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Risk assessment for migration of styrene oligomers into food from polystyrene food containers

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ABSTRACT

Regulation EU 10/2011 requires a risk assessment of Non Intentionally Added Substances (NIAS) migrating into food for food contact plastics within the EU. Styrene oligomers are important potential components of NIAS in polystyrene used for food packaging and so far only dimers and trimers have been identified. They are not genotoxic in vitro, and there is good evidence that they are not endocrine disruptors. Hazard characterization to establish "safe" exposure levels is based on 1. The No Adverse Effect Level (NOAEL) of 1 mg/kg bw/d in an oral rat study during pregnancy and lactation and 2. The concept of Threshold of Toxicological Concern (TTC). Likely human exposure is derived from 1. the concentrations of dimers and trimers in food simulants or 2. in food and 3. the probabilistic FACET exposure estimation based on dimer and trimer concentrations in polystyrene and their potential for migration. The Margin of Safety as the relation of potential consumer exposure and the "safe" exposure level was always above 1 (apart from migration with 95% ethanol which is no longer recommended as an official food simulant for overall migration into fatty food) demonstrating that dimers and trimers in PS food packaging present a low risk for consumers.

1. Introduction/scope

Polystyrene (PS) is widely used in food contact materials. General Purpose Polystyrene (GPPS) and High Impact Polystyrene (HIPS) are used for a wide range of food packaging purposes, with the biggest single application being yogurt pots. Further applications are single use disposable kitchenware such as plates and knives and forks. GPPS and HIPS are also employed in kitchen equipment, with applications in refrigerators being the most significant. Expanded Polystyrene (EPS) is also used in food contact materials, mainly for refrigerated and frozen food, but these specific applications are not the focus of the present assessment because.

- only relatively few analytical data are available
- and its use in different product types with different applications.

Therefore, the present paper concentrates on non-expanded PS.

Oligomers of styrene are known to be present in PS. These are either formed as by-products by incomplete polymerization during production of PS or by degradation after irradiation or thermal treatment of PS within downstream applications (Hoppe et al., 2016). Kawamura et al. (1998b) identified and characterized the quantitatively most important dimers (Di) and trimers (Tri) in food containers (Fig. 1). Generally the abbreviations assigned by the researchers of Nissin Food Products Co., Ltd (e.g. Yamada, 1999) will be used throughout this review. In the large number of studies available, only the Di and Tri of Fig. 1 were found, whereas higher oligomers have not been identified in PS. After dissolution of PS and subsequent precipitation of the polymeric material, Di and Tri accounted for ~90% (Prinsen and Gouko, 2001; Nakai et al., 2014) of all oligomers in PS while Weel (2016a) did not observe any higher oligomers apart from Di and Tri up to the molecular weight

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List of abbreviations

ADI	Acceptable Daily Intake
AF	Assessment Factor
D1	1,3-diphenylpropane
Di	dimers of styrene
EPS	Expanded Polystyrene
gd	gestation day
GLP	Good Laboratory Procedures
GPPS	General Purpose Polystyrene
HIPS	High Impact Polystyrene
IAS	Intentionally Added Substances
Lab-report	full laboratory report of the study of Nagao et al. (2000).
LOAEL	Low Adverse Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification
MoS	Margin of Safety
MTD	Maximally Tolerable Dose
N	number of experimental animals
NA-study	publication of Nagao et al. (2000).
NIAS	Non Intentionally Added Substances
NOAEL	No Adverse Effect Level
NSD-01	2,4-diphenyl-1-butene
NSD-08	cis-1,2-diphenylcyclobutane
NSD-09	trans-1,2-diphenylcyclobutane
NST-01	2,4,6-triphenyl-1-hexene
NST-02	1,3,5-triphenyl-cyclohexane (mixture of isomers)
NST-03	1-phenyl-4-(1-phenylethyl) tetralin
NST-03-1	1e-phenyl-4e-(1-phenylethyl) tetralin
NST-03-2	1a-phenyl-4e-(1-phenylethyl) tetralin
NST-03-3	1a-phenyl-4a-(1-phenylethyl) tetralin
NST-03-4	1e-phenyl-4a-(1-phenylethyl) tetralin
NST-12	1-phenyl-4-(2-phenylethyl) tetralin (mixture of isomers)
NST-13	(1S*,6R*,7S*,8S*,11R*)-6,11-diphenyltricyclo[6,2,2,0 $2^{2,7}$] dodeca-2,9-diene
OECD TG	Test Guidelines of the OECD
OPS	Oriented Polystyrene
pnd	postnatal day
POD	Point of Departure
PS	polystyrene
PSP	Polystyrene Paper
SDT	Sum of dimers and trimers of styrene
SML	Specific Migration Limit
Tri	trimers of styrene
TTC	Threshold of Toxicological Concern
XPS	Extruded Polystyrene

range equivalent to styrene hexamers. Thus, at most only low amounts of higher oligomers are expected to be present in PS used for food packaging and their resulting relevance for consumer exposure is expected to be negligible as migration decreases with increasing molecular weight. Therefore the present assessment concentrates on Di and Tri.

PS used in applications with food contact is subject to an in-depth safety assessment according to EU (2011). PS will contain Intentionally Added Substances (IAS) which are known ingredients such as residual monomeric styrene, solvents, suspension media, etc. Besides IAS, all polymers also contain Non Intentionally Added Substances (NIAS) comprised of reaction/degradation products formed during the manufacturing process. Oligomers, and in this context especially Di and Tri, are an important part of NIAS that may significantly contribute to the total migrate from PS. According to Article 19 of EU (2011) the potential risk to health of NIAS also has to be assessed which requires a safe level of exposure to be determined in terms of an Acceptable Daily Intake (ADI) or a Specific Migration Limit (SML). Safety assessment of NIAS is challenging because very often comprehensive toxicological data are not available comparable to those that are to be provided for existing substances, for example registered under REACH. In addition, there may be challenges in isolating NIAS in sufficient purity and quantities for toxicity testing. Fortunately, there are other approaches that may be used such as the concept of the Threshold of Toxicological Concern (TTC). A valuable guidance on how to deal with and assess NIAS was published by ILSI (2015). A general cut-off criterion given by EU (2011) is that migration of a substance should not be detectable at a Limit of Detection of 10 µg/kg food (or food simulant). This limit was not derived on the basis of toxicological considerations, but rather on the current state of analytical technology. Because the concentrations of Di and Tri and the sum of both (SDT) often exceed this limit, especially for migration into certain more aggressive food simulants, a toxicological assessment is performed.

-This paper describes the safety assessment of Di, Tri and SDT by the following approaches: first“safe” exposure levels are established using both a NOAEL of 1 mg/kg bw/d (established in a comprehensive rat study with oral exposure during pregnancy and lactation) and by applying the concept of TTC according to EFSA (2012b). Then the likely consumer exposure levels are determined in a tiered approach with 3 levels of increasing refinement. The relationship of the “safe” exposure level to the potential consumer exposure determines the Margin of Safety (MoS). A MoS of > 1 indicates a low risk for consumers.

A literature search was carried out with the key words styrene oligomers, dimers and trimers to completely cover the published literature on these substances up to January 2018. The following databases were used: Chemlist, Registry, Embase and Toxcenter. Only studies measuring Di, Tri and SDT in food simulants, food items and PS were used for the present purpose as well as investigations in experimental animals excluding screening studies related to potential endocrine effects that were the basis of a separate review (Gelbke et al., 2018). Meeting abstracts were excluded from this assessment.

2. The study of Nagao et al. (2000)

Nagao et al. (2000) (referred to subsequently as the NA-study) published an investigation with a mixture SDT obtained by dissolution of GPPS in acetone, precipitation of the polymeric material with methanol and subsequent liquid chromatographic fractionation with methanol and methanol/acetone (1:1) to separate SDT from higher molecular weight polymers. The relationship of Di and Tri was approximately 1:10 and about 10% of the test material consisted of higher oligomers. The NA-study primarily aimed at investigating potential endocrine effects of SDT. Pregnant female rats (F0) were exposed to the SDT mixture from gestation day (gd) 6 to postnatal day (pnd) 21 at weaning. The offspring (F1) was not further exposed and a variety of developmental parameters was recorded. F1 animals were mated at the age of 11 weeks and their offspring (F2) was kept until weaning at pnd 21. SDT was administered daily by gavage in corn oil at dose levels of 0, 0.04, 0.2 and 1.0 mg/kg bw/d. The highest dose level was by a factor of 1000 higher than the estimated maximum daily intake of humans from instant noodles prepared in PS noodle cups in Japan based on the study of Kawamura et al. (1998a). No adverse effects were observed in the NA-study. However, the publication concentrated mainly on effects related to potential endocrine activities of SDT and further details were not provided. Therefore more details were extracted from the original laboratory (Lab) report (Ono et al., 1999) obtained from the Japan Styrene Industry Association (JSIA).

The Lab-report enabled a comparison of the test design with current OECD test guidelines (details are given in the electronic supplement).

OECD TG 443 (2015a), extended 1-generation study.

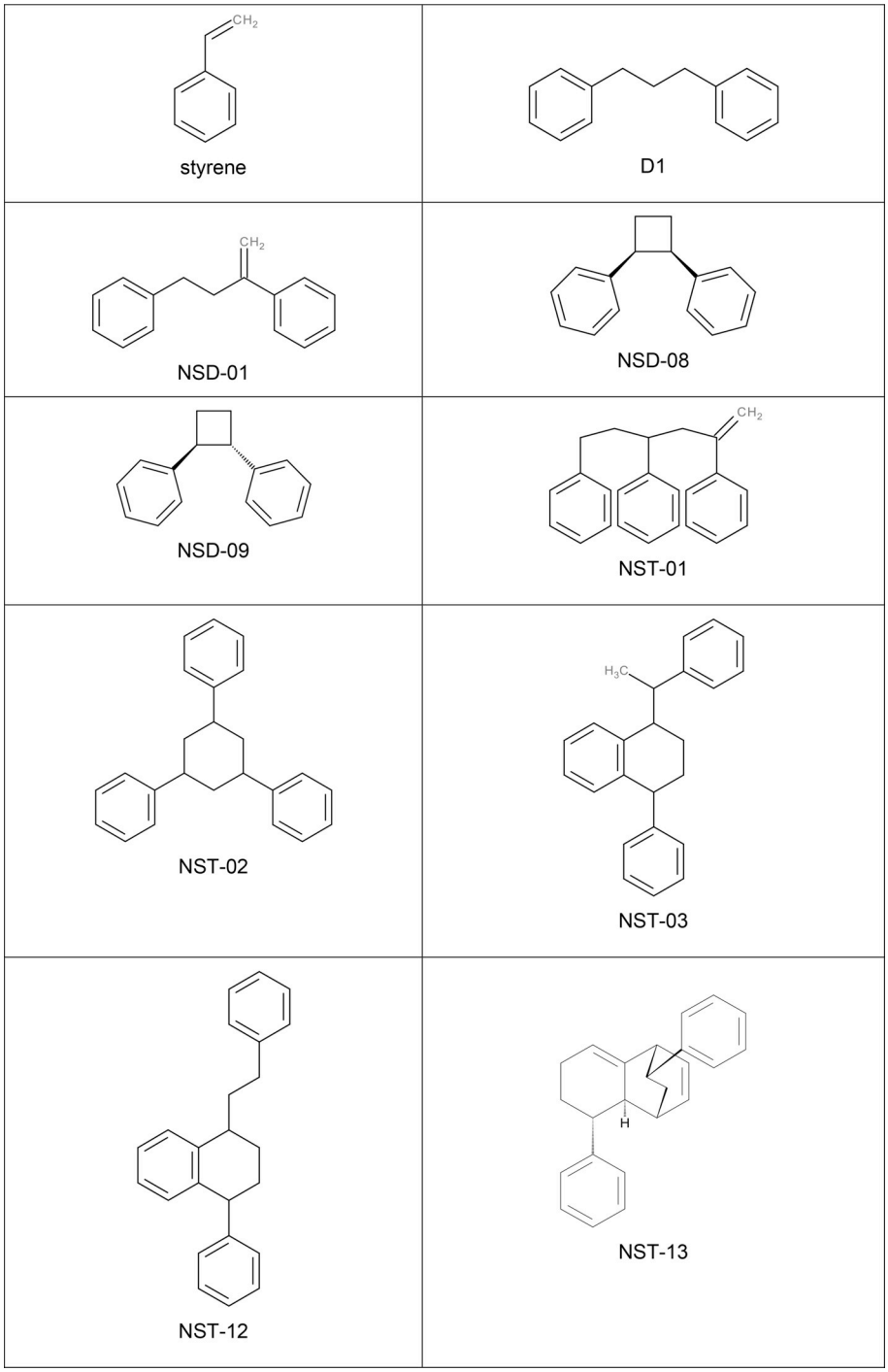

Fig. 1. Chemical structures of styrene dimers and trimers. The percentages of the different Di and Tri in relation to the sum of Di + Tri in PS food packaging materials are given in square brackets for non-expanded PS.
LOQ: limit of quantification D1: 1,3-diphenylpropane (CAS: 1081-75-0) [0.15–0.53%; often below LOQ]
NSD-01: 2,4-diphenyl-1-butene (CAS: 16 606-47-6) [0.16–7.96%]
NSD-08: cis-1,2-diphenylcyclobutane (CAS: 7694-30-6) [0.12–0.80%; often below LOQ]
NSD-09: trans-1,2-diphenylcyclobutane (CAS: 20071-09-4) [1.31–5.96%]
NST-01: 2,4,6-triphenyl-1-hexene (CAS: 18964-53-9) [11.3–38.3%]
NST-02: 1,3,5-triphenyl-cyclohexane (mixture of isomers) (CAS: 28336-57-4 for cis/trans isomer) [0.26–6.2%; often below LOQ]
NST-03: 1-phenyl-4-(1-phenylethyl) tetralin (CAS: 26681-79-8), sum of isomers [50.2–88.5%] with the following isomers if specifically analyzed
NST-03-1: 1e-phenyl-4e-(1-phenylethyl) tetralin [15.0–26.1%]
NST-03-2: 1a-phenyl-4e-(1-phenylethyl) tetralin [20.0–33.9%]
NST-03-3: 1a-phenyl-4a-(1-phenylethyl) tetralin [8.22–18.2%]
NST-03-4: 1e-phenyl-4a-(1-phenylethyl) tetralin [6.5–19.3%]
NST-12: 1-phenyl-4-(2-phenylethyl) tetralin: (mixture of isomers) [3.49–10.7%]
NST-13: (1S*,6R*,7S*,8S*,11R*)-6,11-diphenyl-tricyclo [6,2,2,0 $2^{2,7}$] dodeca-2,9-diene [only measured in 1 PSP and one HIPS sample being below LOQ and 0.70%, respectively].

