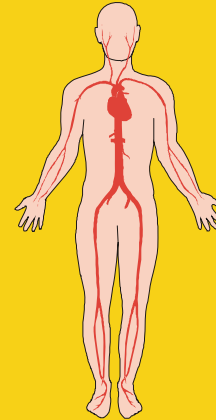




# Mammalian Toxicology & Toxicokinetics of Mineral Oil

Studies in animals  
and human  
volunteers

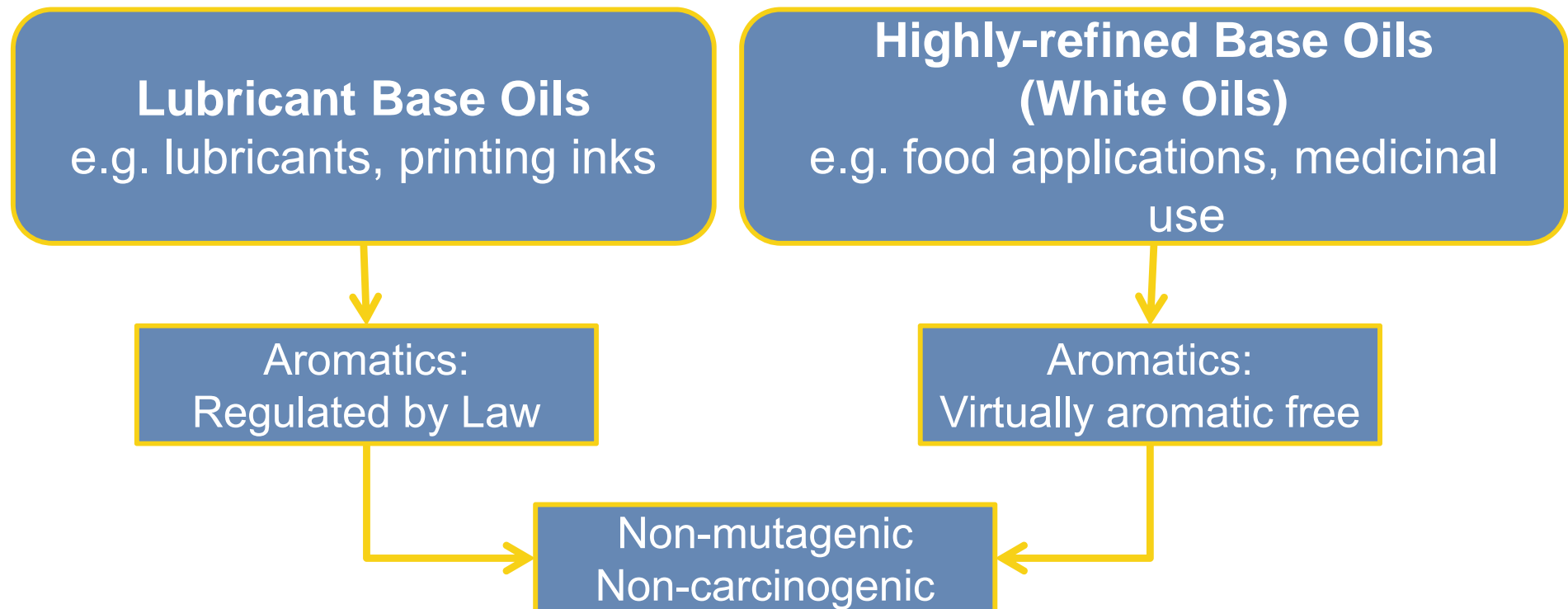


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# Mineral oils

Aim of this presentation is to provide an overview of

- how the toxicity of mineral oils (= UVCBs, hydrocarbons  $C_{15}$ - $C_{50}$ , derived from crude oil) is assessed
- give an overview of the available toxicological data



# Crude oils, refining, and lubricating oils

- Crude oils are carcinogenic, due to the presence of benzene and polycyclic aromatic hydrocarbons
  - ‘Simple’ refining doesn’t alter the molecules, just separates them into different fraction: benzene ends up in the naphtha (gasoline), polycyclic aromatics end up in heavy fuel oil and lubricating oil
  - ‘Poorly’ refined lubricating base oils are dermal carcinogens:
    - Human experience (note: dermal is only relevant route of exposure)
    - Animal studies → chronic mouse ‘skin painting studies’
  - More severe refining: solvent-dewaxing, hydrotreatment → removal/conversion of unsaturated and aromatic compounds
- Note: poorly refined oils reflect the composition of the crude oil they are derived from, but with more severe refining this variation disappears*

# Carcinogenicity assay for mineral oils

Two-year dermal mouse skin painting study:  
“golden standard”



- Biological assay

- Simultaneous assessment of all PAC present

- Undiluted oil applied to the shorn back of mice



- Endpoint:

- formation of tumours (benign/malignant)
  - time to tumour
  - benzo[a]pyrene as positive control



*note: tumourigenicity of oils is a function of PAC concentration in tissue level and not of dose volume on the skin area (Roy et al. 1988)*

