Mineral oil hydrocarbons: OVERVIEW OF METABOLIC DIFFERENCES

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Part of the data are coming from the EFSA Negociated Procedure NP/EFSA/CONTAM/2011/03*

**The views expressed herein are those of the author and do not necessarily reflect the views of the European Food Safety Authority or of any other institution, agency or body of the European Union*

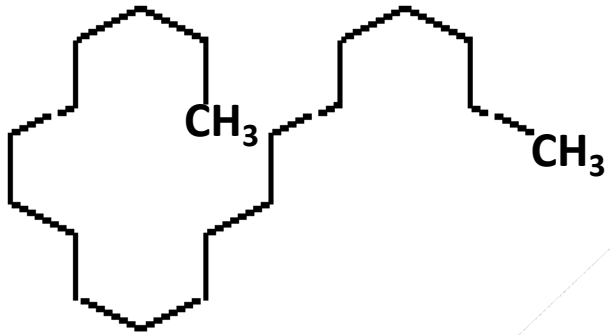
Rationale

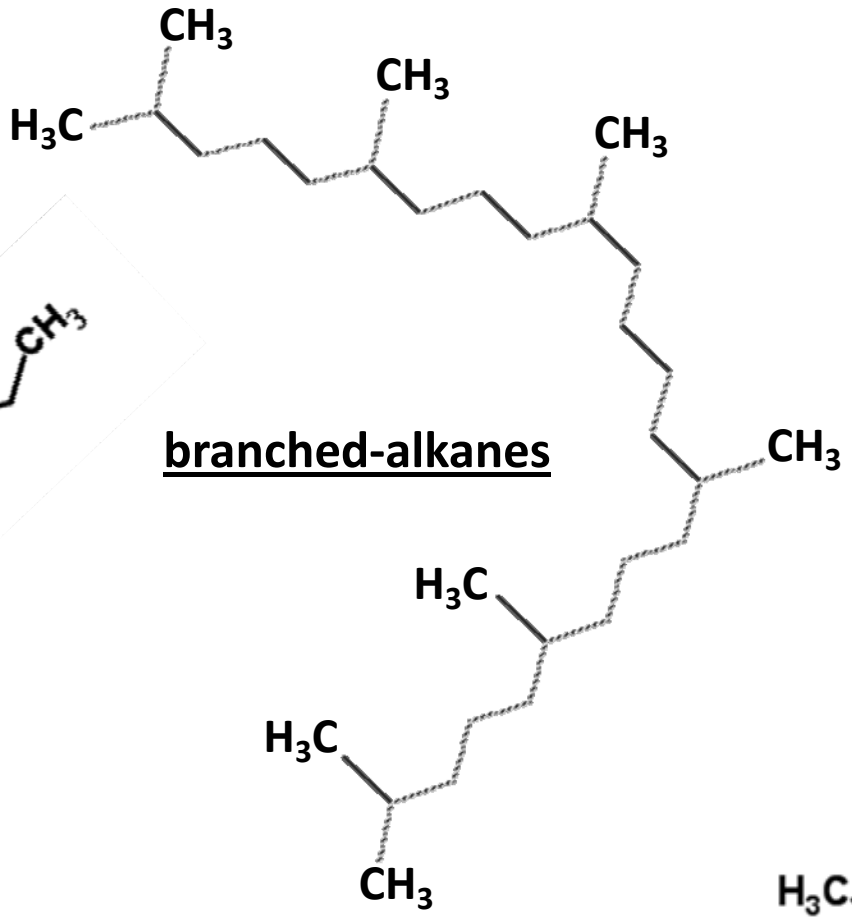
- MOH may accumulate to a significant extent in animals and humans and substantial differences in the toxicity of these hydrocarbons have been reported between rat strains.

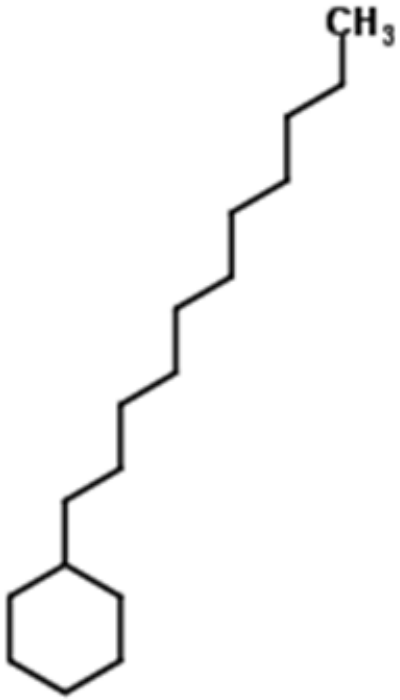
- Metabolism is the major defense mechanism in living organisms to prevent accumulation of lipophilic compounds such as MOH.

- Both biotransformation pathways and metabolic rates are considered to have an impact on the capacity of an organism to eliminate MOH

Some examples of mineral oil saturated hydrocarbons (MOSH)