OECD TG 415 (1983), 1-generation study.

OECD TG 422 (2015b), combined repeated dose/reproduction/developmental screening test.

OECD TG 421 (2015c), reproduction/developmental screening test.

A comparison with current OECD test guidelines adopted in 2015 revealed many strengths but also some significant weaknesses that may limit its value for some regulatory purposes beyond the scope of this risk assessment. Important strengths include:

- Three dose levels allowing a decision whether effects at low doses may have occurred possibly by chance
- A larger number of pregnant F0 females to assess reproductive success in comparison to OECD TG 443 and 415 or TG 422 and 421
- Detailed investigations in F1 offspring (albeit only exposed in utero and during lactation) for neurophysiological behavior and developmental landmarks, determination of T3, T4 and TSH and reproductive success.

Significant deficiencies include:

- The highest dose (1 mg/kg bw/d) was derived by risk considerations based on estimated consumer exposures in Japan, but a level leading to slight toxicity (in the sense of MTD) was not included
- No exposure or investigations of male F0 animals
- No premating exposure of F0 females with exposure starting on gd 6
- Exposure of F1 offspring only in utero and during lactation

- No hematology or clinical biochemistry in F0 animals
- Limited histopathology and organ weight determinations

In summary, the NA-study and Lab-report enables the conclusion that SDT do not affect reproductive health after in utero and lactational exposure up to the production of the F2 generation at dose levels exceeding likely consumer exposure by a factor of 1000. It is recognized that it is not possible to draw conclusions about the inherent hazard of SDT since standard parameters required to evaluate the impact of SDT after subacute/subchronic exposure of adult animals are missing to a large extent. However, this does not detract from the adequacy of this study for the objective of this assessment. In addition, it is noted that external and visceral abnormalities were assessed in a large number of F1 offspring. Although skeletal abnormalities could not be evaluated, this data supports the conclusion that SDT do not affect prenatal development to a significant extent at the dose levels administered.

2.1. Derivation of a safe exposure level according to ECHA (2012) and EFSA (2012a) based on the NA-study and the lab-report

First an assessment is necessary on whether SDT need to be considered as genotoxic or not. As EFSA (2012b) primarily asks for structural alerts for genotoxicity, the database of Ashby and Tennant (1991) was consulted and no clear structural alerts could be identified. Only NSD-01 and NST-01 might be associated with the group of "compounds classed as non-alerting in structure but with minor concerns" because of an allylic-like substructure. In case of structural alerts EFSA proposes genotoxicity testing (or read across). EFSA (2012b) further discussed the use of genotoxicity prediction tools without being able to give guidance which ones to use. As the predictive value of most of these tools are comparable to the Ames test we decided to rely on actual results of in vitro tests.

Grifoll et al. (1990) did not observe mutagenic activity of Di and Tri isomers (no further specification) when tested in *S. typhimurium* strain TA 98 with metabolic activation up to a concentration of 1 mg/plate. Nakai et al. (2014) studied SDT obtained by extraction of GPPS with acetone and precipitation of higher molecular weight oligomers and polymers with methanol. The SDT mixture was investigated in the Ames test using the pre-incubation method with the *S. typhimurium* strains TA 100, TA 1535, TA 98 and TA 1537 and the *E. coli* strain WP2uvrA and in the in vitro chromosomal aberration test with Chinese hamster lung cells according to the OECD guidelines TG 471 and 473, respectively. The tests were carried out with and without metabolic activation. The concentrations reached 5 mg/plate in the bacterial test and led to substance precipitation in the chromosomal aberration test. The concentrations of Di and Tri in the extract were 540 and 13,431 ppm, a relation of ~1:24 in a similar range as that in the NA-study. Of the total extract, about 88% could be assigned to specific structures of known Di and Tri and 12% were of tetrameric or greater size. As no indication for genotoxicity (including absence of aneugenicity) was obtained, the application of numerical assessment factors (AF) for risk assessment is justified.

Derivation of a long-term oral DNEL for the general population potentially exposed to SDT migrating from PS food containers into food follows the approach of ECHA (2012) with the interspecies AF for rats to humans of 10 (containing the factor of 4 for allometric scaling and that of 2.5 for remaining differences) and the intraspecies AF of 10 for the general population. While the NA-study and the Lab-report comprehensively addressed apical reproductive endpoints, also including to a major part reproductive toxicity, the study was not designed to investigate repeated dose toxicity per se although the F0 females were exposed from gd 6 to pnd 21 over a period of about 5 weeks, corresponding roughly to the duration of a subacute study. Parental F0 males were not exposed. The point of departure (POD) for repeated dose toxicity assessment will therefore be based upon the NOAEL for effects on weights of sex organs and of potential major target organs, i.e. brain, liver and kidney in parental females. Extrapolation from subacute to chronic exposure would require an AF of 6 according to ECHA (2012). It is proposed to increase this AF to 10 to account for uncertainties arising from the limited observations in adult animals. In this respect the very low NOAEL of 1 mg/kg bw/d should also be taken into consideration; as in this study no LOAEL was obtained, using the highest dose without adverse effect clearly is a conservative approach per se as the "real" NOAEL may be much higher. In addition, the structure of SDT does not contain any element indicative of a propensity for specific toxicity (Fig. 1). The factor of 10 is also considered sufficiently conservative for males, because no effects were noted in F1 males at the age of 10 weeks after exposure during the very vulnerable life phases in utero and during lactation. In these F1 males, organ weight and histopathology data are available for the most sensitive organs, namely sex organs, brain, liver, kidneys, adrenal glands, thyroid glands and pituitary gland. These subfactors lead to a total AF of 1000 that is the same as that used in the NA-study for definition of the highest exposure dose. Under these considerations the long-term oral DNEL for the general population would be 1 μg/kg bw/d.

Similar considerations apply using the EFSA (2012a) guidance for default values. Again for inter- and intraspecies variability an AF (here called uncertainty factor, UF) each of 10 is appropriate. In contrast to the approach used by ECHA, no specific factors are proposed by EFSA to account for study duration i.e. extrapolation from subacute to chronic exposure, or for deficiencies in the database. Reflecting the considerations above, an additional factor of 10 is again considered appropriate. Thereby also the approach of EFSA (2012a) would lead to a "safe" exposure level via food of 1 μg/kg bw/d.

A minor uncertainty should be mentioned for dose transformation from a μg/kg bw/d basis to μg/person/d based on differences of default body weights. EFSA (2012a) proposed to use a body weight of 70 kg for adults. EU (2011) takes the consumption of 1 kg food/d by a person of 60 kg body weight for setting migration limits. A body weight of 60 kg (for adults) also is the basis of the TTC concept, and ECHA (2012) proposes 60 kg for the general population and 70 kg for workers. Therefore, in the context of this assessment the default approach according to EU (2011) is used, i.e. food uptake of 1 kg/d and a body weight of 60 kg for adults.

3. Application of the Threshold of Toxicological Concern (TTC) based on EFSA (2012b)

In the NA-study the highest dose level was a NOAEL and a LOAEL was not obtained. Under consideration that the NOAEL of 1 mg/kg bw/d is quite low for SDT with no obvious toxicophores, this has to be considered as an important data gap leading to major uncertainties for the definition of the POD and risk characterization. Therefore the TTC concept was used as a second independent approach. EFSA (2012b) recommended the TTC approach as a useful screening tool for substances with few or no relevant toxicity data for deciding whether the probability of adverse health effects is very low, for example for the assessment of impurities, reaction products etc.

EFSA (2012b) lists several categories of substances to which the TTC should not be applied that are not relevant to SDT, such as metals, high potency carcinogens, or nanomaterials. This list also includes substances with a potential for bioaccumulation. The physical chemical properties of SDT do not indicate in this direction and experimental data show a rapid excretion: Nagao et al. (2000) refer to an abstract (Aiba et al., 1998; in Japanese language) reporting that after oral administration of 20 mg/kg bw of Di or Tri to rats, plasma levels of the unchanged substances reached a maximum after 2–4 h and then decreased rapidly. After 24 h the plasma concentrations were below the limit of detection (LOD). Therefore, a potential for bioaccumulation is not to be expected.

Furthermore, the TTC concept is only applicable for substances for which the chemical structure is known. Therefore the question arises as

to the chemical structure of the SDT mixture. It was shown by Nakai et al. (2014) that of the mixture they tested for genotoxicity, about 88% could be assigned to known structures of 4 dimers and 5 trimers and that 12% accounted for tetramers or higher oligomers. Therefore, although SDT is a mixture of several substances possibly with some quantitative variability of composition, the structures of the main components are well defined and the relation of Di to Tri are in a similar range when comparing the SDT composition of the studies of Nagao et al. (2000) and Nakai et al. (2014). For mixtures it is further required that these should contain only substances with closely related chemical structures (and mixtures with substances of unknown structure should be excluded), but then dose additivity should be assumed and the exposures should be summed as was done for the groups of Di and Tri.

Next it has to be decided what would be the appropriate TTC level. As SDT are not genotoxic (Nakai et al., 2014) a decision remains as to which Cramer class SDT should be assigned to. The original TTC concept contained the Cramer classes I, II and III with decreasing TTCs of 30, 9 and 1.5 μg/kg bw/d (Kroes et al., 2004). According to EFSA (2012b), class II is not well supported and it was therefore merged with class III using the lower TTC of 1.5 μg/kg bw/d. By their structure containing 2 or 3 aromatic rings, SDT would fall into the merged class with a TTC of 1.5 μg/kg bw/d.

EFSA (2012b) concluded that the TTC approach can also be applied to endpoints of specific concern, like effects on the nervous system, immune system, endocrine system and reproduction/development that partly are not covered in sufficient detail by the NA-study and the Lab-report. In order to use this system for all age groups, including children at an age of < 6 months with their higher food uptake, exposure should be related to body weight, i.e. for class II/III 1.5 μg/kg bw/d.

Finally, a decision has to be made whether a safe exposure level should be based on the TTC concept (1.5 μg/kg bw/d) or on the basis of the NOAEL of the NA-study (1 μg/kg bw/d) using AFs of ECHA (2012) or EFSA (2012a). The latter approach is partly compromised by the incomplete database for F0 animals, the necessary extrapolation from subacute to chronic exposure and especially by uncertainties of the NOAEL in to the absence of a LOAEL. Starting from the highest dose as NOAEL implicitly implies a conservativism, the magnitude of which cannot be estimated. In this context the observation of EFSA (2012b) should be taken into consideration that when "a TDI has been set by the SCF or by EFSA on the basis of oral toxicity data … the TTC approach was found to be more conservative than the risk assessment based oral toxicity data". Therefore, derivation of an ADI or SML according to the TTC concept is considered the preferred approach.

3.1. Applicability of TTC for SDT and endocrine effects

The question whether SDT may exhibit endocrine mediated toxicity warrants a more detailed discussion since Kroes et al. (2004) concluded that for such effects the TTC approach might not be applicable. EFSA (2012b) describes two circumstances that might be relevant for SDT:

- "If there are data showing that a substance has endocrine activity, but the human relevance is unclear, then these data should be taken into consideration, case-by-case, in deciding whether or not to apply the TTC approach.
- If there are data showing that a substance has endocrine-mediated adverse effects, then, as would be the case for adverse data on any other endpoint, the risk assessment should be based on the data, rather than the TTC approach."