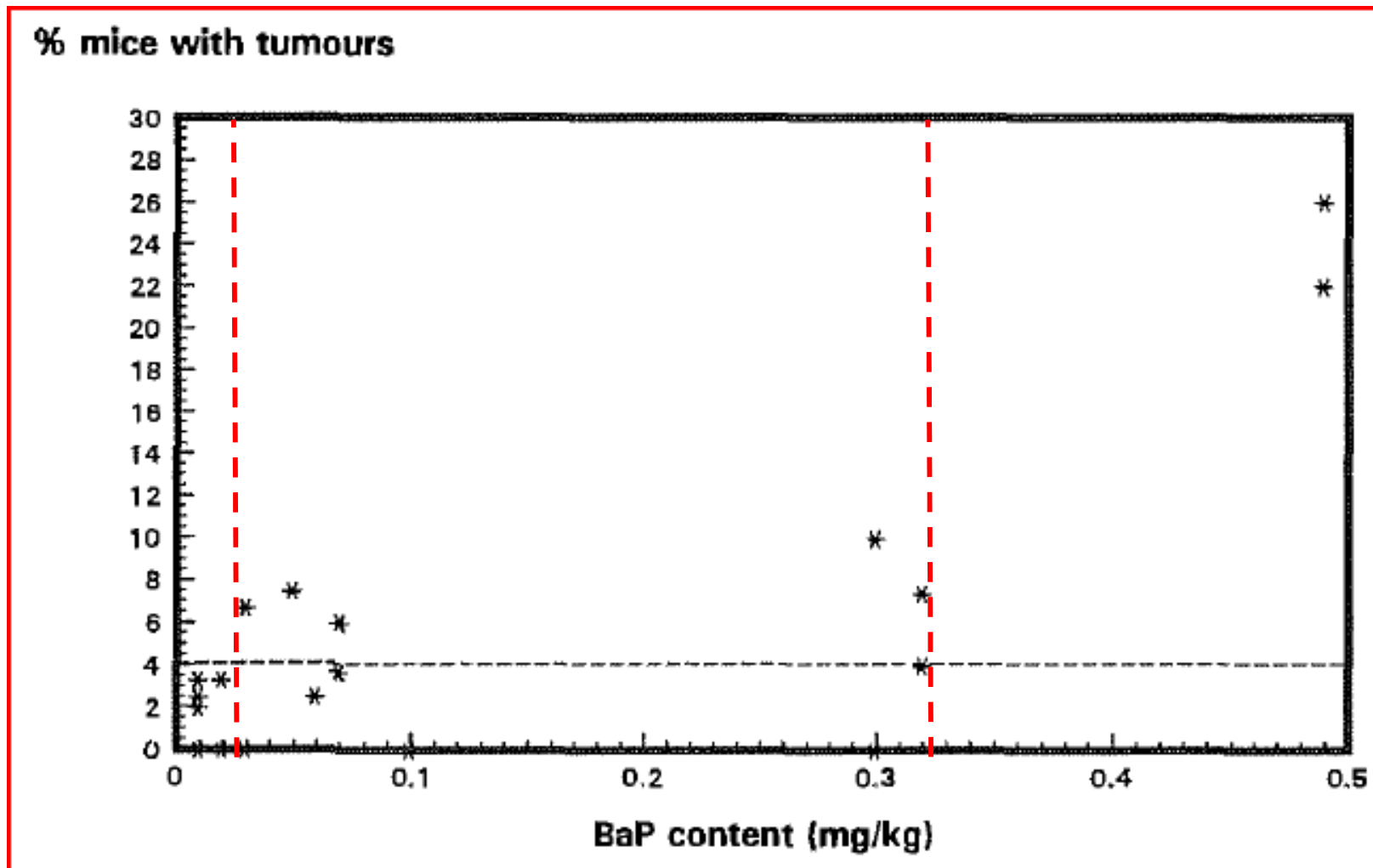
- No dose response obtained → no DMEL
- The mouse skin painting study is a pass/fail test
- Not for routine checking since it is too time-consuming

# Alternatives for mouse skin painting assay (1)

Oil Industry developed two assays for routine testing in the 80's, both based on DMSO extracts: **IP346 method** and **modified Ames' test**

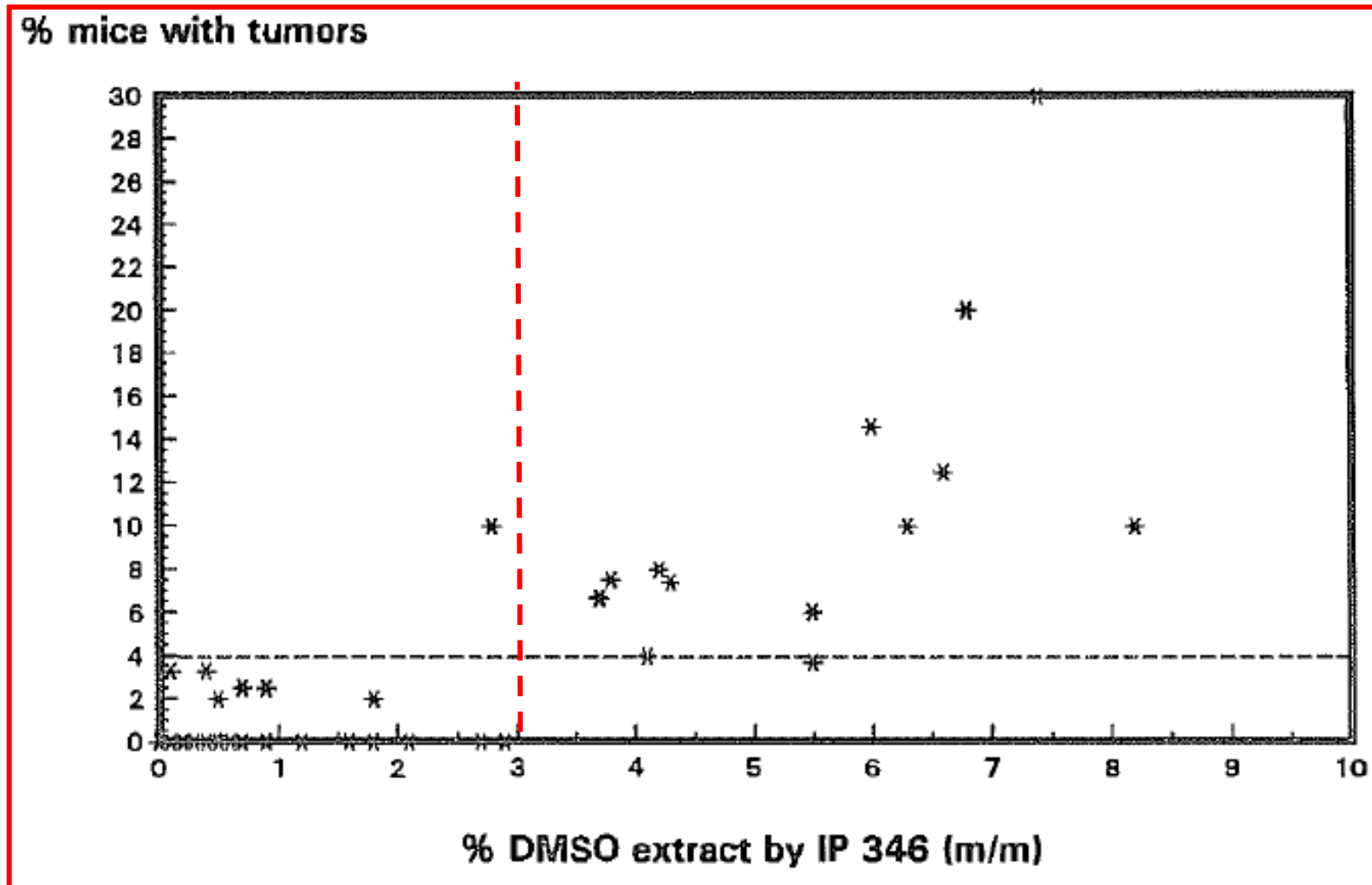
- IP 346 (legally binding in the EU since 90's; now in REACH): drives hazard assessment, classification & labelling
  - Reliable, gravimetric (mass%) routine testing method for carcinogenicity of mineral oils
  - Oil sample is extracted twice with DMSO: efficient extraction of PAC with 3 to 7 rings (plus other materials)
  - IP 346 < 3% → oil is considered safe
  - IP 346 ≥ 3% → oil is considered carcinogenic (Cat. 1B)
  - Validated against the 'golden standard' (76 studies)

