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MOSH Toxicology Considerations

Hepatic Granulomas

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Session Outline

- Introduction
- F344 Hepatic Granuloma
- Strain-Dependent Differences
- Human Lipogranuloma
- Points of Uncertainty
- Conclusions

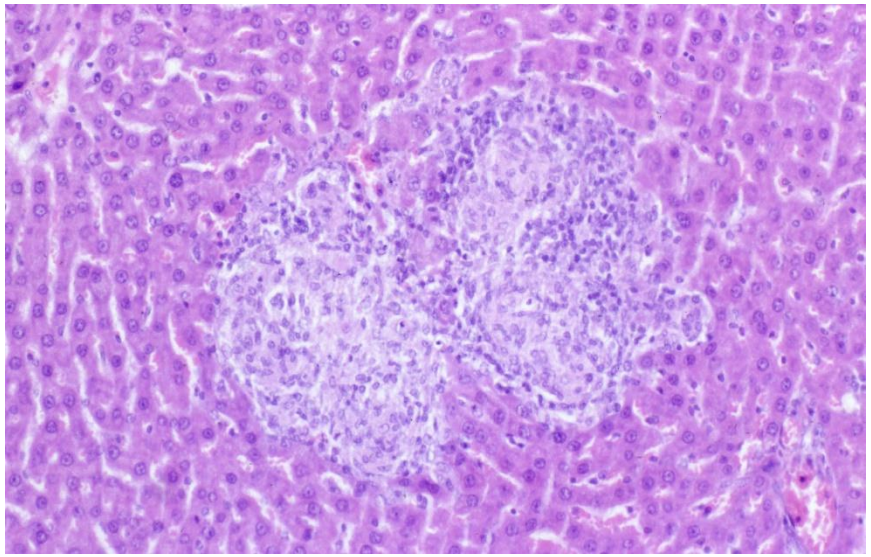
Introduction

- Mineral hydrocarbons (MHC) are used in food, cosmetic, and pharmaceutical applications

- Human health hazards and realistic exposure scenarios need to be well understood
- Food-grade, high molecular weight, saturated MHC have generally been considered safe for intended uses
  - Absence of evidence of human toxicity
  - Large body of evidence from toxicology data showing negligible systemic effects from long-term oral exposure

Hepatic Granulomas Associated with MHC

- Subchronic feeding studies of MHC in Fischer 344 (F344) rats have shown a dose-related increase in histopathologic observations in some treatment groups.
  - Observations include granulomas and microgranulomas in the liver
  - Appear to result from an inflammatory response



- **Are the observations made in F344 rats typical for and relevant to other rat strains?**
- **Are the observations made in F344 rats typical for and relevant to humans?**

Use of Animal Models in Toxicity Testing

- Animal models can help predict biological effects in humans resulting from exposures to exogenous substances.

- Test model selection considerations:
  - Sensitive indicator of exposure
  - Treatment related effects must be discernable from the background pathology that is spontaneous and unrelated to treatment
  - Observed biological effects should have some relevance and significance for human health

There is a marked contrast in granuloma occurrence between rat strains

- Subchronic feeding studies of MHC in the F344 rat have observed the occurrence of liver epithelioid granulomas.
  - *Granulomas occurring in F344 rats occurred in only certain treatment groups. Not all MHC studied produced granuloma in F344 livers.
  - *Differences in granulomatous response within F344 appear to be dependent on substance composition
- Marked contrast to the negative findings reported in numerous subchronic and chronic toxicity studies on MHC conducted in several animal models, including:
  - Sprague–Dawley (SD)
  - Long-Evans rats
  - Beagle dogs

