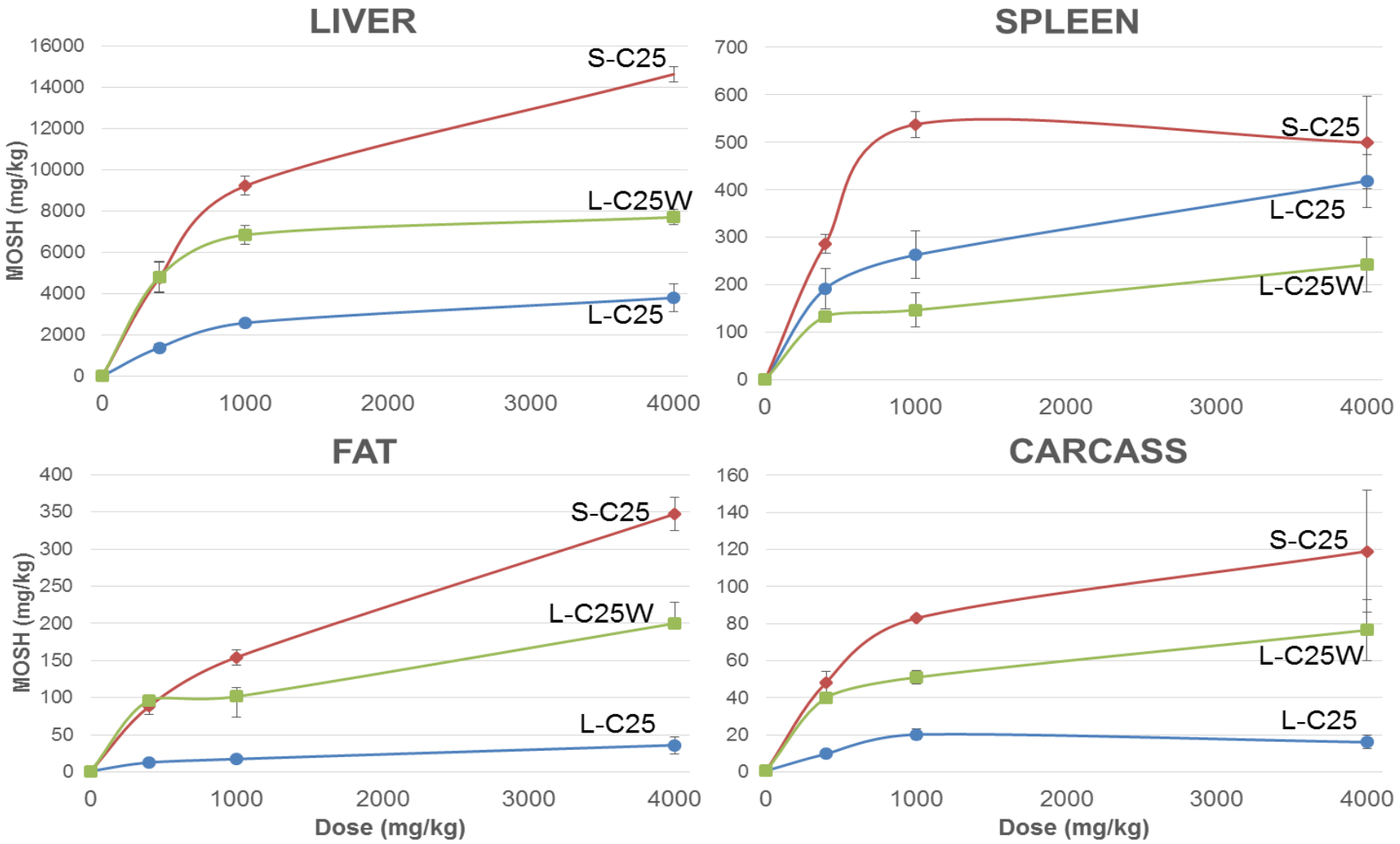
Spleen

Remaining carcass

microgranulomas

MOSH analysis

MOSH concentrations (mg/kg) in tissues and carcass



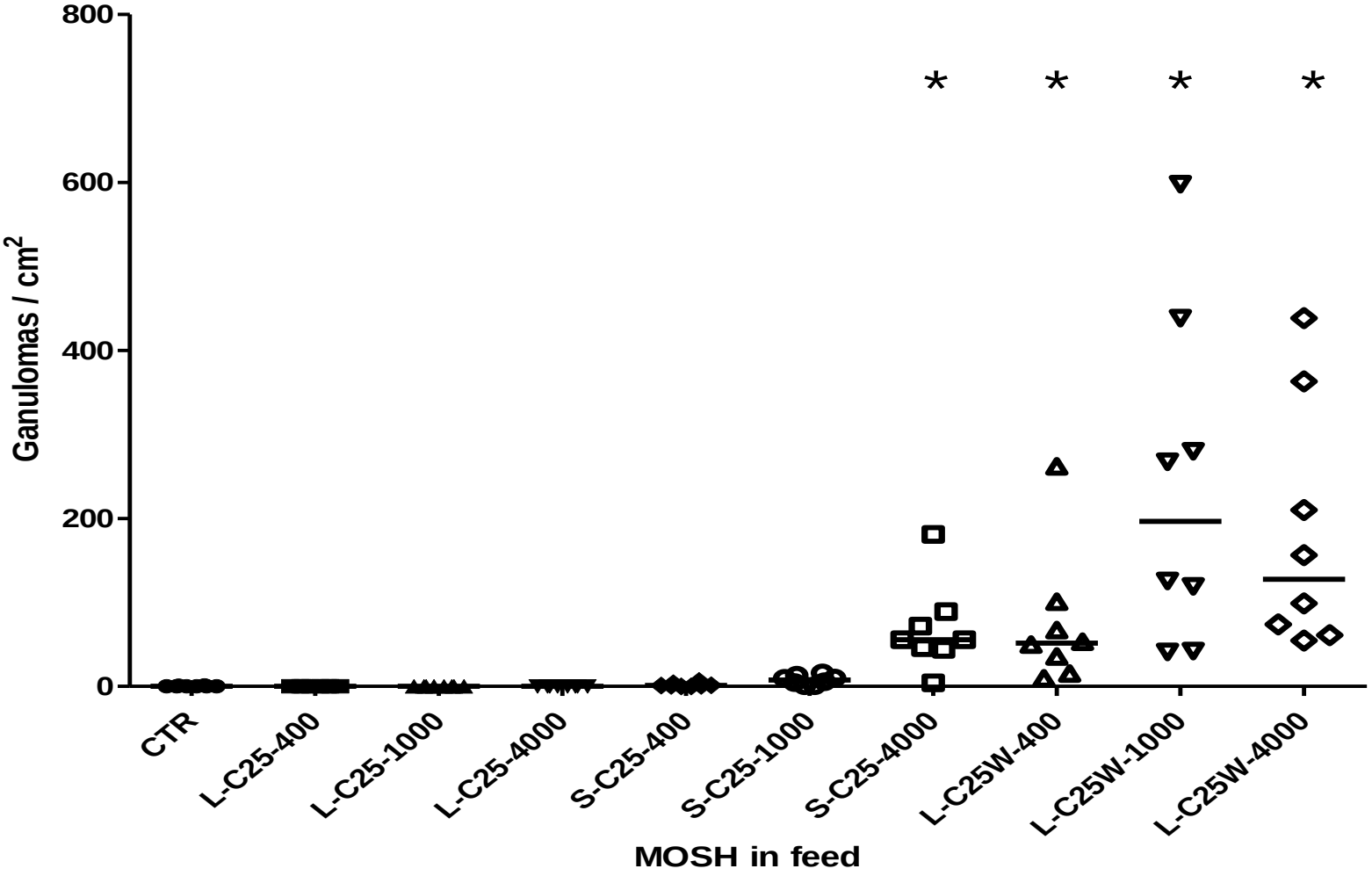
Effect of MOSH on the weight of animals and organs

