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Derivation of safe health-based exposure limits for potential consumer exposure to styrene migrating into food from food containers

Heinz-Peter Gelbke [a,*], Marcy Banton [b], Eric Faes [c], Edgar Leibold [d], Mark Pemberton [e], Sophie Duhayon [f]

[a] CinTox, 381, Avenue de Pessicart, F-06100 Nice, France
[b] Lyondell Chemical Company, 1221 McKinney Street, Houston, TX 77010, USA
[c] Styrenics Steering Committee, Cefic and PlasticsEurope, Av. E. van Nieuvenhuise 4, B-1160 Brussels, Belgium
[d] BASF SE, GUP/P – Z570, D-67056 Ludwigshafen, Germany
[e] Systox Limited, 84 Hazelwood Rd, Wilmslow, Cheshire SK92QA, United Kingdom
[f] Total Research & Technology Feluy, Zone Industrielle C, B-7181 Seneffe (Feluy), Belgium

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ABSTRACT

Residual styrene present in polystyrene food packaging may migrate into food at low levels. To assure safe use, safe exposure levels are derived for consumers potentially exposed via food using No/Low Adverse Effect Levels from animal and human studies and assessment factors proposed by European organisations (EFSA, ECHA, ECETOC). Ototoxicity and developmental toxicity in rats and human ototoxicity and effects on colour discrimination have been identified as the most relevant toxicological properties for styrene health assessments. Safe exposure levels derived from animal studies with assessment factors of EFSA and ECHA were expectedly much lower than those using the ECETOC approach. Comparable safe exposure levels were obtained from human data with all sets of assessment factors while ototoxicity in rats led to major differences. The safe exposure levels finally selected based on criteria of science and health protection converged to the range of 90–120 mg/person/d. Assuming a consumption of 1 kg food/d for an adult, this translates to 90 mg styrene migration into 1 kg food as safe for consumers. This assessment supports a health based Specific Migration Limit of 90 ppm, a value somewhat higher than the current overall migration limit of 60 ppm in the European Union.

1. Introduction

Polystyrene (PS) is extensively used as food packaging material mainly in the form of GPPS (general purpose polystyrene), HIPS (high impact polystyrene) and EPS (expanded polystyrene) or in copolymerisation with other monomers, especially acrylonitrile and butadiene. Due to the production process styrene monomer (S) is found in residual amounts in such polymer food containers, due either from unreacted S in the starting polymer before converting or from thermal depolymerisation during the converting process. In current S-based polymer grades, the amount of the monomers may reach about 500 mg S/kg PS (O'Brian, 2001).

In the present EU regulation for plastic materials and articles intended to come into contact with food (EU, 2011) S is listed without any restriction. Especially no Specific Migration Limit (SML) is defined; consequently the overall migration limit of 60 mg/kg food would apply. In 2003, WHO (2003) published a Tolerable Daily Intake (TDI) of 7.7 μg/kg bw/d, corresponding to 0.46 mg/person/d (based on 60 kg body weight). This latter TDI was based upon reduced body weight at 250 ppm in female rats in a 2-year drinking water study (Beliles et al., 1985) and derived by dividing the

Abbreviations: AF, assessment factor; bw, body weight; DNEL, Derived No Effect Level; ECETOC, European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA, European Chemicals Agency; EFSA, European Food Safety Authority; EH, epoxide hydrolase; EPS, expanded polystyrene; GPPS, general purpose polystyrene; HED, human equivalent dose; HIPS, high impact polystyrene; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LOAEL, Low Adverse Effect Level; LWAE, lifetime weighted average exposure; MA, mandelic acid; MAK, Maximale Arbeitsplatz Konzentration; NOAEL, No Adverse Effect Level; PGA, phenylglyoxylic acid; PS, polystyrene; RAC, RISK Assessment Committee of ECHA; RAR, Risk Assessment Report of the EU; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; RfD, Reference Dose; S, styrene; SEL, safe exposure level; SML, Specific Migration Limit; SO, styrene-7,8-oxide; SSC, Styrenics Steering Committee; TDI, Tolerable Daily Intake; TWA, time weighted average exposure; UK, United Kingdom; US FDA, Food and Drug Administration of the U.S. Department of Health and Human Services; WHO, World Health Organisation.

* Corresponding author. Tel.: +33 (0)983253688, mobile: +33 (0) 659 13 88 31.
E-mail addresses: heinz-peter.gelbke@gmx.de (H.-P. Gelbke), marcy.banton@lyondellbasell.com (M. Banton), efa@cefic.be (E. Faes), edgar.leibold@basf.com (E. Leibold), markpemberton@systox.com (M. Pemberton), sophie.duhayon@total.com (S. Duhayon).

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NOAEL (125 ppm/7.7 mg/kg bw/d for males) by 1000. Health Canada (1993) and RIVM (2001) derived a TDI of 120 μg/kg bw/d based on the same NOAEL (125 ppm/12 mg/kg bw/d for females) but using an AF of 100. The US EPA (1990) derived an oral Reference Dose (RfD) of 200 μg/kg bw/d based on effects observed on haematology and liver in the dog at exposure concentrations higher than 200 mg/kg bw/d (oral intubation) in a 19 months study (Quast et al., 1979). An AF of 1000 was applied.

With the advent of the REACH regulation in Europe a procedure has been prescribed to establish Derived No Effect Levels (DNELs) for long-term exposure of the general population. Although DNELs are not intended to specifically address direct consumer ingestion these may provide a useful approach for the safety assessment of food contaminants. A DNEL (long term – oral route – general population) of 2.1 mg/kg bw/d was proposed for S (IUCLID, 2013). The calculation of DNELs is based on toxicity benchmarks, usually the No/Low Observed Adverse Effect Levels (NOAEL/LOAEL) found in experimental animals or epidemiological studies and the application of assessment factors (AFs).

There is a broad, albeit very low exposure of consumers to S via food packaging materials and some recent exposure estimates are reported. Safe exposure levels (SEL) are derived for consumers exposed to S via food. The toxicological endpoints that are nowadays considered as most relevant are taken into consideration, namely ototoxicity and colour vision disturbance in humans as well as postnatal developmental effects in rats. Especially the database for ototoxicity is robust in experimental animals and humans and concordant under qualitative and even quantitative aspects. Division of the NOAELs/LOAELs for these toxicity benchmarks by appropriate AFs leads to different SELs. This paper concentrates on AFs recently proposed by EFSA (2012), ECHA (2012) and ECETOC (2010) and therefore the results primarily relate to the European regulatory environment. The SELs will then allow defining a Specific Migration Limit (SML) for the migration of S into food. Since only toxicity data are taken into consideration this SML is solely based on health effects.

