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"ToxOligo": Characterization of toxicological properties of oligomers from food contact materials

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Key words
Oligomers; Food contact materials; Toxicology; Hazard assessment; Exposure assessment; Chemical risk assessment

Summary and aim of the study

The objective of this study was to perform a comprehensive evaluation of oligomers present in food contact materials (FCMs) to provide information for chemical risk assessment. Oligomers are molecules formed mainly during the production and recycling of FCMs and are present in all polymers. They are considered non-intentionally added substances (NIAS) and may pose a potential risk to consumer health if they transfer from FCMs into consumed food. It has so far been assumed that the toxicity of oligomers is covered by their monomers. However, this assumption has to be questioned as there is insufficient data available on the toxicology of oligomers. Their structures are often not solved, they are often not commercially available, and there are no clear guidelines for evaluating their safety. This makes it difficult to perform toxicological tests according to regulatory standards. To address this knowledge gap, this study employed a range of methods, including *in silico* and *in vitro* toxicological approaches, systematic evidence mapping, and migration modeling. Systematic evidence mapping involved the review and synthesis of existing data on the physico-chemical properties, migration, and biological activity of oligomers to address research needs. Migration modeling was utilized to systematically predict and evaluate the migration of oligomers from FCM into food or beverages under realistic application conditions. *In silico* methods were used to predict the physico-chemical properties of oligomers and to identify potential areas of toxicological concern, with a focus in this project on endocrine disturbances. Oligomers of potential concern were then prioritized for further testing and *in vitro* evaluation in the follow-up project. In addition, areas were identified that require further research to perform a risk assessment of oligomers. This provides an important knowledge base for future research.

Material and methods

Identification of oligomers reported in the scientific literature

In collaboration with the Food Packaging Forum Foundation (FPF), the internal version of the FCCmigex database that was recently developed by the FPF, was used for the identification of known oligomers in the scientific literature (FCCmigex, 2022). The search was conducted as follows: (I) Titles, abstracts, and food contact chemicals of identified scientific literature within the FCCmigex database were searched using "oligomer" as a search term; (II) Food contact chemicals (FCC) were searched using the following terms: dimer, dimers, trimer; (III) CAS numbers were searched using the terms (internal use of terms, when CAS RN was not reported): nan and TBD. Form all three searches, FCC entries and their corresponding articles were extracted and manually screened at the full-text level for oligomer relevant data. The identified oligomers were recorded within a MS Access database and deduplicated. Oligomers were selected based on the following criteria: high to medium level of confidence of the structure (structures indicated as e.g., tentative, were excluded); acronyms and the associated sequence of monomers to build the oligomer must be unambiguous; isomers must be defined; stereoisomers are included if available, otherwise the oligomer is included without stereochemistry; oligomers must be derived from materials with intended food contact application (with exception of oligomers from styrene-acrylonitrile (SAN)); the source of the oligomer must be defined with exception of the plasticizers; self-synthesized materials or conditions with special treatment of the material were excluded (e.g., ionization and oxidation). There is as yet no official definition of the term oligomer, and for some substances it is therefore not possible to clearly decide whether they are oligomers or not. In such cases, substances that could also be interpreted as degradation products or (modified) monomers were also included.

To every oligomer recoded in the MS Access database, additional information was added, including material of origin, CAS RN, SMILES, molecular formula, molecular weight, supplier availability, hydrolysis, and toxicology and/or bioactivity. The material of origin was assigned as described in the respective literature. CAS RN, SMILES, molecular weight, molecular formula, and supplier availability were extracted from SciFinder-n, or if not available, SMILES, molecular formula, and molecular weight were generated by using ChemDraw 21.0.0. Based on the collected chemical information in PubChem, an exploratory data search was performed to obtain a preliminary overview of toxicology and bioactivity information. It is important to note that the additional information on each of the substances covered was collected in an exploratory and not systematic approach.

In silico methods

Physico-chemical and toxico-pharmacokinetic parameters were characterized for all oligomers, using Schrodinger QikProp (https://www.schrodinger.com/products/qikprop), Marvin Sketch (Chemaxon Ltd., https://chemaxon.com/products/marvin), and ADMET predictor (Simulations Plus, https://www.simulations-plus.com/software/admetpredictor/). Toxicological characterization was performed by using VirtualToxLab (VTL) (http://www.biograf.ch/index.php?id=projects&subid=virtualtoxlab).