# Benzo[a]pyrene not an appropriate discriminator



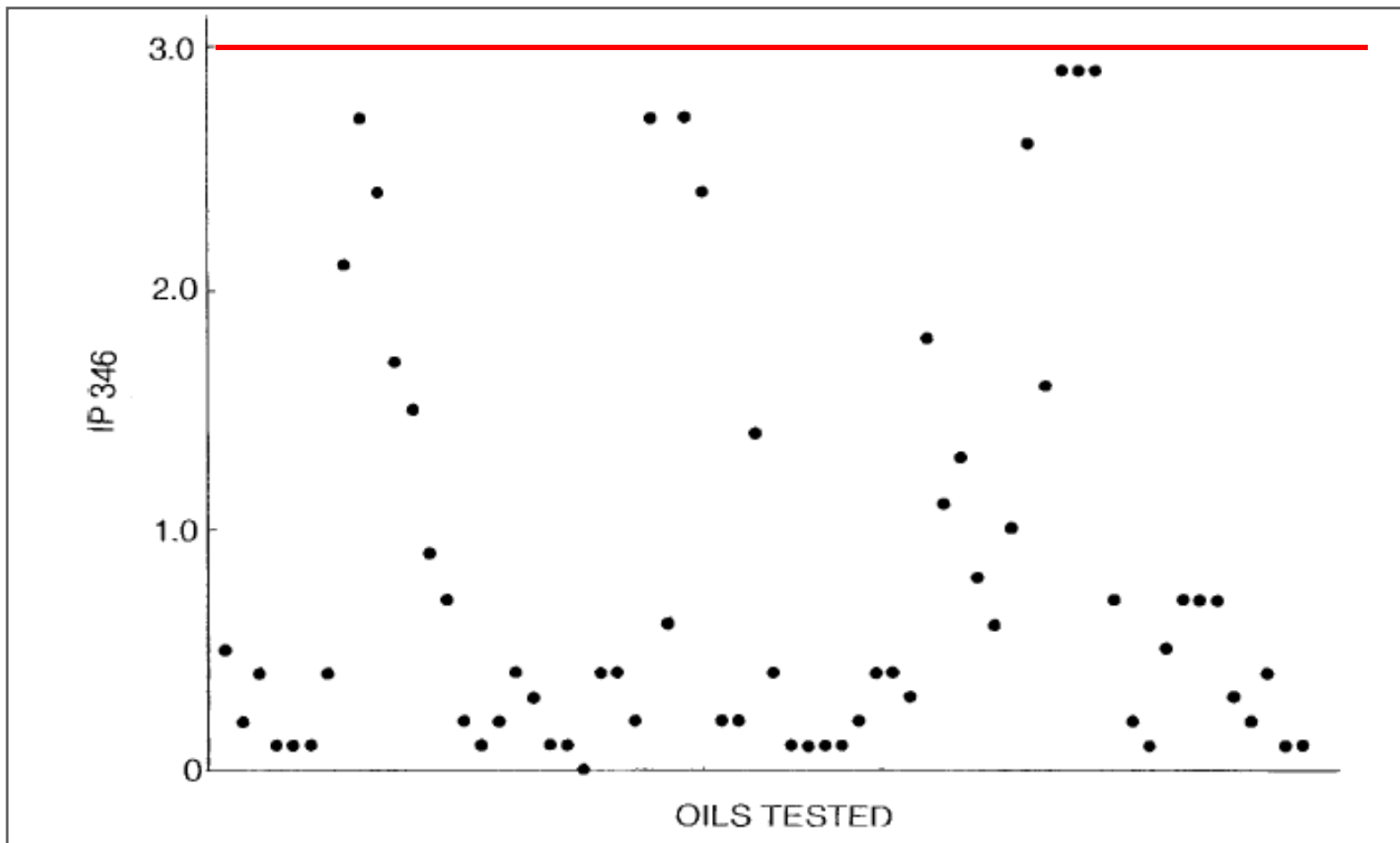
- Hazard determination by single components is not adequate!
- Benzo[a]pyrene **alone** as a marker in oils delivers **ambiguous results**

# DMSO extract by IP-346 as discriminator



- Hazard determination should always be on the whole stream!
- DMSO extract by IP 346 → one false negative, two false positives