2. General considerations

2.1. Potential consumer exposure to styrene

The SELs and the SML finally arrived at in this paper should be put into context with actual data for potential consumer exposure via migration of S from PS food containers. Exposures have been measured or estimated by methods of different complexity and some more recent examples are given below:

1. *Use of food simulants under standardised conditions*: GPPS, HIPS and EPS were extracted with food simulants prescribed by European Directives (EC, 1997; EU, 2011), namely 3% acetic acid, 10% aqueous ethanol and olive oil for 2 h at 70 °C or 10 days at 40 °C (GPPS, HIPS) or 10 days at 5 °C (EPS), representing most closely the conditions of use for these polymers. The PS surface to food ratio was 6 dm^2/1 kg food equating the EU "standard cube" (1 kg food packaged in 6 dm^2 plastics packaging material). For GPPS and HIPS migration of S into 3% acetic acid or 10% ethanol was generally below 100 μg/kg, but reached 75–590 μg/kg in olive oil after 10 days at 40 °C. With EPS extracted for 2 h/70 °C somewhat higher concentrations were found (up to 340 μg/kg in olive oil), but clearly lower concentrations at 10 d/5 °C (up to 40 μg/kg in sunflower oil) (O'Brian, 2001).
2. *Food surveillance*: In 5 sets of a total diet study covering 100 categories of food (UK MAFF, 1999) S was detected at low levels of up to 14 μg/kg. Using consumption data from the National Food Survey the dietary exposure of consumer was estimated to be 0.03–0.05 μg/kg bw/d (1.8–3 μg/person/d). In a previous study (UK MAFF, 1994) covering 248 food samples packed in a variety of pack types the concentrations of S were generally in the range of <1–60 μg/kg with the exception of a "low fat" table spread (97 μg/kg) and milk and cream products sold as small portions (~10 g) for tea and coffee (23–230 μg/kg). In this latter case the high surface to volume ratio and the high fat content explain these higher concentrations. Subsequently 22 samples of such "coffee creamers" were specifically studied and relatively high concentrations of S were verified with a range of 13–316 μg/l (Offen et al., 1995).
3. *Compilation of data*: Tang et al. (2000) assessed human exposure to S on the basis of literature data. The average consumption profile of the general German population was utilised under the assumption that milk, milk products, fat and oil were all packed in PS materials. The daily S intake was estimated to reach 2–12 μg/person/d. A comparable estimate (9 μg/person/d) was obtained by Lickly et al. (1995) for people in the United States based on a consumption of 3 kg food/d. A probabilistic approach was used by Holmes et al. (2005) to calculate a median exposure for adults of 0.039 μg/kg bw/d (~2.4 μg/person/d). Vitrac and Leblanc (2007) used a probabilistic method to estimate S exposure via consumption of yogurt, a food item often packed in PS. They assumed an average S concentration for the food containers of 500 mg/kg and calculated for the daily uptake a 50th percentile of 12 μg/person/d.

To estimate the exposure of children (5–12 years) to S from food containers Duffey and Gibney (2007) used the type and amount of food consumed by children from the Irish National Children's Food Survey, including the type of packaging for these foods. A food was assumed to contain S if it was packaged in a material that might release the monomer. Migration values were taken from the literature and exposure estimates used either the 90th percentile or the maximum migration values. The mean intake of S was calculated to be 0.122 μg/kg bw/d or 0.169 μg/kg bw/d. A comparison with the provisional Tolerable Daily Intake (TDI) of 40 μg/kg bw/d (established by JECFA in 1984) led to the conclusion that exposure to S via food is of no concern for children.

Apart from exposures via food packaging materials, consumers are also exposed to S in natural food items. S occurs in unpackaged food at concentrations near the limit of detection (0.1 μg/kg food) to typically 200 μg/kg (e.g. in olive oil) although levels up to 5 mg/kg have been reported in some mouldy cheeses (Tang et al., 2000). The highest concentrations have been found in cinnamon, ranging from 170 μg/kg to 34 mg/kg (Steele et al., 1994).

2.2. Point of departure

The first step in the derivation of a SML is the definition of SELs for consumers to migrants (herein S) from packaging into food. The calculation of a SEL is generally based on the NOAELs/LOAELs, the points of departure, obtained in experimental animals or epidemiological studies. In studies on experimental animals the selection of a NOAEL or LOAEL is straightforward, as these are defined by the doses selected for the experiment.

Both ECHA (2012) and ECETOC (2010) specifically note that differences exist in the nature of data from animal versus epidemiological studies. In animal tests the doses are predefined leading to precise values for the NOAEL and the LOAEL. The true threshold apparently lies somewhere between the two values. In the case of epidemiological studies, exposures generally cannot be exactly defined and rather exposure categories forming a continuum are estimated. As a consequence, epidemiological data do not directly allow to establish the exact NOAEL, but only to approximate the

exposure range that should encompass the NOAEL. Generally the upper exposure limit of the no-effect category is the same as the lower limit of the category showing an effect and the true threshold lies within one of these categories. Therefore the NOAELs/LOAELs will not be displayed at discrete exposure concentrations but within exposure categories/ranges.

ECHA (2012) proposes that generally the lower boundary/limit of the lowest exposure category with an effect should be considered the LOAEL and the upper boundary/limit of the exposure range with no statistically or biologically significant effects should be considered the NOAEL for epidemiological studies. If the number of individuals in the NOAEL category is small or if the exposure distribution is skewed towards the lower end of the category, a more conservative NOAEL may be justified, e.g. the average of the lower and the upper limit value of the NOAEL category, the average exposure of the individuals, or the median exposure value of the NOAEL group. A similar approach is proposed by ECETOC (2010) based on the consideration that the LOAEL will be located within the lowest exposure range in which an adverse effect is observed. In the absence of a high prevalence of responders in this exposure category, its lower limit is likely to reflect the NOAEL. No guidance is given by EFSA (2012) how to define a NOAEL from epidemiological studies.

2.3. Assessment factors (AF)

After the NOAEL/LOAEL has been identified from experimental animal or epidemiological studies, AFs are applied for various extrapolation steps to arrive at a sufficiently protective SEL:

- route-to route (e.g. inhalation to oral);
- interspecies (e.g. rats to humans);
- intraspecies (e.g. workers to the general population);
- exposure duration (e.g. 6–24 h/d; subchronic to chronic exposure);
- dose–response relationship (e.g. NOAEL to LOAEL);

AFs are available from several authority/scientific organisations and are considered in this assessment, including:

- EFSA (2012).
- ECHA (2012).
- ECETOC (2010).

Important differences exist between these organisation's guidances and AFs that may affect the derivation of a SEL, especially for inter- and intraspecies extrapolation, and are briefly discussed here.

2.3.1. AF for interspecies extrapolation

In the absence of chemical-specific data on toxicokinetics and/or –dynamics EFSA (2012) applies a total AF of 10, subdivided into a factor of 4 for toxicokinetics and of 2.5 for toxicodynamics. EFSA uses body weight as a scale for interspecies extrapolation while other organisations, including ECHA (2012) and ECETOC (2010), have proposed allometric scaling based on differences in metabolic rate per body weight.

