Hydrocarbon Waxes:
The ”MOSH” that are no “MOSH”

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Natural n-Alkanes

"Cuticular wax" coats external surfaces of most fruits and vegetables

Composition: n-Alkanes, wax esters, fatty acids, ketones, fatty alcohols

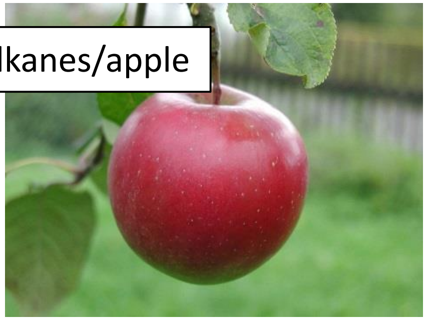
Typically carbon chain length 29 to 31 can be up to 50%

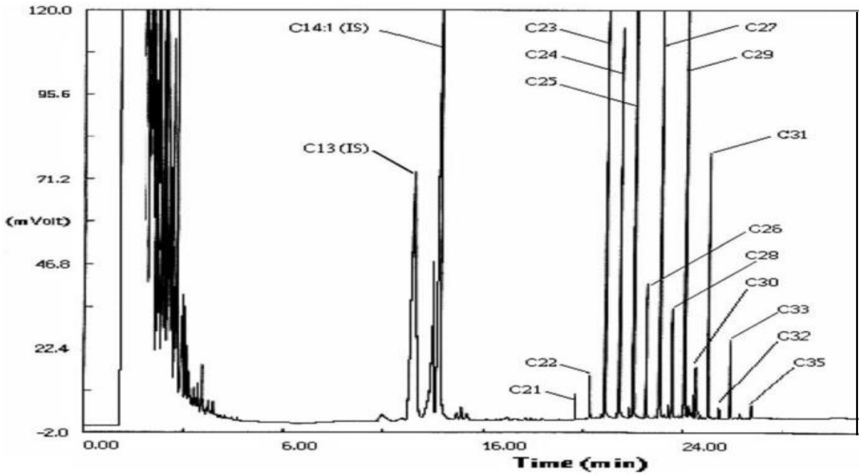
Odd carbon number compounds dominate

Number of samples	*Edible oil*	n-*alkane* (Σ_{15}^{33}) *content*[a] *(mg kg^{-1} oil)*
6	Olive and extra virgin olive	28–99
5	Sunflower	105–166
4	Sesame	22–82
4	Vegetable	62–96
3	Corn	26–33
3	Walnut	7–30
3	Peanut/groundnut	27–40
1	Hazelnut	14
1	Sweet almond	44
1	Pistachio	21
1	Mustard seed	74
1	Safflower	61
1	Grapeseed	52
1	Olive and sunflower	100
1	Soya	17
1	Cod liver oil	16
1	Encapsulated cod liver oil	22
1	Encapsulated halibut liver oil	33
3	Specialist	
	barbecue	106
	for fish	49
	baking spray	11

n-Alkane content in edible oils (Source McGill et al J. Sci Food Agri. 1993 **61** 357-362)

A single apple: 25-50 mg n-alkanes/apple





n-Alkanes in olive oil (Source: O. Koprivnjak et al. 2005)

Functions of Cuticular Wax in Plants

- Seals completely the surface of most leaves, fruits and seeds
- Reduces water loss
- Mechanical barrier against wear and tear
- Prevents invasion by fungi and micro-organisms
- Protective boundary layer between the plant and the outside world

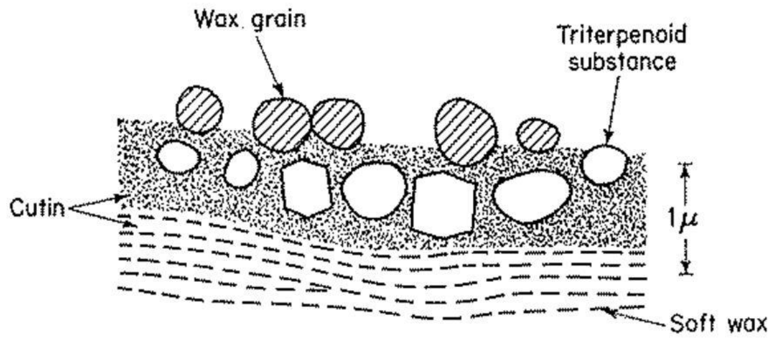


Figure 22. Schematic representation of the superficial area of the epidermal outer cell wall of apples (variety *Calville blanc*) [from Mazliak[131,134]].

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EXTERNAL SCIENTIFIC REPORT

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Bioaccumulation and toxicity of mineral oil hydrocarbons in rats - specificity of different subclasses of a broad mixture relevant for human dietary exposures

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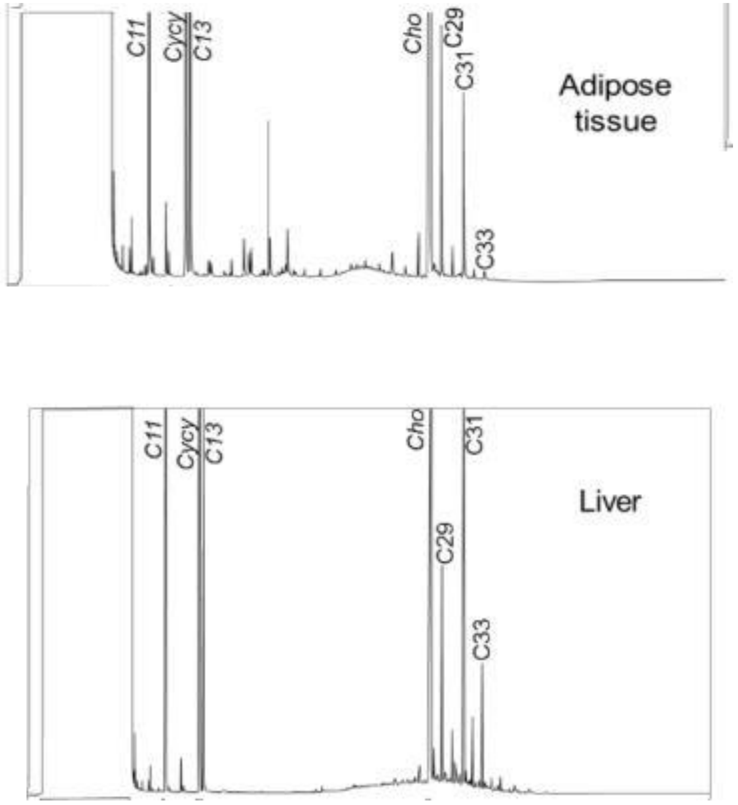
[c]Norwegian Institute of Public Health (NIPH), Oslo, Norway

