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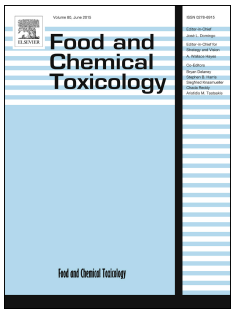

GTL synthetic paraffin oil shows low liver and tissue retention compared to mineral oil

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Juan-Carlos Carrillo [1]; Hua Shen [2]; Fayaz Momin [2]; Olaf Kral [3]; Holger Schnieder [4]; Susanne Kühn [5]

1. Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition
2. Conceptualization, Writing - Review & Editing,
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6. Investigation, Methodology, Validation, Writing - Original Draft, Writing - Review & Editing, Visualization

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Abstract

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Synthetic Gas to liquid (GTL) oils offer an alternative to petroleum derived mineral oils, because they do not contain naphthenic structures. To demonstrate this point, SD rats were fed either GTL oil (99% iso-alkanes) or naphthenic mineral oil (84% cycloalkanes) at 200 mg/kg bw /day for 90 or 134 days with a recovery group. Liver, fat and mesenteric lymph nodes were analyzed for alkane sub-type levels using Online-HPLC-GC-FID and GCxGC-TOF-MS.

Results indicate that at equal external dose, GTL hydrocarbons result in lower tissue levels and more rapid excretion than MOSH. GTL retained hepatic fractions were also qualitatively different than MOSH constituents. Because chemical composition differences, GTL oil show low absorption and tissue retention potential and thus an advantageous alternative to conventional mineral oil.

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Key words: iso-alkane, MOSH, naphthenic, cycloalkane, paraffin, mineral oil, GTL, accumulation

Abstract

Oral exposure to mineral oil may result in a narrow fraction of mineral oil saturated hydrocarbon (MOSH) being retained in tissues. Excess of MOSH hepatic retention may lead to the formation of lipogranuloma caused by predominantly multiring cycloalkanes (naphthenics) in a critical range of C_{25}-C_{35}. Although hepatic lipogranuloma is of low pathological concern, MOSH tissue deposition could be minimized by using an oil of similar quality but devoid of naphthenic structures to decrease hepatic retention.

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1. Introduction

Mineral oils are petroleum derived vacuum distillates which have a complex composition and are thus designated as UVCB's (EU 2006; Rasmussen et al. 1999). Their composition is determined by crude oil feedstock (paraffinic or naphthenic) and distillation initial and final boiling points that will determine the oil's carbon number range. The level of polycyclic aromatic compounds (PAC) is controlled by refining processes such as solvent extraction, acid treatment or/and hydrogenation. When mineral oils are highly refined to meet pharmacopeia purity standards, the presence of PAC is negligible to be able to comply with pharmacopoeia purity standards (EDQM 2016). This type of mineral oils are commonly known as white oils and consist virtually of only three types of alkanes; normal, iso (paraffins) and cyclic (naphthenics). The proportion of paraffins and naphthenics in white oil is related to the crude oil origin. Composition is controlled by manufacturing distillation boiling points, where high boiling points yield oils which have high molecular weight constituents (e.g. long carbon chains typically in the range from 25 to 50 carbon number) that is reflected in high viscosities. Therefore, viscosity is an important technical specification because it is indirectly related to composition and well correlated to molecular weight and boiling point range of an oil.

Viscosity was introduced as a distinguishing parameters for white oils for the first time in 1995 (JECFA; 1995; SCF 1995) dividing white oils into 'classes' according to their respective viscosities measured at 100°C, average molecular weight and corresponding carbon number at 5% distillation point . For example, high viscosity oils should have a viscosity of >11 mm^2/s, an average molecular weight > 500 g/mol and a carbon number of $\geq$28 at 5% distillation point (see table 1 for the other white oil classes)

Table 1. *Classification of white mineral oils according to JECFA (JECFA; 2002)*

Name	Viscosity at 100°C in mm^2/s	Average molecular weight g/mol	Carbon number at 5% distillation	Examples[1]
High viscosity	> 11	> 500	$\geq$ 28	P100H
Class I	8.5 – 11	480 – 500	$\geq$ 25	P70H
Class II	7.0 – 8.5	400 – 480	$\geq$ 22	N70H
Class III	3.0 – 7.0	300 – 400	$\geq$ 17	P15H, N15H

Safety evaluations have established a group ADI (12 mg/kg bw day) for white mineral oils with viscosities > 8.5 mm^2/s and >11.0 mm^2/s (EFSA 2009b; EFSA 2013b). The key study for this assessment was a 2-year dietary study in the F-344 fed with P70H and P100H mineral oils that included a 1 year recovery period, which in addition to toxicological evaluations also included the assessment of total hydrocarbon retention (alkane content) in the liver among other organs assessed (Trimmer et al. 2004).
Treatment related effects included mesenteric lymph node histiocytosis and increased organ weight which reversed to baseline, both of which are not considered adverse (EFSA 2013b). The NOAEL was thus established at 1200 mg/kg bw.
Regarding the retention of alkanes from mineral oil origin in the liver, it was observed that while there was retention over time a maximum was achieved at about 3 months for P70H and 18 months for P100H with minimal further increase. After switching to clean diet, complete reversibility to near

[1] The white oil nomenclature used is based on the oil's crude origin, viscosity at 40°C and refining method. Thus, a P100 oil is from paraffinic crude (P), viscosity of 100 mm^2/s @ 40°C and purified by hydrotreatment (H). Similarly, N70A, would be an oil obtained from naphthenic crude, with 70 mm^2/s @ 40°C and purified by acid treatment (A).

background levels (i.e. not statistically different from control) was measured at 12 months recovery (Trimmer et al. 2004).
While the study measured hepatic increase and decrease of "mineral hydrocarbons" no detailed analysis of the retained alkane material was provided. However, this can be inferred from the type of oils used and from the literature which indicates a retention range in the liver of C_{20}-C_{35} with a critical range at about C_{25}-C_{30} (Barp et al. 2017; Biedermann et al. 2015; Cravedi et al. 2017; Scotter et al. 2003). The term "mineral hydrocarbons" in the context of Trimmer (Trimmer et al. 2004) refers thus to white oils that consist virtually of only two types of hydrocarbons; iso and cyclo-alkanes. The presence of n-alkanes in these oils is negligible. Thus is this study, the observed increase of hydrocarbon levels in the F-344 rat liver after mineral oil exposure is related to the temporary retention of iso and cycloalkanes consistent with observations in humans where the retained hydrocarbon material was similar in composition and carbon number range (Biedermann et al. 2015; Boitnott and Margolis 1970) indicating that cycloalkanes with a peak around the C_{28}-C_{30} have higher retention compared to iso-alkanes. This observation is supported by comparative studies of mineral oil surrogates where F-344 rats exposed to multiring cycloalkanes (naphthenics) show higher oral absorption and tissue burden than normal or iso-alkanes (Low L. 1992). This may be due to naphthenic having slower elimination than normal or iso-alkanes (Tulliez and Bories 1975). Consequently retained mineral oil saturated hydrocarbons (MOSH) fractions consisting of mostly multiring cycloalkanes (naphthenics) in a narrow critical range of C_{25}-C_{30} (Barp et al. 2014; Biedermann et al. 2015), may result in the formation of liver lipogranuloma (Cruickshank and Thomas 1984; Dincsoy et al. 1982). Mineral oil related hepatic lipogranuloma is not of pathological concern and shows an internal threshold of about 200 mg/kg liver tissue (Boitnott and Margolis 1970; Fleming and Carrillo 2018), but it is not desired and thus should be minimized (EFSA 2008).

Although the oral route is considered the most frequent source of MOSH , mineral oil based adjuvants in vaccines may also contribute the overall exposure (Aucouturier et al. 2002). In rats residues of mineral oil after intraperitoneal injection have been found after 24 hrs. of injection in the liver and fat (Ebert et al. 1966), with residues retained at the site of injection after 10 months (Bollinger 1970). Because mineral oils are also used as adjuvants in animal vaccines (Aucouturier et al. 2001), their residues in meat production may contribute to overall MOSH burden and thus have to comply with established minimal residual levels (MRL) (EMEA 1995).

2. GTL oil vs mineral oil

Given this background and the advent of synthetic paraffin oils by gas to liquid – (GTL) technology as alternatives to white mineral oil applications in sensitive applications (e.g., cosmetics, food contact and vaccine adjuvants), it was of our interest to study the tissue retention properties of GTL oils compared to conventional petroleum derived white oils. We speculated that GTL oils would show lower hydrocarbon retention and residues in tissues because they have virtually no cycloalkane constituents, which are the alkane types with the highest retention potential.
To test our assumption, a dietary study to assess GTL hydrocarbon retention in rat tissues was therefore carried out as complementary information to the already available GTL oil toxicology data for systemic (OECD 408), prenatal and reproductive toxicity (OECD 416) that showed no adverse effects (Boogaard et al. 2017; Dunster 2009; Faiola 2011; Senn 2014). For comparison, a naphthenic white mineral oil with similar carbon number range distribution was also tested.

The main difference between these two oils is that the GTL oil consists of virtually only branched paraffins (~99%) which are rather simple branched iso-alkanes, whereas the naphthenic mineral oil consists of mostly (~84%) naphthenic hydrocarbons (poly-cycloalkanes), with only about 10% of iso-

alkanes and low levels of n-alkanes (Figure 1). Because these two oils represent two extremes of alkane type composition, it is expected that clear retention differences between alkane types will be observed in biological tissues.

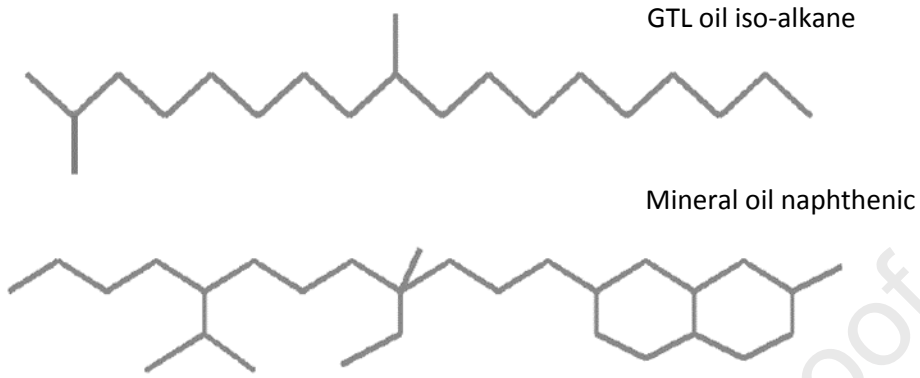


Figure 1. Representative main GTL oil and mineral oil constituents.

In the context of this paper, the use of the term MOSH refers to the chromatographic fraction that encompasses hydrocarbons from mineral oil origin, including iso and poly-cycloalkanes (naphthenics), but excluding n-alkanes. Further, because the same chromatography technique is applied to both oils, it must be realized that the term "MOSH" as such does not apply to GTL products as there are clear alkane composition and origin differences. Hence results of the analysis are presented using the neutral term "saturated hydrocarbons". The term MOSH is only used in instances where we specifically refer to mineral oil.

Hypothesis

The study design tried to address the following hypothesis:

- Because naphthenics show higher hepatic retention than iso-alkanes, GTL oils which are virtually free from naphthenics will show less residues and more rapid excretion than mineral oil after repeated exposure.
- Because of its synthetic origin, GTL iso-alkanes have a different branching pattern than their mineral oil counterparts and with no prior knowledge to their in-vivo uptake, we conservatively assumed that this type of structures will show higher uptake resulting in higher measured values in the liver.

3. Material and Methods

Oil selection

The selected oils compositional bulk was centered around the C_{25}-C_{35} range which has been shown to be the critical range for human retention of MOSH (Barp et al. 2014). Compositional information by two-dimensional gas chromatography (GCxGC) and simulated distillation was used to confirm this requirement. To maximize the effect of hepatic retention of naphthenics, a white mineral oil from naphthenic crude oil was selected over one from paraffinic origin. This was compared to a highly

isoparaffinic GTL oil with comparable carbon number distribution containing only trace levels of naphthenic (cycloalkane) constituents. Both oils were obtained from Shell Deutschland Oil GmbH.

White mineral oil (CAS 8042-47-5)
A medicinal grade naphthenic oil, N70H: ~84% cycloalkanes (naphthenics) with about 3% normal alkanes, 7% mono/dimethyl alkanes, 4% multi-branched iso-alkanes including those with tertiary carbons. These constituents are in the C_{18}- C_{48} range and an initial and final boiling points of 340 °C and 530°C respectively. Pharmacopeia purity achieved by hydrogen treatment.

GTL base oil (CAS 1262661-88-0)
A medicinal grade synthetic oil produced by GTL technology: ~99% iso-alkanes, which about 3% are mono/dimethyl and the rest are multi-branched iso-alkanes with no tertiary structures present and <1% cycloalkanes. These constituents are the C_{20}- C_{42} range with initial and final boiling points of 379 °C and 537°C. Pharmacopeia purity achieved by hydrogen treatment. Mineral Oil aromatic hydrocarbons (MOAH) content was below detection limit.

Animals and husbandry

Female Sprague Dawley (SD) rats 8-10 weeks old were obtained from Envigo, Frederick, Maryland, USA. Animals had a weight range at first dose of 168.6-201.7 grams, acclimated to laboratory conditions for at least seven days prior to the first dose and released from acclimation by a staff veterinarian.
Feed and water (via automatic watering system) were provided at libitum. Animals were housed individually or paired in polysulfone individually ventilated cages (IVC) suspended on stainless steel racks. Each cage was ventilated with the standard 50-60 air exchanged per hour and a positive differential pressure. Each cage was affixed with a cage card containing pertinent animal and study information. Temperature ranged between 20 to 26°C; humidity range 30 to 70%; light cycle 12-hour light/12-hour dark, interrupted as necessary for study-related events air changes minimum of 10 air changes per hour. All environmental parameters were monitored continuously and kept withing protocol requirements.

Dose selection

Previous studies on GTL base oil including a 90-day study with doses of 50, 200, or 1000 mg/kg bw indicate a NOAEL of 1000 mg/kg bw (Boogaard et al. 2017). In addition, the second postulate of the hypothesis was considered in the selection of the dose. Thus, the dose of 3000 ppm (0.3% in the feed which is approx. 200 mg/kg bw /day) the “intermediate” dose of the existing repeated dose studies was selected for the assessment of mineral oil vs GTL alkane retention. The pre-requisite for the dose selection is that during the recovery period, significant elimination would be observed. The high dose was not selected because it was assumed that because GTL could show higher absorption and thus higher hepatic levels than conventional oil, the recovery period would be too long to see either oil going back to baseline; this would make the study too long and outside budget. The low dose was not selected because it was assumed that GTL would be eliminated too fast, so that it would not be possible to measure 3 timepoints of the recovery period. Therefore, the mid dose was considered a reasonable choice to ensure enough hydrocarbon retention and a relative short recovery time to measure at least 3 timepoints. In this way the study would be manageable in time and cost effective.

Study design

Animal experimentation was carried out at BASi (former Smithers Avanza Toxicology Services) 13 First field Road Suite 110 Gaithersburg, Maryland 20878, USA.

Two groups of Sprague Dawley rats received for 13 weeks (92 days) or 19 weeks (134 days) a diet spiked with 3000 ppm of either white mineral oil (N70H) or GTL oil. Control was given a clean diet (Teklad 2018 certified).
Groups F2 and F4 received GTL oil, while groups F3 and F5 received N70H mineral oil. Group F1 fed on a clean diet. Groups F2 and F3 switched to a clean diet at day 92 and were terminated at day 134. Groups F4 and F5 continued the spiked diet beyond day 92 and were also terminated at day 134, and thus considered as positive control when compared to the recovery groups. Thus, at the end of the experimental time for each oil there was a group that had an exposure of 92 and 134 days and a recovery period from day 92-134.
Groups of 5 rats of each group were sacrificed at determined timepoints for the assessment of target tissues (table 2).

Table 2. Necropsy Schedule. Study day (SD) and number of rats sacrificed.

	Main Phase					Recovery Phase		
Feed Group	**SD 1**	**SD 29**	**SD 57**	**SD 92**	**SD134**	**SD 106**	**SD 120**	**SD 134**
Group F1 (Control)	5	-	-	5	-	-	-	5
Group F2 (GTL oil)	-	5	5	5	-	5	5	5
Group F3 (mineral oil)	-	5	5	5	-	5	5	5
Group F4 – continuous (GTL oil)	-	-	-	-	5	-	-	-
Group F5 – continuous (mineral oil)	-	-	-	-	5	-	-	-

The liver was considered the most important tissue to be assessed because of the potential of MOSH to be retained in this organ. To ensure low variability of sampling, the caudate lobe was selected, as it has been suggested that this area of the liver most targeted by hydrocarbon retention (Butler 1992). Samples were collected at study days, 1, 29, 57, 92 and 134 and during recovery at 106, 120 and 134. The other tissues had a semi quantitative analysis to merely observe trends. The mesenteric lymph node was assessed at main phase time points 1, 29, 92, 134 and at recovery day 134. In the original study plan the hydrocarbon analysis in visceral fat was not foreseen, but before the in life phase of the study was finished this omission was partially amended; hence hydrocarbon analysis for this tissue is only given for the end of the study (SD-134) for both main phase and recovery groups.

Necropsy and tissue collection.

Animals were necropsied as soon as possible after the time of death. Gross necropsy included examination of the external surface of the body, all orifices and the cranial, thoracic, and abdominal cavities and their contents. Tissues were collected using stainless steel instruments and weighed in foil weigh boats as soon as possible. Contact with nitrile glove and/or any plastic material was avoided to the extent possible. A sample of one representative nitrile glove used during tissue collections was wrapped in aluminum foil and shipped with the frozen tissue samples for hydrocarbon analysis.
For the liver at least 250 mg of each liver lobe (caudate, right lateral, right medial, left lateral, left medial) were weighed and then collected into a glass tube and stored at -75 ± 15°C. The remaining portion of the liver (stock and remaining segments of each lobe) was preserved in 10% neutral buffered formalin (NBF).

Observations

Physical examinations, cage side observations, body weights and food consumption were recorded.

Clinical Pathology

Animals were fasted overnight (with water available) prior to sample collection. Blood samples were collected for serum clinical chemistry (> 1ml) and hematology (>0.5 ml) analysis.

Histopathology

As the aim of the study was to assess tissue retention of hydrocarbons, no histopathology was carried out because for GTL oil there is a full 90-day study available (Boogaard et al. 2017). In the case of mineral oil of this viscosity several studies have been carried out in the F-344 rat (Nygaard et al. 2019; Scotter et al. 2003; Shoda et al. 1997; Trimmer et al. 2004) and recently reviewed (Pirow et al. 2020).

Dose formulation and sampling

The vehicle/control substance, basal diet Teklad 2018 (certified, in meal form) was used as received.
Oil formulations (3000 ppm in feed) were prepared biweekly by mixing the appropriate amount of oil premixes for approximately 10 minutes at 15 RPM. Formulations were given a 14-day shelf life and stored at room temperature until used for dosing.
Formulations were sampled (5 g) for homogeneity (first mix from top, middle and bottom stratum) and concentration verification (weeks 1, 4, 8, 13 and 19 from middle stratum).
Analysis of concentration verification was carried out by BASI and for confirmation shipped to Berlin where the bioanalytical sample analysis for hydrocarbon was carried out.

Bioanalytical analysis for saturated hydrocarbons

Biological and feed formulations were analyzed for “MOSH” at the Institut Kirchhoff Berlin GmbH; Oudenarder Straße 16 / Carrée Seestraße D-13347 Berlin, Germany.
The term MOSH refers to ‘mineral oil saturated hydrocarbons’ for which the analytical method was originally developed. Because GTL is a synthetic oil, the “MOSH” method applied for the analysis of groups F2 and F4 should be interpreted as the synthetic hydrocarbons’ equivalents of MOSH found in groups F1, F3 and F5. Therefore, because the method does not distinguish between “mineral oil” or “synthetic” origin we use to the neutral term “saturated hydrocarbons” in the chromatograms whose interpretation is given separately.

Sampling: At least 3 individual samples (1 sample per rat) were measured. Measurements in duplicate were not feasible since pilot measurements showed inhomogeneities in the liver tissue in case that livers were subsampled. Therefore, whole liver sample was prepared and measured only once. For other samples total sample amount was prepared to keep LoQ at acceptable range. When it was observed that the standard deviation exceeded 50% of the mean, additional samples were measured.
Quality control measures: Each batch of solvents was tested to avoid blanks. Glassware was treated specially to clean it prior to usage. Each sample was spiked with a set of internal standards to monitor accurate sample preparation and assure qualification of LC-GC-FID system.

