GOED

INDUSTRY ADVISORY:
MICRO- AND NANOPLASTICS PARTICLE CONTAMINATION IN EPA/DHA OMEGA-3 OILS

Edition: December 10, 2018

Purpose

Technical memorandum that can be used to inform members and clients looking for information on this topic.

Summary

- Given the extensive application of filtration in the manufacturing of EPA/DHA oils, it can be said with a high level of certainty that microplastic particles with a diameter larger than 0.5-1 micrometer are not present in refined fish oils and other EPA/DHA oils used for consumer finished products.
- No known measurements on plastic particle contamination in EPA/DHA oils have been made to date.
- The hazard of microplastic particle exposure from EPA/DHA oils is deemed to be low.
- It is possible that nanosized plastic particles with a diameter smaller than 0.5 micrometer may be present in oils, but there are currently no methods to assess this.
- The hazard of nanoplastic particle exposure from EPA/DHA oils is unknown.
- By the best practice in oil refining and independent analysis, the contamination of GOED member EPA/DHA oils with pollutants originating from microplastics is of low concern.

Introduction

Concern about the presence of micro- and nanoplastics in the environment and in food has increased in recent years. Due to the widespread use and durability of synthetic polymers, plastic debris is littering the environment globally. Uncertainties exist about the potential hazard of small micron- and nano-size plastic particles entering the food chain, especially if they have low or restricted biodegradability. The release of plastics into the environment should be reduced in a broad and global effort regardless of proof of an environmental risk. This summary addresses some important points about microplastics and nanoplastics with respect to fish oil and other EPA/DHA omega-3 oils.

Primary microplastics are plastic micrometer-sized particles in the range of 0.1 µm – 5 mm (5.000 µm), which originate from a variety of sources. Microplastics are manufactured for use in cosmetics, personal care products, and detergents. Microplastics can also be released from fabrics during washing and are produced around sites of human activity such as roads.

Secondary microplastics are formed by the fragmentation of larger pieces of plastic.

Microplastics enter the marine environment through wastewater discharges and runoff into the ocean, as well as from plastics used in marine events like shipping, fisheries and off-shore activity. Quantitative information on the relevance of these sources is generally lacking, but abrasion/fragmentation of larger plastic materials containing synthetic polymers is estimated to be most relevant.

Nanoplastics are formed by degradation of larger micron-sized plastic particles. They are defined as a material with any external dimension in the nanoscale (0.001-0.1 µm) or having an internal or surface structure in the nanoscale. Little is known about the fate and effects of nanoplastics on the marine environment and human health, primarily because no methods exist to measure these within biological organisms.

Given the near complete absence of information about nanoplastics, much of the present report will focus on microplastics.

Microplastics and nanoplastics can be made up of any commonly used polymers, such as low-density polyethylene (LDPE), linear LDPE, high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), nylon 6, acrylonitrile-butadiene-styrene (ABS), polycarbonate, polyurethane, polystyrene (PS), polyvinyl alcohol (PA), polyamide (PA) and polymethylmethacrylate (PMMA). Their lifetimes in the environment depend on the rate of degradation, which varies significantly

with material type, exposure to UV light, temperature and oxygen concentration, and possible microbial degradation, which all depend on locally prevailing conditions. Biodegradable plastics such as poly-lactic acid, lactones and polyalcohols are also used, with shorter expected lifetimes and a lower expected environmental impact. Microplastics can have a large variety of shapes, such as fragments, beads, filaments, fibers and films. Fragments and fibers have been reported to dominate in surface waters and sediments, while bead-shaped particles make up a smaller proportion.

Microplastics have been detected in marine organisms, such as fish, shellfish and other organisms living on or within sea floor sediments. The lowest microplastic concentrations affecting marine organisms exposed via water are much higher than levels measured in marine water. Microplastics have been detected in the gut and gills of filter feeders such as mussels. Several studies have determined microparticles in the stomach and gut of fish. Marked differences in microparticle loads between fish species have been noted. Fish obtain microparticles through consumption of organisms at lower trophic levels, whereas filter feeders obtain microplastics directly from water. The contribution of absorption of microplastics via the gills in fish has not been addressed. To date, there is no clear evidence of bioaccumulation or biomagnification of microplastics in living organisms. Concentrations of microplastics in internal tissues are generally much lower than loads present in the gastrointestinal tract, although for a limited number of fish species the tissue levels have been reported to exceed the levels in viscera and gills.

Although the currently available data suggest that at the concentrations of microplastics detected in surface water, microplastics are not causing harm to the environment (feeding, reproduction, growth, tissue inflammation, and mortality in organisms), there is a mismatch between the particle types, size ranges, and concentrations of microparticles used in laboratory tests and those measured in the environment. New research is expected to provide improved insight during the coming years.