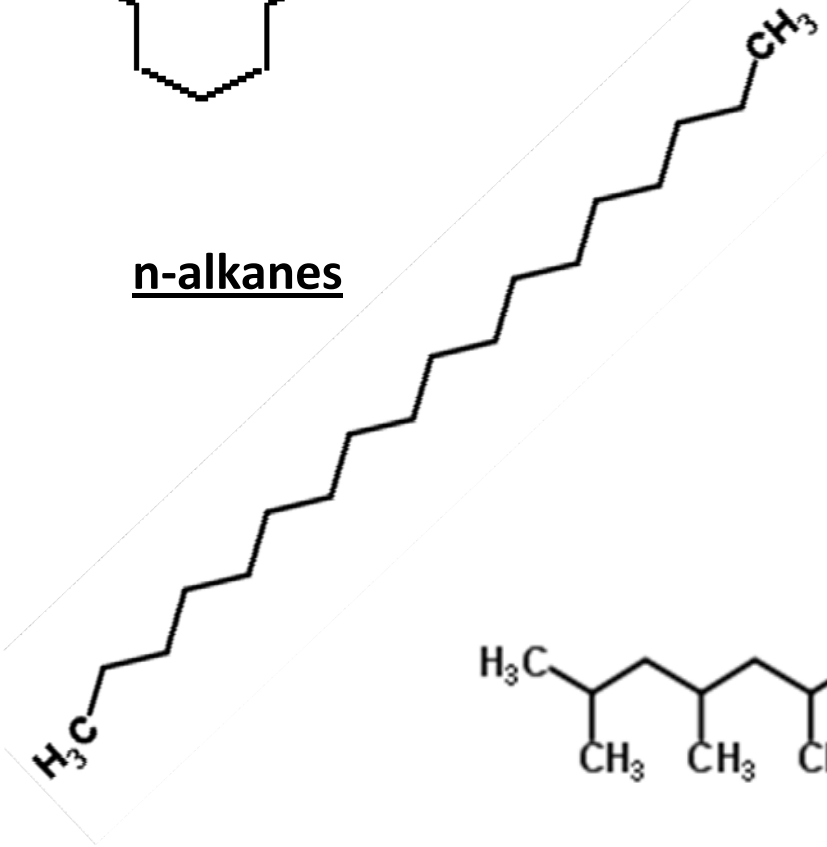


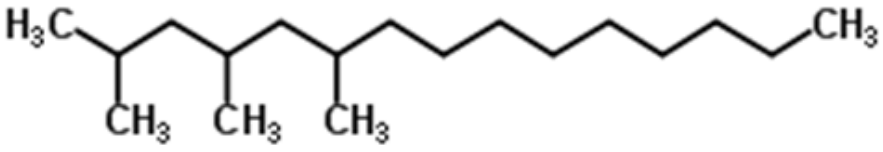


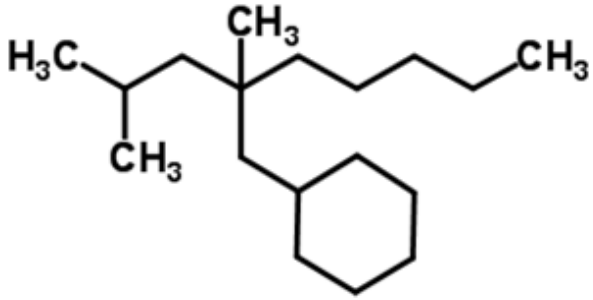
n-alkanes

branched-alkanes

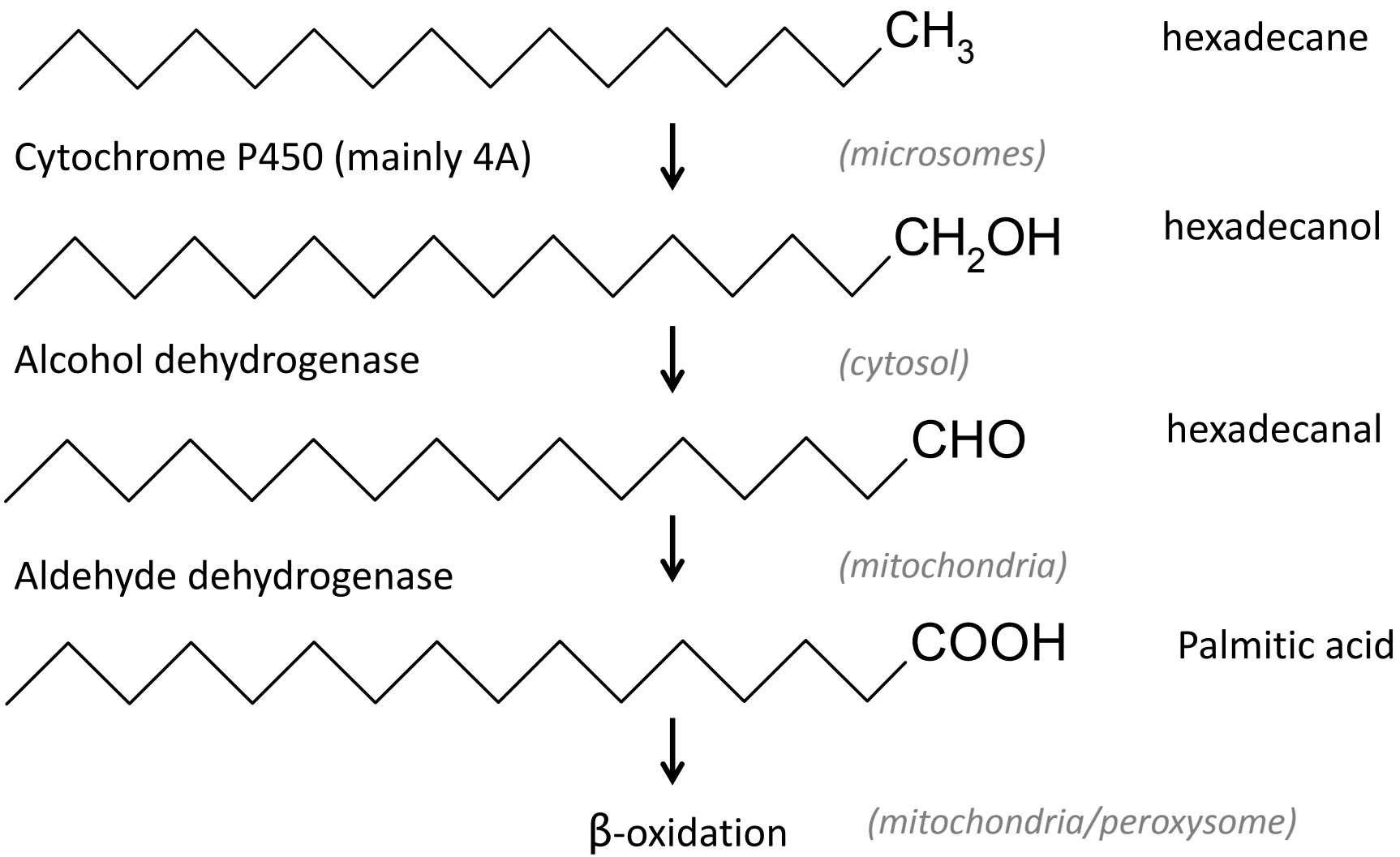