For the oral exposure route the same total default AF of 10 is proposed by ECHA and EFSA for the extrapolation from rats to humans. This factor is subdivided by ECHA for rats into a subfactor of 4 for allometric scaling, but this subfactor varies according to the species under consideration, for example 7 for mice. The factor for allometric scaling is not applicable if both, experimental animals and humans are exposed by inhalation. A further subfactor of 2.5 accounts for toxicokinetic differences not related to metabolic rate (small part) and toxicodynamics differences (larger part). ECETOC (2010) reviewed ECHA's 2008 guidance on this subfactor (note: in this respect there is no difference between the ECHA guidances of 2008 and 2012) and the available literature and concluded that the inclusion of the factor for allometry is justified, but not the application of the factor of 2.5. They presented evidence that the multiplicative association between interspecies and intraspecies AFs is already overly conservative per se, so the inclusion of the factor of 2.5 for remaining differences is unnecessary.

2.3.2. AF for intraspecies extrapolation based on animal data

For the majority of chemicals reliable human data are not available and the assessment has to rely on information from animals only. This is likely the basis for the default AF of 10 proposed by EFSA (2012) to calculate SELs for consumers exposed via food. The same AF of 10 is used by ECHA (2012) for intraspecies extrapolation to the general population.

To derive an AF for intra-human variability based on actual data and not only on default assumptions, ECETOC (2010) analysed the distribution of human data available from the literature and arrived at the conclusion that an AF of 5 is appropriate for extrapolations from animal data to the general population.

If the point of departure is the NOAEL of a study in rats, the procedure of EFSA (2012) and ECHA (2012) leads to a total AF of 100 while that of ECETOC to 20. But other organisations/agencies may favour different approaches. For example, the US FDA (2005) uses a scaling factor of 6.2 for interspecies extrapolation from the NOAEL in an oral study in rats to a human equivalent dose (HED) on the basis of differences in body surface area. The resulting HED is then divided by a default safety factor of 10. Thus, starting from an oral rat study this leads to a total factor of 62 that lies in between those of EFSA/ECHA and ECETOC.

2.3.3. AF for intraspecies extrapolation based on human data

If human data are used as the SEL basis at least part of the intra-human variability is addressed, depending on the size and heterogeneity of the study population. No specific AFs are mentioned by EFSA (2012) for this situation.

ECHA (2012) requires an integration of animal and human data although a commonly agreed formalised procedure is not yet available. The AF finally used would strongly depend on the quality of the human data. If the quality is poor (e.g. a small, homogenous sample in the study of humans) the standard default factors should be taken, 5 for workers and 10 for the general population. An example given is a small occupational surveillance study with only 10–20 workers who might have been selected so that the healthy worker effect applies. On the other hand, AFs lower than the standard assessment factors may be used when some of the factors that cause the intraspecies variation are covered in the target population. But apart from referring to expert judgment, no further guidance is provided what numerical AFs may be used in specific situations.

More guidance on this issue is given by ECETOC (2010). For extrapolation from worker data to the general population an AF of 3 is proposed (note: in the accompanying table an AF of 2 is given). Additional AFs may be necessary for a study substantially influenced by healthy worker effect that may result in an overestimation of the NOAEL (AF 2) or for a small study size (AF 3). For this latter case an example is given, namely the derivation of an occupational exposure limit based on volunteer studies with a limited number of participants.

3. Relevant toxicological endpoints

On the basis of the UK Risk Assessment Report (RAR, 2008) and the literature update provided in the Chemical Safety Report

(according to the REACH requirements) a number of studies have been identified that are potentially relevant to human health assessments.

A large database is available for S to assess its internal disposition in the body and toxicological properties. Of particular importance for evaluating toxicity concerns for long term exposures such as ingestion in food include considerations for mutagenicity/genotoxicity, carcinogenicity, reproductive and developmental toxicity and repeated exposure target organ effects.

The metabolic profile of S in mice, rats and humans has been well defined in a large number of studies and provides the basis for extrapolation of toxicity data from experimental animals to humans. The RAR (2008) notes that the "data indicate significant differences in the metabolism of S between species and between tissues" and that "the specifics of the local metabolism in a target tissue must be considered when extrapolating findings in animals to assess the likely hazard and risks in the equivalent human tissues." This relates to the reactive metabolites formed from S, i.e. styrene oxide (SO) and the downstream metabolites of 4-vinylphenol. The RAR (2008) concludes: "that the human tissues investigated – apart from the liver – produce very little SO, if any, and have a greater capacity to hydrolyse SO with epoxide hydrolase (EH) than rodents. This difference is most pronounced in human nasal and lung tissues where production of SO is minimal or undetectable, and is also associated with a greater capacity to hydrolyse SO by EH. The mouse lung and nasal tissues produce the greatest amount of SO among the species tested, and, in general, have less EH activity, suggesting that significantly high local concentrations of SO will be present in these tissues. It is also evident that other toxic metabolites, particularly 4-vinylphenol and its reactive downstream products, are produced to a far higher extent in mouse lung than in rat ... or human lung ..." The overall conclusion was that "the data suggests that the mouse is a poor model for predicting the effects of repeated inhalation exposure to S in humans." Therefore data derived from investigations especially in mice must be interpreted with caution and generally are of limited relevance for the risk assessment for humans.

The specifics of metabolism in mice have to be taken into account for the assessment of carcinogenicity. There is clear evidence that S exposure leads to lung tumours in mice while a carcinogenic response has not been observed in rats (Cruzan et al., 1998, 2001). After a detailed evaluation of the mechanism leading to these lung tumours in mice the RAR (2008) comes to the following conclusion: "All of the key events of (the) postulated mode of action are less operative in the non-responsive rat (which does not develop lung tumours at exposure concentrations up to 1000 ppm) and even less operative in humans."

Mutagenicity/genotoxicity properties of S have been thoroughly studied in in vitro and animal investigations as well as in studies in humans. The weight of evidence assessment in the RAR (2008) leads to the conclusion that "there is no convincing evidence that S possesses significant mutagenic/clastogenic potential *in vivo* from the available data in experimental animals" and that "there is no convincing evidence that S has shown mutagenic activity in humans".

Repeated dose studies have identified several organs/tissues that may be targets for S toxicity. The RAR (2008) concludes that "four well characterised target sites of toxicity have been identified: the nasal epithelium (in rats and mice), the lung (in the mouse), the liver (in the mouse) and the ear (in the rat)." These target sites were analysed in regard to their relevance to humans and it was found that "the available inhalation repeated dose toxicity studies have identified ototoxicity as the most sensitive and relevant effect of S repeated inhalation exposure." Ototoxicity was also demonstrated to occur after oral exposure in rats and in workers at the workplace by the inhalation route. Furthermore, impairment of colour vision was reported for workers. Finally, in a 2-generation study in rats some slight effects on postnatal development were described. Based on these effects the Risk Assessment Committee (RAC) of the European Chemicals Agency (ECHA) has proposed to classify S as a category 2 "suspected human reproductive toxicant" for developmental effects (RAC, 2012). Although it has been questioned whether these effects represent a direct and specific expression of S developmental toxicity (SSC, 2011), this information is taken into account to calculate a safe level based on the highest dose for which no effect on either reproduction or neurodevelopment could be found. Therefore, for derivation of safe oral exposure levels and a SML, the following effects are considered:

- Ototoxicity in rats after inhalation and oral exposure.
- Delays in postnatal development in rats after inhalation exposure.
- Ototoxicity in workers after inhalation exposure.
- Colour vision impairment in workers after inhalation exposure.

