

# The Toxicology Of Mineral Oil At Dermal Exposure

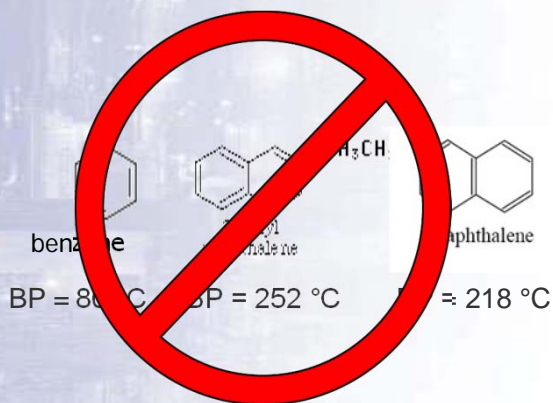
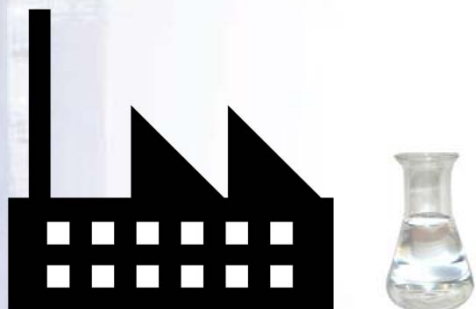
**Juan-Carlos Carrillo (Shell)**

**BfR –December 7-8, 2017**



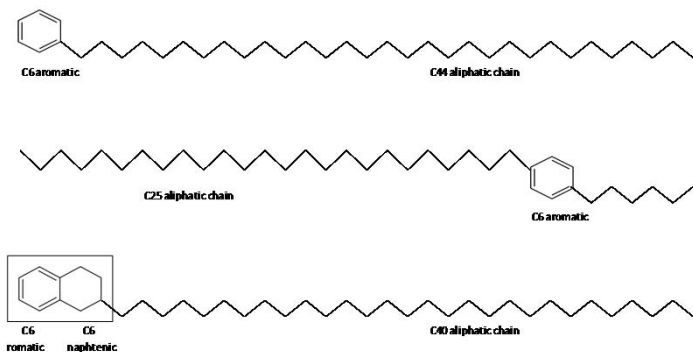
## What Type Of MOAH Can Be In Mineral Oil?