Naphthenic hydrocarbons



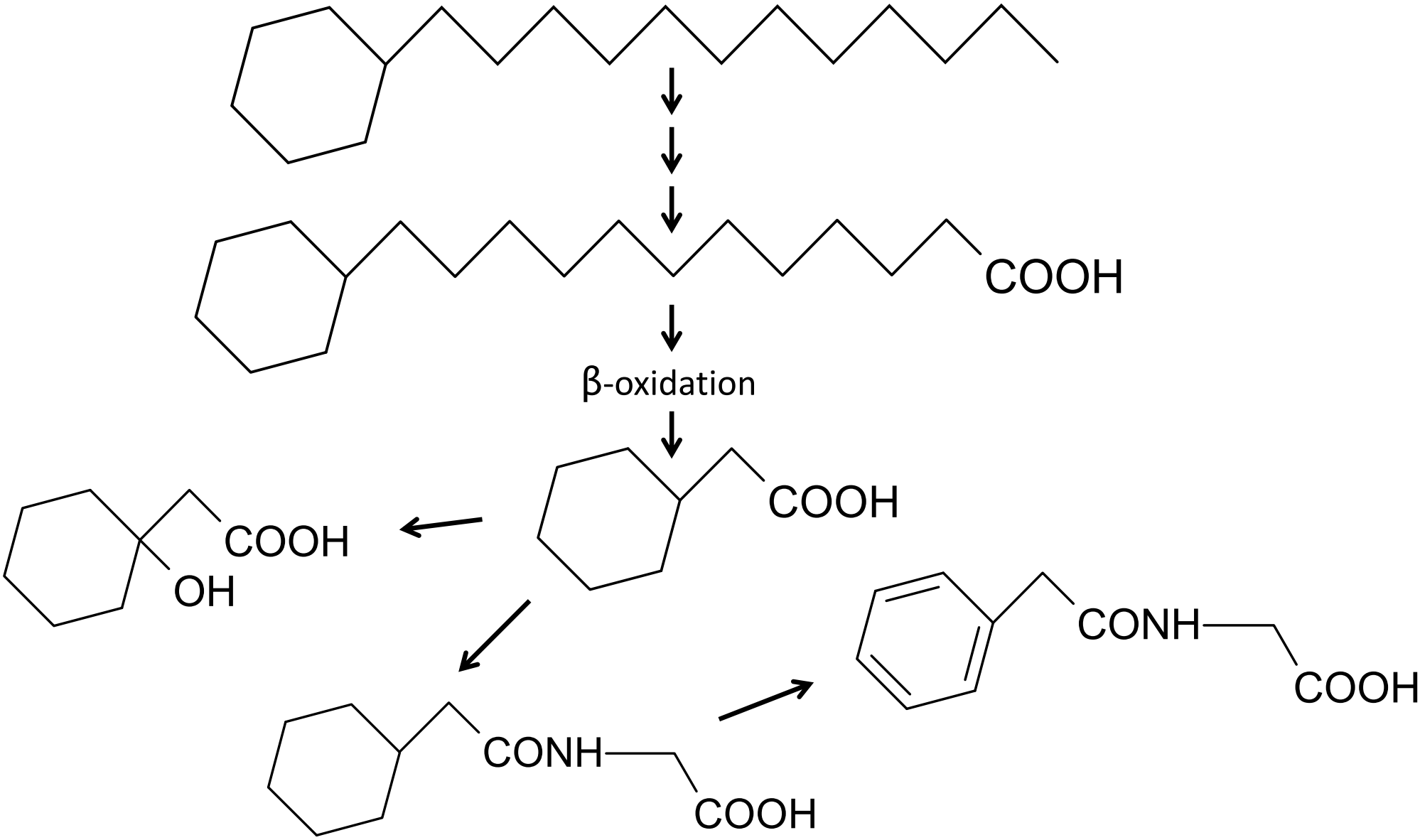




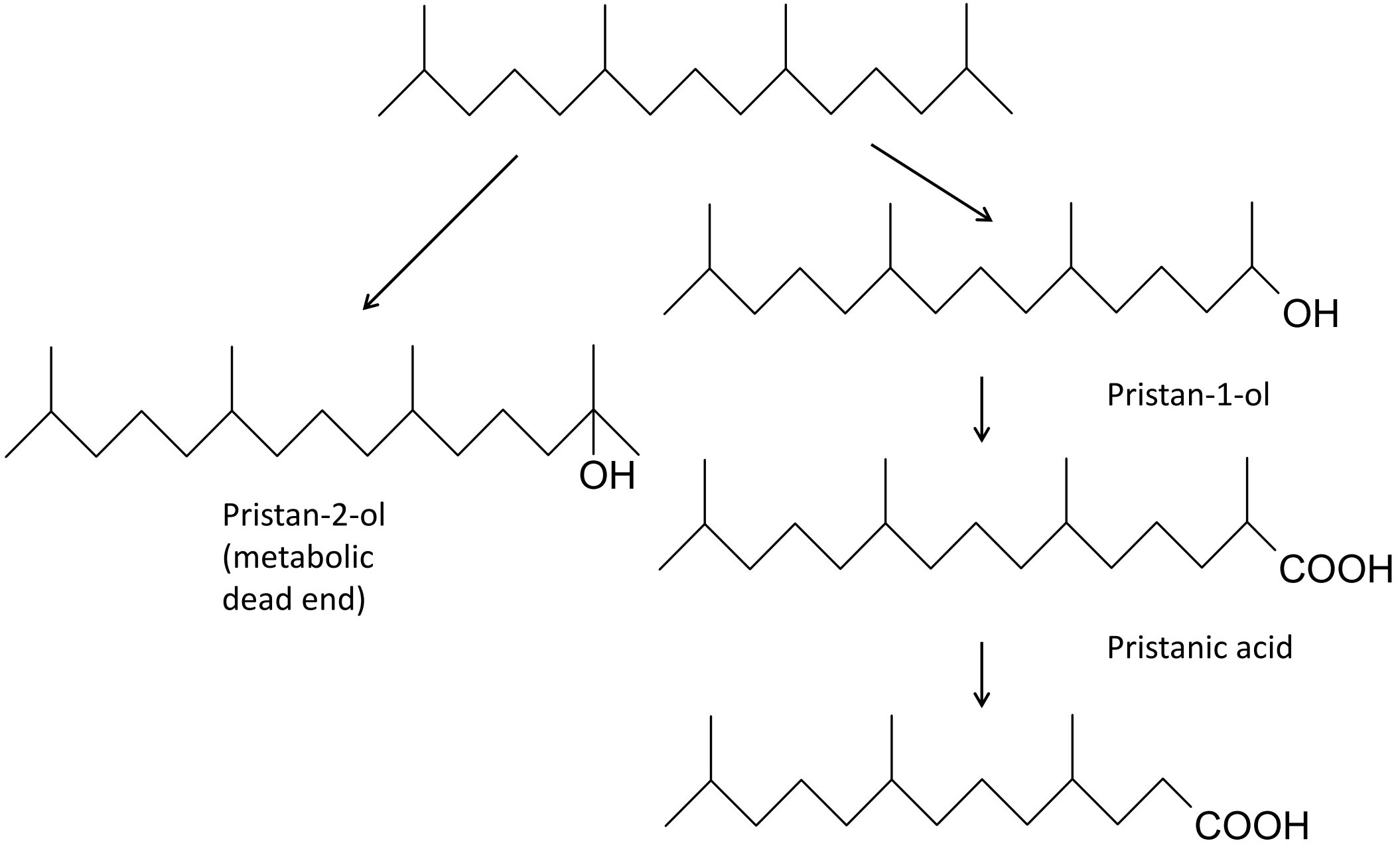
Metabolic pathways of n-alkanes (e.g. hexadecane)