The quality control measures included for each sequence the measurement of blank and quality control sample (incl. comparison to control chart), control of used internal standard mix as well as check of GC system through ratio of n-C_{10} to n-C_{20} and n-C_{50} to n-C_{20} (acceptance criterion >= 80%).

For each sample the check of standard recovery rate, check of HPLC separation efficiency via internal standards, control of HPLC separation by UV signal and GC separation (separation of n-C_{11} and solvent) was performed.

Chemicals

n-Undecane (*C_{11}*; Sigma Aldrich, Steinheim, Germany), Bicyclohexyl (*Cycy*; Sigma Aldrich, Steinheim, Germany), n-tridecane (*C_{13}*; Sigma Aldrich, Steinheim, Germany), n-tetracontane (*C_{40}*; Sigma Aldrich, Steinheim, Germany), 5-alpha-Cholestane (*5-Cho*; Sigma Aldrich, Steinheim, Germany), n-pentyl benzene (*5B*; Sigma Aldrich, Steinheim, Germany), 1-methylnaphthalene (*1-MN*; Sigma Aldrich, Steinheim, Germany), 2-methylnaphthalene (*2-MN*; Sigma Aldrich, Steinheim, Germany) and perylene (*Per*; Sigma Aldrich, Steinheim, Germany) were used as reference substances (Biedermann and Grob 2009). Potassium hydroxide, ethanol, n-hexane and dichloromethane were from Th. Geyer (Renningen, Deutschland).
A stock solution of 0.5 mg/mL was prepared including all reference substances and diluted to a standard mix containing 50 µg/mL of all substances.

Analytical Method

For total saturated hydrocarbon quantification, the GC-FID was preferred since this detector offers a similar response for all saturated hydrocarbons compared to using GC-TOF-MS where the response factor is substance dependent. Thus, for the complex hydrocarbon composition of the oils under investigation it is not feasible to independently calibrate the GC-TOF-MS because contrary to a GC-FID set up where a simple hydrocarbon can be used, for GC-TOF-MS an exactly characterized calibration mixture would be needed which is not feasible. Therefore GC-FID was used for quantification.

We chose m/z 71 as typical fragment for a n-alkane and iso-alkanes in order to omit the shorter hydrocarbon side chains of branched alkanes. For cyclo-alkanes we chose m/z 82, although m/z 83 could also be used but here more non cyclic molecules form the same fragment. Thus the analysis focused on the fragments m/z 71 and 82 based on the internal database.

For saturated hydrocarbon determination in caudate liver lobes and mesenteric lymph nodes, tissue was digested by addition of 3 ml hydrochloric acid and 25 µL internal standard mix (50 µg/mL) and shaking for 60 minutes at 40 °C. After cooling to room temperature, the acidic solution was diluted with 5 mL water

and 5 mL ethanol and extracted twice with 10 mL n-hexane. The combined n-hexane phases were cleaned through 1 g of silica gel and 0.5 g sodium sulfate in a column and concentrated to 250 µL.
For fatty tissue 100 µL internal standard mix (50 µg/mL) and 25 mL n-hexane/ ethanol (1:1, v/v) mix was added to sample. Mixture was constantly shaken at 60 °C for 30 minutes. Triglycerides were saponified by addition of 2.5 mL potassium hydroxide solution in water (50 %, m/m) under shaking for 30 minutes at 60 °C. 5 mL water and 5 mL n-hexane were added, and the mixture was shaken. Afterwards, the organic phase was washed with 5 mL water/ethanol mixture (1:1, v/v), dried with sodium sulfate, cleaned with 1 g silica gel and 0.5 g sodium sulfate in a column and concentrated to 375 µL.

A volume of 50 µL was used for on-line LC-GC-FID analysis. For all sample preparations the saturated hydrocarbons were quantified according to the method of the internal standard (Biedermann et al. 2009).

Saturated hydrocarbon analysis was performed by on-line HPLC-GC-FID and GCxGC-TOF-MS. Briefly, 50 µL sample was injected into a 250 mm x 2,1 mm i.d. HPLC silica gel column, using n-hexane as starting eluent and a flow rate of 300 µL/min. MOSH and MOAH fraction were separated using a gradient up to 35 % dichloromethane. GC separation was performed after large volume on column injection using an uncoated precolumn (Restek MXT 10 m x 0.53 i.d.) followed by a steel t-piece union connecting to SVE (solvent vapour exit) and a nonpolar separation column (Restek MXT-1, 15 m x 0.25 mm i.d. x 0.25 µm).

A volume of 1 to 8 µL of the sample extract was used to further investigate the saturated hydrocarbon fraction using GCxGC-TOF-MS after HPLC separation as described above. For the reverse system setting a 30 m x 0.25 mm i.d. x 0.15 µm DB-17HT (Agilent Technologies, Waldbronn, Germany) as the first dimension column was connected via the ultimate union connection system (Agilent Technologies, Waldbronn, Germany) to the 1.5 m x 0.25 mm i.d. x 0.1 µm DB-5HT (Agilent Technologies, Waldbronn, Germany) which was used as second dimension column. The characterization of the saturated hydrocarbon fraction by GCxGC-TOF-MS was performed as described by Biederman et al. (Biedermann and Grob 2009). For the evaluation of results the mass-filtering approach was used, and the substance classes were identified by their retention time and mass spectra.

LC-GC-FID parameters

A summary of the methodology described elsewhere is provided (Koch et al. 2020). The MOSH were measured by an on-line HPLC-GC-FID system (Axel Semrau GmbH, Sprockhövel, Germany), using a PAL CTC sampler (CTC Analytics AG, Zwingen, Switzerland) on a 1260 Infinity HPLC instrument (Agilent Technologies, Waldbronn, Germany). A silica gel column (Restek Allure Silica 5 µm, 250 mm x 2.1 mm) was connected via a Y- interface to a DANI Master GC (DANI Instruments S.p.A., Cologno Monzese, Italy) equipped with an uncoated precolumn (Restek MXT 10 m x 0.53 i.d.) followed by a steel t-piece union connecting to SVE (solvent vapour exit) and a nonpolar separation column (Restek MXT-1, 15 m x 0.25 mm i.d. x 0.25 µm). A gradient of n-hexane with dichloromethane was used with backflush after the elution of the MOAH, started at 0.3 mL/min with 100% n-hexane, reaching 35% dichloromethane after 1.5 min, backflush initiated after 6.2 min with 100% dichloromethane at 0.5 mL/min for 9 min, followed by a recondition with 100% n-hexane for 10 min at a flow rate of 0.5 mL/min and 5 min at 0.3 mL/min. The injection volume was 90 µL for the TPAF (40 µL were dissolved to 100 µL after the GCxGC-TOF-MS injection), and 10-50 µL for mineral oil aromatic hydrocarbons (MOAH) and MDA fractions. Hydrogen was used as a carrier gas with 90 kPa applied during the fraction transfer from LC to GC through the Y-interface and 150 kPa after the partially concurrent solvent evaporation and closure of SVE valve. GC started at 58 °C (11 min), followed by a temperature program of 5 °C/min to 80 °C, then at 15 °C/min to 110 °C and at 25 °C/min to 370 °C (7 min), resulting in a total run time of 34 min.

GCxGC-Parameters

A summary of the methodology described elsewhere is provided (Koch et al. 2020). For GCxGC-TOF-MS (Figure 2), a Leco Pegasus 4D (Leco Instrumente GmbH, Mönchengladbach, Germany) was used, controlled by Leco Chroma TOF acquisition software. The instrument consisted of a 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a split/splitless injector, a PAL combi XT autosampler (CTC Analytics AG, Zwingen, Switzerland), a secondary internal oven, a cryogenic consumable-free (CF) nitrogen-cooled (FC100 chiller from SP Scientific-FTS Systems, Warminster, PA, USA) jet modulator and a TOF mass spectrometer. The column configuration was of the reversed polarity type, with a 30 m x 0.25 mm i.d. x 0.15 µm DB-17HT (Agilent Technologies, Waldbronn, Germany) first dimension column connected via the ultimate union connection system (Agilent Technologies, Waldbronn, Germany) to a 1.5 m x 0.25 mm i.d. x 0.1 µm DB-5HT (Agilent Technologies, Waldbronn, Germany) second dimension column. These columns were temperature-programmed from 60 °C to 370 °C at 3 °C/min without secondary oven offset. The modulator offset was 20 °C. Helium was used as a carrier gas in constant flow mode (1 mL/min). Modulation was in staged mode, from 9 s to 14 s at the end of chromatographic separation in order to avoid the wrap-around of high boiling compounds. Spectra were collected in the m/z range from 35 to 650, with a scan rate of 50 spectra/s. The ion source was at 250 °C, the transfer-line at 340 °C; a detector voltage of 1600 V was applied after the solvent delay of 450 s. To lower the detection limit, pooled TPA fractions (2.2) were evaporated to 40 µL. Injection volumes were between 1-3 µL in pulsed spitless mode.

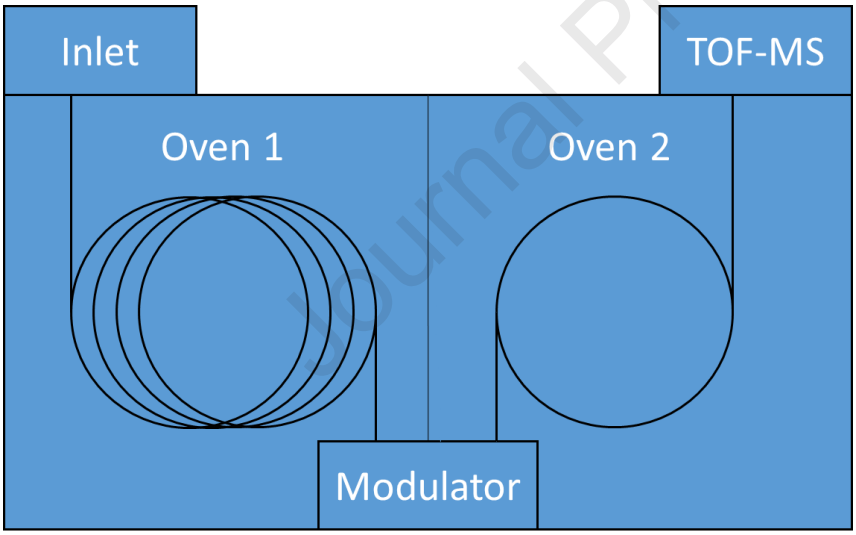


Figure 2. Connection diagram of the two dimensional chromatography instrumentation (GCxGC)

Statistical analysis

Toxicological parameters

In consideration of ethical concerns of animal use and resource utilization, our study used a low number of animals per group after stratification by treatment group, study day, and tissue type. To minimize detected differences occurring by chance due to low sample size, the statistical methods used for hypothesis testing were more conservative (Kruskal-Wallis versus one-way ANOVA).

Group means of collected biological measures (i.e., body weight, body weight changes, food consumption, clinical pathology, and organ weight) were compared across all treatment groups and the control group to detect statistical differences. One-way Analysis of Variance (ANOVA) was used where untransformed data were normally distributed and variances were homogeneous, as determined by Shapiro-Wilk test and Levene's test, respectively. If log-transformed data also did not meet these two assumptions, the non-parametric Kruskal-Wallis ANOVA was used. Dunnett's t-test was used for post-hoc testing where mean differences between groups was significant ($p \leq 0.05$). An arcsine square root transformation was used for some proportion/percentage data that do not meet the assumptions of parametric statistical tests in the attempt to normalize the data; this data was transformed prior to the ANOVA analysis.

Hydrocarbon analysis.

Given very low sample sizes, uneven group sizes, and variation from normality in some study groups, the Kruskal-Wallis test, which is more conservative compared to the one-way ANOVA, was used. Where applicable Dunn's post-hoc test correcting for multiple comparisons was used to investigate statistical differences between groups.

4. Results

Formulation

The spiked feed formulations were homogeneous and within an acceptable range of 82 to 115% of the target concentration of 3000 ppm, re-confirmed during the biological sample analysis.

Animal Treatment

Treatment of animals with either oil had no effect on mortality, physical examinations, or cage side observations. All animals survived until the scheduled termination. No effect on body weights or body weight changes were observed and all the animals gained weight over the course of the study. No overall effect on food consumption was observed, although increases and decreases were recorded at intervals. These changes were not test substance related and considered incidental as there was no corresponding effect on body weight or body weight changes. There was no effect on food efficiency and the approx. achieved dosage was 200 mg/kg bw/day. No substance related alternations in clinical chemistry were noted.

Group 2 (GTL) animals had a higher red cell distribution width than the control on SD 92. Group 5 (mineral oil) animals had lower absolute monocytes than the control on SD 134. These differences were statistically significant but were not considered test substance-related due to the small magnitude of the change and lack of correlation with other groups. Treatment with either oil had no effect on gross pathology. No visible lesions were observed in any of the animals during necropsy. All the organ weights were comparable across the groups and no test substance-related changes were observed.

Thus, at the tested concentration of 200 mg/kg bw/day, no observed adverse effects were seen in any of the treated groups.

Hydrocarbon analysis results

Control and spiked feed.

It was observed that the control feed had a 90-ppm background contamination consisting of saturated hydrocarbons likely from mineral oil origin (Figure 3), with a bulk carbon number range of C_{16}-C_{25} but also a smaller fraction visible around C_{25}-C_{40}. The bulk contamination was thus also present in the in the spiked feed and biological samples analysed.

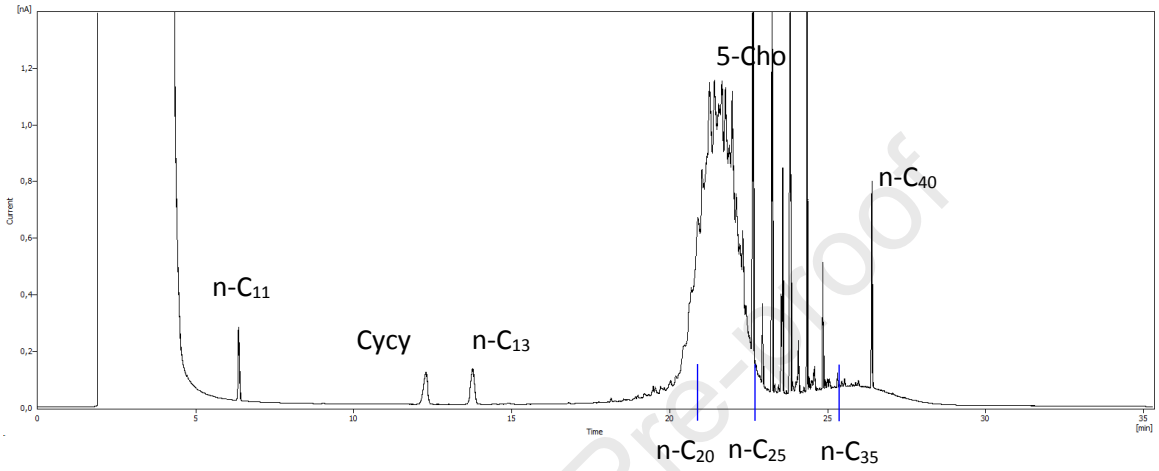


Figure 3. *Online-HPLC-GC-FID-Chromatogram of the background mineral oil contamination found in control feed of about 90 ppm. Reference substances undecane (n-C11); bicyclohexyl (Cycy), tridecane (n-C13), cholestane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

Online-HPLC-GC-FID of the feed spiked with either the GTL oil (Figure 4a), or mineral oil (Figure 4b) showed a good correlation with the original material (data not shown). It was noted that both spiked feeds contained the background mineral oil contamination detected in the control feed. In the case of the GTL spiked feed (F2 and F4) this is visible as a bimodal hump of an adjacent hump in the C_{20}-C_{25} range on the left side of the main GTL hump. In the case of the mineral oil spiked feed (F3 and F5) the background contamination becomes totally integrated within the range of the mineral oil's main hump C_{20}-C_{35}, and therefore not visible.

Figure 4a. GTL oil spiked in feed F2 and F4

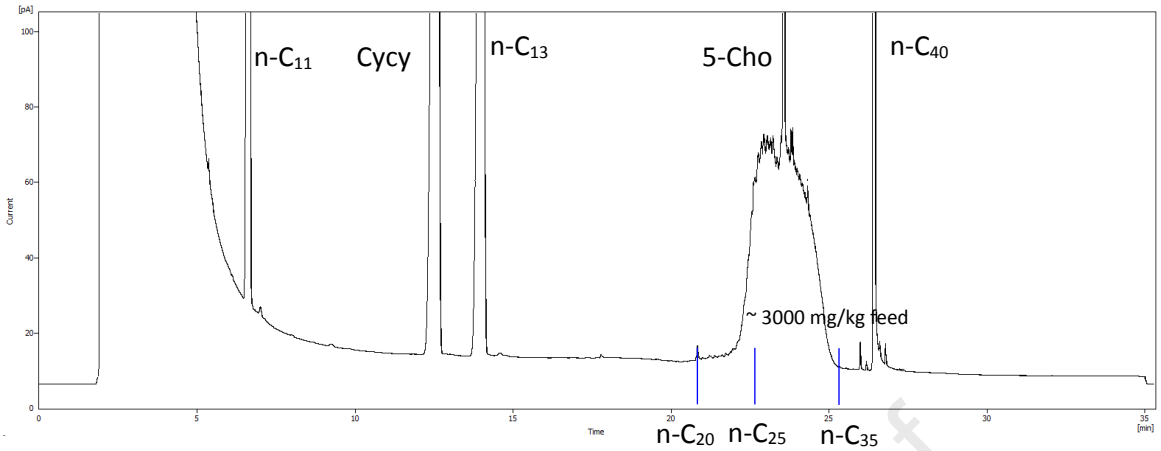
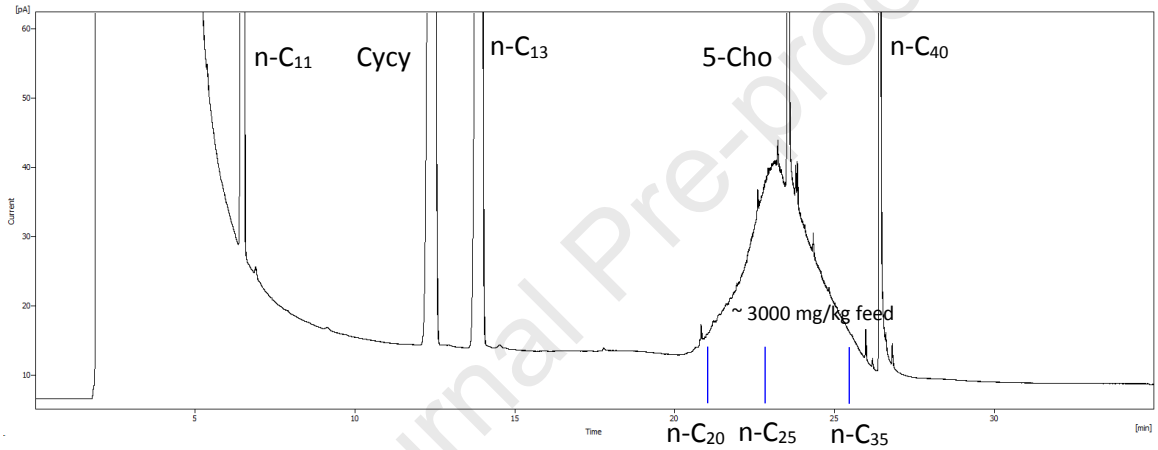
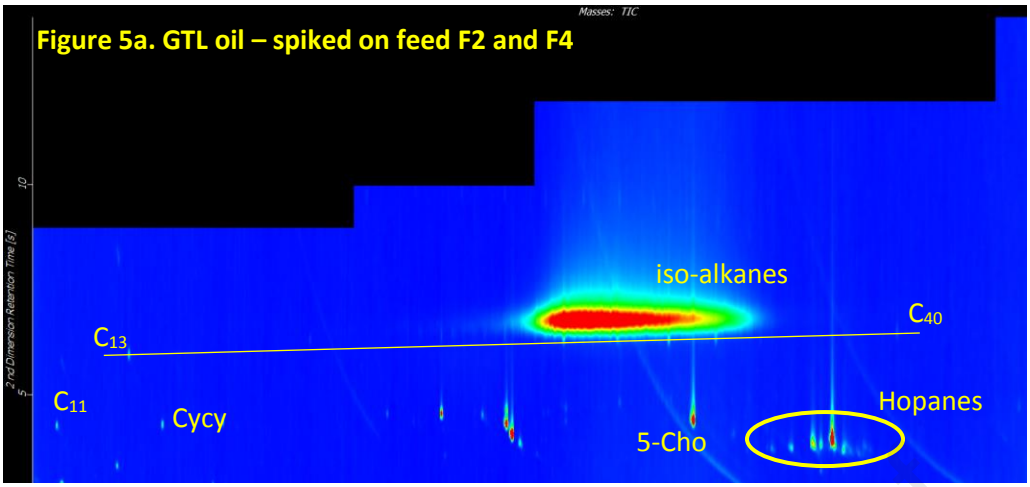