- Study on the fate and effects of mineral oil saturated hydrocarbons in female Fischer 344 rats (MOSH)
- Feed with 40 to 4000 mg/kg for up to 120 days (oil with broad carbon range distribution and oil/wax mixtures
  - Hydrocarbons analyzed in liver, spleen, adipose tissue and remaining carcass at different sampling times
  - Additional end-points checked: clinical effects, liver granulomas, hepatic inflation and disruption of immune function

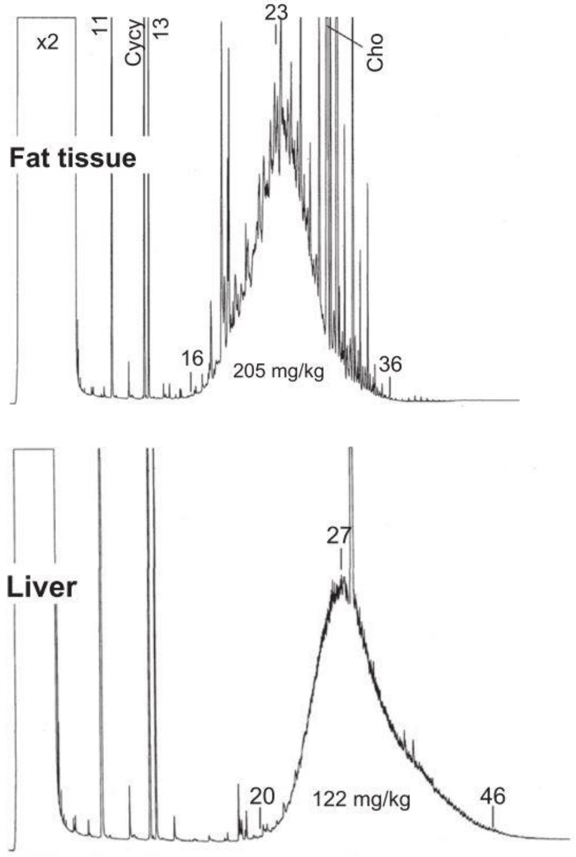
Natural n-Alkanes in the F-344 Rats and Humans

F-344 Rat Data (Control Group)



Presence of n-alkanes in the tissues of the F-344 rats of the control group (Cravedi et al. 2017 p 69)

Human Data



Barp et al 2014. p 316

Accumulation of Natural n-Alkanes in the F-344 Rat

Table 25: Concentrations (mg/kg) of the natural *n*-alkanes in the control feed and the tissues of the rats fed with the control feed

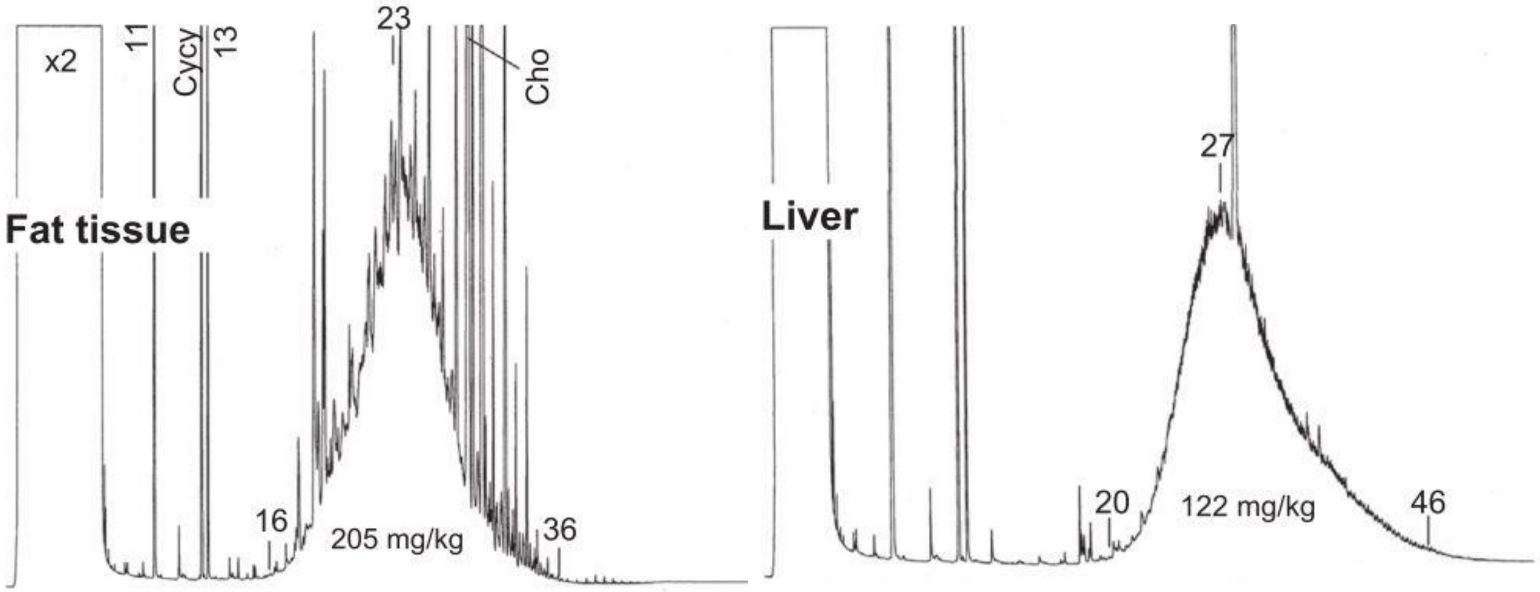
	Concentrations (mg/kg)				
	Feed	Liver	Spleen	Adipose tissue	Carcass
n-C_{29}	0.05	1.64	0.19	0.42	0.56
n-C_{30}	0.02	0.37	0.11	0.06	0.05
n-C_{31}	0.12	4.57	0.25	0.39	0.52
n-C_{32}	0.02	0.38	0.08	0.03	0.02
n-C_{33}	0.02	0.75	0.06	0.00	0.05
Total	0.22	7.72	0.70	0.89	1.20

Source: Cravedi et al. 2013 EFSA Supporting Publication 2017:EN-1090 Page 70-71

- Data obtained on animals in the control group: No exposure to oils and/or waxes other than those present in the feed
- Concentration in the F-344 liver almost 9 times higher than in adipose tissue
- This is opposite to what is seen in other species and humans where n-alkanes are found to be the easiest hydrocarbons to metabolize

The “enriching” of n-alkanes at very low concentrations is telltale for the F-344’s inadequacy as model

No "Accumulation" of n-Alkanes in Humans



- In the fat tissue, some n-alkanes, mainly the odd-numbered ones,
- These components were considered transient as continuous accumulation would end up in fat higher concentrations. In the liver, no n-alkanes (natural or synthetic ones – including those from POSH) were observed in the liver samples of any the 37 subjects of the study
- Lipogranulomas were seen in only one of the livers
- The presence of n-alkanes in the fat tissue demonstrates the absence in the liver is not caused by lack of exposure