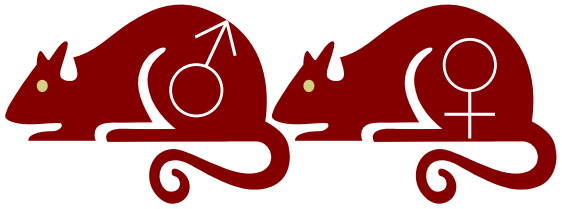
Metabolic pathways of cyclo-alkanes (e.g. dodecylcyclohexane)



Metabolic pathways of branched-alkanes (e.g. pristane)



Metabolic rate of alkanes : in vitro approach



WISTAR SPRAGUE DAWLEY F-344

LIVER
(triplicates)

PREPARATION OF SUBCELLULAR FRACTIONS

6 different pools (3 males and 3 females) of microsomes from at least 10 donors each

Microsomes (2 mg proteins)
+
^{14}C-heptadecane (3 conc.)
or
^{3}H-pristane (3 conc.)
or
^{3}H-dodecylcyclohexane (3 conc.)

0.5 mL incubations with 1mM NADPH, 1mM NADP
1mM NAD, 3 mM glucose-6-phosphate,
2IU glucose-6-phosphate dehydrogenase,
carried out at 37°C for 2 hr

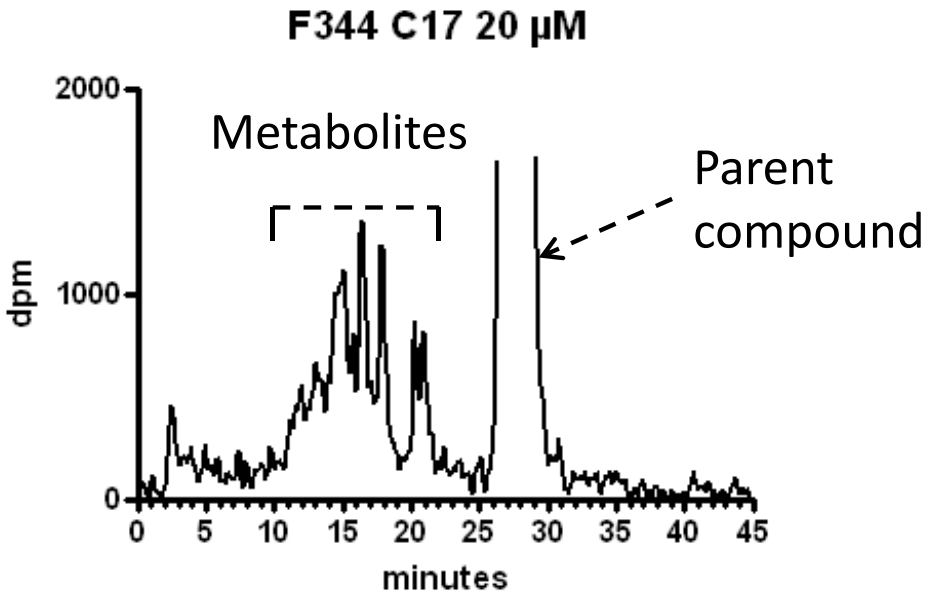
Analytical issues

INCUBATES

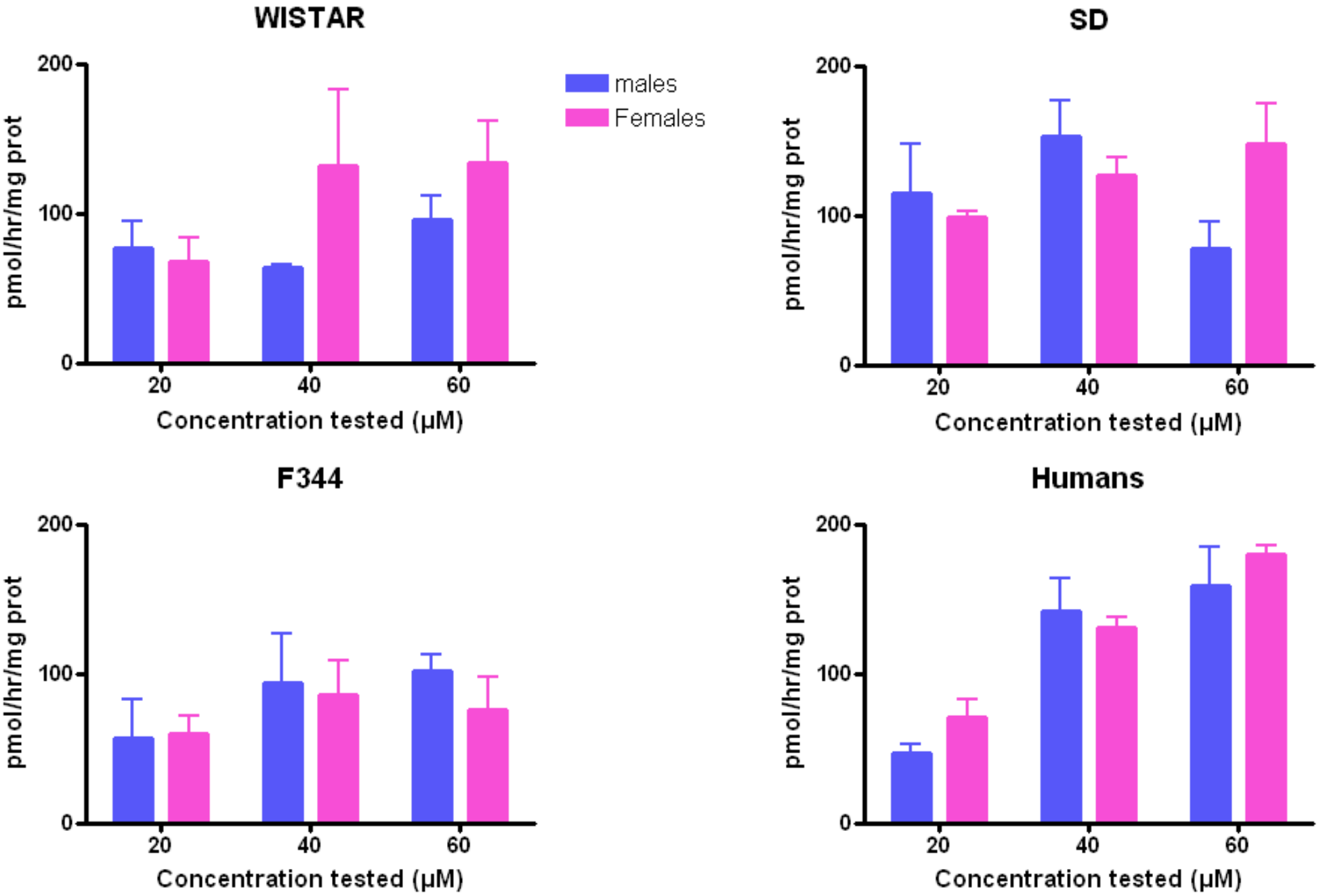
Ethyl acetate extraction

Radioactivity counting

Radio-chromatography profiling



Metabolic rate of heptadecane incubated with hepatic microsomes from rats and humans