By the definition of EFSA (2013) the first indent corresponds to an Endocrine Active Substance and the second one to an Endocrine Disruptor. The major discriminating criterion is that endocrine disruptors lead to adverse effects while an endocrine active substance may have the inherent ability to interfere with hormonal regulation without clear adverse effects.

While the NA-study did not give any indication for endocrine mediated effects of SDT, there are two investigations claiming endocrine activity for some specific Tri, namely those of Ohyama et al. (2007) and Yanagiba et al. (2008). One important difference in comparison to the NA-study is that in the NA-study a mixture of SDT was investigated that consumers may be exposed to while Ohyama et al. (2007) and Yanagiba et al. (2008) concentrated on specific Tri not reflecting the potential impact of "real world" exposures. The basic study designs were as follows:

- Ohyama et al. (2007): test compounds NST-01 (47.4% of SDT in NA-study), NST-03-2 (23.3% of SDT in NA-study) and NST-03-4 (4.4% of SDT in NA-study). Subcutaneous injection to pregnant rats from gd 11 to 17 at dose levels of 0, 10, 100 and 1000 μg/kg bw/d. Number of dams between 4 and 8 depending on dose and test substance. Male offspring were kept without further treatment until pnd 101–103.
- Yanagiba et al. (2008): test compound NST-01 (47.4% of SDT in NA-study). Oral dosing of mice over 4 days at dose levels of 0, 32 and 64 μmol/kg (corresponding to 10 and 20 mg/kg bw/d). Eight wild type mice and 5 transgenic mice/dose level (the results with transgenic mice are not further taken into consideration).

The major findings reported by Ohyama et al. (2007) were related to NST-03-4 being quantitatively the least important Tri. The effects noted (decreased anogenital distance, decreased male genital organ weights, reduced blood levels of LH and FSH accompanied by increased serum testosterone) were interpreted by the authors as an indication for estrogenicity, although a clear dose response relationship was mostly missing. Yanagiba et al. (2008) observed an increase of circulating thyroxine (T4) levels not accompanied by alterations of T3 or TSH.

We compared the results of the studies of Nagao et al. (2000), Ohyama et al. (2007) and Yanagiba et al. (2008) in a separate publication (Gelbke et al., 2018). The weight of evidence assessment, which also included the broad database from in vitro and in vivo screening studies, led to the conclusion that SDT are not endocrine disruptors. However, using the definitions of EFSA (2013) it cannot be excluded that some of the SDT may have endocrine active properties, albeit these results came from non-guideline screening studies at high concentrations and hence have questionable reliability and relevance. On the basis of the total database described by Gelbke et al. (2018) and the robust and very detailed NA study, the application of the TTC concept is sufficiently justified.

4. Application of a more recent TTC proposed by Leeman et al. (2014)

Already EFSA (2012b) acknowledged that "removing organophosphate and carbamate substances from Cramer Class III (being the most potent substances in that class) would have an impact on the existing TTC value for this Class III." But it was decided that "pending any future revision of the TTC approach, …it would be prudent to maintain the value for Cramer Class III at 90 μg/person per day." The Cramer class III contains among others organophosphate insecticides that are potent cholinesterase inhibitors. For this subgroup a lower TTC of 0.3 μg/kg bw/d was derived (Munro et al., 1999; Kroes et al., 2004). If this subgroup is subtracted from the rest of the Cramer class III substances, a higher TTC would be obtained for the remaining chemicals (Munro et al., 2008) but neither Kroess et al. (2004) nor EFSA (2012b) defined a TTC for groups of substances without organophosphates.

Leeman et al. (2014) calculated TTCs for organophosphates + carbamates (40 substances) and organohalogens (166 substances) as groups of substances with higher toxicity to be 0.30 and 1.5 μg/kg bw/d. As expected, for the remaining Cramer class III substances (242 substances) the TTC was clearly higher with 4.0 μg/kg bw/

Table 1
Mean amounts of different Di and Tri measured in food simulants and food (in mg/kg food; mg/6 dm^2 for food simulant). Their percentages related to the sum of SDT are given in brackets.

Author	Media	Sum of SDT	Dimers, mean (% of SDT)				
			D1	NSD-01	NSD-08	NSD-09	Sum of NSD-08 + 09
Concentrations of SDT by extraction of PS materials with food simulants							
OFI (2017a)	GPPS, 50%, 40 °C, 10 d	0.234	< 0.011	Nd	Nd	0.034	
	GPPS, 95%, 60 °C, 10 d	0.732	< 0.011	Nd	Nd	0.332	
OFI (2017b)	HIPS, 50%, 40 °C, 10 d	0.9	< 0.011	Nd	Nd	0.3	
	HIPS, 95%, 60 °C, 10 d	2.4	0.01	Nd	Nd	1.4	
Weel (2016c)	GPPS, 20%, 40 °C, 10 d	0.018	0.0005[1] (2.8)	0.0005[1] (2.8)	Nsa	Nsa	0.005 (27.8)
	GPPS, 50%, 40 °C, 10 d	0.162	0.0005[1] (0.31)	0.001 (0.62)	Nsa	Nsa	0.014 (8.6)
	HIPS, 20%, 40 °C, 10 d	0.006	0.0005[1] (8.3)	0.0005[1] (8.3)	Nsa	Nsa	0.001 (17)
	HIPS, 50%, 40 °C, 10 d	0.197	0.0005[1](0.25)	0.001 (0.51)	Nsa	Nsa	0.021 (10.7)
Weel (2016a)	HIPS, rod 95%, 40 °C, 10 d	0.936	< 0.01 (< 1.1)	0.016 (1.71)	Nsa	Nsa	0.13 (13.9)
	HIPS, sheet 95%, 40 °C, 10 d	0.265	< 0.01 (< 3.8)	Nd	Nsa	Nsa	0.069 (26.0)
Concentrations of SDT in food							
Genualdi et al. (2014)	7 food items	< 0.0013	Up to 0.005 (38.5)	0.001[2] (7.7)[b]7/7	Nsa	0.001[2] (7.7)[b]7/7	
Kawamura et al. (1998d)	8 noodle samples	0.030	Nsa	0.0025[3] (8.3)[b]8/8	Nsa	0.0025[3] (8.3)[b]8/8	
Kawamura et al. (1998a)	34 instant foods	0.024	Nsa	0.0025[3] (10.4)[b]34/34	Nsa	0.0025[3] (10.4)[b]34/34	
Yamada et al. (2000b)	17 noodle soups in conventional PS	0.0142	Nsa	0.0007[4] (4.9)[b]4/17	0.0001[4] (0.7)[b]17/17	0.0004[4] (2.8)[b]6/17	
	9 noodle soups in improved PS	0.004	Nsa	0.0004 (10)[b] 4/9	0.0001 (2.5) 9/9[b]	0.0001 (2.5) 8/9[b]	
Kaneko et al. (2003) [c]	30 noodles	0.0205	0.0005[5] (2.44)[b]30/30	0.0006[5] (2.9)[b]20/30	0.0005[5] (2.44)[b]30/30	0.0006[5] (2.9)[b]18/30	
	24 soups	0.0307	0.0005[5] (1.63)[b]24/24	0.0009[5] (2.93)[b]8/24	0.0005[5] (1.63)[b]24/24	0.0009[5] (2.93)[b]14/24	

Author	Media	Sum of SDT	Trimers, mean (% of SDT)												
			NST-01	NST-02	ST-6	ST-7	NST-03	NST-03-1	NST-03-2	NST-03-3	NST-03-4	NST-12	NST-12-1	NST-12-1	NST-13
Concentrations of SDT by extraction of PS materials with food simulants															
OFI (2017a)	GPPS, 50%, 40 °C, 10 d	0.234	Nd	Nd	Nd	Nd	0.2	Nsa	Nsa	Nsa	Nsa	Nd	Nd	Nd	Nd
	GPPS, 95%, 60 °C, 10 d	0.732	Nd	Nd	Nd	Nd	0.4	Nsa	Nsa	Nsa	Nsa	Nd	Nd	Nd	Nd
OFI (2017b)	HIPS, 50%, 40 °C, 10 d	0.9	Nd	Nd	Nd	Nd	0.6	Nsa	Nsa	Nsa	Nsa	Nd	Nd	Nd	Nd
	HIPS, 95%, 60 °C, 10 d	2.4	Nd	Nd	Nd	Nd	1.0	Nsa	Nsa	Nsa	Nsa	Nd	Nd	Nd	Nd
Weel, 2016c	GPPS, 20%, 40 °C, 10 d	0.018	0.001 (5.6)	0.0005[a] (2.8)	Nsa	Nsa	0.011 (61.1)	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nd
	GPPS, 50%, 40 °C, 10 d	0.162	0.013 (8.0)	0.009 (5.6)	Nsa	Nsa	0.125 (77.2)	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nd
	HIPS, 20%, 40 °C, 10 d	0.006	0.0005[a] (10)	0.0005[a](10)	Nsa	Nsa	0.003 (60)	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nd
	HIPS, 50%, 40 °C, 10 d	0.197	0.022 (11.2)	0.012 (6.1)	Nsa	Nsa	0.141 (71.6)	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nd
Weel (2016a)	HIPS, rod 95%, 40 °C, 10 d	0.936	0.101 (10.8)	0.079 (8.4)	Nsa	Nsa	0.610 (65.1)	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nd
	HIPS, sheet 95%, 40 °C, 10 d	0.265	0.023 (8.7)	0.013 (4.9)	Nsa	Nsa	0.160 (60.4)	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nsa	Nd

(*continued on next page*)

Table 1 (*continued*)

Author	Media	Sum of SDT	Trimers, mean (% of SDT)												
			NST-01	NST-02	ST-6	ST-7	NST-03	NST-03-1	NST-03-2	NST-03-3	NST-03-4	NST-12	NST-12-1	NST-12-1	NST-13
Concentrations of SDT by extraction of PS materials with food simulants															
Concentrations of SDT in food															
Genualdi et al. (2014)	7 food items	< 0.0013	0.001[2] (7.7)[b] 7/7	0.001[2] (7.7)[b]7/7	Nsa	Nsa		0.001[2] (7.7)[b]7/7	0.001[2] (7.7)[b]7/7	0.001[2] (7.7)[b]7/7	0.001[2] (7.7)[b]7/7	Nd	Nd	Nd	Nd
Kawamura et al. (1998d)	8 noodle samples	0.030	0.006 (20.0)[b] 5/8	Nsa	Nsa	Nsa	0.019 (63.4)[b]3/8	Nsa	Nsa	Nsa	Nsa	Nd	Nd	Nd	Nd
Kawamura et al. (1998a)	34 instant foods	0.024	0.005 (20.8)[b] 20/34	Nsa	Nsa	Nsa	0.014 (58.3)[b]15/34	Nsa	Nsa	Nsa	Nsa	Nd	Nd	Nd	Nd
Yamada et al. (2000b)	17 noodle soups in conventional PS	0.0142	0.0021 (14.8)[b] 1/17	Nsa	Nsa	Nsa	0.01 (70.4)	Nsa	Nsa	Nsa	Nsa	0.0009 (6.3)[b]3/17	Nsa	Nsa	Nd
	9 noodle soups in improved PS	0.004	0.001 (25)[b] 1/9	Nsa	Nsa	Nsa	0.0022 (55)	Nsa	Nsa	Nsa	Nsa	0.0002 (5) 8/9[b]	Nsa	Nsa	Nd
Kaneko et al. (2003) [c]	30 noodles	0.0205	0.0038 (18.5)	0.0005[5] (2.44)[b]30/30	Nsa	Nsa		0.004 (19.5)[b] 3/30	0.0046 (22.4)[b] 5/30	0.0018 (8.78)[b] 6/30	0.002 (9.76)[b] 5/30	0.0016 (7.8)[b]10/30	Nsa	Nsa	Nd
	24 soups	0.0307	0.0076 (24.8)[b] 1/24	0.0005[5] (1.63)[b]24/24	Nsa	Nsa		0.0056 (18.2)[b] 3/24	0.0068 (22.1)[b] 5/24	0.0027 (8.79)[b] 4/24	0.0029 (9.45)[b] 4/24	0.0018 (5.86)[b]9/24	Nsa	Nsa	Nd

The column Media indicates.

- For "Concentrations of SDT by extraction of PS materials with food simulants" the extraction conditions: % aqueous ethanol, extraction temperature and time.
- For "Concentrations of SDT in food" the food items analyzed.

Concentrations below limit of quantification (LOQ) were set at 0.5 x LOQ, if the oligomer was specifically analyzed.

D1: 1,3-diphenylpropane; NSD-01: 2,4-diphenyl-1-butene; NSD-08: cis-1.2-diphenyl-cyclobutane; NSD-09: trans-1.2-diphenyl-cyclobutane.

