

COMPOSITIONS OF AND METHODS OF MAKING ULTRA-PURE TRICAPRILIN

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of the U.S. provisional application number 63/472,229, filed June 09, 2023 and 63/607,779, filed December 8, 2023, the disclosures of which are each herein incorporated by reference in their entirety.

BACKGROUND

1. FIELD

[0002] This invention generally relates to compositions of and methods of making ultra-pure tricaprillin.

2. DISCUSSION OF RELATED ART

[0003] Tricaprilin (which is synonymously called tricaprylin, trioctanoylglyceride, tricaprylyl glycerol, glycerol trioctanoin, tricapryloyl glycerol, octanoic acid-1,2,3-tricaprylyl-ester, Glycerol tricaprylate, tricaprylyl Glycerol, glycerol tricaprylate, glycerol trioctanoate, glyceryl tricaprylate, caprylic triglyceride, tricaprillin, tricaprylyl glycerol etc.) is the triglyceride of caprylic acid which is usually manufactured by esterification of caprylic acid with Glycerol (glycerol).

[0004] Tricaprilin is used in pharmaceutical preparations, for example as a neutral carrier or excipient, as an absorption promoter and as a solubilizer for active drugs. It is also used as an oily phase to prepare water-in-oil-in-water multiple emulsions for incorporating water-soluble drugs and also for obtaining stable microcapsules. Being readily miscible with natural oils and surfactants, tricaprillin is used as fat component in two-phase foam baths, and in products like sunscreen creams, oils, fixative for perfumes and fragrances. High dose tricaprillin has also been used as active ingredients in pharmaceutical compositions for Alzheimer's diseases.

[0005] However, conventional production methods for manufacturing fatty acid polyol esters, particularly fatty acid glycerol esters, such as medium-chain triglycerides (MCTs), especially tricaprillin but also others, lead to relatively high degrees of impurities and by-products. These impurities are known to be toxic, especially toxic halide-based by-products and impurities. In particular, presence of these impurities and toxic by-products is not conducive to high dosage use. There is therefore an un-met need for production methods for ultra-pure tricaprillin, which may be broadly applicable to all MCTs.

SUMMARY

[0006] In an aspect, the current disclosure encompasses an ultra-pure composition comprising at least about 99.2% by weight tricaprilin and about 0.8% by weight or less of impurities, wherein the tricaprilin is a tri-ester tricaprilin; and wherein the impurities comprise one or more of 2-monochloropropanediol, 2-monochloropropanediol monoester, 2-monochloropropanediol diester, 3-monochloropropanediol, 3-monochloropropanediol monoester, 3-monochloropropanediol diester, or any combination thereof. In an aspect, the ultra-pure composition comprising at least about 99.5% by weight tricaprilin and about 0.5% by weight or less of impurities. In an aspect, the ultra-pure composition comprising at least about 99.7% by weight tricaprilin and about 0.3% by weight or less of impurities. In an aspect, the ultra-pure composition may comprise 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester in a concentration of 35 ppb or less. In an aspect, the ultra-pure composition may comprise less than 35 ppb of each of 3-monochloropropanediol and 3-monochloropropanediol diester. In an aspect, the impurities may further comprise glycidol, mono-ester tricaprilin, di-ester tricaprilin, or tetra-ester tricaprilin. In an aspect, the impurities may further comprise unreacted reactants.

[0007] In an aspect, the current disclosure also encompasses a method of making an ultra-pure composition of tricaprilin, the method comprising: (a) feeding caprylic acid, a concentrated acid, glycerol, and water into a first reactor; (b) reacting caprylic acid, the concentrated acid, glycerol, and water to form a product mixture comprising tricaprilin and impurities in the first reactor, wherein the impurities comprise unreacted reactants and optionally undesired side products; (c) adding an organic solvent and an aqueous solution of a basic salt to a second reactor forming a biphasic mixture comprising a top organic phase and a bottom aqueous phase; (d) adding portions of the product mixture from step (b) into the biphasic mixture in step (c) to remove the unreacted reactants, wherein the unreacted reactants are in the aqueous phase; (e) removing the bottom aqueous phase from the second reactor; (f) treating the top organic phase with charcoal; (g) distilling off the organic solvent and leaving tricaprilin and impurities in the second reactor; and (h) recrystallizing the tricaprilin and impurities to remove the impurities and form the ultra-pure tricaprilin. In some aspects, step (a) may further comprise: (ai) co-feeding caprylic acid and concentrated acid into the first reactor to form a mixture; (aii) heating the mixture to a first temperature under an inert atmosphere; and (aiii) adding Glycerol and water to the mixture over a first specified time. In some aspects, the step (b) may further comprise: (bi) stirring the product mixture for a second specified time at a second temperature; (bii) removing a first in process sample from the first reactor; (biii) checking the first in process sample for conversion, wherein conversion is reaction of hydroxyl groups in the glycerol to ester groups in tricaprilin ; and (biv) repeating

step (bii) to step (biii) until conversion occurs. In some aspects, the step (g) may further comprise (gi) removing a second in process sample from the second reactor; (gii) measuring amount of the organic solvent remaining in the tricaprilin and impurities; and (giii) repeating steps (gi) to (gii) until the amount of the organic solvent remaining is 500 ppm or less. In some aspects, the step (h) may further comprise: (hi) adding a polar solvent to the tricaprilin and impurities forming a second mixture comprising a polar solvent, tricaprilin, and impurities; (hii) cooling the second mixture to a third temperature; (hiii) adding seeding crystals to the second mixture forming a third mixture comprising seeding crystals and the second mixture; (hiv) stirring the third mixture for a third specified time at a third temperature; (hvi) cooling the third mixture to a fourth temperature in a fourth specified time; (hvi) maintaining the third mixture at the fourth temperature for a fifth specified time; (hvii) adding water to the third mixture; (hviii) maintaining the third mixture at the fourth temperature; (hix) filtering the third mixture on a filter cooled at the fourth temperature; (hx) washing the third mixture with an aqueous solution of the polar solvent at a fifth temperature; (hxi) repeating the washing step (hx); (hxii) collecting crystals of tricaprilin; and (hxiii) evaporating residual solvent from the crystals using a rotary evaporator. In some aspects, the concentrated acid comprises sulfuric acid. In some aspects of the method disclosed herein, the first temperature ranges from about 90 °C to about 120 °C. In some aspects, the first specified time ranges from about 3 hours to about 5 hours. In some aspects, the second temperature ranges from about 90 °C to about 120 °C. In some aspects, the second specified time ranges from about 15 hours to about 24 hours.

[0008] In an aspect, the current disclosure also encompasses a pharmaceutical composition comprising the ultra-pure composition provided herein. Also, disclosed herein is a method of prophylactically or therapeutically treating a disease or a disorder in a subject in need thereof, the method comprising administering a composition comprising ultra-pure tricaprilin to the subject. Non-limiting examples of diseases or disorders craniocerebral trauma, stroke, hypoxia, myocardial infarction, refeeding syndrome, anorexia, epilepsy, neurodegenerative diseases such as dementia, Alzheimer's disease, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis, fat metabolic diseases such as glucose transporter defect (GLUT1 defect), VL-FAOD and mitochondriopathies such as mitochondrial thiolase defect, Huntington's disease, cancers such as T-cell lymphomas, astrocytomas and glioblastomas, HIV, rheumatic diseases such as rheumatoid arthritis and arthritis urica, chronic inflammatory bowel diseases, ulcerative colitis, Crohn's disease, lysosomal storage diseases, sphingolipidosis, especially Niemann-Pick disease, diabetes mellitus, migraine and migraine headaches, epilepsy, especially infantile spasms as well as effects or side-effects of chemotherapy. In an aspect, the ultra-pure composition of tricaprilin is administered orally.

BRIEF DESCRIPTION OF THE FIGURES

[0009] FIG. 1 shows a schematic of the reaction resulting in generation of monochloropropanediol esters during production of tricaprilin.

[0010] FIG. 2 shows a schematic of the reaction scheme for the production of tricaprilin.

[0011] FIG. 3 shows a general process of making an ultra-pure tricaprilin composition, in accordance with a representative aspect of the present invention.

[0012] FIG. 4 shows a general process of making an ultra-pure tricaprilin composition, in accordance with a representative aspect of the present invention.

[0013] FIG. 5 provides a schematic for production of tricaprilin (TARI-01).

[0014] FIG. 6 shows the technical set up for a lab scale experiment. The reactor was equipped (from left to right) with a N₂ Inlet, a mechanical stirred, a thermocouple and a Dean Stark receiver.

[0015] FIG. 7 shows a lab scale apparatus for the recrystallization of tricaprilin.

[0016] FIG. 8A shows a CG-MS assay for glycidol for Exp23 AA8219-3.

[0017] FIG. 8B shows a CG-MS assay for 3-monochloropropanediol (3-MCPD) and 2-monochloropropanediol (2-MCPD) for Exp23 AA8219-3.

[0018] FIG. 8C shows a CG-MS assay for glycidol for Exp23 AA5433-1.

[0019] FIG. 8D shows a CG-MS assay for 3-MCPD and 2-MCPD for Exp23 AA5433-1.

[0020] FIG. 9 shows a manufacturing flow chart for a process of manufacturing an ultra-pure tricaprilin composition, in accordance with a representative aspect of the present invention.

DETAILED DESCRIPTION

[0021] The following description of the aspects of the invention is not intended to limit the invention to these aspects but rather to enable a person skilled in the art to make and use this invention.

[0022] In an aspect, the current disclosure is based on extensive experimentation to develop a method for producing ultra-pure tricaprilin. These experiments provided several unexpected results and insights and led to the development of a novel method for production of tricaprilin with minimal impurities.

[0023] Conventional production methods for manufacturing fatty acid polyol esters, particularly fatty acid glycerol esters, such as medium-chain triglycerides (MCTs), especially tricaprilin but also others, including the subsequent multistage refinement treatment (above multistage distillation at elevated temperatures), lead to relatively high degrees of impurities and by-products particularly due to high process temperatures needed and the used starting materials and reagents, particularly halogen-containing substances (e.g., originating from hydrochloric acid HCl or metal-based catalysts used in the esterification process). Especially,

when triglycerides with low acid and hydroxyl values and high esterification degrees are required, the mandatory drastic esterification conditions, especially high temperatures over 180 °C and up to 230 °C, the necessary subsequent multistage refinement treatment (above multistage distillation at elevated temperatures) and the use of metal-based catalysts lead to the formation of high contents of toxic by-products and impurities, especially toxic halide-based by-products and impurities.

[0024] Above all, conventional production methods for manufacturing fatty acid polyol esters, particularly fatty acid glycerol esters, including the subsequent multistage refinement treatment (above multistage distillation at elevated temperatures), usually produce relatively high levels of impurities and by-products, especially toxic impurities and by products, such as, genotoxic glycidol esters of fatty acids (synonymously also called glycidyl esters of fatty acids or simply GEs of fatty acids or only GEs) and nephrotoxic fatty acid esters of monochloropropanediols (synonymously called MCPD fatty acid esters or simply MCPD esters), especially fatty acid esters of 3- monochloropropanediol (3-MCPD fatty acid esters or 3-MCPD esters) and fatty acid esters of 2-monochloropropanediol (2-MCPD fatty acid esters or 2-MCPD esters).

[0025] The exemplary reaction scheme of **FIG. 1** illustrates the formation of the three aforementioned toxic by-products, i.e., 3-MCPD fatty acid ester, 2-MCPD fatty acid ester and glycidol fatty acid ester for the triglyceride tricaprilin (selected as a non-limiting example), whose formation occurs when high temperatures (e.g., temperatures of 200 °C or more) are applied to the fatty acid triglyceride (e.g., tricaprilin) in the presence of chlorine-containing substances originating from the production process. The substance 3-chloro-1,2-propanediol originates from glycerol when reacting to 3-MCPD in the presence of chlorine-containing substances or chlorides, respectively (see **FIG. 1**).

[0026] Above all with respect to pharmaceutical and nutritional applications, controlling the impurity profile is crucial and compulsory, especially for known toxic by-products such as the three aforementioned toxic by-products, i.e., 3-MCPD fatty acid ester, 2-MCPD fatty acid ester and glycidol fatty acid ester.

[0027] Although it has been well known for decades that, due to the harsh process conditions occurring during the conventional esterification/condensation methods of fats and oils, undesired and toxic by-products are formed, no attempts have been made to avoid such harsh conditions by improving or modifying the production processes. Mild esterification conditions using biobased catalysts and lower esterification temperatures reduce the formation of toxic by-products, such as the three aforementioned toxic by-products (*i.e.*, 3-MCPD fatty acid ester, 2-MCPD fatty acid ester and glycidol fatty acid ester), but at the same time only lead to triglycerides with undesirably high acid and hydroxyl values and relatively low triglyceride contents at low conversion rates and yields.

[0028] Consequently, instead of trying to avoid the formation of undesired and toxic by-products, rather cost-intensive and burdensome pre- and/or post-treatment methods are affected in order to remove these undesired and toxic by-products from the desired final product obtained under harsh esterification conditions (i.e., especially high temperatures over 180 °C and up to 230 °C and use of metal-based catalysts, etc.). Such burdensome post treatment methods comprise, inter alia, e.g., the addition of strongly alkaline reagents such as KOH, multistage distillation cascades, heat-treatment with strongly alkaline aqueous solutions etc. (as described in, for example, WO 2019/038320 A1 and WO 2014/012548 A1 which are each incorporated herein by reference in their entirety). These post-treatment methods also affect and deteriorate the product quality as such and overall yields and efficiency. For instance, WO 2021/070209 A1, incorporated herein by reference in its entirety, discloses a complex multistage extraction process for the purification of triglycerides, using a saline / organic solvent two-phase extraction system also with chlorinated organic solvents.

[0029] However, these post-treatment methods mostly fail to remove last traces of these undesired and toxic by-products, and these traces then remain in the final product. This is of particular danger when applying high dosages of the fatty acid polyol esters, particularly fatty acid glycerol esters, e.g., MCTs such as tricaprilin, in pharmaceutical and nutritional applications (above all in case of parenteral application), which leads to an undesired accumulation of such traces of undesired and toxic by-products in the human body, thus also exceeding the authorized threshold levels of the undesired and toxic by-products as regulated by law. Moreover, such cost-intensive and burdensome post-treatment methods which aim to effect a only subsequent or retroactive removal of the undesired and toxic by-products are also inefficient since these post-treatment methods often lead to an undesired entrainment of product and do also not increase or influence product quality or product purity such as the esterification degree or the residual content of other impurities (such as, e.g., the total halogenated substance content or the total acid content).

[0030] It would be desirable to conceive efficient production processes which directly produce high-grade fatty acid polyol esters, particularly fatty acid glycerol esters, such as MCTs (e.g., tricaprilin), having high degrees of purity or minimum levels of by-products and impurities, respectively, as well as exhibiting at the same time high esterification degrees and good stabilities, especially storage-stabilities, and high active ingredient contents, especially without the necessity of additional cost-intensive and burdensome post-treatment operations. Consequently, the prior art has not lacked attempts to find efficient production processes but failed to improve the efficiency and performance of existing processes. But so far, however, no efficient production processes have been conceived in the prior art. Also, access to such high-grade fatty acid polyol esters, particularly fatty acid glycerol esters, such as MCTs (e.g., tricaprilin), is not or not easily possible according to the prior art.

[0031] The problem underlying the present invention is thus providing an efficient process for producing triglycerides of fatty acids (i.e., fatty acid glycerol triesters), above all tricaprilin, especially with a high purity degree, wherein the aforementioned disadvantages and/or drawbacks of the prior art should be at least partially avoided or even at least essentially overcome.

[0032] Such production process should especially make the respective fatty acid polyol esters, particularly fatty acid glycerol esters, such as MCTs (above all tricaprilin), accessible in an efficient way, especially with improved qualities and without significant amounts of toxic by-products or impurities, which production process should be feasible on an industrial level, especially on a large-scale level, and without excessive and cost-intensive post-treatment as envisaged in the prior art processes.

[0033] Accordingly, the disclosure herein provides an ultra-pure composition comprising at least about 99.2% by weight tricaprilin and about 0.8% by weight or less of impurities as well as methods of preparing an ultra-pure composition comprising at least about 99.2% by weight tricaprilin and about 0.8% or less by weight of impurities. In an aspect, the disclosure herein provides an ultra-pure composition comprising at least about 99.5% by weight tricaprilin and about 0.5% by weight or less of impurities as well as methods of preparing an ultra-pure composition comprising at least about 99.5% by weight tricaprilin and about 0.5% or less by weight of impurities.

DEFINITIONS

[0034] The terms “a” and “an” and “the” and similar referents as used herein refer to both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

[0035] The term “about” as used herein refers to greater or lesser than the value or range of values stated by 1/10 of the stated values but is not intended to limit any value or range of values to only this broader definition. For instance, a value of “about 30%” means a value of between 27% and 33%. Each value or range of values preceded by the term “about” is also intended to encompass the aspect of the stated absolute value or range of values.

[0036] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. All patents, applications, published applications and other publications are incorporated by reference in their entirety. In the event that there are a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

[0037] Throughout this specification, unless the context requires otherwise, the word “comprise” and “include” and variations (e.g., “comprises,” “comprising,” “includes,”

“including”) will be understood to imply the inclusion of a stated component, feature, element, or step or group of components, features, elements or steps but not the exclusion of any other integer or step or group of integers or steps.

[0038] As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations where interpreted in the alternative (“or”).

[0039] Moreover, the present disclosure also contemplates that in some aspects, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0040] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise-indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure.

[0041] “Pharmaceutical composition” means a mixture of substances suitable for administering to an individual that includes a pharmaceutical agent. As used herein a pharmaceutical composition comprises one or more of the compounds as disclosed herein compounded with suitable pharmaceutical excipients.

[0042] As used herein, the term “patient”, “subject”, including “test subject” refers to any organism to which provided compound or compounds described herein are administered in accordance with the present disclosure e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, humans, insects, worms etc.). In an aspect, a subject is a human. In some aspects, a subject may be suffering from a tumor and/or cancer as disclosed herein. In some aspects, the cancer is a leukemia. In some aspects, the cancer is colorectal cancer.

[0043] The term “effective amount” as used herein is defined as the amount of the molecules of the present disclosure that are necessary to result in the desired physiological change in the cell or tissue to which it is administered. The term “therapeutically effective amount” as used herein is defined as the amount of the molecules or compositions of the present disclosure that achieves a desired effect with respect to cancer. In this context, a “desired

effect" is synonymous with "an antitumor activity" or "an anti-cancer activity". A skilled artisan readily recognizes that in many cases the molecules may not provide a cure but may provide a partial benefit, such as alleviation or improvement of at least one symptom or parameter. In some aspects, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some aspects, an amount of molecules that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount."

[0044] The term "ultra-pure" as used herein refers to tricaprilin that comprises at least about 99.2% by weight tricaprilin. Ultra-pure active pharmaceutical ingredient (API) contains the highest purity with lowest related substances including lowest level of genotoxic impurities compared to any currently available MCT oils and other sources of tricaprilin. In an aspect, the tricaprilin comprises less than 0.8% by weight of impurities. The term "impurities" as used herein and unless otherwise specified herein, refers to undesired compounds and elements, such as, for example, unreacted reactants and undesired side and/or by-products. In some instances, the impurity(s) may have negative and/or deleterious effects on a subject; especially when the subject is exposed to amounts of the impurity(s) that exceed the authorized threshold levels; either in a single instance or over time. In an aspect, the ultra-pure tricaprilin disclosed herein is suitable for administration to a subject at a high dose of at least about 20 mg/day. In an aspect, the ultra-pure tricaprilin is suitable for administration to a subject at a high dose of at least about 30 mg/day. In an aspect, the ultra-pure tricaprilin is suitable for administration to a subject at a high dose of at least about 40 mg/day. The per day amount may be split into multiple doses or be administered as a single dose.

I. Compositions

[0045] In an aspect, the current disclosure encompasses an ultra-pure composition comprising at least about 99.2% by weight tricaprilin and about 0.8% by weight or less of impurities, wherein the tricaprilin is a tri-ester tricaprilin. In an aspect, the ultra-pure composition of the present disclosure may include at least about 99.3% by weight tricaprilin and about 0.7% by weight or less of impurities. In some aspects, the ultra-pure composition may include at least about 99.4% by weight tricaprilin and about 0.6% by weight or less of impurities. In an aspect, the ultra-pure composition of the present disclosure may include at least about 99.5% by weight tricaprilin and about 0.5% by weight or less of impurities. In some aspects, the ultra-pure composition may include at least about 99.6% by weight tricaprilin and about 0.4% by weight or less of impurities. In some aspects, the ultra-pure composition may include at least about 99.7% by weight tricaprilin and about 0.3% by weight or less of impurities. In some aspects, the ultra-pure composition may include at least about 99.8% by weight tricaprilin and about 0.2% by weight or less of impurities. In some aspects, the ultra-pure composition may include at least about 99.9% by weight tricaprilin and about 0.1% by

weight or less of impurities. In some aspects, the ultra-pure composition may include at least about 99.99% by weight tricaprilin and about 0.01% by weight or less of impurities.

[0046] In some aspects, the impurities may comprise any one or more of 2-monochloropropanediol, 2-monochloropropanediol monoester, 2-monochloropropanediol diester, 3-monochloropropanediol, 3-monochloropropanediol monoester, 3-monochloropropanediol diester, or any combination thereof. In some aspects, the impurities may include unreacted reactants and optionally undesired side products. In some aspects, the impurities may include 2-monochloropropanediol, 2-monochloropropanediol monoester, 2-monochloropropanediol diester, 3-monochloropropanediol, 3-monochloropropanediol monoester, 3-monochloropropanediol diester, may include unreacted reactants and optionally undesired side products, or any combination thereof.