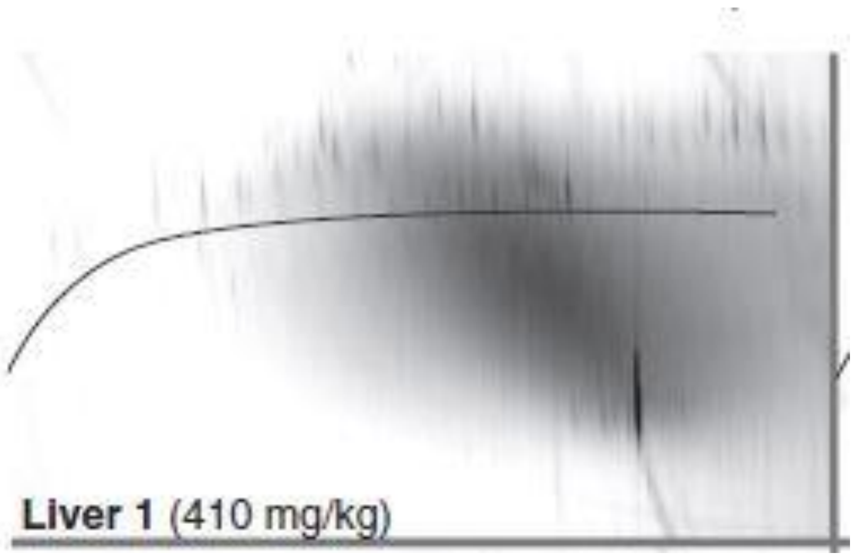
Source: Barp et al. 2014, K.Grob, 2017 Personal Communication

Hydrocarbons in Human Tissues

- Margolis and Boitnott (1966-1970)
  - Large oil droplets were identified in livers, spleens and MLN
  - Oil droplets are largely deposits of saturated hydrocarbons
  - The saturated hydrocarbon content was determined on 60 livers and 34 spleens obtained from 63 patients (4 children and 59 adults) at the time of autopsy
  - Identification of hydrocarbons present with TLC and molecular sieves
    - Large amounts of branched and/or cyclic compounds including high proportions of polycycloalkanes
    - No hydrocarbons of vegetable or animal origin:

      "These hydrocarbons consist predominantly of n-alkanes with small amounts of branched and monocyclic hydrocarbons" (Author's quote)

Composition of the Hydrocarbons in the Human Liver



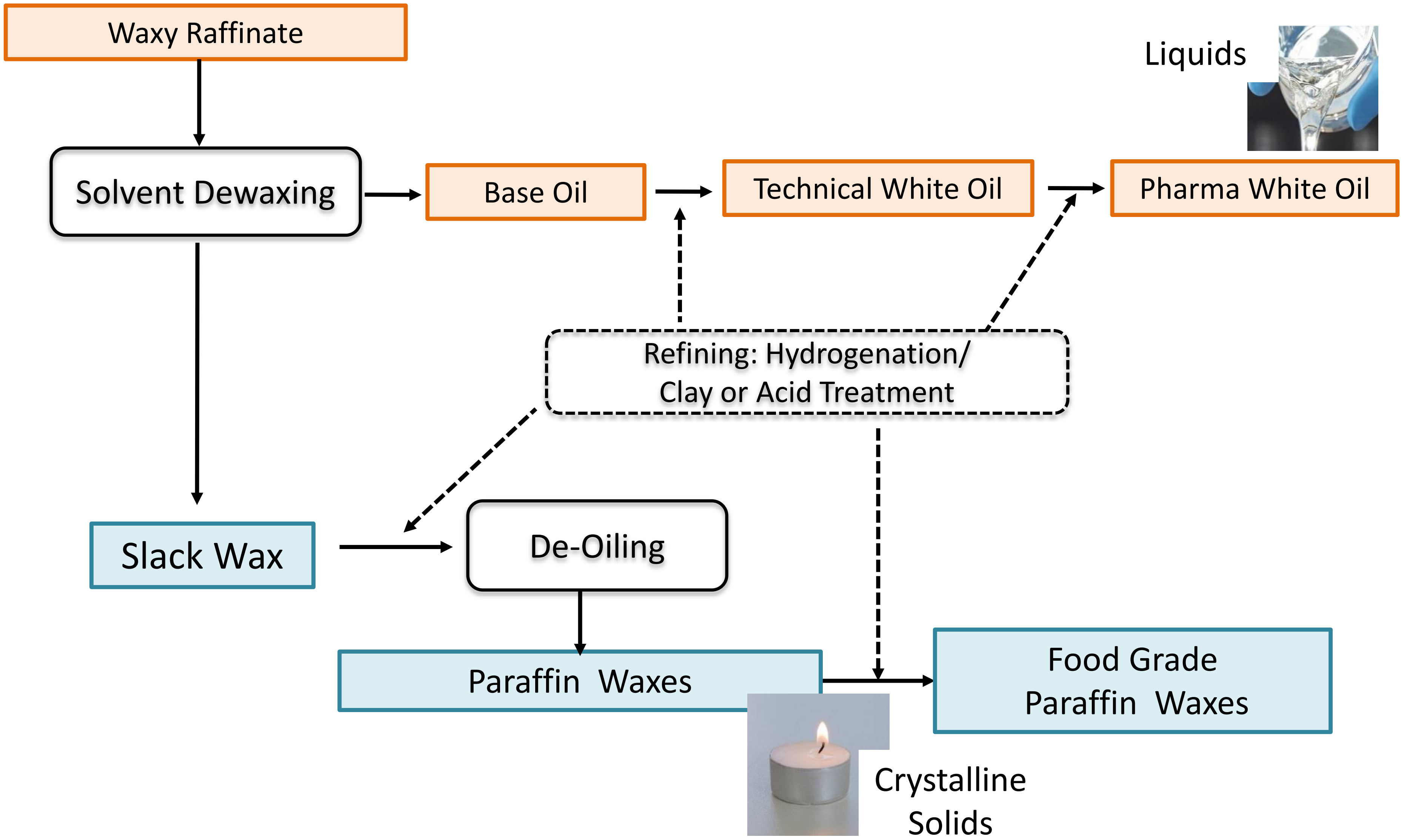
- Absence of n-alkanes (both those from natural and mineral oil products)
- All hydrocarbons components forming distinct signals are essentially removed. This includes many (little branched) and cyclic hydrocarbons including steranes and hopanes
- The persistent hydrocarbons are mainly represented in the gray cloud of unresolved, highly isomerized and (poly)cyclic compounds

Source: Biedermann et al. 2015,

Hydrocarbon Waxes

- Definition
  - Solid at 20° C, varying in consistency from mostly brittle and hard to sometimes soft and plastic
  - Coarse to fine white crystalline solids, transparent to opaque, but never glass-like
  - Melt above ca. 40° C without decomposition
  - Low viscosity just above the melting point
- Manufacturing
  - Crude oil refining – Group 1 Refinery
  - Fischer-Tropsch synthesis