# Technical grade oils in the market, IP 346



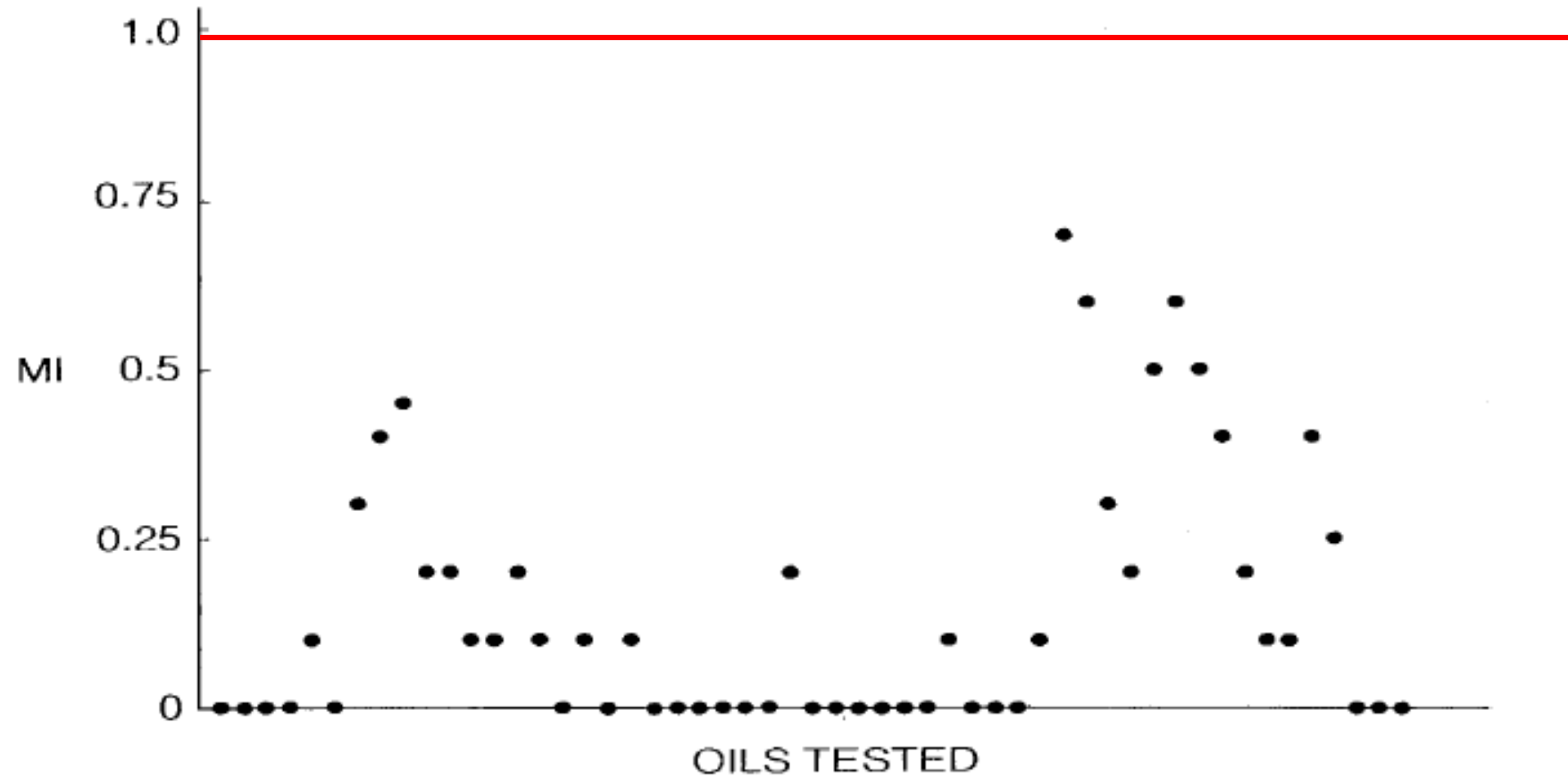
Mackerer et al, 2003



## Alternatives for mouse skin painting assay (2)

- Modified Ames' Test (ASTM E1687-10)
  - Based on the 'standard' Ames' test
  - DMSO extract (i.e. extraction of 3- to 7-ring PAC)
  - Metabolic activation with induced hamster liver S<sub>9</sub>
  - Validated against 104 mouse skin painting studies
  - Routinely applied, protocolised in American Standard Technical Method (ASTM) and updated regularly
  - Mutation Index (MI):
    - $MI < 1.0 \rightarrow$  not expected to be carcinogenic
    - $1 \leq MI < 2.0 \rightarrow$  more data needed (PAC profile etc.)
    - $MI \geq 2.0 \rightarrow$  expected to be carcinogenic (Cat. 1B)

# Technical grade oils in the market, MI



# Historical oral studies on white mineral oils

Oral repeated-dose studies in Long Evans rats and Beagle dogs with several highly-refined mineral oils (white oils) confirmed earlier studies from the 1950's and 1960's (Smith et al., 1995):

- No test-item related adverse effects (apart from mild laxative effects in dogs)
- Special staining (oil red O) of liver, kidney, spleen, mesenteric lymph nodes and gastro-intestinal tract indicated the absence of deposition or accumulation of mineral hydrocarbons (MHC)
- Overall: data support the previous conclusions that white oils are non-hazardous in nature

Note: this is in agreement with the use of white oils as 'medicinal oil' in humans for decades.

## More recent studies indicate potential problems (1)

90-Day repeated dose studies in Fischer 344 rats, which were fed 0, 20, 200, 2000, and 20000 ppm of seven highly refined base oils and five highly refined waxes, gave different results (Smith et al., 1996):

- Histiocytosis of mesenteric lymph nodes was seen with low- & medium viscosity oils & wax at all dose levels (~2 to ~1900 mg/kg/day) and inflammatory hepatic granulomata at doses > ~19 mg/kg/day
- The effects appeared more severe in female than male rats
- The severity of these effects appeared inversely related to viscosity
- Chronic dietary studies in F344 rats with high-viscosity white oils showed minor, reversible effects (Trimmer et al., 2004)

## More recent studies indicate potential problems (2)

Comparative feeding studies in Fischer 344 and Sprague-Dawley rats, which were fed low-viscosity highly-refined mineral oil for 30, 61 or 92 days at 0.2 or 2.0 %, showed distinct strain differences (Miller et al., 1996):

- Fischer 344 rats: enlarged hepatic and mesenteric lymph nodes with inflammatory microgranulomata (and increase of  $\gamma$ -GT, but not other liver enzymes) and histiocytosis of mesenteric lymph nodes (at both dose levels)
- CRL:CD rats: only indications for hepatic chronic inflammation at highest dose
- Dose dependent increase in hepatic MHC levels significantly less in CRL:CD rats than Fischer 344 rats.