NST-01: 2,4,6-triphenyl-1-hexene; NST-02: 1,3,5-triphenyl-cyclohexane (mixture of isomers); ST-6: 1e, 3e, 5a isomer of NST-02 according to Ohyama et al. (2007); ST-7: 1e, 3e, 5e isomer of NST-02 according to Ohyama et al. (2007); NST-03: 1-phenyl-4-(1'-phenylethyl)tetralin (mixture of isomers); NST-03-1: 1e, 4e isomer of NST-03; NST-03-2: 1a, 4e isomer of NST-03; NST-03-3: 1a, 4a isomer of NST-03; NST-03-4: 1e, 4a isomer of NST-03; NST-12: 1-phenyl-4-(2-phenylethyl)tetralin (mixture of isomers); NST-12-1: 1e, 4a isomer of NST-12; NST-12-2: 1a, 4a isomer of NST-12; NST-13: (1S*,6R*,7S*,8S*,11R*)-6,11-diphenyltricyclo(6,2,2,$0^{2,7}$)dodeca-2,9-diene.

Nsa: isomers not specifically analyzed; Nd: no data and not specifically analyzed.

They also reported the concentrations of 2 Tri (T7 and T8) with unknown structures not listed in the table: Tri T7: mean 0.0011 (*10/30) in noodles and 0.0012 (*9/24) in soup; Tri T8: mean 0.0005 (*26/30) in noodles and 0.0006 (*19/24) in soup. This shows that T7 and T8 are quantitatively of minor importance. The amounts of T7 were less than those of any one isomer of NST-03 and T8 could not be quantified in most cases. Yamada et al. (2000a) identified 2 Tri with unknown structure as isomers of NST-12 (NST-12-1 and NST-12-2). Therefore the sum of T6 and T7 are reported here tentatively under NST-12.

[a] LOQ taken as 0.001 mg/6 dm^2;[2]: LOQ reported as 0.002 mg/kg food;[3]: LOQ reported as 0.005 mg/kg food;[4]: LOQ 0.0002–0.00004 mg/kg food depending on food sample;[5]: LOQ 0.0001 mg/kg food.

[b] x/y: x samples of the total of y samples below LOQ.

[c] Kaneko et al. (2003) determined the Di and Tri separately in noodles and the soups after separation from the solid material. Here the results obtained for the noodles and the remaining soups are listed separately. The authors assume that the different results reflect differences of fat content of the noodles and the soup.

Table 2
Mean amounts of different Di and Tri measured in PS in mg/kg PS. The percentages related to the sum of SDT are given in brackets. Concentrations below limit of quantification (LOQ) were set at 0.5 x LOQ, if the oligomer was specifically analyzed.

Author	PS material	Sum of SDT	Dimers, mean (% of SDT)				
			D1	NSD-01	NSD-08	NSD-09	Sum of NSD-08 + 09
Weel (2016b)	HIPS rod	12620	Nd	20 (0.16)	Nsa	Nsa	740 (5.9)
	GPPS rod	12430	Nd	20 (0.16)	Nsa	Nsa	690 (5,5)
	HIPS sheet	12390	Nd	20 (0.16)	Nsa	Nsa	710 (5.7)
Kawamura et al. (1998c) [c]	6 GPPS	8035	18.3[a] (0.22)[b]2/6	173 (2.06)	10[1] (0.12)[b]4/6	142 (1.69)	
	6 HIPS	11959	17.5[a] (0.15)[b]3/6	165 (1.38)	30.8[1] (0.26)[b]1/6	284[1] (2.37)[b]1/6	
Kawamura et al. (1998a)	1 HIPS food container	11350	Nd	70 (0.62)	Nd	310 (2.7)	
	1 PSP	6600	Nd	60 (0.91)	Nd	170 (2.58)	
	18 HIPS/PSP[d]	8019	Nd	124 (1.55)	Nd	153 (1.91)	
	3 HIPS/PSP/HIPS[d]	7917	Nd	93.3 (1.18)	Nd	140 (1.77)	
	1 PSP/HIPS[d]	5530	Nd	70 (1.27)	Nd	180 (3.26)	
Yamada (1999)	1 PSP	3890	Nd	150 (3.7)	5[1] (0.13)[b]1/1	60 (1.5)	
	1 HIPS	4395	Nd	20 (0.46)	20 (0.46)	180 (4.1)	
Yamada et al. (2000a)	1 PSP	3830	Nd	150 (3.92)	10[2] (0.26)[b]1/1	50 (1.31)	
	1 HIPS	4380	Nd	20 (0.46)	10[2] (0.23)[b]1/1	100 (2.28)	
Sakamoto et al. (2000)	2 HIPS	2281	Nd	36 (1.6)	Nd	Nd	
	2 PSP	5687	Nd	92 (1.62)	Nd	Nd	
	9 GPPS	4962	Nd	123 (2.48)	Nd	Nd	
Nakada et al. (2000)	18 PS, not specified	8286	44 (0.53)	95 (1.15)	36 (0.44)	133 (1.61)	
Hirano et al. (2001)	1 PSP	980	Nd	210 (21,4)	5[1] (0.51)[b]1/1	5[1] (0.51)[b]1/1	
	1 HIPS	1435	Nd	70 (4.88)	5[1] (0.35)[b]1/1	30 (2.09)	
Kaneko et al. (1999)	51 PS samples for food use (not further specified)	4290	13 (0.30) [b]13/51	146 (3.40) [b]1/51	14 (0.33) [b]25/51	163 (3.8) [b]1/51	
Kaneko et al. (2003)	21 SB/PSP[d]	3090	5.7[a] (0.18)[b]18/21	118 (3.81)	7.1[1] (0.23)[b]18/21	74.3 (2.40)	
	6 PSP/SB[d]	2818	0.5[a] (0.02)[b]6/6	28.3 (1.00)	22.5[1] (0.80)[b]1/6	168 (5.96)	
Genualdi et al. (2014)	17 HIPS	7539	17.2 (0.23) [b]1/17	281 (3.73) [b]2/17	Nd	296 (3.93)	
	1 GPPS	7259	30.8 (0.42)	578 (7.96)	Nd	200 (2.76)	
	3 XPS	2490	13.0 (0.52)	156 (6.27)	Nd	40.0 (1.61)	

Author	PS material	Sum of SDT	Trimers, mean (% of SDT)								
			NST-01	NST-02	NST-03	NST-03-1	NST-03-2	NST-03-3	NST-03-4	NST-12	NST-13
Weel (2016b)	HIPS rod	12620	1440 (11.5)	760 (6.1)	9660 (77.3)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
	GPPS rod	12430	740 (5.9)	740 (5.9)	10240 (81.9)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
	HIPS sheet	12390	1410 (11.3)	770 (6.2)	9.480 (75.8)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
Kawamura et al., 1998c [c]	6 GPPS	8035	2038 (24.3)	21.7[a] (0.26)[b]2/6	5632 (67.0)	Nsa	Nsa	Nsa	Nsa	357[a] (4.25)[b]1/6	Nd
	6 HIPS	11959	2420 (20.2)	68.3 (0.43)	8300 (69.4)	Nsa	Nsa	Nsa	Nsa	673[a] (5.63)	Nd
Kawamura et al. (1998a)	1 HIPS	11350	1800 (15.8)	Nd	9170 (80.7)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
	1 PSP	6600	1480 (22.5)	Nd	4890 (74.3)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
	18 HIPS/PSP[d]	8019	1545 (19.3)	Nd	6197 (77.5)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
	3 HIPS/PSP/HIPS[d]	7917	1127 (14.2)	Nd	6557 (82.8)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
	1 PSP/HIPS[d]	5530	1530 (27.7)	Nd	3750 (67.9)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
Yamada (1999)	1 PSP	3890	480 (12.3)	5[a] (0.13)[b]1/1	3190 (82.0)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
	1 HIPS	4395	290 (6.6)	5[a] (0.11)[b]1/1	3880 (88.5)	Nsa	Nsa	Nsa	Nsa	Nd	Nd
Yamada et al. (2000a) [c]	1 PSP	3830	980 (25.6)	Nd	2450 (64.0)	Nsa	Nsa	Nsa	Nsa	190 (4.96)	Nd
	1 HIPS	4380	540 (12.3)	Nd	3430 (78.2)	Nsa	Nsa	Nsa	Nsa	280 (6.38)	Nd
Sakamoto et al. (2000)	2 HIPS	2281	260 (11.4)	Nd	1985 (87)	595 (26.1)	775 (33.9)	285 (12.5)	330 (14.5)	Nd	Nd
	2 PSP	5687	890 (15.7)	Nd	4705 (82.7)	1380 (24.3)	1855 (32.6)	650 (11.4)	820 (14.4)	Nd	Nd
	9 GPPS	4962	812 (16.4)	Nd	4027 (81.4)	1149 (23.2)	1617 (32.7)	538 (10.9)	723 (14.6)	Nd	Nd
Nakada et al. (2000)	18 PS, not specified	8286	2872 (34.8)	Nd	5106 (61.5)	1643 (19.9)	1760 (21.3)	679 (8.22)	1024 (12.4)	Nd	Nd

(*continued on next page*)

Table 2 (*continued*)

Author	PS material	Sum of SDT	Trimers, mean (% of SDT)								
			NST-01	NST-02	NST-03	NST-03-1	NST-03-2	NST-03-3	NST-03-4	NST-12	NST-13
Hirano et al. (2001)	1 PSP	980	640 (65.3)	Nd	110 (11.2)	Nsa	Nsa	Nsa	Nsa	5[a] (0.51)[b]1/1	5[a] (0.51)[b]1/1
	1 HIPS	1435	550 (38.3)	Nd	720 (50.2)	Nsa	Nsa	Nsa	Nsa	50 (3.49)	10 (0.70)
Kaneko et al. (1999) [c]	51 PS samples for food use (not further specified)	4290	994 (23.2)	33 (0.77) [b]11/51		794 (18.5)	916 (21.4) [b]5/51	567 (13.2) [b]4/51	444 (10.3) [b]3/51	206 (4.80) [b]7/51	Nd
Kaneko et al. (2003) [c]	21 SB/PSP[d]	3090	770 (24.9)	10.2[a] (0.33)[b]7/21	1897 (61.2)	530 (17.1)	620 (20.0)	363 (11.7)	384 (12.4)	208 (6.72)	Nd
	6 PSP/SB[d]	2818	577 (20.5)	33.3 (1.18)	1686 (59.9)	498 (17.7)	133 (4.72)	512 (18.2)	543 (19.3)	302 (10.7)	Nd
Genualdi et al. (2014)	17 HIPS	7539	2492 (33.1)	0.59 (0.01) [b]16/17		1188 (15.8)	2024 (26.8)	730 (9.68)	510 (6.76)	Nd	Nd
	1 GPPS	7259	1601 (22.1)	0.001 (0.00001) [b]1/1		1182 (16.3)	2343 (32.3)	800 (11.0)	524 (7.22)	Nd	Nd
	3 XPS	2490	840 (33.7)	0.0001 (0.000004) [b]3/3		373 (15.0)	672 (27.0)	234 (9.40)	162 (6.50)	Nd	Nd

D1: 1,3-diphenylpropane; NSD-01: 2,4-diphenyl-1-butene; NSD-08: cis-1.2-diphenyl-cyclobutane; NSD-09: trans-1.2-diphenyl-cyclobutane. NST-01: 2,4,6-triphenyl-1-hexene; NST-02: 1,3,5-triphenyl-cyclohexane (mixture of isomers); NST-03: 1-phenyl-4-(1'-phenylethyl)tetralin (mixture of isomers); NST-03-1: 1e, 4e isomer of NST-03; NST-03-2: 1a, 4e isomer of NST-03; NST-03-3: 1a, 4a isomer of NST-03; NST-03-4: 1e, 4a isomer of NST-03; NST-12: 1-phenyl-4-(2-phenylethyl)tetralin (mixture of isomers); NST-13: (1S*,6R*,7S*,8S*,11R*)-6,11-diphenyltricyclo(6,2,2,$0^{2,7}$)dodeca-2,9-diene.

Nsa: isomers not specifically analyzed; Nd: no data and not specifically analyzed.

[a] LOQ reported as 10 mg/kg PS;[2]: LOQ reported as 20 mg/kg PS.

[b] x/y: x samples of the total of y samples below LOQ.

[c] Kawamura et al. (1998c) reported the concentrations of 2 Tri (T6 and T7) with unknown structures not listed in the table: T6: mean 280 (*1/6) in GPPS, 465 in HIPS. T7: mean 77 (*1/6) in GPPS, 208 in HIPS. Similarly, Kaneko et al. (1999, 2003) observed 2 unknown Tri, named T7 and T8. Yamada et al. (2000a) identified 2 Tri with unknown structure as isomers of NST-12 (NST-12-1 and NST-12-2). The relation of NST-12-1 to NST-12-2 was reported to be 5:1 and the sum of both isomers is listed under NST-12. Similarly, the sum of T6 and T7 and of T7 and T8 are reported here tentatively under NST-12.