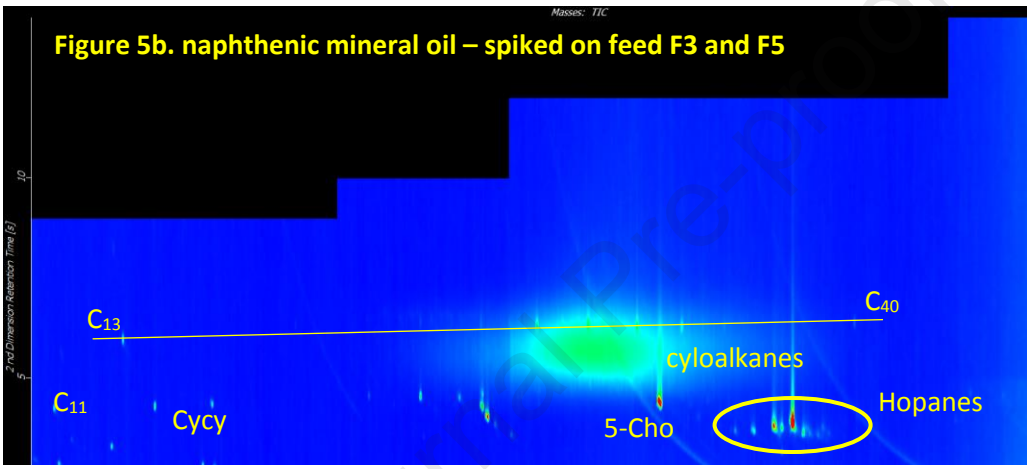
Figure 4b. Mineral oil spiked in feed F3 and F5



Figures 4a, 4b. *Online-HPLC-GC-FID-Chromatogram of the extracts from spiked feed with GTL oil (2a) or mineral oil (2b) shown with their corresponding carbon number ranges. Note the bimodal hump profile from the GTL oil spiked feed (2a) due to background mineral oil contamination found in the control feed. Reference substances undecane (n-C11); bicyclohexyl (Cycy), tridecane (n-C13), cholestane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

Compositional analysis by GCxGC TOF-MS of the spiked feed shows that although both oils show a similar carbon number range distribution these oils consist of contrasting alkane compositions. By connecting the reference n-alkanes substances (n-C_{13} and C_{-40}) with a straight line it can be visualized that F2 and F4 groups fed on GTL oil were exposed to virtually only iso-alkanes (Figure 5a) which appear above the n-alkane reference line. The groups F3 and F5 fed on mineral oil were exposed to mostly cyclo-alkanes (naphthenic hydrocarbons) as only a low level of n-alkanes and iso-alkanes was observed (Figure 5b) where a cloud of cycloalkanes appears below the n-alkane reference line. Hopanes are marker compounds for petroleum derived mineral oil products. Thus, the hopanes visible in the total ion count (TIC) plot for the GTL oil (and in the naphthenic sample) are indicative of the background control feed contamination as these were not originally present in the GTL oil.





Figures 5a, 5b. *GCxGC-TOF-MS, total ion count (TIC) of the spiked feed with either GTL oil (3a) or mineral oil of naphthenic origin (3b). The presence of hopanes in GTL feed indicates presence of background contamination in the control feed. The horizontal lines connecting C_{13} and C_{40} indicates the relative position of the n-alkanes (on the line) and the multi branched iso-alkanes (above) or cycloalkanes (below).*

Biological samples

Liver results

Liver caudate lobe analysis showed a large measurement spread, especially the groups fed mineral oil (F3 and F5). Mean liver hydrocarbon concentrations and standard deviations (as measured in the caudate lobe) are presented in Table 3, expressed as mg/kg tissue and depicted in Figure 6. The control feed showed a background average hydrocarbon contamination of 27.4 mg/kg tissue. Already day 29, statistically higher values were observed for group F3 compared to F2 of about a 3-fold increase.
At day 92 the liver alkane levels in F3 group raised to about 660 mg/kg compared to 150 mg/kg in group F2 which was fed GTL oil. However, compared to control only the group fed mineral oil (F3) was significantly higher than the base line concentration. This increase over base line was not seen in the group fed GTL oil (F2) indicating that this type of oil shows less retention at comparable external doses.

At the end of the recovery period, we did not see any significant difference between the recovery groups and the control (maybe due to the large spread in measurements) suggesting that at day 134 both treated groups did not significantly differ from baseline levels. However, at study day 106 and 120 the GTL group (F2) is closer to base line levels (within measurement error) compared to the group fed mineral oil (F3) with significantly higher levels at these two timepoints. Therefore, faster elimination is seen for GTL hydrocarbon constituents (iso-alkanes) than those found in mineral oil (cyclo-alkanes).

When comparing the groups kept on the spiked diet beyond day 92 until study day 134, only the group fed mineral oil (F5) showed significantly higher levels compared to the control group (F1). The group fed GTL (F4) showed higher levels than control but did not achieve statistical significance. Thus, in general GTL oil fed uninterruptedly shows lower retention than mineral oil which is eliminated at a slower rate.

Table 3. *Total saturated hydrocarbon mean levels ($\bar{x}$) and standard deviations (sd) expressed in mg/kg tissue found in caudate liver lobes of rats fed control diet (F1), GTL oil (F2 and F4), or naphthenic mineral oil (F3 and F5), with respective sample size (n). F2 and F3 groups were switch to clean diet after day 92 until study day 134, where F3 and F5 continued with the spiked food till day 134.*

Group	Day 1			Day 29			Day 57			Day 92			Day 106			Day 120			Day 134		
	n	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***
Group F1 Group (Control)	3	34.8	9.2							4	21.3	16.5							3	28.3	14.8
Group F2 (GTL)				3	156.6	22.1	3	141.0	7.7	5	148.6	82.9	3	65.6	28.2	3	37.1	3.2	4	55.5	23.9
Group F3 (mineral oil)				3	***532.4****	***129.4***	5	399.7	164.5	4	***659.7****	***268.3***	3	***379.4****	***78.7***	4	***325.4****	***145.1***	4	183.5	148.2
Group F4 (GTL)																			3	299.4	86.2
Group F5 (mineral oil)																			5	***653.3****	***414.1***

*Significant difference in hydrocarbon retention by Kruskal-Wallis Test (α=.05)

* At SD-92, significant difference in hydrocarbon retention compared to Control group (F1) by Dunn's test for multiple comparisons (Bonferroni-corrected α*=.0167)

* At SD-134, significant difference in hydrocarbon retention compared to Control group (F1) by Dunn's test for multiple comparisons (Bonferroni-corrected α*=.005)

Note: all other pairwise comparisons were not significant

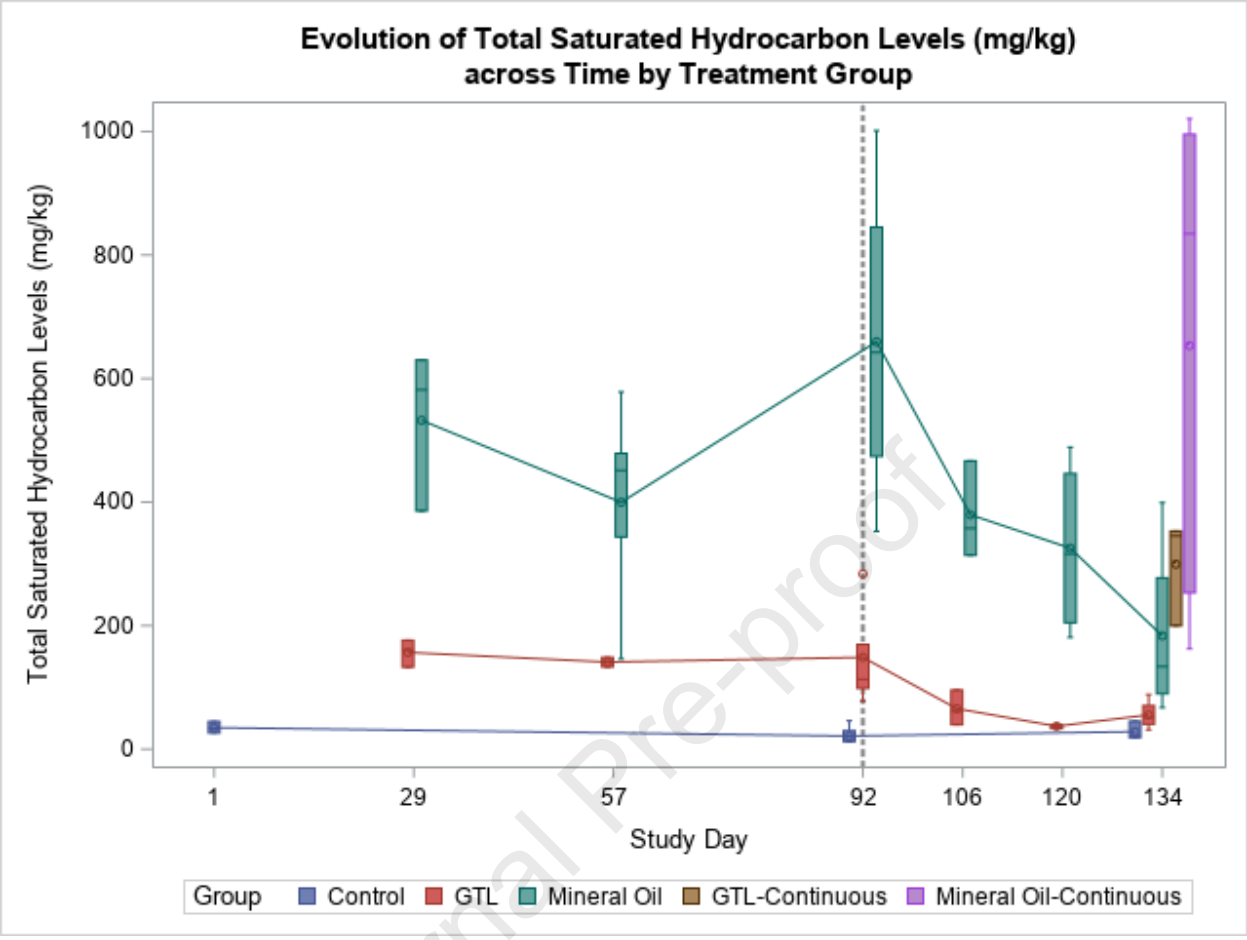


Figure 6. *Evolution of total saturated hydrocarbon levels (mg/kg liver tissue) measured at different timepoints. GTL groups F2 and F4 vs mineral oil groups F3 and F5 fed continuously until study day 92 or 134 with a recovery period until study day 134 (F2 and F3).*

Alkane types retained in the liver

At study day 92, the caudate liver lobe in the GTL group (F2) had hydrocarbon residues with a composition similar to that of the original GTL oil as shown in the inserted figure (Figure 7a, top panel), albeit with a small "foot" in the C_{20}-C_{25} range originating from background contamination. At the end of the recovery period (study day 134) a bimodal hump was observed (middle panel), clearly revealing the background contamination of the control feed found in the C_{20}-C_{25} range (highlighted in light blue) which has an average saturated hydrocarbon amount of ~ 30 mg/kg liver throughout the study (bottom panel). Considering that at study day 92 the liver in the F2 group has an average amount of 149 mg/kg of total hydrocarbons, the approximate amount corresponding to the background contamination could be about 20% of the total hydrocarbon hepatic retention. At study day 134, this is about 50% of the total of hydrocarbons retained, clearly visible as an adjacent C_{20}-C_{25} hump to the left on the GTL C_{25}-C_{35} hump range. Thus, the background contamination contributed significantly to the total hydrocarbon hepatic retention detected in the group fed GTL oil.

The group fed mineral oil showed a broader hump under which the background contamination is covered and therefore no bimodal hump is observed during the recovery phase (Figure 7b).
As comparison, a single analysis of the medial liver lobe of the F5 (continuously fed mineral oil) revealed a similar composition and amounts as in the caudate lobe, indicating that there might not be qualitative nor quantitative differences in within the liver.
Virtually no n-alkanes are present in the liver of groups fed either oil, indicating their rapid excretion and preferential retention of certain types of alkanes.

Compositional GCxGC-TOF-MS analysis of the liver caudate lobe indicates that GTL fed groups (F2) at the end of the recovery period (SD-134) show predominantly iso-alkanes (m/z 71) residues from synthetic origin above to the reference n-alkane line n-C_{11} and n-C_{40}, with virtually no presence of cycloalkanes (Figure 8). Qualitatively these synthetic hydrocarbons are likely lightly branched iso-alkanes due to their relative position to the normal alkane reference line.
In contrast at the end of the recovery period, liver residues from naphthenic mineral oil group (F3) consist of both, iso and cyclo alkanes (m/z 71 and 82 respectively), with the cyclo structures eluting close to the 5-cholastane internal standard indicative of their cyclic structure and still significantly present by showing a strong "cloud" signal (Figure 9). Based on their relative position below the n-alkane reference line, the residual iso-alkanes from the mineral oil seem to be qualitatively different to those in the GTL oil, these are likely cycloalkanes with branched substituents (i.e. alkylated cycloalkanes). Some lightly branched iso-alkanes are also visible crossing over the n-alkane reference line.

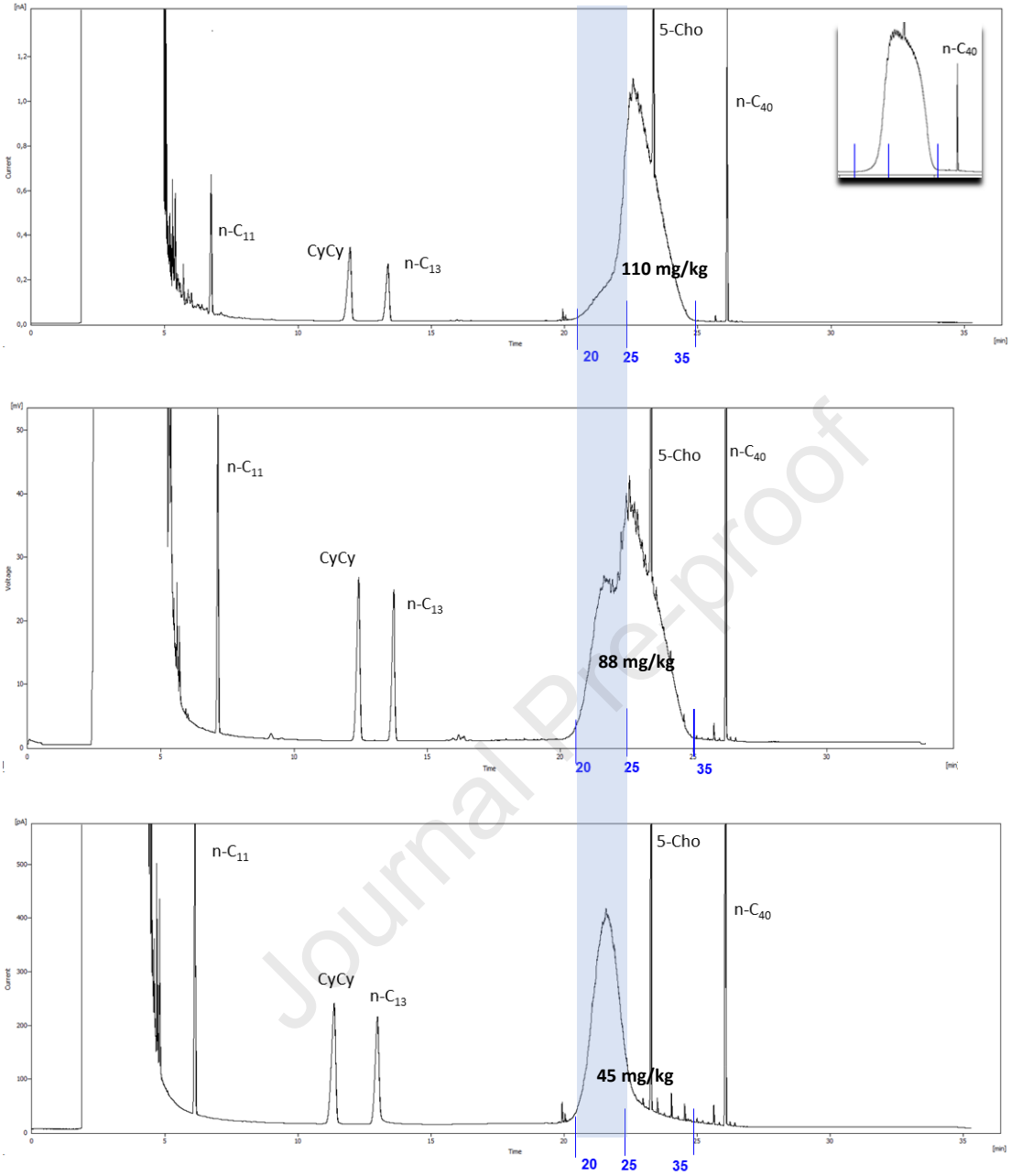


Figure 7a. *Liver caudate lobe online-HPLC-GC-FID-Chromatogram of GTL oil fed to F2 group. Saturated hydrocarbon humps are aligned to background mineral oil contamination of the control group. Top panel, hydrocarbons at study day 92 (SD-92) correlated with the original material's profile shown in the inserted figure. Middle panel, end of the recovery period (SD-134) seen as a bimodal hump consisting of the background contamination hump on the left-hand side of the GTL residual hump. Bottom panel, background mineral oil contamination of the control group at SD-134 in the range of C_{20} -C_{25} marked with a light blue strip across all three panels indicating its corresponding position. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholestane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