In vitro methods

Transactivation assays have been established for androgen receptor (AR), estrogen receptor-alpha (ERα), ERβ, glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and progesterone receptor (PR), using recombinant human receptor expressed together with a receptor-response element linked to a luciferase reporter in HEK-293 cells. The conditions for these assays, including suitable concentrations of positive and negative controls, were optimized to test for agonist and antagonist activity of a given compound. In addition, an assay for retinoid-related orphan receptor-gamma (RORγ), kindly provided by Prof. Anton Jetten, NIEHS, was optimized for testing oligomers. Another, newly established enzyme assay included 17β-HSD6, an enzyme converting androsterone to androstanedione as well as 3-androstanediol to the potent androgen dihydrotestosterone (DHT). In this assay tritium-labeled androsterone is used to measure the formation of androstanedione in the presence of vehicle or various concentrations of the test compounds. To assess cytotoxicity, the XTT assay was applied. In addition, cell morphology was manually checked prior to measuring luciferase activity in receptor assays.

Systematic evidence mapping

The systematic evidence map (SEM) protocol describing the methodology of the SEM was initially registered on Zenodo (Schreier, Appenzeller-Herzog, et al., 2022a). Methodological details are described and can be found online in the peer-reviewed SEM protocol (Schreier, Appenzeller-Herzog, et al., 2022b).

Migration modeling
Details of the methods used for migration modeling of PET oligomers are described in detail in the corresponding peer-reviewed and published study (Schreier, Odermatt, et al., 2022). AKTS SML software version 4.54 and 6.41 (AKTS AG Siders, Switzerland) was used for the modeling of all migrations. The molinspiration online tool (https://www.molinspiration.com/) was used for the calculation of molecular volumes (M_V) for all PET oligomers. For the activation energy-based E_A model the validated parameters for the prediction of the diffusion coefficient were: a = 1.93 10^{-3} 1/K, b = 2.37 cm^2/s, c = 11.1 $Å^3$ and d = 1.50 10^{-5} 1/K [9,14]. For the prediction of diffusion coefficients with the A_P model the following parameter were used: A'_P = 3.1 and τ = 1577 (realistic case) or A'^*_P = 6.4 and τ = 1577 (upper limit).

Results and significance

A literature search was performed to identify oligomers in literature that had previously been characterized. This was a crucial step of the project as it allowed the evaluation and identification of oligomers of interest, as well as substances that could be used as representative substances for toxicological testing. The search was conducted in collaboration with the FPF using their recently established FCCmigex database (Food Packaging Forum Foundation, 2022; Geueke, 2022). As a result, a list of all structurally characterized oligomers was compiled and 514 oligomers were recorded in a MS Access database. Additional information about each oligomer was collected, including its material of origin, CAS RN, SMILES, molecular weight, molecular formula, supplier availability, and hydrolysis information. Using the structural information (CAS RN and SMILES), an exploratory data search was conducted in PubChem to obtain a preliminary overview of toxicology and bioactivity information to potentially identify oligomers of interest.

Given the high number of oligomers that are already known, it was necessary to prioritize the oligomers for our study. As it was not feasible to cover all oligomers in this study, the project was split into two parts: (I) A pilot project covering PET oligomers; (II) evaluate all oligomers using *in silico* tools to prioritize substances for future research. Given the existing knowledge base and tools for studying PET oligomers, it was decided to focus on these oligomers in this project. The knowledge gained and tools developed in this project can be applied to the study of other oligomers in the future. This will allow to further deepen the understanding of oligomers and their potential impact on human health and the environment.

In silico evaluation of all collected oligomers
The 514 collected oligomers were used to perform physico-chemical and toxicological *in silico* predictions for all identified oligomers. The *in silico* predictions in combination with the previously collected information was used to further prioritize 30 oligomers originating from different FCMs. These molecules will be studied *in vitro* and *in silico* in an extension period of the project. The focus will be on assessment of bioactivity of these substances. Hazard profiles will be established using various *in vitro* assays to assess general toxicity, cellular stress pathways, macrophage activation and endocrine effects. These investigations aim at assessing the level of concern of such substances and providing recommendations for prioritization of future toxicological testing.