[d] X/Y/Z = outside/middle/inside; SB: styrene-butadiene copolymer.

d as compared to 1.5 μg/kg bw/d proposed EFSA (2012b). Leeman et al. (2014) concluded that the TTC of 4.0 μg/kg bw/d was also compatible with TTCs recently published for other enlarged datasets (including developmental toxicity in rats and rabbits, repeated dose toxicity of industrial chemicals or plastic food contact materials). TTCs based on the larger datasets were equal or even higher than the one calculated by Leeman et al. (2014) on the basis of the original Munro database after exclusion of organophosphates, carbamates and organohalogens. Although this refinement of TTCs by Leeman et al. (2014) has not been officially endorsed by EFSA, the approach is scientifically well justified and is considered by the authors as conservative as the original one. It also corresponds to the proposal of EFSA (2012b) and WHO (2016) to analyze organophosphates and carbamates separately and evaluate the consequence for Cramer class III chemicals. Therefore, this refinement of the original TTC will be applied here to calculate the MoS of SDT.

5. Exposure assessment

5.1. General considerations

The next step in risk assessment is the evaluation of potential consumer exposure via migration of SDT into food from PS food containers. Exposures can be measured or estimated by methods of different complexity:

a. Migration of SDT into food simulants under standardized conditions (Tier 1)
b. Determination of SDT in food packaged in PS containers (Tier 2)
c. Application of the FACET (2017) approach based on the amounts of SDT in PS materials (Tier 3)

If a risk assessment is based on these approaches it must be ascertained that the total amounts of SDT were reliably determined. This is the case for the study of Klärner et al. (1998) who determined the sum of Di and Tri after extraction of PS with 50% ethanol. But for the other studies the sum of SDT has to be calculated on the basis of the different Di and Tri chemical entities that were separately analyzed. It must be ascertained that by such separate analyses of Di and Tri at least the quantitatively most important SDT were accounted for.

5.2. Determination of different Di and Tri in food or food simulants

In Table 1 the concentrations of the different Di and Tri measured in food simulants (upper part) and packaged food (lower part) are given. In order to develop an analytical method for identification and quantitation of Di and Tri, Weel (2016a) extracted HIPS rods and sheets with i-octane (over 2 d at 20 °C) and 95% ethanol (over 10 d at 40 °C). With i-octane no Di, Tri or styrene monomer were found. In the 95% ethanol extracts Di and Tri could be identified that had already been described by Kawamura et al. (1998b), but because appropriate standards were not available the gas chromatographic peaks could not be assigned to specific isomers. The following Di and Tri were found by Weel (2016a, c): D1, 1,2-diphenyl cyclobutane (no differentiation of the cis and trans isomers, NSD-08, NSD-09), NSD-01, NST-01, NST-02 (no assignment to different isomers), NST-03/NST-12 (no differentiation between NST-03 and NST-12 and between the different diastomers of NST-03). Higher oligomers than Tri were not observed although oligomers up to 690 m/z could be detected in a commercial oligomer standard.

The analytical data described by the different authors in Table 1 show a consistent pattern (for more details cf. electronic supplement):

- when comparing the absolute amounts of SDT, those analyzed in Japanese food items (4–30.7 μg/kg food) corresponded to those extracted by 20% ethanol at 40 °C over 10 days for HIPS and GPPS (6–18 μg/6 dm^2). Much higher amounts of SDT were extracted with 50% ethanol (197 and 162 μg/6 dm^2) and 95% ethanol extracted even 265 and 936 μg/6 dm^2 from HIPS sheet and HIPS rod, respectively. Therefore, migration into 20% ethanol may better represent the situation for actual food items than higher ethanol concentrations.

- The analyses of specific Di and Tri showed, especially for Di in food items, that the amounts often were below the limit of quantification (LOQ) and using 0.5 x LOQ as defaults presents an upper bound. In these cases the percentages related to all SDT may be misleading and are excluded from the calculation of the sum of SDT if more than 50% of samples were below LOQ.
- The percentages for the sum of all Di were always clearly lower (~9–30%) than those for the sum of all Tri (~70–90%) irrespective of the extraction medium or the food analyzed.
- The highest percentages were observed for the sum of the NST-03 isomers (55–77.2%) with NST-03-2 being quantitatively the most important isomer (~22%).

The observation of much lower Di than Tri amounts is strongly supported by Klärner et al. (1998). After extraction of HIPS with 50% ethanol, Klärner found that Di amounted to 11–30% and Tri to 70–89% related to SDT. For GPPS most Di were below LOQ. Only in the case of 1 GPPS sample the amount of Di and the relationship of Di and Tri (29%:71%) could be reliably determined.

5.3. Determination of different Di and Tri in PS used for food packaging

If the FACET (2017) method is used as tier 3 for exposure assessment, it has to be verified whether the quantitatively most important Di and Tri have been determined. In Table 2 the results are listed for the different food packaging PS materials. In total, data from 179 PS food packaging materials can be used. In addition, analytical data are available in literature for 16 EPS and 13 PS foam materials that are not included in the present assessment. A comparison of the total amounts of SDT in Table 1 (migrating in μg/kg food or μg/6 dm^2) and Table 2 (in mg/kg PS) shows that obviously only small amounts of SDT migrate.

For the non-expanded PS samples the isomers of NST-03 were quantitatively predominant with in total 50–88.5% (apart from one sample with ~11%), NST-03-2 was the most important isomer followed by NST-03-1 and NST-01. Details for the percentages of different SDT in PS food packaging materials are given in the electronic supplement. All studies analyzed NSD-01 and NSD-09 as well as the quantitatively most important Tri (NST-01 and NST-03). NST-02 was studied in 116 and NST-12 (all isomers) in 94 samples. As NST-02 and NST-12 were quantitatively much less important than the other Tri, the sum of SDT is comprehensively addressed such that these studies are a robust basis for the assessment by the FACET (2017) methodology. Furthermore, the most prevalent Di and Tri in PS packaging materials were also the quantitatively most important ones extracted by food simulants or food. The sum of Di was always much lower than that of Tri as had been shown by Fail et al. (1998). They analyzed the total amounts of Di and Tri after dissolution of 7 PS samples (no further specification given) in dichloromethane, precipitation of polymeric material with isopropanol and GC analysis of the oligomer solution. Related to SDT, Di comprised 7–14% and Tri 86–93%.

6. Determination of Margin of Safety (MoS)

By comparison of exposure estimates with safe exposure limits the MoS is obtained. Three different approaches to define safe exposure levels can be used:

a. Starting from the NA-study and applying AFs proposed by ECHA (2012) and EFSA (2012a). This led to 1 μg/kg bw/d.
b. Using the TTC of EFSA (2012b), i.e. 1.5 μg/kg bw/d.
c. Using the modified TTC of Leeman et al. (2014), i.e. 4 μg/kg bw/d.

The first approach a) is surrounded by significant uncertainties. The

NA-study cannot be considered a comprehensive repeated dose study although it comprised many important endpoints related to reproductive health and potential endocrine effects. The important data gaps have been described above, especially the uncertainty of the POD based on the NOAEL in absence of a LOAEL. For substances with few or no relevant toxicity data EFSA (2012b) recommends to apply the TTC, in this case 1.5 μg/kg bw/d (approach b)). But because SDT do not belong to organophosphates, carbamates or organohalogens, it is well justified to use the modified TTC of Leeman et al. (2014), i.e. 4 μg/kg bw/d (approach c)). Therefore, the MoS will be based on 4 μg/kg bw/d, corresponding to 240 μg/person/d (60 kg adult body weight), or 240 μg/kg food (1 kg food consumed per day), or 240 μg/6 dm^2 (1 kg food packaged in 6 dm^2 PS). The results thereby obtained are put into perspective to those using the most conservative safe exposure limit derived from the NA-study, i.e. 1 μg/kg bw/d or 60 μg/kg food for adults. It is reassuring that the safe exposure level obtained by the standard approach a) using AFs is in the same order of magnitude as the TTCs of b) and c).

6.1. SDT migration into food simulants and determination of Margin of Safety (MoS) (cf. Table 3, upper part)

Tier 1 of exposure assessment is based on migration into food simulants, the least refined and most conservative approach. As all analyses comprised the quantitatively most important individual Di and Tri, a MoS can be calculated based on the sum of all Di and Tri found. The total amounts of Di, Tri and SDT that migrated into food simulants and the MoS are given in Table 3 upper part. The MoS obtained by this approach is certainly extremely conservative and should be considered as an upper bound as it is based on the premise that the total food of 1 kg/d is packaged in PS every day over life time without taking into account the mix of different food items, different consumer habits or their change over seasons and life time. The advantage is that standardized data can be easily obtained for different materials. Migration not only depends on temperature and time of exposure but to a large extent also on the ethanol concentration. The MoS calculations are based on 20% and 50% ethanol as food simulant. Data based on 95% ethanol are not considered as the EU (2011) no longer recommends its use for determining overall migration into fatty foods. Since an indication was obtained that 20% ethanol may better represent migration into actual packaged food, 50% ethanol may be regarded as a worst case scenario.

Genualdi et al. (2014) determined the diffusion coefficients of the different Di, including D1, and Tri in 10%, 50% and 95% ethanol for different PS packaging materials. As the amounts of Di and Tri migrating were expected to be very low, only the results obtained with 95% ethanol were reported although it was acknowledged that migration testing for PS with 95% ethanol is no longer recommended by US FDA (2007). By the partition coefficient with 95% ethanol the authors concluded that Di and Tri are likely to stay in the food packaging

Table 3
Exposure assessment and margin of Safety (MoS) based on TTC of 240 μg SDT/person/d for adults and migration of SDT into food simulants or food. Shaded figures indicate a MoS < 1 based on the most conservative safe exposure level of 60 μg/person/d derived from the NOAEL of the NA-study.

Author	Sample	Method (extraction solvent with time and temperature or determination in food)	Estimated exposure (μg/6dm² or μg/person/d) for sum of			MoS for SDT
			Di	Tri	SDT	
Exposure estimation and MoS for migration into food simulants						
Klärner et al. 1998	9 GPPS	50% ethanol, 10 d, 40 ^{0}C	<5.4-8.6	<5.4-21.4	<11-30	8->22
	8 HIPS	50% ethanol, 10 d, 40 ^{0}C	11.8-69.6	40.7-166	52.5-236	1.02-4.6
Weel, 2016c	1 GPPS	20% ethanol, 10 d, 40 ^{0}C	5.5	12.5	18	13
	1 GPPS	50% ethanol, 10 d, 40 ^{0}C	15.5	147	162.5	1.5
	1 HIPS	20% ethanol, 10 d, 40 ^{0}C	1.5	4	5.5	44
	1 HIPS	50% ethanol, 10 d, 40 ^{0}C	22	175	197	1.2
Weel, 2016a	1 HIPS rod	95% ethanol, 10 d, 40 ^{0}C	194	906	1100	0.22
	1 HIPS sheet	95% ethanol, 10 d, 40 ^{0}C	79	196	275	0.87
OFI, 2017a***	GPPS	50% ethanol, 10 d, 40 ^{0}C	34	200	234	1.03
	GPPS	95% ethanol, 10 d, 60 ^{0}C	332	400	734	0.33
OFI, 2017b***	HIPS	50% ethanol, 10 d, 40 ^{0}C	300	600	900	0.27
	HIPS	95% ethanol, 10 d, 60 ^{0}C	1400	1000	2400	0.1
BfR, 2016	Polystyrene cup	50% ethanol, 2h, 70 ^{0}C	11.4	39.3	50.9	4.7
	Polystyrene cup	50% ethanol, 2h, room temp.	nr	nr	13	18.5
Exposure estimation and MoS for migration into food						
Genualdi et al. 2014	7 food items in PS	Direct determination in food	<5**	nd**	<5**	>48
Kawamura et al. 1998d	7 noodle soups in PS	Direct determination in food	<5	<5-62	<5-62	3.9->48
Kawamura et al. 1998a	32 instant foods in PS	Direct determination in food	<5	<5-62.4	<5-62.4	3.8->48
Yamada et al. 2000b*	17 noodle soups in conventional PS	Direct determination in food	<0.2-1.2	1.3-29.2	1.3-31.6	7.6-185
	9 noodle soups in improved PS	Direct determination in food	<0.2-1.7	1.6-6.9	1.6-7.5	32-150
Kaneko et al. 2003	30 noodle products in PS	Direct determination in food	<0.2-3.3	0.5-61	0.5-63	3.8-480

nd: not detected at LOQ of 2 μg/kg food; nr: LOQ not reported.
(Klärner et al., 1998; Weel, 2016a, 2016c; OFI, 2017a, 2017b, BfR, 2016; Genualdi et al., 2014; Kawamura et al., 1998a, 1998d; Yamada et al., 2000b; Kaneko et al., 2003)
[a]Recoveries of different Di and Tri in spiked food samples varied between 54 and 135% and are not taken into account for calculation of Di and Tri amounts analyzed.
[b]Only 1,3-diphenyl propane observed.
[c]Potential confounding by aging of the test samples leading to higher migration cannot be excluded.

material and resist transfer to oily food. The transfer of Di and Tri into 50% and 10% ethanol representing fatty and aqueous food was expected to be even lower.