[0047] In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 50 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 45 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 40 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 35 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 30 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 25 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 20 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 15 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 14 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 13 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 12 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 11 ppb or less. In some aspects, 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester may be present in a concentration of 10 ppb or less. In some

aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 12 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 11 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 10 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 9 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 8 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 7 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 6 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 5 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 4 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 3 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 2 ppb or less. In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 1 ppb or less.

[0051] In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 35 ppb or less.

[0052] In some aspects, of each of 3- monochloropropanediol and 3-monochloropropanediol diester may be present in a concentration of 15 ppb or less.

[0053] In some aspects, the impurities may include glycidol, mono-ester tricaprilin, di-ester tricaprilin, or tetra-ester tricaprilin. In some aspects, the impurities may include glycidol, mono-ester tricaprilin, di-ester tricaprilin, or tetra-ester tricaprilin. In an aspect, glycidol impurities may be present at 50 ppb or less. In an aspect, glycidol impurities may be present at 40 ppb or less. In an aspect, glycidol impurities may be present at 35 ppb or less. In an aspect, glycidol impurities may be present at 30 ppb or less. In an aspect, glycidol impurities may be present at 20 ppb or less.

[0054] In an aspect, the ultra-pure tricaprilin conforms to the limits as provided in **Table 1** below.

Table 1: Characteristics and impurity limits on an exemplary ultra-pure tricaprilin composition

Parameter	Limit
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Appearance	clear, colorless or slightly yellowish liquid
Color	max. 100 APHA
Identity (FT-IR)	conforms with reference spectrum
Identity (GC-FID)	retention time conforms
Assay (GC-FID)	97.0 – 103.0 % (w/w)
Purity	Not less than 99.2%
Related Substances (GC-FID, %area)	
Glycerol-monoheptanoate dioctanoate	NMT 0.5%
Any unspecified related substance	max. 0.2 %
Total related substances	max. 0.8 %
Acid Value	max. 0.2 mg KOH/g
Residue on Ignition (Sulphated Ash)	max. 0.1 %
Elemental impurities (ICP-MS)	
Cd	0.08 ppm
Pb	0.08 ppm
As	0.25 ppm
Hg	0.50 ppm
Co	1 ppm
V	2 ppm
Ni	3 ppm
Water (K.F.)	max. 0.2 %
Residual solvents	
Methyl tert-butyl ether (MTBE)	max. 500 ppm
Methanol	max. 300 ppm
Microbiology	
Total Aerobic Microbial Count	≤ 100 CFU/g
Total Yeasts and Molds Count	≤ 10 CFU/g
<i>E. coli</i>	absent in 1g
MCPD and Glycidol (GC-MS)	
Glycidyl equivalent	max. 35 ppb
2-MCPD equivalent	max. 35 ppb
3-MCPD equivalent	max. 35 ppb

[0055] In some aspects the impurities may be measured by suitable analytical techniques. Suitable analytical techniques include but are not limited to gas chromatography, mass spectrometry, or chemical analysis.

[0056] In some aspects, the ultra-pure composition of tricaprilin may be further characterized by measuring any one of or a combination of acid value, saponification value, refractive index, water content, total aerobic microbial count, total yeast and mold count, elemental impurities by ICP-MS.

II. Method of making ultra-pure composition

[0057] The present disclosure further encompasses methods of making an ultra-pure composition of tricaprilin as disclosed herein. The general reaction is an esterification reaction in which an acid may react with an alcohol resulting in the formation of an ester.

[0058] The method generally includes reacting an alkyl carboxylic acid with an alkyl triol at a specified temperature to form a product mixture and purifying the product mixture to obtain the ultra-pure composition of tricaprilin. The product mixture includes tricaprilin and impurities. The purifying step includes separating the tricaprilin and impurities in an organic solvent, distilling the organic solvent, and recrystallizing the product mixture to obtain the ultra-pure composition of tricaprilin.

[0059] FIG. 2 shows the general reaction according to the present disclosure. The reacting step includes (a) feeding caprylic acid, a concentrated acid, glycerol, and water into a first vessel and (b) reacting caprylic acid, concentrated acid, glycerol, and water to form a product mixture comprising tricaprilin and impurities in a first vessel. The purification step generally includes (c) adding an organic solvent and an aqueous solution of a basic salt to a second vessel forming a biphasic mixture comprising a top organic phase and a bottom aqueous phase; (d) adding portions of the product mixture from step (b) into the biphasic mixture in step (c) to remove the unreacted reactants; (e) removing the bottom aqueous phase from the second vessel; (f) treating the top organic phase with charcoal; (g) distilling off the organic solvent and leaving tricaprilin and impurities in the second vessel; and (h) recrystallizing the tricaprilin and impurities to remove the impurities and form the ultra-pure tricaprilin. FIG. 3 illustrates the steps in the overall process. FIG. 4 further elaborates on which vessel each step may be conducted in.

[0060] In some aspects, the impurities in the product mixture may include unreacted reactants and optionally undesired side products. In some aspects, the unreacted reactants may be in the aqueous phase.

The reacting step

[0061] In some aspects, the reacting step comprises (a) feeding caprylic acid, a concentrated acid, glycerol, and water into a first vessel and (b) reacting caprylic acid, concentrated acid, glycerol, and water to form a product mixture comprising tricaprilin and

impurities in the first vessel. In some aspects, the feeding step may further comprise co-feeding caprylic acid and concentrated acid into the first vessel to form a mixture; heating the mixture to a first temperature under an inert atmosphere; and adding glycerol and water to the mixture.

[0062] In some aspects, the reacting step may further include stirring the product mixture at the first temperature for a specified time; removing a first in process sample from the first vessel; checking the first in process sample for conversion; and repeating the steps until conversion occurs.

[0063] In some aspects, the vessel may be an ordinary reactor, such as a tubular reactor or a tank reactor.

[0064] In an aspect, the caprylic acid used in the current method may be at least about 99% pure. In an aspect, the caprylic acid has a profile as provided below in **Table 2**.

Table 2: Exemplary profile of caprylic acid for use in the disclosed method.

Parameter	Limit
Appearance	clear, colorless or slightly yellowish liquid
Identity (FT-IR)	practically identical with reference spectrum
Assay (Titration)	99 – 101 %
Purity (GC-FID, %area)	
Caprylic acid (C8:0)	min. 99.5 %
Hexanoic acid (C6:0)	max. 0.5 %
Heptanoic acid (C7:0)	max. 0.5 %
Nonanoic acid (C9:0)	max. 0.5 %
Decanoic acid (C10:0)	max. 0.5 %
Dodecanoic acid (C12:0)	max. 0.5 %
Each unspecified impurity	max. 0.3 %
Residue on ignition, 1 g sample	max. 0.1 %

[0065] Similarly, the glycerol for use with the disclosed method may be of high purity, for example at least about 98% pure. In an exemplary aspect, the glycerol for use herein has a profile as provided in **Table 3**.

Table 3: Exemplary profile of glycerol for use in the disclosed method.

Parameter	Limit
Appearance	clear liquid
Color (Appearance of solution)	colorless

Identity (Refractive Index, n _{D20})	1.470 - 1.475
Identity (GC, Retention time)	retention time conforming
Water (KF, 2 g sample)	max. 2.0 %
Assay (Titration, on anhydrous)	98.0 - 101.0 %
Chemical purity (GC, %norm.area)	
Diethylene glycol (Imp. A)	max. 0.1 %
Ethylene glycol	max. 0.1 %
Each unspecified impurity	max. 0.1 %
Total impurities with retention time greater than glycerol	max. 0.5 %
Chloride	max. 10 ppm

[0066] The concentrated acid may be selected based on the dehydrating properties of the acid. The dehydrating property may be crucial in esterification reactions. The water produced as a by-product of the esterification reaction may be removed through dehydration, shifting the equilibrium of the esterification reaction towards the formation of the ester.

[0067] In some aspects, the acid may be concentrated sulfuric acid, phosphoric acid, or a combination thereof. In an aspect, the concentrated acid may be sulfuric acid.

[0068] In some aspects, the concentrated sulfuric acid may be between about 95 weight percent (wt%) and under about 99.99 wt%. For example, the concentrated sulfuric acid may be about 95.1 wt%, about 95.2 wt%, about 95.3 wt%, about 95.4 wt%, about 95.5 wt%, about 95.6 wt%, about 95.7 wt%, about 95.8 wt%, about 95.9 wt%, about 96.0 wt%, about 96.1 wt%, about 96.2 wt%, about 96.3 wt%, about 96.4 wt%, about 96.5 wt%, about 96.6 wt%, about 96.7 wt%, about 96.8 wt%, about 96.9 wt%, about 97.0 wt%, about 97.1 wt%, about 97.2 wt%, about 97.3 wt%, about 97.4 wt%, about 97.5 wt%, about 97.6 wt%, about 97.7 wt%, about 97.8 wt%, about 97.9 wt%, about 99.0 wt%, about 99.1 wt%, about 99.2 wt%, about 99.3 wt%, about 99.4 wt%, about 99.5 wt%, about 99.6 wt%, about 99.7 wt%, about 99.8 wt%, about 99.9 wt%, or about 99.99 wt%.

[0069] In some aspects, the first temperature may range from about 90 °C to about 120 °C. For example, the first temperature may be about 90 °C, about 91 °C, about 92 °C, about 93 °C, about 94 °C, about 95 °C, about 96 °C, about 97 °C, about 98 °C, about 99 °C, about 100 °C, about 101 °C, about 102 °C, about 103 °C, about 104 °C, about 105 °C, about 106 °C, about 107 °C, about 108 °C, about 109 °C, about 110 °C, about 111 °C, about 112 °C, about 113 °C, about 114 °C, about 115 °C, about 116 °C, about 117 °C, about 118 °C, about 119 °C, or about 120 °C. In a preferred aspect, the first temperature may range from about 95 °C to about 115 °C.

[0070] In some aspects, the inert atmosphere may be nitrogen, argon, or a mixture thereof.

[0071] In some aspects, the first vessel may be maintained at a pressure of between about 250 mbar to about 600 mbar. For example, the pressure may be about 260 mbar, about 270 mbar, about 280 mbar, about 290 mbar, about 300 mbar, about 310 mbar, about 320 mbar, about 330 mbar, about 340 mbar, about 350 mbar, about 360 mbar, about 370 mbar, about 380 mbar, about 390 mbar, about 400 mbar, about 410 mbar, about 420 mbar, about 430 mbar, about 440 mbar, about 450 mbar, about 460 mbar, about 470 mbar, about 480 mbar, about 490 mbar, about 500 mbar, about 510 mbar, about 520 mbar, about 530 mbar, about 540 mbar, about 550 mbar, about 560 mbar, about 570 mbar, about 580 mbar, about 590 mbar, or about 600 mbar.

[0072] In some aspects, the glycerol to water ratio may range from 2:1 to 1:2. For example, the ratio may be around 2:1, around 1.5:1, around 1:1, around 1:1.5, or around 1:2.

[0073] In some aspects, the specified time may range from about 2 hours to about 17 hours. In some aspects, the specified time may range from about 3 hours to about 16 hours. In some aspects, the specified time may range from about 4 hours to about 15 hours. In some aspects, the specified time may range from about 5 hours to about 14 hours. In some aspects, the specified time may range from about 6 hours to about 13 hours. In some aspects, the specified time may range from about 7 hours to about 12 hours. In some aspects, the specified time may range from about 8 hours to about 11 hours. In some aspects, the specified time may range from about 9 hours to about 10 hours. In some aspects the specified time may range from about 3 hours to about 5 hours.

[0074] The conversion occurs when the hydroxyl groups in the glycerol react to form ester groups in tricaprilin. After conversion is achieved, the product mixture may undergo purification to produce the ultra-pure composition of tricaprilin.

The purifying step

[0075] In an aspect, the purification step may comprise one or more of (c) adding an organic solvent and an aqueous solution of a basic salt to a second vessel forming a biphasic mixture comprising a top organic phase and a bottom aqueous phase; (d) adding portions of the product mixture from step (b) into the biphasic mixture in step (c) to remove the unreacted reactants; (e) removing the bottom aqueous phase from the second vessel; (f) treating the top organic phase with charcoal; (g) distilling off the organic solvent and leaving tricaprilin and impurities in the second vessel; and (h) recrystallizing the tricaprilin and impurities to remove the impurities and form the ultra-pure tricaprilin. In an aspect, the purification step may comprise all of (c) adding an organic solvent and an aqueous solution of a basic salt to a second vessel forming a biphasic mixture comprising a top organic phase and a bottom aqueous phase; (d) adding portions of the product mixture from step (b) into the biphasic

mixture in step (c) to remove the unreacted reactants; (e) removing the bottom aqueous phase from the second vessel; (f) treating the top organic phase with charcoal; (g) distilling off the organic solvent and leaving tricaprilin and impurities in the second vessel; and (h) recrystallizing the tricaprilin and impurities to remove the impurities and form the ultra-pure tricaprilin

[0076] In some aspects, the product mixture from the first vessel may be added to a second vessel. In an aspect, the purification step may be conducted in the second vessel. An organic solvent and a basic aqueous solution may be added to the second vessel. The basic solution may be added to remove excess concentrated acid. The organic solvent may be added to extract the organic compounds in the product mixture into the organic phase. The compounds may include tricaprilin, monoesters, diesters, tetraesters, 3-MCPD, 2-MCPD, glycidol, or any combination thereof.

[0077] The second vessel may be maintained at a second temperature. In some aspects, the second temperature may range from about 25 °C to about 50 °C. For example, the first temperature may be about 26 °C, about 27 °C, about 28 °C, about 29 °C, about 30 °C, about 31 °C, about 32 °C, about 33 °C, about 34 °C, about 35 °C, about 36 °C, about 37 °C, about 38 °C, about 39 °C, about 40 °C, about 41 °C, about 42 °C, about 33 °C, about 44 °C, about 45 °C, about 46 °C, about 47 °C, about 48 °C, about 49 °C, or about 50 °C.

[0078] In some aspects, the second vessel may be maintained at an atmospheric pressure.

[0079] In some aspects, the organic solvent in step (c) may include an ether. Suitable ethers include but are not limited to dimethoxyethane, diethyl ether, methyl tert-butyl ether, dioxane, THF, 2-methylTHF, CPME, 4-MeTHP, or combinations thereof.

[0080] In some aspects, any suitable basic salt may be used to obtain a basic solution for use in step (c). In some aspects, the aqueous solution of a basic salt in step (c) may include a solution of sodium carbonate.

[0081] In some aspects, the purification step may further include removing a second in process sample from the second vessel and measuring the conductivity of the aqueous layer. In process samples may be taken under the desired conductivity is reached. In some aspects, the desired conductivity may be less than 100 µS/cm.

[0082] After the desired conductivity is reached, the product mixture may be subject to charcoal treatment. In some aspects, charcoal may be added to the second vessel. The charcoal treatment may be conducted at room temperature at 3 g/min.

[0083] The next step in the purification process may include removing organic solvent from the product mixture. In some aspects, the organic solvent may be removed by distillation. The distillation may be conducted at a third temperature and pressure. In some aspects, the third temperature may range from about 40 °C to about 80 °C. For example, the first temperature may be about 40 °C, about 41 °C, about 42 °C, about 43 °C, about 44 °C, about 45 °C, about

46 °C, about 47 °C, about 48 °C, about 49 °C, about 50 °C, about 51 °C, about 52 °C, about 53 °C, about 54 °C, about 55 °C, about 56 °C, about 57 °C, about 58 °C, about 59 °C, or about 60 °C, about 61 °C, about 62 °C, about 63 °C, about 64 °C, about 65 °C, about 66 °C, about 67 °C, about 68 °C, about 69 °C, about 70 °C, about 71 °C, about 72 °C, about 73 °C, about 74 °C, about 75 °C, about 76 °C, about 77 °C, about 78 °C, about 79 °C, or about 80 °C. In some aspects, the pressure may be 1 mbar.

[0084] In some aspects, a second in process sample may be removed during the distillation step to measure the organic solvent remaining in the product mixture. The distillation may be continued until the amount of organic solvent in the product mixture is 500 ppm or less. The amount of solvent may be less than about 500 ppm, less than about 400 ppm, less than about 100 ppm, less than about 50 ppm or less than about 10 ppm.

[0085] In some aspects, the purification step may further include a recrystallization step. In an aspect, the recrystallization step may comprise the steps of: adding a polar solvent to the tricaprilin and impurities forming a second mixture comprising a polar solvent, tricaprilin, and impurities, cooling the second mixture to a third temperature, adding seeding crystals to the second mixture forming a third mixture comprising seeding crystals and the second mixture, stirring the third mixture for a third specified time at a third temperature, cooling the third mixture to a fourth temperature in a fourth specified time, maintaining the third mixture at the fourth temperature for a fifth specified time, adding water to the third mixture, maintaining the third mixture at the fourth temperature, filtering the third mixture on a filter cooled at the fourth temperature, washing the third mixture with an aqueous solution of the polar solvent at a fifth temperature, repeating the washing step, collecting crystals of tricaprilin, and evaporating residual solvent from the crystals using a rotary evaporator.

[0086] In an aspect, the recrystallization step may include adding one or more polar solvent to the product mixture and cooling the product mixture to a fourth temperature. In some aspects, the fourth temperature may range from about -20 °C to about 0 °C. For example, the first temperature may be about -20 °C, about -19 °C, about -18 °C, about -17 °C, about -16 °C, about -15 °C, about -14 °C, about -13 °C, about -12 °C, about -11 °C, about -10 °C, about -9 °C, about -8 °C, about -7 °C, about -6 °C, about -5 °C, about -4 °C, about -3 °C, about -2 °C, about -1 °C, or about 0 °C.

[0087] The one or more polar solvents may include water, acetone, acetonitrile, dimethylformamide (DMF), dimethylsulfoxide (DMSO), isopropanol, methanol, or a combination thereof. In some aspects, the one or more polar solvents may be a mixture of water and methanol.

[0088] To obtain a composition of ultra-pure tricaprilin, the solvent may be removed from the product mixture by heating the product mixture to a fifth temperature. In some aspects, the fifth temperature may range from about 40 °C to about 80 °C. For example, the fifth

temperature may be about 40 °C, about 41 °C, about 42 °C, about 43 °C, about 44 °C, about 45 °C, about 46 °C, about 47 °C, about 48 °C, about 49 °C, about 50 °C, about 51 °C, about 52 °C, about 53 °C, about 54 °C, about 55 °C, about 56 °C, about 57 °C, about 58 °C, about 59 °C, or about 60 °C, about 61 °C, about 62 °C, about 63 °C, about 64 °C, about 65 °C, about 66 °C, about 67 °C, about 68 °C, about 69 °C, about 70 °C, about 71 °C, about 72 °C, about 73 °C, about 74 °C, about 75 °C, about 76 °C, about 77 °C, about 78 °C, about 79 °C, or about 80 °C. In some aspects, the pressure may be 1 mbar.

III. Pharmaceutical Compositions

[0089] Further aspects of the present disclosure are directed to pharmaceutical compositions comprising the ultra-pure tricaprilin provided herein. These pharmaceutical compositions may comprise one or more pharmaceutically appropriate carriers or excipients. The ultra-pure tricaprilin provided herein may be utilized as one or more active pharmaceutical ingredients in a pharmaceutical composition. These pharmaceutical compositions may be used in prophylactic or therapeutic methods of treating various diseases or disorders as described herein below. Alternatively, or in addition, these pharmaceutical compositions may be prepared as a food product and/or the nutritive composition which may, in an aspect, be a dietary supplement, a functional food, a novel food, a food additive, a food supplement, a dietary food, a power snack, an appetite suppressant or a strength and/or endurance sports supplement. In other instances, the ultra-pure tricaprilin provided herein may be used as an excipient in a pharmaceutical composition comprising one or more active pharmaceutical ingredients.

Pharmaceutically acceptable carriers and excipients

[0090] Hereinafter, the phrases “physiologically acceptable carrier” and “pharmaceutically acceptable carrier” which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases. In various aspects, compositions disclosed herein may further comprise one or more pharmaceutically acceptable diluent(s), excipient(s), or carrier(s). As used herein, a pharmaceutically acceptable diluent, excipient, or carrier, refers to a material suitable for administration to a subject without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained. Pharmaceutically acceptable diluents, carriers, and excipients can include, but are not limited to, physiological saline, Ringer’s solution, phosphate solution or buffer, buffered saline, and other carriers known in the art. Pharmaceutical compositions may also include stabilizers, anti-

oxidants, colorants, other medicinal or pharmaceutical agents, carriers, adjuvants, preserving agents, stabilizing agents, wetting agents, emulsifying agents, solution promoters, salts, solubilizers, antifoaming agents, antioxidants, dispersing agents, surfactants, and combinations thereof. Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols. Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition, which is incorporated herein by reference.

[0091] In various aspects, pharmaceutical compositions described herein may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries to facilitate processing of genetically modified endothelial progenitor cells into preparations which can be used pharmaceutically. In other aspects, any of the well-known techniques, carriers, and excipients may be used as suitable and as understood in the art.