Pilot project on PET oligomers
As part of the pilot project on PET oligomers, various approaches to comprehensively evaluate these substances were established. This part of the project contained a systematic evidence map to identify existing knowledge and gaps related to hazard and exposure information of PET oligomers. Migration modeling was used as a systematic approach for exposure assessment. Finally, in the third phase of the project, *in silico* and *in vitro* tests were performed.

Systematic evidence mapping
PET oligomers have been detected in food and beverages, but their toxicological properties are often unknown. There is currently no harmonized risk assessment framework or guidance for safety assessment of oligomers, and the use of the threshold of toxicological concern (TTC) to estimate safety levels for PET oligomers with unknown toxicity has been proposed. However, the TTC concept may not be appropriate for oligomers, and there is a lack of knowledge about their risk potential. This part of the study aimed to collect existing data on 34 previously prioritized PET oligomers in order to improve access to this information and inform chemical risk assessment. The systematic evidence mapping method was used to identify and analyze available information, and

identify knowledge gaps and recommend future research activities. The corresponding methods are available in the SEM protocol that is published and available online (Schreier, Appenzeller-Herzog, et al., 2022b). The results of the SEM are currently being summarized in a manuscript to be submitted for peer-review in February 2023.

Migration modeling

The study results led to the following conclusions, which were published as follows (Schreier, Odermatt, et al., 2022): Experimental migration tests on PET oligomers are time consuming and the results are associated with considerable uncertainty, mainly due to the lack of reference standards for most oligomers. The low concentrations in the migration solutions as well as potential swelling effects or hydrolysis when using aqueous ethanolic solutions as food simulants, make the interpretation of the results very difficult.

The use of migration models represents a useful tool for the safety evaluation of PET oligomers. However, diffusion coefficients need to be available for all PET oligomers. Experimentally determined diffusion coefficients are rare in the scientific literature and only available at very high temperatures for the PET first series cyclic trimer. Therefore, prediction models for diffusion coefficients D_P are necessary for the migration modeling approach. The results for the diffusion coefficients depend on the prediction model for the diffusion coefficients, based on the over-estimative character of the applied prediction model.

Small oligomers with high diffusion coefficients are the most critical substances that can also pose the greatest risk to consumers, especially when the storage conditions include high temperatures, e.g. in microwave or ovenable trays. In these cases, the prediction model for the diffusion coefficients should include the influence of temperature on the diffusion coefficients. This makes the E_A-based model more suitable for a realistic evaluation of the consumer exposure. In addition, the E_A-based prediction model is validated for many different substances and temperatures, resulting in a more realistic modeling approach and therefore a more reliable exposure prediction of oligomers. However, the over-estimative A_P prediction model can be also applied, resulting in a more conservative evaluation of the results, when typical concentrations available from the literature are applied.

The second major parameter influencing the migration process, the partition coefficient $K_{P,F}$, plays a minor role and can be neglected in most cases. This is due to the slow diffusion of high molecular weight compounds like PET oligomers in PET. If the partition coefficient can be neglected, the procedure is simplified, because $K_{P,F} = 1$ can be used for worst-case prediction. In case of doubts, e.g. for small molecules and/or high temperatures a second modeling test should be performed with $K_{P,F} = 1000$ in order to investigate the influence on partitioning on the predicted migration results.

The third important parameter in the exposure evaluation of PET is the concentration of oligomers in PET ($C_{P,0}$). These values are often not available from the scientific literature or might change in the future due to increase recyclate content or optimized production conditions. Calculation of the maximum concentration of oligomers in PET solves this problem, and as a positive side effect, reliable quality control parameters are available for production control. The evaluation of $C_{P,0}$ required to reach safety threshold can be a very useful way of exposure assessment by helping to identify conditions that are more or less likely to be reached and therefore associated with higher or lower risk for the consumer, respectively. This approach can also be readily applied to evaluate the relevance of concentrations associated with observed adverse effects or for other populations with lower safety limits. Additionally, it can further be used for other migrants in PET as PET oligomers.

As an overall conclusion of this study, migration modeling represents a very helpful tool for a systematic exposure evaluation and prioritization of oligomers and/or conditions of concern. It provides a fast, comparable, and comprehensive overview that can easily be used for risk assessment purposes on the migration properties of PET oligomers besides the lack of migration testing data. In addition to the generally known evidence that heating applications and small molecules are of greater concern than low temperature conditions and large oligomers, this study could provide specific numbers needed for a comprehensive exposure assessment for this particular group of substances.