Klärner et al. (1998) investigated representative PS samples intended for food packaging applications from European producers. Test samples were prepared by injection molding of bulk materials which generally gives the highest stresses leading potentially to higher levels of residuals and oligomers. Nine GPPS and 8 HIPS samples were extracted with 50% aqueous ethanol over 10 days at 40 °C. If a quantification was not possible, the LOQ (0.05 mg/l) was taken as upper value for migration of Di or Tri. The extracted SDT amounted to < 11–30 for GPPS and to 52.5–236 μg/6 dm^2 for HIPS. With the TTC of 240 μg/person/d this corresponds to a MoS of 8- > 22 and 1.02–4.6, respectively.

Weel (2016a, c) used a GPPS and a HIPS representative commercial sample sold in the European market for food packaging selected from an anonymized list of materials submitted by European producers. As to be expected, extraction by 50% ethanol yielded higher amounts of SDT as compared to 20% ethanol. The total SDT extracted by the more aggressive 50% ethanol amounted to 162.5 for GPPS and to 197 μg/6 dm^2 for HIPS, respectively. Higher migration of SDT was reported by OFI (2017a, b) using the same GPPS and HIPS samples as Weel (2016a, c): with 50% ethanol total SDT migration was 234 μg/6 dm^2 for GPPS and 900 μg/6 dm^2 for HIPS, and migration into 95% ethanol was clearly higher (734 for GPPS and 2400 μg/6 dm^2 for HIPS). The reason for the higher levels reported by OFI remain unclear. A possible explanation that may need further investigation could be aging of the PS samples as the study of OFI was carried out with the same samples but about 0.5–1 year later than that of Weel.

For development of the analytical method to determine Di and Tri, extraction of PS with 95% ethanol was used (Weel, 2016a). Under these conditions much higher SDT concentrations for a rod and a sheet shaped HIPS sample were found: rod: 1100 μg SDT/6 dm^2; sheet: 275 μg SDT/6 dm^2. Interestingly, migration from the rod sample led to higher Di and Tri (and SDT) concentrations as compared to the sheet sample. An explanation was proposed by Weel (2016a) as that the rod sample had been prepared by injection molding, but the plate sample by the milder compression molding. For preparation of containers for milk products, such as the yogurt pot, the stresses are closer to those for compression molding and hence the data obtained for sheets are considered more relevant. For reasons given above, data obtained with 95% ethanol should only be regarded as benchmarks for extreme conditions and are not suitable for risk assessment.

Based on 50% ethanol as simulant for fatty foods as proposed by regulatory agencies, the following total amounts of SDT and the corresponding MoS (related to 240 μg SDT/kg food for adults) were obtained (the values in brackets represent the data from OFI because potential confounding by aging of these samples cannot be excluded):

- GPPS: SDT < 11–162.5 (234) μg/kg food; MoS 1.5 (1.03)- > 22
- HIPS: SDT 52.5–236 (900) μg/kg food; MoS 1.02 (0.27)-4.6

With 20% ethanol the following migrations and MoS were obtained:

- GPPS: SDT 18 μg/kg food; MoS 13
- HIPS: SDT 5.5 μg/kg food; MoS 44

Therefore, apart from the study of OFI (2017b) with HIPS the MoS always was > 1, although sometimes only slightly above 1. The MoS by extraction with 20% ethanol was clearly higher (GPPS 13; HIPS 44). Only with 95% ethanol the MoS became < 1 (HIPS rod 0.22; HIPS sheet 0.87). The arguments why this aggressive food simulant is not appropriate were given above. In relation to the most conservative safe exposure level derived from the NOAEL of the NA-study (60 μg/person/d) a MoS < 1 would be obtained after extraction with 50% ethanol for most HIPS samples (Klärner et al., 1998; Weel, 2016c) and for 2 GPPS samples (Weel, 2016c; OFI, 2017a) (cf. Shaded figures in Table 3).

In former years a number of migration studies have been carried out in Japan with water, 20% ethanol, 50% ethanol and n-heptane at temperatures varying between 25 °C and 90 °C depending on the solvent. Often D1 was included in the analyses. But the exposure durations were limited to 30 or 60 min mainly or to short microwave treatment because these specific applications prevail in Japan, i.e. ready-to-prepare noodle soups in PS containers. Therefore, the data obtained by these studies are not included here (Kawamura et al., 1998a, c; Yamada, 1999, 2000a; Nakada et al., 2000; Sakamoto et al., 2000; Hirano et al., 2001).

6.2. SDT migration into food packaged in PS containers and determination of MoS (cf. Table 3, lower part)

Tier 2 of exposure assessment is based on migration of SDT into actual food items as listed in Table 3, lower part, including the MoS. In comparison to tier 1, this approach will provide an indication of consumer exposure by “real world” food items. But the uncertainty of extrapolation from single data points to life time exposure still exists with the conservative assumption that the consumer will be exposed daily and over life time by the food item (as 1 kg food/d) with the highest SDT migration.

Genualdi et al. (2014) determined the concentrations of different Di (including D1, the smallest oligomer with the highest water solubility) and Tri in 7 food items packaged in PS (cookies, yoghurt, chocolate candy, raw chicken, raw beef, bakery croissants and noodle soup). The LOQ was 2 μg/kg food. Only D1 was detected in 3 of these foods with concentrations less than 5 μg/kg food leading in total to < 5 μg SDT/kg food. Assuming that 1 kg of these food items is consumed daily by an adult, the MoS is > 48.

Several Japanese investigators reported Di, Tri and SDT migration from PS food containers into noodle soups and other ready-to-use food items. Kawamura et al. (1998d) studied 7 noodle soup preparations with a LOQ for Di and Tri of 5 μg/kg food (note: it is not clear for many of the Japanese studies whether the LOD or LOQ was given; therefore in the following such limits will always be taken as LOQ). The Di concentrations were below LOQ and the Tri concentrations ranged from non-detectable to 62 μg/kg food giving a range for SDT from < 5 to 62 μg/kg food. This study was expanded by Kawamura et al. (1998a) to 32 instant food items including the samples already analyzed by Kawamura et al. (1998d). Again, Di were not detectable and Tri varied between < 5 and 62.4 μg/kg food with SDT between < 5–62.4 μg/kg food. The MoS obtained by these 2 datasets were between 3.8 and > 48.

Yamada et al. (2000b) analyzed 17 noodle soups prepared in conventional PS containers and 9 samples prepared in PS containers modified to achieve lower migrations of Di and Tri. The LOQ varied between 0.2 and 0.4 μg/kg food. There was some indication for a reduced migration from the improved containers, but this could not be further substantiated because the study is described in Japanese language. With the conventional containers, Di concentrations were between < 0.2 and 1.2 μg/kg food, Tri concentrations between 1.3 and 29.2 μg/kg food and total SDT between 1.3 and 31.6 μg/kg food. In the improved containers the concentrations were for Di < 0.2–1.7, for Tri 1.6–6.9 and for SDT 1.6–7.5 μg/kg food. Thus, the MoS was 7.6–185 for conventional and 32–150 for improved food containers.

Kaneko et al. (2003) analyzed 30 instant noodle products in PS containers. This is the only Japanese investigation that tried to quantify D1 in food, but D1 was not detectable. Di was detected in 22 of the 30 samples with < 0.2–3.3 μg/kg food, Tri in all samples with 0.5–61 μg/kg food and SDT with 0.5–63 μg/kg food. The MoS was in the range of 3.8–480.

It should be recognized, however, that all MoS derived from Japanese studies reflected food consumption habits that are more popular in Japan than elsewhere. In the Japanese studies ready-to-use

food items, such as noodle soups or rice products, were prepared in PS containers according to the procedures mentioned by the producers, generally by heating for a few minutes in a microwave oven or by pouring hot water into the container. The products were then left at room temperature for 15–30 min before consumption. In contrast, in Western or European countries PS containers are mainly used for longer storage times in the refrigerator or at room temperature. Thus, in the Japanese studies high temperatures were applied that would lead to higher short-term migration but storage time while cooling at room temperature was only limited to about 30 min. Therefore a decision is not possible whether Western or Japanese conditions lead to higher migration. Only Genualdi et al. (2014) studied Western type food packaged and stored in PS food containers. Therefore, the MoS derived from Japanese food items have only limited relevance for Western applications of PS food containers. Based on the most conservative safe exposure level derived from the NA-study (60 μg/person/d) a few samples from Japan have a MoS slightly below 1 (cf. Shaded figures in Table 3, lower part).

In summary, the amounts of SDT migrating from PS packaging materials into real food yield MoS in relation to the safe exposure level of 240 μg SDT/kg food between (Table 3).

- 3.8 and 480 for real food items with the caveat that most MoS derived from food items relate to Japanese consumption habits
- > 48 for real Western-type food items packaged in PS containers
- 13 for GPPS, 20% ethanol
- 44 for HIPS, 20% ethanol.

The MoS being always > 1 for real food items strongly suggests that SDT migration from food packaging materials is unlikely to adversely affect consumer health. In addition, extraction with 20% ethanol (added here for comparison) seems to more closely resemble migration into real Japanese or Western type food items than 50% ethanol that is the preferred food simulant proposed by US FDA (2007) and EU (2011). Based on the most conservative safe exposure level (60 μg/person/d) a MoS slightly below 1 would be obtained only for 1/32 samples (Kawamura et al., 1998a, d) and 1/30 samples (Kaneko et al., 2003) (shaded in Table 3).

6.3. Determination of MoS via SDT content in PS materials and the FACET approach (Table 4)

Tier 3 of exposure assessment is based on the FACET approach. The FACET (Flavourings, Additives, and food Contact materials Exposure Tool) probabilistic modelling tool was developed by a collaborative team consisting of regulators, industry and modelling experts in response to a call by the European Commission to produce a risk management tool consisting of a database containing information on concentrations of food additives and food flavorings, potential migrants in food contact materials and food consumption data. Derivation of a MoS based on exposure assessments of SDT via migration into food simulants or measurements in real food items both suffer from the same shortcoming, namely that the highest concentrations found in the media are taken as the daily consumer exposure over life time. Furthermore, as already mentioned many of the MoS derived from migration into food relate to Japanese and not to Western or European dietary habits. These shortcomings can be accommodated by the FACET (2017) approach. FACET Software version 3.0.2 was used for the exposure calculations. For exposure to migrants from food packaging, FACET uses European Union-wide industry-supplied data on the occurrence of substances in the packaging, their concentrations and construction of the packaging, and food consumption data supplied by European national database managers, which are then combined into probabilistic dietary exposure models. This results in estimated exposure to packaging migrants at the level of the individual consumer. The starting point is the probabilistic modelling of food consumption and thereby of the uptake of food contaminants (SDT) from the non-expanded PS in packaging materials taking into consideration the physical properties of the migrants. The concentration and occurrence of non-expanded PS in packaging materials and related food consumption data are contained in FACET.

The different PS materials are commodity polymers produced worldwide and over many years by essentially the same manufacturing processes. Therefore the SDT concentrations in present day's PS polymers in Europe are comparable to those in former years in the different regions, e.g. especially in Japan with a large dataset. In total, a dataset is available for HIPS (31 samples), GPPS (16 sample), PSP (6 samples), unspecified PS containers (77 samples), and multilayer materials (49 samples). The 90th percentile of the concentrations in the various PS materials was used as input for the FACET assessments. In every article only the maximum of the concentration range described for a specific material has been considered during the statistical analysis. This has been done in order to correct for the influence of the variation in number of the various datasets in the different articles.

The 90th percentile of the Di and Tri concentrations in PS were introduced separately as new substance in FACET using the NIAS (Non-Metal) wizard. Migration properties from non-expanded PS in food are determined in FACET by the molecular weight and partitioning coefficient of the Di and Tri of PS. The Molecular weight of the Di and Tri of styrene (208.3 and 312.5 resp.) and their average logKow (5.52 and 7.84 resp.) were introduced for NIAS details. Because the migration of the Di and Tri is determined by the molecular weight and partitioning coefficient which are not the same for the Di and Tri, no separate exposure assessment has been made for the SDT. The NIAS were associated with non-expanded PS via the replacement method assuming a market share of 100%.

In a challenge test the boundary conditions of the FACET output have been fully explored for consumer exposure to Di and Tri from non-expanded PS. The exposure calculation of SDT from non-expanded PS to any food type, was not affected by parameters such as inclusion or exclusion of consumer loyalty and set-off migration. Further it did not make a difference whether the calculation was done for the full population, or for the food consumers only. This is explained by the fact that the majority of the population regularly consumes PS packed foods, and that set-off migration is not relevant for SDT migration. When comparing results of exposure calculations using different national dietary surveys, the French (INCA)-2 dataset (Individual National Survey on

Table 4
Calculation of MoS according to the FACET methodology based on Di, Tri and SDT in PS and the TTC of 4 μg/kg bw/d (corresponding to 240 μg/person/d).