Due in large part to the difficulty in measuring microplastics in the human body, the risk of microplastics to human health is unknown. Currently, there is insufficient data on the occurrence, toxicity and fate of these materials for a full risk assessment. In 2011, the European Food Safety Authority (EFSA) published guidance on the risk assessment of nanoscience and nanotechnologies in the food chain (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2011.2140) which applies across all EFSA's scientific areas of competence. An update of the guidance is scheduled for 2018. In 2016, EFSA published an informative Scientific Statement on Microplastics and Nanoplastics in Food and Seafood.

Relevant questions about microplastics and EPA/DHA omega-3 oils

1. Are there any reports of microplastics and nanoplastics in fish oils and EPA/DHA omega-3 oils?

At present, there have not been any reports of the presence of microplastics or nanoplastics in EPA/DHA omega-3 oils.

2. Is it possible that microplastics and nanoplastics could be present in fish oils and EPA/DHA omega-3 oils?

From a practical perspective, nobody has yet made measurements to assess the possible presence of micro- or nanoplastics in EPA/DHA omega-3 oils. From a theoretical perspective, there are some aspects that need to be considered about the potential presence of micro- and nanoplastics in EPA/DHA, see point 3 and 4 below.

Microplastics can also enter foods from the food processing steps themselves, as well as from aerial exposure. These routes need to be taken into account as a potential source of contamination, and controlled when measurements will be made, to draw accurate conclusions about the origin of microplastics contamination.

3. Do microplastic particles survive the processing steps used to produce oils destined for EPA/DHA finished products?

Filtration and centrifugation applied during omega-3 oil processing steps preclude the entry of particles, including microplastics, into refined EPA/DHA omega-3 oils. Several filtration and centrifugation steps are used during both fish oil and EPA/DHA-oil refining and concentration. Typical process steps where filters and centrifuges are used are the following:

A. Crude oil: When the crude oil is pumped into the factory (crude tank farm), and before refining starts, the oil goes through a self-cleaning metal filter (pipe sieve) to remove mechanical particles. Typical pore size: 100 micron.

B. Refining step 1 (wash/degumming): Crude oil producers rely predominantly on centrifuges for separating oil from press water and particulate material. One or two centrifugation steps are employed. Different plastic materials have different densities, with some below 1 g/cm3, such as PE (0.93-0.98 g/cm3), and PP (0.89-0.91), or above, such as PS (1.04-1.11), PVC (1.20-1.45), PA (1.13-1.5), PET (1.38-1.39) and PVA (1.19-1.35). Depending on how differential centrifugation is carried out, the selective removal of particles that are heavier or lighter than the specific oil being processed is likely to occur. If any particles come through, these are very small, although the exact size cut-off is not possible to define.

   Some crude oil processors employ an active carbon treatment step to remove certain contaminants, such as polyaromatic hydrocarbons, PCBs and dioxins, under mild conditions. After treatment, the crude oil either will be filtered or centrifuged to remove the fine carbon particles, a process which will also remove other small particles. A typical filter has a pore size of 80 micron, but this can be varied depending on the particle size of the activated carbon used. Particles will furthermore be removed by the active carbon material packed on the filter screen, but it is not possible to exactly define the effective size of particles that are removed. Some processors use a carbon column for this step.

C. Refining step 2 (bleaching): Refining often involves the use of bleaching earth, optionally together with a certain proportion of added (*e.g.* 10%) active charcoal, forming a filter cake through which the oil is filtered using leaf filter frames. A typical pore size of a leaf filter can be 80 micron, but the effective pore size of the filter cake is unknown. The particle size of the bleaching earth can range from approximately 1.5 micron to 200 micron (for example, with 62% of the particles larger than 25 micron), and well-defined bleaching earths can have a narrow particle size range (*e.g.* 20-30 micron) with a defined portion of particles larger and smaller than this range (*e.g.* maximally 10% of particles larger than 5 micron). Since the oil is visibly clear after filtration, a significant portion of particles larger than 1.5 micron will likely have been removed. Following bleaching, the oil is filtered again using bag filters with pore sizes of 1 micron. Other manufacturers use a 5 micron bag filter (absolute type, i.e. 99% of pores are 5 micron). Some manufacturers employ a second bag filtration step here with a bag filter with 0.5 micron pore size.

D. Winterization: Producers that employ winterization (*e.g.* for triglyceride 180/120 oils) to remove certain classes of lipids (such as stearine), also employ filtration through the formation of a "filter cake" of saturated fat on the filter (usually plate and frame type filters, or pressure leaf filters) that will also remove microparticles. The effective pore size for such packed filters is unknown.

E. Ethylization: Manufacturers that produce EPA/DHA ethyl esters employ centrifugation to remove glycerol following the conversion of triglycerides to ethyl esters. It is unknown what effective size of particles will also be removed in this step.