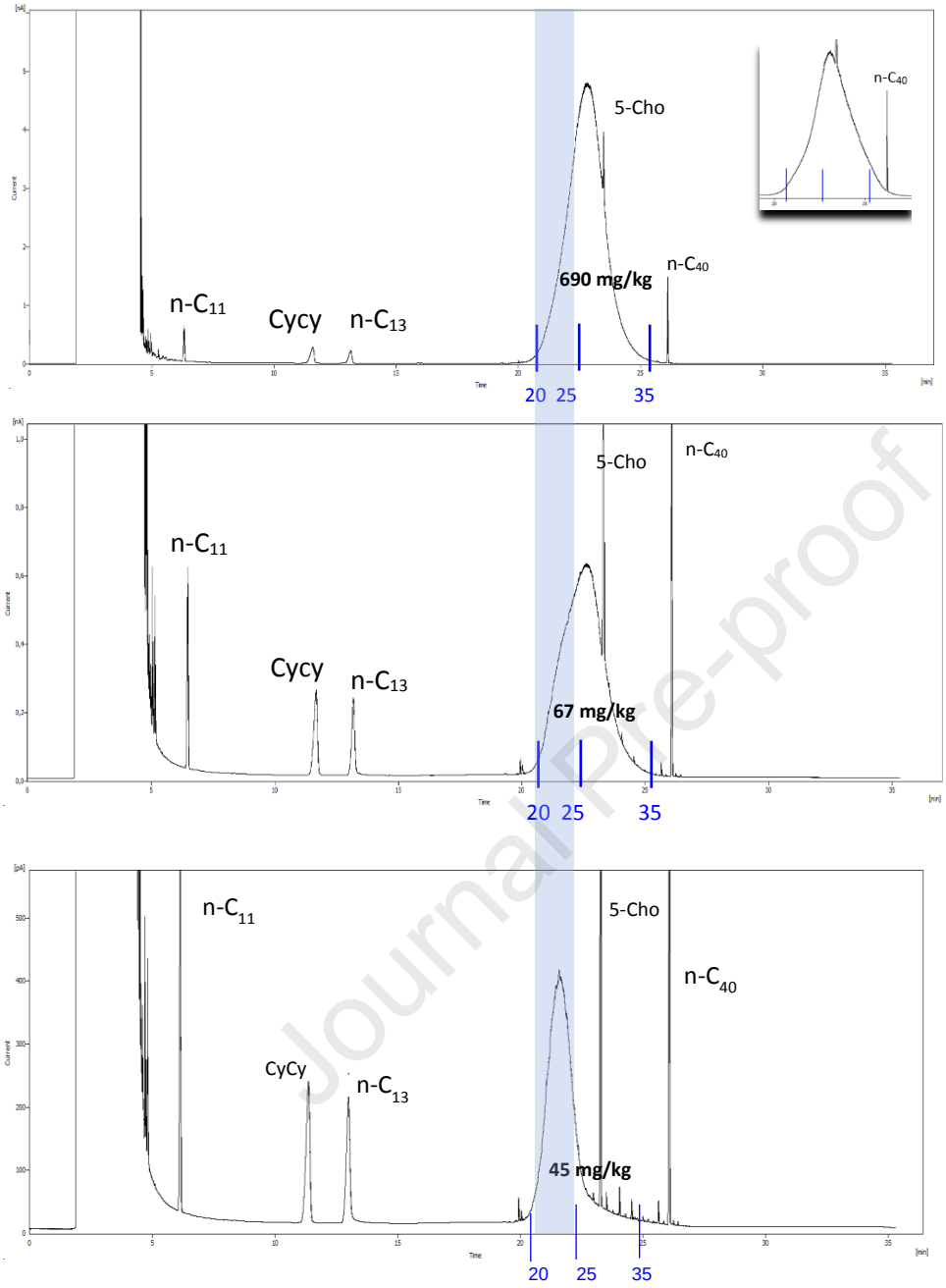
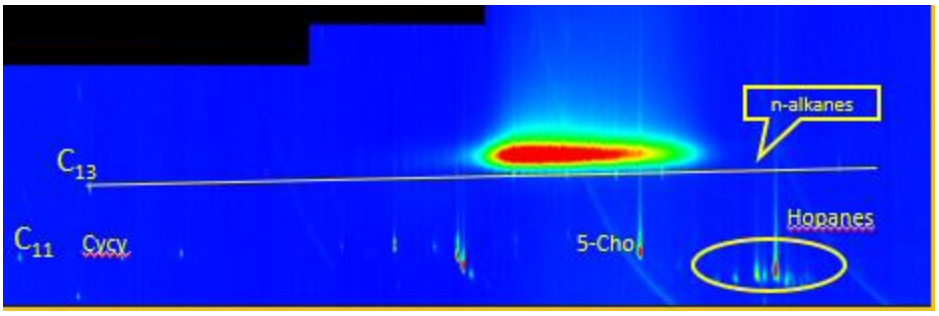
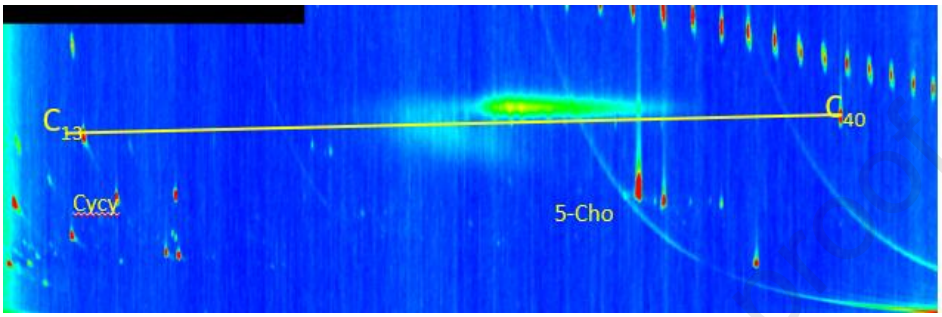


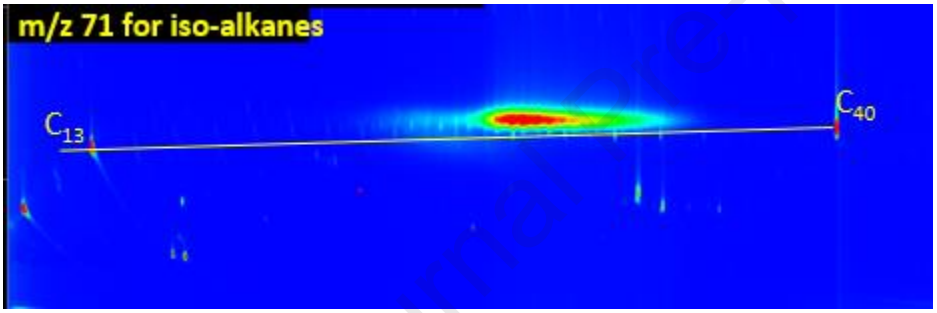
Figure 7b. *Liver caudate lobe Online-HPLC-GC-FID-Chromatogram of naphthenic mineral oil fed to group F3. Saturated hydrocarbon humps are aligned to background mineral oil contamination of the control group. Top panel, hydrocarbons at study day 92 (SD-92) correlated with the original material's profile shown in the inserted figure. Middle panel, end of the recovery period (SD-134) seen a single hump consisting of the background contamination that lies under the main residual naphthenic mineral oil hump. Bottom panel, background mineral oil contamination of the control group at SD-134 in the range of C_{20} -C_{25} marked with a light blue strip across panels indicating its corresponding position. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholestane (5-Cho), and tetracontane (n-C_{40}) are indicated.*



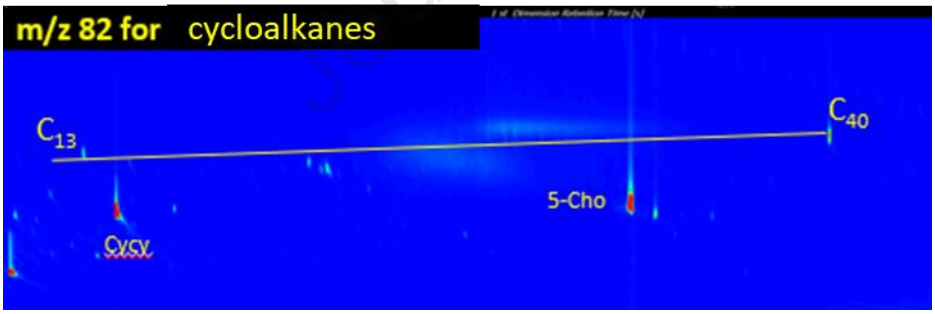
GTL oil spiked feed (F2)



GTL oil liver residues at SD-134 of recovery period (F2)

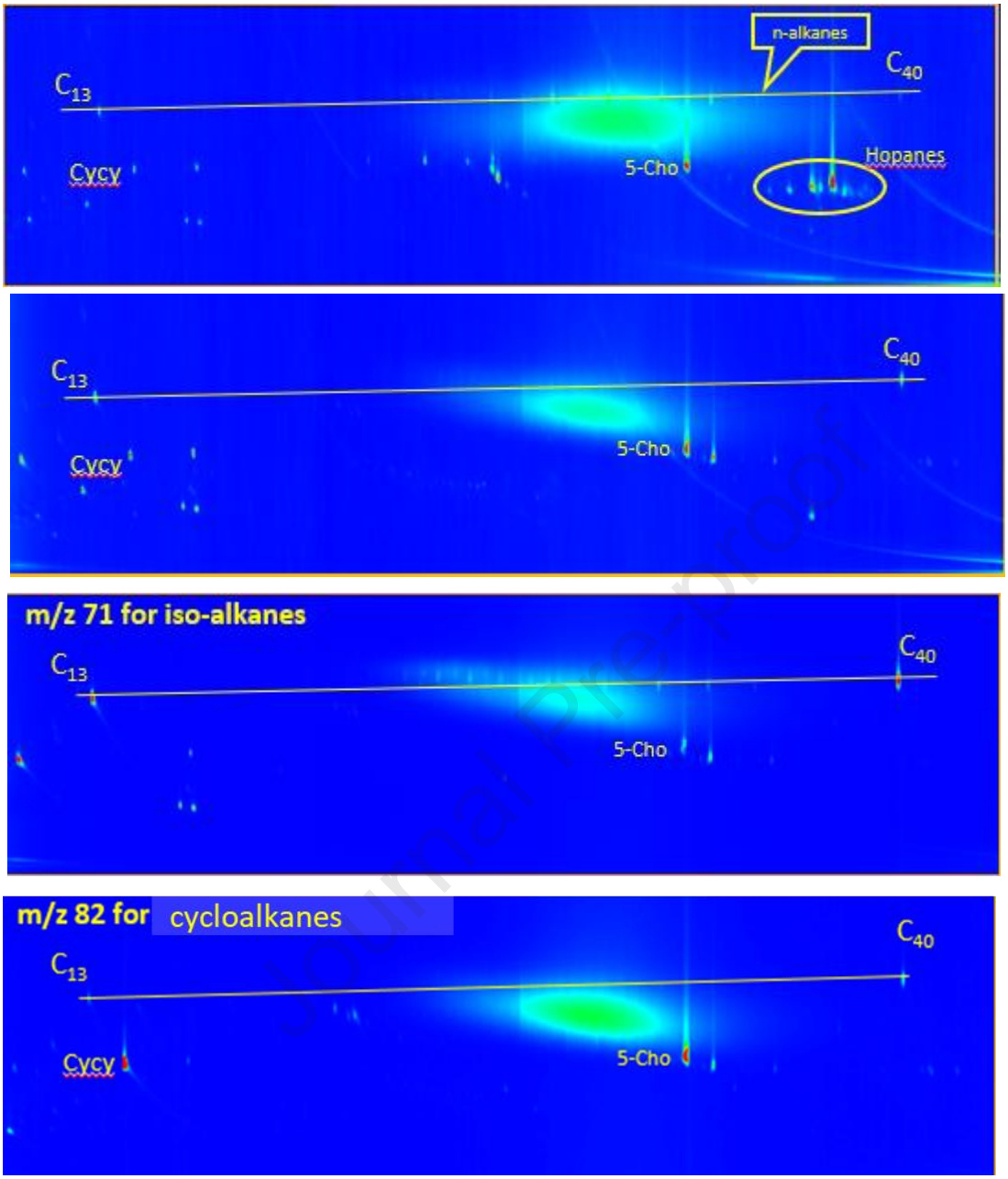


Iso-alkanes liver residues at SD-134 of recovery period (F2)



Cyclo-alkanes liver residues at SD-134 of recovery period (F2)

Figure 8. *Comparison of GCxGC-TOF-MS plots of liver extracts from F2 group. The position of the GTL residual hydrocarbons is observed relative to that of the reference substances normal undecane (C_{11}) and tetracontane (C_{40}), bycyclohexyl (Cycy) and cholestane (5-Cho). At the end of the recovery period the hydrocarbon liver residues from GTL fed animals consist of iso- alkanes and virtually no cyclo-alkanes reflecting the original composition of the GTL oil.*



Mineral oil spiked fed (F3)

Mineral oil liver residues at SD-134 of recovery period (F3)

Iso-alkanes liver residues at SD-134 of recovery period (F3)

Cyclo-alkanes liver residues at SD-134 of recovery period (F3)

Figure 9. *Comparison of GCxGC-TOF-MS plots of liver extracts from F3 group [2]. The position of the naphthenic mineral oil residual hydrocarbons is observed relative to that of the reference substances normal undecane (C_{11}) and tetracontane (C_{40}), bycyclohexyl (Cycy) and Cholestane (5-Cho). At the end of the recovery period the hydrocarbon liver residues from naphthenic mineral oil fed animals consist of predominantly cyclo-alkanes and alkylated cycloalkanes*.

[2] Because of the low sample volume, there was no material left for the mineral oil group GCxGC analysis at study day 134, so that for this time point only, the medial lobe was measured with no apparent qualitative or quantitative difference.

Mesenteric lymph node

Mesenteric lymph node (MLN) samples were also analyzed but because the focus was the evaluation of hydrocarbon residues in the liver, a less rigorous, low sample size analysis was taken aiming at a semi quantitative approach. This approach is supported by the EFSA 2012 opinion on mineral oil hydrocarbons where the liver is considered the relevant target tissue (EFSA 2012). Therefore, a similar statistical analysis done for the liver was not conducted, but the mean values are shown in tables 5 and depicted in Figure 10 to visualize the trend.

Generally, and in line with the liver results, hydrocarbon residue levels measured in the MLN of the GTL fed group were lower compared to those found in the groups fed mineral oil, and in the same range as the background levels measured in the control group. In the control group an increase after 92 and 134 days was observed compared to study day 1. For both groups fed either oil, an increasing trend was observed with longer exposure times. No apparent decrease was observed in the recovery groups of either oil.

Table 5: *Mean levels ($\bar{x}$) and standard deviations (sd) of total saturated hydrocarbons found in mesenteric lymph node expressed in mg/kg tissue with respective sample size (n).*

Mean Sum MOSH (mg/kg)	Day 1			Day 29			Day 92			Day 134		
	n	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***
Group F1 (Control)	2	15.7	0				2	68.1	14.3	2	68.3	27.5
Group F2 (GTL oil)				2	26.1	6.2	2	58.4	30.5	2	87.9	9.7
Group F3 (mineral oil)				2	67.4	36.6	2	203.4	105.2	3	262.9	118.9
Group F4 (GTL oil)										3	282.9	100.8
Group F5 (mineral oil)										3	422.0	274.6

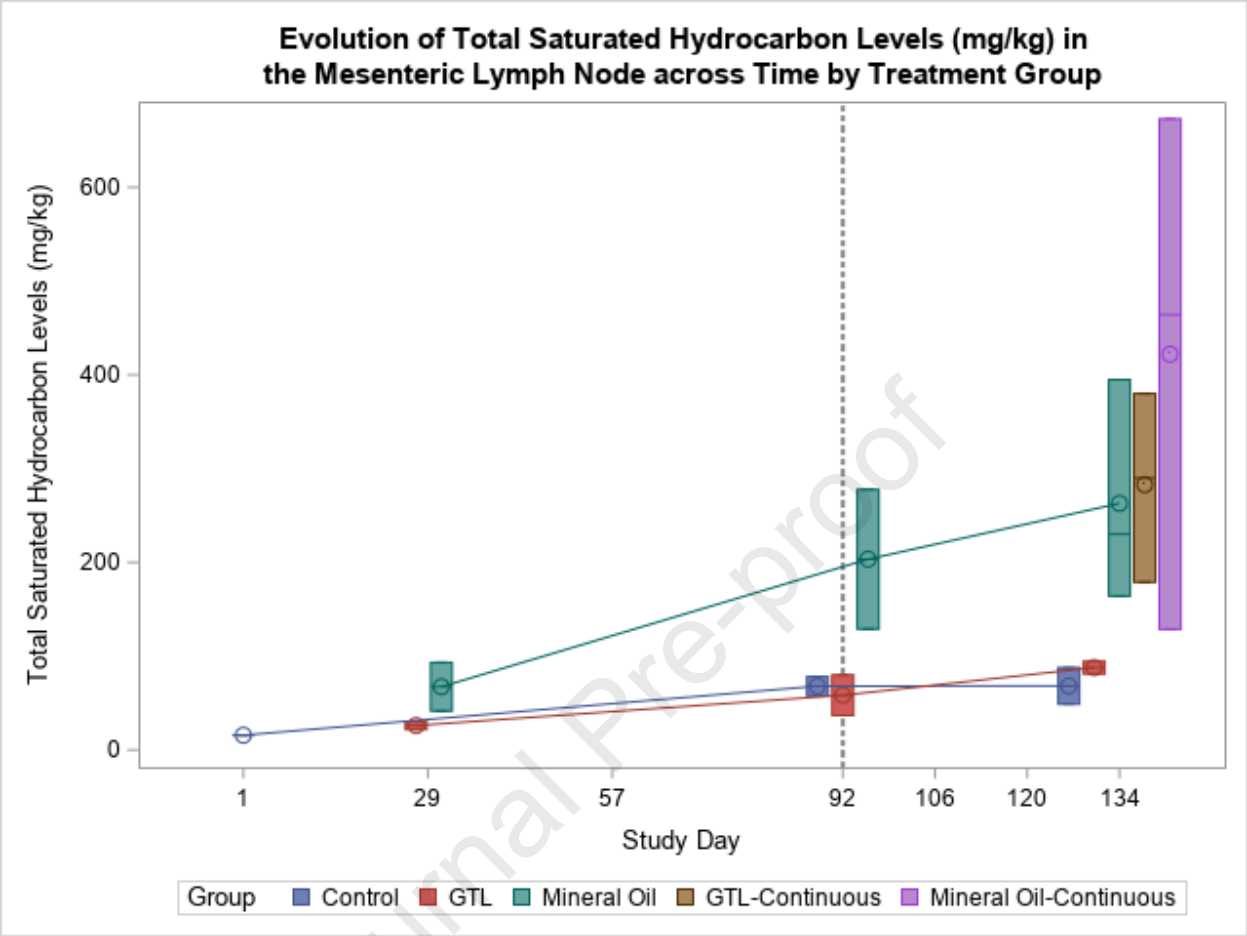


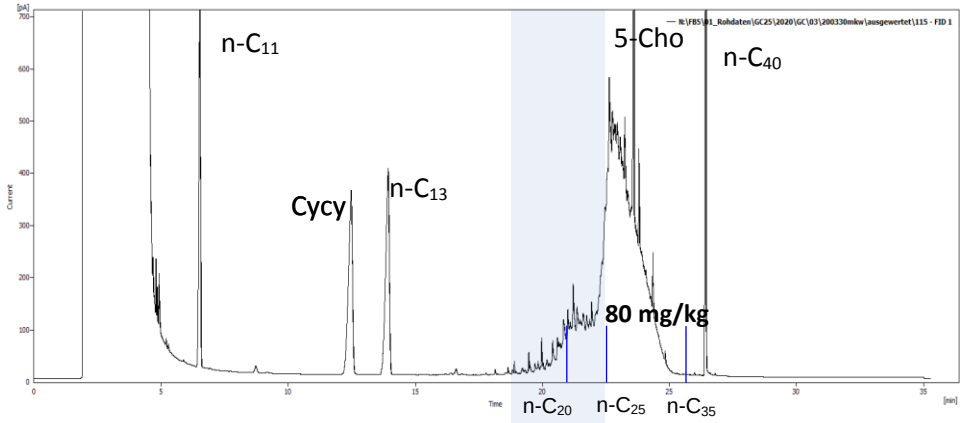
Figure 10. *Evolution of total saturated hydrocarbon levels (mg/kg mesenteric lymph node tissue) measured at different timepoints. GTL groups F2 and F4 vs. mineral oil groups F3 and F5 fed until day 92 or 134 with a recovery period until study day 134.*

As also observed in the liver, at the end of the recovery period a bimodal hydrocarbon hump distribution is seen in the MLN of the GTL oil group (F2) as a result of the background hydrocarbon contamination contained in the feed (Figure 11a). In the MLN, however, the background contamination showed a shorter carbon number range of C_{16}-C_{25} with a maximum at about C_{20} and a transition to the GTL hump at C_{25}. At study day 92, the GTL oil hump predominates over a small contamination "foot" which was also observed when GTL oil continuously for 134 days (group F4, not shown). The bimodal hump becomes clear at the end of the recovery period (SD-134) where the GTL residues go down to background concentrations.

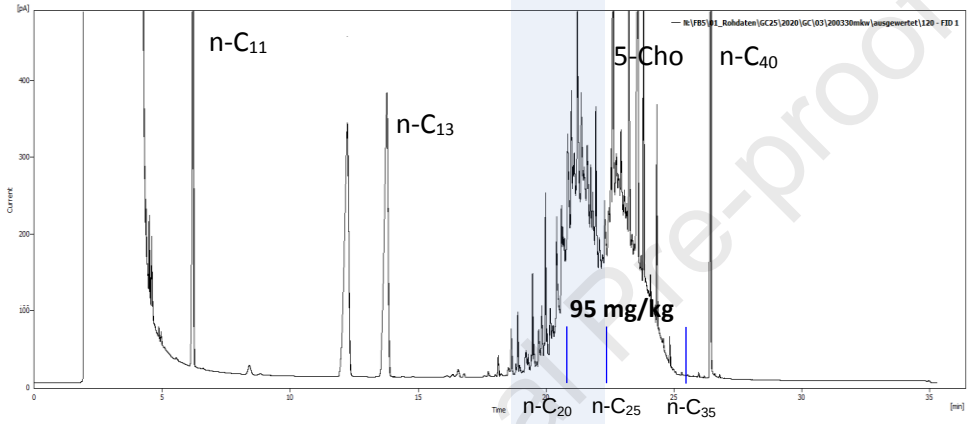
For groups fed mineral oil (F3) the background contamination is less visible, at study day 92 it totally integrates into the profile of the retained mineral oil hydrocarbons becoming a broad mineral oil hump. Only at the end of the recovery period (SD-134) the presence of the background contamination is somewhat visible but not as sharply split as in the case of the GTL oil (Figure 11b).