Values are in grams and are means from 8 animals ± SD; HSI = hepatosomatic index (liver/body weight ratio).
*= significantly different from control ($p<0.05$); ** = significantly different from control ($p<0.01$);
• *** = significantly different from control ($p<0.001$)

Groups	Dose (mg/kg)	Body weight	Liver	Spleen	HSI
Control	0	211.3 ± 6.78	6.76 ± 0.38	0.62 ± 0.03	0.0320 ± 0.0019
S-C25	400	208.8 ± 14.57	6.82 ± 0.80	0.63 ± 0.04	0.0325 ± 0.0019
	1000	210.5 ± 13.20	6.91 ± 0.90	0.64 ± 0.03	0.0327 ± 0.0026
	4000	205.1 ± 9.16	6.87 ± 0.70	0.64 ± 0.05	0.0335 ± 0.0025
L-C25	400	213.0 ± 13.89	7.43 ± 0.49	0.70 ± 0.05**	0.0349 ± 0.0012**
	1000	208.9 ± 10.71	7.86 ± 0.60	0.87 ± 0.15***	0.0376 ± 0.0020***
	4000	211.3 ± 13.16	8.43 ± 0.70**	1.17 ± 0.11***	0.0399 ± 0.0015***
L-C25W	400	203.3 ± 9.41	8.10 ± 1.14*	1.07 ± 0.35**	0.0399 ± 0.0063**
	1000	207.3 ± 9.00	9.05 ± 1.66***	1.22 ± 0.20***	0.0437 ± 0.0082**
	4000	209.8 ± 4.95	9.09 ± 1.29***	1.25 ± 0.23***	0.0433 ± 0.0060***

Granulomas/cm² in livers from rats exposed to various MOSH mixtures

(*dietary exposure to 400, 1000 and 4000 mg/kg feed for 120 days)*. The dots represent the value for individual animals while the lines represent the group median value. Significant differences between controls and exposed rats are denoted by * ($p<0.05$).



Effects of the exposure to various MOSH mixtures on other targets (liver, immune system)

- The numbers of lymphoid cell clusters in the liver parenchyma and in the liver portal tract were not affected by any dose of L-C25, but were significantly increased for the highest dose of the S-C25 mixture.
- For the L-C25W mixture a significant increase of the numbers of lymphoid cell clusters in the parenchyma was observed at low and medium doses, whereas the effect was significant only at the highest dose in the portal tract
- Irrespective to the mixture tested, no significant differences were observed for the KLH-specific IgM concentrations in serum.

Conclusions (I)

- Accumulation of MOSH occurs mainly in the liver and to a lesser extent in adipose tissue.
- Accumulation depends on the composition of the MOSH mixture.
- Strong differences exist between liver and adipose tissue in terms of accumulation profile: whereas in adipose tissue the accumulated fraction corresponds to the most volatile part of the administered mixture, in the liver, the most volatile as well as the highest boiling part of the mixture are almost absent.
- The depuration period results in a significant decrease of the MOSH concentration in the liver, but not in the adipose tissue.
- Not only n-alkanes, but also iso-alkanes accumulate in liver and adipose tissue

Conclusions (II)

- As previously shown MOSH exposure results in a significant increase in absolute and relative liver weight.
- There were large differences in the ability of the different MOSH mixtures to induce liver granulomas.
- 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.
- This pattern appeared to be similar for the lymphoid cell clusters in the parenchyma and the portal tract.
- No significant differences were observed for the KLH-specific IgM concentrations in serum due to MOSH exposure.
- The classification of MOSH by molecular masses below and above n-C25 (with high tolerance for those above n-C25) is not supported by the present findings.

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