Manufacturing Paraffin Waxes



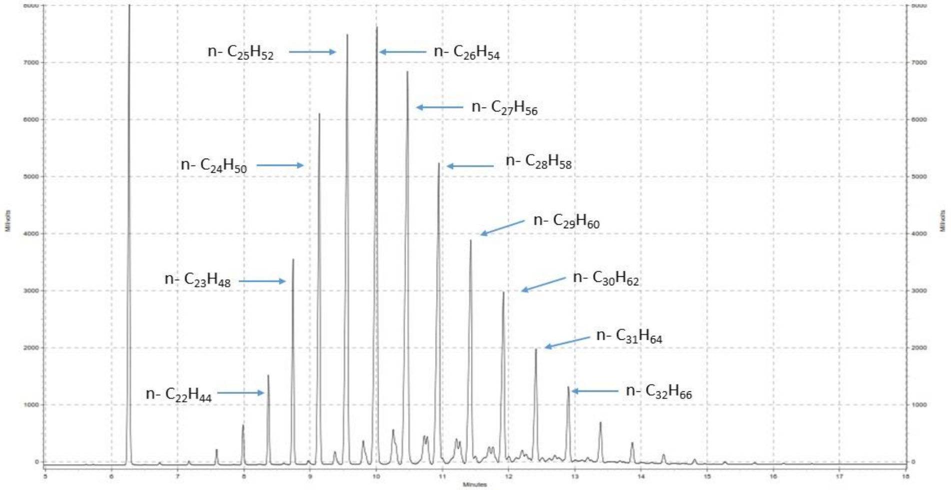
Hydrocarbon Waxes Overview

Source	Group 1 Refinery		
	Vacuum distillate mineral oil		Residu vacuum distillation mineral oil
Abbreviation	LMPW	IMPW	Micro
Carbon distribution	C20 - C35	C25 - C45	C30 - C90
N-alkane content (%)	85-90	50 - 70	20 - 60
(Drop)Melting point (°C)	>50	<70	62-99
Viscosity 100 °C (cSt)	>2.5	<11	>11
Average Mol weight	360	450	600-800

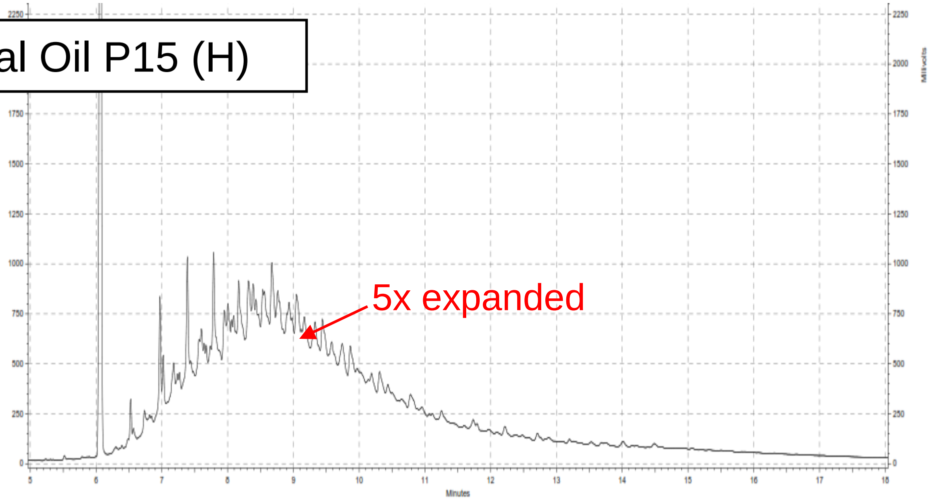
Source	FT process	
Abbreviation	LMSP	HMSP
Carbon distribution	C20 - C50	C30 - C90
N-alkane content (%)	85-90	85-90
Melting point (°C)	50-90	90-120
Viscosity 100 °C (cSt)	2.5-11	>6 (120 °C)
Average Mol weight	360 - 550	600-800

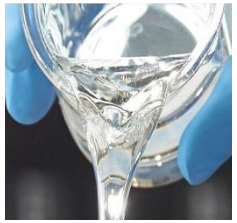
High Temperature GC of LMPW and White Oil

LMPW: Paraffin 52-54

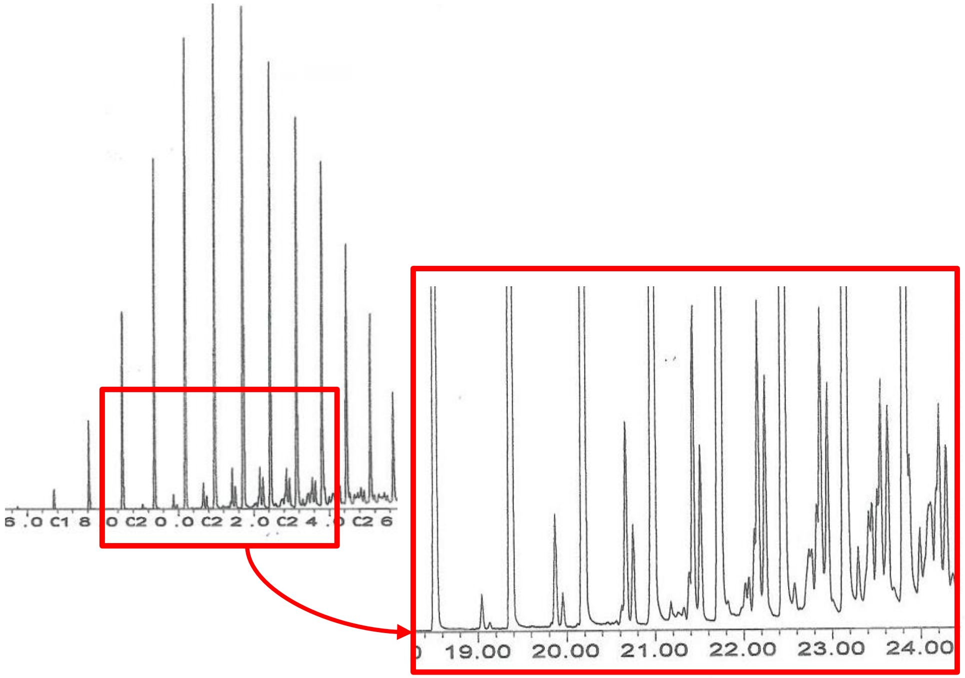


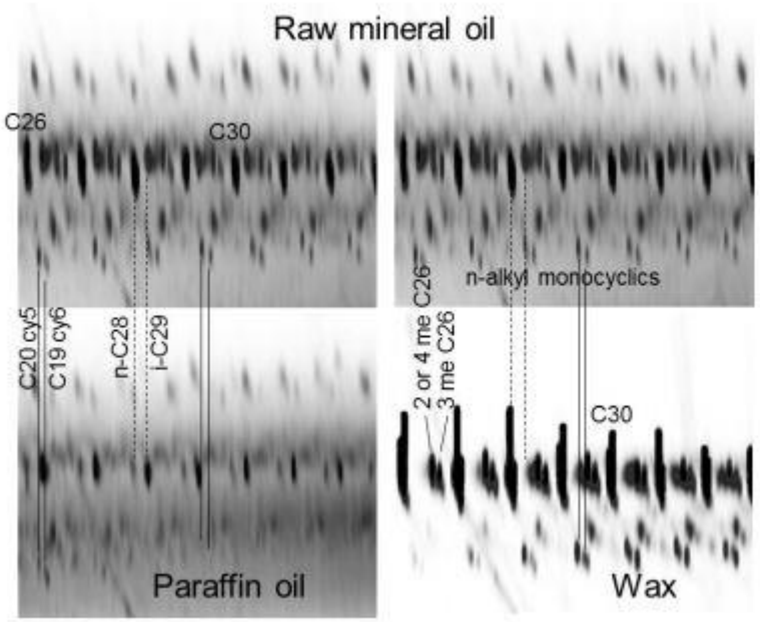
White Mineral Oil P15 (H)