[0092] In various aspects, pharmaceutical compositions described herein may be an aqueous suspension comprising one or more polymers as suspending agents. In some aspects, polymers that may comprise pharmaceutical compositions described herein include: water- soluble polymers such as cellulosic polymers, e.g., hydroxypropyl methylcellulose; water- insoluble polymers such as cross-linked carboxyl-containing polymers; mucoadhesive polymers, selected from, for example, carboxymethylcellulose, carbomer (acrylic acid polymer), poly(methylmethacrylate), polyacrylamide, polycarbophil, acrylic acid/butyl acrylate copolymer, sodium alginate, and dextran; or a combination thereof. In other aspects, compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of polymers as suspending agent(s) by total weight of the composition. **[0189]** In various aspects, pharmaceutical compositions disclosed herein may comprise a viscous formulation. In some aspects, viscosity of the composition may be increased by the addition of one or more gelling or thickening agents. In other aspects, compositions disclosed herein may comprise one or more gelling or thickening agents in an amount to provide a sufficiently viscous formulation to remain on treated tissue. In still other aspects, compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of gelling or thickening agent(s) by total weight of the composition. In yet other aspects, suitable thickening agents can be hydroxypropyl methylcellulose, hydroxyethyl cellulose, polyvinylpyrrolidone, carboxymethyl cellulose, polyvinyl alcohol, sodium chondroitin sulfate, sodium hyaluronate. In other aspects, viscosity

enhancing agents can be acacia (gum arabic), agar, aluminum magnesium silicate, sodium alginate, sodium stearate, bladderwrack, bentonite, carbomer, carrageenan, Carbopol, xanthan, cellulose, microcrystalline cellulose (MCC), ceratonia, chitin, carboxymethylated chitosan, chondrus, dextrose, furcellaran, gelatin, Ghatti gum, guar gum, hectorite, lactose, sucrose, maltodextrin, mannitol, sorbitol, honey, maize starch, wheat starch, rice starch, potato starch, gelatin, sterculia gum, xanthum gum, gum tragacanth, ethyl cellulose, ethylhydroxyethyl cellulose, ethylmethyl cellulose, methyl cellulose, hydroxyethyl cellulose, hydroxyethylmethyl cellulose, hydroxypropyl cellulose, poly(hydroxyethyl methacrylate), oxypolygelatin, pectin, polygeline, povidone, propylene carbonate, methyl vinyl ether/maleic anhydride copolymer (PVM/MA), poly(methoxyethyl methacrylate), poly(methoxyethoxyethyl methacrylate), hydroxypropyl cellulose, hydroxypropylmethyl-cellulose (HPMC), sodium carboxymethylcellulose (CMC), silicon dioxide, polyvinylpyrrolidone (PVP: povidone), Splenda® (dextrose, maltodextrin and sucralose), or combinations thereof. In some aspects, suitable thickening agent may be carboxymethylcellulose.

[0093] In various aspects, pharmaceutical compositions disclosed herein may comprise additional agents or additives selected from a group including surface-active agents, detergents, solvents, acidifying agents, alkalizing agents, buffering agents, tonicity modifying agents, ionic additives effective to increase the ionic strength of the solution, antimicrobial agents, antibiotic agents, antifungal agents, antioxidants, preservatives, electrolytes, antifoaming agents, oils, stabilizers, enhancing agents, and the like. In some aspects, pharmaceutical compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more agents by total weight of the composition. In other aspects, one or more of these agents may be added to improve the performance, efficacy, safety, shelf-life and/or other property of the muscarinic antagonist composition of the present disclosure. In some aspects, additives will be biocompatible, and will not be harsh, abrasive, or allergenic.

[0094] In various aspects, pharmaceutical compositions disclosed herein may comprise one or more acidifying agents. As used herein, "acidifying agents" refers to compounds used to provide an acidic medium. Such compounds include, by way of example and without limitation, acetic acid, amino acid, citric acid, fumaric acid and other alpha hydroxy acids, such as hydrochloric acid, ascorbic acid, and nitric acid and others known to those of ordinary skill in the art. In some aspects, any pharmaceutically acceptable organic or inorganic acid may be used. In other aspects, compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more acidifying agents by total weight of the composition.

[0095] In various aspects, pharmaceutical compositions disclosed herein may comprise one or more alkalizing agents. As used herein, "alkalizing agents" are compounds used to provide

alkaline medium. Such compounds include, by way of example and without limitation, ammonia solution, ammonium carbonate, diethanolamine, monoethanolamine, potassium hydroxide, sodium borate, sodium carbonate, sodium bicarbonate, sodium hydroxide, triethanolamine, and trolamine and others known to those of ordinary skill in the art. In some aspects, any pharmaceutically acceptable organic or inorganic base can be used. In other aspects, compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more alkalizing agents by total weight of the composition.

[0096] In various aspects, pharmaceutical compositions disclosed herein may comprise one or more antioxidants. As used herein, “antioxidants” are agents that inhibit oxidation and thus can be used to prevent the deterioration of preparations by the oxidative process. Such compounds include, by way of example and without limitation, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium bisulfite, sodium formaldehyde sulfoxylate and sodium metabisulfite and other materials known to one of ordinary skill in the art. In some aspects, compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more antioxidants by total weight of the composition.

[0097] In other aspects, pharmaceutical compositions disclosed herein may comprise a buffer system. As used herein, a “buffer system” is a composition comprised of one or more buffering agents wherein “buffering agents” are compounds used to resist change in pH upon dilution or addition of acid or alkali. Buffering agents include, by way of example and without limitation, potassium metaphosphate, potassium phosphate, monobasic sodium acetate and sodium citrate anhydrous and dihydrate and other materials known to one of ordinary skill in the art. In some aspects, any pharmaceutically acceptable organic or inorganic buffer can be used. In another aspect, compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more buffering agents by total weight of the composition. In other aspects, the amount of one or more buffering agents may depend on the desired pH level of a composition. In some aspects, pharmaceutical compositions disclosed herein may have a pH of about 6 to about 9. In other aspects, pharmaceutical compositions disclosed herein may have a pH greater than about 8, greater than about 7.5, greater than about 7, greater than about 6.5, or greater than about 6. In a preferred aspect, compositions disclosed herein may have a pH greater than about 6.8.

[0098] In various aspects, pharmaceutical compositions disclosed herein may comprise one or more preservatives. As used herein, “preservatives” refers to agents or combination of agents that inhibits, reduces, or eliminates bacterial growth in a pharmaceutical dosage form.

Non-limiting examples of preservatives include Nipagin, Nipasol, isopropyl alcohol and a combination thereof. In some aspects, any pharmaceutically acceptable preservative can be used. In other aspects, pharmaceutical compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more preservatives by total weight of the composition.

[0099] In other aspects, pharmaceutical compositions disclosed herein may comprise one or more surface-acting reagents or detergents. In some aspects, surface-acting reagents or detergents may be synthetic, natural, or semi-synthetic. In other aspects, compositions disclosed herein may comprise anionic detergents, cationic detergents, zwitterionic detergents, ampholytic detergents, amphoteric detergents, nonionic detergents having a steroid skeleton, or a combination thereof. In still other aspects, pharmaceutical compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more surface-acting reagents or detergents by total weight of the composition.

[0100] In various aspects, pharmaceutical compositions disclosed herein may comprise one or more stabilizers. As used herein, a “stabilizer” refers to a compound used to stabilize an active agent against physical, chemical, or biochemical process that would otherwise reduce the therapeutic activity of the agent. Suitable stabilizers include, by way of example and without limitation, succinic anhydride, albumin, sialic acid, creatinine, glycine and other amino acids, niacinamide, sodium acetyltryptophonate, zinc oxide, sucrose, glucose, lactose, sorbitol, mannitol, glycerol, polyethylene glycols, sodium caprylate and sodium saccharin and others known to those of ordinary skill in the art. In some aspects, pharmaceutical compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more stabilizers by total weight of the composition.

[0101] In other aspects, pharmaceutical compositions disclosed herein may comprise one or more tonicity agents. As used herein, a “tonicity agents” refers to a compound that can be used to adjust the tonicity of the liquid formulation. Suitable tonicity agents include, but are not limited to, glycerin, lactose, mannitol, dextrose, sodium chloride, sodium sulfate, sorbitol, trehalose and others known to those of ordinary skill in the art. Osmolarity in a composition may be expressed in milliosmoles per liter (mOsm/L). Osmolarity may be measured using methods commonly known in the art. In preferred aspects, a vapor pressure depression method is used to calculate the osmolarity of the compositions disclosed herein. In some aspects, the amount of one or more tonicity agents comprising a pharmaceutical composition disclosed herein may result in a composition osmolarity of about 150 mOsm/L to about 500 mOsm/L, about 250 mOsm/L to about 500 mOsm/L, about 250 mOsm/L to about 350 mOsm/L,

about 280 mOsm/L to about 370 mOsm/L or about 250 mOsm/L to about 320 mOsm/L. In other aspects, a composition herein may have an osmolality ranging from about 100 mOsm/kg to about 1000 mOsm/kg, from about 200 mOsm/kg to about 800 mOsm/kg, from about 250 mOsm/kg to about 500 mOsm/kg, or from about 250 mOsm/kg to about 320 mOsm/kg, or from about 250 mOsm/kg to about 350 mOsm/kg or from about 280 mOsm/kg to about 320 mOsm/kg. In some aspects, a pharmaceutical composition described herein has an osmolality of about 100 mOsm/L to about 1000 mOsm/L, about 200 mOsm/L to about 800 mOsm/L, about 250 mOsm/L to about 500 mOsm/L, about 250 mOsm/L to about 350 mOsm/L, about 250 mOsm/L to about 320 mOsm/L, or about 280 mOsm/L to about 320 mOsm/L. In still other aspects, pharmaceutical compositions disclosed herein may comprise at least 5%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% total amount of one or more tonicity modifiers by total weight of the composition.

Dosage formulations

[0102] Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially trans-nasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as, intravenous, intraperitoneal, intranasal injections, intraocular (e.g., via eye drops) or topical (e.g., creams or ointments). Due to the ultra-purity of the tricaprilin disclosed herein, it is especially suitable for oral administration.

[0103] Pharmaceutical compositions of the present disclosure may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0104] Pharmaceutical compositions for use in accordance with the present disclosure thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

[0105] Pharmaceutical compositions suitable for use in context of the present disclosure include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. In some aspects, a therapeutically effective amount means an amount of active ingredients (i.e., the ultra-pure tricaprilin disclosed herein) effective to prevent, slow, alleviate or ameliorate symptoms of a disorder or prolong the survival of the subject being treated.

[0106] Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0107] For any preparation used in the methods of the present disclosure, the therapeutically effective amount or dose can be estimated initially from in vitro and in vivo assays and or screening platforms disclosed herein. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

[0108] Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition.

[0109] Dosage amount and interval may be adjusted individually to brain or blood levels of the active ingredient that are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

[0110] Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved. The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc. Effective doses may be extrapolated from dose- responsive curves derived from in vitro or in vivo test systems.

IV. Methods of Treatment

[0111] As noted above, the present disclosure provides for an ultra-pure composition of tricaprilin. This ultra-pure composition is particularly appropriate for applications requiring high purity grades and at the same time high active ingredient levels, e.g., for nutritional and for pharmaceutical applications and uses (e.g., for applications in high dosages and/or with high concentrations).

[0112] Accordingly, in various aspects, a method of treating a subject in need thereof is provided. In an aspect, a method of prophylactically or therapeutically treating a disease in a subject is provided. In an aspect the disease to be treated (prophylactically or therapeutically) may be associated with a disorder of the energy metabolism, especially ketone body

metabolism, such as especially craniocerebral trauma, stroke, hypoxia, cardiovascular diseases such as myocardial infarction, refeeding syndrome, anorexia, epilepsy, neurodegenerative diseases such as dementia, Alzheimer's disease, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis, fat metabolic diseases such as glucose transporter defect (GLUT1 defect), VL-FAOD and mitochondriopathies such as mitochondrial thiolase defect, Huntington's disease, cancers such as T-cell lymphomas, astrocytomas and glioblastomas, HIV, rheumatic diseases such as rheumatoid arthritis and arthritis urica, diseases of the gastrointestinal tract such as chronic inflammatory bowel diseases, especially ulcerative colitis and Crohn's disease, lysosomal storage diseases such as sphingolipidosis, especially Niemann-Pick disease, diabetes mellitus, migraine and migraine headaches, epilepsy, especially infantile spasms as well as effects or side-effects of chemotherapy. In some aspects, a method of prophylactically or therapeutically treating Alzheimer's disease, especially mild to moderate forms of Alzheimer's disease; migraine and migraine headaches; as well as epilepsy, especially infantile spasms in a subject in need thereof.

[0113] In accord with the foregoing, a pharmaceutical composition comprising the ultra-pure tricaprilin is provided for the use of prophylactically or therapeutically treating a subject with a disease is provided, where the disease is be associated with a disorder of the energy metabolism, especially ketone body metabolism, such as especially craniocerebral trauma, stroke, hypoxia, cardiovascular diseases such as myocardial infarction, refeeding syndrome, anorexia, epilepsy, neurodegenerative diseases such as dementia, Alzheimer's disease, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis, fat metabolic diseases such as glucose transporter defect (GLUT1 defect), VL-FAOD and mitochondriopathies such as mitochondrial thiolase defect, Huntington's disease, cancers such as T-cell lymphomas, astrocytomas and glioblastomas, HIV, rheumatic diseases such as rheumatoid arthritis and arthritis urica, diseases of the gastrointestinal tract such as chronic inflammatory bowel diseases, especially ulcerative colitis and Crohn's disease, lysosomal storage diseases such as sphingolipidosis, especially Niemann-Pick disease, diabetes mellitus, migraine and migraine headaches, epilepsy, especially infantile spasms as well as effects or side-effects of chemotherapy. In some aspects, a pharmaceutical composition comprising the ultra-pure tricaprilin is provided for use in prophylactically or therapeutically treating Alzheimer's disease, especially mild to moderate forms of Alzheimer's disease; migraine and migraine headaches; as well as epilepsy, especially infantile spasms in a subject in need thereof.

[0114] In accord with the foregoing, in an aspect a high dose of ultra-pure tricaprilin is administered to a subject in need. The high dose may be administered according to any of the dose formulations described above. In particular aspects, the high does may be administered orally. In some aspects, the high dose may be administered systemically. In an aspect, a

suitable dosage regimen may comprise administering at least about 20 mg/day to about 100 mg/day of the ultra-pure tricaprilin to a subject in need thereof. In an aspect, a suitable dosage regimen may comprise administering at least 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg tricaprilin per day. The tricaprilin composition may be combined with suitable excipients or combined with other active ingredients, such that the total amount of tricaprilin ingested per day is in the range of 20 mg/day to 100 mg/day. Further, several divided dosages as well as staggered dosages, can be administered daily or sequentially, or the dose can be continuously infused, or can be a bolus injection. Further, the dosages of the compound(s) described herein can be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation. The selected dosage level will depend upon a variety of factors including the route of administration, the time of administration, and the rate of excretion, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0115] A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds described herein employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0116] If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. Similarly, the daily dose may be administered for any suitable duration, for example for at least a week, 2-weeks, 3-weeks, 1-month, 2-month, 3-month, 4 months, 5-months, 6-months, 7-months, 8-months, 9-months, 10-months, 11-months, 1 year or more.

[0117] While it is possible for a compound described herein to be administered alone, it may be administered in combination with other active composition.

[0118] By the term "combination" is meant either a fixed combination in one dosage unit form, or a kit of parts for the combined administration where a composition described herein and a combination partner may be administered independently at the same time or separately within time intervals that especially allow that the combination partners show a cooperative, e.g., synergistic, effect, or any combination thereof. The compositions described herein may be administered, simultaneously or sequentially, with an anti-inflammatory, antiproliferative,

antibiotics, NSAIDs, painkillers, chemotherapeutic agent, immunosuppressant, other anti-cancer drugs, cytotoxic agent or salt thereof.

[0119] As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes may be made to the aspects of the invention without departing from the scope of this invention as defined in the following claims.

EXAMPLES

EXAMPLE 1: Experiments

[0120] Tricaprilin is synthesized by condensation of glycerol under acidic conditions with three equivalents of octanoic acid (caprylic acid), the latter being used in excess. Octanoic acid is treated with H_2SO_4 -conc. and the mixture is heated to approx. 115 °C. Glycerol is charged slowly and the mixture is then stirred overnight. Subsequently, the mixture is cooled to room temperature and diluted with MTBE (or an alternative solvent that does not lead to methyl esters in a subsequent batch). The solution is then filtered over active charcoal for decolorization. The organic phase is washed several times with Na_2CO_3 -aq. to deplete residual octanoic acid and concentrated H_2SO_4 , followed by extractions with water to remove any residual salts. Afterwards MTBE is distilled off under reduced pressure to obtain Tricaprilin as a clear colorless liquid (see FIG. 5).

[0121] Experiments were conducted to test the significance of various parameters and novel steps were included to improve yield and purity of the samples. **Table 4** provides descriptions and analysis of the experiments conducted and the results obtained. The Examples below describe the conclusions from these sets of experiments and describe in detail the unexpected nature of some of the findings.

Table 4

Exp No	Short description	Result
EXP-22-AA3620	Familiarization experiment 1 <u>Raws:</u> - Glycerol : 20.2 g, Fisher Bioreagents - Octanoic acid: 4.1 mol-eq. - H₂SO₄-conc.: 0.5 mol% <u>Reaction:</u> - ~260 mbar, 115 °C, N2 stream through reaction mixture, reaction overnight - continuous removal of water <u>Workup:</u> - divided: 1) work-up in toluene, 2) work-up in MTBE	<u>Reaction:</u> - Formation of black particles during reaction; reaction mixture clear brown solution at the end - Caprylic acid / TARI-01 was found in cooler; based on DSC data it is most probably TARI-01 - As not full conversion was given, the work-up was not representative (issues with water washes observed: no phase separation; toluene was used for work-up) → not further processed → Reaction without vacuum possible?
EXP-22-AA3622	<u>No nitrogen purging, water not cont. removed</u> <u>Raws:</u> - Glycerol - octanoic acid: 4.2 mol-eq. - H₂SO₄-conc.: 2 mol% <u>Reaction:</u> - ~260 mbar, 120 °C, no N2 stream through reaction mixture, reaction overnight - no work-up	<u>Reaction:</u> - Black reaction mixture overnight → degradation of glycerol → <i>Water removal of utmost importance</i>
EXP-22-AA3623	<u>No vacuum</u> <u>Raws:</u> - glycerol: - octanoic acid: 4.2 mol-eq. - H₂SO₄-conc.: 2 mol% <u>Reaction:</u> - 120 °C, N2 stream through reaction mixture, no vacuum - continuous removal of water - no work-up	<u>Reaction:</u> - Black reaction mixture already during dosing of glycerol → Degradation of glycerol → Vacuum necessary for sufficient removal of water

Exp No	Short description	Result															
EXP-23-AA6314	<u>Familiarization experiment 2</u> <u>Raws:</u> - Glycerol (10 g) from Fisher - Bioreagents - Octanoic acid: 65.8 g, 4.2 eq. from Thermo Scientific - H₂SO₄-conc.: 55 µL, 0.9 mol% from Merck <u>Reaction:</u> - 23 h, ~150 mbar, 115 °C, 60 NL/h N₂ stream - continuous removal of water <u>Workup:</u> - MIBE (10.6+20 g) - 3x Basic wash (20 g Na₂CO₃ 10%) - Water washes (40 g needed)	<u>Reaction:</u> - Some white solid found in cooler: caprylic acid (22.2 g, confirmed by TLC) <u>Workup:</u> - Bad phase separation after addition of water -> increasing amount helped phase separation - White precipitate present (Na Caprylate) -> filtration and basic treatment needed <u>Isolation:</u> - After solvent evaporation, 33.9 g of a pale-yellow oil were obtained - Yield is ~66% (~1% of caprylic acid, purity 72.6%, from qNMR) Purity analysis: <table> <tr> <td>- Monoctanoyl Glycerol:</td> <td>RT 12.21:</td> <td>0.07 %a</td> </tr> <tr> <td>- 1,2-Dioctanoyl Glycerol:</td> <td>RT 19.76:</td> <td>2.51 %a</td> </tr> <tr> <td>- 1,3-Dioctanoyl Glycerol:</td> <td>RT 19.91:</td> <td>10.20 %a</td> </tr> <tr> <td>- TARI-01</td> <td>RT 25.07:</td> <td>75.37 %a</td> </tr> <tr> <td>- Tetraester</td> <td>RT 31.79 + 31.92:</td> <td>2.40 + 4.14 %a</td> </tr> </table> Isolated product is TARI-01 (confirmed via NMR); traces of caprylic acid present. MCPD analysis of product: - 3-MCPD: 1500 ppb - 2-MCPD: ca. 110 ppb - Glycidol: ca. 100 ppm. MPCD analysis of glycerin submitted, comparable values expected <u>Conclusions:</u> - Avoid caprylic acid deposition on the cooler (increasing temperature, reducing N₂ stream and vacuum) - Improve efficiency of caprylic acid removal in aqueous workup (increase amount of base or number of washings)	- Monoctanoyl Glycerol:	RT 12.21:	0.07 %a	- 1,2-Dioctanoyl Glycerol:	RT 19.76:	2.51 %a	- 1,3-Dioctanoyl Glycerol:	RT 19.91:	10.20 %a	- TARI-01	RT 25.07:	75.37 %a	- Tetraester	RT 31.79 + 31.92:	2.40 + 4.14 %a
- Monoctanoyl Glycerol:	RT 12.21:	0.07 %a															
- 1,2-Dioctanoyl Glycerol:	RT 19.76:	2.51 %a															
- 1,3-Dioctanoyl Glycerol:	RT 19.91:	10.20 %a															
- TARI-01	RT 25.07:	75.37 %a															
- Tetraester	RT 31.79 + 31.92:	2.40 + 4.14 %a															
EXP-23-AA6315	<u>Familiarization experiment 3</u> <u>Raws:</u> - Glycerol (10 g), 2mL/h, Fisher - Bioreagents - Octanoic acid: 65.8 g, 4.2 mol-eq., Thermo Scientific - H₂SO₄-conc.: 55 µL, 0.9 mol%, Merck <u>Reaction:</u> - 21 h, ~350 mbar, 130 °C, N₂ stream (30 NL/h) - Dosing pump used <u>Workup:</u>	<u>Reaction:</u> - Amber mixture <u>Workup:</u> - Emulsion formation, NaCl aq. sol. needed to clean phase separation <u>Isolation:</u> - Amber colored turbid oil was obtained (41.29 g, 80%), NaCl crystals present MCPD analysis submitted Purity analysis submitted: <table> <tr> <td>- Monoctanoyl Glycerol:</td> <td>RT 12.21:</td> <td>n.d.</td> </tr> <tr> <td>- 1,2-Dioctanoyl Glycerol:</td> <td>RT 19.76:</td> <td>1.55 %a</td> </tr> <tr> <td>- 1,3-Dioctanoyl Glycerol:</td> <td>RT 19.91:</td> <td>4.30 %a</td> </tr> <tr> <td>- TARI-01:</td> <td>RT 25.07:</td> <td>87.02 %a</td> </tr> <tr> <td>- Tetraester:</td> <td>RT 31.79 + 31.92:</td> <td>1+1.77 %a</td> </tr> </table> qNMR analysis submitted -> 83.33%w	- Monoctanoyl Glycerol:	RT 12.21:	n.d.	- 1,2-Dioctanoyl Glycerol:	RT 19.76:	1.55 %a	- 1,3-Dioctanoyl Glycerol:	RT 19.91:	4.30 %a	- TARI-01:	RT 25.07:	87.02 %a	- Tetraester:	RT 31.79 + 31.92:	1+1.77 %a
- Monoctanoyl Glycerol:	RT 12.21:	n.d.															
- 1,2-Dioctanoyl Glycerol:	RT 19.76:	1.55 %a															
- 1,3-Dioctanoyl Glycerol:	RT 19.91:	4.30 %a															
- TARI-01:	RT 25.07:	87.02 %a															
- Tetraester:	RT 31.79 + 31.92:	1+1.77 %a															