When comparing the hydrocarbon retention of the two oils at 90 days the bulk of the GTL constituents (70%) is narrow and in the C_{25}-C_{35} range (consistent with the original material), while the mineral oil shows a wider distribution between C_{20}-C_{35}, with about equal amounts split between C_{20}-C_{25} and C_{25}-C_{35} and therefore totally integrating the background contamination. At recovery, levels of GTL hydrocarbons in the C_{25}-C_{35} range decrease resulting in a bulk shift towards C_{25}. A similar trend is observed for the mineral oil (F3 group), where the distribution is shifted towards C_{25} and lower carbon numbers but low levels of hydrocarbons around C_{35} are still observed.

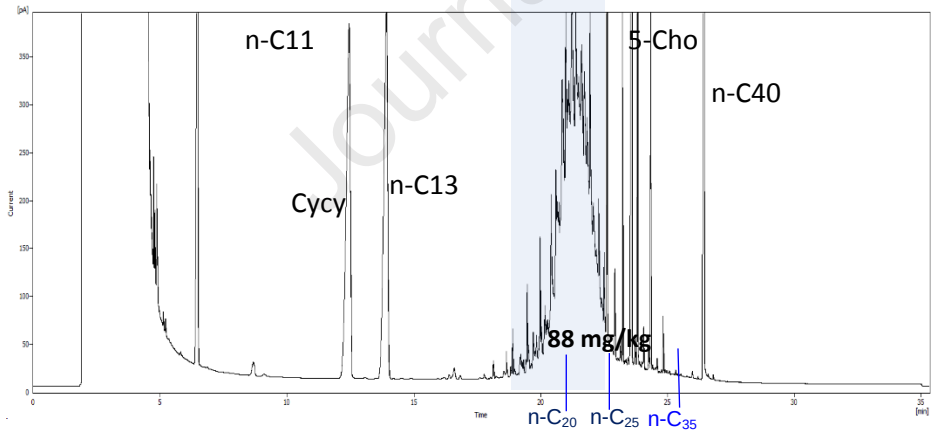
For both groups the presence of n-alkanes is visible at SD 134, which were virtually not present in the liver of either of the exposed groups.



GTL oil,
F2 group at SD-92



GTL oil,
F2 group at SD-134



Control F1 group at SD-134
with background mineral oil
contamination

Figure 11a. M*esenteric lymph node online-HPLC-GC-FID-Chromatogram of GTL oil fed to F2 group. Saturated hydrocarbon humps are aligned to background mineral oil contamination. Top panel, hydrocarbon residues at study day 92 (SD-92). Middle panel, end of the recovery period (SD-134) seen as a bimodal hump consisting of the background contamination on the left-hand side of the GTL residual hump. Lower panel, background mineral oil contamination in the control group in the range of C_{16}-C_{25} marked with a light blue strip across all three panels indicating its corresponding position in the bimodal hump. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholastane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

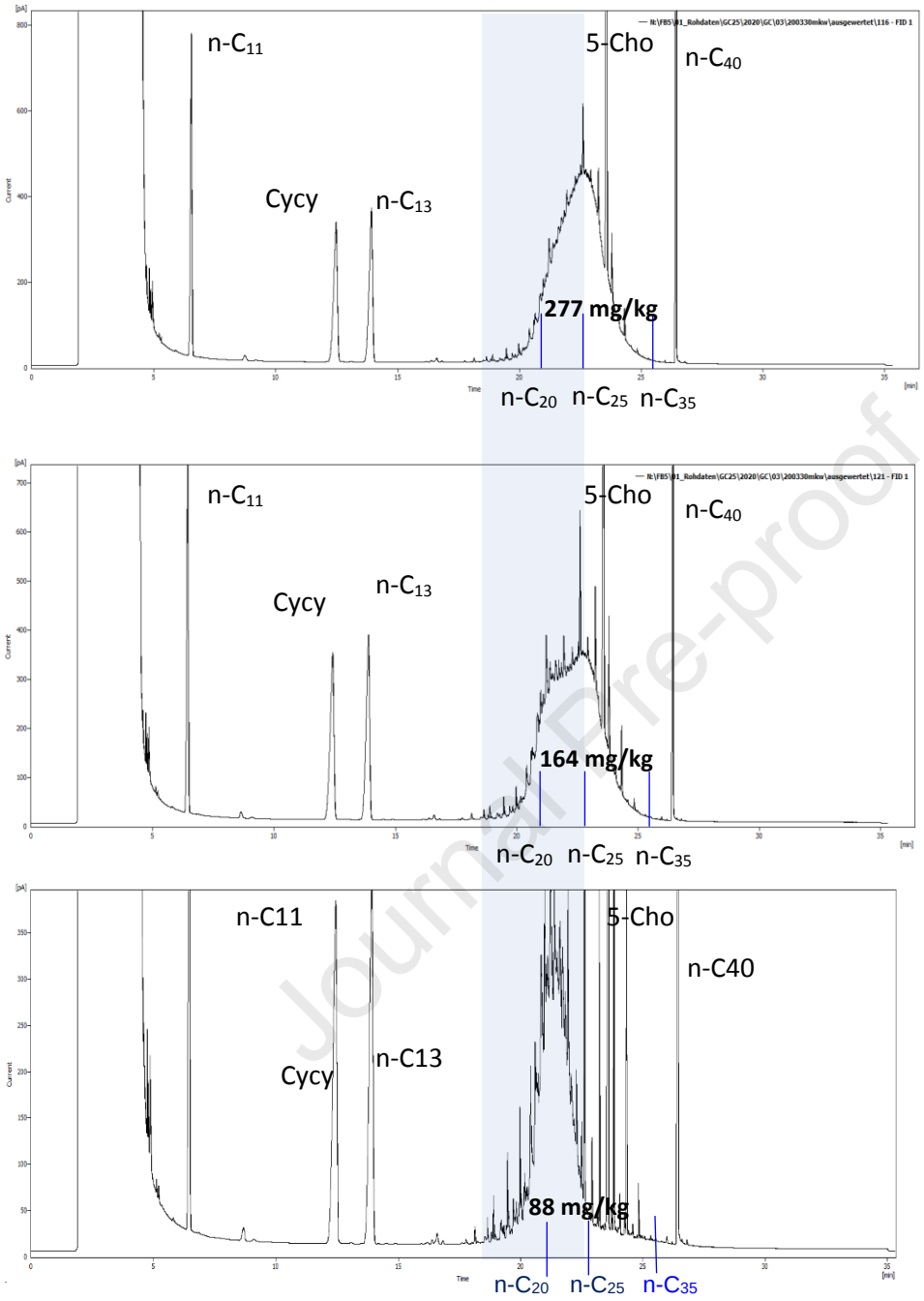


Figure 11b. *Mesenteric lymph node online-HPLC-GC-FID-Chromatogram of GTL oil fed to F3 group. Saturated hydrocarbon humps are aligned to background mineral oil contamination. Top panel, mineral oil residues at study day 92 (SD-92), contamination integrated as one hydrocarbon hump. Middle panel, end of recovery period (SD-134) seen as almost two humps consisting of background contamination on the left-hand side of the naphthenic mineral oil residual hump. Lower panel, background contamination in the control group in the range of C_{16}-C_{25} marked with a light blue strip across all three panels indicating its corresponding position in the humps. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholastane (Cho), and tetracontane (n-C_{40}) are indicated.*

Visceral fat

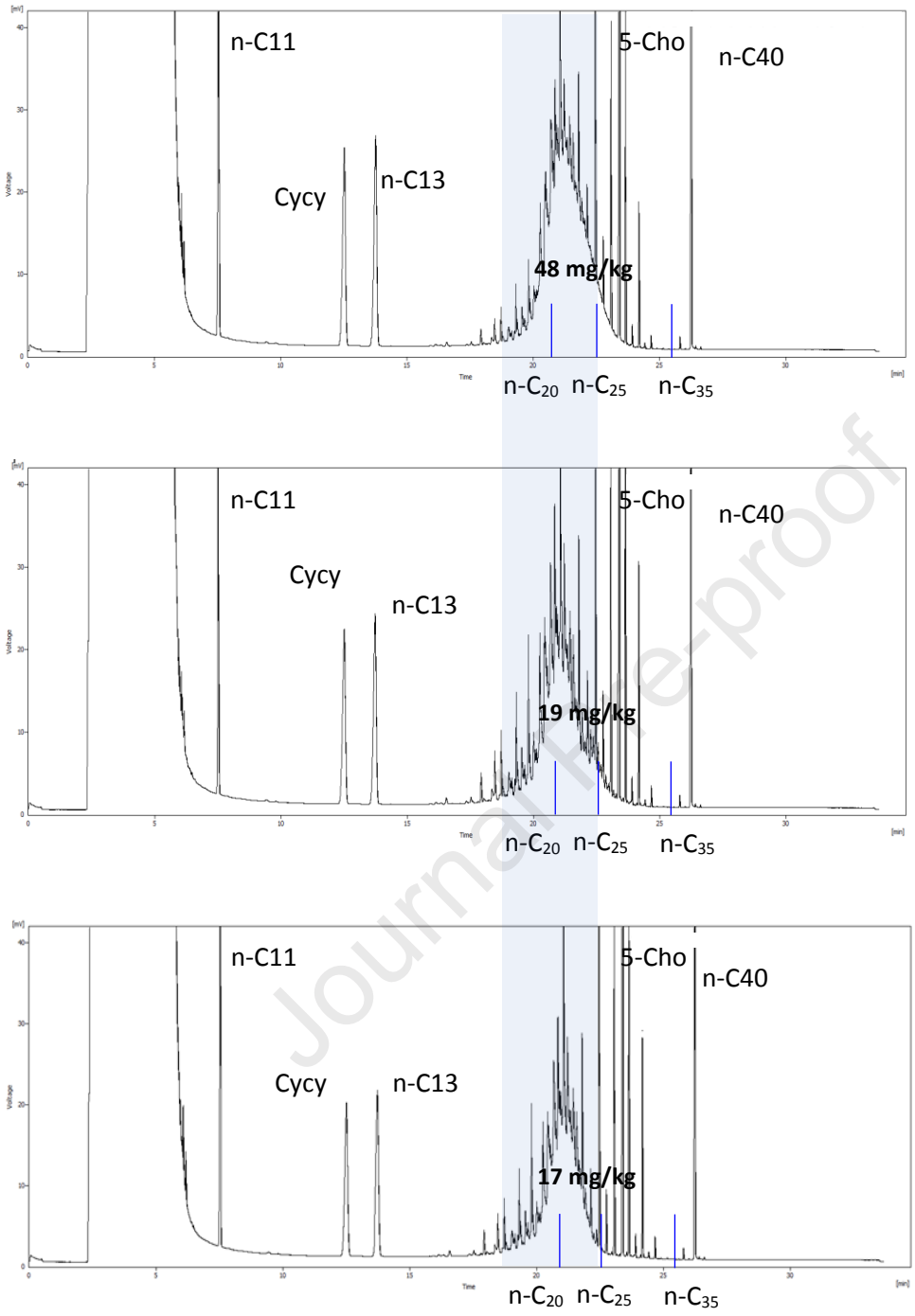
Samples of visceral fat collected at the end of the main and recovery phases (SD-134). The group fed continuously mineral oil (F5) showed statistically higher values than the control group (F1) and the GTL recovery group (F2). Differences between the other groups did not achieve statistical significance, although the difference between GTL vs mineral oil recovery groups (F2 vs F3) were close to be statistically different (Table 6).

Table 6: *Mean levels and standard deviations (sd) of total hydrocarbons found in visceral fat expressed in mg/kg tissue with respective sample size (n).*

Mean Sum MOSH (mg/kg)	**Study Day 134**		
	n	***x̄***	***sd***
Group F1 (Control)	5	19.0	2.3
Group F2 (GTL oil)	5	18.2	2.4
Group F3 (mineral oil)	5	44.0	8.8
Group F4 (GTL oil)	5	*28.0*	*8.0*
Group F5* (mineral oil)	5	*51.0*	*7.7*

Dunn's test for multiple comparisons (Bonferroni-corrected α=.0050) found a significant difference in mean saturated hydrocarbon levels (mg/kg) in the following pairwise comparisons: Group F1 -- Group F5 (p-value= .0014) and Group F2 -- Group F5 (p-value= .0005)

For all visceral fat sample chromatograms, the background contamination was the dominant profile which resulted in overlapping bimodal distributions for the GTL groups (F2 and F4) with a high-profile variation among these groups. At the end of the recovery period the hydrocarbons in the GTL recovery group (F2) consisted of virtually only those originating from the background contamination, confirmed by GCxGC analysis (data not shown). Samples of the mineral oil continuous feeding and recovery group (F5 and F3 respectively) showed similar profiles for all test animals and totally overlapping with the background contamination (Figure 12). The retained carbon number range for all groups, GTL and mineral oil, is comparable to that of the mesenteric lymph nodes namely a shift towards shorter carbon chains in the C_{16}-C_{25} range.



Mineral oil,
F3 group at SD-134

GTL oil,
F2 group at SD-134

Control F1 group at SD-134
with background mineral oil
contamination

Figure 12: *Visceral fat online-HPLC-GC-FID-Chromatogram of saturated hydrocarbons at the end of recovery period at study day 134 from mineral oil (F3) and GTL group (F2) compared to control group (F1). The blue stripe across panels marks the relative position of the background mineral oil contamination in the control feed in the range of C_{16} -C_{25}. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholastane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

5. Discussion

The oral route is probably the biggest source of mineral oil entry in the human body. In 1970, Boitnott and Margolis demonstrated that the spleen and liver were correlated in the type of mineral oil hydrocarbons retained (Boitnott and Margolis 1970) with the liver retaining a higher level of these hydrocarbons. It was concluded that the mineral oil hydrocarbons (alkanes) showing highest retention in the liver (and thus also in the spleen) were the multiring cycloalkanes (naphthenics) and highly isomerized alkanes which were associated with the formation of hepatic lipogranuloma when a critical internal dose was exceeded. Lipogranuloma related to mineral oil exposure is not considered adverse (Fleming and Carrillo 2018; Fleming et al. 1998), but it is undesired. EFSA has indicated that for the assessment of mineral oil, one should focus on the liver as the target organ (EFSA 2012).

In a study of 2014 that analyzed mineral oil residues in human tissues provided state of the art compositional analysis to characterize the type of mineral oil alkanes retained in different tissues, including the liver. These retained hydrocarbon factions were referred to as 'mineral oil saturated hydrocarbons' – MOSH (Barp et al. 2014). None of the MOSH fractions retained in human livers contained n-alkanes although this alkane type is clearly present in the fat tissue on top of the MOSH hump. This observation indicates that despite exposure to n-alkanes, these are effectively metabolized in the liver and thus do not form part of the hepatic MOSH fraction. On the other hand, all livers showed a preferential retention of nonlinear alkanes consisting of highly branched and polycyclic naphthenics within a carbon range of C_{20}-C_{35} with a critical narrow range of ~ C_{25}-C_{30}.

Therefore, there is human evidence of the hepatic retention of a narrow fraction of saturated hydrocarbons from mineral oil origin consisting mostly of cycloalkanes (naphthenics) in the C_{20}-C_{35} range which should be referred to as "MOSH". MOSH may trigger the formation of liver *lipogranuloma* if a critical internal dose is exceeded (Boitnott and Margolis 1970; Fleming and Carrillo 2018; Fleming et al. 1998).

Because of the potential use of GTL synthetic paraffin oil in food contact processes and as vaccine adjuvants as alternatives of conventional mineral oil we investigated its retention potential in the liver and other tissues after repeated dose using state of the art analytical techniques.

As products of Shell's Fischer-Tropsch technology that converts natural gas to liquid hydrocarbons; medicinal grade Gas to Liquid (GTL) oils consist virtually of only iso-paraffins where levels of n-alkanes, aromatics, and cycloalkanes (naphthenics) are negligible.

Therefore, our study compared two oils with extreme composition GTL to a conventional mineral oil of naphthenic origin (high naphthenic content). The choice of these oils is based not only on the compositional contrast between iso-alkanes vs cycloalkanes, but also because the bulk of the oil's carbon number range distribution is in the C_{25}-C_{30} range which has been shown to be the critical range for hepatic hydrocarbon retention. The study focused primarily on the liver, which is considered the critical organ to assess MOSH deposition (EFSA 2012; Nygaard et al. 2019), however complementary analysis was also done for the mesenteric lymph nodes (MLN) and visceral fat.

The results of the study allow us to draw conclusions on the two points postulated in our hypothesis.

1. Because GTL oil is virtually free from naphthenics, it will show less residues and more rapid excretion than mineral oil.
2. The type of branching of GTL iso-alkanes will result higher absorption than conventional mineral oil.

Despite the high variability in the liver sample measurements, we can draw clear conclusions. Contrary to the expected higher levels of absorption of GTL oil, repeated exposure at equal external dose resulted in lower hepatic levels of GTL oil than mineral oil. In the liver, we could also observe lower levels of GTL

residues during recovery, close to those of the control base line which we attribute to faster elimination. In the fat and MLN, a trend towards base line levels during recovery was also observed. Apart from faster removal from tissues, lower tissue levels may be explained by lower gut absorption of iso-alkanes. Radiotracer studies indicate that cycloparaffins are more extensively absorbed than iso-paraffins or n-alkanes for a white oil tested (Low L. 1992). This suggests that GTL iso-alkanes show low tissue retention because of two forces at play namely rapid excretion and lower gut absorption. Conventional mineral oil on the other hand, showed higher hepatic retention of hydrocarbons indicating that mineral oil naphthenics (cycloalkanes) are preferentially retained and absorbed. Cycloalkane constituents are also eliminated during recovery, albeit at lower rate. No n-alkanes in the liver were observed in either group indicating rapid elimination of these type of alkanes and hence preferential retention of certain types of mineral oil saturated hydrocarbons.

This selectivity is driven by the structural differences between GTL vs mineral oil hydrocarbon constituents as clearly seen from the GCxGC liver chromatograms. These structural differences can be inferred from the relative position of the liver hydrocarbon residues to the n-alkane reference line drawn between n-C_{15} and n-C_{40}. GTL oil iso-alkanes are found above the n-alkane line in contrast to mineral oil constituents (mostly cycloalkanes but also iso-alkanes) which are situated below the line. Even those iso-alkanes found in mineral oil are largely below the n-alkane reference line indicative of structural differences between iso-alkanes of mineral oil origin vs those found in GTL oils. These data falsify our second hypothesis that "*the different branching pattern of the iso-alkanes in GTL oils would result in a higher in-vivo uptake causing higher hydrocarbon levels in the liver"*. Based on the present experimental data, we reformulate this hypothesis as: The type of branching present in synthetic GTL oils confers to them a lower gut absorption, lower hepatic hydrocarbon levels and a faster excretion, compared to iso-alkanes and the multiring alkene structures (naphthenics) typical of mineral oil.

Structural differences between GTL iso-alkanes and hydrocarbons from mineral oil origin provide a new perspective into the interpretation of the MOSH fraction found in human tissues. This is clearly seen at the end of the recovery period when the background mineral oil contamination becomes visible in all tissues from the GTL oil group. As the GTL fraction (C_{25}-C_{35}) decreases and the MOSH background contamination becomes the main hydrocarbon bulk (C_{20}-C_{25}) a bimodal hump is formed consisting of a background "MOSH" and a "GTL" hump. In the mineral oil group, the background contamination becomes integrated under a single "MOSH" hump indicative of the structural affinity between the "MOSH contamination" and the "MOSH test material".

