THE USE OF EVERTED RAT SMALL INTESTINAL SACS *IN VITRO* TO ESTIMATE RELATIVE ABSORPTION POTENTIAL OF A SERIES OF ALPHA OLEFINS

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Introduction

Higher Olefins (HO) range from C6 to greater than C32 olefins (with ≥ 1 carbon number) with an alpha or internal double bond and with linear and / or branched carbon chains. They are used in a wide range of processes and applications from polyolefin production to lubricants and waxes.

Member companies of the Higher Olefin and Polyalpha Olefin REACH consortium (HOPA) (http://www.hopaconsortium.com) registered over 40 individual HOs under the EU legislation in 2010.

In order to meet the extensive information requirement of REACH[1], data read-across was used between the HOs using a category approach as described in Annex 1 of the Regulation and used in other regulatory fora. The use of category approaches required that supporting data and hypotheses are presented to support the read-across between one substance and another. In the overall approach the chemistry and structure of the HOs olefins was analysed to ensure that the available data and future testing covered the possible bounds of the chemistry in terms of linearity, branching and positioning of double bonds.

In support of the category, one of the hypotheses for HOs was that the mammalian bioavailability would decrease as molecular weight increased. There was some supporting data for this hypothesis from Albro and Fishbein (1970)[2] but the data were not specifically on the higher olefins of interest. If this hypothesis could be supported experimentally to determine the cut off for absorption, then the case for read-across would be strengthened. Thus, reducing the need for sub-chronic, developmental and reproductive toxicity on the whole series of materials.

Experimental

Male Han Wistar rats (approx. 8 - 12 weeks old) were obtained from Harlan, Bicester, UK.
The alpha olefins examined were supplied by HOPA and are detailed in Table 1.

Table 1: Alpha Olefins examined

HOPA code	Name	Total Olefin Absorbed by Sac per hour (Mean±SD)			CN
		mg	nmoles	%	
1	hex-1-ene	63.1 ± 5.9	751.3 ± 69.9	11.7±1.1	6
2	Oct-1-ene	57.4 ± 4.7	512.6 ± 41.9	10.0±0.8	8
3	dec-1-ene	47.9 ± 8.3	342.2 ± 59.1	8.1±1.4	10
4	1-tetradecene-MC	0.8 ± 0.4	4.1 ± 2.1	0.1±0.1	14
5	1-tetradecene-UVCB	3.5 ± 0.7	17.7 ± 3.6	0.6±0.1	14
6	dodec-1-ene	14.0 ± 1.2	83.3 ± 7.0	2.3 ± 0.2	12
7	hexadec-1-ene - MC	1.2 ± 1.2	5.3 ± 5.4	0.2±0.2	16
8	hexadec-1-ene - UVCB	0	0	0	16
9	octadec-1-ene - MC	0	0	0	18
10	octadec-1-ene - UVCB	0	0	0	18
11	Alkenes, C20-24 α-	Insoluble*	Insoluble	Insoluble	22
12	Alkenes, C20-24 α-	0	0	0	22
13	Alkenes, C24-28 α-	Not arrived	Not arrived	Not arrived	
14	Alkenes, C26-28 α-	Insoluble*	Insoluble	Insoluble	27
15	Alkenes, C20 + α-	Insoluble*	Insoluble	Insoluble	
16	Octene	51.4 ± 0.7	459 ± 6.3	8.6 ± 0.1	8
17	Decene	48.1 ± 7.9	343.9 ± 56.4	8.1±1.3	10
18	Hexadecene	0	0	0	16
19	Nonene	47.2 ± 1.8	374.8 ± 13.9	8.1 ± 0.3	9
20	octadecene	Not arrived	Not arrived	Not arrived	18
21	octadecene	0	0	0	18
22	Alkenes, C6-8 (aka Alkenes, C6-8, even and odd, linear and branched)	Not arrived	Not arrived	Not arrived	7
23	Alkenes, C10-14	47.5 ± 25.3	282.5 ± 150.5	7.9 ± 4.2	12
24	Alkenes C11/C13/C14 (aka tridecene) (aka Alkenes C13-14)	6.8 ± 1.7	35.9 ± 9.1	1.1 ± 0.3	13
25	Alkenes C10/C11/C12/C13 (aka Alkenes C11-12)	6.2 ± 0.7	38.5 ± 4.5	1.0 ± 0.1	12
26	ALKENES, C8-10, C9-RICH	64.2 ± 7.3	509.2 ± 57.6	10.9±1.2	9
27	ALKENES, C15-18	0	0	0	16
28	Alkenes, C16-18	0	0	0	17
29	Alkenes, C19-23 (aka Hydrocarbons, C12-30, olefin-rich, ethylene polymn. by-product)	0	0	0	21
30	Alkenes, C20-24 (aka C20-C22 (even numbered, linear and branched) and C24(branched) alkenes)	0	0	0	23
31	Alkenes, C24-28 (aka Alkenes, C21-32 linear and branched)	Insoluble*	Insoluble	Insoluble	26
32	Hexadecene	1.8 ± 1.0	8.0 ± 4.7	0.3 ± 0.2	16
33	Iso-octene	92.8 ± 5.0	828.7 ± 44.4	16.2 ± 0.9	8