Exp No	Short description	Result
	- MIBE (106 g) - 4x 20 % Na₂CO₃ aq. sol. (58 g) - Water (105 g) + Brine (30 g) until pH was 7 - Water (105 g) + Brine (30 g)	MCPD analysis of product <u>before</u> Celite treatment: - 3-MCPD: 10923 ppb - 2-MCPD: 766 ppb - Glycidol: 71534 ppb MCPD analysis of product <u>after</u> Celite treatment overnight: - 3-MCPD: 10504 ppb - 2-MCPD: 708 ppb - Glycidol: 59651 ppb Conclusions: Filtration on Celite resulted in a glycidol decrease of 17%; no effect on MCPD levels Problems: - Caprylic acid still on the cooling trap (19.9 g, 1.3 Equiv.) - Emulsion formation in the workup step (time consuming) Next attempt: - Tackle the octanoic acid issue (less vacuum and N₂ flow) - Investigate different solvents for the workup (heptane, toluene, no solvent) - Avoid emulsion
EXP-23-AA6317	Familiarization experiment 4 Raws: - Glycerol: 20 g, 2mL/h, Fisher Bioreagents - Octanoic acid: 134.6 g, 4.2 mol-eq., Thermo Scientific - H₂SO₄-conc.: 291 mg, 0.9 mol%, Merck Reaction: - 23.5 h, ~350 mbar, 130 °C, N₂ stream (20 NL/h) - Dosing pump used Workup - Filtration on Celite, charcoal treatment (with 100g heptane and 100 g toluene) - 100 mL org. sol. + 400 mL of aq. phase (20% Na₂CO₃, Water, Brine, Water)	Reaction: - Turned black overnight - Condenser obstructed with Caprylic acid -> water not efficiently removed Workup: - Filtration worked well - No emulsion formed Isolation: - Yield: 47.3+48.1 = 95.4 g (92%) - Colorless oil (from heptane) and yellowish oil (from toluene) were isolated Purity analysis: From Heptane workup - Monooctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.74: 0.03 %a - 1,3-Dioctanoyl Glycerol: RT 19.86: 0.06 %a - TARI-01: RT 25.10: 97.81 %a - Tetraester: RT 31.77 + 31.90: 0.24+0.52 %a MCPD analysis submitted: - 3-MCPD: 4500 ppb - 2-MCPD: 430 ppb - Glycidol: ca. 69.6 ppm From toluene workup

Exp No	Short description	Result
EXP-23-AA6318	<i>Familiarization experiment 5</i> <u>Raws:</u> - Glycerol: 10 g, 2mL/h, Fisher Bioreagents - Octanoic acid: 65.9 g, 4.2 mol-eq., Thermo Scientific - H₂SO₄-conc.: 55 µL, 0.9 mol%, Merck <u>Reaction</u> - 21 h, 500 mbar, 115 °C, N₂ stream (20 NL/h) - Dosing pump used <u>Workup:</u> - without organic solvent + 4x semi saturated solution of NaCl + Na₂CO₃ 1M, (80 g) - 2x semi saturated NaCl washes (110 g) until neutral pH - 2x water wash (50 mL), - Addition of heptane, active charcoal filtration	- Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.73: 0.05 %a - 1,3-Dioctanoyl Glycerol: RT 19.86: 0.10 %a - TARI-01: RT 25.10: 97.73 %a - Tetraester: RT 31.77 + 31.90: 0.24+0.52 %a MCPD analysis submitted: - 3-MCPD: 4840 ppb - 2-MCPD: 601 ppb - Glycidol: ca. 67.8 ppm <i>Problems:</i> - Caprylic acid on the condenser <i>Next attempt:</i> - Further reduce vacuum (500mbar) - Further reduce N₂ flow to 20 L/h
EXP-23-AA6319	<i>Familiarization experiment 6</i> <u>Raws:</u> - Glycerol (10 g), 4mL/h instead 1mL/h, Verbio	<i>Reaction:</i> - Glycerol was added in 2 h - Amber reaction mixture with small amount of black residue <i>Workup:</i>

Exp No	Short description - Octanoic acid: 66.2 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%, Merck (Chloride ≤ 0.1 ppm) <u>Reaction:</u> - 600 mbar, 115 °C, N₂ stream (5-10 NL/h) - Dosing pump used <u>Workup:</u> - Without organic solvent + semi saturated solution of NaCl + Na₂CO₃ (1M) - Semi sat. NaCl washes until neutral pH - Addition of heptane, active charcoal filtration	Result - Fast phase separation <u>Isolation:</u> - Isolated yield 43.1 g (84%) Purity analysis: <table border="0"> <tr> <td>- Monoctanoyl Glycerol:</td><td>RT 12.20:</td><td>0.0%</td></tr> <tr> <td>- 1,2-Dioctanoyl Glycerol:</td><td>RT 19.75:</td><td>0.88 %a</td></tr> <tr> <td>- 1,3-Dioctanoyl Glycerol:</td><td>RT 19.90:</td><td>2.00 %a</td></tr> <tr> <td>- TARI-01:</td><td>RT 25.08:</td><td>94.36 %a</td></tr> <tr> <td>- Tetraester:</td><td>RT 31.79 + 31.92:</td><td>0.36+0.77 %a</td></tr> </table> MCPD analysis of product: - 3-MCPD: 755 ppb - 2-MCPD: 47 ppb - Glycidol: ca. 47.7 ppm <u>Problems:</u> - Amount of caprylic acid of the condenser was reduced but still relevant (16.6 g, 1.05 equiv.) - Amount of MCPD and glycidol critical <u>Next attempt:</u> - Premixing glycerin with caprylic acid and add them with dropping funnel -> avoid evaporation of caprylic acid	- Monoctanoyl Glycerol:	RT 12.20:	0.0%	- 1,2-Dioctanoyl Glycerol:	RT 19.75:	0.88 %a	- 1,3-Dioctanoyl Glycerol:	RT 19.90:	2.00 %a	- TARI-01:	RT 25.08:	94.36 %a	- Tetraester:	RT 31.79 + 31.92:	0.36+0.77 %a
- Monoctanoyl Glycerol:	RT 12.20:	0.0%															
- 1,2-Dioctanoyl Glycerol:	RT 19.75:	0.88 %a															
- 1,3-Dioctanoyl Glycerol:	RT 19.90:	2.00 %a															
- TARI-01:	RT 25.08:	94.36 %a															
- Tetraester:	RT 31.79 + 31.92:	0.36+0.77 %a															
EXP-23-AA6320	Familiarization experiment 7 <u>Raws:</u> - Glycerol (15 g), Verbio - Octanoic acid: 98.7 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 82.5 µL, 0.9 mol%, Merck <u>Reaction:</u> - 500 mbar, 120 °C, N₂ stream (5-10 NL/h) - 1 eq. of caprylic acid was added to the reactor, the rest was mixed with glycerin - Reagents premixed and added over several hours dropping funnel <u>Workup:</u> - Without organic solvent + semi saturated solution of NaCl + Na₂CO₃ (1M)	<u>Reaction</u> Mixture of glycerin and octanoic acid were added with dropping funnel over 5 h <u>Workup:</u> - Nothing particular to note - Filtration over activated charcoal <u>Isolation:</u> - Slight yellow product obtained (65.7 g) Purity analysis: <table border="0"> <tr> <td>- Monoctanoyl Glycerol:</td><td>RT 12.20:</td><td>0.0%</td></tr> <tr> <td>- 1,2-Dioctanoyl Glycerol:</td><td>RT 19.75:</td><td>0.24 %a</td></tr> <tr> <td>- 1,3-Dioctanoyl Glycerol:</td><td>RT 19.90:</td><td>0.58 %a</td></tr> <tr> <td>- TARI-01:</td><td>RT 25.08:</td><td>87.89 %a</td></tr> <tr> <td>- Tetraester:</td><td>RT 31.79 + 31.92:</td><td>2.13+5.33 %a</td></tr> </table> <u>Problems:</u> - Starting material are poorly miscible → Accumulation of glycerin occurred - Caprylic acid deposited on the condenser <u>Next attempt:</u> - Use of Dean-Stark apparatus. Water from reaction will be collected and caprylic acid would return to the reaction	- Monoctanoyl Glycerol:	RT 12.20:	0.0%	- 1,2-Dioctanoyl Glycerol:	RT 19.75:	0.24 %a	- 1,3-Dioctanoyl Glycerol:	RT 19.90:	0.58 %a	- TARI-01:	RT 25.08:	87.89 %a	- Tetraester:	RT 31.79 + 31.92:	2.13+5.33 %a
- Monoctanoyl Glycerol:	RT 12.20:	0.0%															
- 1,2-Dioctanoyl Glycerol:	RT 19.75:	0.24 %a															
- 1,3-Dioctanoyl Glycerol:	RT 19.90:	0.58 %a															
- TARI-01:	RT 25.08:	87.89 %a															
- Tetraester:	RT 31.79 + 31.92:	2.13+5.33 %a															

Exp No	Short description	Result
	- Semi saturated NaCl washes until neutral pH - Addition of heptane, active charcoal filtration	- First neutralization, then salt addition (NaCl or other salt to increase ion strength and avoid emulsion formation/ facilitate phase separation)
EXP-23-AA8203	<u>Raws:</u> - Glycerol (10 g), 8 mL/h, from Verbio - Octanoic acid: 20.2+46 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%, Merck <u>Reaction:</u> - 500 mbar, 115 °C, N2 stream (5-10 NL/h) above reaction surface - 1 eq. of caprylic acid was added to the reactor, the rest was dosed with dosing pump - Glycerol was added with dosing pump - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - Without organic solvent + semi saturated solution of NaCl + Na₂CO₃ (1M) - Semi saturated NaCl washes until neutral pH - Aqueous wash - Addition of heptane, active charcoal filtration	<u>Reaction</u> - Yellowish reaction mixture - 2.7 mL of water, 0.5 mL octanoic acid collected in Dean Stark Apparatus <u>Workup:</u> - Foam development after addition of Na₂CO₃ + NaCl solution - Gelification observed, addition of water solved the problem - Filtration over activated charcoal <u>Isolation:</u> - Colorless liquid obtained (40.7 g, 86.5 %) Purity analysis: - Monooctanoyl Glycerol: RT 12.20: 0.0% - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.0 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.56 %a - TARI-01: RT 25.08: 97.84 %a - Tetraester [%a]: RT 31.79 + 31.92: 0.09+0.22 MCPD analysis of product: - 3-MCPD: 314 ppb - 2-MCPD: 117 ppb - Glycidol: <2.5 ppm <u>Problems:</u> - Intensive foam formation during neutralization - Gelification occurred <u>Next attempt:</u> - Keep same reaction condition, Dean Stark apparatus, increase vacuum to test efficacy of Dean-Stark apparatus. - Avoid use of NaCl.
EXP-23-AA8204	<u>Raws:</u> - Glycerol (10 g), 1 mL/h, from Verbio - Octanoic acid: 65.9 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%,	<u>Reaction</u> - Yellowish reaction mixture - 2.5 mL of water, 1.5 mL octanoic acid collected in Dean Stark Apparatus <u>Workup:</u> - Foam development after addition of Na₂CO₃, good phase separation, aqueous layer clear, organic layer turbid even after overnight.

Exp No	Short description	Result
	Merck <u>Reaction:</u> - 250 mbar, 115 °C, N2 stream (5-10 NL/h) above reaction surface - All octanoic acid was added to the reactor - Glycerol was added with dosing pump - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - Without organic solvent + solution of Na₂CO₃ (1M), - Heptane addition, aq. wash washes until neutral pH - Active charcoal filtration + rinse with heptane	- Filtration over activated charcoal, heptane <u>Isolation:</u> - Colorless liquid obtained (43.4 g, 89.1 %) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: 0.0 %a - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.0 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 1.13 %a - TARI-01: RT 25.08: 97.03 %a - Tetraester: RT 31.79 + 31.92: 0.06+0.14 %a MCPD analysis of product: - 3-MCPD: 334 ppb - 2-MCPD: 111 ppb - Glycidol: <6.5 ppm <u>Problems:</u> - Intensive foam formation was observed → slow dosing of basic wash needed - Organic phase without solvent is not clear, addition of unpolar solvent increase hydrophobicity <u>Next attempt:</u> - Accelerate conversion: increase acid loading - Speed up phase separation: implement solvent in work-up (product concentration from 10 to 50%) OR - addition of NaCl after neutralization of reaction mixture and filtration of NaCl crystals during charcoal treatment
EXP-23-AA8206	<u>Raws:</u> - Glycerol (10 g), 1 mL/h, from Verbio - Octanoic acid: 65.9 g, 4.2 mol-eq., Metadynaea - H₂SO₄-conc.: 273 µL, 4.5 mol%, Merck <u>Reaction:</u> - 500 mbar, 115 °C, N2 stream (5-10 NL/h) above reaction surface - All octanoic acid was added to the reactor - Glycerol was added with dosing pump over 8h - Dean-Stark apparatus used	<u>Reaction:</u> - Brown reaction mixture <u>Workup:</u> - No foam development after addition of Na₂CO₃, good phase separation with carbonate, slower during aqueous washings - Filtration over activated charcoal, heptane <u>Isolation:</u> - Colorless liquid obtained (47.0 g, 92.9%) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.81 %a - TARI-01: RT 25.08: 97.55 %a - Tetraester: RT 31.79 + 31.92: 0.17+0.44 %a MCPD analysis of product: - 3-MCPD: 165 ppb

Exp No	Short description	Result
	- Condenser temperature at 20 °C <u>Workup:</u> - Without organic solvent + solution of Na₂CO₃ (1M), - Heptane addition, aq. wash washes until neutral pH - Active charcoal filtration + rinse with heptane	- 2-MCPD: 50 ppb - Glycidol: 31.4 ppm Problems: - phase separation between unpolar organic solvent (heptane) and pure water is not ideal. Next attempt: - Bubbling nitrogen through the solution
EXP-23-AA8207	<u>Raws:</u> - Glycerol (10 g), 1 mL/h, from Verbio - Octanoic acid: 65.9 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%, Merck <u>Reaction:</u> - 500 mbar, 115 °C, N₂ stream (5-10 NL/h) below reaction surface - All octanoic acid was added to the reactor - Glycerol was added with dosing pump over 8h - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - Heptane + solution of Na₂CO₃ (1M), - Aq. wash washes until neutral pH - Back-extraction of aq. layer - Active charcoal filtration + rinse with heptane	<u>Reaction:</u> - Golden yellow reaction mixture - In Dean Stark apparatus: 4.5 mL water + 1 mL Caprylic acid <u>Workup:</u> - Foam development after addition of Na₂CO₃, good phase separation with carbonate, organic layer not clear when only water is used. - Filtration over activated charcoal, heptane <u>Isolation:</u> - Pale yellowish liquid obtained (49.7 g, 96.3%) Purity analysis: - Monooctanoyl Glycerol: RT 12.20: n.d - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.08 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.30 %a - TARI-01: RT 25.08: 97.82 %a - Tetraester: RT 31.79 + 31.92: 0.05+0.11 %a MCPD analysis of product: - 3-MCPD: 220 ppb - 2-MCPD: 69 ppb - Glycidol: 4.34 ppm Problems: - phase separation between unpolar organic solvent (heptane) and pure water is not ideal. Next attempt: - Can the reaction be accelerated without increasing tetraesters?
EXP-23-AA8208	<u>Raws:</u> - Glycerol: 10 g, Verbio - Octanoic acid: 65.9 g, 4.2 mol-eq., Metadynea	<u>Reaction:</u> - Golden orange reaction mixture - after 3 hours 5 mL water were collected in Dean Stark apparatus <u>Workup:</u>

Exp No	Short description	Result
	- H₂SO₄-conc.: 55 µL, 0.9 mol%, Merck <u>Reaction:</u> - 500 mbar, 115 °C, N₂ stream (6 NL/h) below reaction surface - All octanoic acid preadded to the reactor - Glycerol added with dosing pump over 1h - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - Na₂CO₃ (1M, 10 Vol) + MtBE (4.5 Vol) - Semi sat. NaCl. sol.(10 Vol) washes until neutral pH - Active charcoal filtration + rinse with MtBE	- Foam development after addition of Na₂CO₃, first separation took less than 10 min.; following were fast <u>Isolation:</u> - Pale yellowish liquid obtained (47.7 g, 97.5%) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.05 %a - TARI-01: RT 25.08: 98.4 %a - Tetraester: RT 31.79 + 31.92: 0.04 + 0.11 %a MCPD analysis of product: - 3-MCPD: 193 ppb - 2-MCPD: 85 ppb - Glycidol: 11.12 ppm <u>Problems:</u> - Phase separation appears to be good for all extraction, except the first one where most of the caprylic acid excess is removed <u>Next attempt:</u> - Can the reaction be performed below 115 °C? Does the reduced temperature reduce side product formation? - Is the workup better with MtBE, Na₂CO₃ followed by NaCl?
EXP-23-AA8209	<u>Raws:</u> - Glycerol: 15 g, Verbio - Octanoic acid: 100 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 82,5 µL, 0.9 mol%, Merck <u>Reaction:</u> - 500 mbar, 105 °C, N₂ stream (6 NL/h) below reaction surface - All octanoic acid preadded to the reactor - Glycerol added with dosing pump over 8h - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - Na₂CO₃ (1M, 10 Vol) + MtBE	- Very pale-yellow reaction mixture - Dosing started at 18:00, at 08:30 8mL water were collected in Dean Stark apparatus, meaning 10 hours reaction including 8 hours of dosing <u>Workup:</u> - Foam development after addition of Na₂CO₃. - First two separation took 3 minutes; the following phase separation took about 1 minute <u>Isolation:</u> - Colorless liquid obtained (72.9 g, 96.5%) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: 1.8 %a - TARI-01: RT 25.08: 96.99 %a - Tetraester: RT 31.79 + 31.92: 0.03 + 0.06 %a A sample of the end product was analysed after solvent removal at rotary evaporator MCPD analysis of product: - 3-MCPD: 260 ppb

Exp No	Short description	Result
	(4.5 Vol) - Semi sat. NaCl sol. (10 Vol) - washes until neutral pH - Active charcoal filtration + rinse with MtBE	- 2-MCPD: 68 ppb - Glycidol: 1.04 ppm Another sample was dried at rotary evaporator at 70 °C @1 mbar for 4 h MCPD analysis of product: - 3-MCPD: 271 ppb - 2-MCPD: 72 ppb - Glycidol: 1.50 ppm <u>Problems:</u> - The reaction time was shortened (only 2 hour after dosing was completed), resulting in increased amount of diesters. Tetraesters level are very low. No changes in MCPD and glycidol content after distilling the sample at 70 °C <u>Next attempt:</u> - Keeping the reaction temperature low and increase the dosing speed of glycerine will lead to tetraester formation?
EXP-23-AA8210	<u>Raws:</u> - Glycerol: 10 g, Verbio - Octanoic acid: 66.3 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 55 µL, 0.9 mol%, Merck <u>Reaction:</u> - 500 mbar, 105 °C, N₂ stream (6 NL/h) below reaction surface - All octanoic acid preadded to the reactor - Glycerol added with dosing pump over 1h - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - Na₂CO₃ (1M, 10 Vol) + MtBE (3 Vol) - Semi sat. NaCl. sol. (10 Vol) - washes until neutral pH - Active charcoal filtration + rinse with MtBE	<u>Reaction</u> - very pale-yellow reaction mixture with black particles - 3 mL of water in D.S apparatus. <u>Workup:</u> - Foam development after addition of Na₂CO₃, - First two separations took about 2 minutes; the following phase separation took about 1 minute <u>Isolation:</u> - Colourless liquid obtained (47.0 g, 94.9%) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.15 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.42 %a - TARI-01: RT 25.08: 98.25 %a - Tetraester: RT 31.79 + 31.92: 0.04 + 0.11 %a MCPD analysis of product: 3-MCPD: 301 ppb 2-MCPD: 90 ppb - Glycidol: 5510 ppb <u>Problems:</u> - Fast addition of glycerine lead to formation of dark brown droplets - Lower yield probably due to glycerine decomposition - Low temperature does not promote tetraesters formation even if glycerine is added fast. <u>Next attempt:</u>

Exp No	Short description	Result
EXP-23-AA6322	<u>Raws:</u> - Glycerol: 10.1 g, Verbio - Octanoic acid: 65.6 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 55 μL, 0.9 mol%, Merck <u>Reaction:</u> - 250 mbar, 107 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol added with dosing pump (2mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> 1st part with solvent - 4x Na₂CO₃ (10%, 10 Vol) to 25% sol. in MIBE - Water (10 Vol) 2nd part without solvent - 4x Na₂CO₃ (10%, 10 Vol) - Water (10 Vol) at 50 °C - Active charcoal filtration + rinse with MIBE	Result - Is phase separation acceptable when TARI-01 is diluted in MIBE and no NaCl is used? - Is possible to improve the phase separation without solvent by increasing the temperature? <u>Reaction</u> - yellow reaction mixture - 9 mL of caprylic acid in D.S apparatus, no water - The set up is not vacuum tight → increased air in the gas flow <u>Workup:</u> - Foam development strongly reduced when solvent in present - Without solvent, phase are not clear even if the water washing is carried out at 50 °C <u>Isolation:</u> - Colourless liquid obtained Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.49 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 1.6 %a - TARI-01: RT 25.08: 94.6 %a - Tetraester: RT 31.79 + 31.92: 0.57 + 1.3 %a <u>Problems:</u> - Apparatus not tight resulted in removal of caprylic acid / additional water in the reaction mixture → incomplete conversion - Addition of glycerine from above resulted in increased tetra esters formation - Workup without solvent does not yield clear phases even if performed at 50 °C <u>Next attempt:</u> - Is a workup in portions a good solution to avoid the foaming and ensure a quick phase separation without using NaCl? - Will the use of NaHCO₃ improve the MCPD / glycidol removal efficacy of the work up?
EXP-23-AA8211	<u>Raws:</u> - Glycerol: 10.1 g, Verbio - Octanoic acid: 65.6 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 55 μL, 0.9 mol%, Merck <u>Reaction:</u> - 250 mbar, 107 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol added with dosing pump (3mL/h) above reaction mixture	<u>Reaction</u> - Almost colorless reaction mixture - 5 mL of water in D.S apparatus, 0.5 mL octanoic acid - The set up is vacuum tight and reliable <u>Workup:</u> - No foam development upon dosing of reaction mixture due to MIBE presence (low surface tension) - Clear and fast phase separation in all the washings <u>Isolation:</u> - Colourless liquid obtained Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d.