Liver Granuloma Occurrence in MHC Studies				
Study	**F344**		**Sprague-Dawley**	**Long Evans**
90 Day Studies				
Baldwin, 1992	Granuloma		N/A	N/A
Firriolo, 1995	Granuloma		No granuloma	N/A
Smith, 1995	N/A		N/A	No granuloma
Smith, 1996	Granuloma	No granuloma	N/A	N/A
Hoglen, 1998	Granuloma		No granuloma	N/A
Griffis, 2010	Granuloma		No granuloma	N/A
McKee, 2012	Granuloma		N/A	N/A
2 Year Studies				
Shubik, 1962	N/A		No granuloma	N/A
Shoda, 1997	No granuloma		N/A	N/A
Trimmer, 2004	No granuloma		N/A	N/A

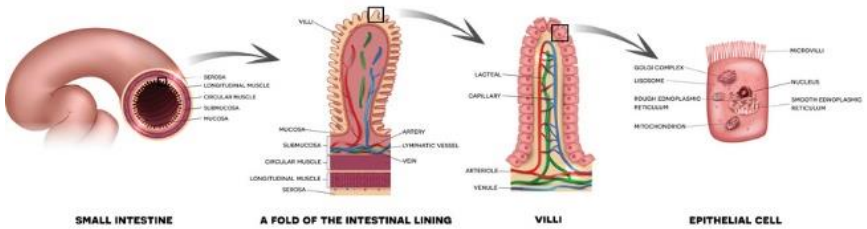
Evidence suggests that F344 rats are prone to spontaneous granuloma formation

- US National Toxicology Program (NTP) conducted a database review of 152 subchronic and chronic studies in F344 rats
  - Liver granulomas were commonly observed in untreated control F344 rats
    - Reported in control F-344 rats in 57% of the NTP studies
    - Background incidence of granulomas: 0-78%
    - Similar to those seen in F344 livers from MHC studies
    - No long term consequences on animal health or survival
    - Like with MHC studies, treatment with other test materials enhanced these observations conducted by NTP

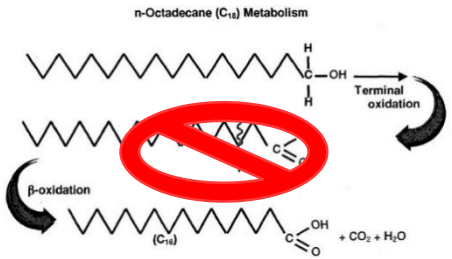
- NTP concluded: Granulomatous response in F344 rats may not be a relevant endpoint for predicting health effects in humans.

The unique etiology of MHC effects seen in F344 rats is not fully elucidated

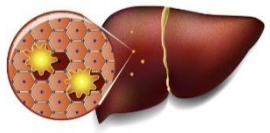
- MHC appear to be absorbed, distributed, and metabolized in a manner similar to other naturally occurring saturated hydrocarbons.
- The underlying mechanisms for species/strain differences in response to MHC is unknown, but is hypothesized to result from differences in:
  - Absorption