## Two main toxicological effects in F344 rat

- Histiocytosis of mesenteric lymph nodes →  
JECFA (2009): most probably a non-adverse effect, representing an attempt by the histiocytes of the mesenteric lymph nodes to degrade small amounts of absorbed test article ('corpus alienum effect')
- Inflammatory hepatic granulomata →  
EFSA (2012): evidence from chronic studies indicate that there is no prolonged inflammatory response, nor are there pathological changes; however, a precautionary approach is taken by assuming that microgranulomata, as observed in the F344 rats, might be relevant for humans and therefore be the most critical effect

# JECFA request for more data

- Methodological limitations: analytics do not allow to measure mineral oil hydrocarbons in blood or tissues at low concentration (< ~10 µg/ml).
- Study with surrogate marker (<sup>14</sup>C-eicosanyl-cyclohexane, C<sub>26</sub>H<sub>52</sub>) confirmed significant higher bioavailability in Fischer 344 compared to the Sprague-Dawley rats (Halladay et al., 2002).
- New method (2010): n-hexane extraction (with d<sub>34</sub>-hexadecane as standard to check efficiency/recovery), followed by solid phase extraction, and GC×GC-MS analysis and quantification of C<sub>19</sub>–C<sub>24</sub> alkanes against d<sub>42</sub>-eicosane as internal standard: LOQ was 0.36 µg/ml for rat blood and 0.16 µg/ml for human blood.
- Accuracy at 0.5 µg/ml was 1.9 %; repeatability was 18 % for 0.5 µg/ml and <9 % for the higher concentrations; reproducibility was 23 % for 0.5 µg/ml and <11 % for the higher concentrations.

# Toxicokinetics studies in F344 and SD rats

Based on the observed effects in Fischer 344 rats in the available studies, three dose levels of low-viscosity white oil were chosen:

- 20 mg/kg body weight – the NOAEL for hepatic granulomata
- 200 mg/kg body weight – the LOAEL
- 1500 mg/kg body weight – a clear effect dose

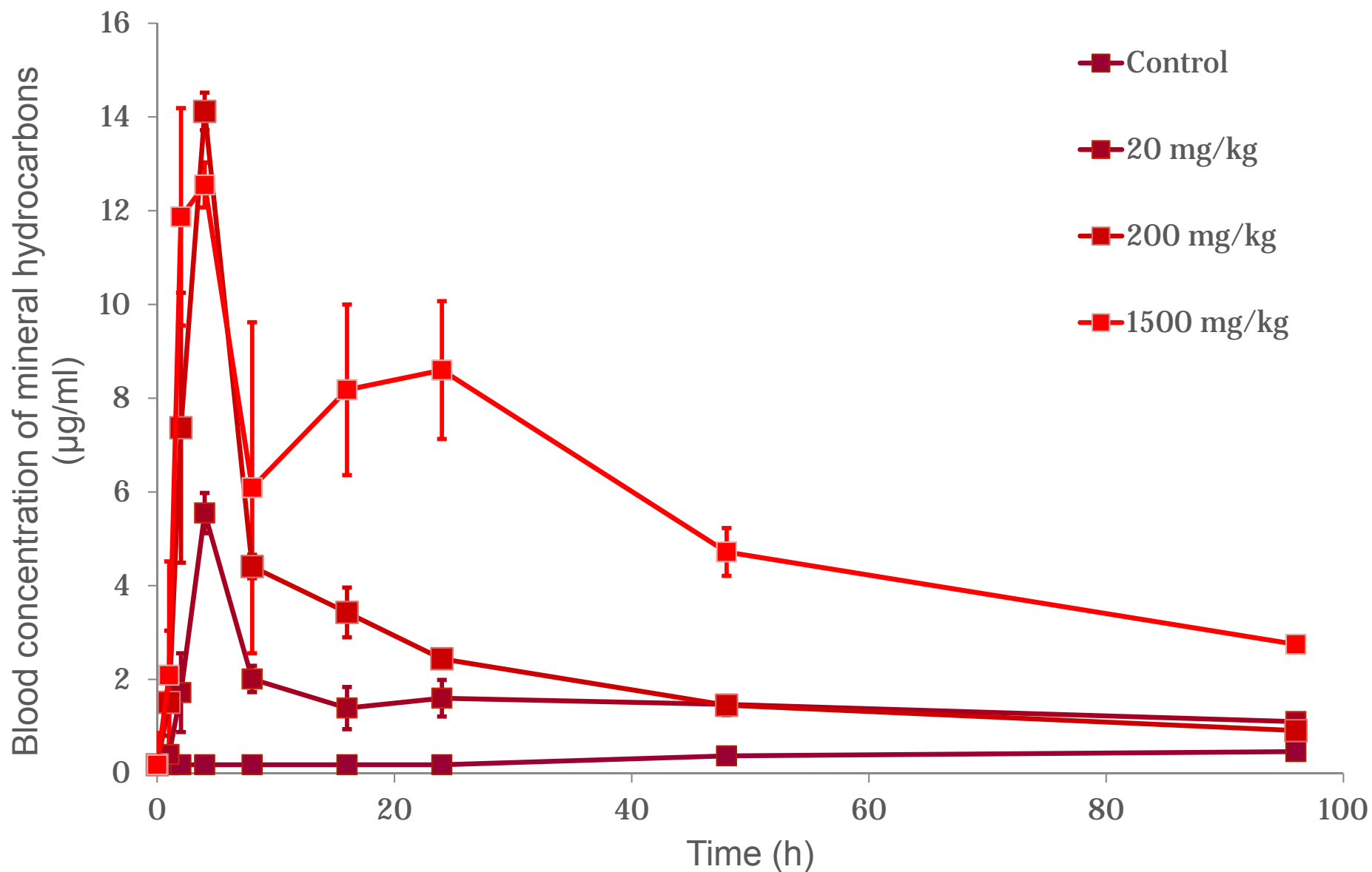
Note: the 200 and 1500 mg/kg body weight are NOAELs in the Sprague-Dawley rat.