4. Derivation of SMLs

4.1. Point of departure: ototoxicity in rats

4.1.1. Inhalation route

When using ototoxicity data derived from rat inhalation studies, the following aspects are necessarily considered:

- Species sensitivity: the rat is the most sensitive species as compared to the other species investigated (hamsters). These species differences could be related to the metabolic turnover of the parent organic solvent that is the ultimate chemical moiety causing hearing deficits. There is a close similarity in metabolism between rats and humans, as a single inhalation exposure to S led to very similar blood levels for rats and humans (Ramsey and Young, 1978). Therefore, rat data will be used for this endpoint.
- Exposure duration: In the RAR (2008) it is concluded that "ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response." Therefore, an exacerbation of hearing deficits is not to be expected by prolongation of exposure beyond a few weeks to life time. Data derived from studies with an exposure duration of a few weeks can be used for the assessment of long term effects.
- Sensitivity of histopathological and (electro)physiological effects: The NOAEL for ototoxicity as derived from auditory dysfunction is generally higher than that defined by histopathological effects on the outer hair cells of the cochlea. It is mentioned in the RAR (2008) that "the destruction of the hair cells is irreversible and occurs at slightly lower exposure concentrations than those producing the audiometric hearing threshold shifts". But hearing deficits are the toxicological endpoint of interest. Hence, taking very small losses of outer hair cells in row 3 as the decisive endpoint would be a very conservative approach for risk assessment based upon hearing impairment.
- Anatomical/histological similarities of target tissues between rats and humans: Ototoxicity is governed by direct impact of the (unmetabolised) parent compound on the hair cells of the cochlea. Passage to these target cells occurs via diffusion of the solvent from blood through Hensen's and Deiters cells. Taking into account the identical target cells (outer hair cells, Hensen's cells, Deiters cells) and the identical structure of the target organ (cochlea) and its blood supply, the toxicodynamics for ototoxicity caused by aromatic solvents are very similar between rats and humans.

Regarding the animal inhalation studies, the RAR (2008) defined for ototoxicity "NOAEL values of 500 ppm (2165 mg/m^3) and 300 ppm (1300 mg/m^3) for 4 weeks in sedentary/ordinary and active rats respectively." These NOAELs are derived from the study of Lataye et al. (2005) with male rats exposed 6 h/d, 5 d/week over 4 weeks and are based on electrophysiological measurements of hearing thresholds. For a risk assessment for the general population or for consumers exposed via food the NOAEL for sedentary rats should be used. Minor histopathological alterations (loss of hair cells) were still found in the cochlea of sedentary rats at 500 ppm without an objective hearing deficit. The toxicological significance of such a minor histopathological finding without a physiological hearing effect for human health and risk assessment is questionable.

To estimate the body burden via the inhalation route, an assumption about the absorption via the respiratory tract is necessary. According to the RAR (2008) "a value of 100% for absorption (for humans) (brackets added) via the inhalation route of exposure is taken forward to the risk characterisation", and "the absorption rate in humans is approximately the same as in rats". This would mean 100% absorption for rats and humans. However, the RAR (2008) also mentions several studies in humans with absorptions after inhalation of 59–88% and Young et al. (1979) assumed an absorption of 66% via the respiratory tract for rats. S absorption was determined in a study with human volunteers (Engström et al., 1978). An uptake by inhalation of 70% was calculated at rest (as would be relevant for the general population) and of 60% after heavy physical activity of 150 W over 30 min. Therefore a value of 70% is justified for respiratory uptake of humans and rats.

For oral absorption it is stated in the UK RAR (2008) that "a value of 100% for oral absorption is taken forward to the risk characterisation." For the oral route 100% is used here for rats and humans.

To calculate the body burden for consumers exposed orally to S the respiratory volume for rats over 6 h is taken from ECHA (2012) as 0.29 m^3/kg bw. As the rats were only exposed 5 d/week the default assumption is used that this dose is evenly distributed over the whole week. Therefore this daily body burden will by multiplied by 5/7.

Starting from the NOAEL for hearing deficits, the daily body burden by the oral route would be

- 2165 mg/m^3 × 0.29 m^3/kg bw × 5/7 × 0.70 = 314 mg/kg bw.

It may be argued that at the NOAEL for hearing deficits some minor histopathological alteration in the cochlea were observed that should be taken into consideration although their toxicological meaning is unclear. 2165 mg/m^3 should then be regarded as a LOAEL for hair cell loss in the cochlea requiring an additional AF for LOAEL to NOAEL extrapolation. For such an extrapolation both, ECETOC (2010) and ECHA (2012) propose a default AF of 3. ECHA specifies that this AF should be "between 3 (as minimum/majority of cases) and 10 (as maximum/exceptional cases)" and it "should take into account the dose spacing in the experiment (in recent study designs generally spacing of 2–4 fold), the shape and slope of the dose–response curve, and the extent and severity of the effect seen at the LOAEL."

The data of Lataye et al. (2005) should be evaluated based on these criteria:

- Spacing of doses: in this study the spacing is extremely narrow with a factor of only 1.3 between the 3 lowest dose levels (500, 650, 850 and 1000 ppm).
- Shape and slope of the dose–response curve: the most sensitive histopathological endpoint as defined in many studies with S (e.g. Loquet et al., 1999) is hair cell loss in the third row. Comparison of the effects at 500 and 650 ppm shows an extremely steep dose–response curve and the effects at 500 ppm are very small.
- Extent and severity of effects: as regards the extent of effect see above. As the histopathological lesions were not accompanied by an objective hearing deficit (by electrophysiological measurement) the health implication of this effect is at best questionable and by no means severe.

Therefore an AF of 2 should be sufficient especially under consideration that in the study of Maekitie (1997), Maekitie et al. (2002) the NOAEL for hearing deficits as well as for histopathological alterations was 300 ppm.

This additional AF of 2 should be used for the study of Lataye et al. (2005) in addition to all other AFs (EFSA, ECHA, ECETOC) if hair cell loss in the cochlea is taken as the decisive endpoint. Thereby a daily body burden of 157 mg/kg bw would be obtained.

In another study (Maekitie, 1997, Maekitie et al. (2002)) a NOAEL of 300 ppm (corresponding to 1300 mg/m^3) was identified for histopathological lesions and by means of electrophysiological measurements. Male rats were exposed 12 h/d, 5 d/week over 4 weeks. With a respiratory volume for the rat over 12 h of 0.29 × 2 = 0.58 m^3/kg bw this NOAEL would lead to

- 1300 mg/m^3 × 0.58 m^3/kg bw × 5/7 × 0.70 = 377 mg/kg bw for the daily body burden.