Mineral Oil Production Boiling Range > 300°C

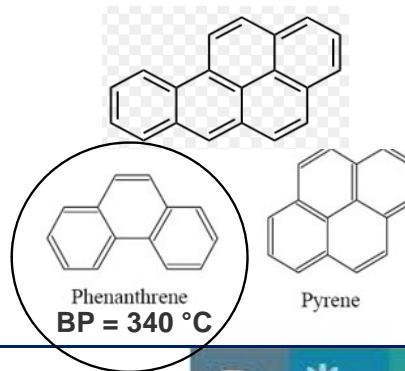


Boiling Point < 300°C → not found in mineral oil

### Highly Alkylated Aromatics



### Polycyclic Aromatic Compounds (PAC) –ring structures removed through refinement



Some PAC are considered dangerous



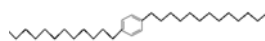
## Key points for discussion



Mouse skin painting studies



DMSO affinity to 3-7 ring PAC – The IP346



Recent MOAH Measurements



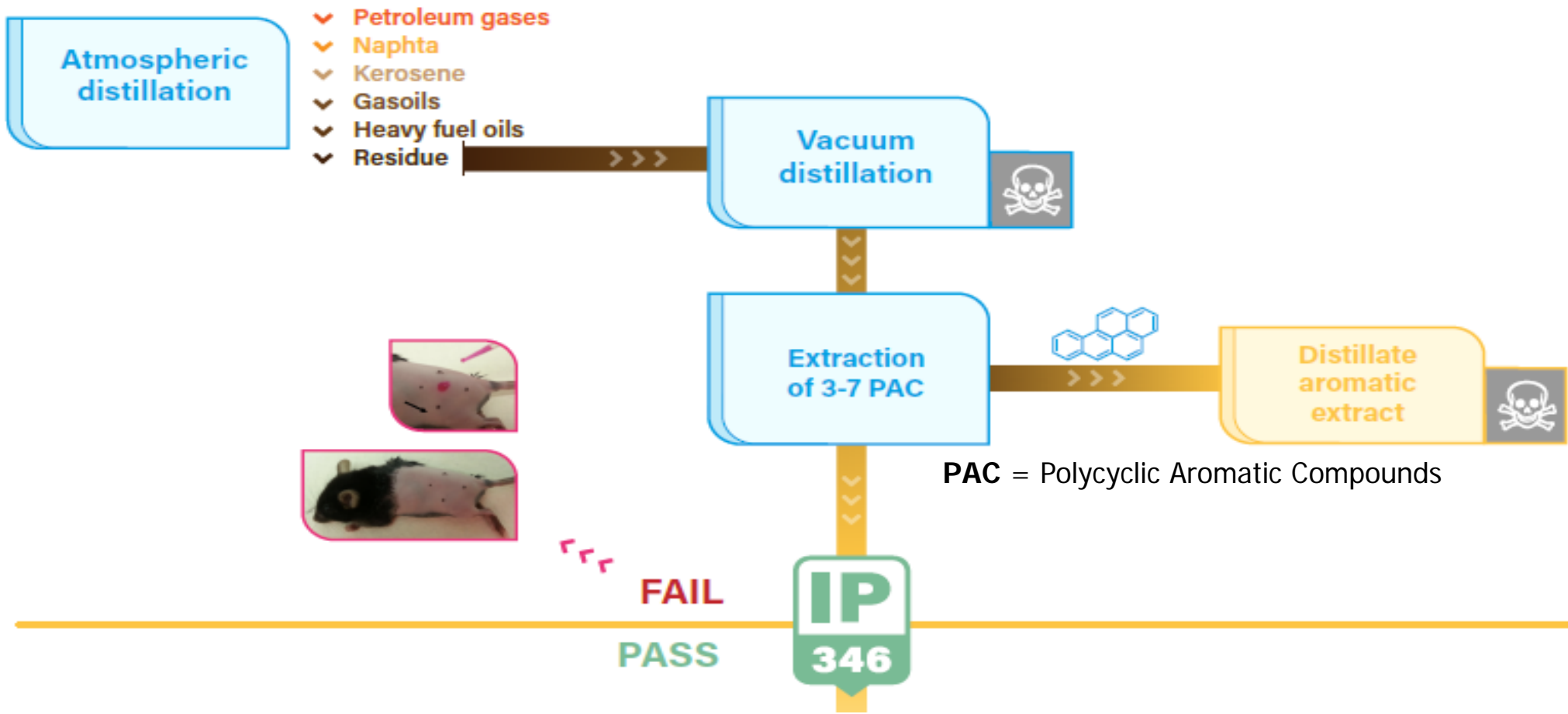
Conclusions



# Mouse skin painting studies

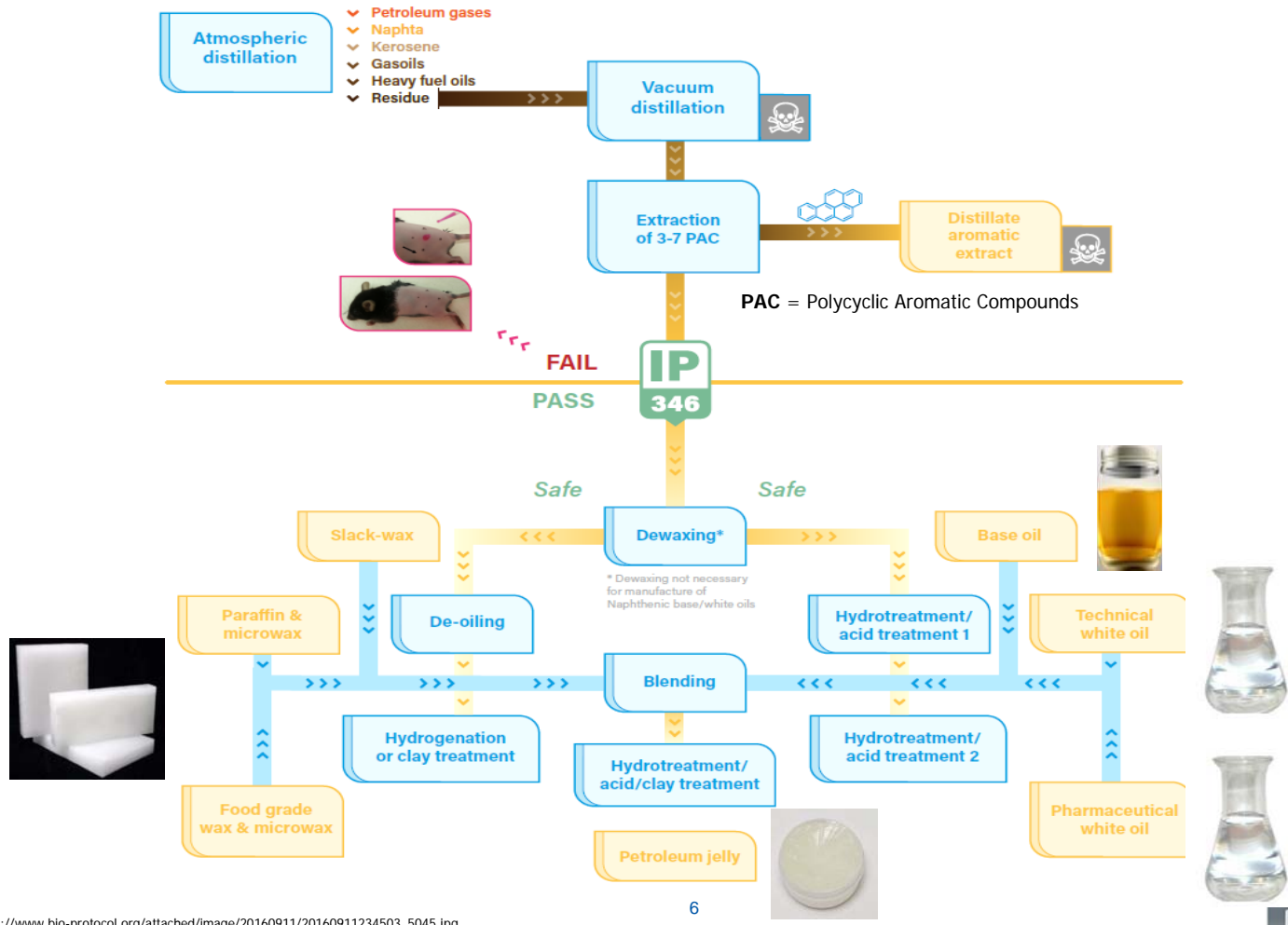


# The Mouse Skin Painting Bioassay In Mineral Oil Manufacture

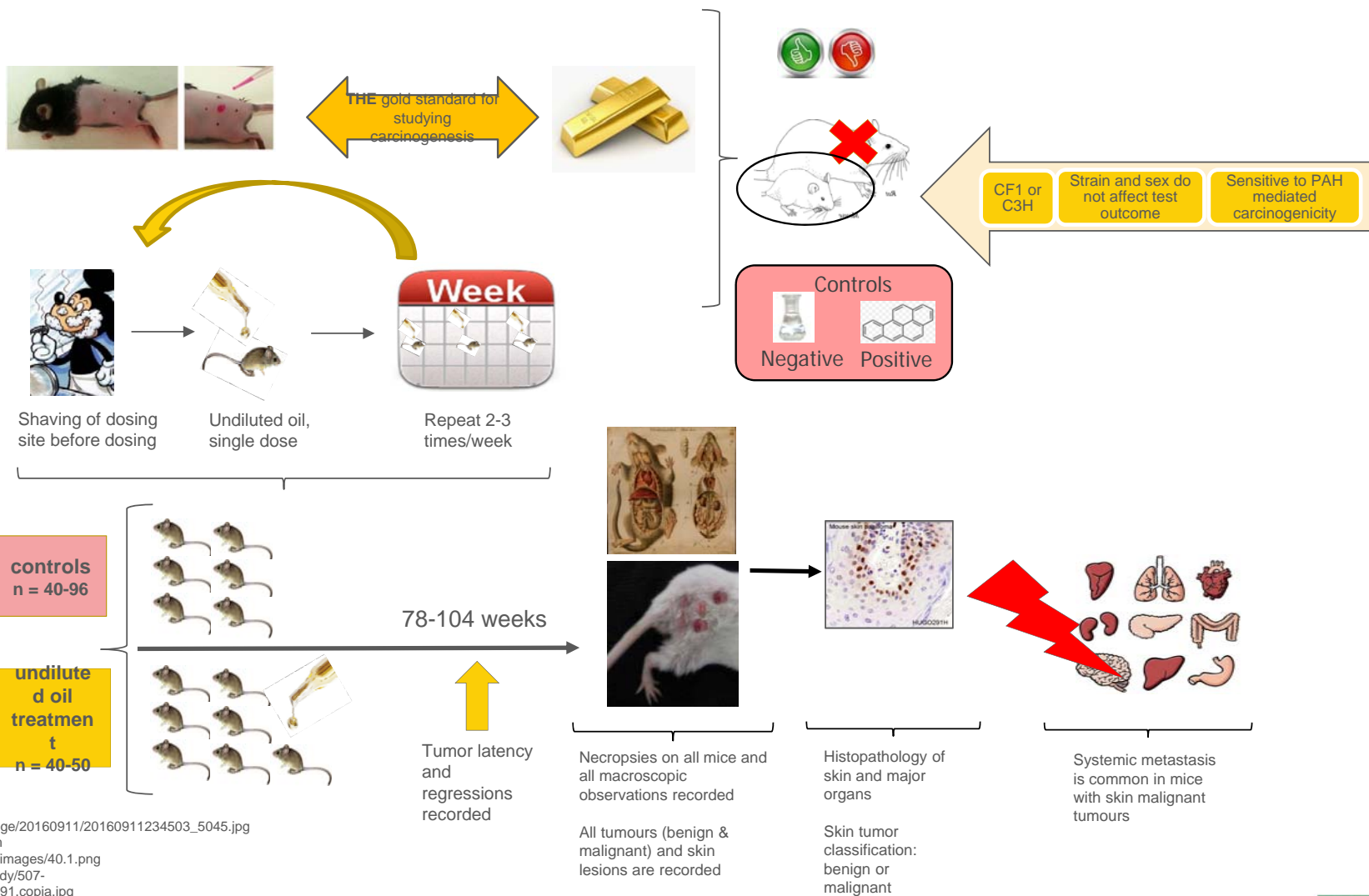


# The Mouse Skin Painting Bioassay In Mineral Oil Manufacture

## Base oil, wax, white oil, Petroleum jelly manufacture



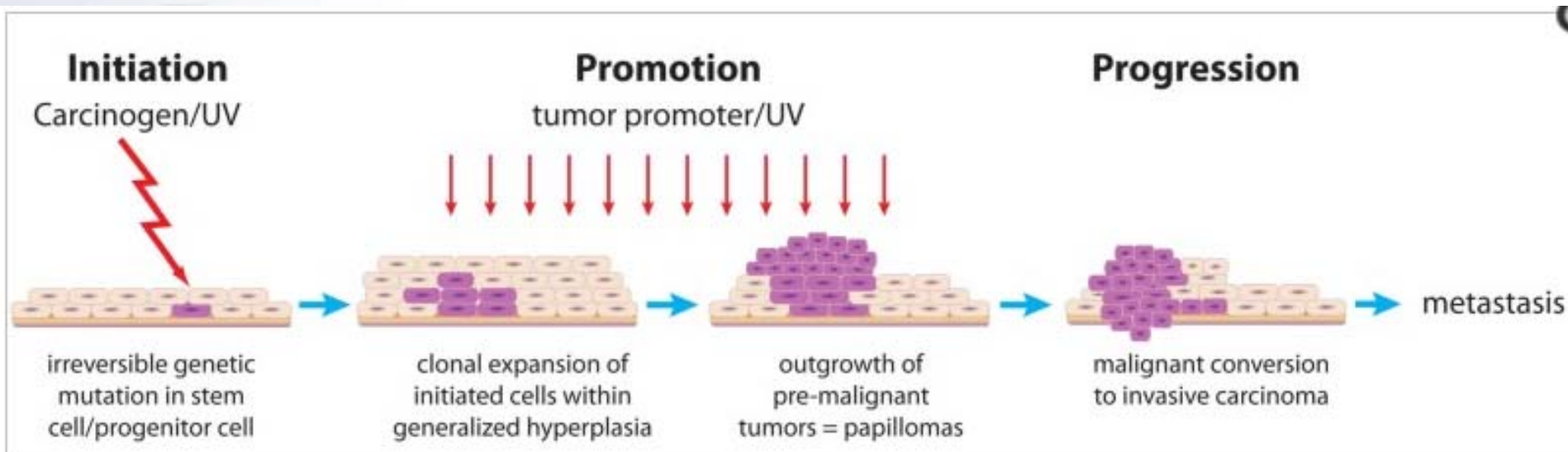
# The Mouse Skin Painting Bioassay – Carcinogenicity Test