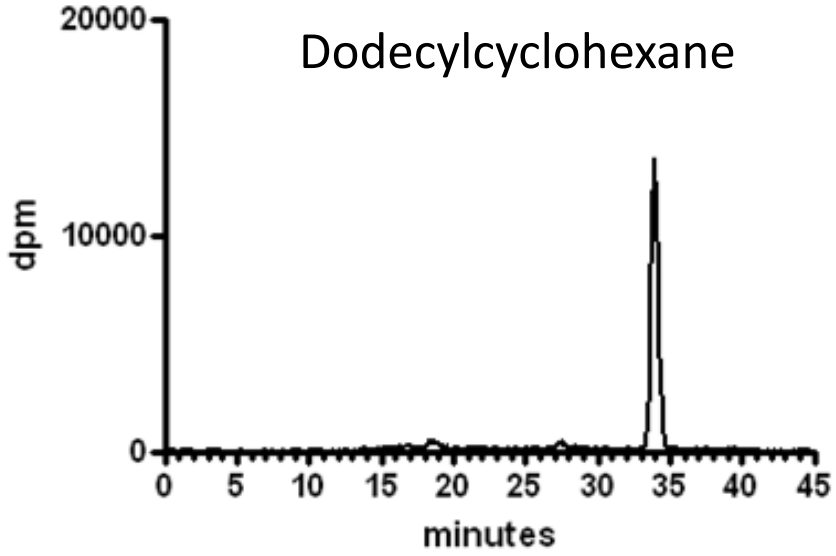


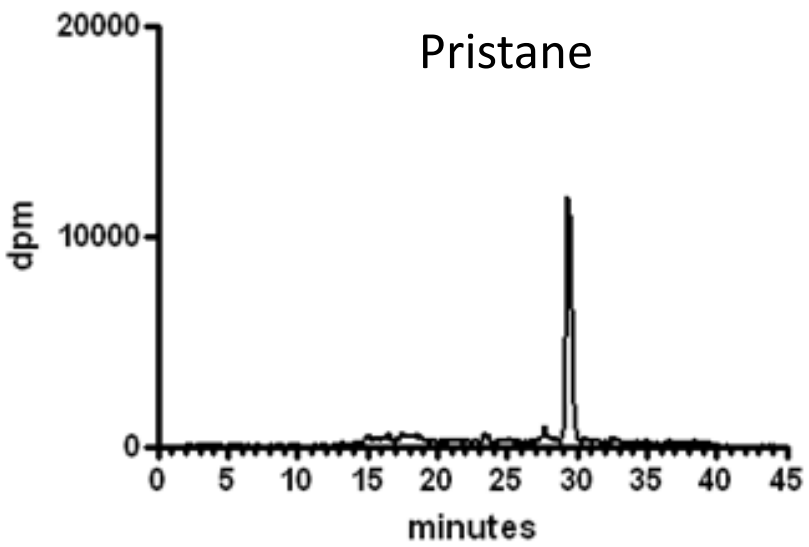
Statistical analysis

Intergroup comparisons (alkane concentration*Biological model or sex) were performed by a two-way ANOVA followed by Bonferroni and Tukey post tests. Differences between groups were considered significant when $p<0.05$.

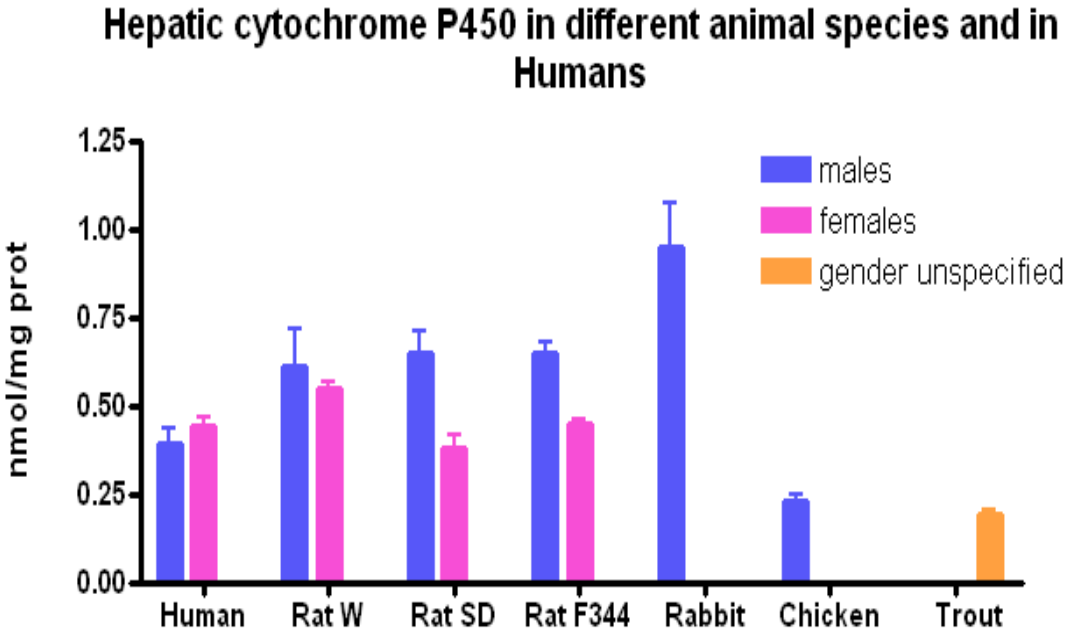
- Males: No significant difference between rat strains or between rats and humans.
- Females: significant difference between F-344 and SD strains, and between Humans and F-344.
- No significant difference between males and females.
- For n-heptadecane microsomal enzyme involved in the hydroxylation were saturated from 20 µM in rats whereas the highest concentration tested (60 µM) was likely below the saturating substrate concentration for humans.