In addition to the bimodal hump effect seen in the GTL group, the MLN and fat tissue showed a selective and limited retention of lower molecular weight alkane constituents in the C_{16}-C_{25} range with a peak at about C_{20} for both type of oils suggesting a shift to the left from the distribution seen in the liver. In the case of GTL, at the end of the recovery period the fat had was virtually devoid of GTL hydrocarbons where the remaining residues had an identical profile as the background MOSH contamination. In contrast to the liver, n-alkanes were visible in MLN of both oil groups. This indicates that qualitatively the fat retains different hydrocarbons than the liver; the n-alkanes present in the feed and the low molecular weight components (< C_{20}) from the background contamination although present in the fat are effectively eliminated in the liver. This confirms that the relevant hydrocarbon carbon range retained in the liver is C_{20}-C_{35} with a peak around C_{25} with clear absorption and metabolic differences between GTL iso-alkanes and mineral oil constituents, including naphthenics (MOSH). Furthermore, because this study tested two oils of extreme compositions, we demonstrated how iso and cylo alkane sub-classes partition into different tissues in the Sprague Dawley rat. n-Alkanes, virtually absent or in negligible amounts in the oils but present in the feed, were retained in the MLN and fat but eliminated

by the liver. This provides the basis for a qualitative comparison with similar qualitative chromatography results from human tissues (Barp et al. 2014; Biedermann et al. 2015; Boitnott and Margolis 1970). The qualitative retention of naphthenic hydrocarbons in liver, MLN and fat observed in the Sprague Dawley rats are also consistent with those reported in the literature for the F344 rat (Barp et al. 2017; Cravedi et al. 2017) suggesting that for oils devoid of n-alkanes, retention of naphthenics is qualitatively comparable across rat strains and humans: virtually total elimination for alkane constituents $<C_{20}$, moderate for C_{20}-C_{25}, slower for C_{25}-C_{35}, and virtually no $>C_{35}$ due to limited bioavailability. The only difference between rat strains is that the F-344 rat strain accumulates also n-alkanes (Cravedi et al. 2017) from biogenic and petrogenic origin which is not relevant for humans and thus must not be regarded MOSH (Carrillo et al. 2021). Therefore, from the results of this study where iso-alkanes originating from GTL show a low absorption and retention potential and faster elimination, the concept of MOSH should focus on (poly)cycloalkanes (naphthenics) of mineral oil origin supported by human and animal studies.

The low accumulation potential of GTL oil has practical consequences. Apart from GTL oil low toxicity shown in repeated dose studies where a NOAEL = 1000 mg/kg was set for sub-chronic, pre-natal and reproductive toxicity studies (Boogaard et al. 2017; Dunster 2009; Faiola 2011; Senn 2014) , its lower potential for tissue hydrocarbon accumulation presents an alternative for applications in the food industry where lubrication and low MOSH contamination is required. As MOSH retention in humans is mostly related to the retention of naphthenic hydrocarbons in the C_{25}-C_{35} range (Biedermann et al. 2015), GTL oils in this carbon number range clearly present an advantage over high viscosity mineral oils currently approved for food contact applications (EFSA 2009a; EFSA 2013a).
In addition, their use as adjuvants in animal vaccines presents an improvement in MRL (minimal residual level) over conventional mineral oil currently used for such purposes (EMEA 1995). Vaccine formulations with GTL adjuvants may provide an alternative so that hydrocarbon residues in slaughtered meat can be kept as low as possible and free from mineral oil hydrocarbons which are more difficult to eliminate.

6. Conclusions

- At the same external dose, the iso-alkanes and multiring cycloalkanes (naphthenics) from mineral oil show higher hepatic retention and slower excretion than GTL iso-alkane constituents with the same carbon number range distribution.
- The lower hepatic levels of GTL hydrocarbons may be explained by lower gut absorption and faster elimination of the GTL iso-alkanes. We attribute these divergences to the structural differences between the iso-alkanes present in synthetic GTL oils versus those iso-alkanes from mineral oil origin.
- Retention of alkane sub-classes in SD rat tissues, including the liver, is qualitatively comparable to that seen in humans. Both SD-rat and human tissues show the same pattern for n-alkane distribution where the F-344 notably shows a deviant pattern. Thus, the present study provides evidence for the relevance of the SD rat strain as a model for the risk assessment of hydrocarbons in humans.
- The study provides further experimental evidence that the alkane sub-class most prone to hepatic retention are cycloalkanes (naphthenics). As these are notably absent in GTL oils, the term “MOSH” should not encompass synthetic GTL oils and be restricted to petroleum products containing this alkane sub-class in the C_{20}-C_{35} range.

- The low accumulation potential of GTL oil offers an alternative in food related applications and vaccine adjuvants where MOSH retention in organs, including the liver, is not desired. This especially includes therapeutic vaccines with multiple vaccinations.

7. Conflict of interest

Juan-Carlos Carrillo; Hua Shen; Fayaz Momin and Olaf Kral are employed by Shell, which has a commercial interest in both mineral oils and GTL products. This study was fully sponsored by Shell as part of a research and development program. Holger Schnieder is a paid consultant advising Shell on the potential use of GTL oils as vaccine adjuvants.

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Table 1. *Classification of white mineral oils according to JECFA (JECFA; 2002)*

Name	Viscosity at 100°C in mm^2/s	Average molecular weight g/mol	Carbon number at 5% distillation	Examples[1]
High viscosity	> 11	> 500	$\geq$ 28	P100H
Class I	8.5 – 11	480 – 500	$\geq$ 25	P70H
Class II	7.0 – 8.5	400 – 480	$\geq$ 22	N70H
Class III	3.0 – 7.0	300 – 400	$\geq$ 17	P15H, N15H

[1] The white oil nomenclature used is based on the oil's crude origin, viscosity at 40°C and refining method. Thus, a P100 oil is from paraffinic crude (P), viscosity of 100 mm^2/s @ 40°C and purified by hydrotreatment (H). Similarly, N70A, would be an oil of naphthenic origin, with 70 mm^2/s @ 40°C and purified by acid treatment (A).

Table 2. *Necropsy Schedule. Study day (SD) and number of rats sacrificed.*

	Main Phase					Recovery Phase		
Feed Group	**SD 1**	**SD 29**	**SD 57**	**SD 92**	**SD134**	**SD 106**	**SD 120**	**SD 134**
Group F1 (Control)	5	-	-	5	-	-	-	5
Group F2 (GTL oil)	-	5	5	5	-	5	5	5
Group F3 (mineral oil)	-	5	5	5	-	5	5	5
Group F4 – continuous (GTL oil)	-	-	-	-	5	-	-	-
Group F5 – continuous (mineral oil)	-	-	-	-	5	-	-	-

Table 3. *Total saturated hydrocarbon mean levels ($\bar{x}$) and standard deviations (sd) expressed in mg/kg tissue found in caudate liver lobes of rats fed control diet (F1), GTL oil (F2 and F4), or naphthenic mineral oil (F3 and F5), with respective sample size (n). F2 and F3 groups were switch to clean diet after day 92 until study day 134, where F3 and F5 continued with the spiked food till day 134.*

Group	Day 1			Day 29			Day 57			Day 92			Day 106			Day 120			Day 134		
	n	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***
Group F1 Group (Control)	3	34.8	9.2							4	21.3	16.5							3	28.3	14.8
Group F2 (GTL)				3	156.6	22.1	3	141.0	7.7	5	148.6	82.9	3	65.6	28.2	3	37.1	3.2	4	55.5	23.9
Group F3 (mineral oil)				3	***532.4****	***129.4***	5	399.7	164.5	4	***659.7****	***268.3***	3	***379.4****	***78.7***	4	***325.4****	***145.1***	4	183.5	148.2
Group F4 (GTL)																			3	299.4	86.2
Group F5 (mineral oil)																			5	***653.3****	***414.1***

*Significant difference in hydrocarbon retention by Kruskal-Wallis Test (α=.05)

* At SD-92, significant difference in hydrocarbon retention compared to Control group (F1) by Dunn's test for multiple comparisons (Bonferroni-corrected α*=.0167)

* At SD-134, significant difference in hydrocarbon retention compared to Control group (F1) by Dunn's test for multiple comparisons (Bonferroni-corrected α*=.005)

Note: all other pairwise comparisons were not significant

Table 5: *Mean levels ($\bar{x}$) and standard deviations (sd) of total saturated hydrocarbons found in mesenteric lymph node expressed in mg/kg tissue with respective sample size (n).*

Mean Sum MOSH (mg/kg)	**Day 1**			**Day 29**			**Day 92**			**Day 134**		
	n	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***	***n***	***$\bar{x}$***	***sd***
Group F1 (Control)	2	15.7	0				2	68.1	14.3	2	68.3	27.5
Group F2 (GTL oil)				2	26.1	6.2	2	58.4	30.5	2	87.9	9.7
Group F3 (mineral oil)				2	67.4	36.6	2	203.4	105.2	3	262.9	118.9
Group F4 (GTL oil)										3	282.9	100.8
Group F5 (mineral oil)										3	422.0	274.6

Table 6: *Mean levels and standard deviations (sd) of total hydrocarbons found in visceral fat expressed in mg/kg tissue with respective sample size (n).*

Mean Sum MOSH (mg/kg)	**Study Day 134**		
	n	***x̄***	***sd***
Group F1 (Control)	5	19.0	2.3
Group F2 (GTL oil)	5	18.2	2.4
Group F3 (mineral oil)	5	44.0	8.8
Group F4 (GTL oil)	5	*28.0*	*8.0*
Group F5* (mineral oil)	5	*51.0*	*7.7*

Dunn's test for multiple comparisons (Bonferroni-corrected α=.0050) found a significant difference in mean saturated hydrocarbon levels (mg/kg) in the following pairwise comparisons: Group F1 -- Group F5 (p-value= .0014) and Group F2 -- Group F5 (p-value= .0005)

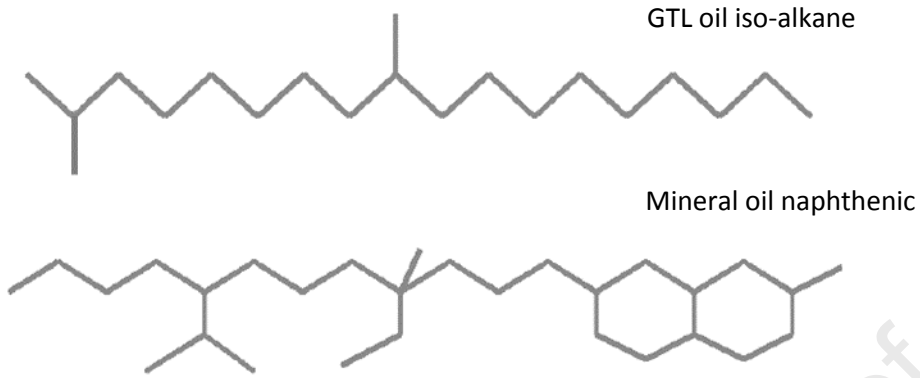


Figure 1. Representative main GTL oil and mineral oil constituents.

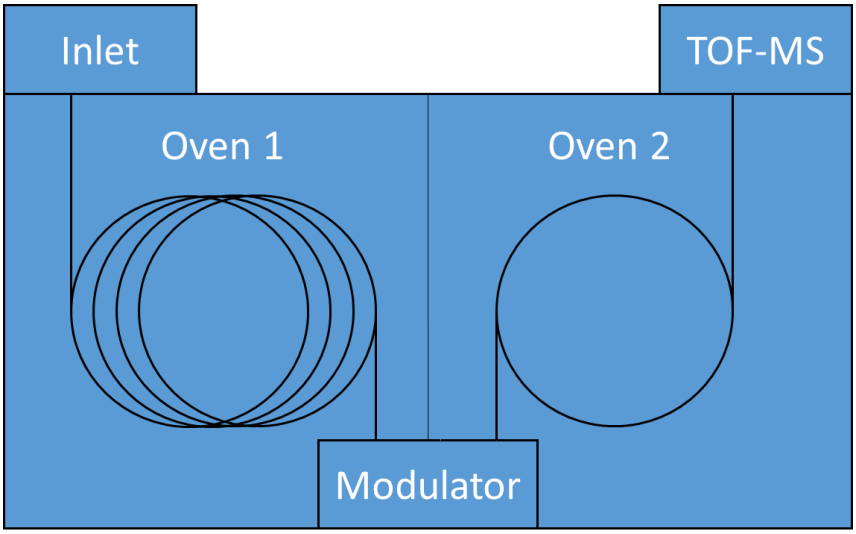


Figure 2. Connection diagram of the two dimensional chromatography instrumentation (GCxGC)

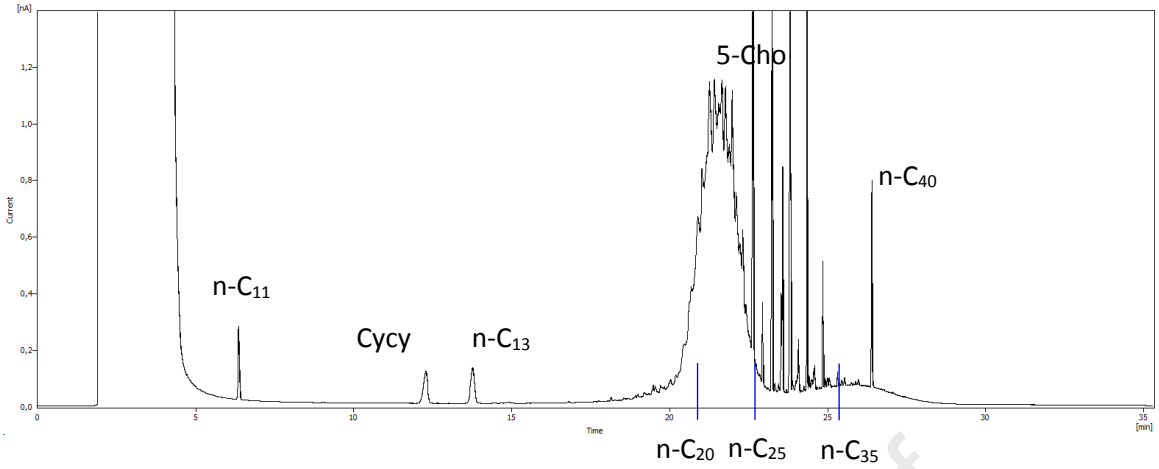


Figure 3. *Online-HPLC-GC-FID-Chromatogram of the background mineral oil contamination found in control feed of about 90 ppm. Reference substances undecane (n-C11); bicyclohexyl (Cycy), tridecane (n-C13), cholestane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

Figure 4a. GTL oil spiked in feed F2 and F4

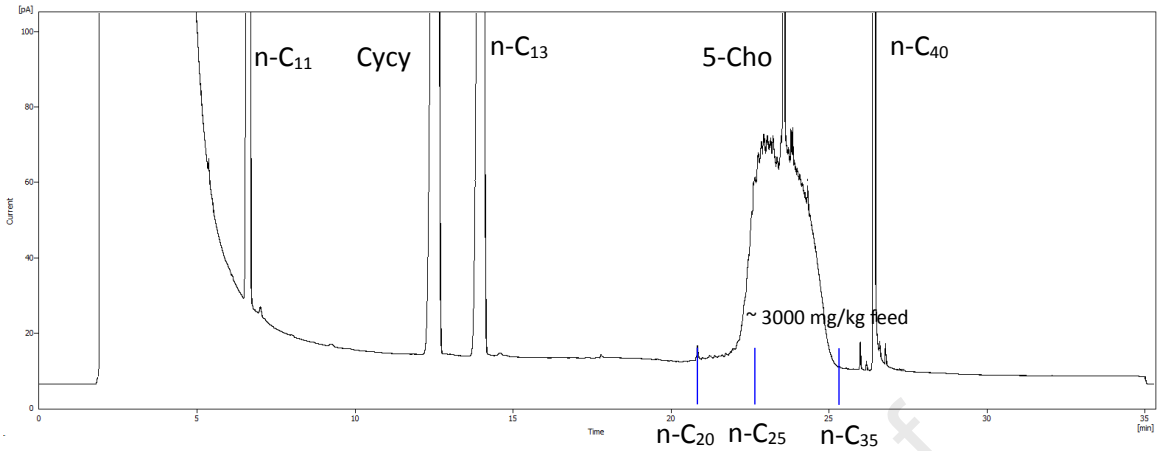
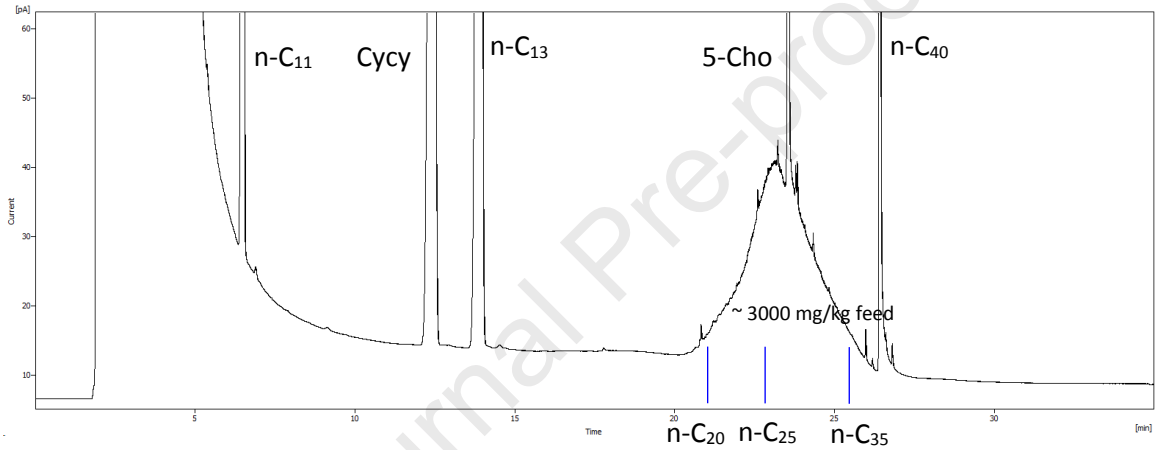
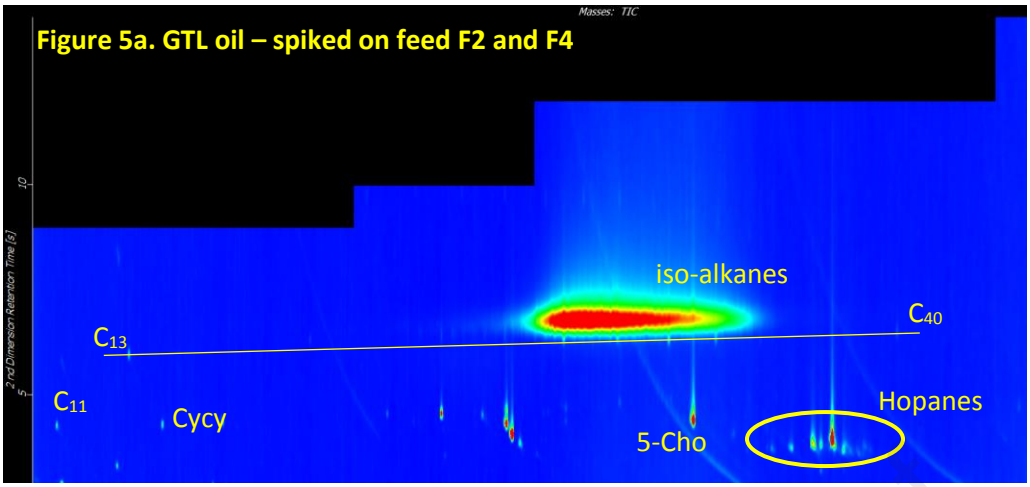


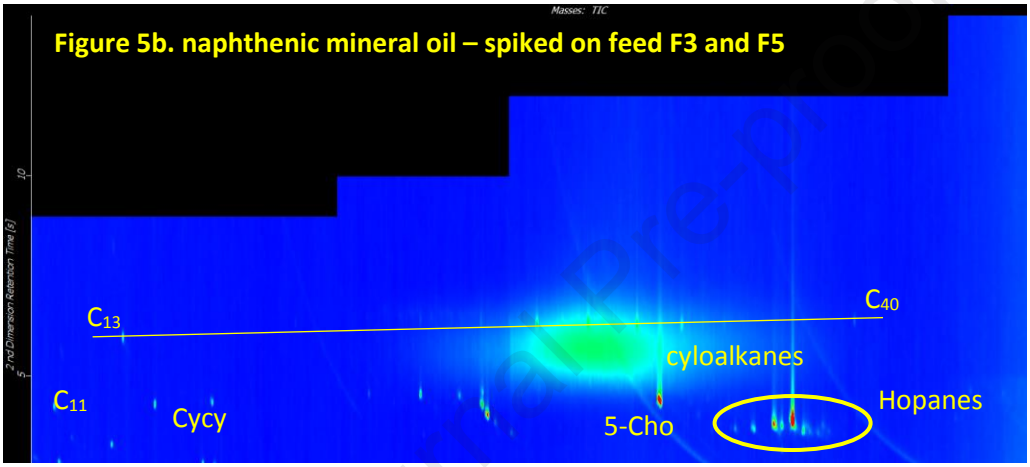
Figure 4b. Mineral oil spiked in feed F3 and F5



Figures 4a, 4b. *Online-HPLC-GC-FID-Chromatogram of the extracts from spiked feed with GTL oil (2a) or mineral oil (2b) shown with their corresponding carbon number ranges. Note the bimodal hump profile from the GTL oil spiked feed (2a) due to background mineral oil contamination found in the control feed. Reference substances undecane (n-C11); bicyclohexyl (Cycy), tridecane (n-C13), cholestane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