Composition of Paraffin Hydrocarbon Waxes





Source: Cravedi et al. 2017 EFSA ESR

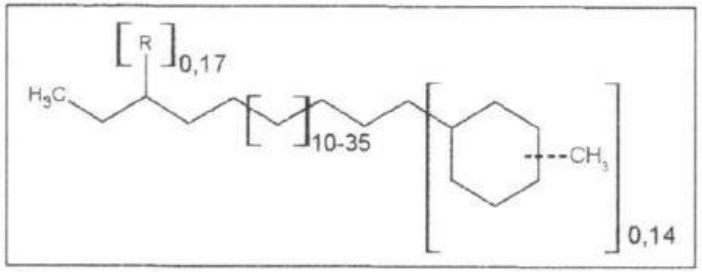


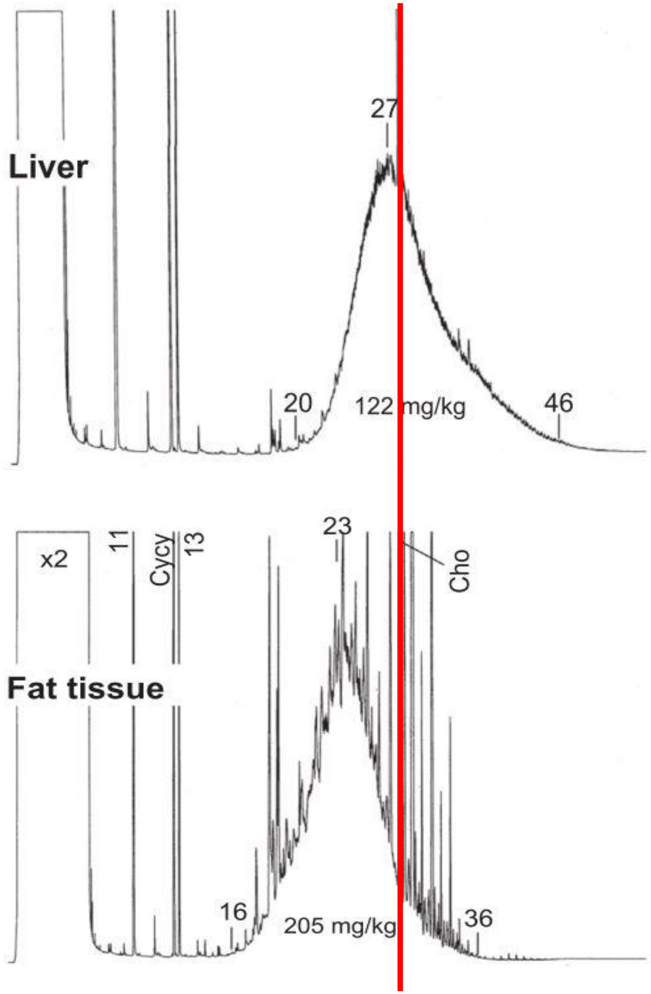
Abb. 6 Hypothetische mittlere Struktur makrokristalliner Paraffine. R = $[-CH_3]_{0,12}$; $[-C_2H_5]_{0,02}$; $[-C_3H_7$ und länger$]_{0,03}$

Source: Matthai et al. 2004

- Mostly linear n-alkanes
- Few short alkyl branches (methyl) per molecule
- Some 5 and 6 membered rings part of the chain
- Discrete peaks, also in gc x gc: No “gray cloud”
- Double crystallization key to molecular composition