  - Metabolism

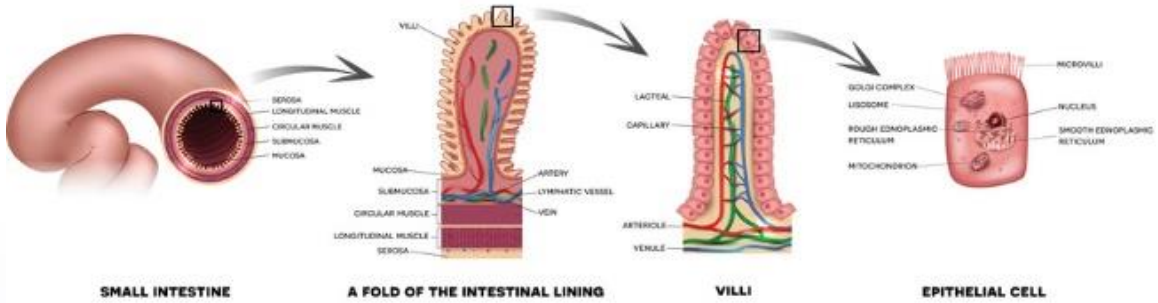


  - Inflammatory Response



Strain-Dependent Differences in Absorption

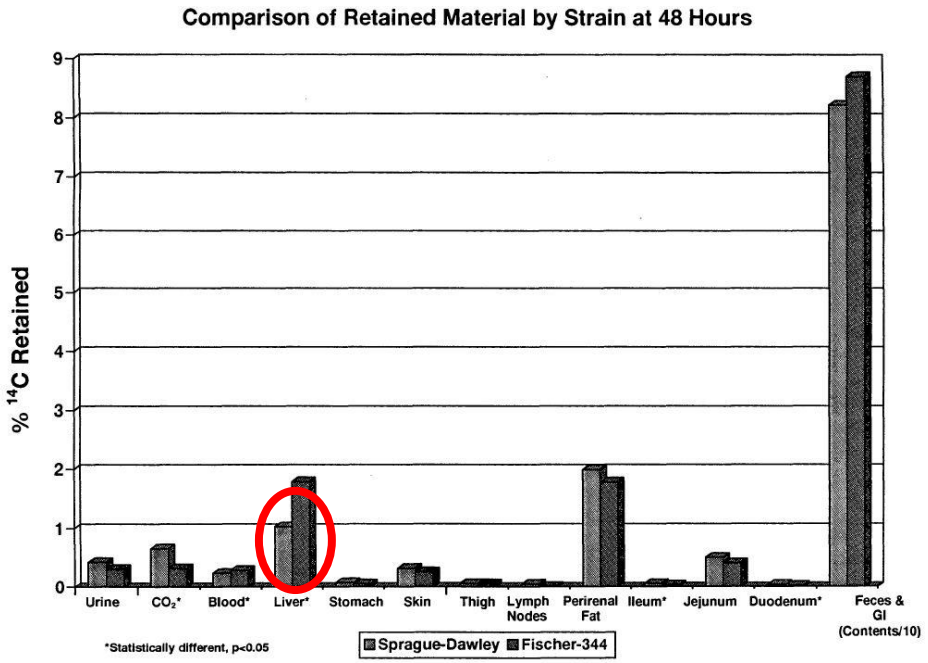
- MHC are primarily absorbed in the small intestine and transported to the body through the lymphatic system (Albro and Fishbein, 1970; Albro and Thomas, 1974;).



- Studies suggest strain-dependent differences in MHC absorption:
  - Radiolabeled MHC values were 2.7-fold higher in F344 rats compared to SD rats (Halladay et al, 2002).
  - MHC absorbed into the systemic circulation at a 4-fold higher concentration in the F344 rats at the same oral dose compared to SD rats (Boogaard et al, 2012).

Strain-Dependent Differences in MHC Metabolism

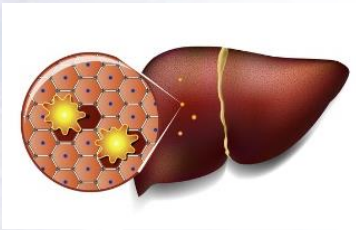
- MHC undergo oxidative metabolism in the liver
- Studies suggest strain-dependent differences in MHC metabolism
  - SD rats have more efficient metabolism of saturated hydrocarbons and are less sensitive to MHC exposure as compared to F344 (Cravedi & Perdu, 2012; Lonardo, 1998)
  - The oxidative metabolism of hydrocarbons shows species differences and appears to be mediated through cytochrome P450 enzymes (Perdue-Durand and Tulliez, 1985).
  - SD rats contain more than twice the specific activity of hepatic microsomal epoxide hydrolase than do F344 rats (Glatt and Oesch, 1987).