The daily oral doses derived above from different NOAELs/LOAELs have then to be divided by the AFs for inter- and intraspecies variability to obtain the SELs. In Table 1 the different AFs and points of departure are given together with the SELs thereby derived. For calculation of SELs on a mg/person/d-basis a standard body weight of 70 kg for adults is used (EFSA, 2012).

Table 1 exhibits a large difference by a factor of 10 between the different SELs. A factor of 5 is due to the interspecies and intraspecies AFs proposed by EFSA and ECHA (100) versus those by ECETOC (20). There is a further factor of 2.4 depending on the NOAELs/LOAELs selected from different endpoints and studies. The first consideration is whether the LOAEL based on histopathology or the NOAEL for hearing deficits should be used. If the decision criterion is to use the most conservative approach without any further considerations, then the LOAEL for histopathological effects should be selected. However their toxicological meaning is unclear as these changes were not accompanied by hearing deficits. This would argue to base SEL derivation on the NOAEL for hearing deficit being the predominant parameter of concern for humans.

A second issue concerns the different NOAELs/LOAELs obtained by Lataye and Maekitie. One explanation may be possible differences in sensitivity of the rat strains (Lataye: Long Evans; Maekitie: Wistar). But the slightly different results obtained by both investigators may also be related to the daily exposure duration. If the same total dose is absorbed within an exposure duration of 6 h higher S blood concentrations will be reached compared to those obtained by an exposure duration of 12 h. Taking into account the protracted food intake of humans over the total daytime, an inhalation exposure duration of 12 h/d might be more meaningful. By this consideration preference for derivation of a SEL may be given to the study of Maekitie et al. (2002) with a daily exposure of 12 h and a NOAEL of 300 ppm for histopathology as well as for electrophysiology.

4.1.2. Oral route

Ototoxic effects were not only noted in rats after inhalation exposure but also after oral application, although the most comprehensive studies were carried out by inhalation. A study by of Wang et al. (2001) examined oral dose levels of 400 and 800 mg/kg bw, however a NOAEL for auditory effects could not be derived from this study (RAR, 2008). Further studies confirmed ototoxicity of S by the oral route including:

Table 1
SELs (in mg/person/d) derived from the different NOAELs/LOAELs based on ototoxicity in rats by inhalation (in mg/kg bw/d) by application of different AFs and a body weight of 70 kg for adult humans.

AFs	EFSA (2012)	ECHA (2012)	ECETOC (2010)
Interspecies variability	10		
–Allometric scaling (rat)		4	4
–Additional subfactor		2.5	
Intraspecies variability (humans/consumers)	10	10	5
Total AFs (for inter-/intraspecies variability)	**100**	**100**	**20**
Calculated oral points of departure (mg/kg bw/d)	SELs in mg/person/d		
377 (Maekitie; histopathology, hearing deficit)	264	264	1320
314 (Lataye; hearing deficit)	220	220	1100
Additional AF for extrapolation LOAEL to NOAEL	**2**	**2**	**2**
Total AFs (incl. LOAEL/NOAEL extrapolation)	200	200	40
Calculated oral point of departure (mg/kg bw/d)	SELs in mg/person/d		
157 (Lataye; histopathology)	110	110	550

- Gagnaire and Langlais (2005), but a NOAEL could not be derived as only one high dose of 880 mg/kg bw/d was given.
- Chen and Henderson (2009) exposed rats to continuous noise (100 dB SPL, 6 h/d, 5 d/week over 3 weeks) superimposed by an impact noise (up to 110 dB SPL, duration 30 ms at a rate of 1/s). 300 or 400 mg/kg bw S were given by gavage (5 d/week over 3 weeks). The exposure levels to S only induced a limited loss of outer hair cells and a very small hearing loss, but a NOAEL was not attained. Exposure to noise alone led to a clear hearing loss while there were only slight losses of outer hair cells. The combined exposures induced hearing and outer hair cell losses that were greater than the sum caused by S or noise alone. Thus, this study showed a S/noise interaction but the high noise levels leading by themselves already to hearing loss have to be taken into consideration.
- Yang et al. (2009) exposed rats by gavage at 400 mg/kg bw, 5 d/week over 3 weeks. Hearing ability was impaired and there was a clear damage of outer hair cells. A NOAEL was not established.
- The study of Chen et al. (2008) did not allow establishing a NOAEL, but the results of Chen et al. (2007) may allow an approximation of a NOAEL.

Chen et al. (2007) exposed male rats orally once per day by gavage to 200, 300, 400, and 800 mg/kg bw/d (5 d/week) over 3 weeks. The dose level of 200 mg/kg bw/d led only to small losses of outer hair cells. Unfortunately no statement is made whether hearing ability was significantly impaired at 200 mg/kg bw in comparison to the control group. But Fig. 2A of the publication indicates that at this dose level no significant or at most a very minor hearing deficit occurred. Thus 200 mg/kg bw is the LOAEL. An AF of 2 is proposed for LOAEL to NOAEL extrapolation, the same as used for the histopathological alterations in the study of Lataye et al. (2005). The application of the same AF is supported by

- the narrow spacing of the dose levels (200, 300, 400 and 800 mg/kg bw) with a factor of 1.5 between the two lowest doses;
- the steep slope of the dose–response curve;
- the questionable health implications of the histopathological findings;
- and finally by a comparison of the cochleograms given by Chen et al. (2007) and Lataye et al. (2005) (for sedentary rats) both showing very similar losses of hair cells at their lowest dose levels.

To account for continuous exposure the LOAEL of 200 mg/kg bw will be multiplied by 5/7 yielding a corrected daily body burden of 143 mg/kg bw/d as point of departure for the application of the AFs. Derivation of SELs (Table 2) is similar to the approach used for ototoxicity by inhalation.

The SEL derived from the LOAEL of the oral study of Chen et al. (2007) is by a factor of ~2 lower than that obtained by the LOAEL for histopathological alterations of the cochlea in the inhalation study of Lataye et al. (2005) and by a factor of ~5 lower compared to Maekitie et al. (2002). Chen and Lataye used the same rat strains (Long Evans), but Maekitie used Wistar rats. Whether these different strains may have a major impact on NOAELs/LOAELs is unknown. But more important is the daily duration of application as already indicated above when comparing the NOAELs of Lataye et al. (2005) and Maekitie et al. (2002). The lowest SEL is obtained by the study of Chen et al. (2007) with oral bolus application, followed by Lataye et al. (2005) with inhalation over 6 h/d and the highest SEL is derived by the study of Maekitie et al. (2002) with an inhalation duration of 12 h/d in. The protracted exposure of humans via food resembles most the latter experimental setup and a SEL based on oral bolus application would be a worst case situation clearly exaggerating any potential risk for humans exposed via food.