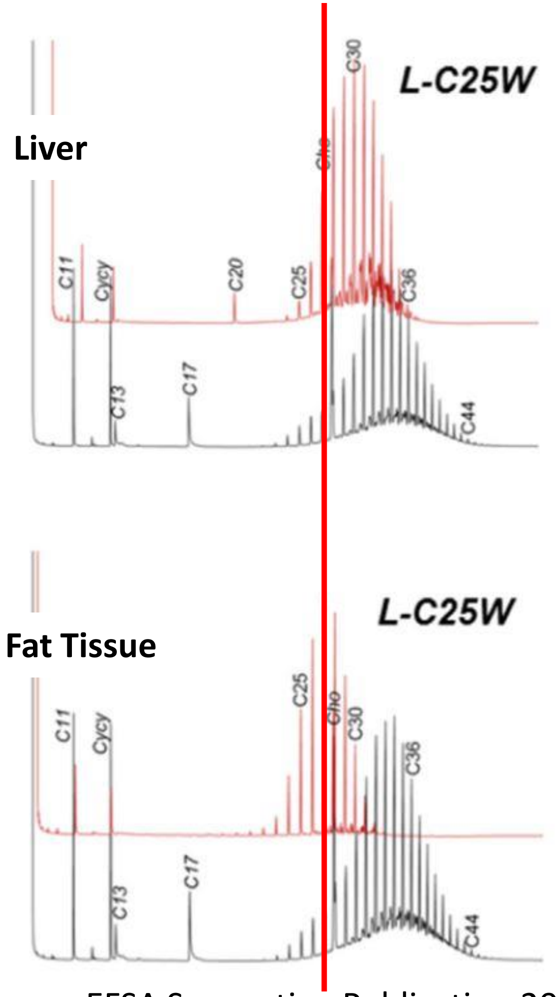
Hydrocarbons in Human and F-344 Tissues

Human Data



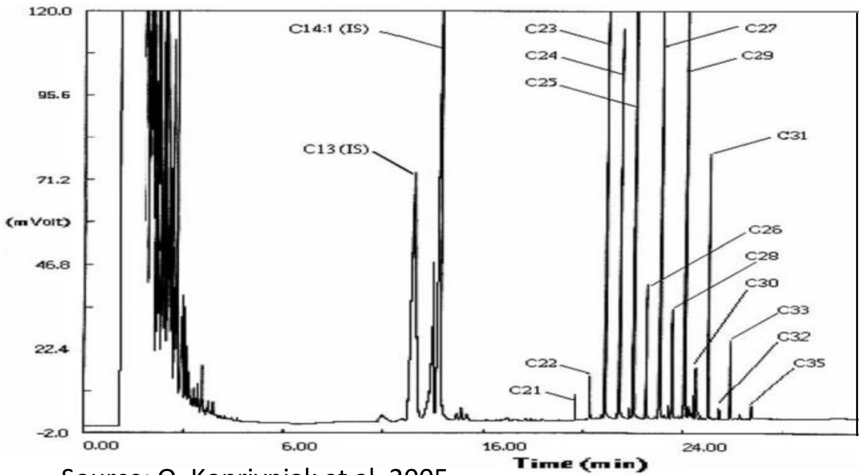
Source: Barp et al. 2014

F-344 Data



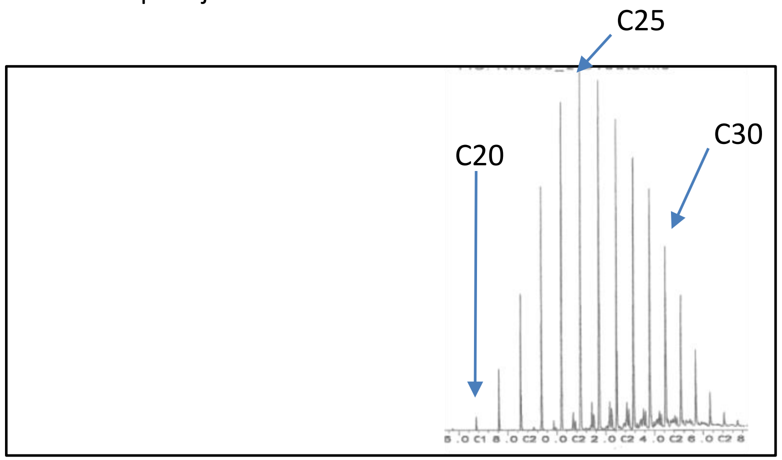
Source: EFSA Supporting Publication 2017:EN-1090

n-Alkane Compositions



Source: O. Koprivnjak et al. 2005

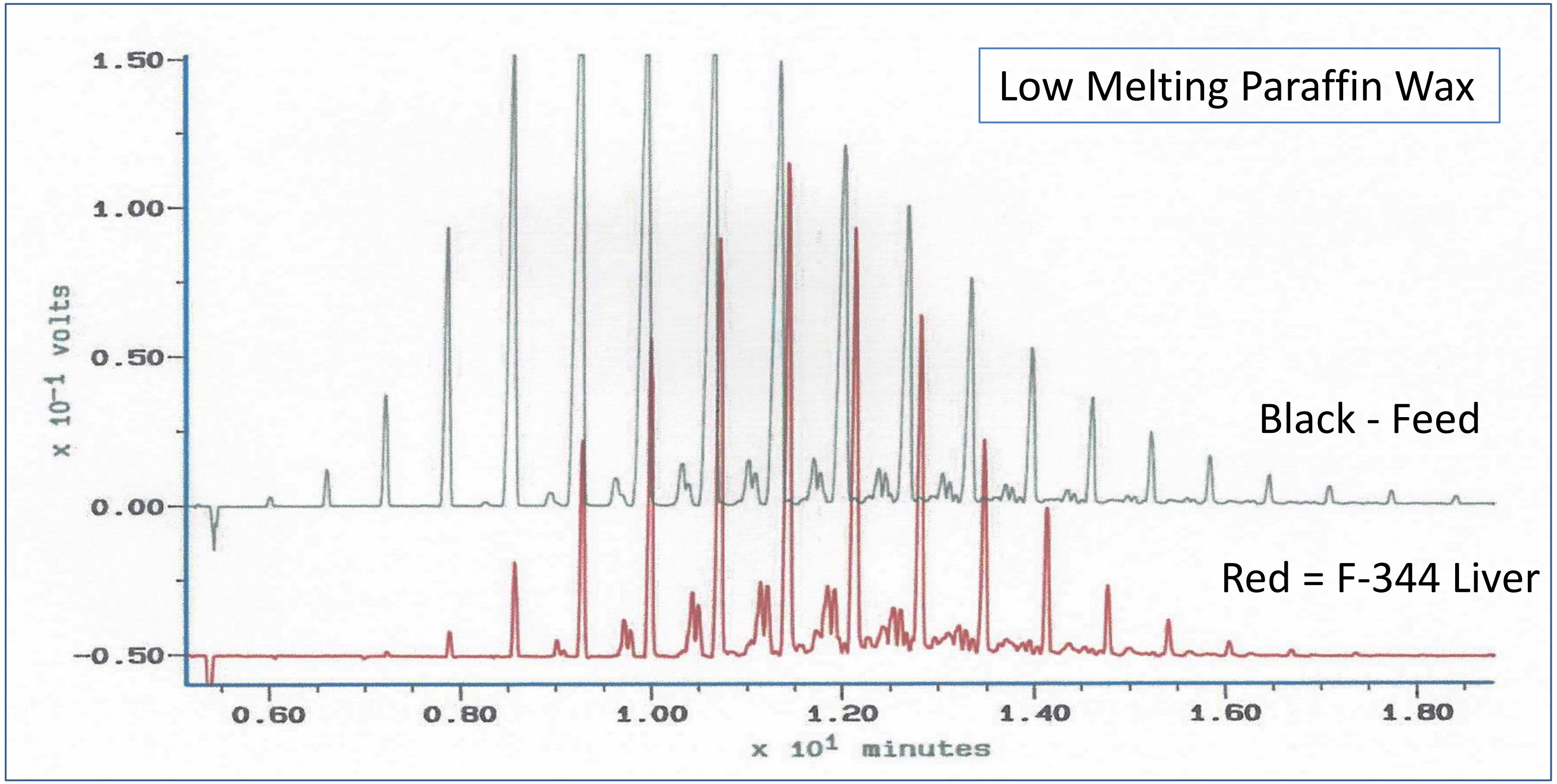
Olive Oil



LMPW: 52-54°C Paraffin Wax

Difference odd vs even distribution of C_{20}-C_{35} in olive oil and LMPW

Metabolisation of Odd or Even Carbon Chains ?