In silico and in vitro evaluation of PET oligomers

Oligomers that cannot be obtained commercially pose a challenge in identifying substances that may pose a risk to consumers. *In silico* approaches can be used to analyze and predict the properties and behaviors of these molecules without the need for experimental testing, allowing for the prioritization of substances for further evaluation and confirmation of the *in silico* results.

Therefore, toxicological *in silico* predictions were performed for 88 PET oligomers using VirtualToxLab (VTL), that have either been unambiguously identified from literature (34 molecules) or based on literature been theoretically defined (54 molecules). The reasoning for the structure of the hypothetical oligomers is based on the molecular weight, reported oligomers, and the chemistry used for synthesis.
Of the 88 PET oligomers analyzed, *in silico* results obtained from the VTL platform predicted that 21 molecules may potentially affect nuclear receptors, warranting further evaluation (Table 3). Of these molecules, seven oligomers seemed to be commercially available and were ordered for further evaluation *in vitro*. The *in vitro* testing is currently ongoing.

Publications, posters and presentations

Publications

Birgit Geueke*, Ksenia J. Groh, Maricel V. Maffini, Olwenn V. Martin, Justin M. Boucher, Yu-Ting Chiang, Frank Gwosdz, Phoenix Jieh, Christopher D. Kassotis, Paulina Łańska, John Peterson Myers, Alex Odermatt, Lindsey V. Parkinson, Verena N. Schreier, Vanessa Srebny, Lisa Zimmermann, Martin Scheringer, and Jane Muncke. **Most Chemicals detected in Food Contact Materials are not listed for Use: Systematic Evidence on migrating and extractable Food Contact Chemicals.** * corresponding author. *Crit. Rev. Food Sci.,* **2022**, 10.1080/10408398.2022.2067828.

Verena N. Schreier*, Christian Appenzeller-Herzog, Beat J. Brüschweiler, Birgit Geueke, Martin F. Wilks, Thomas J. Simat, Benoit Schilter, Martin Smieško, Jane Muncke, Alex Odermatt, Nicolas Roth. **Evaluating the food safety and risk assessment evidence-base of polyethylene terephthalate oligomers: Protocol for a systematic evidence map**. *corresponding author. *Environ. Int*., **2022**, 167, 107387.

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Posters

Conference ONE – Health, Environment, Society, 2022: Systematic mapping of chemicals known to migrate or be extracted from food contact materials and articles. Birgit Geueke, Justin Boucher, Yu-Ting Chiang, Ksenia Groh, Frank Gwosdz, Phoenix Jieh, Christopher Kassotis, Maricel V. Maffini, Olwenn V. Martin, Alex Odermatt, Lindsey Parkinson, Verena N. Schreier, Vanessa Srebny, Lisa Zimmermann, Jane Muncke

***Conference ILSI, 2022**: A systematic evidence map of chemicals known to migrate or be extracted from food contact materials and articles. Birgit Geueke, Justin Boucher, Yu-Ting Chiang, Ksenia Groh, Frank Gwosdz, Phoenix Jieh, Christopher Kassotis, Maricel V. Maffini, Olwenn V. Martin, Alex Odermatt, Lindsey Parkinson, Verena N. Schreier, Vanessa Srebny, Lisa Zimmermann, Jane Muncke. * selected for oral presentation by J. Muncke.

Annual Research Meeting of the University of Basel, 2022: Black Box: Toxicology of Oligomers from Food Contact Materials. Verena N. Schreier, Beat J. Brüschweiler, Benoit Schilter, Martin Smieško, Martin F. Wilks, Thomas J. Simat, Frank Welle, Birgit Geueke, Jane Muncke, Alex Odermatt.

Conference Swiss Society of Toxicology, 2022: Black Box: Toxicology of Oligomers from Food Contact Materials. Verena N. Schreier, Beat Brüschweiler, Benoit Schilter, Martin Smieško, Martin F. Wilks, Thomas J. Simat, Frank Welle, Birgit Geueke, Jane Muncke, Alex Odermatt

Project

Project 4.21.01. “ToxOligo”. Order number 0714001652.

Project duration

The project was conducted over a period of two years with the start date of 01.03.2021 and end date 28.02.2023.

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