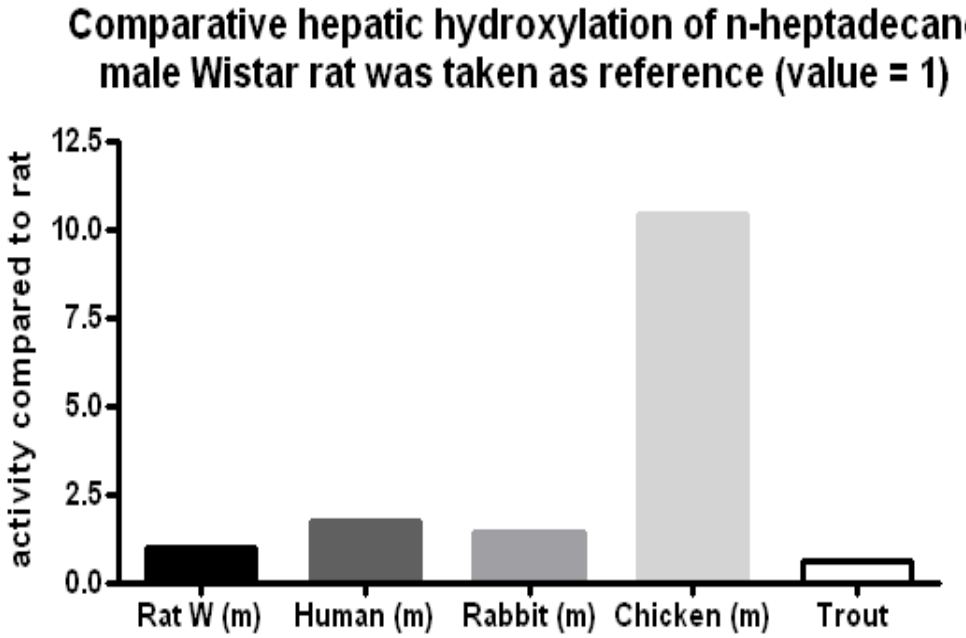
Radio-hplc profiles of dodecylcyclohexane and pristane incubated with male and female rat liver microsomes (20µM)

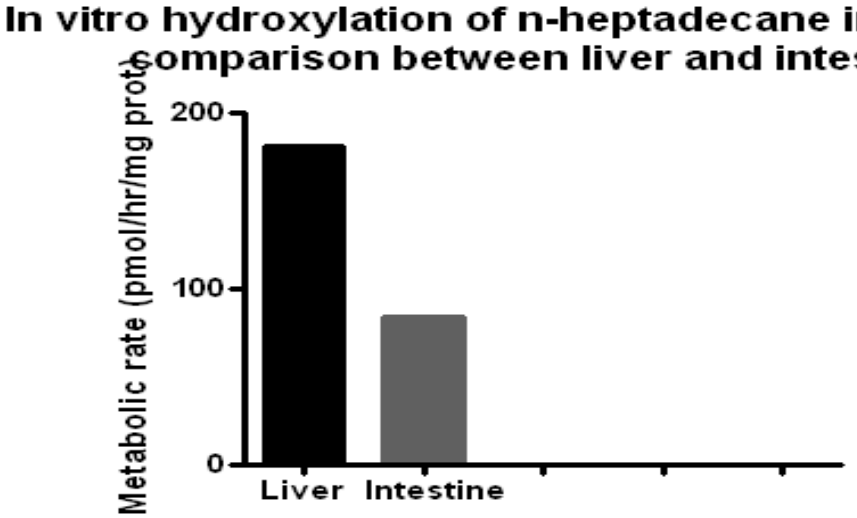




Metabolic differences between animal species and humans and between liver and intestine