[http://www.bio-protocol.org/attached/image/20160911/20160911234503\\_5045.jpg](http://www.bio-protocol.org/attached/image/20160911/20160911234503_5045.jpg)  
<http://www.ratbehavior.org/RatsMice.htm>  
<http://www.phytojournal.com/vol2issue2/images/40.1.png>  
<https://www.euromabnet.com/img/antibody/507-TEJ.RATON.SKIN.PAPILLOMA.HUGO291.copia.jpg>



## Why the Mouse Skin Painting Bioassay?



- ▶ Main model to study different types of PAC
- ▶ Relevant to other epithelial tissues
- ▶ Main route of exposure to mineral oil products

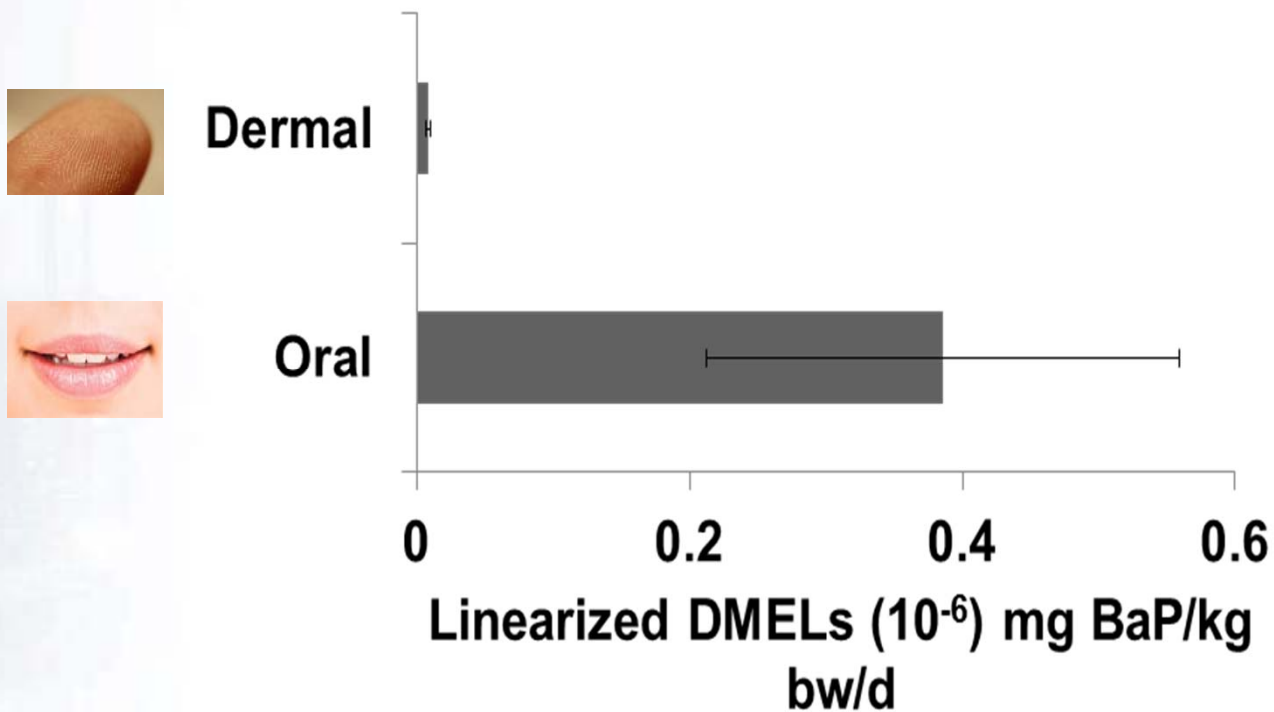
1. Rundhaug et al., 2010. *Cancers*; 2(2): 436–482.
2. La Voie et al., 1985. *Carcinogenesis*; 6(10): 1483-1488.
3. Luch A., 2009. *Mol. Clin. Env. Tox.* (1): 151-179
4. Bingham et al., 1980. *J. Env Path Tox*; (3)483-563.





## Why the Dermal Route?

Based on Derived *Minimal* Effect levels (DMEL), the risk by dermal route is the worst case scenario for PAC mediated carcinogenicity: risk of **one in a million** in developing cancer at a certain dose level.

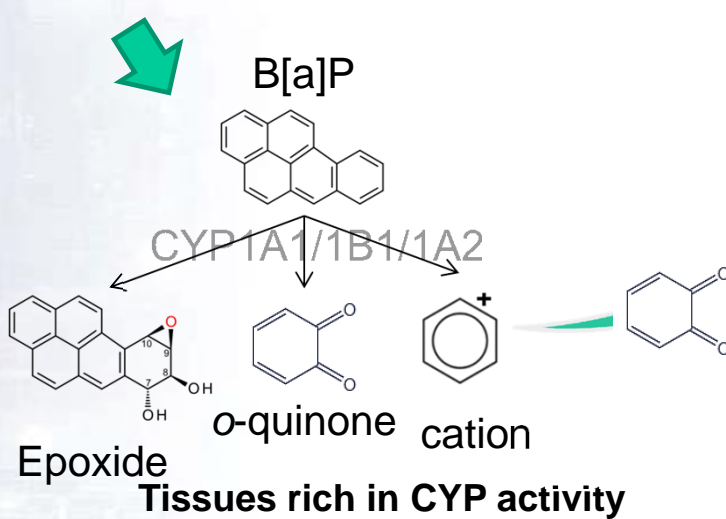


DMELs calculated from BMD<sub>10</sub> values for BaP carcinogenicity tests in rodents.  
 See Table 61 of Baua Annex XV Restriction Report Proposal for a Restriction

Slide credits: D. Adenuga - ExxonMobil



## PAC Tumor Mode of Action Dictates Tumor Location



**Tumor promotion:**  
 Local irritation, cytotoxicity,  
 inflammatory response

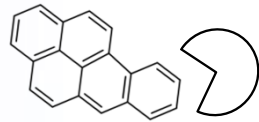
**Most sensitive tumor sites –  
 dermal, oral (forestomach,  
 oral cavity, GIT)**

Slide credits: D. Adenuga - ExxonMobil



## MOAH Molecular Structure Determines Carcinogenicity Steric Hindrance

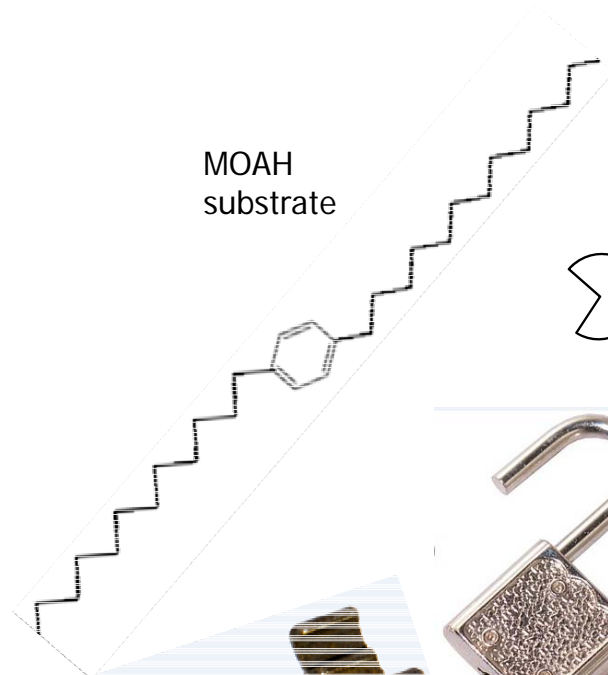
MOAH  
substrate



CYP  
enzymes



MOAH  
substrate



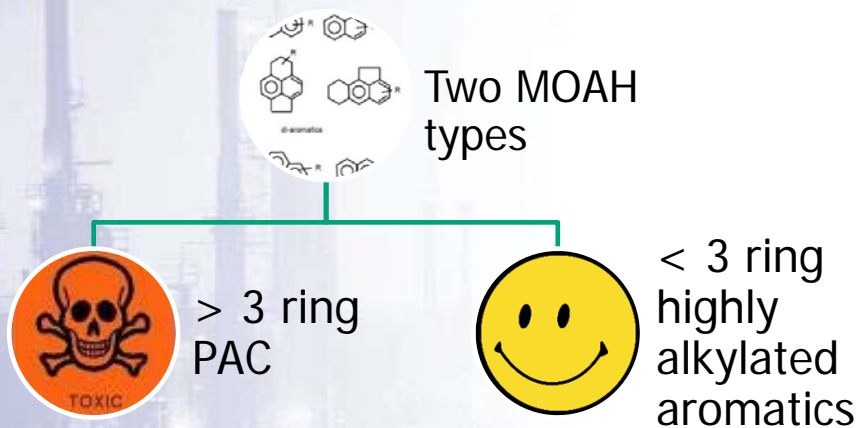
CYP  
enzymes



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# What Type Of MOAH Are Carcinogenic?