F. Bleaching 2: Refiners that employ a second bleaching step may use the same type of bleaching earth and filter frames as for the first bleaching process, although the dimensions of the filter house/bleaching reactor and filter frames are typically smaller. After filtration, the oil is again filtered using bag filters with 0.5 micron pore size.

G. Deodorization: After deodorization, the oil is filtered using a bag filter with 0.5 or 1 micron pore size, or a polishing filter with 1 micron pore size. Deodorization frequently constitutes the last step in the process before drumming or packaging the bulk refined oil.

H. Drumming: Before drumming, the final oil product is filtered using a cartridge filter, size 1 micron - absolute type (which mean that 99.9% of the filtering area is 1 micron). Some manufacturers use a 1 µm absolute filter if activated carbon was used during bleaching, or a 1 µm nominal filter if activated carbon was not used. Other manufacturers work with a polishing filter station, which is installed right before the filling / packaging step and consists of a multi-cartridge filter housing that allocates a number of filter cartridges (for example of 5.00 micron).

A note on filtration: To denominate the sizes of pores in filters, mesh numbers (the number of openings per inch) and pore sizes are used. A useful chart comparing mesh number to pore size (in micron, inch and mm) is provided here: https://www.netafimusa.

com/wp-content/uploads/2016/10/Mesh-vs-Micron.pdf. Mesh terminology is no longer used below approximately 37 micron (400 mesh), and small pore sizes relevant to filtration of oils are given in micrometer. A 1.0 micron pore size would theoretically correspond to approx. 12000 mesh.

A note on heating: Heating steps employed during the isolation of oil from whole fish (such as anchovy), fish tissues, or from other specific marine organisms, as well as during oil refining, are not expected to lead to the melting of micro- or nanoplastic particles that are made of the most common synthetic polymers. These materials generally have high melting points (for crystalline polymers) and high glass transition temperatures (for amorphous polymers), which are substantially above 200°C, and 300°C, respectively. The highest temperatures employed in EPA/DHA omega-3 oil processing typically do not exceed 200 °C. (low-pressure distillation, deodorization), indicating that heating-induced disintegration of nano- and microplastics is unlikely to occur.

Taken together, a number of filtration and centrifugation steps are used in refining and processing steps employed by producers of EPA/DHA omega-3 oils. The smallest filter pore size employed will determine the diameter above which any occurring microplastic particles will be removed. Based on information collected from GOED member producers, this corresponds to a size of 0.5 to 1 µm. It is possible that particles smaller than 0.5 µm will be removed as well, but there are no data on the effective size of particles removed via filtration through the packed, activated carbon or pre-coated filter screens, by means of centrifugation, and via winterization.

4. Is there a different hazard for microplastics contamination of different omega-3 oils?

Oils prepared from whole fish bodies, such as from small pelagic fish, also include the gut. From this perspective, fish body oils are refined from a source that theoretically have a higher microplastics load than oils obtained from specific internal organs, such as fish liver. Oils prepared from other marine species that are filter feeders (shellfish) and sea floor and sediment feeders (marine cucumbers and worms) could in theory contain higher microplastics levels. Microplastic loads in algae or krill are unknown. The accumulation of microplastics in fish gills is unknown.

It is important to take note that irrespective of the biological source of the EPA/DHA-containing oil, the employment of similar refining technologies, and especially filtration using filter bags, will provide final refined oils without particles larger than 0.5-1 µm.

5. Are microplastics absorbed and deposited in the body?

It is considered that a very small portion of microplastics in foods might be absorbed from the gastrointestinal tract into the body, and most of which is excreted again. Inter-trophic transfer of microplastics from mussels to crabs has been shown to occur, with a very small portion of particles being transferred and eliminated from the crab's body over time. In some species of fish, microplastics are higher in tissues than in the gastrointestinal tract, suggesting translocation.

No data for humans is available. On the one hand it is considered that microplastics smaller than 150 µm display the highest risk for being absorbed from the gastrointestinal tract into the body. On the other hand, it is thought that the intestinal wall does not allow the passage of particles larger than 2 to 10 nanometers (through so-called tight junctions that control fluid, solute and particulate transport between cells). Modern pharmaceutical delivery systems, however, aim to make use of the property that tight junctions between epithelial cells of the intestinal tract can further open (or close) in response to particles with specific surface chemistries. Intestinal epithelial cells also have the capability to engulf particles with sizes in the hundreds of nanometer range, but the *in vivo* relevance for particle exposure is unknown. The likelihood that nano- and micrometer-sized plastic particles are absorbed from the gastrointestinal tract will depend on a combination of several factors such as the surface chemistry of the particles, their size, shape and charge, as well as the integrity of the gastrointestinal tract, and no comprehensive data is available on this today. The reported presence of microplastics in human stool indicates that a measurable portion of particles ingested from the diet is excreted and is not absorbed. Furthermore, nanosized particles tend to aggregate, a process that will lower

their availability for absorption. In other words, there is a paucity of information on microplastic and nanoplastic absorption and deposition in the human body, and their potential effects are unknown.