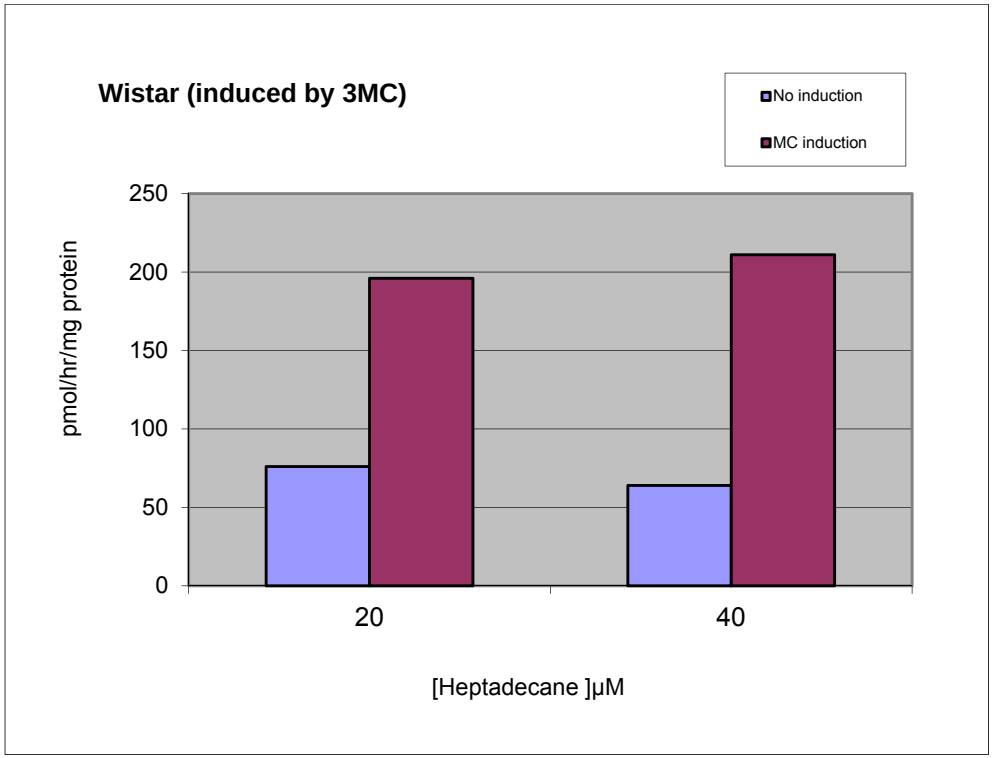


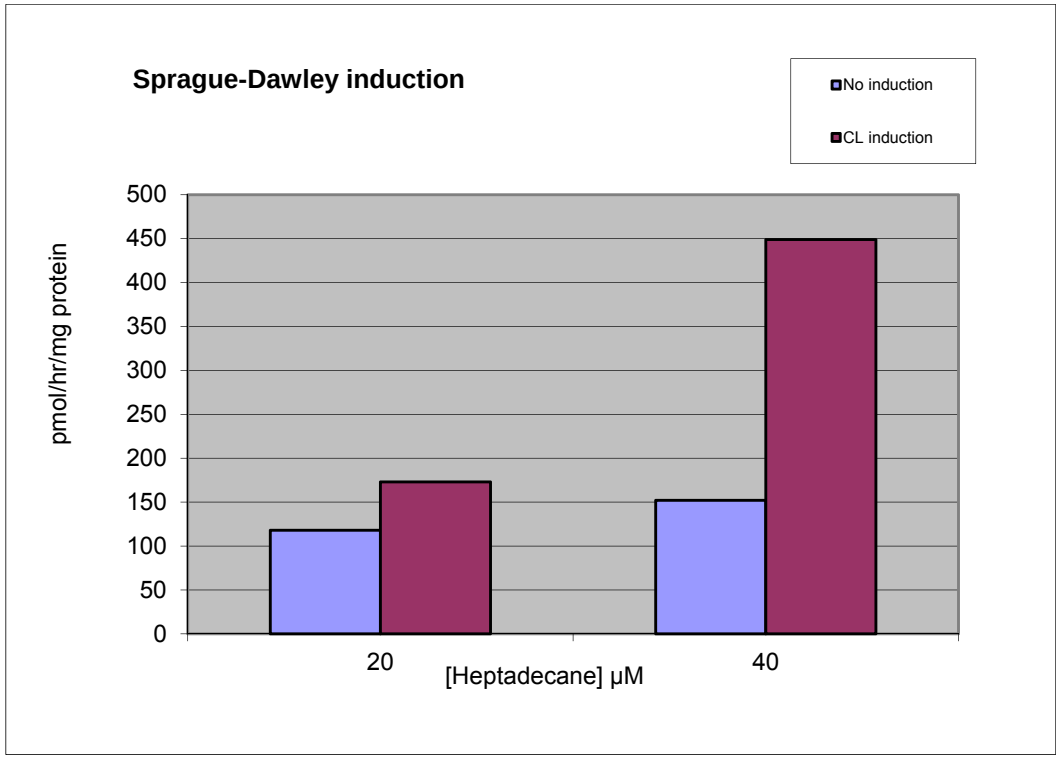
No hydroxylation was observed when octadecane was incubated with liver or intestine microsomes, irrespective of the rat strain investigated (Cravedi et al., Eurotox, 2011)

Metabolism of heptadecane incubated with microsomes from rats treated by two inducers

Induction 3 MC(CYP IA): liver microsomes from animals injected ip with 3MC (40mg/kg bw) at day 1 and 3, and sacrificed at day 4. Induction controlled by measuring EROD activity.

Induction with clofibrate (CYP IV A) : liver microsomes from animals dietary administered clofibrate (4 mg/kg feed) for 15 days before sacrifice. Induction controlled by measuring Lauric acid 12-hydroxylation.





Conclusions

➢ Few *in vivo* metabolic studies on n-, cyclo- and branched alkanes have been carried out in rodents, showing that oxidation of these hydrocarbons can occur in mammals.

➢ More recently, *in vitro* studies funded by EFSA were performed with different MOSH incubated with rat and human hepatic microsomes.

➢ Both rat and human hepatic microsomes showed the ability to metabolise n-heptadecane. No strong differences were observed in the biotransformation of n-heptadecane between the 3 rat strains, and between rats and humans, with the exclusion of females, for which biotransformation of n-heptadecane occurs at a higher extent in humans compared to Fischer 344.

➢ No biotransformation was observed for pristane and dodecylcyclohexane, whatever the type of microsomes used, suggesting that n-alkanes are metabolized at a higher rate that branched- and cyclo-alkanes.

➢ Data on the metabolic fate of alkanes are scarce and only based on few model compounds. No precise information exist on the fate of complex MOH mixtures.