1
2
3





Figures 5a, 5b. *GCxGC-TOF-MS, total ion count (TIC) of the spiked feed with either GTL oil (3a) or mineral oil of naphthenic origin (3b). The presence of hopanes in GTL feed indicates presence of background contamination in the control feed. The horizontal lines connecting C_{13} and C_{40} indicates the relative position of the n-alkanes (on the line) and the multi branched iso-alkanes (above) or cycloalkanes (below).*

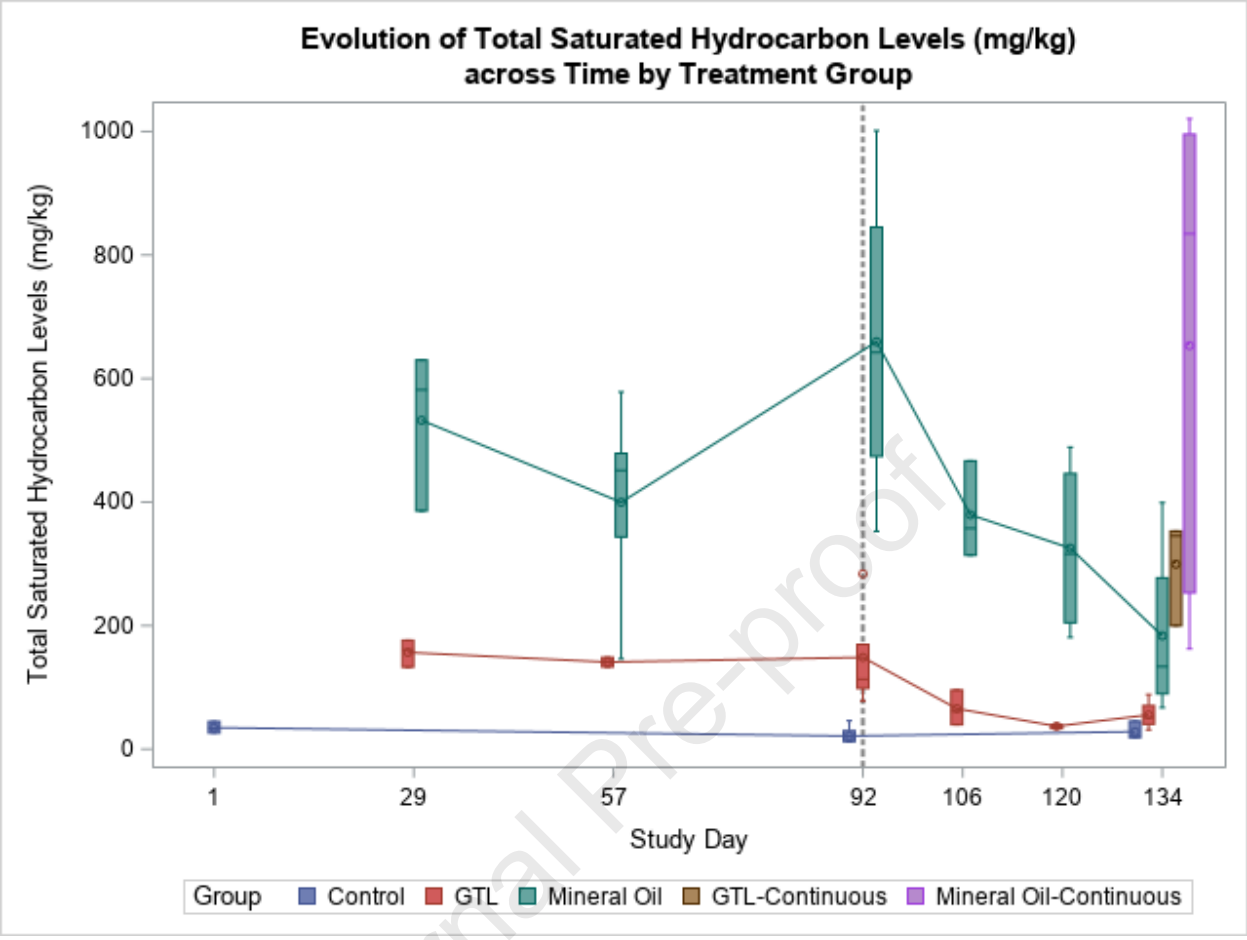
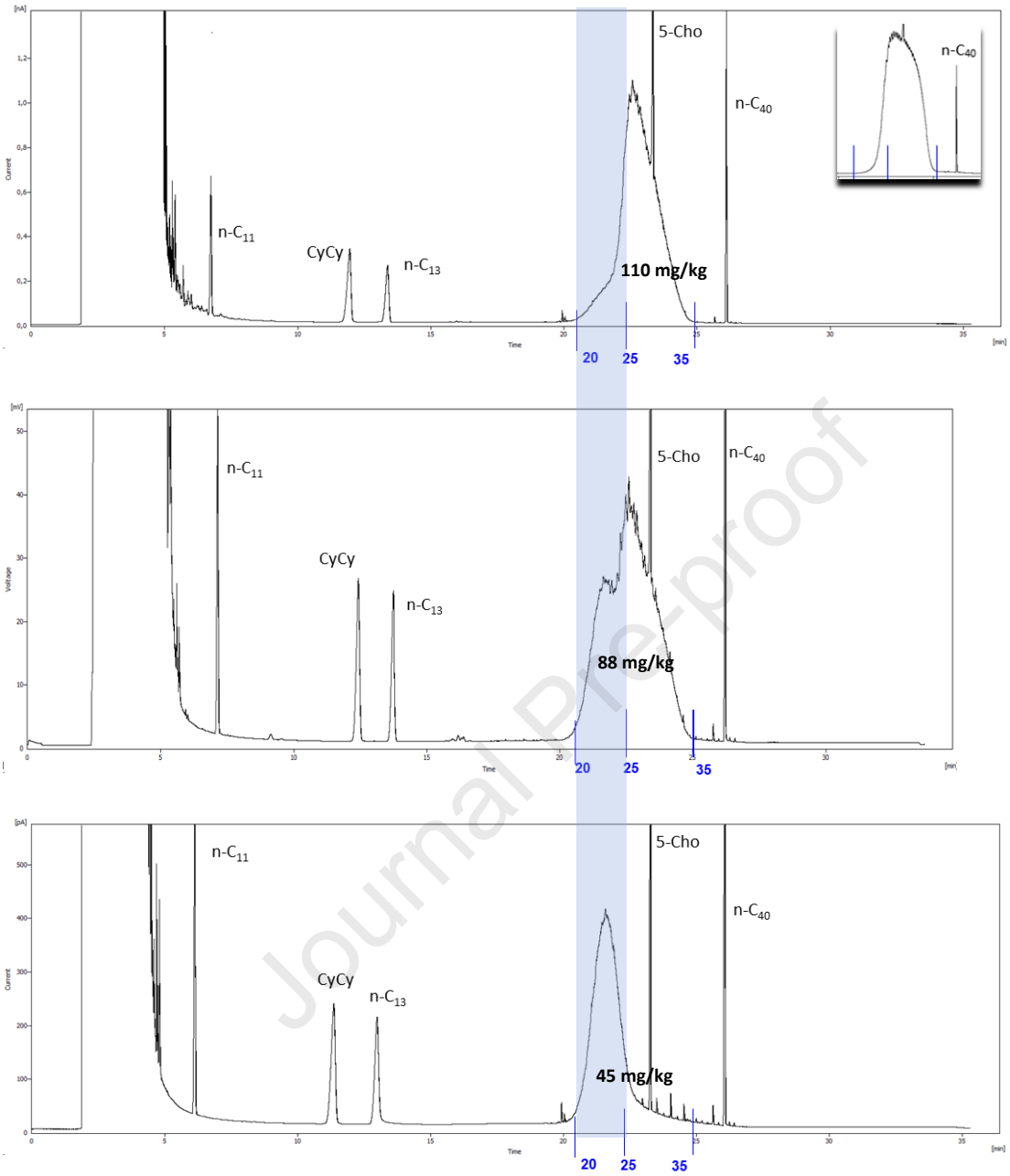


Figure 6. *Evolution of total saturated hydrocarbon levels (mg/kg liver tissue) measured at different timepoints. GTL groups F2 and F4 vs mineral oil groups F3 and F5 fed continuously until study day 92 or 134 with a recovery period until study day 134 (F2 and F3).*

1

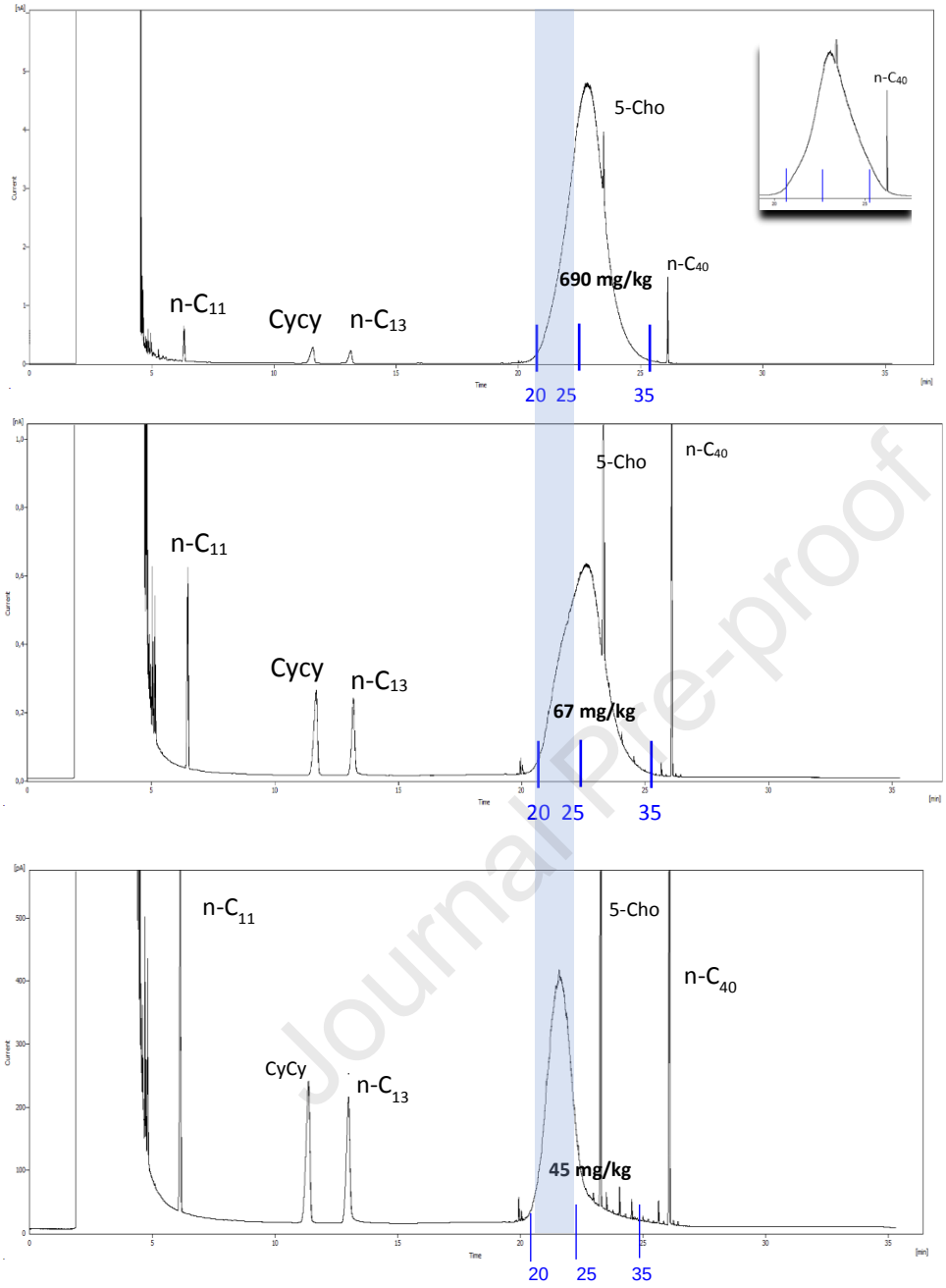


GTL oil, F2 group at SD-92

GTL oil, F2 group at recovery SD-134

Control, F1 group at SD-134, with background mineral oil contamination

Figure 7a. *Liver caudate lobe online-HPLC-GC-FID-Chromatogram of GTL oil fed to F2 group. Saturated hydrocarbon humps are aligned to background mineral oil contamination of the control group. Top panel, hydrocarbons at study day 92 (SD-92) correlated with the original material's profile shown in the inserted figure. Middle panel, end of the recovery period (SD-134) seen as a bimodal hump consisting of the background contamination hump on the left-hand side of the GTL residual hump. Bottom panel, background mineral oil contamination of the control group at SD-134 in the range of C_{20} -C_{25} marked with a light blue strip across all three panels indicating its corresponding position. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholestane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

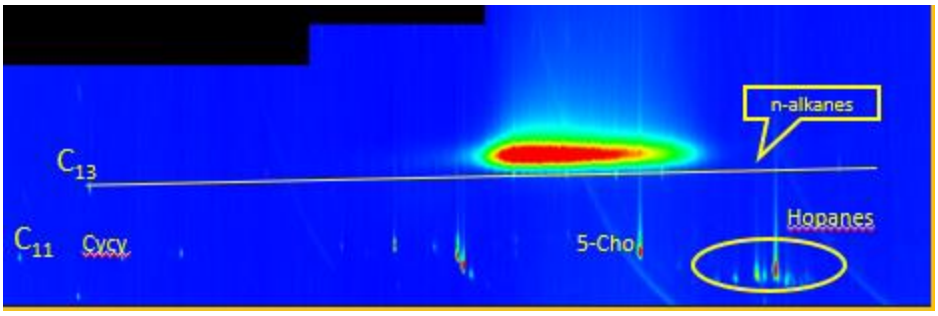


Mineral oil, F3 group at SD-92

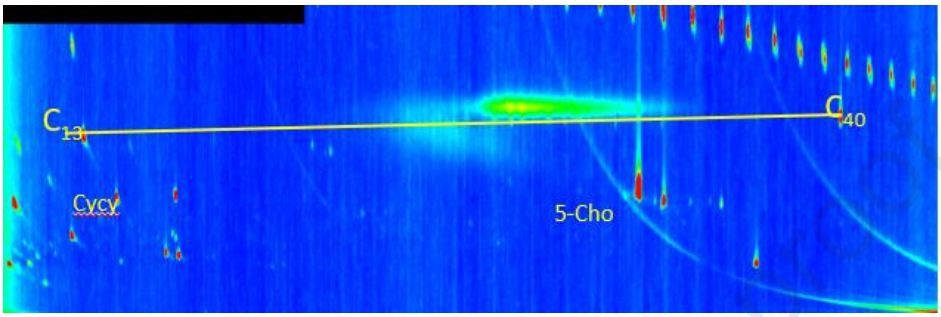
Mineral oil, F3 group at SD-134

Control, F1 group at SD-134, with background mineral oil contamination

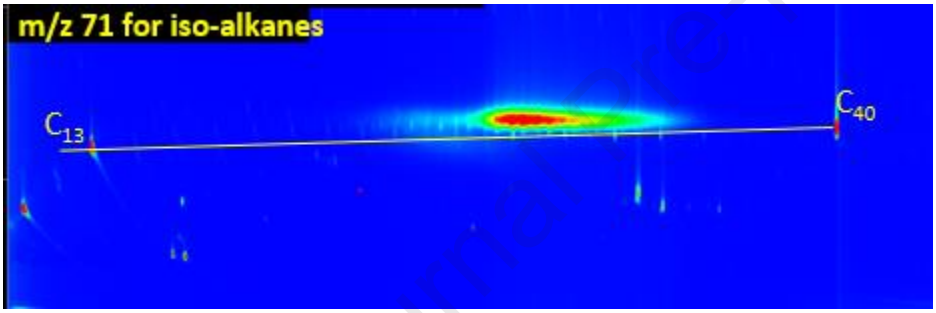
Figure 7b. *Liver caudate lobe Online-HPLC-GC-FID-Chromatogram of naphthenic mineral oil fed to group F3. Saturated hydrocarbon humps are aligned to background mineral oil contamination of the control group. Top panel, hydrocarbons at study day 92 (SD-92) correlated with the original material's profile shown in the inserted figure. Middle panel, end of the recovery period (SD-134) seen a single hump consisting of the background contamination that lies under the main residual naphthenic mineral oil hump. Bottom panel, background mineral oil contamination of the control group at SD-134 in the range of C_{20} -C_{25} marked with a light blue strip across panels indicating its corresponding position. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholestane (5-Cho), and tetracontane (n-C_{40}) are indicated.*



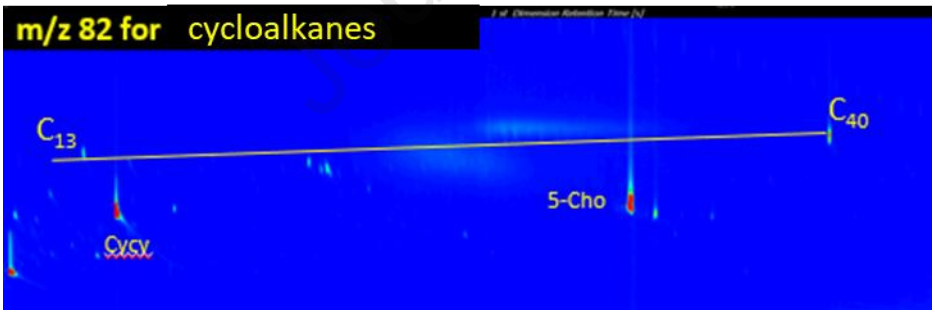
GTL oil spiked feed (F2)



GTL oil liver residues at SD-134 of recovery period (F2)



Iso-alkanes liver residues at SD-134 of recovery period (F2)



Cyclo-alkanes liver residues at SD-134 of recovery period (F2)

Figure 8. *Comparison of GCxGC-TOF-MS plots of liver extracts from F2 group. The position of the GTL residual hydrocarbons is observed relative to that of the reference substances normal undecane (C_{11}) and tetracontane (C_{40}), bycyclohexyl (Cycy) and cholestane (5-Cho). At the end of the recovery period the hydrocarbon liver residues from GTL fed animals consist of iso- alkanes and virtually no cyclo-alkanes reflecting the original composition of the GTL oil.*

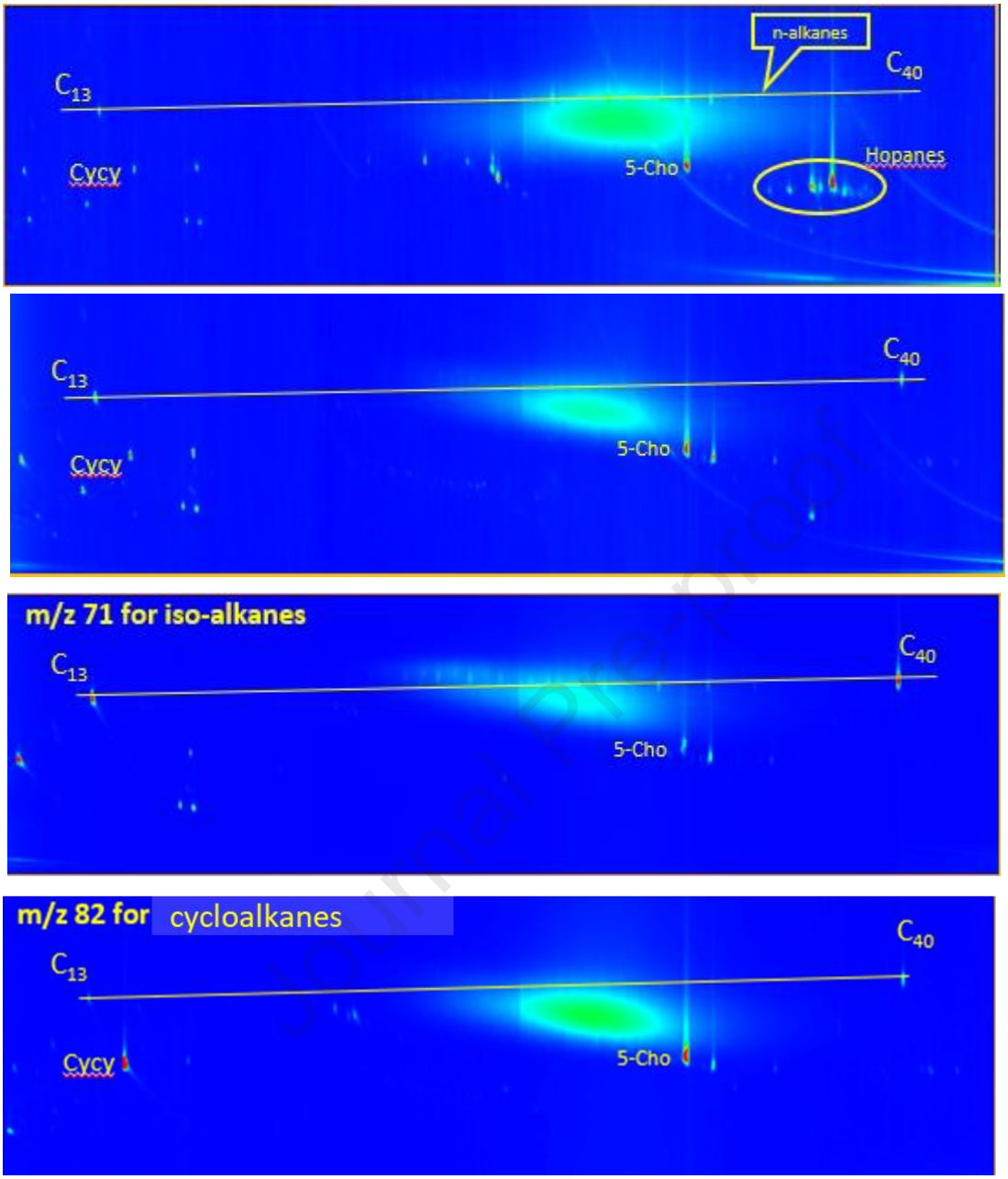


Figure 9. *Comparison of GCxGC-TOF-MS plots of liver extracts from F3 group [1]. The position of the naphthenic mineral oil residual hydrocarbons is observed relative to that of the reference substances normal undecane (C_{11}) and tetracontane (C_{40}), bycyclohexyl (Cycy) and Cholestane (5-Cho). At the end of the recovery period the hydrocarbon liver residues from naphthenic mineral oil fed animals consist of predominantly cyclo-alkanes and alkylated cycloalkanes*.