6. Do microplastics and nanoplastics constitute a relevant vehicle carrying environmental contaminants that pose a risk for human health?

At the present time, it is unclear if micro- and nanoplastics serve as a vehicle carrying environmental contaminants and thus a risk to human health. Interest in this topic has stemmed from observations that microplastics have a relatively large surface area and can adsorb hydrophobic organic pollutants. Recent research has calculated that the contribution of microplastics to deliver environmental contaminants that adsorb to their surface is negligible compared to the dose of contaminants received from our normal intake of food. The evaluation of health risks from exposure to environmental contaminants needs to be considered from the point of the quality of EPA/DHA oils (stipulated for GOED members in the GOED Monograph and determined by the market where products are sold). Plastics typically also contain additives, which in currently-produced plastics typically amount to up to 4% of the weight of the plastic material. These additives include plasticizers such as phthalates and bis-phenols. Traces of unpolymerized plastic (monomers) may also be present within plastics. The health risk of these substances should be evaluated as part of the food type being ingested.

Methods for the measurement of microplastics and nanoplastics

Detection and identification of microplastics in different types of samples requires specialized approaches. No methods for the determination of nanoplastics in oils or biological matrices are reported. Methods for the measurement of number, types and sizes of microplastics are relatively new, and still under development, and a few approaches are provided below. Applications for the measurement of microparticles in oils have not been specifically reported on, and may need to be developed or optimized.

- A Fourier Transformed Infrared (FTIR) spectroscopic imaging was highlighted in an application note by Agilent. Spectra of individual particles are mapped against a reference database of many material types and micrometer-sized particles can be characterized by polymer group, dimensions, and mass. Link: https://www.agilent.com/cs/library/applications/5991-8271EN_microplastics_ftir_application.pdf
- The combination of infrared imaging microscopy, Raman imaging microscopy, image analysis, and matching with polymer libraries by Thermo Scientific offers an approach to analyzing microplastics of a wide size range (1-5000 µm). The technique involves sampling, sample digestion, and collection of microparticles on special filters allowing removal of matrix and biogenic material, drying of filters, imaging using infrared microscopy, and particle material recognition using reference spectra. A semi-quantitative measure of the particulate concentrations is obtained and works best for particles > 10 µm. For particles <10 µm, Raman imaging microscopy can be used with a spatial resolution down to 0.5 µm. Link: http://assets.thermofisher.com/TFS-Assets/MSD/Technical-Notes/TN53006-microplastics-bottled-water.pdf
- Micro-Raman spectroscopy and elemental analysis with energy-dispersive X-ray spectroscopy can be used for the determination of microplastics in fish tissues. Study: Microplastics in eviscerated flesh and excised organs of dried fish. Karami et al. *Sci. Rep.* 2017 Jul 14;7(1):5473. Link: https://www.ncbi.nlm.nih.gov/pubmed/28710445
- The use of reference materials with well-characterized dimension is important in order to achieve accurate measurements of microplastic particles. One example is a study showing fluorescent detection of microplastics and reference particles of different polymeric materials (15-100 µm range). Study: A novel method for preparing microplastic fibers. Cole M. Sci. Rep. 2016 Oct 3;6:34519. Link: https://www.nature.com/articles/srep34519
- Particle size analysis of wet dispersions can be performed by laser diffraction using a particle sizer instrument (*e.g.* Mastersizer). A wide range of sizes can be determined, approximate range 20 nm-2000 µm. This technique can be used in combination with flow imaging microscopy to determine the number, size and morphology of plastic microparticles and agglomerates. For example: Micro-Flow Imaging as a quantitative tool to assess size and

agglomeration of PLGA microparticles. van Beers et al. *Eur. J. Pharm. Biopharm.* 2017 Aug;117:91-104. Link: https://www.ncbi.nlm.nih.gov/pubmed/28392414

Note: Any methods used for measuring microparticles in oils should employ appropriate controls to correct for the contribution of (inadvertent) introduction of particles during sample preparation or refining itself.

Regulations

The US and Canada have called for a ban on the use of microplastics. The US Environmental Protection Agency (EPA) has stated "The toxicity risk from ingesting microplastics and Persistent Organic Pollutants that cling to them, or from consuming prey that has consumed microplastics, requires further study. EPA believes it may be a contributing stressor to the sensitive species in some of the worlds' most valuable ocean and coral ecosystems. EPA is taking action under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) to assess and mitigate this threat to the environment and human health." (Link: https://www.epa.gov/trash-free-waters/statutes-and-regulations-affecting-marine-debris)

The EU may implement regulations in the future.

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