4.2. Point of departure: delays in postnatal development in rats after inhalation exposure

In a guideline 2-generation reproductive toxicity study with rats that included examinations of developmental neurotoxicity (Cruzan et al., 2005a, 2005b) some minor delays in postnatal development were observed only in the F2 offspring predominantly at 500 ppm. Based on these findings the Risk Assessment Committee of ECHA proposed that S should be classified for developmental toxicity (cat. 2, H361d; CLP) (RAC, 2012). After an in-depth assessment of the Cruzan studies the RAR (2008) came to the following conclusion: "For effects on the pups, a NOAEL of 150 ppm can be established based on body weight reductions and effects on related developmental parameters at 500 ppm. Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEL for effects on pups at 50 ppm." Therefore 150 ppm (corresponding to 650 mg/m^3) is used as NOAEL for this effect. 150 ppm also was identified as NOAEL for maternal toxicity.

Sprague–Dawley rats were exposed daily for 6 h/d. The F_0 generation was exposed for 10 weeks prior to mating and throughout the subsequent 2 weeks of mating. The females continued inhalation exposure during gestation and lactation, except from gestation

Table 2
SELs (in mg/person/d) derived from the LOAEL based on ototoxicity in rats after oral exposure (in mg/kg bw/d) by application of different AFs and a body weight of 70 kg for adult humans.

AFs	EFSA (2012)	ECHA (2012)	ECETOC (2010)
Interspecies variability	10		
– Allometric scaling (rat)		4	4
– Additional subfactor		2.5	
Intraspecies variability (humans/consumers)	10	10	5
Extrapolation LOAEL to NOAEL	2	2	2
Total AFs	**200**	**200**	**40**
Calculated oral point of departure (mg/kg bw/d)	SELs in mg/person/d		
143	50	50	250

day 21 through to lactation day 4, when S was administered in olive oil by gavage at dose levels of 66, 120 and 300 mg/kg bw/d divided into 3 equal doses approximately 3 h apart. This was done because this period is critical to pup neurological and neuroendocrine development and hence, there were concerns that stress on the pups arising from the removal of the dams for the 6 h exposure session might have affected pup development. These oral dose levels were chosen to generate peak blood levels of S after each gavage dose that closely matched the predicted blood levels of S from the inhalation exposure levels. At weaning, offspring (25/sex/group) were randomly selected to constitute the F_1 generation. Inhalation exposure of the F_1 animals was initiated on postnatal day 22 and followed exactly the same protocol as for the F_0 generation. The F_2 generation was not directly exposed to the test article but was potentially exposed in utero and through nursing during postnatal day 0–21. At weaning, F_2 pups were selected for further observations up to about 2.5 months. No exposure to S occurred during this period.

This study was specifically designed to detect effects related to reproduction and development of offspring and comprised the whole reproduction cycle. The application of an AF for study duration is therefore not necessary and, as the exposure schedule was seven days a week, a correction factor for daily exposure is also not necessary. Using the respiratory volume for rats over 6 h of 0.29 m^3/kg bw (ECHA, 2012) and the absorption of 70%, a body burden of $0.29 \times 650 \times 0.70 = 132$ mg/kg bw/d for rats is estimated to base SELs. Application of AFs for derivation of SELs corresponds to the approach for ototoxicity by inhalation (Table 3). As can be seen the SELs derived for reproductive toxicity are in the same range or by a factor of ~2.5 lower than those for ototoxicity by the inhalation route.

4.3. Point of departure: ototoxicity in workers after inhalation exposure

Studies in experimental animals and findings from exposed worker population have provided strong evidence that S exposure may lead to hearing deficits. The RAR (2008) summarised the data available as follows: "these human data cannot be used for risk characterisation purposes, nevertheless they indicate that the observations of ototoxicity in animals may be relevant to humans". Since the RAR review, however, further studies became available that support the conclusion that S may induce ototoxicity in humans: Sliwinska-Kowalska et al. (2005), Johnson et al. (2006), Zamyslowska-Szmytke et al. (2009), Triebig et al. (2009), Morata et al. (2011). For studies before 2009, due to co-exposure to noise and other solvents and insufficient exposure data, it is not possible to establish a clear dose–response relationship.

The most comprehensive study was carried out by Triebig et al. (2009). This study is unique as it differentiates between worker populations exposed to high concentrations in former years and those exposed to lower concentrations in recent years. All other investigations only relied on exposure assessments around the time of initiation of the study. Such a differentiation between former and present exposure levels is pivotal as ototoxicity once initiated by high exposure is considered irreversible. In addition, it is well known that in former years high exposures prevailed in reinforced fibreglass plastics industries, especially for laminators, the workforce most often studied for effects on hearing ability. Such high exposures have now generally been reduced to comply with existing occupational exposure limits. Therefore studies without data for former, most probably high exposures cannot be used to reliably define a NOAEL or LOAEL.

Triebig et al (2009) investigated 128 laminators from a boat building plant in comparison to 127 matched controls of the same plant. Exposure analysis was based on biological monitoring (using urinary mandelic and phenylglyoxylic acid; MA + PGA) to avoid any confounding by personal protection measures. The biomonitoring data was then transformed to airborne concentrations of S in ppm. Biological monitoring showed that the controls working in the same plant were exposed to very low levels of S. Thus a comparison was made between three groups of low ($N = 99$), medium ($N = 118$) and high ($N = 31$) exposed workers with calculated mean exposure concentrations of 1.7–3.4, 7.6–15.2 and 38.8–48.5 ppm at the time the investigation took place.

Due to the irreversibility of ototoxic effects, the workplace situation was also analysed retrospectively before hygiene standards were systematically improved. Thereby a high/long exposure

Table 3
SELs (in mg/person/d) derived from the NOAEL based on reproductive toxicity in rats after inhalation (in mg/kg bw/d) by application of different AFs and a body weight of 70 kg for adult humans.

AFs	EFSA (2012)	ECHA (2012)	ECETOC (2010)
Interspecies variability	10		
-Allometric scaling (rat)		4	4
-Additional subfactor		2.5	
Intraspecies variability (humans/consumers)	10	10	5
Total AFs	**100**	**100**	**20**
Calculated oral point of departure (mg/kg bw/d)	SELs in mg/person/d		
132	92.4	92.4	462

group ($N = 17$; mean job tenure 14.6 years) could be defined that was compared to a matched low/short exposure group ($N = 34$; mean job tenure 6.4 years). For these groups, as expected, a large difference was apparent for the chronic exposure index and for the lifetime weighted average exposure (LWAE), but there was only little difference in current exposure levels. Regular measurements in the past had shown former mean exposure concentrations of >30 ppm and exposure ranges up to 150 and 200 ppm were reported.