Source: Lonardo, 1998

Strain-Dependent Differences in Inflammatory Responses

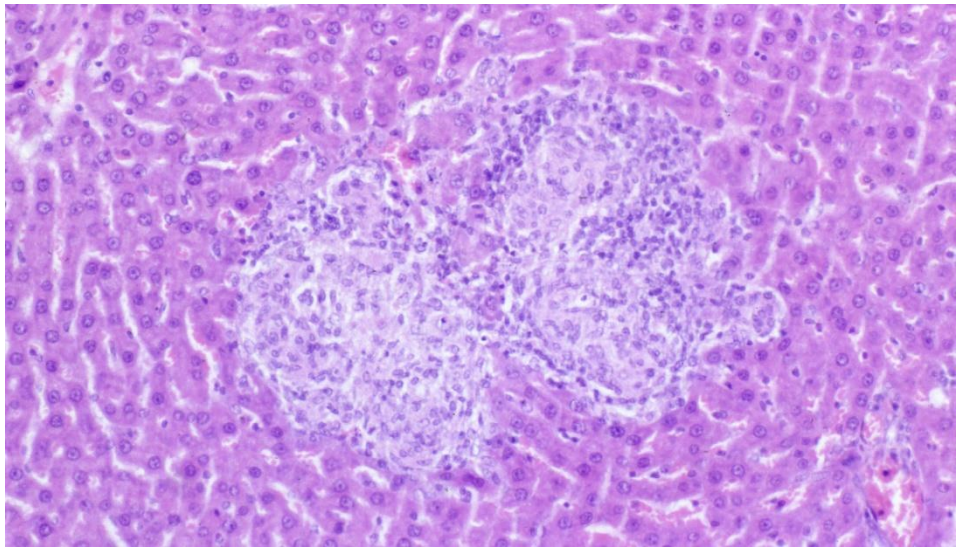
- Studies suggest strain-dependent differences in MHC inflammatory response
  - At similar target tissue concentrations of MHC, F344 rats exhibit inflammatory lesions, whereas no response is seen in SD rats (Griffis et al, 2010).
  - F344 rats showed evidence for severe microgranulomas and increased serum levels of ALT and AST, indicative of liver injury. No comparable effects were found in SD rats (Hoglen, 1998)



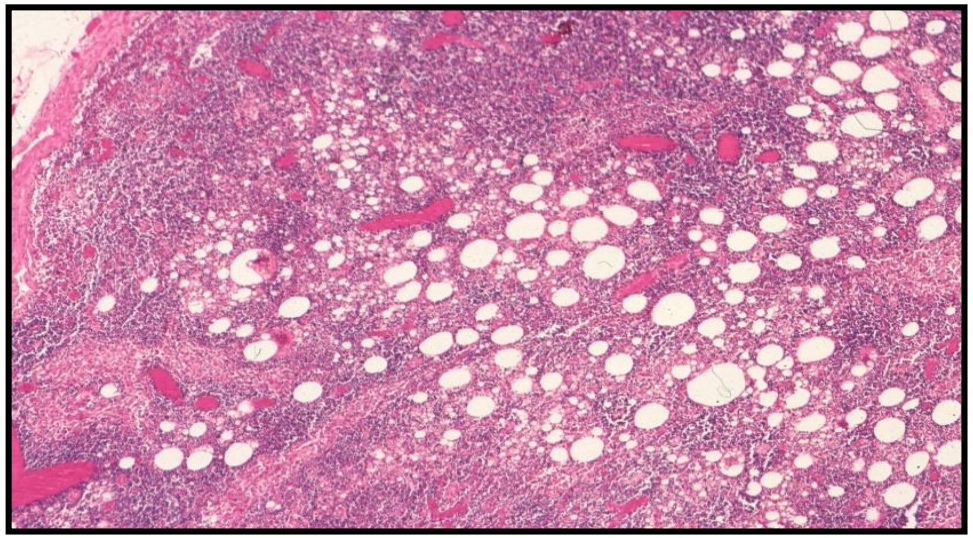
- Differences in resident liver macrophages (Kupffer cells) (Decker, 1990; Matsuo,1985; Flemming, 1998; Carlton, 2001).
  - One of the largest populations of fixed macrophages in the body
  - Phagocytize cells and other particulate material that enter the hepatic sinusoids.
  - Secrete a number of vasoactive and toxic mediators involved in the host defense mechanisms

F344 hepatic granuloma are morphologically distinct from those observed in humans.