[1] Because of the low sample volume, there was no material left for the mineral oil group GCxGC analysis at study day 134, so that for this time point only, the medial lobe was measured with no apparent qualitative or quantitative difference.

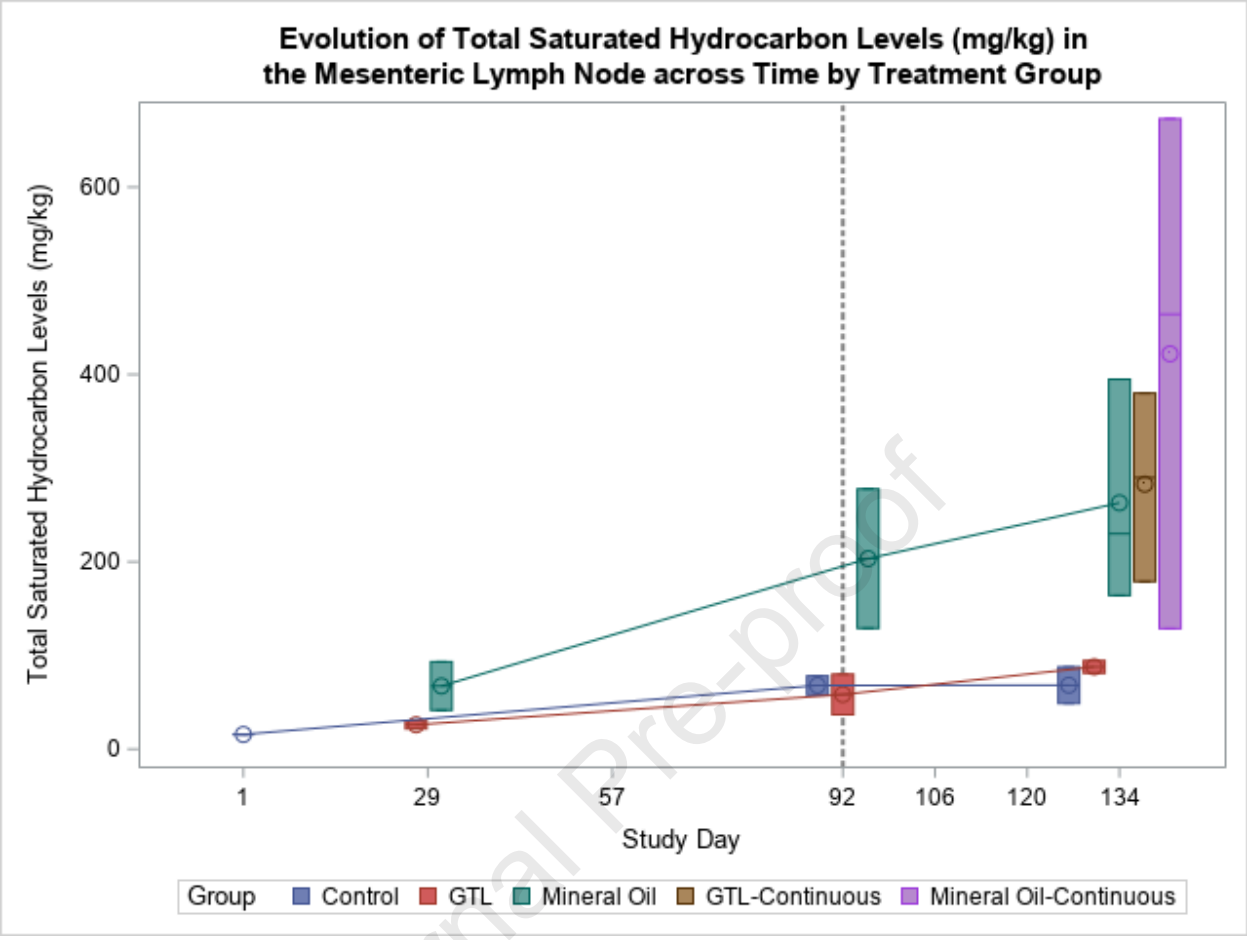
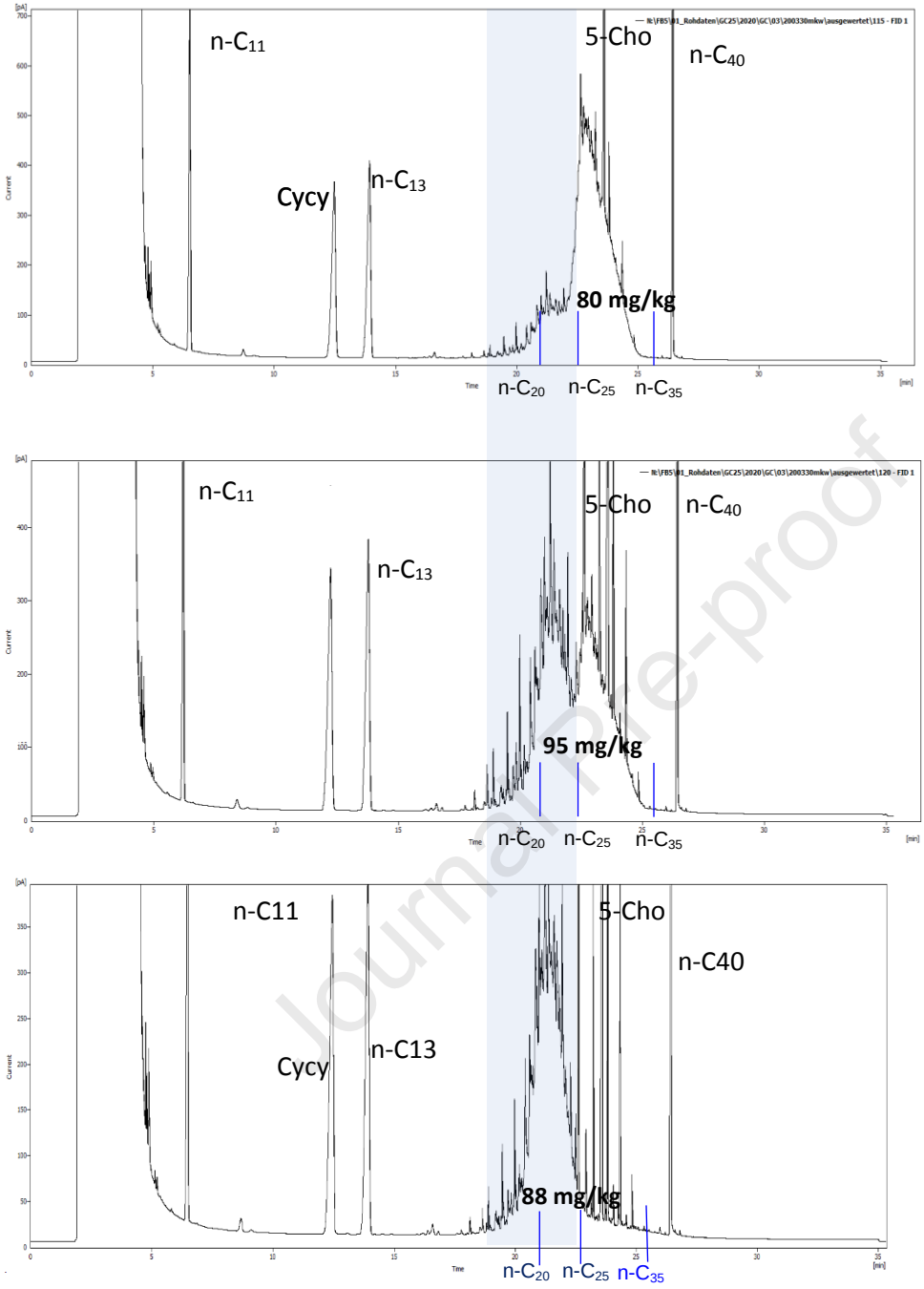


Figure 10. *Evolution of total saturated hydrocarbon levels (mg/kg mesenteric lymph node tissue) measured at different timepoints. GTL groups F2 and F4 vs. mineral oil groups F3 and F5 fed until day 92 or 134 with a recovery period until study day 134.*



GTL oil,
F2 group at SD-92

GTL oil,
F2 group at SD-134

Control F1 group at SD-134
with background mineral oil
contamination

Figure 11a. M*esenteric lymph node online-HPLC-GC-FID-Chromatogram of GTL oil fed to F2 group. Saturated hydrocarbon humps are aligned to background mineral oil contamination. Top panel, hydrocarbon residues at study day 92 (SD-92). Middle panel, end of the recovery period (SD-134) seen as a bimodal hump consisting of the background contamination on the left-hand side of the GTL residual hump. Lower panel, background mineral oil contamination in the control group in the range of C_{16}-C_{25} marked with a light blue strip across all three panels indicating its corresponding position in the bimodal hump. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholastane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

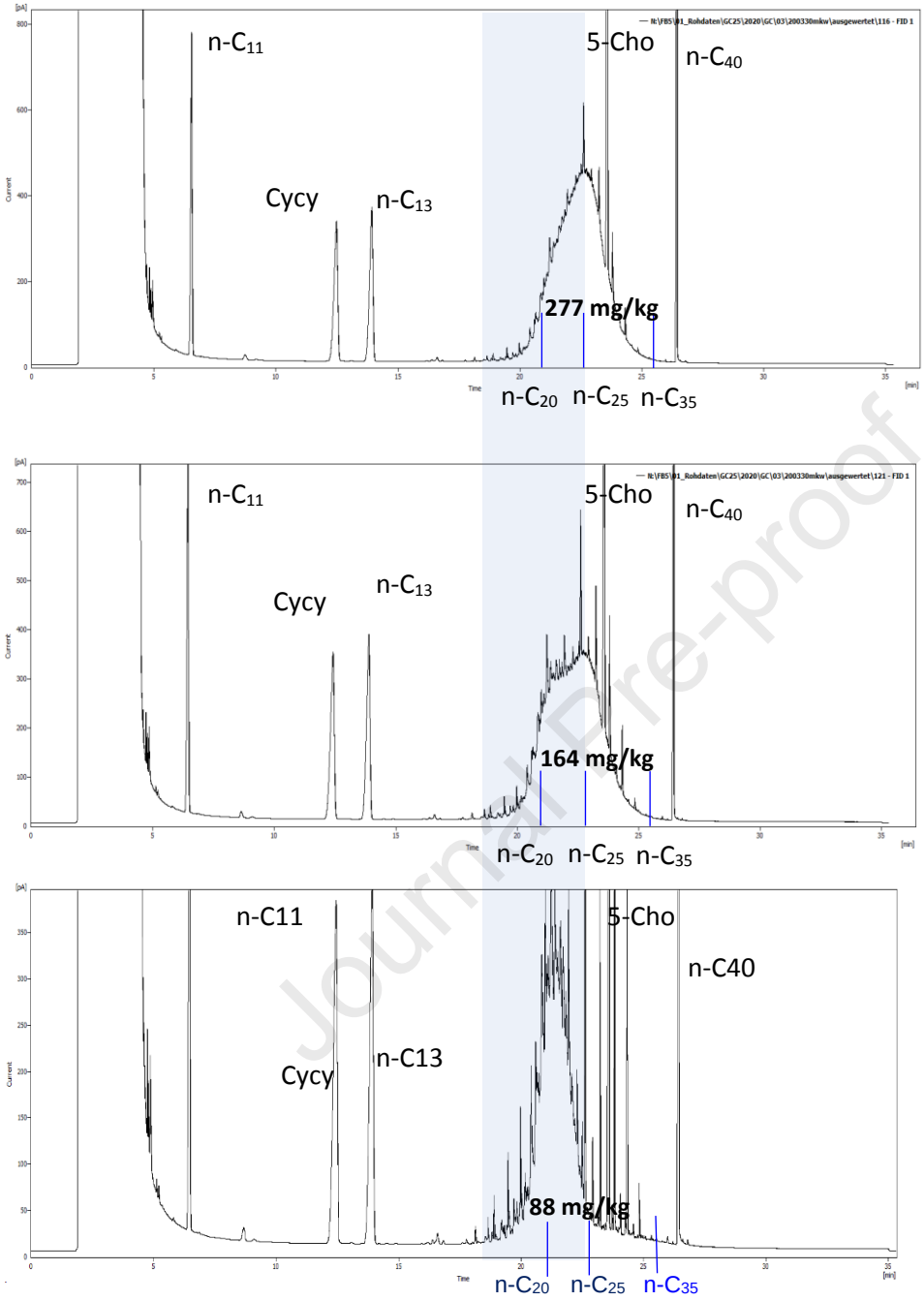
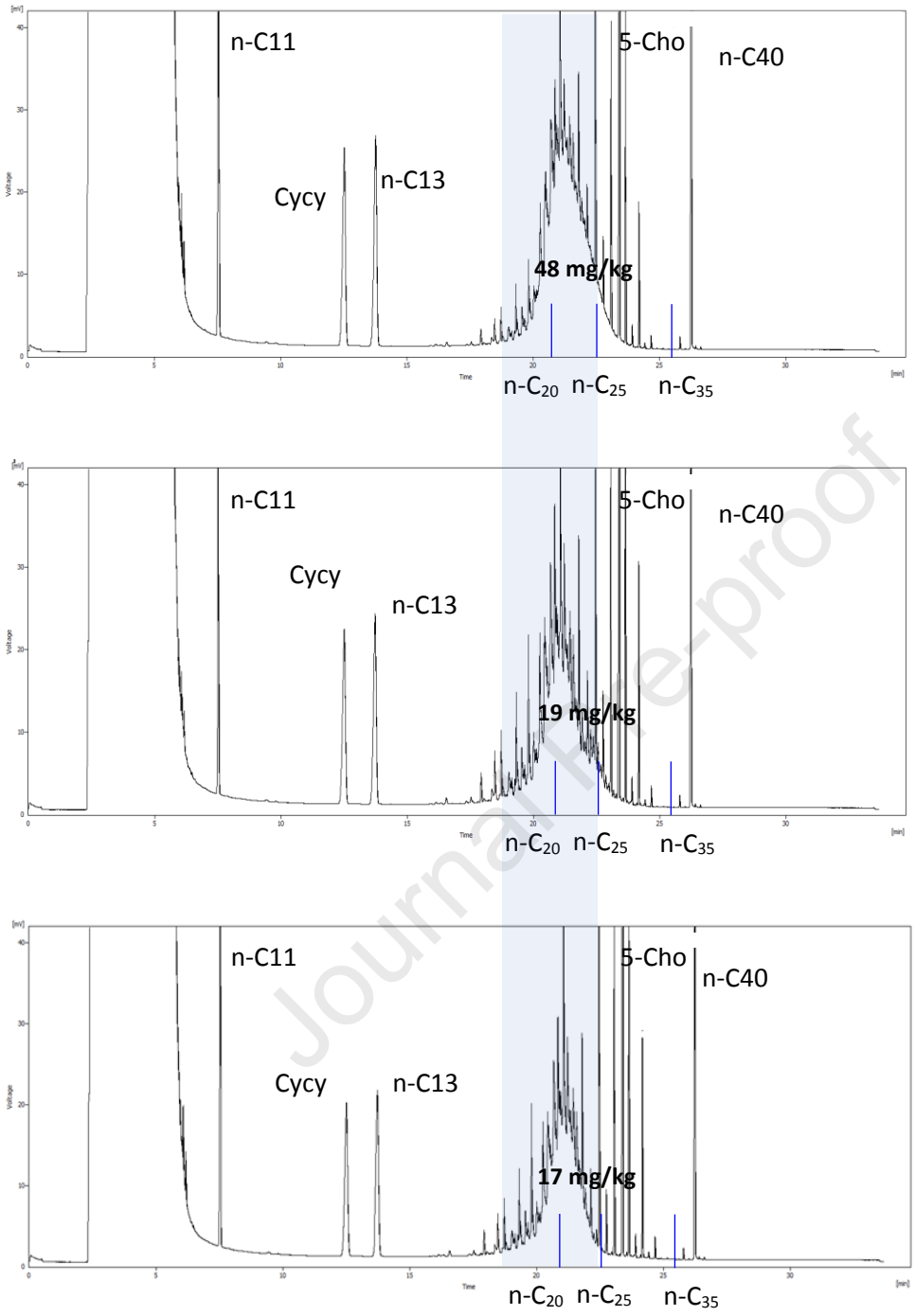


Figure 11b. *Mesenteric lymph node online-HPLC-GC-FID-Chromatogram of GTL oil fed to F3 group. Saturated hydrocarbon humps are aligned to background mineral oil contamination. Top panel, mineral oil residues at study day 92 (SD-92), contamination integrated as one hydrocarbon hump. Middle panel, end of recovery period (SD-134) seen as almost two humps consisting of background contamination on the left-hand side of the naphthenic mineral oil residual hump. Lower panel, background contamination in the control group in the range of C_{16}-C_{25} marked with a light blue strip across all three panels indicating its corresponding position in the humps. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholastane (Cho), and tetracontane (n-C_{40}) are indicated.*



Mineral oil,
F3 group at SD-134

GTL oil,
F2 group at SD-134

Control F1 group at SD-134
with background mineral oil
contamination

Figure 12: *Visceral fat online-HPLC-GC-FID-Chromatogram of saturated hydrocarbons at the end of recovery period at study day 134 from mineral oil (F3) and GTL group (F2) compared to control group (F1). The blue stripe across panels marks the relative position of the background mineral oil contamination in the control feed in the range of C_{16} -C_{25}. Reference substances undecane (n-C_{11}); bicyclohexyl (Cycy), tridecane (n-C_{13}), cholastane (5-Cho), and tetracontane (n-C_{40}) are indicated.*

Highlights

- At the same external dose, the iso-alkanes and multiring cycloalkanes (naphthenics) from mineral oil show higher hepatic retention and slower excretion than GTL iso-alkane constituents with the same carbon number range distribution.
- The lower hepatic levels of GTL hydrocarbons may be explained by lower gut absorption and faster elimination of iso-alkanes found in GTL oil.
- The study provides experimental evidence that the alkane sub-class most prone to hepatic retention are cycloalkanes (naphthenics) which are absent in GTL oils.
- Retention of alkane sub-classes in SD rat tissues, including the liver, is qualitatively comparable to that seen in humans. Both SD-rat and human tissues show the same pattern for n-alkane distribution where the F-344 notably shows a deviant pattern.
- The low accumulation potential of GTL oil offers an alternative in food related applications and vaccine adjuvants where MOSH retention in organs, including the liver, is not desired.

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Holger Schnieder reports a relationship with Sustainable Chemistry Consult that includes: consulting or advisory. Juan-Carlos Carrillo ; Hua Shen ; Fayaz Momin and Olaf Kral are employees of Shell. Shell manufactures and has commercial interests in mineral oil and GTL derived products.