Substance or fraction	Live animals after 40 weeks	Re-treatment of live animals with a tumour promotor
Carcinogenic oil	Tumours in all animals	-
Fraction I (PAC "free")	No tumours	No tumours
Fraction II (2 and 3 rings)	No tumours	No tumours
Fraction III (> 3 rings)	No tumours	Tumours in all animals
Fraction I+II+III	Tumours in all animals	-

1. Agarwal et al., 1988
2. Doak et al., 1985

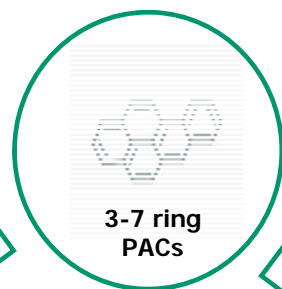
To assess MOAH it is imperative to test SUBSTANCE (the actual oil), and NOT the isolated fractions.



## Key Points: Mouse Skin Painting Bioassay

- ▶ Boiling range of PAC
- ▶ Hydrogenation
- ▶ Solvent extraction
- ▶ Acid treatment

### Refining Methods



### Mouse Skin Painting Bioassay

- ▶ Skin is the most sensitive route
- ▶ Toxicity depends on MOAH Structure
- ▶ Two types of MOAH
- ▶ Bad MOAH is > 3 ring PAC

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# DMSO affinity to 3-7 ring PAC



## The IP346



# The Need To Replace The Mouse Skin Painting Bioassay

**CAN'T CATCH UP WITH  
 MOUSE SKIN PAINTING  
 STUDIES**



- ▶ Time consuming
- ▶ Limits manufacturing flexibility
- ▶ Animal & cost intensive

# wish list



**Fit for purpose to  
 mineral oils**

- rapid, reliable, specific, simple, low cost
- reflect variability in feedstock and manufacturing conditions
- animal free test

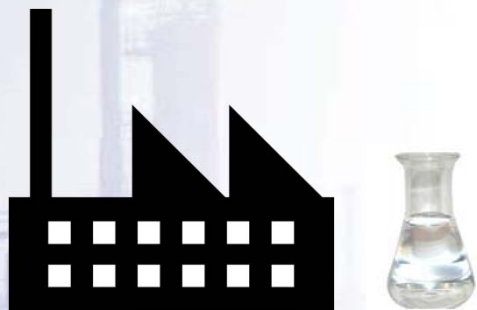


**Reflect toxicological  
 hypothesis**

- potentially hazardous are the 3-7 PAC
- PAC are bare or with few and short alkyl substituents
- highly correlated to mouse skin painting data



Mineral Oil Production Boiling Range > 300°C



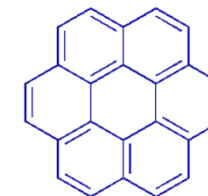
The 3-7 ring PAC found in mineral oil are found in the 340-565°C boiling point range

Through boiling points we can link manufacture to toxicity

### 3 - 7 ring PAC



340 °C  
Phenanthrene



535 °C  
Coronene



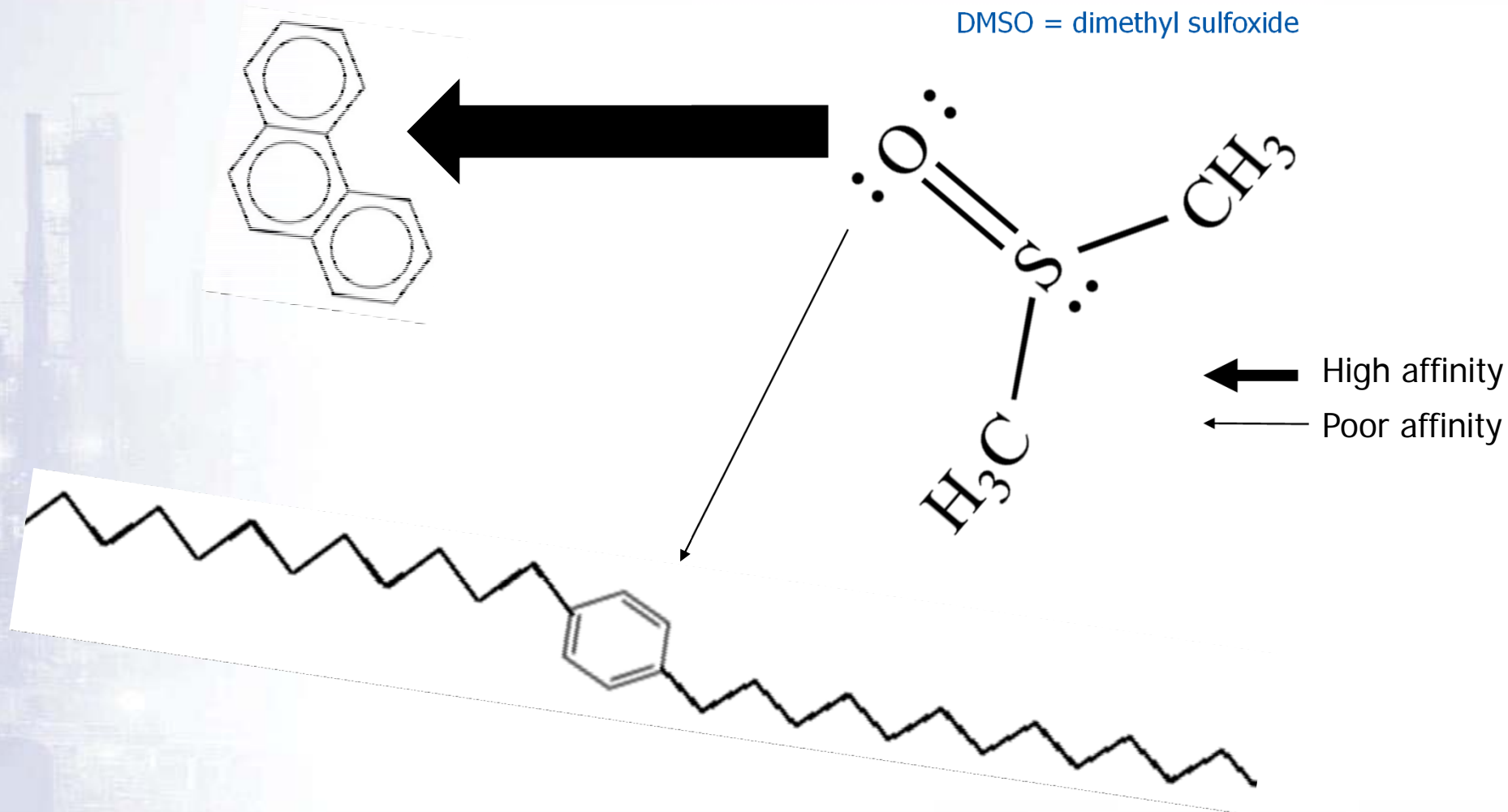
**BOILING POINT**





## Screening Method: Selective Towards 3-7 PAC

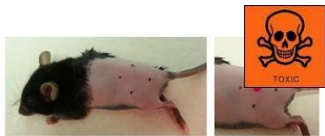
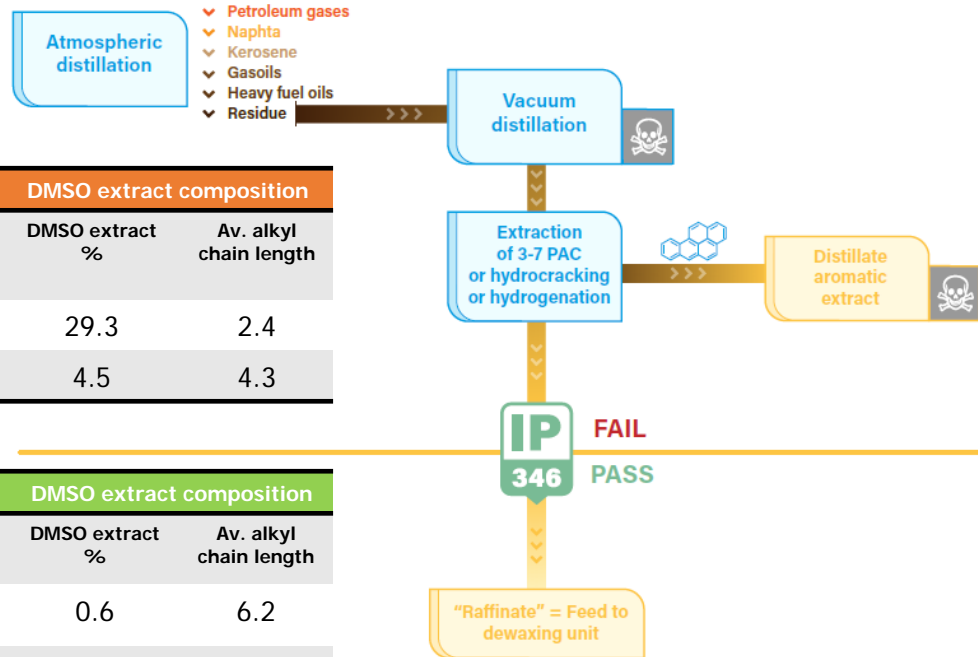
DMSO = dimethyl sulfoxide