A statistical assessment based on current exposure did not show a significant effect when comparing the low versus the high exposure group with calculated mean airborne S exposures of 39–49 ppm and job tenure of about 6 years. On the other hand there was an indication for S induced hearing losses in the subgroup of high/long exposed workers assumed to have been exposed at 25–33 ppm over about 15 years (range up to 26 years). This exposure calculation was based on the LWAE. But such LWAEs only insufficiently reflect short time periods with high exposures and certainly not peak exposures that are important for laminators in the glass reinforced plastic industries. But median exposure concentrations of 80–100 ppm existed for a time more than 10 years before this study was initiated. Taking into account the irreversibility of S induced hearing deficits, the authors concluded that these effects stem from former exposure concentration of more than 50 ppm as an average.

If the study of Triebig et al. (2009) is used to derive a NOAEL based on auditory effects several points need consideration:

1. An important strength of this study is that it is based on biomonitoring data, thus representing the "real" internal dose.
2. The ranges of airborne concentrations were calculated by transformation of urinary MA + PGA to ppm based on the results of several studies leading to ranges like for example 500–600 MA + PGA (mg/g creatinine) correspond to 20 ppm.
3. For a small number of individuals in a NOAEL category ECHA (2012) proposes to use a more conservative approach than the upper limit of this category, for example the average exposure.
4. As regards the representativeness of the study population for the general population it has to be taken into consideration whether only healthy, adult male workers were investigated. In contrast, the authors noted that the study results themselves were not influenced to any major extent by a possible healthy worker effect because a pre-selection with respect to hearing function for the high and low exposure groups can hardly be assumed. In addition, such an effect can hardly be assumed for the small hearing deficits found in this study that will not be noticed in daily life.
5. The key issue is whether the assessment should be based on exposures that had prevailed for about the last 6 years with a NOAEL of a mean exposure of 39–49 ppm or alternatively should the high/long exposure group be used to define a NOAEL/LOAEL based on the LWAE of 25–33 ppm over about 15 years. But the LWAE is not a reliable quantitative indicator for former high exposure levels. It is derived by the life time exposure at different jobs divided by job tenure. This is in principle an average over the total life time exposure situation and high exposures in former years are insufficiently represented. Therefore the LWAE is only suited to a very limited extent to define a NOAEL/LOAEL that stems from high exposures over limited time periods in former years.

Taking into consideration that ototoxicity is induced after relatively short exposures exceeding the threshold for this effect, it is not appropriate to derive the LOAEL from the LWAE. Instead the NOAEL should be based on the transformed biomonitoring data for exposures that had prevailed the 6 years before study initiation. To be conservatively protective, the lower end of the NOAEL category should be selected, i.e. 39 ppm (rounded to 40 ppm, corresponding to 172 mg/m^3). A more conservative approach would be to use 20 ppm (corresponding to 86 mg/m^3) proposed as DNEL (long-term for workers) by industry under the REACH regulation (IUCLID, 2013) in accordance with several national occupational exposure limits, e.g. that of the German MAK commission (Neumann et al., 1997; MAK, 2003).

Based on these two points of departure the total daily body burden is calculated taking into consideration:

- days of exposure per week for workers (5 days) and consumers (7 days) by a factor of 5/7;
- respiratory volume of workers under light activity over 8 h, i.e. 10 m^3/person (ECHA, 2012);
- correction for absorption in the respiratory tract (70%).

resulting in estimations of:

$72 \times 5/7 \times 10 \times 0.7 = 860$ mg/person/d, corresponding to 12.3 mg/kg bw/d (for 40 ppm), and to 430 mg/person/d, corresponding to 6.15 mg/kg bw/d (for 20 ppm).

As these points of departure are derived from data for humans, only an intraspecies AF is applicable that should take into account that part of the total intra-human variability is already covered by the variability within the workforce investigated. As described above this situation is not considered by EFSA (2012) and no detailed guidance is given by ECHA (2012).

Thus guidance on how to address this factor presently can only be obtained from ECETOC (2010). Their default approach is an AF of 3 for extrapolation of worker data to the general population, provided that the study population is representative, the study group is sufficiently large and the study data are adequate. These prerequisites are basically met by the Triebig et al. (2009) data so that the default AF of 3 may be applicable. But there are two specific features mentioned by ECETOC that may lead to a modification (increase) of this default AF:

- If the study may be substantially influenced by a healthy worker effect an additional factor of 2 may become necessary. But as stated above, it cannot be assumed that a healthy worker effect could have played any major role in this study. Thus, there is no need for this additional factor of 2.
- If the study size is small, especially the subgroup defining the NOAEL, an additional factor of 3 may be used. Whether the study size is to be regarded as small (a total 128 exposed laminators and thereof 31 highly exposed subjects) cannot be decided by objective criteria. A conservative approach may apply this additional factor leading to an overall AF of 9.

Therefore based on ECETOC (2010) two AFs are applied: 3 for the standard approach and 9 taking into account the specificity of the cohort of Triebig et al. (2009). Based on ECHA (2012) again two AFs are applied: 10 for the default approach and 9 as an appropriate modification under consideration of the criteria of ECETOC (2010). Based on EFSA (2012) only the unmodified default AF of 10 can be used due to lack of guidance for this situation. The results obtained for the SELs by use of these AFs are summarised in Table 4.

SELs derived from the epidemiological study in workers differ by a factor of ~7. This is slightly lower than the factor of 10 obtained for ototoxic effects in rats after inhalation (Table 1). The broader spread of SELs based on experimental data is explained by uncertainties around the appropriate selection of intra- and interspecies AFs, while the selection of the point of departure (NOAEL) is straight forward when based on animal experiments.

The difference by the factor of ~7 in Table 4 is explained by two uncertainties:

- The selection of the appropriate point of departure, i.e. 40 ppm based on the actual data of the Triebig et al. (2009) study or 20 ppm in accordance with many national occupational exposure limits and the DNEL derivation under REACH by industry. The derivation of national occupational exposure limits may follow different procedures without the explicit use of AFs. Furthermore these limits generally were derived before publication of the Triebig et al. (2009) study. And finally, after ECHA (2012) has recently published its guideline how to derive NOAELs from epidemiological studies, closely resembling that of ECETOC (2010), this should be the basis for future assessments. Therefore, on balance it was decided to place emphasis on actual data, i.e. to accept 40 ppm as the point of departure.
- The appropriate AF for extrapolating worker data to the general population, 10 as the default AF of EFSA (2012) and ECHA (2012), 3 as the standard approach of ECETOC (2010) or 9 derived by a modification in accordance with considerations of ECETOC. The use the factor of 9 appears most appropriate, although this factor might still be too conservative for the basis from the Triebig study.

Thus the SEL of 95.6 mg/person/d (1.37 mg/kg bw/d) is best justified from a scientific point of view.

4.4. Point of departure: colour vision impairment in workers after inhalation exposure

There is an extensive body of data reported in the RAR (2008) on the effects of exposure to styrene on colour vision discrimination obtained from studies using tests specifically designed to examine this endpoint. It was concluded that "given the very mild nature (the affected individuals were not even aware of any deficit) and the likely reversibility of the effect which appears not to affect performance in jobs that require good colour vision, ... that the slight changes in colour discrimination detected should not be considered as an adverse health outcome of styrene exposure". Overall, 50 ppm (215 mg/m^3; 8 h TWA) were considered a NOAEL.