Exp No	Short description	Result
	- Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - 10 VolGly MIBE (80g) and 10 VolGly NaHCO₃ (100g) pre added in the reactor - the crude mixture is added slowly under stirring in 3x portions - NaHCO₃ phase is discarded and added fresh after each washing (total 4x) - 3x washing with only water - Active charcoal filtration	- 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.67 %a - TARI-01: RT 25.08: 95.3 %a - Tetraester: RT 31.79 + 31.92: 0.03+0.08 %a MCPD analysis of product before Celite treatment: 3-MCPD: 888 ppb 2-MCPD: 205 ppb Glycidol: 1690 ppb MCPD analysis of product after active charcoal treatment 3-MCPD: 903 ppb 2-MCPD: 210 ppb Glycidol: 1439 ppb Charcoal treatment resulted in a glycidol decrease of 15%; no effect on MCPD levels MCPD analysis of previous sample after overnight celite treatment 3-MCPD: 877 ppb 2-MCPD: 207 ppb Glycidol: 1276 ppb Celite treatment resulted in a glycidol decrease of 11% The purity of all three samples was analysed: comparable; no increase of any other GC visible impurity. <u>Problems:</u> - Reaction run smoothly - Reversed workup run well - Use of NaHCO₃ resulted in increased MCPD values <u>Next questions:</u> <u>Reaction:</u> - Can the amount of acid be reduced to 0,5%mol? → see EXP-23-AA6324 - is it possible to further reduce the reaction temperature to 95 °C? - With glycerin dosing above the reaction mixture, is it possible to control tetra ester formation with low temperature and/or slow addition and/or low acid catalyst? <u>Workup:</u> - Is it possible to reduce the MIBE amount in the portioned workup to 1gMIBE/gTARI-01? (Currently is 1.4 g/gTARI) → see EXP-23-AA6325 <u>Isolation:</u> - Is it possible to remove glycidol via distillation and/or celite filtration (see EXP-23-AA8211 and EXP-23-AA6315) and/or treatment with ion exchange resins?
EXP-23-AA6324	<u>Raws:</u> - Glycerol: 9.76 g, Verbio - Octanoic acid: 65.8 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 27 µL, 0.45	<u>Reaction</u> - yellowish reaction mixture - 7 mL octanoic acid collected in D.S apparatus <u>Workup:</u> - No foam development

Exp No	Short description	Result
	mol%, Donauchem <u>Reaction:</u> - 250 mbar, 105 °C, N2 stream (6 NL/h) below reaction surface - Glycerol added with dosing pump (3 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - 10 VolGly MBE (80g) and 10 VolGly Na₂CO₃ (100g) pre added in the reactor - the crude mixture is added slowly under stirring in 3x portions - Na₂CO₃ phase is discarded and added fresh after each washing (total 4x) - 5x washing with only water (50g) - Active charcoal filtration	- Clear and fast phase separation in all the washings (1-2 mins) <u>Isolation:</u> - 47 g (94%) slightly yellow liquid obtained Purity analysis: - Monooctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: n.d. - TARI-01: RT 25.08: 97.6 %a - Tetraester: RT 31.79 + 31.92: 0.5+1.3 %a MCPD analysis of product: 3-MCPD: 385 ppb 2-MCPD: 107 ppb Glycidol: 4500 ppb <u>Problems:</u> - Again, high levels of tetraester (glycerol was charged above) - Good reaction progress although less acid was used - Will be discussed when MCPD and glycidol analysis will be available
EXP-23-AA6325	<u>Raws:</u> - Glycerol: 9.76 g, Verbio - Octanoic acid: 66.1 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 55 µL, 0.9 mol%, Donauchem <u>Reaction:</u> - 250 mbar, 115 °C, N2 stream (6 NL/h) below reaction surface - Glycerol added with dosing pump (3 mL/h) below reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C	<u>Reaction:</u> - yellowish reaction mixture - 6.8 mL of water collected, 1 mL octanoic acid <u>Workup:</u> - No foam development - Clear and fast phase separation in all the washings (1-2 mins) <u>Isolation:</u> - 51.1 g (102%) Colourless liquid obtained Purity analysis: - Monooctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.13 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.49 %a - TARI-01: RT 25.08: 98.02 %a - Tetraester: RT 31.79 + 31.92: 0.06+0.14 %a MCPD analysis of product:

Exp No	Short description	Result
	- No heat gun for head of reactor used <u>Workup:</u> - 5 VolGly MtBE (50g) and 10 VolGly NaHCO₃ (100g) pre added in the reactor - the crude mixture is added slowly under stirring in 3x portions - NaHCO₃ phase is discarded and added fresh after each washing (total 5x) - 4x washing with water (100g) until pH <7 - Active charcoal filtration + rinse with MtBE	- 3-MCPD: 361 ppb - 2-MCPD: 110 ppb - Glycidol: 4335 ppb <u>Problems:</u> - Without heat gun, water condensate in the reaction mixture. Set up was more intensely thermally isolated. - Aqueous washings with 10% NaHCO₃ do not efficiently remove the octanoic acid from the product (acid value 3.71 – Limit is 0.2 mg KOH/mg) <u>Next steps:</u> - use the half of the acid catalyst (0.05%mol) to verify effect on glycidol formation - use of carbonate instead of bicarbonate
EXP-23-AA6326	<u>Raws:</u> - Glycerol: 10.4 g, Verbio - Octanoic acid: 66.0 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 27.3 µL, 0.45 mol%, Donauchem <u>Reaction:</u> - 250 mbar, 115 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol added with dosing pump (3 mL/h) below reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C - No heatgun for head of reactor used <u>Workup:</u> - 7 VolGly MBE (70g) and 10 VolGly 10% Na₂CO₃ (100g) pre added - the crude mixture is added	<u>Reaction:</u> - Yellow reaction mixture - Water condensate in the reactor <u>Workup:</u> - First phase separation in 3 minutes <u>Isolation:</u> - 51.3 g (96%) Colorless liquid obtained Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.04 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.08 %a - TARI-01: RT 25.08: 98.47 %a - Tetraester: RT 31.79 + 31.92: 0.07+0.15 %a MCPD analysis of product: - 3-MCPD: 327 ppb - 2-MCPD: 133 ppb - Glycidol: 6776 ppb <u>Problems:</u> - Water condensate on the reactor wall <u>Next steps:</u> - Can tetra ester formation be reduced via dilution of glycerin in water? - Can glycidol and MCPD esters be reduced via bleaching earth treatment / steam distillation / ion exchange resins?

Exp No	Short description	Result
EXP-23-AA8212	slowly under stirring in 3x portions - Na₂CO₃ phase is discarded and added fresh after each washing (total 4x) - 5x washing with water (100g) until pH <7 - Active charcoal filtration + rinse with MIBE <u>Raws:</u> - Glycerol: 10.24 g, Verbio - Octanoic acid: 65.6 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 55 µL, 0.9 mol%, Donauchem <u>Reaction:</u> - 250 mbar, 95 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol added with dosing pump (1 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - 7 VolGly MIBE (70g) and 10 VolGly 10% NaHCO₃ (~100g) pre added - the crude mixture is added slowly under stirring in 3x portions - aqueous phase is discarded and added fresh after each washing (total 4x) - 4x washing with water (100g) until pH <7 - Active charcoal filtration + rinse with MIBE	<u>Reaction:</u> - very pale-yellow reaction mixture <u>Workup:</u> - phase separation within minutes <u>Isolation:</u> - 50.3 g (97%) colourless liquid obtained - Acid value off spec (1.52 mg KOH/g) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.06 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.72 %a - TARI-01: RT 25.08: 95.45 %a - Tetraester: RT 31.79 + 31.92: 0.02+0.05 %a Tetraesters level are very low with a slow addition from above MCPD analysis of product: - 3-MCPD: 172 ppb - 2-MCPD: 40 ppb - Glycidol: 1048 ppb MCPD value are consistent with other reactions, glycidol is low (despite still being off spec) <u>Problems:</u> - Sodium bicarbonate does not promptly remove the octanoic acid excess, resulting in off spec material for acid value <u>Next steps:</u> - Fine-tune the addition of glycerine from above - More focus on the removal of MCPD and glycidol

Exp No	Short description	Result
EXP-23-AA8213	First Recrystallization Experiment TARI-01 (7.1 g from AA8211-1) was recrystallized at -5 °C in MeOH. This experiment was carried out to increase purity as well as to assess feasibility of this technique for glycidol removal.	Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.016 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.045 %a - TARI-01: RT 25.08: 99.44 %a - Tetraester: RT 31.79 + 31.92: 0.01 %a MCPD analysis of product on small scale: - 3-MCPD: <100 (26) ppb - 2-MCPD: <100 (3) ppb - Glycidol: <100 (68) ppb The recrystallization allows to increase the purity of the product. Problem: - Yield is low (2.2 g, 31%)
EXP-23-AA8214	Raws: - Glycerol: 10.18 g, Verbio - Water: 12.41 g - Octanoic acid: 66.0 g, 4.2 mol-eq., ThermoScientific - H₂SO₄-conc.: 27 µL, 0.45 mol%, Donauchem Reaction: - 500 mbar, 100 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (6 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C Workup: - 3.5 VolGly MIBE (35g) and 10 VolGly 10% Na₂CO₃ (100g) pre added - the crude mixture is added slowly under stirring in 3x portions - Na₂CO₃ phase is discarded and added fresh after each washing (total 6x)	Reaction: - Water condensate in the reactor but D. S stark apparatus also full. Workup: - Water washings long and not clear phase separation Isolation: - Colorless liquid obtained, yield not available due to IPC taken Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.22 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.49 %a - TARI-01: RT 25.08: 98.29 %a - Tetraester: RT 31.79 + 31.92: 0.05 + 0.11 %a MCPD analysis of product: - 3-MCPD: 260 ppb - 2-MCPD: <100 (84) ppb - Glycidol: 1858 ppb Problems: - Decrease solvent lead to problem in water washings Next steps: - Can glycidol and MCPD esters be reduced via bleaching earth treatment / steam distillation / ion exchange resins?

Exp No	Short description	Result
	- 4x washing with water (100g) until pH <7 - Active charcoal filtration + rinse with MtBE	
EXP-23-AA8215	Further Recrystallization Experiments TARI-01 (8.24 g from AA8212) was recrystallized at -10 °C in MeOH via addition of 50 mg of seed crystals (EXP-23-AA8210)	Yield: 5.36 g (65%) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d - 1,3-Dioctanoyl Glycerol: RT 19.90: n.d - TARI-01: RT 25.08: 98.65 %a - Tetraester: RT 31.79 + 31.92: n.d MCPD analysis of product before - 3-MCPD: 172 ppb - 2-MCPD: 40 ppb - Glycidol: 1048 ppb MCPD analysis of product after: - 3-MCPD: <100 (n.d.) ppb - 2-MCPD: <100 (n.d.) ppb - Glycidol: <100 (73) ppb Yield was increased; No smell detected from recrystallized samples Next Steps: Further experiments aimed to increase final yield are in progress.
EXP-23-AA6328	Raws: - Glycerol: 10.18 g, Verbio - Water: 10.41 g - Octanoic acid: 65.8 g, 4.2 mol-eq., ThermoScientific - H ₂ SO ₄ -conc.: 55 µL, 0.9 mol%, Donauchem Reaction: - 500 mbar, 100 °C, N ₂ stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (6 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C	Reaction: - pale yellow reaction mixture Workup: - Water washings long and not clear phase separation Isolation: - 49.8 g (96%) Colorless liquid obtained Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.13 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.29 %a - TARI-01: RT 25.08: 98.59 %a - Tetraester: RT 31.79 + 31.92: 0.03 + 0.08 %a MCPD analysis of product: - 3-MCPD: 282 ppb - 2-MCPD: 87 ppb - Glycidol: 1610 ppb

Exp No	Short description	Result			
	<u>Workup:</u> - 3.5 VolGly MIBE (35g) and 10 VolGly 10% Na ₂ CO ₃ (100g) pre added - the crude mixture is added slowly under stirring in 3x portions - Na ₂ CO ₃ phase is discarded and added fresh after each washing (total 4x) - 3x washing with water (100g) until pH <7 - Active charcoal filtration + rinse with MIBE	<u>Problems:</u> - Decrease solvent lead to problem in water washings <u>Next steps:</u> - Can glycidol and MCPD esters be reduced via bleaching earth treatment / steam distillation / ion exchange resins?			
EXP-23-AA4115	TARI-01 in MIBE (1:1) was washed with different basic washing solutions (NaHCO ₃ , K ₂ CO ₃ , KHCO ₃) at 40 °C, extracted and analyzed after solvent evaporation. Treatment with Celite and activated charcoal at 110 °C was tested as well. The effect of these treatments on the MCPD and glycidol level as well as on the purity of the samples was evaluated.		3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]
		Starting Values (EXP-23-AA2362)	184	66	1040
		10% K ₂ CO ₃ (EXP-23-AA4115-1)	204	75	522
		10% KHCO ₃ (EXP-23-AA4115-2)	196	73	574
		10% NaHCO ₃ (EXP-23-AA4115-3)	197	71	558
		Celite + Charcoal @ 110 °C overnight (EXP-23-AA4115-4)	169	64	89
Treatment with Celite and charcoal at increased temperature strongly reduce levels of glycidol. MCPD content remain almost unchanged.					
		Sum of Diesters [%a]	TARI [%a]	Sum of Tetraesters [%a]	
Starting Values (EXP-23-AA2362)		0.36 + 0.79	97.72	0.04 + 0.10	
10% K ₂ CO ₃ (EXP-23-AA4115-1)		0.37 + 0.84	97.67	0.04 + 0.11	

Exp No	Short description	Result				
		10% KHCO3 (EXP-23-AA4115-2)	0.37 + 0.83	97.67	0.04 + 0.11	
		10% NaHCO3 (EXP-23-AA4115-3)	0.37 + 0.83	97.67	0.04 + 0.11	
		Celite + Charcoal @ 110 °C overnight (EXP-23-AA4115-4)	0.33 + 0.69	97.89	0.04 + 0.11	
		Treatment with Celite and charcoal at increased temperature slightly reduces the amount of diesters. No impact on the amount of tetraesters and comparable purity of TARI-01"				
EXP-23-AA6329	A sample of TARI-01 was subjected to steam distillation. 51.1 g TARI-01 were treated with 500 mL water. Temperature of the steam was 150 °C Temperature of the TARI sample was set to 130 °C. Flow of steam calculated from the water was ~10mL/min	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]		
		Starting Values (EXP- 23-AA2362)	184	66	1040	
		Steam distillation (EXP-23-AA6329-2)	195	78	142	
		The steam distillation allows the removal of glycidol esters but not of MCPD. After the treatment, 49.4 g were collected (97%)				
		Sumo of Diesters [%a]	TARI [%a]	Sum of Tetraesters [%a]		
		Starting Values (EXP-23-AA2362)	0.36 + 0.79	97.72	0.04 + 0.10	
		Steam distillation (EXP-23-AA6329-1)	0.42 + 0.87	97.59	0.04 + 0.10	
		The treatment has little impact on the quality of the material.				
EXP-23-AA3252	"Samples of TARI have been treated with different ion exchange resins over weekend at room temperature: - Amberlite IRA 900 Chloride	3-MCPD [ppb]	2- MCPD [ppb]	Glycidol [ppb]		
		Starting Values (EXP-23-AA8212)	172	40	1048	

Exp No	Short description	Result																			
	- Amberlite IRA 402 Chloride"	Amberlite IRA 900 (EXP-23-AA3252-1)	203	54	206																
	- Amberlite IRC 120 H Content of MCPD and glycidol was then determined.	Amberlite IRA 402 (EXP-23-AA3252-2)	185	50	225																
		Amberlite IRC 120 H (EXP-23-AA3252-3)	217	58	1347																
		"The use of anion exchange resin reduces the amount of glycidol but is accompanied by a slight increase in the MCPD species. The cation exchange resin does not appear to be a suitable candidate for the removal of these impurities. <u>Next Steps:</u> - Evaluation of the performances of different bleaching earth in the removal of MCPD and glycidol under different conditions, taking into account suitability for production. - Fine tune crystallization conditions"																			
EXP-23-AA3255	Use of short path evaporator to reduce glycidol / MCPD content. Jacket temperature = 130 °C, 4 mbar	MCPD analysis of product before - 3-MCPD: 184 ppb - 2-MCPD: 66 ppb - Glycidol: 1040 ppb MCPD analysis of product after - 3-MCPD: 167 ppb - 2-MCPD: <100 (59) ppb - Glycidol: 4217 ppb <u>Conclusions:</u> This technique is not suitable for the removal of these impurities as their amount increases during the treatment.																			
EXP-23-AA3256	Treatment of TARI samples with ion exchange resins at 70 °C. The resins used are: - Amberlite IRA 900 Chloride - Amberlite IRA 402 Chloride - Amberlite IRC 120 H	<table><thead><tr><th></th><th>3-MCPD [ppb]</th><th>2-MCPD [ppb]</th><th>Glycidol [ppb]</th></tr></thead><tbody><tr><td>EXP-23-AA8208</td><td>193</td><td><100 (85)</td><td>11123</td></tr><tr><td>Amberlite IRA 900 (EXP-23-AA3256-1)</td><td>201</td><td><100 (92)</td><td>3398</td></tr><tr><td>Amberlite IRA 402 (EXP-23-AA3256-2)</td><td>193</td><td>100</td><td>2795</td></tr></tbody></table>					3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]	EXP-23-AA8208	193	<100 (85)	11123	Amberlite IRA 900 (EXP-23-AA3256-1)	201	<100 (92)	3398	Amberlite IRA 402 (EXP-23-AA3256-2)	193	100	2795
	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]																		
EXP-23-AA8208	193	<100 (85)	11123																		
Amberlite IRA 900 (EXP-23-AA3256-1)	201	<100 (92)	3398																		
Amberlite IRA 402 (EXP-23-AA3256-2)	193	100	2795																		

Exp No	Short description	Result				
		<table><tr><td>Amberlite IRC 120 H (EXP-23-AA3256-3)</td><td>199</td><td>115</td><td>3008</td></tr></table> <i>Conclusions:</i> Despite the amount of glycidol decreases in all the experiments, the amount of 3-MCPD and 2-MCPD remains constant. Overall, the treatment of TARI-01 using ion exchange resins at increased temperature does not appear to be a valuable option.	Amberlite IRC 120 H (EXP-23-AA3256-3)	199	115	3008
Amberlite IRC 120 H (EXP-23-AA3256-3)	199	115	3008			
EXP-23-AA8216	<i>Further crystallization experiments</i> TARI-01 (8.41 g from AA8206) was recrystallized at -10 °C in MeOH via addition of 100 mg seed crystals (EXP-23-AA8211-3)	Yield: 6.01 g (71.5%) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: n.d. - TARI-01: RT 25.08: 99.74 %a - Tetraester: RT 31.79 + 31.92: 0.03 %a MCPD analysis of product before (EXP-23-AA8206) - 3-MCPD: 165 ppb - 2-MCPD: 50 ppb Glycidol: 31400 ppb MCPD analysis of product after: - 3-MCPD: <100 (10) ppb - 2-MCPD: <100 (n.d.) ppb - Glycidol: 1847 ppb Product was obtained as a colorless, odorless oil. <i>Conclusions:</i> The recrystallization can remove effectively glycidol, even if the starting value is in the ppm range. The content of mono-, di-, and tetraesters is extremely low. Yield of first recrystallization: 91.6% Yield of second recrystallization: 96.5% Purity analysis of first recrystallized product: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: n.d. - TARI-01: RT 25.08: 99.21 %a - Tetraester: RT 31.79 + 31.92: 0.04 %a Purity analysis of second recrystallized product: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: n.d. - TARI-01: RT 25.08: 99.35 %a				
EXP-23-AA8217	Recrystallization of TARI-01 (13 g) from EXP- 23 AA2362 from MeOH at -10C using 140 mg of seeds from (AA8211-3), adding 25% water to improve yield.					