PS oligomer	Sum (mg/kg PS) p90	Exposure to Di/Tri of food consumers (mg/kg bw/day)				Margin of Safety (MoS)			
		French population		UK children		French population		UK children	
		Mean	p95	Mean	p95	Mean	p95	Mean	p95
Dimers of Styrene	960	1.95E-06	1.26E-06	1.66E-06	0.00E+00	2048	3174	2415	n/d
Trimers of Styrene	14200	6.89E-06	5.02E-06	7.42E-06	0.00E+00	581	796	539	n/d
SUM (SDT)	15160	n/d	n/d	n/d	n/d	453	637	441	n/d

n/d = Not determined; p90/p95 = 90th and 90th percentiles.

Food Consumption (INCA)-2. ANSES, FRANCE. Download at: https://www.data.gouv.fr/fr/datasets/donnees-de-consommations-et-habitudes-alimentaires-de-letude-inca-2-3/) represents the worst case when modelling the European populations. In addition, the most sensitive subpopulation is listed (UK children at the age of 1–4 years) (UK, 1995).

The FACET (2017) methodology enabled calculation of MoS and the results obtained are given in Table 4 including the assumed 90th percentile concentrations of Di, Tri and SDT in the PS material. A short explanation of statistics for food consumption is given by the following example. Take a group of 100 people and line them up in order of who consumed the most Di, Tri and SDT. In this case the 95th percentile of this group is the amount of Di, Tri and SDT that the 95th highest consumer ate. The mean is the "average" amount consumed, i.e. the total amount consumed by all consumers together divided by the number of people. The mean can thus be higher than the 95th percentile when there is a relative small group with high consumption. For example, if in a group of 10 persons 4 persons eat one cup of yogurt a day and one person eats 10 cups of yoghurt a day, the 90th percentile of the population would eat 1 cups of yoghurt a day, while the mean would eat 1.4 cups of yoghurt a day. Table 4 shows the mean estimated exposure for food consumers to Di and Tri is higher than the 95th percentile, the 95th percentile estimated exposure of the UK Children to the Di and Tri is even zero. For food consumers this value is derived from only non-zero food consumption simulants. This indicates, like in the example, the mean exposure results from a minority of food consumers with relative high exposure. The other food consumers do consume food which could lead to exposure, but the migration from the packaging materials to real food leading to exposure is estimated to be negligible.

The MoS was related to a TTC 4 μg/kg bw/d according to Leeman et al. (2014). Normalization to bodyweight enabled MoS calculation for different age groups as proposed by EFSA (2012b). The MoS of the SDT has been determined by dividing the TTC by the sum of the exposure estimate of the Di and the Tri. The MoS of the 95th percentile estimated exposure of the UK Children to the Di and Tri cannot be calculated as exposure is estimated to be zero. Table 4 indicates the MoS is always well above 1 for both the mean as well as for the 95th percentile for the French population (453 and 637respectively), as well as for the UK children population (441). This strongly suggests that SDT migration from food packaging materials is unlikely to adversely affect consumer health. The FACET reports, describing the input and output in more detail, are given in the electronic supplement.

Facet allows more detailed analyses of the exposure by both Food Group and Packaging materials. Table 5 summarizes the drivers of exposure by both Packaging materials and Food Group of the oligomers.

Table 5 shows the main source of estimated exposure is related to the consumption of fish and fish products, molluscs, crustaceans and echinoderms. The oligomers are estimated to mainly migrate from the Plastic Tray/Pot/Tub/Cup food packaging material. For the UK-children this is almost solely from the PS Flexible Wrapper/Bag/Pouch, whereas for the France population the estimated exposure also results significantly from migration from PS Sealed Lidding/membrane.

7. Discussion

The European food safety regulations have required for many years the assessment of IAS (Intentionally Added Substances) that are for polymeric plastic packaging materials predominantly the starting monomers. In addition, since 2011 (EU, 2011), formal safety evaluation of NIAS (Non Intentionally Added Substances) is required arising, i.e. from initiators, processing aids, additives and their reaction or decomposition products. Oligomers are a quantitatively important group of NIAS, and Di and Tri are by far the most relevant oligomers in the case of PS materials. In this paper the safety of SDT for consumer exposure via GPPS and HIPS food containers is assessed.

Table 5
Drivers of exposure to Di and Tri according to the FACET methodology.

PS oligomer	Population	Plastic Tray/Pot/Tub/Cup food packaging material		Food groups		
		Flexible Wrapper/Bag/Pouch	Sealed Lidding/membrane	Fish and fish products, molluscs, crustaceans and echinoderms	Dairy products and analogues	Desserts (except bakery and fruit desserts)
Dimers of Styrene	French population	55.78%	44.22%	91.47%	0.77%	7.76%
	UK children	99.81%	0.19%	99.90%	0.10%	0.00%
Trimers of Styrene	French population	49.95%	50.05%	89.46%	1.17%	9.37%
	UK children	99.70%	0.30%	99.84%	0.11%	0.05%

Exposure assessment: For SDT in PS food packaging materials concentrations of up to 12600 mg/kg PS were found (Table 2) showing that SDT are quantitatively important NIAS. But such data do not allow any conclusion about potential migration and the investigation of Genualdi et al. (2014) on partition coefficients has shown for example that the transfer of SDT to food simulants is low. Migration of SDT from PS into 20% or 50% ethanol is generally below 200 μg/6 dm^2 (with higher values obtained for 95% ethanol) and below ~60 μg/person/d in different food items (Table 3). This indicates that only a small proportion of SDT present in PS polymer will actually migrate into food and food simulants.

Due to the large number of manufacturers of PS and the range of products in the marketplace a prerequisite for risk assessment of SDT is that virtually all different Di and Tri have been accounted for when analyzing migrates or PS materials. Overall, nearly 30 samples from food simulants, about 100 food items (Table 3) and about 180 different PS materials (Table 2) have been analyzed. The total dataset is complex but overall the Di and Tri identified and the levels reported give a representative picture of all oligomers potentially migrating as NIAS from PS food contact materials.

Klärner et al. (1998) and Fail et al. (1998) measured the total amounts of Di and Tri without further specification of the single chemical entities. On the other hand, the vast majority of the other studies specifically measured each Di and Tri separately. It had been shown by Prinsen and Gouko (2001) and Nakai et al. (2014) that the sum of these Di and Tri represented about 90% of the total SDT and Weel (2016a) could not find any oligomers with a molecular weight higher than that of Tri. It was verified that all studies quantifying the single Di and Tri entities included the quantitatively most important SDT. In PS materials and migrates the concentrations of Di always were much lower than those of Tri and the quantitatively most important single substances was NST-03 (and thereof the isomers NST-03-2 and NST-03-1), followed by NST-01, NSD-01 and NSD-09. On the other hand, the concentrations of D1, NST-02, NSD-08, NST-12, and NST-13 were negligible reaching rarely more than 1% of the sum of SDT. In all studies that were used for this assessment the isomers exceeding about 1% of the total SDT were quantified. Thus, a broad database is available with analytical information for the chemical entities of Di and Tri migrating into food or food simulants and especially for their concentrations in GPPS and HIPS.

Based on these analytical data the likely consumer exposure is estimated in a tiered approach with 3 levels of increased refinement. The first tier is a conservative approach using SDT migration from PS food packaging materials into food simulants, predominantly 50% ethanol, 10 d, 40 °C.a It is assumed that an adult person consumes 1 dm^3 (1 kg) of food packaged in 6 dm^2 of PS food packaging material, every day. Tier 2 is based on measured SDT concentrations in actual food, again using an assumed daily food consumption of 1 kg food/adult/d. This strategy has an important drawback in that most analyses were done on Japanese food items that clearly differ from European or Western dietary habits. Tier 1 and 2 are based on the very conservative and quite unrealistic premise that all food items are packaged in PS and as such are daily ingested over life time while different consumer habits, variations during different seasons and preferences at different ages are not taken into account. Therefore, as tier 3 the FACET (2017) methodology is applied which incorporates a probabilistic modelling of food consumption habits. In the FACET approach measured migration into food or food simulants is substituted by modelling the transfer of Di and Tri from PS packaging into the different food items under consideration. Also, since manufacture of PS food packaging materials is virtually the same in different regions of the world and has not been changed over many years, the FACET methodology can rely on a very large data base of SDT concentrations in PS including many studies from Japan.

Derivation of ADI or SML: In terms of establishing an ADI or SML it is an unavoidable fact that for most NIAS there will not be comprehensive toxicology data available upon which to reliably establish a point of departure. The absence of robust data, e.g. equivalent to that generally becoming available for existing substances, combined with difficulties in isolating test materials in sufficient purity and quantities for conventional toxicity testing forces risk assessors to look to using other, less data demanding, approaches such as the concept of TTC.

In the case of SDT there is a well conducted Reproductive and Developmental Toxicity study which allows a comparison of the "conventional" approach with that of TTC. Basing the ADI or SML solely upon the published study of Nagao et al. (2000) (NA-study) was judged insufficient as the study design did not meet the general requirements for repeated dose studies. But the identified weaknesses were not considered as being of sufficient importance as to preclude the use of the study and a NOAEL of 1 mg/kg bw/d was identified. Application of assessment factors of EFSA (2012a) and ECHA (2012) led to a safe exposure level of 1 μg/kg bw/d. The use of these assessment factors is justified because it has been demonstrated that SDT do not accumulate in mammals and are not genotoxic in bacterial and mammalian cell tests. Similarly, these findings enable using the TTC proposed by EFSA (2012b) and inclusion of SDT into Cramer class II + III with its associated TTC of 1.5 μg/kg bw/d. This is further supported by the weight of evidence assessment that SDT are not to be considered as endocrine disruptors (Gelbke et al., 2018). The modification to the TTC approach proposed by Leeman et al. (2014) is considered justified since SDT are clearly not organophosphates, carbamates and organohalogens. Thereby the TTC of 4 μg/kg bw/d is used in place of 1.5 μg/kg bw/d for Cramer class II + III as currently recommended by EFSA (2012b).

It is noted that the TTC approach has some inbuilt conservativism, too. It is based on the distribution of the NOAELs of single compounds. Such a distribution will overestimate the probability of high toxicity for mixtures since the TTC value of 4 μg/kg bw/d implies that every component will have the NOAEL of 4 μg/kg bw/d. Even if each oligomer of SDT has a 5% chance of having a NOAEL as low as 4 μg/kg bw/d, the probability that all oligomers have such low NOAELs is much smaller than 5%. Price et al. (2009) have analyzed this phenomenon. It is difficult to assess in detail as to how much these considerations may affect the evaluation of SDT. SDT were divided into 2 groups, the Di and Tri, with Tri being quantitatively more important than Di. If the Di and Tri are considered as two groups with similar migration properties within, but different properties between both groups (as may be gleaned by the data of Genualdi et al., 2014) the TTC may be presumed to be driven predominantly by Tri and the modifying effect of Di may be relatively small.

Risk assessment and MoS: The MoS for consumers potentially exposed to SDT is calculated by comparison of the "safe exposure" levels (ADI, SML and TTC) with estimates for potential exposure via food.

As shown in Table 3 MoS > 1 are obtained in all assessments based upon migration measurements and the TTC of 240 μg/person/d, apart from the simulant 95% ethanol which is thought to be unsuitable as it is likely affecting the integrity of the PS samples and apart from a HIPS sample analyzed by OFI (2017b). It is not fully understood why this HIPS sample gave high migration results other than it possibly being due to aging of the sample. Also, the large difference in MoS obtained by migration into 95% ethanol and the FACET methodology strongly indicates that 95% ethanol is not an appropriate food simulant and largely exaggerates migration. Specifically, for exposures derived by migration from GPPS and HIPS into 50% ethanol (10 d, 40 °C) the MoS are between 1.02 and > 22 (apart from the one HIPS sample of OFI, 2017b). As to be expected, migration into 20% ethanol is lower than into 50% ethanol and produced higher MoS for both GPPS and HIPS. With migration conditions selected by BfR (2016), namely 50% ethanol over 2 h at 70 °C, the MoS is 4.7 and at room temperature 18.5 showing that migration, as expected, is temperature dependent. Similarly, MoS of > 1 are obtained for SDT concentrations measured in Japanese-type food items (MoS of 3.8–480) and several food items from the US (MoS > 48).

Most importantly, MoS well above 1 are obtained for the French

population and for UK children using the FACET (2017) methodology (95th percentile/mean) as tier 3 for a large variety of food PS packaging materials. In risk assessment, the 95th upper percentiles are typically viewed as most important. However, Table 4 shows that the mean exposure is higher than the 95th percentile. This indicates the mean exposure results from a minority of food consumers with relative high exposure. Nevertheless, the MoS is always well above 1 for the French population both for the mean as well as for the 95th percentile (453 and 637) and even for the mean of the UK children population (441). This strongly suggests that SDT migration from food packaging materials is unlikely to adversely affect consumer health.