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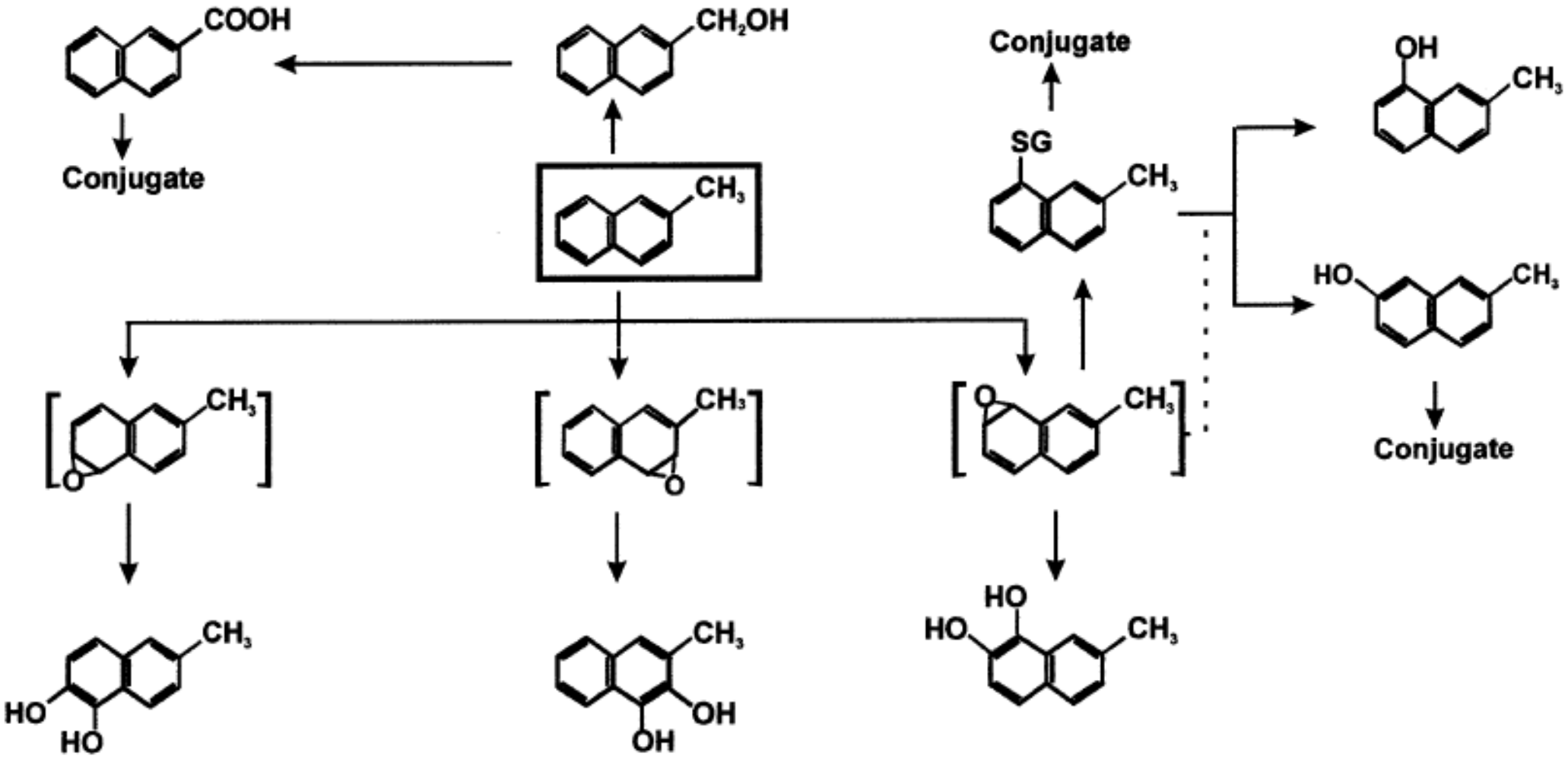
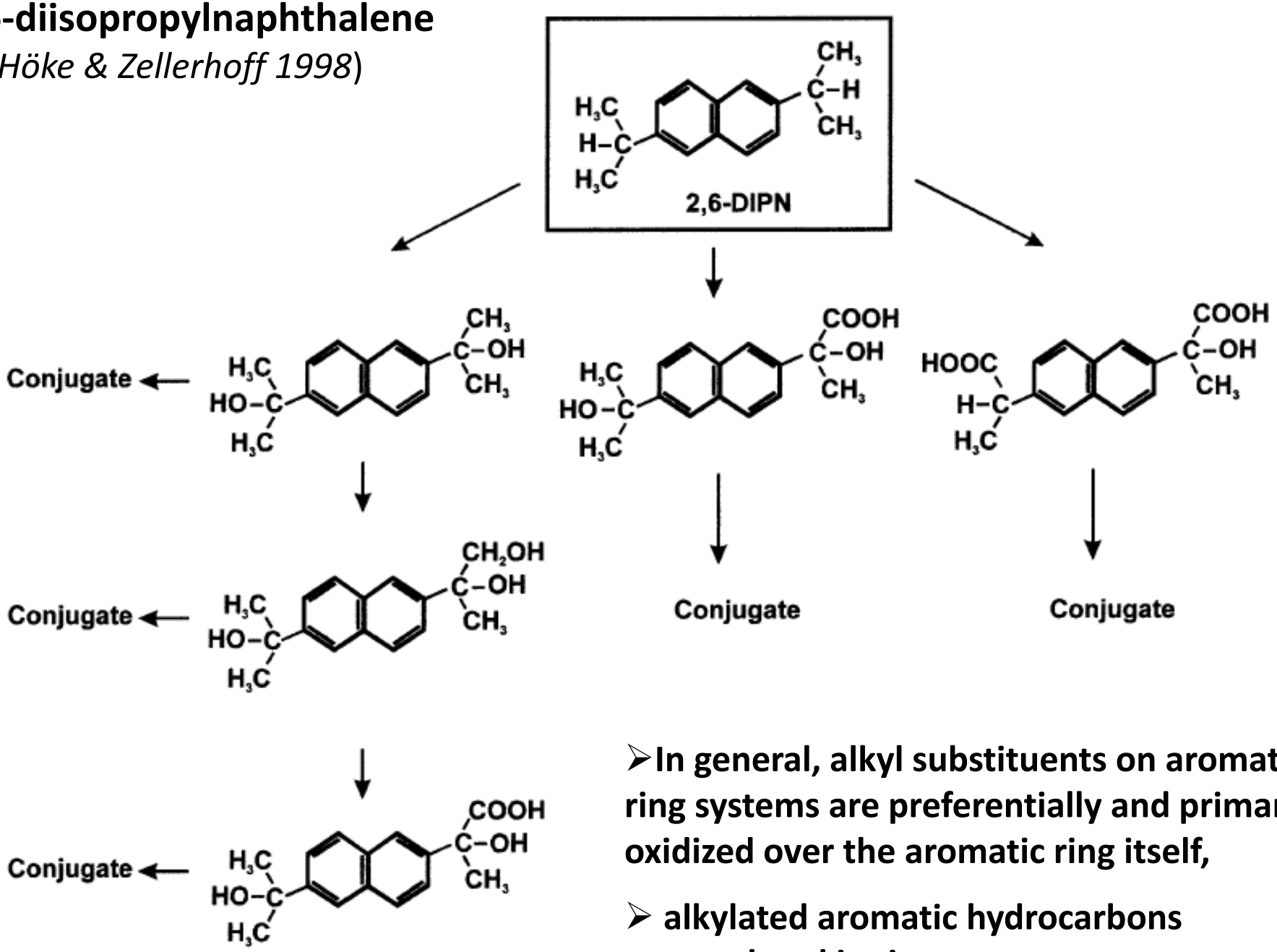
Thank you for your attention

Mammalian metabolic pathways of 2-methylnaphthalene

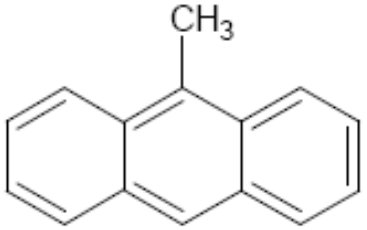
(*from Höke & Zellerhoff 1998*)



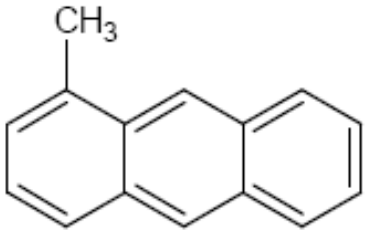
Mammalian metabolic pathways of 2,6-diisopropylnaphthalene
(*from Höke & Zellerhoff 1998*)



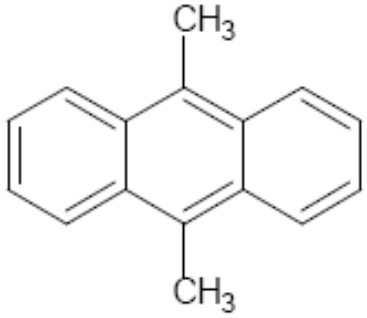
- **In general, alkyl substituents on aromatic ring systems are preferentially and primarily oxidized over the aromatic ring itself,**
- **alkylated aromatic hydrocarbons accumulated in tissues to a greater extent than unsubstituted derivatives**



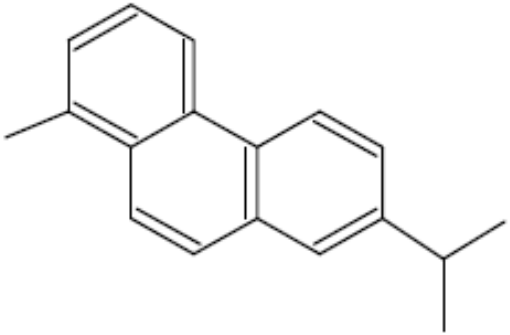
9-MA



1-MA



9,10-DMA



Ret