- No differences in relative metabolisation rates for odd versus even n-alkanes
- From a response perspective, the F-344 would cope the same way with natural n-alkanes as with those from LMPW

Source: Jan Woldhuis, Paramelt

90 Day Studies in the F-344 and S-D Rats

Mineral hydrocarbon concentrations (mg/g) after 90 days

Liver

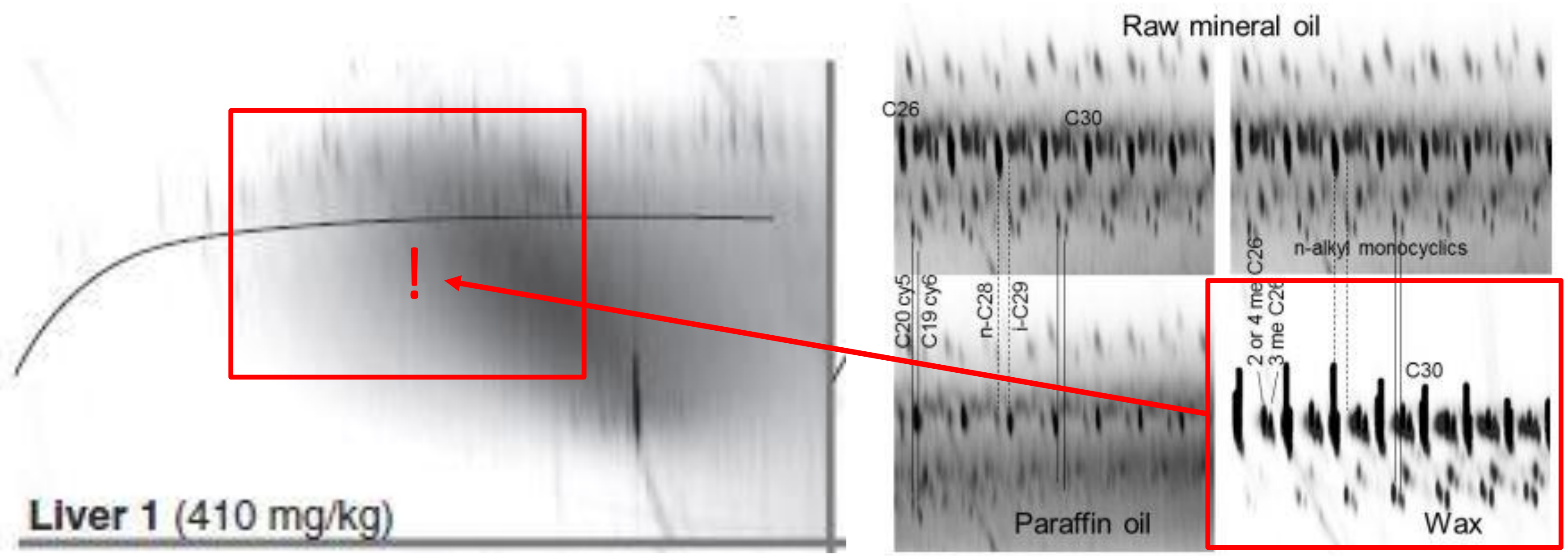
Dose Group	Mean dose %	Mean dose/day mg/kg/day	P 15(H) [b]	LMPW [a]
F-344	0.20% 2.0%	160 1600	5.6 ± 1.2 8.2 ± 1.0	13.3 ± 3.7* 19.8 ± 2.04*
S-D	0.20% 2.0%	160 1600	1.7 ± 1.2 4.1 ± 1.4	<LOQ <LOQ

LOQ Limit of Quantification 0.5 mg/g HC

(b) Firriolo et al 1995 (a) Griffis et al 2010

- Comparison in P15(H) Column: White oil concentration in F-344 2 to 3 times higher than in Sprague-Dawley
- Comparison in F-344 Row: Hydrocarbon concentration 2.5 times higher for LMPW compared to P15(H) oil
- Comparison in the LMPW Column: LMPW in S-D livers below LOQ, high concentrations found in F-344
    - Non-linear dose-response for n-alkanes in F-344 compared to S-D strain
    - The SD rats cope with 2.0 % or 20,000 mg/kg LMPW in their feed without accumulation in the liver
    - F-344 rat is not able to deal with even 0.22 mg/kg natural n-alkanes in its feed (See slides 5 and 6)

Wax Constituents in Human Livers



- The first condition for lipogranuloma formation is the presence of the relevant hydrocarbons
- In human livers containing saturated hydrocarbons no hydrocarbon wax constituents are observed
- This absence is not due to lack of exposure: Transient presence of n-alkanes in fat tissue demonstrate exposure to wax constituents
- Waxes don't contribute to the "accumulation" of hydrocarbons in humans

Conclusions

- F-344 a wrong model for assessing the safety of n-alkanes
  - Contrary to other species (including humans) the F-344 rats are of metabolizing n-alkanes very slowly (Genetic deficiency?)
  - This results in the formation of wax crystals further reducing n-alkane bioavailability and triggering a unique set of pathological effects: inflammatory epitheloid granulomas
  - The effects seen for LMPW in the F-344 have no relevance for human safety assessment of hydrocarbons. They are unique to the F-344
- Hydrocarbon waxes show no "MOSH" concern
  - n-Alkanes and other constituents of hydrocarbon waxes are fully metabolisable and they are not found in human livers
  - Those saturated hydrocarbon subclasses that have been identified in human livers are absent in hydrocarbon waxes
  - Absent hydrocarbons don't create granulomas

LMPW: not the "most potent" but the "most innocent" MOSH

Fischer Lessons

Consequences and possible next Steps

- From the “most potent” to the “most innocent” MOSH
  - LMPW in the Plastics Regulation EU 10/2011
  - General MOSH concern
  - Low Viscosity Mineral Oil

Hydrocarbon Waxes in Annex 1 of EU 10/2011

FCM 93	**FCM 94**
Waxes, paraffinic, refined, derived from petroleum based or synthetic hydrocarbon feedstocks, low viscosity	Waxes, refined, derived from petroleum based or synthetic hydrocarbon feedstocks, high viscosity
Average molecular weight no less than 350 Dalton	Average molecular weight no less than 500 Dalton
Visocity at 100°C not less than 2.5 cSt	Visocity at 100°C not less than 11 cSt
Content of hydrocarbons with Carbon Number less that 25, not more than 40% (w/w)	Content of hydrocarbons with Carbon Number less that 25, not more than 5 % (w/w)
SML: 0.05 mg/kg	None
Not to be used for articles in contact with fatty foods for which similant D is laid down	

Listing of LMPW in the Plastics Regulation (I)

- 2005: Listing of LMPW in the Plastics Regulation
  - Petition by the EWF to obtain listing of LMPW in Annex 1
  - The migration of LMPW, used as an additive in plastic in the intended applications, is extremely low
  - Mesenteric lymph node granulomas were still considered toxicologically relevant
  - EWF submitted a TIER 1 dossier and obtained listing of LMPW in Annex 1
  - Restrictions: SML 0.05 mg/kg and not to be used in contact with fatty food

Listing of LMPW in the Plastics Regulation (II)

- Situation Today
  - The critical effects to be considered in the safety assessment of hydrocarbons are hepatic microgranulomas. The granulomas seen in the mesenteric lymph nodes lack toxicological relevance (EFSA Contam Panel 2012)
  - Compelling evidence that the F-344 is the wrong model for n-alkanes
  - Absence of accumulation for n-alkanes and other hydrocarbon wax constituents in humans clearly demonstrated
  - SML of 0.05 mg/kg creates problems:
    - Customers have identified LMPW applications in plastics with higher experimental specific migration values
    - Compliant use of FCM 94 could lead to surpassing 0.05 mg/kg SML of FCM 93