Exp No	Short description	Result																								
		- Tetraester: RT 31.79 + 31.92: 0.02 %a MCPD analysis of product before (EXP-23-AA2362) - 3-MCPD: 184 ppb - 2-MCPD: 66 ppb - Glycidol: 1040 ppb MCPD analysis of product after first recrystallization: - 3-MCPD: <100 (19) ppb - 2-MCPD: <100 (n.d.) ppb - Glycidol: 188 ppb MCPD analysis of product after second recrystallization: 3-MCPD: <100 (8) ppb 2-MCPD: <100 (n.d.) ppb Glycidol: <100 (90) ppb <u>Conclusions:</u> Purification by recrystallization allow to achieve very high level of purity and very low level of MCPD / glycidol. These values are reduced by around 50 % when the material is recrystallized twice. The yield of the process could be increase to more than 90 °C by using water as antisolvent.																								
EXP-23-AA6323	The effect of different bleaching earth has been tested. A sample of TARI-01 in MiBE (5 Vol) was stirred overnight under inert atmosphere in presence of the adsorbent (2 weight equivalent). After filtration and solvent removal, the sample were analysed for MCPD / glycidol content.	<table><tr><th>@ RT</th><th>3-MCPD [ppb]</th><th>2-MCPD [ppb]</th><th>Glycidol [ppb]</th></tr><tr><td>Start material (EXP-23-AA8209)</td><td>260</td><td>68</td><td>1044</td></tr><tr><td>Bentonite</td><td>266</td><td><100 (62)</td><td>574</td></tr><tr><td>Zeolite</td><td>247</td><td><100 (65)</td><td>2601</td></tr><tr><td>Fullers Earth</td><td>254</td><td><100 (68)</td><td>1299</td></tr><tr><td>Mol. Sieves 12X</td><td>248</td><td><100 (61)</td><td>1035</td></tr></table> <u>Conclusions:</u> The only adsorbent that resulted in a decrease of glycidol was bentonite. In this case 3- and 2-MCPD remained constant. The other adsorbent did not increase the product quality, and in the case of zeolite, an increase of glycidol was observed.	@ RT	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]	Start material (EXP-23-AA8209)	260	68	1044	Bentonite	266	<100 (62)	574	Zeolite	247	<100 (65)	2601	Fullers Earth	254	<100 (68)	1299	Mol. Sieves 12X	248	<100 (61)	1035
@ RT	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]																							
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Mol. Sieves 12X	248	<100 (61)	1035																							
EXP-23-AA6331	The effect of different bleaching earth at increased temperature (120 °C) and vacuum (30 mbar) have been tested. A sample of concentrated TARI-01 was treated for 1 hour with	<table><tr><th>@ 120 °C / 30 mbar</th><th>3-MCPD [ppb]</th><th>2-MCPD [ppb]</th><th>Glycidol [ppb]</th></tr><tr><td>Start material (EXP-23-AA8209)</td><td>260</td><td>68</td><td>1044</td></tr><tr><td>Bentonite (12.5%w)+ Active charcoal</td><td>326</td><td><100 (87)</td><td>306</td></tr></table>	@ 120 °C / 30 mbar	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]	Start material (EXP-23-AA8209)	260	68	1044	Bentonite (12.5%w)+ Active charcoal	326	<100 (87)	306												
@ 120 °C / 30 mbar	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]																							
Start material (EXP-23-AA8209)	260	68	1044																							
Bentonite (12.5%w)+ Active charcoal	326	<100 (87)	306																							

Exp No	Short description	Result																																
	different adsorbent in presence of 7%w activated charcoal.	<table><tr><td>Zeolite (6%w) + Active charcoal</td><td>340</td><td><100 (87)</td><td>640</td></tr><tr><td>Fullers Earth (6%w) + Active charcoal</td><td>346</td><td><100 (89)</td><td>187</td></tr><tr><td>Mol. Sieves 12X (9%w)+ Active charcoal</td><td>283</td><td><100 (68)</td><td>317</td></tr></table>	Zeolite (6%w) + Active charcoal	340	<100 (87)	640	Fullers Earth (6%w) + Active charcoal	346	<100 (89)	187	Mol. Sieves 12X (9%w)+ Active charcoal	283	<100 (68)	317																				
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		Mol. Sieves 12X (9%w)+ Active charcoal	283	<100 (68)	317																													
<u>Conclusions:</u> In general, this treatment was accompanied by a decrease of glycidol amount, with the Fullers earth performing the best. A slight increase in the formation of 2-MCPD and a more marked increase in 3-MCPD was observed as well. Due to this fact, this treatment as well is not sufficient to achieve material of the desired quality."																																		
EXP23-AA6330	Replacing sulfuric acid by phosphoric acid (85%) as the catalyst for the esterification.	Yield: 13.0 g (27%) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: 0.7 %a - 1,2-Dioctanoyl Glycerol: RT 19.75: 24.9 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 52.6 %a - TARI-01: RT 25.08: 22.0 %a - Tetraester: RT 31.79 + 31.92: n.d. MCPD analysis of product: - 3-MCPD: 421 ppb - 2-MCPD: 158 ppb - Glycidol: 412 ppb Product was obtained as a yellow oil. <u>Conclusions:</u> Phosphoric acid performs much worse than sulfuric acid as the catalyst for this esterification reaction.																																
EXP23-AA5430	The effect of activated carbon filters was tested. Solutions of TARI-01, either undiluted or diluted with 50 wt% MtBE were filtered through various activated carbon filters. - CA: mostly macropores (>25 nm) - PA: meso-macropores (1-25 nm) - PXS: micropores (<1 nm)	<table><tr><td></td><td>3-MCPD [ppb]</td><td>2- MCPD [ppb]</td><td>Glycidol [ppb]</td></tr><tr><td>Starting material (EXP-23-AA2366)</td><td>231</td><td><100 (78)</td><td>465</td></tr><tr><td>Carbofil CA (diluted)</td><td>196</td><td><100</td><td>215</td></tr><tr><td>Carbofil PA (diluted)</td><td>204</td><td><100</td><td>224</td></tr><tr><td>Carbofil PXS (diluted)</td><td>197</td><td><100</td><td>234</td></tr><tr><td>Carbofil CA</td><td>192</td><td><100</td><td><100</td></tr><tr><td>Carbofil PA</td><td>202</td><td><100</td><td>246</td></tr><tr><td>Carbofil PXS</td><td>195</td><td><100</td><td>285</td></tr></table>		3-MCPD [ppb]	2- MCPD [ppb]	Glycidol [ppb]	Starting material (EXP-23-AA2366)	231	<100 (78)	465	Carbofil CA (diluted)	196	<100	215	Carbofil PA (diluted)	204	<100	224	Carbofil PXS (diluted)	197	<100	234	Carbofil CA	192	<100	<100	Carbofil PA	202	<100	246	Carbofil PXS	195	<100	285
			3-MCPD [ppb]	2- MCPD [ppb]	Glycidol [ppb]																													
		Starting material (EXP-23-AA2366)	231	<100 (78)	465																													
		Carbofil CA (diluted)	196	<100	215																													
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Carbofil PXS (diluted)	197	<100	234																															
Carbofil CA	192	<100	<100																															
Carbofil PA	202	<100	246																															
Carbofil PXS	195	<100	285																															

Exp No	Short description	Result						
EXP23-AA8218	Crystallization of TARI-01 (8-13 g EXP-23 AA2362) from MeOH at 0 °C using approximately 140 mg of seeds from (AA8211- 3), then cooling to -10 °C and finally adding 10% water to improve yield.		Yield (%)	Purity (%)	3-MCPD [ppb]	2- MCPD [ppb]	Glycidol [ppb]	
		Starting material		97.7	184	<100 (66)	1040	
		As described	92.4	99.43	<100	<100	<100	
		2nd crystallization	93.1	99.57	<100 (14)	<100 (14)	<100 (3)	
		Directly from 10% water	90.2	99.17	<100 (50)	<100 (23)	146	
		Adding water before cooling to -10C	87.8	99.44	<100 (20)	<100 (21)	<100 (27)	
		Higher quality seeds	86.6	99.40	<100 (20)	<100 (17)	<100 (13)	
		Celite instead of seeds	87.8		117	<100	335	
Better quality seeds (AA8218-2):								
Seeds:	AA8211-3							
Purity:		95.3%	Purity:	99.57%				
3-MCPD:		877 ppb	3-MCPD:	<100 (14) ppb				
2-MCPD:		207 ppb	2-MCPD:	<100 (14) ppb				
Glycidol:		1276 ppb	Glycidol:	<100 (3) ppb				
EXP23-AA8219	Crystallization of TARI-01 (8-13 g) from EXP- 23 AA2362 from various solvents at 0 °C using approximately 140 mg of seeds from (AA8218- 2), then cooling to -10 °C and finally adding water to improve yield.		Yield (%)	3-MCPD [ppb]	2- MCPD [ppb]	Glycidol [ppb]		
		As in AA8218 with double the scale	92.6	<100	<100	<100		
		Using 15% water in MeOH	92.3	<100 (10)	<100 (n.d.)	<100 (30)		
		Using 25% water in Acetonitrile	80.2	<100 (n.d.)	<100 (n.d.)	<100 (49)		
		Using 25% water in EtOH	87.0	<100 (n.d.)	<100 (n.d.)	<100 (61)		
		Better quality seeds (AA8218-2):						

Exp No	Short description	Result
EXP-23-AA6332	<u>Raws:</u> - Glycerol: 10.1 g, Verbio - Water: 10 g - Octanoic acid: 65.8 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%, Donauchem - 500 mbar, 100 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (10 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - MBE (70 g) and 10% Na₂CO₃ (100g) pre added - the crude mixture is added slowly under stirring in 3x portions - Na₂CO₃ phase is discarded and added fresh after each washing (total 5x) - 3x washing with only water (100g) at 45 °C until pH <7 - Active charcoal filtration + rinse with MBE	<u>Reaction:</u> - Yellow reaction mixture <u>Workup:</u> - Water washings at 45 °C between 5 and 7 minutes <u>Isolation:</u> - 46.0 g (89%) colourless liquid obtained (4 IPCs taken) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: 0.02 %a - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.45 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 1.11 %a - TARI-01: RT 25.08: 94.63 %a - Tetraester: RT 31.79 + 31.92: 0.07 + 0.16 %a MCPD analysis of product: - 3-MCPD: <100 ppb - 2-MCPD: <100 ppb - Glycidol: 5071 ppb Acid Value: 0.26mg KOH/g <u>Next steps:</u> - Repetition standard procedure with recrystallization
EXP-23-AA6334	<u>Raws:</u> - Glycerol: 10.2 g, Verbio - Water: 10 g - Octanoic acid: 66.1 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%, Donauchem <u>Reaction:</u>	<u>Reaction:</u> - pale yellow reaction mixture <u>Workup:</u> - all washings within 2 minutes <u>Isolation:</u> - 49.8 g (96%) colorless liquid obtained (1x IPC taken) Purity analysis (IPC): - Monoctanoyl Glycerol: RT 12.20: 0.01 %a

Exp No	Short description	Result																
	- 500 mbar, 100 °C, N2 stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (6 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - MtBE (70 g) and 10% Na₂CO₃ (100g) pre added - the crude mixture is added slowly under stirring in 3x portions - Na₂CO₃ phase is discarded and added fresh after each washing (total 5x) - 5x washing with only water (100g) until pH <7 - Active charcoal filtration + rinse with MtBE	- 1,2-Dioctanoyl Glycerol: RT 19.75: 0.51 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: n.d - TARI-01: RT 25.08: 98.09 %a - Tetraester: RT 31.79 + 31.92: 0.03 + 0.07 %a MCPD analysis of product after recrystallization (EXP-23-AA8221): - 3-MCPD: <100 (n.d.) ppb - 2-MCPD: <100 (n.d.) ppb - Glycidol: <100 (n.d.) ppb Yield recrystallization: 94% (45.9 g)																
EXP-23-AA5433	"The effect of activated carbon filters on MCPD / glycidol removal was tested. TARI-01 was filtered through various activated carbon filters at 70 °C and a slow rate (~2g / min) - CA: mostly macropores (>25 nm) - PA: meso-macropores (1-25 nm) - PXS: micropores (<1 nm)"	<table><thead><tr><th></th><th>3-MCPD [ppb]</th><th>2-MCPD [ppb]</th><th>Glycidol [ppb]</th></tr></thead><tbody><tr><td>Carbofil CA (starting values from EXP-23-AA2370)</td><td>177 (197)</td><td><100 (58) (<100 (56))</td><td>354 (1092)</td></tr><tr><td>Carbofil PA (starting values from from EXP-23-AA2370)</td><td>168</td><td><100 (62)</td><td>295</td></tr><tr><td>Carbofil PXS (starting values from from EXP-23-AA2368)</td><td>208 (235)</td><td><100 (60) (<100 (68))</td><td>278 (892)</td></tr></tbody></table> MCPD remains unchanged, glycidol is removed to ~300 ppb <u>Workup:</u> - all washings within 2 minutes <u>Isolation:</u>		3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]	Carbofil CA (starting values from EXP-23-AA2370)	177 (197)	<100 (58) (<100 (56))	354 (1092)	Carbofil PA (starting values from from EXP-23-AA2370)	168	<100 (62)	295	Carbofil PXS (starting values from from EXP-23-AA2368)	208 (235)	<100 (60) (<100 (68))	278 (892)
	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]															
Carbofil CA (starting values from EXP-23-AA2370)	177 (197)	<100 (58) (<100 (56))	354 (1092)															
Carbofil PA (starting values from from EXP-23-AA2370)	168	<100 (62)	295															
Carbofil PXS (starting values from from EXP-23-AA2368)	208 (235)	<100 (60) (<100 (68))	278 (892)															
EXP-23-AA3527	<u>Raws:</u> - Glycerol: 10.2 g, Verbio - Water: 8 g - Octanoic acid: 66 g, 4.2 mol-																	

Exp No	Short description	Result
	eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%, Donauchem Reaction: - 500 mbar, 100 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (6 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - MIBE (70 g) and 10% Na₂CO₃ (100g) pre-added - the crude mixture is added slowly under stirring in 3x portions at 40 °C - Na₂CO₃ phase is discarded and added fresh after each washing (total 4x) - 5x washing with only water (100g) until pH <7 - Active charcoal filtration + rinse with MIBE	- 50.26 g (98%) colorless liquid obtained (1x IPC taken) Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.36 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.74 %a - TARI-01: RT 25.08: 97.64 %a - Tetraester: RT 31.79 + 31.92: 0.06 + 0.16 %a MCPD analysis of product: 3-MCPD: <100 (17) ppb 2-MCPD: <100 (n.d.) ppb Glycidol: 2236 ppb Purity analysis of crystallized sample: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: n.d. - TARI-01: RT 25.08: 99.65 %a - Tetraester: RT 31.79 + 31.92: n.d. MCPD analysis of product after recrystallization (EXP-23-AA8222): - 3-MCPD: <100 (n.d.) ppb - 2-MCPD: <100 (n.d.) ppb - Glycidol: <100 (n.d.) ppb Yield of recrystallization: 94%
EXP-23-AA6333	<i>Reaction in uncleaned reactor</i> <u>Raws:</u> - Glycerol: 10.1 g, Verbio - Water: 10 g - Octanoic acid: 66 g, 4.2 mol- eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%, Donauchem <u>Reaction:</u> - 500 mbar, 100 °C, N₂ stream (6 NL/h) below reaction surface	<u>Reaction:</u> - Yellow clear solution - 10 mL Water + 2 mL Octanoic acid <u>Workup:</u> - all washings in 3 minutes or less <u>Isolation:</u> - 51.1 g (99%) colorless liquid obtained Purity analysis: - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: 0.14 %a - 1,3-Dioctanoyl Glycerol: RT 19.90: 0.34 %a

Exp No	Short description	Result
	- Glycerol/water added with dosing pump (6 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - MIBE (70 g) and 10% Na₂CO₃ (100g) pre-added - the crude mixture is added slowly under stirring in 2x portions at RT - Na₂CO₃ phase is discarded and added fresh after each washing (total 4x) - 4x washing with only water (100g) until conductivity is <100 µS/cm - Active charcoal filtration + rinse with MIBE	- TARI-01: RT 25.08: 98.2 %a - Tetraester: RT 31.79 + 31.92: 0.04+0.1 %a MCPD analysis of product: - 3-MCPD: 149 ppb - 2-MCPD: <100 (43) ppb - Glycidol: 1326 ppb Purity analysis of crystallized sample (EXP-23-AA8223): - Monoctanoyl Glycerol: RT 12.20: n.d. - 1,2-Dioctanoyl Glycerol: RT 19.75: n.d. - 1,3-Dioctanoyl Glycerol: RT 19.90: n.d. - TARI-01: RT 25.08: 99.65 %a - Tetraester: RT 31.79 + 31.92: n.d. MCPD analysis of product after recrystallization (EXP-23-AA8223): - 3-MCPD: <100 (n.d.) ppb - 2-MCPD: <100 (n.d.) ppb - Glycidol: 126 ppb Yield of recrystallization: ... - Glycidol: <100 (64); analysis repeated: <100 (57) ppb Yield of recrystallization: 88.5 %a
EXP-23-AA6338	<u>Standard Experiment</u> <u>Raws:</u> - Glycerol: 10.1 g, Verbio - Water: 10 g - Octanoic acid: 66 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 55 µL, 0.9 mol%, Donauchem <u>Reaction:</u> - 500 mbar, 100 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (6 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - MIBE (70 g) and 10% Na₂CO₃	<u>Reaction:</u> - Yellow clear solution - 10 mL Water + 1 mL Octanoic acid <u>Workup:</u> - all washings within 2 minutes <u>Isolation:</u> - 46.2 g (90%) colorless liquid obtained Purity analysis: - Monoctanoyl Glycerol: RT 12.20: 0.02 %a - Dioctanoyl Glycerol: RT 19.75: 13.43%a - TARI-01: RT 25.08: 84.13 %a - Tetraester: RT 31.79 + 31.92: 0.06+0.02 %a MCPD analysis of product: - 3-MCPD: 217 ppb - 2-MCPD: <100 (71) ppb - Glycidol: 3499 ppb Purity analysis of crystallized sample (EXP-23-AA8230): - Monoctanoyl Glycerol: RT 12.20: 0.02 %a - Dioctanoyl Glycerol: RT 19.75: 13.4 %a

Exp No	Short description	Result
	(100g) pre- added - the crude mixture is added slowly under stirring in 2x portions at 40 °C - Na₂CO₃ phase is discarded and added fresh after each washing (total 4x) - washing with only water (100g) until conductivity is <100 µS/cm - Active charcoal filtration + rinse with MTBE	TARL-01: RT 25.08: 84.12%a Tetraester: RT 31.79 + 31.92: 0.05+0.02 %a MCPD analysis of product after recrystallization (EXP-23-AA8230): 3-MCPD [ppb]: n.d. 2-MCPD [ppb]: n.d. Glycidol [ppb]: 180 Yield of recrystallization: 76.2%
EXP-23-AA6339	<u>Standard Experiment Raws:</u> - Glycerol: 20.1 g, Verbio - Water: 20 g - Octanoic acid: 131.8 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 0.22g, 0.9 mol%, Donauchem <u>Reaction:</u> - 500 mbar, 100 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (12 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - MTBE (140 g) and 10% Na₂CO₃ (200 g) pre- added - the crude mixture is added slowly under stirring in 2x portions at 40 °C - Na₂CO₃ phase is discarded and added fresh after each washing (total 5x) - washing with only water (100 g) until conductivity is <100 µS/cm	<u>Reaction:</u> - Yellow clear solution - 25 mL Water + 2 mL Octanoic acid <u>Workup:</u> - all washings within 1 minutes <u>Isolation:</u> - 83.2 g (81%) colorless liquid obtained Purity analysis: - Monoctanoyl Glycerol: RT 12.20: 0.20 %a - Dioctanoyl Glycerol: RT 19.75: 27.83 %a TARL-01: RT 25.08: 70.85 %a Tetraester: RT 31.79 + 31.92: 0.02+0.04 %a MCPD analysis of product: - 3-MCPD: 3112 ppb - 2-MCPD: 1002 ppb - Glycidol: 2651 ppb Purity analysis of crystallized sample (EXP-23-AA8229): - Monoctanoyl Glycerol: RT 12.20: n.d. - Dioctanoyl Glycerol: RT 19.80: 0.05 %a TARL-01: RT 25.08: 99.6 %a Tetraester: RT 31.79 + 31.92: n.d. MCPD analysis of product after recrystallization (EXP-23-AA8229): - 3-MCPD: <100 (36) ppb; analysis repeated: <100 (34) ppb - 2-MCPD: <100 (n.d.) ppb - Glycidol: <100 (9) ppb; analysis repeated: <100 (13) ppb Yield of recrystallization: 66.0%a