Study: single oral dose of white oil at 20, 200 and 1500 mg/kg body weight in Fischer 344 rat, and at 200 and 1500 mg/kg in Sprague-Dawley rats

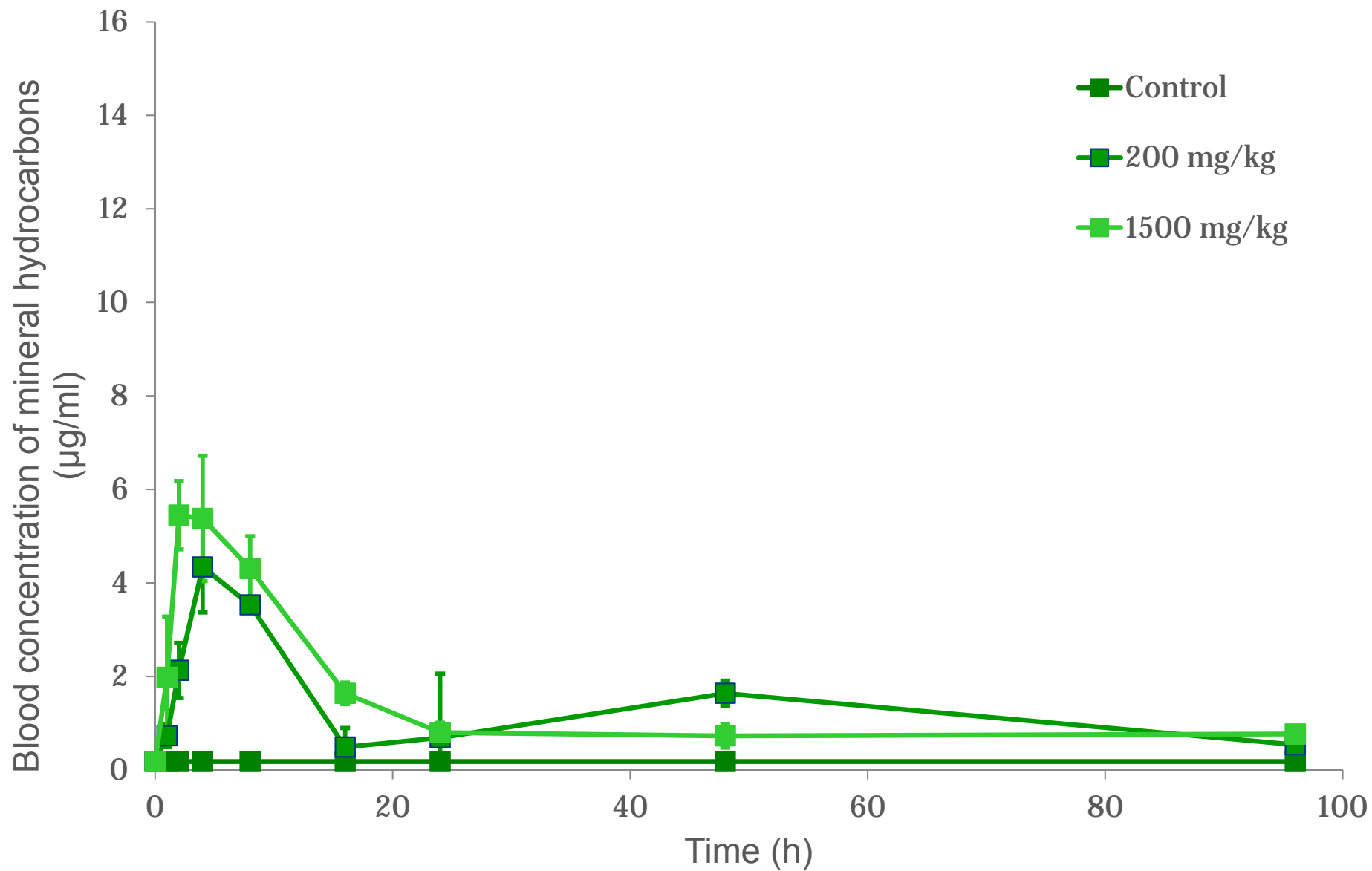
Measure mineral hydrocarbons in blood and liver over time (up to 72 h after dosing)