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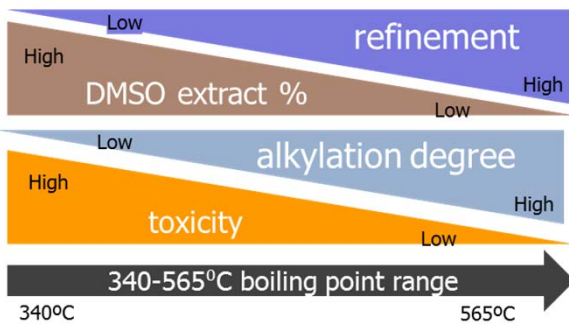



# Screening Method: Reflect Toxicological Hypothesis



DMSO extract composition		
Aromatic extracts*	DMSO extract %	Av. alkyl chain length
Extract A	29.3	2.4
Extract B	4.5	4.3

DMSO extract composition		
Base Oil*	DMSO extract %	Av. alkyl chain length
A. Low viscosity	0.6	6.2
B. High viscosity	0.1	12.5



 = DMSO affinity test

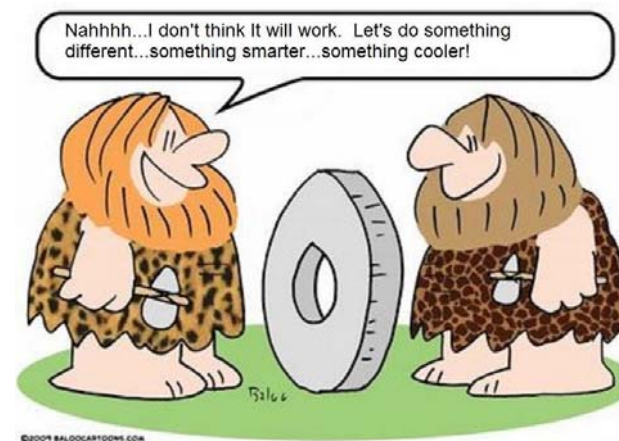
\*Note:  
At equivalent cut extraction



## Problems with MOAH Chromatography\*

- ▶ Does not distinguish MOAH types
- ▶ Over estimates because of "tail" length
- ▶ No correlation to toxicity
- ▶ Not easy to transfer or reproduce

Oil type	PAC Analysis (1970)			Cancer
	Chromatography	DMSO extract %	Av. alkyl chain length	
Oil N2 Low viscosity	2.9	6.8	3.4	YES
Oil B. High viscosity	5.7	0.1	12.5	NO

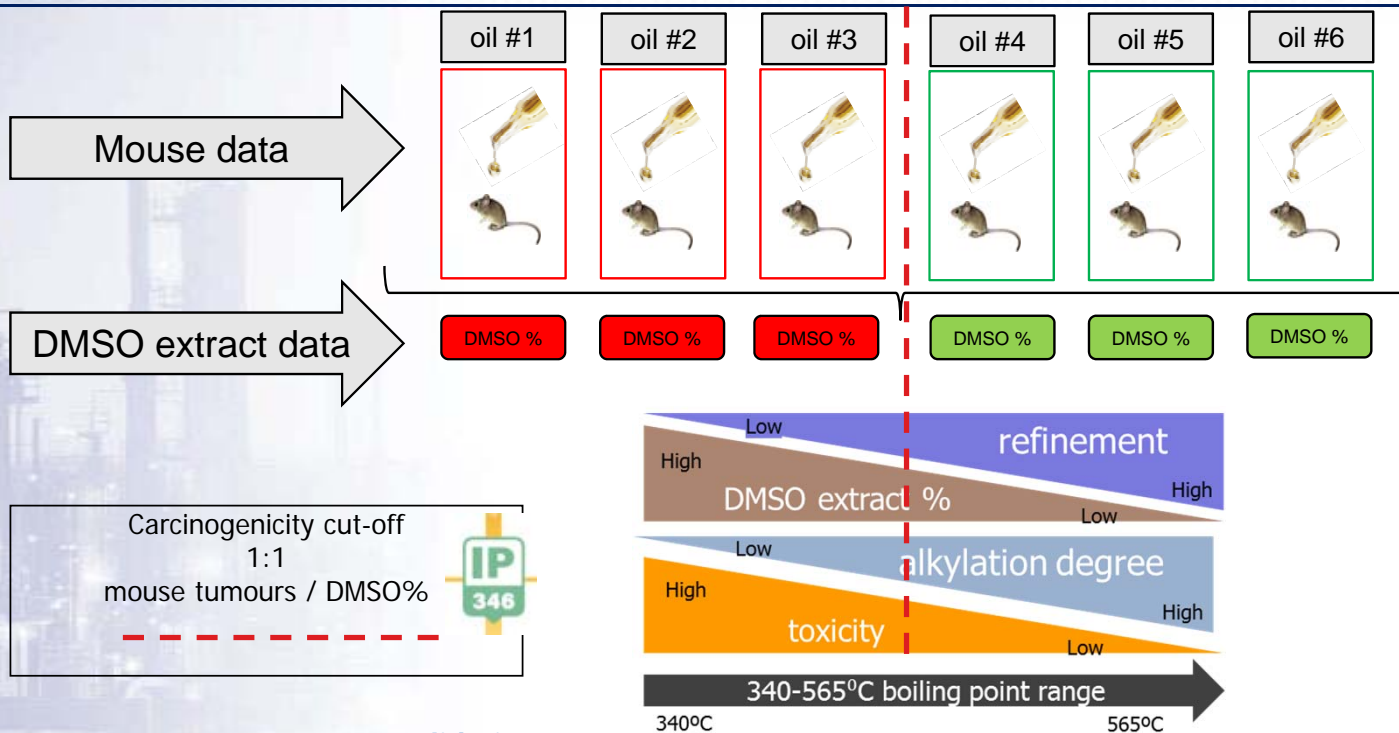


\*PLC-MS = preparative thin layer chromatography followed by mass spectrometry

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# IP346: The Mouse Dermal Bioassay vs. DMSO% PAC Affinity



### Carcinogenicity criteria for validation:

- ▶ No discrimination between benign or malignant tumours
- ▶ Potency (time of appearance of first skin tumour) is not considered
- ▶ Tumour incidence (4%) and not tumour formation stages used for IP346 validation

### IP346 validation:

- ▶ DMSO-based screening method validated with animal data
- ▶ 1:1 relationship same oil mouse skin painting studies and its own DMSO-extract
- ▶ Determine a "cut-off": % DMSO extract that is correlated to non-carcinogenic oil
- ▶ **Cut-off solely on a hazard basis:**
  - ▶ Pass/fail in carcinogenicity assessment
  - ▶ Pass/fail is binary. "Pass" means safe (and not safer, safest, etc...)

What is the DMSO% cut-off number?