Exp No	Short description	Result
	- Active charcoal filtration + rinse with MIBE	
EXP-23-AA6340	Standard Experiment <u>Raws:</u> - Glycerol: 20.1 g, Verbio - Water: 20 g - Octanoic acid: 132.1 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 0.22g, 0.9 mol%, Donauchem <u>Reaction:</u> - 500 mbar, 100 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (12 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C <u>Workup:</u> - MIBE (140 g) and 10% Na₂CO₃ (200 g) pre-added - the crude mixture is added slowly under stirring in 2x portions at 40 °C - Na₂CO₃ phase is discarded and added fresh after each washing (total 5x) - Washing with water (100 g) until conductivity is <100 µS/cm - Active charcoal filtration + rinse with MIBE	<u>Reaction:</u> - gold-colored clear solution - 27 mL Water + 1 mL Octanoic acid <u>Workup:</u> - all phase separation time within 1-2 minutes <u>Isolation:</u> - 99.1 g (97%) colorless liquid obtained <u>Purity analysis:</u> - Monooctanoyl Glycerol: RT 12.20: 0.02 %a - Dioctanoyl Glycerol: RT 19.75: 2.07 %a - TARI-01: RT 25.08: 96.66 %a - Tetraester: RT 31.79 + 31.92: 0.08+0.03 %a MCPD analysis of product: 3-MCPD: 718 ppb 2-MCPD: 198 ppb Glycidol: 1099 ppb (the analysis was repeated due to an error in the calculation; corrected value is 1370 ppb) Purity analysis of crystallized sample (EXP-23-AA8233): Monooctanoyl Glycerol: RT 12.20: n.d. Dioctanoyl Glycerol: RT 19.75: n.d. TARI-01: RT 25.08: 99.66 %a Tetraester: RT 31.79 + 31.92: n.d. MCPD analysis of product after recrystallization (EXP-23-AA8233): - 3-MCPD: <100 (29) ppb - 2-MCPD: <100 (n.d.) ppb - Glycidol: <100 (71) ppb; analysis repeated: <100 (46) ppb Yield of recrystallization: 92.5 %a
EXP-23-AA6341	Standard Experiment <u>Raws:</u>	<u>Reaction:</u> - gold-coloured clear solution

Exp No	Short description	Result
	- Glycerol: 10.1 g, Verbio - Water: 10 g - Octanoic acid: 65.8 g, 4.2 mol-eq., Metadynea - H₂SO₄-conc.: 0.55 µL, 0.9 mol%, Donauchem Reaction: - 500 mbar, 100 °C, N₂ stream (6 NL/h) below reaction surface - Glycerol/water added with dosing pump (6 mL/h) above reaction mixture - Dean-Stark apparatus used - Condenser temperature at 20 °C Workup: - MIBE (70 g) and 10% Na₂CO₃ (100 g) pre-added - the crude mixture is added slowly under stirring in 2x portions at 40 °C - Na₂CO₃ phase is discarded and added fresh after each washing (total 5x) - washing with only water (100 g) until conductivity is <100 µS/cm - Active charcoal filtration + rinse with MIBE	- 12 mL Water + 1 mL Octanoic acid Workup: - all phase separation time within 1 minute Isolation: - 49.9 g (98%) colorless liquid obtained Purity analysis: n.d. - Monoctanoyl Glycerol: RT 12.20: 0.22 % aa - Dioctanoyl Glycerol: RT 19.75: 98.76% aa - TARI-01: RT 25.08: 0.07 + 0.02% a - Tetraester: RT 31.79 + 31.92: MCPD analysis of product: - 3-MCPD: 173 ppb - 2-MCPD: <100 (60) ppb - Glycidol: 4141 ppb (the analysis was repeated due to an error in the calculation; corrected value is 3822 ppb) Purity analysis of crystallized sample (EXP-23-AA8232): - Monoctanoyl Glycerol: RT 12.20: n.d. - Dioctanoyl Glycerol: RT 19.90: n.d. - TARI-01: RT 25.08: 99.66 %a - Tetraester: RT 31.79 + 31.92: n.d. MCPD analysis of product after recrystallization (EXP-23-AA8232): - 3-MCPD: <100 (13) ppb; analysis repeated: n.d. - 2-MCPD: <100 (n.d.) ppb - Glycidol: 513 ppb; analysis repeated: 621 ppb Yield of recrystallization: 94.8 %a

EXAMPLE 2: Minimizing condensation of caprylic acid on the condenser

[0122] In the initial experiments (EXP-23-AA6314, EXP-23-AA6315, EXP-23-AA6317, EXP-23-AA6318, EXP-23-AA6319), an undesired condensation of the caprylic acid on the condenser between the reactor and the water receiving flash was observed. This phenomenon was considered critical not only because it reduced the amount of reagents available for the reaction, but also because it could lead to temporary blockage of the vacuum line (as in EXP-23-AA6317), reducing the efficacy of the water removal and leading to a potentially dangerous overpressure of the reactor caused by the nitrogen inlet. This observation was unexpected since the boiling point of the reagent at different pressure values (reported in Table 2) was always above the temperature of the reaction.

[0123] In the attempt to solve this issue, the addition of premixed Glycerol (glycerol) and octanoic acid were tested, (EXP-23-AA6320). This reduced the total residence time of the caprylic acid in the reactor. This approach did not avoid the condensation of the reagent on the condenser; additionally, it led to a biphasic mixture during addition and during the reaction, due to the low miscibility of these reagents.

[0124] Decreasing the nitrogen flow from 60 to 6 NL/h was not sufficient to resolve this problem (EXP-23-AA6320). Surprisingly using an apparatus with a longer tube, for example a Dean Stark apparatus instead of a Dimroth cooler (EXP-23-AA-8203), suggesting the importance of the apparatus geometry. Using a Dean Stark apparatus resulted in a longer way for the vapors to go before reaching the condenser; consequently, the caprylic acid recondensed in the reaction mixture before leaving the reactor to reach the condenser. Water, on the other hand, at the given values of temperature and pressure, is sufficiently volatile to be removed from the reaction mixture. In almost all the experiments where the Dean Stark apparatus was used, in addition to the water released from the reaction, the amounts of caprylic acid recollected were in the order of few mL, ensuring the excess of caprylic acid in the reactor. A representation of the apparatus used is reported in FIG 6.

EXAMPLE 3: Maximizing conversion to productMode of addition of glycerol (Glycerol): from above

[0125] If chemicals do not leave the reactor, the reaction can proceed to completion upon gradual formation of mono ester, di ester and ultimately of the product, glyceryl trioctanoate. Glycerol, the limiting reagent, was dosed from above into a stirred and heated mixture of octanoic

acid and sulfuric acid. The dosing speed under these conditions strongly influenced the tetraester formation; this impurity was quantified beyond the originally specified limit of 0.4% in almost all the experiments. Decreasing the catalyst loading to 0.5% is not sufficient to reach specification limit (see **Table 6**).

Table 6: Overview of results when pure glycerol is added from above the heated and stirred octanoic acid / sulfuric acid mixture.

Entry	Reaction name	Temperature [°C]	Dosing speed [mL/h]	H ₂ SO ₄ loading [%mol]	TRICAPRILIN Purity [%a]	Tetraesters [%a]
1	EXP-23-AA6315	130	2.6	1	87.01	1.05
2	EXP-23-AA6317	130	2.1	1	97.81	0.74
3	EXP-23-AA6314	115	8	1	75.36	6.53
4	EXP-23-AA6318	115	3	1	83.85	2.77
5	EXP-23-AA6319	115	1	1	95.92	1.05
6	EXP-23-AA6322	105	2	1	94.6	0.69
7	EXP-23-AA6324	105	3	0.5	96.19	1.85
8	EXP-23-AA8211	105	3	1	95.3	0.11
9	EXP-23-AA8212	95	1	1	95.45	0.07

[0126] The experiments where the tetra ester level was below the original specification of 0.4% are as listed in the last entries 8 and 9. In general, the head of the reactor in the lab scale requires additional heating due to the distance from the heated jacket. When this additional heating is absent, the water produced from the reaction is removed more slowly and the tetra ester formation is inhibited (EXP-23-AA8211). Additionally, if the reaction temperature is reduced to 95 °C and the addition speed of pure glycerol is reduced to 1 mL/h, the reaction proceeds with neglectable by-product formation product (EXP-23- AA8212).

Mode of addition of glycerol (Glycerol): from above

[0127] Addition of glycerol below the reaction surface unexpectedly resulted in an improved mixing with reaction components, resulting in a decreased tetraester formation. All the experiments carried out using such addition method resulted in tetraester levels below 0.5%;

Addition of glycerol directly in the reaction mixture resulted in an overall increase of the robustness of the reaction. Fast addition did not result in a strong by-product increase (EXP-23-AA8208); increasing the catalyst loading to 4.5 %mol resulted in a only moderate tetraester increase (EXP-23-AA8206) where lower catalyst loading had basically no apparent influence (EXP-23-AA6326). Temperature appears to be less critical as well: comparable results in terms of yield and purity have been obtained when the reaction was carried out at 115, 105 or 95 °C. Surprisingly, decreasing the reaction temperature from 115 °C to 105 °C with fast addition (EXP-23-AA8210) had a small but positive effect on the by-product formation and no influence on the conversion. Decreasing at the same time temperature and catalyst yield did not result in noticeable change in the purity profile and final yield of the product (EXP-23-AA6327); unexpectedly, in this case the tetraester detected in the final product was relatively high if compared with the other experiments in this series (**Table 7**).

Table 7: Overview of results when pure glycerol is added directly within the heated and stirred octanoic acid / sulfuric acid mixture.

Entry	Reaction name	Temperature [°C]	Dosing speed [mL/h]	H ₂ SO ₄ loading [%mol]	TRICAPRILIN Purity [%a]	Tetraesters [%a]
1	EXP-23-AA8208	115	8	1	98.4	0.17
2	EXP-23-AA8206	115	1	4.5	97.55	0.44
3	EXP-23-AA6325	115	3	1	98.02	0.20
4	EXP-23-AA6326	115	3	0,5	98.47	0.22
5	EXP-23-AA8210	105	8	1	98.24	0.15
6	EXP-23-AA6327	95	3	0.5	97.73	0.44

Addition mode of Glycerol: above but diluted

[0128] In order to allow the dosing from above but to keep the tetraesters level low, it was rationalized that decreasing the starting concentration of Glycerol would reduce the possibility of acid catalyzed ether formation. Hence, Glycerol was diluted with an equivalent amount of water and then added dosed to the reaction; the additional water would be removed just as the water

formed during the reaction to TRICAPRILIN. This approach resulted in a more controlled tetraester formation in all the experiments, as summarized in the table below.

[0129] Results suggest that tetraester formation can be minimized if the dosing speed is low (EXP-23-AA6328). Increasing the dosing speed (EXP-23-AA3527 or EXP-23-AA6332) or decreasing the catalyst loading (EXP-23-AA358214) do not result in an improve in the quality of the material. A non-exhaustive list of experiment with this dosing mode is summarized in **Table 8**.

Table 8: Non exhaustive overview of results after dosing the glycerol/water mixture

Entry	Experiment	Temperature [°C]	Dosing speed [mL/h]	H ₂ SO ₄ loading [%mol]	TRICAPRILIN Purity [%a]	Tetraesters [%a]
1	EXP-23-AA6328	100	3	1	98.59	0.11
2	EXP-23-AA3527	100	3.5	1	97.64	0.21
3	EXP-23-AA6332	100	5	1	94.64	0.24
4	EXP-23-AA8214	100	3	0.5	98.29	0.16

Use of other acid catalysts

[0130] Phosphoric acid (85%) was tested as an alternative to sulfuric acid used in other experiments. The conversion to the product was much slower and after almost 23 h of reaction at 100 °C and subsequent workup, analysis of isolated material showed 75%a of diesters and only 20% product (EXP-23-AA6330).

Table 9: Reaction outcome when using phosphoric acid instead of sulfuric acid.

Experiment	Temp. [°C]	Dosing speed [mL/h]	H ₃ PO ₄ loading [%mol]	Sum of Diester [%a]	TRICAPRILIN Purity[%a]	Tetraesters [%a]
EXP-23-AA6330	100	6	1	75	20	n.d.

[0131] Glycidol amount is influenced by the combination of different parameters such as the quality of the starting materials as well as the applied reaction conditions. In general, its formation requires harsh conditions, in order to form the strained 3-member ring of the epoxide. As observed

in a few experiments, a higher reaction temperature was associated with an increase in the glycidol levels (EXP-23-AA6319 and EXP-23-AA8206).

[0132] Additional considerations can be made: although milder conditions (EXP-23-AA8212) and slower glycerol addition (EXP-23-AA8209) mitigate its formation, glycidol is formed if the catalyst loading is increased, even if slow addition (EXP-23-AA8206) or increase temperature (EXP-23-AA6319 and EXP-23-AA8206) is applied. Decreasing the catalyst loading appears to have a beneficial effect (EXP-23-AA6322 and AA6324) even when the dosing speed was slightly increased.

[0133] Quite interestingly, mixing glycerol with water appears to be useful in reducing the glycidol formation and an increased dosing speed does not correlate to an increase in this side product (EXP-23-AA6328 and EXP-23-AA8214).

[0134] It might be worth to note that despite certain trend can be identified, these values are not always consistent due to the very low amount considered and the high sensitivity of the measure. These results are summarized in the following **Table 10**.

Table 10: Effect of reaction condition on glycidol formation

Experiment	Addition of Glycerol	Addition of Glycerol [mL/h]	Temperature [°C]	Catalyst loading [%mol]	Reaction Time	Glycidol [ppb]
EXP-23-AA6319	above	1	115	1	17 h	47700
EXP-23-AA8212	above	1	95	1	20 h	1048
EXP-23-AA6327	below	3	95	0.5	19 h	3506
EXP-23-AA6322	above	2	105	1	23 h	7677
EXP-23-AA6324	above	3	105	0.5	23 h	4500
EXP-23-AA8209	below	1.5	105	1	15 h	1044
EXP-23-AA8206	below	1	115	4.5	20 h	31400
EXP-23-AA6328	above, with water	3	100	1	19 h	1610
EXP-23-AA8214	above, with water	3	100	0.5	19 h	1858

EXAMPLE 4: Work-up procedure

[0135] During the workup, treatment of the reaction mixture with a basic aqueous solution allow to quench the acidity and wash the excess of octanoic acid by converting it to the more water-soluble sodium octanoate. Experiments were conducted to test various work-up procedures.

Different Base

[0136] In addition to the intensively used sodium carbonate, sodium bicarbonate was evaluated as well. NaOH was not tested due to the increased risk of saponification represented by treating an ester with a such reagent. Experimental results showed that natrium bicarbonate does not remove promptly the fatty acid excess, resulting in a very high acid value in the final product. **Table 11** provides a comparison between these two reagents.

Table 11: Removal of octanoic acid during the work up by sodium carbonate and bicarbonate (constant conditions).

Experiment	Organic phase	Base	Number of washings	Acid value [mg KOH/g]
EXP-23-AA6328	MtBE	Na ₂ CO ₃	4	0.16
EXP-23-AA8211	MtBE	NaHCO ₃	4	17.79

[0137] The effect of different basic washing solutions such as, K₂CO₃, KHCO₃ and NaHCO₃) at 40 °C in the glycidol and MCPD removal was evaluated as well. Results are summarized in **Table 12**.

Table 12: Effect of different bases on the MCPD and glycidol removal.

Experiment	Base	3-MCPD [ppb]	2- MCPD [ppb]	Glycidol [ppb]
EXP-23-AA2362	Starting Values	184	66	1040
EXP-23-AA4115-1	10% K ₂ CO ₃	204	75	522
EXP-23-AA4115-2	10% KHCO ₃	196	73	574
EXP-23-AA4115-3	10% NaHCO ₃	197	71	558

[0138] Different bases tested have no impact on the removal of MCPD and little impact on the glycidol amount. Considering the reduced efficacy of sodium bicarbonate in the removal of excess of octanoic acid during the work up, natrium carbonate was identified as base of choice.

Different solvents

[0139] Next, the organic solvent during the workup was investigated. The reaction outcome was split in two and the same procedure was applied for both the fractions. Toluene and heptane performances were compared and results are summarized in **Table 13**.

Table 13: Purity profile of TRICAPRILIN after workup with heptane or toluene.

Experiment	Organic phase	Monoester [%a]	Sum of Diester [%a]	TRICAPRILIN [%a]	Tetraester [%a]	MCPD	MCPD	Glycidol
EXP-23-AA6317	Heptane (100 g)	n.d.	0.09	97.81	0.76	4500	430	69600
	Toluene (100 g)	n.d.	0.15	97.73	0.76	4840	601	67800

[0140] No change in the purity profile of the sample was observed; it is therefore concluded that both of them are equally suitable.

No solvent

[0141] A workup was attempted without any additional organic solvent as well (EXP-23-AA6318). In this case, different washings solutions were tested: (a) very good phase separation within seconds was obtained using a mixture of saturated NaCl and 10 % Na₂CO₃. The high amount of salt contained ensured fast phase separation and clear phases; (b) The basic wash was followed by treating the reaction crude with a NaCl saturated aqueous solution. phase separation was very good and achieved within seconds; and (c) the organic residue was washed with only water. In this case, after 30 minutes the organic layer was still turbid and contained copious amount of water.

[0142] It was concluded that during the workup, if no organic solvent is present, water phase with a strong ionic strength (e.g., NaCl solution) is needed to achieve a satisfactory phase separation.

EXAMPLE 5: Refining

[0143] After workup, the material can be further processed to improve its appearance, odor or purity profile. Different materials and techniques were tested and evaluated.

Activated Charcoal

[0144] The activated charcoal treatment allows to remove impurities in the material, improving its appearance. Different types of active charcoal have been tested under different conditions. The results as summarized in **Table 14**.

Table 14: Summary of the different active charcoal filter used.

Experiment	Material	Condi tions	Tem p.	Amo unt proce ssed	Flow (ml/min)	3- MCPD [ppb]	2- MCPD [ppb]	Glyci dol [ppb]
EXP-23-AA2366	Starting Material					231	<100 (78)	465
EXP-23-AA5430	Carbofil CA	concent rated	RT	15 mL	3	192	<100	<100
		50% in MtBE	RT	15 mL	6	196	<100	215
	Carbofil PA	concent rated	RT	15 mL	3	202	<100	246
		50% in MtBE	RT	15 mL	6	204	<100	224
	Carbofil PXs	concent rated	RT	15 mL	3	195	<100	285
		50% in MtBE	RT	15 mL	6	197	<100	234
EXP-23-AA2370	Starting Material					197	<100 (56)	1092
EXP-23-AA5433	Carbofil CA	concent rated	70 °C	150 g	~4 g/min	177	<100 (58)	354
	Carbofil PA	concent rated	70 °C	150 g	~3 g/min	168	<100 (62)	295
	Carbofil PXS	concent rated	70 °C	150 g	~1.5 g/min	208	<100 (60)	278
EXP-23-AA3341	Starting Material					122	<100 (45)	1510 9
EXP-23-AA3343	BECODISC	concent rated	RT	7 mL	1 drop/sec	161 5	678	2914
	BECODISC	50% in MtBE	RT	14 mL	1 drop/sec	151 5	598	9219

[0145] The best results were obtained treating a concentrated sample of TRICAPRILIN with CARBOFIL CA at RT (EXP- 23-AA5430). The same material did not perform comparably when the temperature was increased to 70 °C, but this might be caused by the amount treated, which was increased 10 times (EXP-23-AA5433) and might have led to a saturation of the pad. Treating TRICAPRILIN with BECODISC resulted in a decrease of glycidol levels and surprisingly to a

strong increase in the MCPD amount. TRICAPRILIN was also treated with celite and active charcoal in powder at 110 °C overnight. The results are presented in **Table 15**.

Table 15: Treatment of TRICAPRILIN with celite and charcoal at 110 °C

Experiment	Material	Sum of Diester [%a]	TRICAPRILIN [%a]	Tetraester [%a]	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]
EXP-23-AA2362	Starting Values	0.36 + 0.79	97.72	0.04 + 0.10	184	66	1040
EXP-23 AA4115-4(50 g TRICAPRILIN)	Celite (3g) and Charcoal (2.5g)	0.33 + 0.69	97.89	0.04 + 0.11	169	64	<100 (89)

[0146] Interestingly, this treatment resulted in a marked decrease in the glycidol content. No impact on the quality of the material nor on the amount of chlorinated by-products was observed. In conclusion, treatment of concentrated TRICAPRILIN at RT with active charcoal can significantly decrease the amount of glycidol in the sample (EXP-23-AA4115-4 and EXP-23-AA5430). Unfortunately, none of the experiments influenced the MCPD species.

Diatomaceous earth

[0147] Analogous to active charcoal, diatomaceous earth are also commonly employed as filtering aid or adsorption material due to their high porosity and low density. Different types of material and conditions have been tested and the results are summarized in **Table 16**.