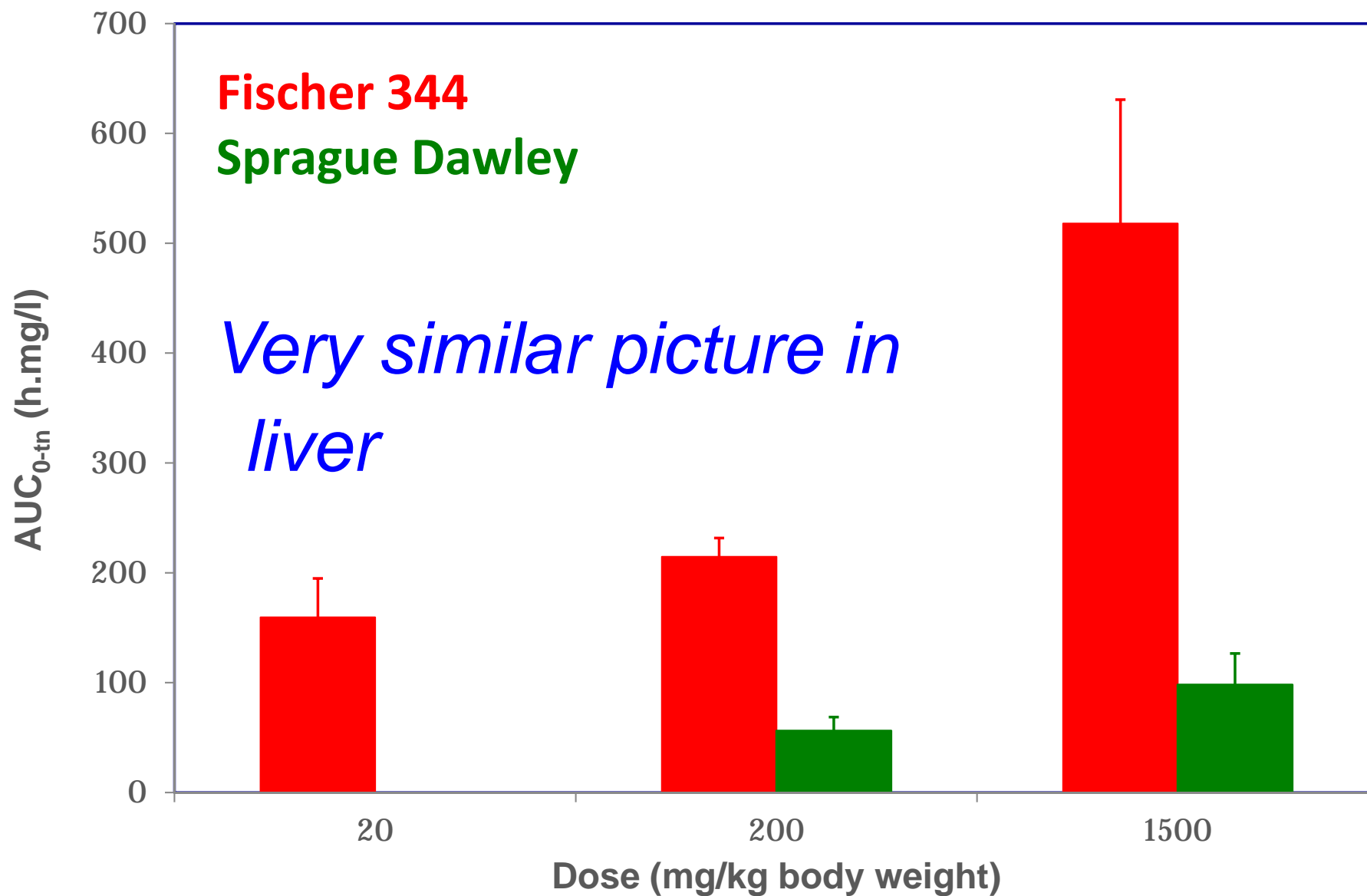
# Blood concentrations MHC in F344 rats



# Blood concentrations MHC in SD rats



# AUC of MHC in blood



# Conclusions from rat toxicokinetics studies

Studies confirm hypotheses built on experiments with surrogate markers:

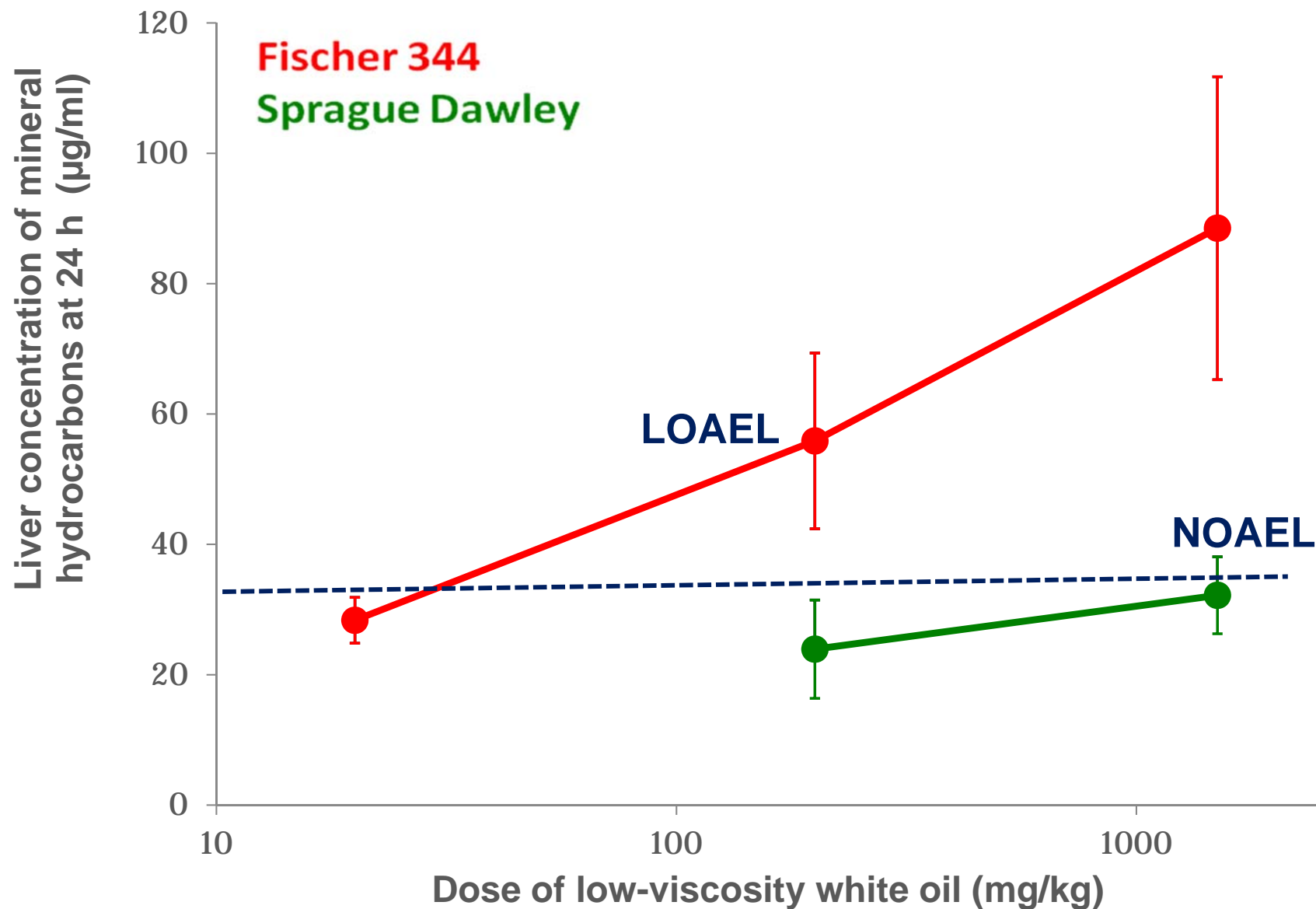
- The bioavailability of low-viscosity white oils is significantly higher in Fischer 344 rats than in Sprague-Dawley rats
- The blood concentrations of MHC reflect the hepatic concentration of MHC very well → *blood concentrations may therefore be used as an internal marker for hepatic dose* (i.e. dose in the target organ)

At (different) dose levels *without* adverse effects blood and liver concentrations of MHC are very similar in Fischer 344 and Sprague-Dawley rats.