- ▶ **133 data** pairs support the IP346
- ▶ Completely eliminated carcinogenicity testing on animals
- ▶ Adopted in the 90's in the EU and in other countries (e.g. Australia, Malaysia) as regulatory standard for carcinogenicity assessment
- ▶ **It is the only validated analytical method with biological significance**

IP 346 < 3% oil is not carcinogenic  
 IP 346 ≥ 3% is carcinogenic

**Three** is the number thou shall count !

Reference	Data points (2 year studies)
<b>CONCAWE 6/16</b> <b>CONCAWE 94/51</b>	<b>133 *</b> <b>104</b>
Chasey et al., 1993	94
McKee et al., 1989	9
Doak et al., 1983 and (1985)	12 (6)
Blackburn et al., 1996	120
Roy et al., 1988	39

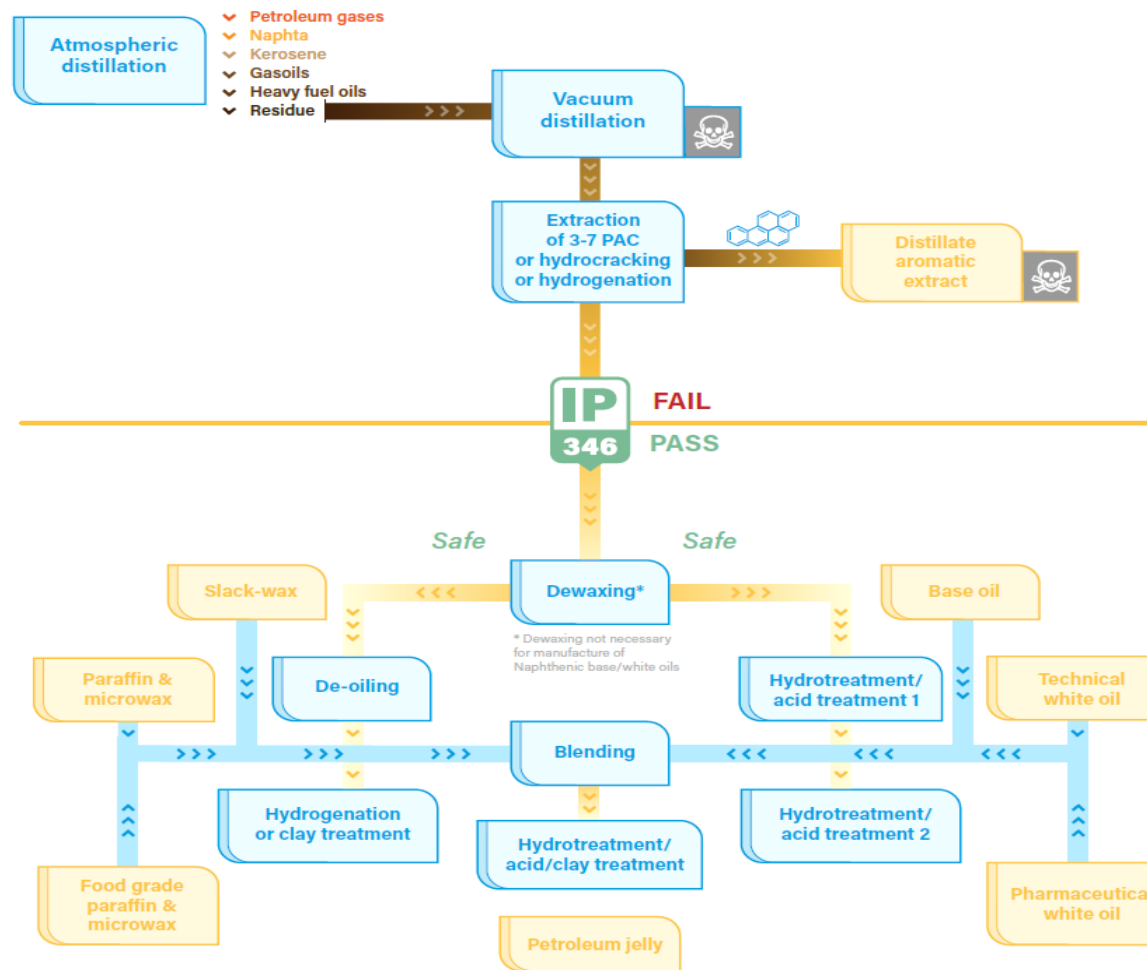
Negative predictivity = 95%  
 Accuracy = 89% (because of false positives)



\*Including all studies cited, without repetitions



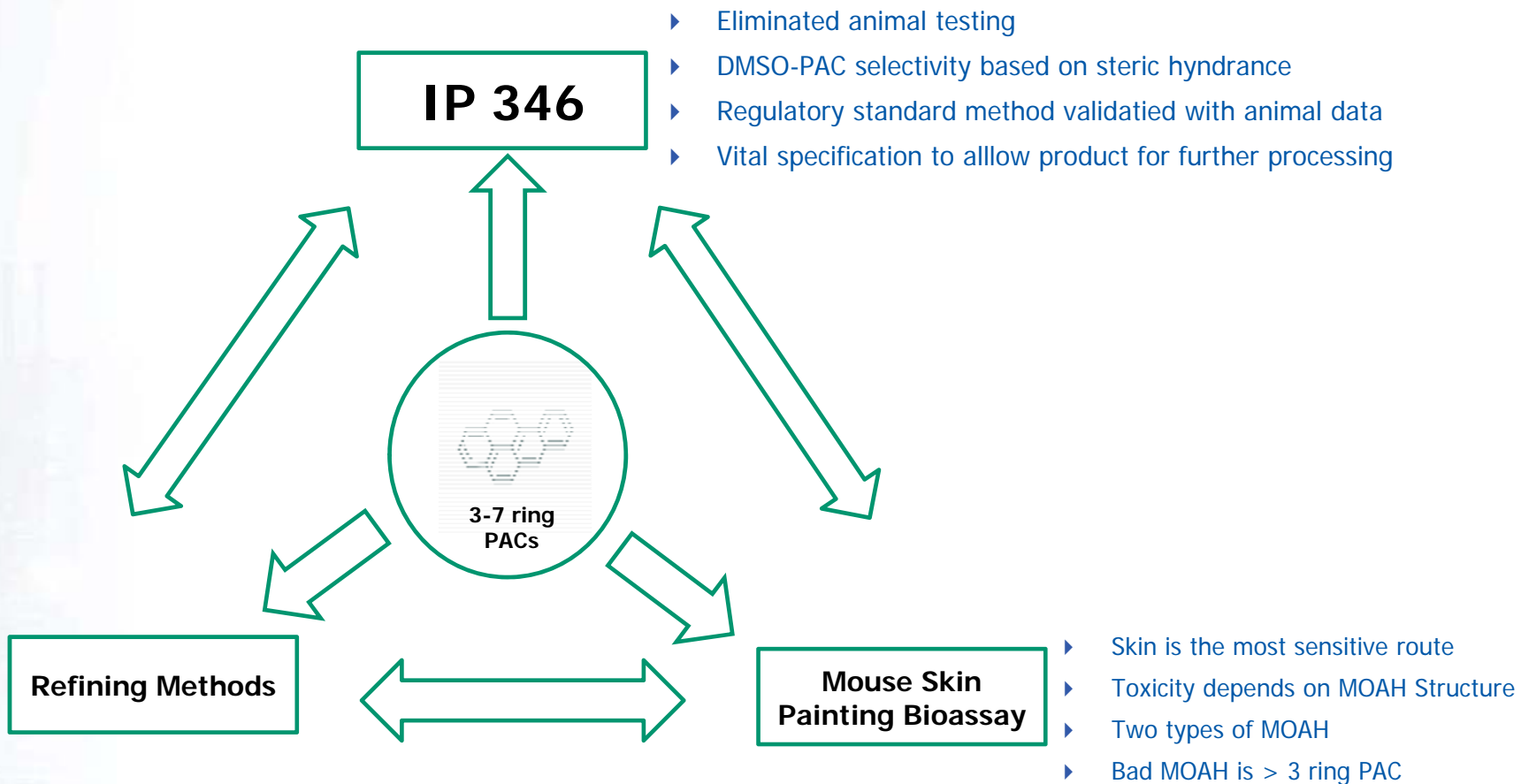
## Base oil, wax, white oil, petroleum jelly manufacture



EU Pharmacopeia: DMSO based

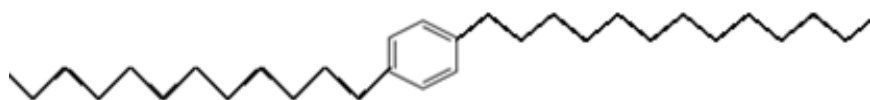


## Key Points Mineral Oil Dermal Toxicity



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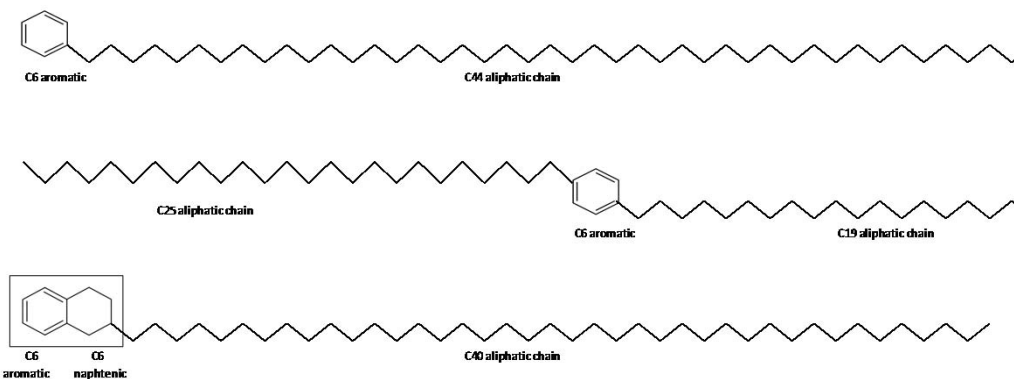
# Recent MOAH Measurements





## Why Is MOAH “High”? – The MOAH Paradox Example Microcrystalline Wax

- ▶ **MOAH (HPLC-GC FID) typical levels:**
  - ▶ 1-5 %.
- ▶ **MOAH content < C35**
  - ▶ virtually absent
- ▶ **Content of aromatic protons (NMR):**
  - ▶ ~ 0,1 – 0,5 %
- ▶ **Typical av. mol weight microwax:**
  - ▶ 700 (C50H102)
- ▶ **3-7 rings aromatics:**
  - ▶ trace levels (specific UV test / Grimmer etc.)