Calculation of an SML for LMPW

- No pathological findings attributable to the LMPW seen in life time study at 10% exposure in Sprague-Dawley rats (Shubik et al. 1962)
- Subchronic studies (oral 90 days in Sprague-Dawley for LMPW found a NOAEL of at least 1644mg/kg b.w. per day
  - No LMPW hydrocarbons present in S-D rat livers
  - No granulomas seen in the S-D rat livers

- In-vitro studies of enzymatic hydroxylation rates:
  - Cravedi et al 2011: F-344 < S-D < Wistar
  - Cravedi and Perdu 2012: No differences between rats strains. For human females higher (p 0.05) then F-344

- TDI calculated with a Safety Factor of (100 for intra/interspecies uncertainty):

  → 16.44 mg/kg b.w. per day

  Resulting SML: 968 mg/kg food

- This is 16 times higher than the overall migration limit: A higher SML for FCM 93 in Annex 1 of the Plastics Regulation (EU) 10/2011 would be justified

General MOSH Concern

SCIENTIFIC OPINION

Scientific Opinion on Mineral Oil Hydrocarbons in Food[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Consumers are exposed to a range of mineral oil hydrocarbons (MOH) via food. Mineral oil saturated hydrocarbons (MOSH) consist of linear and branched alkanes, and alkyl-substituted cyclo-alkanes, whilst mineral oil aromatic hydrocarbons (MOAH) include mainly alkyl-substituted polyaromatic hydrocarbons. Products, commonly specified according to their physico-chemical properties, may differ in chemical

and liver. Hepatic microgranulomas associated with inflammation in Fischer 344 rats were considered the critical effect. The no-observed-adverse-effect level for induction of liver microgranulomas by the most potent MOSH, 19 mg/kg b.w. per day, was used as a Reference Point for calculating margins of exposure (MOEs) for background MOSH exposure. MOEs ranged from 59 to 680. Hence, background exposure to MOSH via food in

and liver. Hepatic microgranulomas associated with inflammation in Fischer 344 rats were considered the critical effect. The no-observed-adverse-effect level for induction of liver microgranulomas by the most potent MOSH, 19 mg/kg b.w. per day, was used as a Reference Point for calculating margins of exposure (MOEs) for background MOSH exposure. MOEs ranged from 59 to 680. Hence, background exposure to MOSH via food in

- The NOAEL of 19 mg/kg b.w. is an artefact caused by the very low metabolisation rate in the F-344 combined with the crystallizability of waxes. It lacks human relevance.
- It should therefore not be used as a Reference Point as for the assessment of Mineral Oil Exposure

90 Day Studies on White Oils

Table 16: NOAELs observed in female Fischer 344 rats exposed to white mineral oils, based on MLN histiocytosis and liver microgranulomas.

Test item identification: P indicates a paraffinic white oil, mainly containing branched alkanes, no or minor amounts of aromatics. N indicates a naphthenic white oil, mainly containing cyclo alkanes, no or minor amounts of aromatics. The following number indicates the approximate viscosity (expressed in mm^2/s) at 40 °C. The letter between brackets indicates the refining method applied (A: acid treatment; H: hydrogenation treatment). OTWO: oleum treated white oil (acid treatment), containing alkanes and cyclo alkanes, minor amounts of aromatics. HTWO: hydrotreated white oil, containing alkanes and cyclo alkanes (cyclo-alkanes in higher proportions than OTWO), no or minor amounts of aromatics.

Test item	Physico-chemical properties	Duration	Concentration in diet (mg/kg)	Dose (mg/kg b.w. per day)	NOAEL (mg/kg)		Reference
					MLN histiocytosis	Liver granulomas	
Oils							
N10(A)	Viscosity at 40 °C (mm^2/s): 13.3 Viscosity at 100 °C (mm^2/s): 3.1 Average MW: 320, C number range: 15-30	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	2	190	Smith et al, 1996
N15(H)	Viscosity at 40 °C (mm^2/s): 16.6 Viscosity at 100 °C (mm^2/s): 3.4 Average MW: 330 C number range: 17-30	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	< 2	190	Smith et al, 1996
P15(H)	Viscosity at 40 °C (mm^2/s): 15.0 Viscosity at 100 °C (mm^2/s): 3.5 Average MW: 350 C number range: 18-30	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	2	190	Smith et al, 1996
	Viscosity at 40 °C (mm^2/s): 14.8	90 days	2000, 20 000	161, 1582	< 161	< 161	Firriolo et al., 1995
OTWO	Viscosity at 40 °C (mm^2/s): 26	90 days	10, 100, 500, 5 000, 10 000, 20 000	0.93, 9.3, 46, 440, 940, 1 800[1]	0.93	46[2]	Baldwin et al. 1992
N70(A)	Viscosity at 40 °C (mm^2/s): 76.4 Viscosity at 100 °C (mm^2/s): 7.9 Average MW: 410 C number range: 21-35	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	2	190	Smith et al, 1996
N70(H)	Viscosity at 40 °C (mm^2/s): 68.0 Viscosity at 100 °C (mm^2/s): 7.6 Average MW: 420 C number range: 22-37	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	2	190	Smith et al, 1996
HTWO	Viscosity at 40 °C (mm^2/s): 69	90 days	10, 100, 500, 5 000, 10 000, 20 000	0.93, 9.0, 45, 450, 940, 1 800[1]	45	45[2]	Baldwin, 1992

Low and Medium Viscosity Mineral Oil

- Oils should be evaluated on their own data set
- It is the liver and not the MLN the basis for this evaluation
- Only the F344 (and not the SD) shows granulomatous effects
- In the F344 rat the NOAEL is 190 mg/kg bw
- In the SD the NOAEL is at least 10x higher. > 2000 mg/kg bw
- An ADI can thus be justified at > 20 mg/kg

- There is thus no reason to believe that low and medium viscosity oils can’t be supported via a new ADI.

Summary

- The Fischer 344 rats are very inefficient in digesting n-alkanes and this leads to pathological consequences that are not seen in any other species
- They are definitely the wrong model to assess the human safety of n-alkanes, both those from natural origin or from hydrocarbon waxes
- LMPW is not the "most potent MOSH". Considering they don't accumulate waxes don't have the related concerns
- The currenty available tox data support a higher SML for FCM 93 in the Plastics Regulation
- Medium and low viscosity oils can be regulated via an ADI based on their own data set

Next Steps

- Publication in peer reviewed journals of new scientific evidence on F-344 inadequacy as a model for n-alkane safety assessment
- Preparation of a dossier supporting the removal of the SML for LMPW (FCM 93) In Annex 1 of the Plastics Regulation
- Extensive communication program with supply chain partners and other stakeholders

Thank you for you attention

Any further questions?

Many thanks to Juan-Carlos Carrillo (Shell) and Jan Woldhuis (Paramelt) for their support and discussions