The oligomers are estimated to mainly migrate from the Plastic Tray/Pot/Tub/Cup food packaging material. As shown in summary Table 5 the main source of the estimated exposure is related to the consumption of fish and fish products, molluscs, crustaceans and echinoderms. This might be surprising as the biggest single application of PS is yogurt pots. This may be explained by the high logKow of Di and Tri, which would make migration to low-fat content foods such as yogurt negligible. The added value of the FACET exposure assessment has hereby been demonstrated, as FACET allows to easily explore the relevance of other minority food-groups for the risk assessment related to Di and Tri.

In summary, notwithstanding the high migration observed into 95% ethanol, irrespective of the exposure assessment method used the MoS always was clearly > 1 demonstrating that SDT in PS food packaging do not present a risk for consumers.

This investigation demonstrated the utility of the TTC approach and the FACET methodology to assessing NIAS such as oligomers. In addition, the value of the Leeman et al. (2014) modification has been demonstrated here supporting the recommendation that if the chemical structures allow, the 4 μg/kg bw/d threshold for Cramer class II + III as proposed by Leeman et al. (2014) should be more widely adopted.

Conflicts of interest

Heinz-Peter Gelbke and Mark Pemberton are private consultants working for the Styrenics Steering Committee, a sector group within PlasticsEurope. Marcy Benton, Gordon Dawkins, Ralf Eisert, Edgar Leibold and Atsushi Yasukawa work for companies that manufacture styrene and/or polystyrene. Atsunobu Sakoda works for the Japanese Styrene Industry Association (JSIA), Christian Block for PlasticsEurope. Iris Maria Puijk is employed by Triskelion B.V. who prepared the FACET calculations for the Styrenics Chain. The authors have sole responsibility for the content and the writing of the paper. The interpretation and views expressed in the paper are not necessarily those of the author's employers.

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Appendix A. Supplementary data

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Transparency document

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References

Aiba, T., Nagao, Y., Hatakeyama, O., et al., 1998. Metabolic fate of styrene oligomers: blood levels in rats after a single oral or intravenous administration. In: 4th Annual Joint Meeting of the Japanese Society of Environmental Toxicology and the Society of Bioassay Studies. Abstract, pp. 29 (in Japanese).

Ashby, J., Tennant, R.W., 1991. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. Mut. Res. 257, 229–306.

BfR, 2016. Gemessene Gehalte an Styrol-Oligomeren in Lebensmittelsimulanzien: Gesundheitliche Risiken sind unwahrscheinlich. Aktualisierte Stellungnahme 023/2016 des BfR vom 21. April 2016.

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.8: Characterisation of Dose [concentration]-response for Human Health.

EFSA, 2012a. SCIENTIFIC OPINION. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA J. 10 (3), 2579 2012.

EFSA, 2012b. Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA Journal 10 (7), 2750. https://doi.org/10.2903/j.efsa.2012.2750. 2012. www.efsa.europa.eu/efsajournal.

EFSA, 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. EFSA J. 11 (3), 3132.

EU, 2011. Commission Regulation (EU) No. 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food.

FACET, Oldring, P.K.T., O'Mahony, C., Dixon, J., Vints, M., Mehegan, J., Dequatre, C., Castle, L., 2017. Development of a new modelling tool (FACET) to assess exposure to chemical migrants from food packaging (2013). Food Addit. Contam. Part A Chem. Anal. Control, Exposure Risk Assess. 31, 444–465.

Fail, P.A., Hines, J.W., Zacharewski, T., Wu, Z.F., Borodinsky, L., 1998. Assessment of polystyrene extract for estrogenic activity in the rat uterotrophic model and in vitro recombinant receptor reporter gene assay. Drug Chem. Toxicol. 21 (Suppl. 1), 101–121.

Gelbke, H.P., Banton, M., Block, C., Dawkins, G., Leibold, E., Pemberton, M., Sakoda, A., Yasukawa, A., 2018. Oligomers of styrene are not endocrine disruptors. Crit. Rev. Toxicol. https://doi.org/10.1080/10408444.2018.1447547.

Genualdi, S., Nyman, P., Begley, T., 2014. Updated evaluation of the migration of styrene monomer and oligomers from polystyrene food contact materials to food and food simulants. Food Additives Comtam. 31 (Part A), 723–733.

Grifoll, M., Solanas, A.M., Bayona, J.M., 1990. Characterization of genotoxic components in sediments by mass spectrometric techniques combined with Salmonella/microsome test. Arch. Environ. Contam. Toxicol. 19, 175–184.

Hirano, S., Tanaka, M., Date, K., Ohno, K., Kobayashi, K., Sakurai, T., Nagao, Y., Nobuhara, Y., Yamada, T., 2001. Identification, determination, and biological evaluation of a novel styrene trimer contained in polystyrene food containers. 2. J. Agric. Food Chem. 49, 4127–4131.

Hoppe, M., de Voogt, P., Franz, R., 2016. Identification and quantification of oligomers as potential migrants in plastics food contact materials with focus in polycondensates – a review. Trends Food Sci. Technol. 50, 118–130.

ILSI, 2015. Guidance of Best Practices on the Risk Assessment of Non Intentionally Added Substances (NIAS) in Food Contact Materials and Articles. Report Commissioned by the ILSI Europe Packaging Materials Task Force. Published July 2015; Version January 2016. (D/2015/10.996/39; ISBN: 9789078637424; ISSN: 2294-5504.

Kaneko, R., Watanabe, Y., Funayama, K., Kabashima, J., Saito, K., 1999. Survey of styrene dimers and trimers in polystyrene equipment and packages for food. Ann. Rep. Tokyo Metr. Res. Lab. P.H. 50, 208–214.

Kaneko, R., Funayama, K., Haneishi, N., Watanabe, Y., Ogino, S., 2003. Survey of amount of styrene dimers and trimers in instant-noodles contained in polystyrene cups. Ann. Rep. Tokyo Metr. Inst. P.H. 54, 235–241.

Kawamura, Y., Nishi, K., Maehara, T., Yamada, T., 1998a. Migration of styrene dimers and trimers from polystyrene containers into instant foods. J. Food Hyg. Soc. Jpn. 39, 390–398.

Kawamura, Y., Sugimoto, N., Takeda, Y., Yamada, T., 1998b. Identification of unknown substances in food contact polystyrene. J. Food Hyg. Soc. Jpn. 39, 110–119.

Kawamura, M., Kawamura, Y., Takeda, Y., Yamada, T., 1998c. Determination of styrene dimers and trimers in food contact polystyrene. J. Food Hyg. Soc. Jpn. 39, 199–205.

Kawamura, Y., Nishi, K., Sasaki, H., Yamada, T., 1998d. Determination method of styrene dimers and trimers in instant noodles contained in polystyrene cups. J. Food Hyg. Soc. Jpn. 39, 310–314.

Klärner, P., Klenz, R., Eder, R., Volz, W.E., Schnell, H.W., Leyendecker, D., Güntner, A., Nießner, N., Morris, C.R., Christian, M.S., 1998. Preparation and analysis of styrene oligomers containing migrates from various polystyrenes used in food packaging. Drug Chem. Toxicol. 21 (Suppl. 1), 31–49.

Kroes, R., Renwick, A.G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., Schlatter, J., van Schothorst, F., Vos, J.G., Würtzen, G., 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem. Toxicol. 42, 65–83.

Leeman, W.R., Krul, L., Houben, G.F., 2014. Reevaluation of the Munro dataset to derive more specific TTC thresholds. Regul. Toxicol. Pharmacol. 69, 273–278.

Munro, I.C., Kennepohl, E., Kroes, R., 1999. A procedure for the safety evaluation of flavouring substances. Food Chem. Toxicol. 37, 207–232.

Munro, I.C., Renwick, A.G., Danielewska-Nikiel, B., 2008. The threshold of toxicological concern (TTC) in risk assessment. Toxicol. Lett. 180, 151–156.

Nagao, T., Wada, K., Kuwagata, M., Ono, H., 2000. Effects of prenatal and postnatal exposure to styrene dimers and trimers on reproductive function in rats. Reprod. Toxicol. 14, 403–415.

Nakada, Y., Sakai, M., Hinata, M., Suzuki, T., 2000. Determination of the migration

amount and total amount of styrene monomer, dimers and trimers in polystyrene food containers by GC/MS. Bunseki Kagaku 49, 775–800.

Nakai, M., Tsubokura, M., Suzuki, M., Fujishima, S., Watanabe, Y., Hasegawa, Y., Oyama, K., Ogura, S., 2014. Genotoxicity of styrene oligomers extracted from polystyrene intended for use in contact with food. Toxicol. Rep. https://doi.org/10.1016/j.toxrep.2014.11.007.

OECD TG 415, 1983. One-generation Reproductive Toxicity Study. adopted 26 May 1983.

OECD TG 443, 2015. Extended One-generation Reproductive Toxicity Study. adopted 28 July 2015.

OECD TG 422, 2015. Combined Repeated Dose Toxicity Study with the Reproduction/developmental Toxicity Screening Test. adopted 28 July 2015.

OECD TG 421, 2015. Reproduction/developmental Toxicity Screening Test. adopted 28 July 2015.

OFI, 2017a. Analysis of Polystyrene Migrates for Endocrine Active Substances. Project report 418.935 of 2017-06-07. .

OFI, 2017b. Analysis of Polystyrene Migrates for Endocrine Active Substances. Project report 419.955 of 2017-06-07. .

Ohyama, K., Satoh, K., Sakamoto, Y., Ogata, A., Nagai, F., 2007. Effects of prenatal exposure to Styrene trimers on genital organs and hormones in male rats. Exp. Biol. Med. 232, 301–308.

Ono, H., Wada, K., Kuwagata, M., 1999. Effects of Orally Administered Styrene Dimers and Trimmers on Reproductive and Developmental Function in Rats. Hatano Research Institute, Food and Drug Safety Center Study No.: R-98-023. Commissioned by Japan Styrene Industry Association.

Price, P.S., Hollnagel, H.M., Zabik, J.M., 2009. Characterizing the noncancer toxicity of mixtures using concepts from the TTC and quantitative models of uncertainty in mixture toxicity. Risk Anal. 29, 1534–1548.

Prinsen, M.K., Gouko, N., 2001. Determination of the oestrogenic (uterotrophic) activity of extracts of 'general purpose polystyrene (GPPS)' using immature female rats. J. Appl. Toxicol. 21, 235–239.

Sakamoto, H., Matsuzaka, A., Itoh, R., Tohyama, Y., 2000. Quantitative analysis of styrene dimers and trimers migrated from disposable lunch boxes. J. Food Hyg. Soc. Jpn. 41, 200–205.

UK, 1995. Office of population censuses and surveys. Social survey division, medical research council. Dunn nutrition unit, department of health, ministry of agriculture, fisheries and food. (1995). National diet, nutrition and dental survey of children aged 1 1/2 to 4 1/2 Years, 1992-1993. [data collection]. UK Data Service. SN: 3481. http://doi.org/10.5255/UKDA-SN-3481-1.

US FDA, 2007. Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations. April 2002; December 2007. (Accessed June 2016). http://www.fda.gov/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm081818.htm.

Weel, K.G.C., 2016a. Investigation of the Migration of Oligomers from Polystyrene: Type of Oligomers and Their Migration Levels. TNO Triskelion Report V20633. .

Weel, K.G.C., 2016b. Investigation of the Migration, Residual Content and FACET Exposure of Oligomers from Polystyrene. TNO Triskelion Report V20766. .

Weel, K.G.C., 2016c. Investigation of the Migration of Oligomers from Polystyrene: Migration into 20% and 50% Ethanol. TNO Triskelion Report V20820. .

Yamada, T., 1999. Synthesis, analysis and biological evaluation of styrene oligomers. Yuki Gosei Kagaku Kyokaishi 57, 58–64.

Yamada, T., Hirano, S., Kobayashi, K., Sakurai, T., Takaki, I., Tanaka, M., Nagao, Y., Azuma, Y., Date, K., Ohno, K., Chiba, M., Nobuhara, Y., 2000a. Identification, determination and biological evaluation of novel styrene trimer in polystyrene container. Bunseki Kagaku 49, 493–501.

Yamada, T., Tanaka, M., Hirano, S., Nagao, Y., Kobayashi, K., Sakurai, T., Furukawa, Y., Nobuhara, Y., 2000b. Determination of styrene oligomers in instant noodles contained in a polymer container. Bunseki Kagaku 49, 857–867.

Yanagiba, Y., Ito, Y., Yamanoshita, O., Zhang, S.Y., Watanabe, G., Taya, K., Li, C.M., Inotsume, Y., Kamijima, M., Gonzalez, F.J., Nakajima, T., 2008. Styrene trimer may increase thyroid hormone levels via down-regulation of the aryl hydrocarbon receptor (AhR) target gene UDP-glucuronosyltransferase. Environ. Health Perspect. 116, 740–745.