Table 16: Screening of different diatomaceous earth under different conditions and the related impact on MCPD / Glycidol levels

Experiment	Material	Conditions	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]
EXP-23-AA8209	Start material		260	68	1044
EXP-23-AA6323	Bentonite (2 weight equivalents)	@ RT overnight	266	<100 (62)	574
EXP-23-AA6331	Bentonite (12.5%w)+ Active charcoal (7%w)	@ 120 °C / 30 mbar	326	<100 (87)	306
EXP-23-AA6323	Zeolite (2 weight equivalents)	@ RT overnight	247	<100 (65)	2601
EXP-23-AA6331	Zeolite(6%w) + Active charcoal (7%w)	@ 120 °C / 30 mbar	340	<100 (87)	640

EXP-23-AA6323	Fullers Earth (2 weight equivalents)	@ RT overnight	254	<100 (68)	129 9
EXP-23-AA6331	Fullers Earth (6%w) + Active charcoal (7%w)	@ 120 °C / 30 mbar	346	<100 (89)	187
EXP-23-AA6323	Mol. Sieves 12X (2 weight equivalents)	@ RT overnight	248	<100 (61)	103 5
EXP-23-AA6331	Mol. Sieves 12X (6%w) +Active charcoal (7%w)	@ 120 °C / 30 mbar	283	<100 (68)	317
EXP-23-AA3529	Starting material		197	<100 (56)	109 2
	Tonsil210FF (10%w)	@ RT overnight	176	<100 (77)	113
	Tonsil310FF (10%w)	@ RT overnight	184	<100 (79)	251
	Vulcobent Protex Ü (10%w)	@ RT overnight	172	<100 (75)	355
	Vulcobent Aktiv G (10%w)	@ RT overnight	171	<100 (77)	351
	Sorbit R 40 (10%w)	@ RT overnight	167	<100 (75)	351
	Prosolv 730 (10%w)	@ RT overnight	169	<100 (67)	317
	Prosolv 50 (10%w)	@ RT overnight	179	<100 (74)	377

[0148] The general observed trend is that the glycidol removal efficacy of all these materials increase with the temperature. The MCPD amount remains basically unvaried. In presence of activated charcoal and at increased temperature, the material that allowed glycidol removal at a bigger extend is the so called “Fullers Earth” (EXP-23-AA6331).

[0149] Another series of commercially available diatomaceous earth have been tested (EXP-23-AA3529). In general, this treatment resulted in a good decrease of the glycidol amount, with the best results given by the Tonsil210FF. MCPD species remained unchanged after all the treatments.

Steam distillation

[0150] Steam distillation was tested for the removal of volatile components, such as odors, by treating the material with a flow of water steam under vacuum conditions. Treating a sample of TRICAPRILIN of 51 g with 500 mL water at 130 °C with a flow of 10 mL/min resulted a strong decrease in the glycidol amount, as summarized in **Table 17**.

Table 17: Purity and MCPD / Glycidol content on a sample of TRICAPRILIN after steam distillation

Experiment	Sum of Diester [%a]	TRICAPRILIN [%a]	Sum of Tetraester [%a]	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]
Starting Values (EXP-23-AA2362)	0.36 + 0.79	97.72	0.04 + 0.10	184	66	1040
EXP-23-AA6329	0.42 + 0.87	97.59	0.04 + 0.10	195	78	142

[0151] Steam distillation appear to be a suitable way to efficiently remove glycidol in TRICAPRILIN samples; MCPD levels remains unchanged. The purity profile remains comparable after the treatment.

Ion Exchange

[0152] In an attempt to reduce the amount of MCPD and glycidol in the isolated TRICAPRILIN, the material was treated with different ion exchange resins at different conditions. The results are summarized in **Table 18**.

Table 18: TRICAPRILIN treatment with ion exchange resin at RT for prolonged time.

Experiment	Ion exchange	Conditions	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]
EXP-23-AA8212	Starting Values		172	40	1048
EXP-23-AA3252-1	Amberlite IRA 900	RT@56h	203	<100 (54)	206
EXP-23-AA3252-2	Amberlite IRA 402	RT@56h	185	<100 (50)	225
EXP-23-AA3252-3	Amberlite IRC 120 H	RT@56h	217	<100 (58)	1347

[0153] The use of anion exchange resin caused a decrease in glycidol but was accompanied by a slight increase in the MCPD species. The cation exchange resin used in EXP-23-AA3252-3 did not appear to be a suitable candidate for the removal of these impurities. Despite the positive results obtained with the anion exchange resins, their removal capability was insufficient to achieve levels of glycidol in the single-digit ppb.

[0154] The same resins were tested at an increase temperature (70 °C) but reduced reaction time (2h) and results are summarized in **Table 19**.

Table 19: Treatment of TRICAPRILIN with ion exchange resin at increased temperature for 2 hours.

Experiment	Ion exchange	Conditions	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]
EXP-23-AA8208	Starting Values		193	<100 (85)	11123
EXP-23-AA3256-1	Amberlite IRA 900	70 °C @ 2h	201	<100 (92)	3398

[0155] Despite the decrease in glycidol amount observed in the three experiments, the amount of 3-MCPD and 2-MCPD remains more or less constant. Overall, the treatment of TRICAPRILIN using ion exchange resins at increased temperature does not appear to be a valuable option.

Short path distillation

[0156] TRICAPRILIN was subjected to a short path distillation with a jacket temperature of 130 °C and 4 mbar vacuum. The MCPD and glycidol profile of the sample was then analyzed, as summarized in **Table 20**.

Table 20: The MCPD and glycidol profile

Experiment	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]
Starting material	184	<100 (66)	1040
EXP-23-AA3255	167	<100 (59)	4217

[0157] Considering that the glycidol level increased after the treatment, the use of short path distillation may not be advised as a purification method.

Recrystallization

[0158] TRICAPRILIN can be recrystallized at a temperature close to 0 °C from MeOH. The presence of water as antisolvent is beneficial to the process in terms of yield and purity.

[0159] In general, the product is mixed with MeOH (8 Vol) and cooled to 0 °C. Addition of seeding crystals was followed by controlled cooling from 0 ° to – 10 °C within 3 hours. Water (1

Vol) is added slowly to the cold mixture and stirred for an additional hour. The crystals are then filtrated on a cooled filter and washed with a cold mixture of MeOH / Water (9:1). The crystals are collected and the solvent is removed at rotary evaporator. The set-up used is presented in **FIG. 7** and the results are presented in **Table 21**.

Table 21: Exhaustive list of the crystallization experiment with purity and MCPD / glycidol content.

Experiment	Yield of crystallization [%]	Diester [%a]	TRICAPRI LIN [%a]	Tetraester [%a]	3-MCPD [ppb]	2-MCPD [ppb]	Glycidol [ppb]
EXP-23-AA8213-1 (small scale)	51	0.02	99.54	n.d.	26	3	68
EXP-23-AA8213-2 (big scale)	31	0.06	99.44	0.02	120	29	183
EXP-23-AA8215 (big scale, better cooling)	65	n.d.	98.6	n.d.	n.d.	n.d.	<100 (73)
EXP-23-AA8216 (From material with 31400 ppb glycidol)	71	n.d.	99.75	0.03	10	n.d.	1847
EXP-23-AA8217-1 (Stepwise cooling, 25% water added)	92	n.d.	99.21	0.05	19	n.d.	188
EXP-23-AA8217-2 (Second crystallization with same conditions)	96	n.d.	99.36	0.02	8	n.d.	90
EXP-23-AA8218-1 (Standard: 10% water at -10 °C)	92	n.d.	99.43	n.d.	n.d.	n.d.	<100 (70)
EXP-23-AA8218-2 (More pure)	93	n.d.	99.57	n.d.	<100 (14)	<100 (14)	<100 (3)

starting material)							
EXP-23-AA8218-3 (Water added at the beginning)	90	0.04	99.17	0.05	<100 (50)	<100 (23)	146
EXP-23-AA8218-4 (High quality seeds)	88	n.d.	99.44	n.d.	<100 (20)	<100 (17)	<100 (13)
EXP-23-AA8218-5 (Water added at 0 °C, then further cooled)	87	n.d.	99.40	n.d.	<100 (20)	<100 (21)	<100 (27)
EXP-23-AA8218-6 (Using Celite to induce nucleation)	88	0.23	96.2	0.11	117	<100	335
EXP-23-AA8219-1 (Scale up)	92	n.d.	99.07	n.d.	n.d.	n.d.	<100 (55)
EXP-23-AA8219-2 (Using ACN instead of MeOH and 25 % water)	80	n.d.	99.26	n.d.	n.d.	n.d.	<100 (49)
EXP-23-AA8219-3 (Increasing water to 15 %)	92	n.d.	99.37	0.01	<100 (10)	n.d.	<100 (30)
EXP-23-AA8219-4 (Using EtOH instead of MeOH and 25 % water)	87	n.d.	99.47	n.d.	n.d.	n.d.	<100 (61)
EXP-23-AA8221 (From MeOH at 0 °C, then water addition at –	94	n.d.	99.65	n.d.	n.d.	n.d.	n.d.

10 °C – standard conditions)							
EXP-23-AA8222 (From MeOH at 0 °C, then water addition at – 10 °C)	94	n.d.	99.65	n.d.	n.d.	n.d.	n.d.
EXP-23-AA8223-1 (Standard conditions, after 1 wash)	n.a.	0.03	99.57	n.d.	n.d.	n.d.	216
EXP-23-AA8223-2 (Standard condition, after 2 washings)	91.1	n.d.	n.d.	n.d.	n.d.	n.d.	126
EXP-23-AA8224-1 (Standard conditions, after 1 wash)	n.a	0.28	99.36	n.d.	n.d.	n.d.	194
EXP-23-AA8224-2 (Standard conditions, after 2 wash)	n.a.	0.07	99.59	n.d.	n.d.	n.d.	124
EXP-23-AA8224-3 (Standard conditions, after 3 wash)	88	n.d.	99.66	n.d.	n.d.	n.d.	<100 (57)
EXP-23-AA8225 (Standard conditions)	79	0.04	99.54	0.02	n.d.	n.d.	130
EXP-23-AA8226 (Standard conditions)	76	0.02	99.63	n.d.	n.d.	n.d.	194
EXP-23-AA8227 (Standard conditions)	92	0.02	99.37	n.d.	n.d.	n.d.	<100 (60)

EXP-23-AA8228 (Standard conditions)	82	0.14	99.46	0.07	n.d.	n.d.	1033
EXP-23-AA8229 (Standard conditions)	66	0.05	99.60	n.d.	<100 (34)	n.d.	<100 (13)
EXP-23-AA8230 (Standard conditions)	76	0.02	99.64	n.d.	n.d.	n.d.	180
EXP-23-AA8231 (Standard conditions)	89	n.d.	99.64	n.d.	<100 (7)	n.d.	181
EXP-23-AA8232 (Standard conditions)	95	n.d.	99.66	n.d.	<100 (n.d.)	n.d.	621
EXP-23-AA8233 (Standard conditions)	92	n.d.	99.66	n.d.	<100 (29)	n.d.	<100 (46)

[0160] After optimizing the crystallization condition, it was possible to obtain material in 50 g scale with very high and consistent purity (equal or above 99 %a). As shown in the table above, this technique allows the removal of diesters, tetraesters, as well as MCPD and glycidol (below the detection limit of 100 ppb).

EXAMPLE 6: Exemplary work flow

[0161] Glycerol was obtained from Verbio. Caprylic acid was obtained from Fisher Scientific. Na_2CO_3 was obtained from Thermo Scientific. Concentrated sulfuric acid was obtained from Merck. The chloride content in the reactants was all under <10 ppm or not detectable.

[0162] FIG. 9 shows a process to make an ultra-pure composition of tricaprilin. Caprylic acid (99.6% purity) and concentrated sulfuric acid (96%) were added to a first vessel. The vessel was heated to 100 °C and pressurized to 500 mbar under a stream of nitrogen. A 1:1 ratio of glycerol (>99.5%) and water were added to the first vessel and stirred. The vessel was maintained at 100 °C and 500 mbar. In-process samples were taken for the vessel to check for conversion. After conversion was observed, a portion of the reaction mixture from vessel 1 was added to a second vessel. MTBE (>99%) and an aqueous solution of Na_2CO_3 (>99%) were also added to the second vessel and the vessel was heated to 40 °C. The pressure of the second vessel was 1 atm. The

contents of the second vessel were stirred and the aqueous phase discharged. A second portion of the reaction mixture was vessel 1 was added to vessel 2, MTBE, and an aqueous solution of Na_2CO_3 were added to vessel 2 and the vessel was maintained at 40 °C and 1 atm. The contents of the second vessel were stirred and the aqueous phase discharged. Additional water was added to vessel 2, the vessel was maintained at 40 °C and 1 atm. The aqueous phase discharged. In-process samples were removed from vessel 2 and their conductivity measured. Water added, stirring, and removal of the aqueous phase was continued until the conductivity of the aqueous phase was less than 100 $\mu\text{S}/\text{cm}$. After the desired conductivity was reacted, charcoal was added to the second vessel for charcoal treatment of the remaining organic phase at 3 g/min. The charcoal treatment was performed at room temperature. After the charcoal treatment, MTBE was distilled off by heating the second vessel to 50 °C and 1 mbar pressure. In process samples were tested for MTBE by gas chromatography. The distillation was continued until the amount of residual MTBE was 500 ppm. The remaining product mixture was recrystallized by cooling the vessel to a temperature of between -10 °C to about 0 °C. After recrystallization, any residual solvent was removed by heating the vessel to 50 °C and 1 mbar. The resulting product was analyzed by gas chromatography.

[0163] Table 22 shows the results of 6 batches of tricapriliin produced according to the Exemplary procedure. FIGs. 8A-8D provide CG-MS analysis of the products.

Table 22: List of experiment according to exemplary procedure

Batch	Yield [%]	Monoesters [%a]	Sum of Diesters [%a]	Tricaprilin [%a]	Tetraesters [%a]	3-MCPD [%a]	2-MCPD [%a]	Glycidol [ppb]
EXP-23-AA3527 (10g scale)	97.8	n.d.	0.36 + 0.75	97.6	0.06 + 0.15	<100	<100	2236
Crystallized product EXP-23-AA8222	94.2	n.d.	n.d.	99.6	n.d.	<100	<100	<100
EXP-23-AA6336 (10g scale)	96.1	0.01	4.9	93.7	0.06 + 0.15	<100	<100	2236
Crystallized product EXP-23-AA8224	88.5	n.d.	n.d.	99.7	n.d.	<100	<100	<100
EXP-23-AA6338 (10g scale)	96.1	0.02	13.4	84.1	0.05	217	<100 (71)	3449

Crystallized product EXP-23-AA8230	76.2	tba	tba	tba	tba	<100 (n.d.)	<100 (n.d.)	180
EXP-23-AA6339 (20g scale)	81.1	0.2	27.8	70.8	0.06	<100	<100	2236
Crystallized product EXP-23-AA8229	66	n.d.	0.05	99.6	n.d.	<100	<100	<100
EXP-23-AA6340 (20g scale)	97	0.02	2.07	96.66	0.08 + 0.03	718	198	1099
Crystallize dproduct EXP-23-AA8233	92.5	n.d.	0.05	99.66	n.d.	<100	<100	<100
EXP-23-AA6341 (10g scale)	98	n.d.	0.22	98.76	0.07 + 0.03	173	<100	4141
Crystallized product EXP-23-AA8232	94.8	n.d.	n.d.	99.66	n.d.	<100	<100	621

CLAIMS

What is claimed is:

1. An ultra-pure composition comprising at least about 99.2% by weight tricaprilin and about 0.8% by weight or less of impurities,
wherein the tricaprilin is a tri-ester tricaprilin; and
wherein the impurities comprise one or more of 2-monochloropropanediol, 2-monochloropropanediol monoester, 2-monochloropropanediol diester, 3-monochloropropanediol, 3-monochloropropanediol monoester, 3-monochloropropanediol diester, or any combination thereof.
2. The ultra-pure composition of claim 1, comprising at least about 99.5% by weight tricaprilin and about 0.5% by weight or less of impurities.
3. The ultra-pure composition of claim 1, comprising at least about 99.7% by weight tricaprilin and about 0.3% by weight or less of impurities.
4. The ultra-pure composition of claim 1, wherein 3-monochloropropanediol, 3-monochloropropanediol monoester, and 3-monochloropropanediol diester are present in a concentration of 35 ppb or less.
5. The ultra-pure composition of claim 1, comprising less than 35 ppb of each of 3-monochloropropanediol and 3-monochloropropanediol diester.
6. The ultra-pure composition of claim 1, wherein the impurities further comprise glycidol, mono-ester tricaprilin, di-ester tricaprilin, or tetra-ester tricaprilin.
7. The ultra-pure composition of claim 6, wherein the impurities further comprise less than 35 ppb of glycidol.
8. The ultra-pure composition of claim 1, wherein the impurities further comprise unreacted reactants.
9. A method of making an ultra-pure composition of tricaprilin, the method comprising:

- (a) feeding caprylic acid, a concentrated acid, glycerol, and water into a first reactor;
 - (b) reacting caprylic acid, the concentrated acid, glycerol, and water to form a product mixture comprising tricaprillin and impurities in the first reactor, wherein the impurities comprise unreacted reactants and optionally undesired side products;
 - (c) adding an organic solvent and an aqueous solution of a basic salt to a second reactor forming a biphasic mixture comprising a top organic phase and a bottom aqueous phase;
 - (d) adding portions of the product mixture from step (b) into the biphasic mixture in step (c) to remove the unreacted reactants, wherein the unreacted reactants are in the aqueous phase;
 - (e) removing the bottom aqueous phase from the second reactor;
 - (f) treating the top organic phase with charcoal;
 - (g) distilling off the organic solvent and leaving tricaprillin and impurities in the second reactor; and
 - (h) recrystallizing the tricaprillin and impurities to remove the impurities and form the ultra-pure tricaprillin.
10. The method of claim 9, wherein step (a) further comprises:
- (ai) co-feeding caprylic acid and concentrated acid into the first reactor to form a mixture;
 - (aii) heating the mixture to a first temperature under an inert atmosphere; and
 - (aiii) adding Glycerol and water to the mixture over a first specified time.
11. The method of any one of claims 9-10, wherein step (b) further comprises:
- (bi) stirring the product mixture for a second specified time at a second temperature;
 - (bii) removing a first in process sample from the first reactor;
 - (biii) checking the first in process sample for conversion, wherein conversion is reaction of hydroxyl groups in the Glycerol to ester groups in tricaprillin ; and

- (biv) repeating step (bii) to step (biii) until conversion occurs.
12. The method of any one of claims 9-11, wherein step (g) further comprises
- (gi) removing a second in process sample from the second reactor;
 - (gii) measuring amount of the organic solvent remaining in the tricaprilin and impurities;
 - and
 - (giii) repeating steps (gi) to (gii) until the amount of the organic solvent remaining is 500 ppm or less.
13. The method of any one of claims 9-12, wherein step (h) further comprises
- (hi) adding a polar solvent to the tricaprilin and impurities forming a second mixture comprising a polar solvent, tricaprilin, and impurities;
 - (hii) cooling the second mixture to a third temperature;
 - (hiii) adding seeding crystals to the second mixture forming a third mixture comprising seeding crystals and the second mixture;
 - (hiv) stirring the third mixture for a third specified time at a third temperature;
 - (hv) cooling the third mixture to a fourth temperature in a fourth specified time;
 - (hvi) maintaining the third mixture at the fourth temperature for a fifth specified time;
 - (hvii) adding water to the third mixture;
 - (hviii) maintaining the third mixture at the fourth temperature;
 - (hix) filtering the third mixture on a filter cooled at the fourth temperature;
 - (hx) washing the third mixture with an aqueous solution of the polar solvent at a fifth temperature;
 - (hxi) repeating the washing step (hx);
 - (hxii) collecting crystals of tricaprilin; and
 - (hxiii) evaporating residual solvent from the crystals using a rotary evaporator.
14. The method of claim 9, wherein the concentrated acid comprises sulfuric acid.
15. The method of claim 10, wherein the first temperature ranges from about 90 °C to about 120 °C.
16. The method of claim 10, wherein the first specified time ranges from about 3 hours to about 5 hours.

17. The method of claim 11, wherein the second temperature ranges from about 90 °C to about 120 °C.
18. The method of claim 11, wherein the second specified time ranges from about 15 hours to about 24 hours.
19. A pharmaceutical composition comprising the ultra-pure composition of claim 1.
20. A method of prophylactically or therapeutically treating a disease or a disorder in a subject in need thereof, the method comprising administering a composition comprising ultra-pure tricaprilin to the subject.
21. The method of claim 20, wherein the disease or disorder is any one or more of craniocerebral trauma, stroke, hypoxia, myocardial infarction, refeeding syndrome, anorexia, epilepsy, neurodegenerative diseases such as dementia, Alzheimer's disease, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis, fat metabolic diseases such as glucose transporter defect (GLUT1 defect), VL-FAOD and mitochondriopathies such as mitochondrial thiolase defect, Huntington's disease, cancers such as T-cell lymphomas, astrocytoma and glioblastoma, HIV, rheumatic diseases such as rheumatoid arthritis and arthritis urica, chronic inflammatory bowel diseases, ulcerative colitis, Crohn's disease, lysosomal storage diseases, sphingolipidosis, especially Niemann-Pick disease, diabetes mellitus, migraine and migraine headaches, epilepsy, especially infantile spasms as well as effects or side-effects of chemotherapy.
22. The method of claim 20, wherein the method comprises administering the ultra-pure composition of tricaprilin of claim 1 to the subject.
23. The method of claim 20, wherein the method comprises administering ultra-pure composition of tricaprilin prepared by the method of claim 7 to the subject.
24. The method of claim 20, wherein the ultra-pure composition of tricaprilin is administered orally.