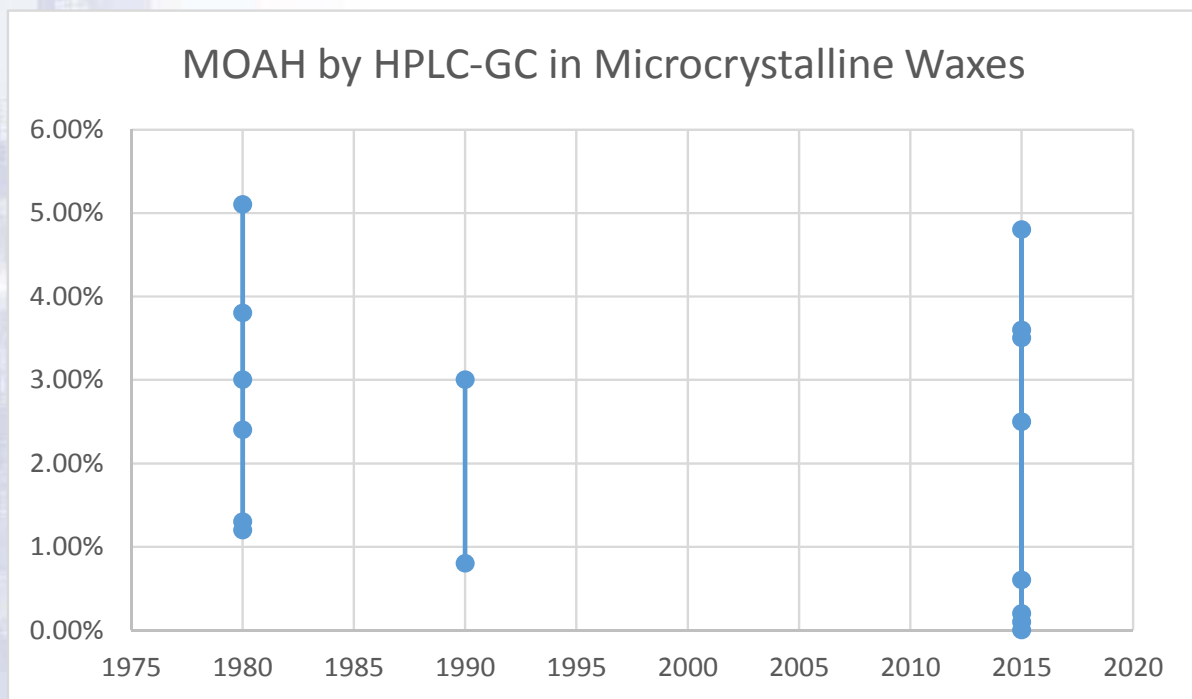
- ▶ High alkylation of a small number of aromatic carbons leads to high MOAH values (everything is interpreted as aromatic)
- ▶ The higher the MW the greater the MOAH

**MOAH paradox: the more aliphatic, the more “aromatic”**



## Former Material Is Representative For Today – Decades Long Consistency In Manufacturing

- ▶ Recent HPLC-GC measurements on old and new production samples of several (EU) manufacturers (2015) confirm that MOAH was always present – nothing new!
- ▶ Historic concentrations used for fundamental toxicological studies were at least as high or even higher than those in products presently on the market



- <1980 Concaawe 84-60 Samples
- 1990 BIBRA Study Samples
- 2015 Recent production samples of several EU Manufacturers

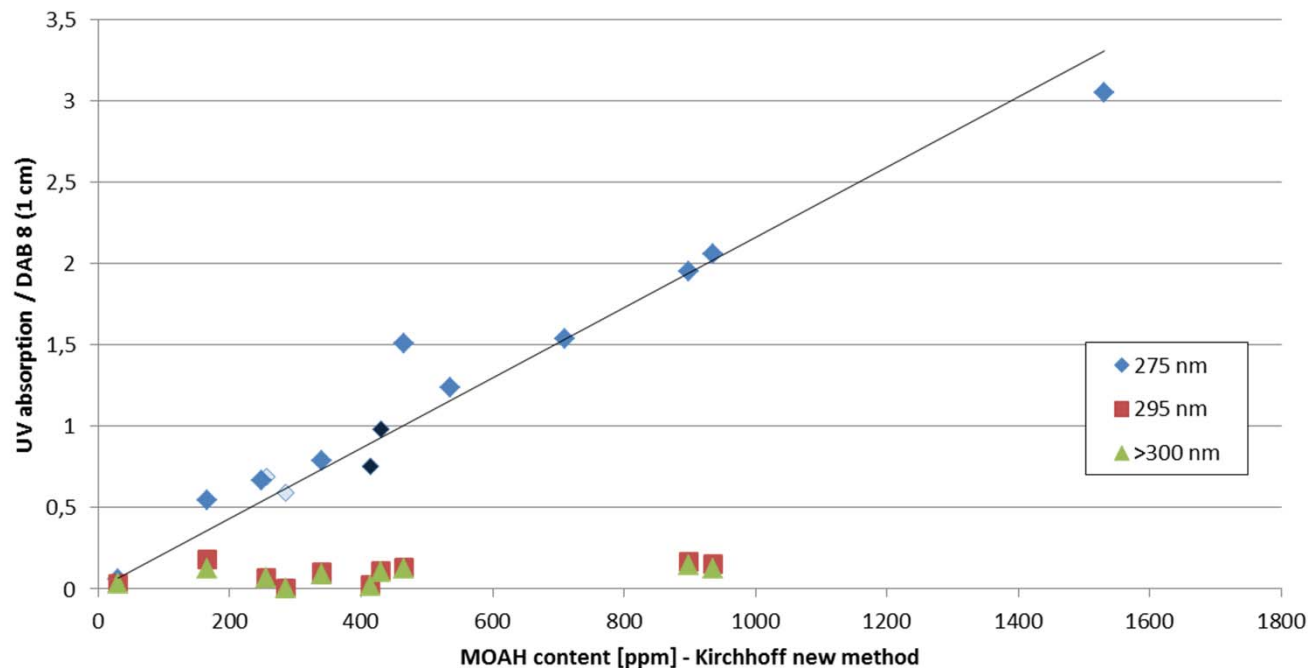
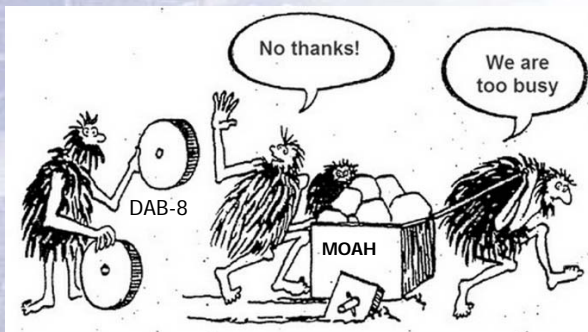
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## Do We Need A Sophisticated MOAH Method?