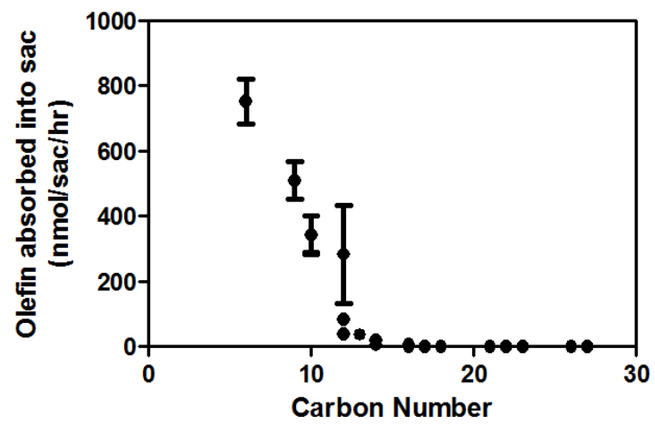
* Compounds with codes 11, 14, 15 and 31 were insoluble in the FeSSIF media and were not included in the data analysis.

The everted intestinal sacs were prepared by everting a freshly excised rat proximal small intestine over a glass stirring rod, rinsing with TC-199 media and filling the everted intestine with oxygenated "Fed State"-Simulated Intestinal Fluid (FeSSIF) medium at 37 °C.

The intestine was divided into sacs approximately 2.5 cm in length using braided suture silk. Sacs were incubated in flasks containing 10 mL FeSSIF medium at 37 °C with added individual alpha-olefins (20 µL). The incubations were performed in triplicate at 37 °C for 1 hour. After 1 hour the individual sacs were removed, washed and blotted dry. The sacs were cut open and the serosal fluid collected.
The contents of each sac (400 µL) and a sample of the external medium after incubation (400 µL) were extracted into isooctane (or n-hexane for C8 and C9) prior to analysis by GC-FID.

Results

Figure 1: Relationship between Olefin Absorption and Mean Carbon Number



Conclusions

The C6, C8-C10 (C9 Rich) and C10 molecules were readily absorbed into the intestinal sacs.

Marked inter-compound differences were observed, with the amount of absorption generally decreasing with increasing Carbon number. Olefins with 14 carbons and over were either not absorbed or very poorly absorbed.

These data will be validated by an *in vivo* radiolabeled study on selected compounds.

1. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

2. Albro, PW and Fishbein, L (1970) Absorption of aliphatic hydrocarbons by rats. Biochimca et Biophysica Acta 219, 437-446.

The extensive support of the members Higher Olefins Technical Committee are acknowledged.