F-344 High Dose Liver
Epithelioid Granuloma



Human Autopsy
Lipogranuloma



Human Lipogranuloma

- Saturated hydrocarbons are found in human livers (Boitnott and Margolis, 1970; Cruickshank, 1984; Dincsoy, 1982; Wanless & Geddie, 1985; Salvayre, 1988; Duboucher, 1988)
  - From MHC and natural plant sources
- Human hepatic lipogranulomas are benign, circumscribed lesions, containing lipids in the center
  - No evidence of inflammation or fibrosis
  - Not associated with any adverse clinical effects
- These findings are in contrast to the liver granulomas observed in F-344 rats
  - Human exposure to MHC does not result in F-344 rat-type epithelioid granulomas in liver (Carlton, 2001; Duboucher, 1988; Fleming, 1998; Nochomovitz, 1975)
- The European Food Safety Authority (EFSA) reviewed the data on the incidence of human hepatic lipogranuloma
  - "The current incidence is very low and do not appear to have any adverse consequences".

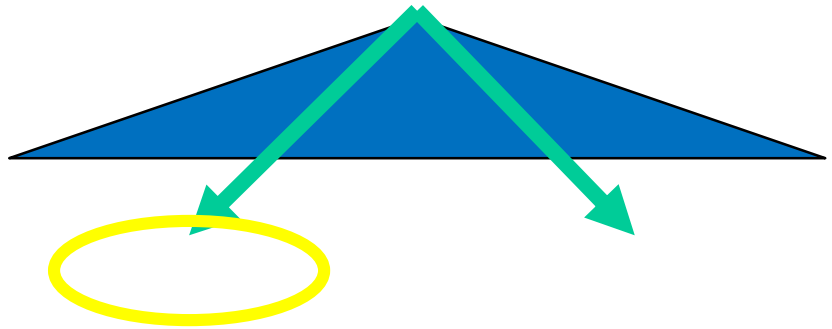
Points of Uncertainty

- Hypothesized differences in the pharmacokinetics of MHC, between F344 rats and humans, remains to be fully elucidated

- Underlying mechanisms responsible for the unique response in F344 rats have been suggested
  - Suggested rates of MHC absorption differ between rats and humans
  - F-344 rat appears to have a less efficient rate of MHC metabolism compared to other species/strains, including humans.
    - Differences in enzymatic induction
  - F-344 rat appears to be particularly sensitive in its inflammatory response to MHC compared to other species/strains, including humans.
    - Differences in Kupffer cell activity

Conclusions (1/3)

- Risk is a function of both hazard and exposure



- Safety assessments should be based on the most relevant animal model to humans.
  - The occurrence of strain/species differences in response to MHC complicates the extrapolation of animal toxicity data to humans.
  - Extrapolations are often conservatively based on data obtained from the most sensitive animal species, unless it can be shown that the response in that species is not relevant to humans

Conclusions (2/3)

- **Are the observations made in F344 rats typical for and relevant to other rat strains?**
  - Subchronic feeding studies of MHC in the F344 rat have shown a dose-related increase in observations of liver epithelioid granulomas in some treatment groups.
  - Marked contrast to the negative findings reported in numerous subchronic and chronic toxicity studies on MHC conducted in several animal models.
  - The underlying mechanisms for species/strain differences in response to MHC is unknown, but is hypothesized to result from differences in absorption, metabolism, and/or inflammatory responses.

Conclusions (3/3)

- **Are the observations made in F344 rats typical for and relevant to humans?**
  - Epithelioid granulomas observed in F-344 rats exposed to MHC are morphologically different from lipogranulomas observed in humans.
  - Human hepatic lipogranuloma incidence is low and they have not been associated with any adverse clinical effect.
  - It is unlikely that extrapolation of hepatic granuloma effects from F-344 rats are informative to human health risk assessments.

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Questions

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