→ Confidence that a human volunteer study can be done at safe levels

→ Toxicokinetic data allow to estimate steady-state concentrations upon continuous dosing (accumulation): 0.12, 0.20 and 0.38 mg/g liver for the F344 rat dosed with 20, 200, 1500 mg/kg day, and 0.08 and 0.15 mg/g liver for SD rats dosed with 200, 1500 mg/kg day.

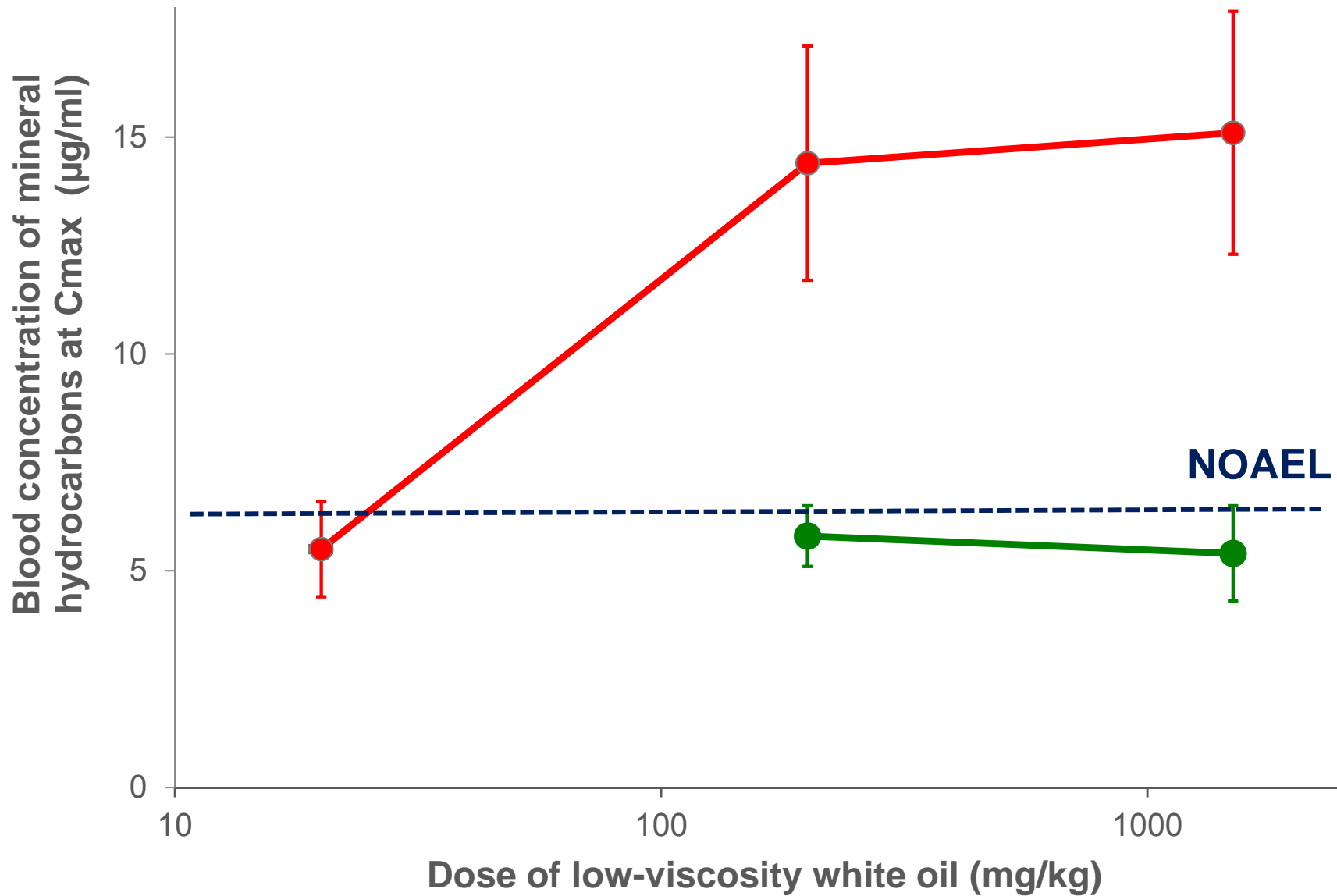
# Maximum liver concentrations of MHC in rats



# Human volunteer study

- A human volunteer study was designed based on the results from the rat studies, considering that blood concentrations in human volunteer studies may be used as internal exposure markers for liver as well.
- Based on linear extrapolation from rat data, it was anticipated that a dose of 1.0 mg/kg would result in blood concentrations that were at least 10-fold lower than threshold concentration for liver effects of 6 µg/ml observed in F344 rats (at 20 mg/kg) and SD rats (at 1500 mg/kg)
- Study was done in 9 female volunteers – females are probably more sensitive and have higher exposure to mineral oils (cosmetics).
- Selected dose level was 1 mg/kg (~ 5 times normal average daily intake).

# Maximum MHC levels in rat blood vs. effects



# Results human volunteer study

- Human volunteers were dosed with  $1.09 \pm 0.12$  mg/kg body weight (range: 0.87 to 1.26 mg/kg body weight).
- Measured blood concentrations in human volunteers were below the LOQ (0.16  $\mu\text{g/ml}$ ) at all time points in all volunteers.
- These results indicate negligible absorption at a dietary exposure of 1.0 mg/kg and, based on rat data, also negligible liver exposure.
- A blood level of  $< 0.16$   $\mu\text{g/ml}$  implies a margin-of-safety of at least 37-fold (based on NOAEL at  $\sim 6$   $\mu\text{g/ml}$  in blood).



# Conclusions

- All mineral oils on the EU market are treated to reduce/remove aromatics and comply with IP346 and the modified Ames' test; they are not expected to be a mutagenic or carcinogenic hazard
- There is a significant difference between Fischer 344 rats and other rat strains and other mammalian species in bioavailability of saturated hydrocarbons, with Fischer 344 rats being most sensitive (and humans least).
- Blood concentrations of mineral hydrocarbons track liver concentrations of mineral hydrocarbons and can therefore be used as an internal exposure marker for mineral hydrocarbons.
- In human volunteer studies large safety margins were found for an oral dose of 1 mg/kg bw
- Based on the single dose kinetics, accumulation of hydrocarbons in SD rats is significantly less than in F344 rats; in humans it is expected to be even lower.