The measurement of Total Aromatics (MOAH) is nothing new

- **DAB 8 UV-method** did the same
- Best correlation with Oils
- Oils have shorter MOAH`s
- Longer MOAH chains are not toxicologically relevant
- Replaced by UV-methods including DMSO extraction to focus on PAH
- not biased by MW



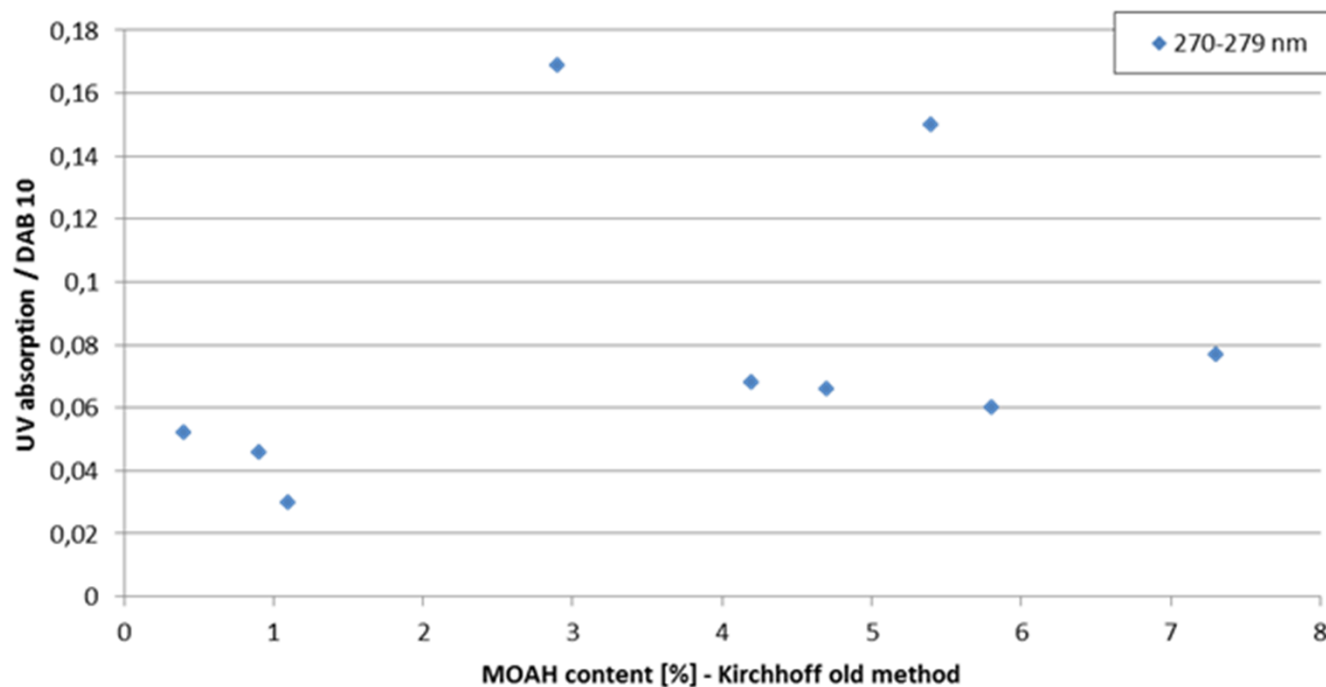
DAB 8 UV = Deutsche Arzneibuch 8, ultra violet method

Data source: H&R



## MOAH Does Not Correlate With DMSO-PAC Measurements

- ▶ No correlation between MOAH content\* and UV absorption according to the pharmacopoeia PAC test
- ▶ Amount of PACs found in products is independent of measured MOAH content



DAB 10 UV = Deutsche Arzneibuch 10; ultra violet-DMSO method

\*Kirchhoff method, July 2015

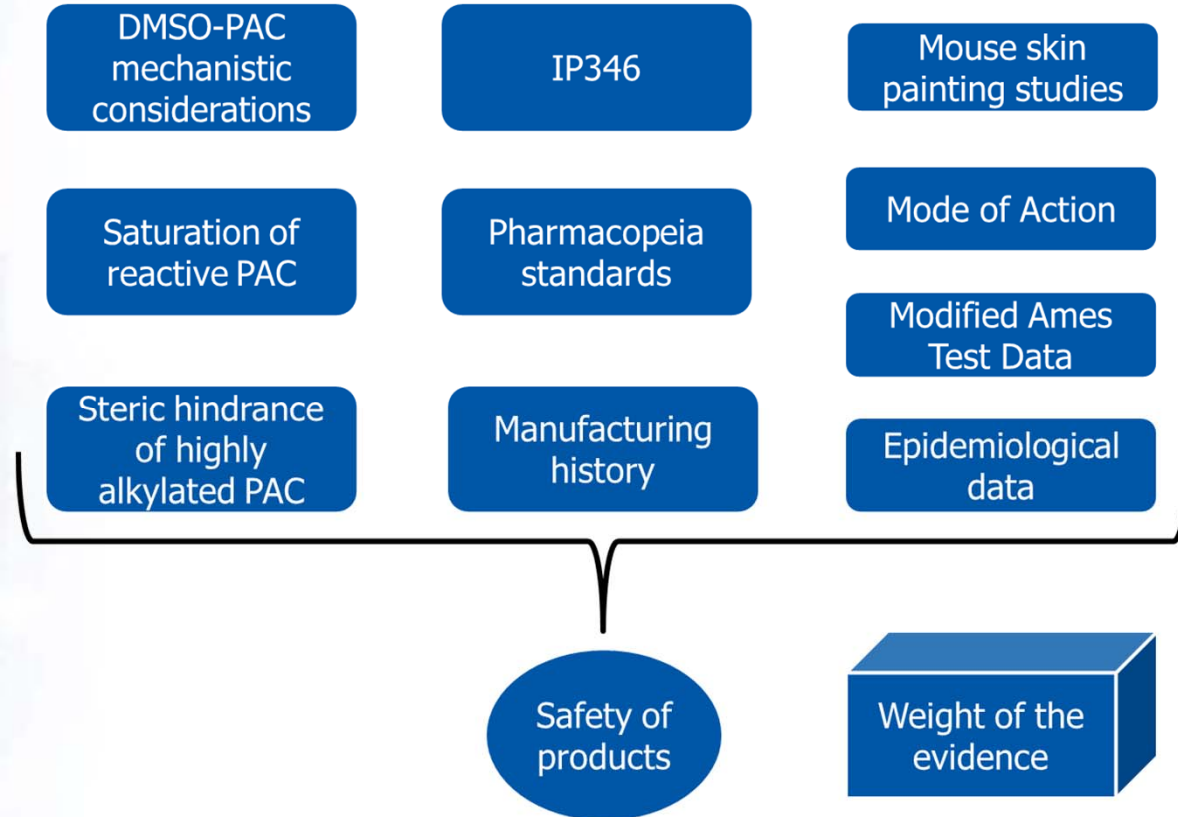
Data source: H&R



# Conclusions



# The Mineral Oil Carcinogenicity Weight of Evidence



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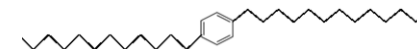
## MOAH Take Home Message

▶ The term “MOAH” does not describe the quality of the substance, because there are two types:

▶ **Bad MOAH:** 3-7 ring PAC (eliminated through refinement)



▶ **Harmless MOAH:** highly alkylated aromatics (what is left after 3-7 PAC elimination)



▶ Refinement, toxicologists and compliance tests (IP346, Pharmacopeia) focus on **Bad MOAH:** 3-7 ring PAC

▶ **Refined mineral oil** products have an impeccable history of safety: even if “MOAH” is present



**“Don’t focus on what you can measure,  
measure what you need to focus on”**

*Dirk Danneels*





**concaawe**

ENVIRONMENTAL SCIENCE FOR THE EUROPEAN REFINING INDUSTRY

European Wax Federation



**paramelt**



**ExxonMobil**

**Dirk Danneels** – EWF  
**Jan Woldhuis** – Paramelt

Olaf Kral – Shell  
Klaus Suedkamp - Shell  
Hans Ketelslegers – CONCAWE  
David Adenuga – ExxonMobil  
Daniela Heber – H&R

STF 33- CONCAWE

Mineral oils are safe  
for human health?

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# Thank You





IP 346/92  
(2004)



BS 2000 : Part 346 :1996

## Determination of polycyclic aromatics in unused lubricating base oils and asphaltene free petroleum fractions — Dimethyl sulfoxide extraction refractive index method

### REGULATIONS

REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL  
of 16 December 2008

on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

(Text with EEA relevance)

Note L:

The classification as a carcinogen need not apply if it can be shown that the substance contains less than 3 % DMSO extract as measured by IP 346 'Determination of polycyclic aromatics in unused lubricating base oils and asphaltene free petroleum fractions — Dimethyl sulphoxide extraction refractive index method', Institute of Petroleum, London. This note applies only to certain complex oil-derived substances in Part 3.

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
649-501-00-1	Lubricating oils (petroleum), base oils, paraffinic; Baseoil — unspecified; [A complex combination of hydrocarbons obtained by refining of crude oil. It consists predominantly of aromatics, naphthenics and paraffinics and produces a finished oil with a viscosity of 120 SUS at 100 °F (23cSt at 40 °C).]	297-474-6	93572-43-1	Carc. 1B	H350	GHS08 Dgr	H350			H L