The study of Iregren et al. (2005) was not discussed in RAR (2008). Based on their findings the authors concluded that even levels below 90 mg/m^3 may affect colour vision negatively. A major criticism of this study is that a control group consisting of unexposed workers was not included. This deficit renders the conclusion very unreliable taking into account the high variability of the test procedure used (Lanthony D15 desaturated panel test) under different experimental settings (Paramei et al., 2004).

Two more recent investigations using the Lanthony D15 desaturated panel test give strong support to the conclusion of RAR (2008) that 50 ppm should be taken as a NOAEL. Seeber et al. (2009) studied the same workforce as Triebig et al. (2009). No impairment of colour vision was found in relation to the exposure levels at the time of investigation nor for the long/high exposed subgroup. Vyskocyl et al. (2012) subdivided 104 workers in the reinforced plastics industry into three groups based on their average exposure concentration during the work shift: a "Control group" exposed to 7 mg/m^3 (1.6 ppm), an "Average group" exposed to 137 mg/m^3 (32 ppm), and a "High group" exposed to 333 mg/m^3 (78 ppm). The maximum exposure was 520 mg/m^3. The workers were also classified according to their exposure to peaks. Colour vision was not affected. In addition, in both of these latter studies no adverse effects on contrast sensitivity were found.

The derivation of the SEL based on the NOAEL of 50 ppm (215 mg/m^3) follows the approach for ototoxic effects. The total daily body burden as point of departure is $215 \times 5/7 \times 10 \times 0.7 = 1075$ mg/person/d, corresponding to 15.4 mg/kg bw/d.

With the different AFs discussed above the SELs given in Table 5 are obtained:

The differences obtained for the SELs based on colour vision can be explained as done for ototoxicity. Again, the SEL of 119.4 mg/person/d (1.71 mg/kg bw/d) based on an AF of 9 is best justified from a scientific point of view.

4.5. Other considerations for point of departure

There are some "classical" effects that might also be discussed for derivation of a "safe exposure level":

According to RAR (2008), in mice, the most reliable information comes from a cancer bioassay (NCI, 1979), in which increased mortality and hepatic necrosis were observed at the highest dose of 300 mg/kg bw/d, and a NOAEL of 150 mg/kg bw/d was identified. But it was cautioned by RAR (2008) that in extrapolation to humans careful consideration has to be taken of the specifics of mouse metabolism and the high sensitivity of this species for liver toxicity as compared to e. g. the rat. Therefore this finding will not be used for derivation of a SEL.

In the assessment of the 2-generation reproductive toxicity study the RAR (2008) stated that 50 ppm can be identified as the

Table 4
SELs (in mg/person/d) based on the Triebig et al. (2009) study on ototoxicity in workers derived by application of different intraspecies AFs.

	EFSA (2012)	ECHA (2012)		ECETOC (2010)	
AF	**Standard 10**	**Standard 10**	**Modified 9**	**Standard 3**	**Modified 9**
	SEL based on point of departure 40 ppm, corresponding to 860 mg/person/d				
	86	86	95.6	286.7	95.6
	SEL based on point of departure 20 ppm, corresponding to 430 mg/person/d				
	43	43	47.8	143.3	47.8

Table 5
SELs (in mg/person/d) based on the NOAEL for colour vision impairment in workers derived by application of different intraspecies AFs.

	EFSA (2012)	ECHA (2012)		ECETOC (2010)	
AF	**Standard 10**	**Standard 10**	**Modified 9**	**Standard 3**	**Modified 9**
	SEL based on point of departure 50 ppm, corresponding to 1075 mg/person/d				
	107.5	107.5	119.4	358.3	119.4

NOAEL for parental toxicity based on body weight reductions at 150 and 500 ppm and on the degeneration of the nasal olfactory epithelium at 500 ppm. It was shown that the nasal effects cannot be extrapolated to humans. As regards the body weight reductions this finding is rather to be considered as being without toxicological significance, as similar effects at corresponding exposure concentrations were not described in the 90-day (Cruzan et al., 1997) nor in the carcinogenicity study inhalation studies in rats (Cruzan et al., 1998). Therefore, this finding is not used for derivation of a SEL.

5. Discussion

SELs are derived for consumers potentially exposed to S migrating from packaging into food. AFs of EFSA (2012), ECHA (2012) and ECETOC (2010) are applied for different toxicity effects identified from studies on exposed workers or experimental animals. The results are summarised in Table 6.

Table 6 shows that the SELs span a wide range from 43 to 1320 mg/person/d (factor of ~30) depending on the health endpoints and AFs selected. The impact of the health endpoints alone is clearly smaller as compared to that of the AFs as can be seen if only the SELs are compared which are either obtained with the conservative AFs of EFSA (2012) and ECHA (2012) (range 43–264; factor of ~6) or with the standard AFs of ECETOC (2010) (range 143.3–1320; factor of ~9).

The first consideration regards the AFs applied for the derivation of SELs. The conservative AFs of EFSA and ECHA have been used over decades but these lack conclusive scientific justification. Alternatively, ECETOC presents sound scientific evidence for the selection of their AFs and for any deviations from those of ECHA. Therefore for SELs that reflect a conservative, precautionary approach, the AFs of EFSA and ECHA may be appropriate, while for a science based approach those of ECETOC are preferred.

Scientifically more challenging is the selection of the most appropriate health endpoint basis for the SEL as a variety of effects have been observed in experimental animal and human studies. When comparing the animal studies on ototoxicity the following criteria are considered:

1. Daily exposure schedule in comparison to the protracted exposure of consumers via food intake: the exposure schedule of Maekitie et al. (2002) (inhalation, 12 h/d, 5 d/week) comes closest to the consumer exposure, followed by Lataye et al. (2005) (inhalation, 6 h/d, 5 d/week) and finally by Chen et al. (2007) (oral bolus by gavage, once/d, 5 d/week).
2. Endpoint of concern: for humans hearing loss is of major concern while the toxicological relevance of minor histopathological changes in the cochlea without hearing loss is of questionable toxicological relevance. Maekitie et al. (2002) did not observe hearing loss or histopathological alterations at 300 ppm. Lataye et al. (2005) found a NOAEL for hearing loss (in sedentary rats) at 500 ppm but this exposure concentration represented the LOAEL for histopathological alterations in the cochlea. Regarding the study of Chen et al. (2007) the LOAEL was 200 mg/kg bw but a clear decision is not possible whether this relates only to hair cell loss in the cochlea or in addition to hearing deficits.
3. Presentation of results: Lataye et al. (2005) presented their findings much more in detail as compared to Chen et al. (2007) or Maekitie et al. (2002). Furthermore, a wealth of studies with similarly detailed data presentation is available from the group of Lataye and coworkers.

Under these aspects preference is given to the inhalation study of Lataye et al. (2005) with its protracted exposure over 6 h/d. Although in the study of Chen et al. (2007) the oral route was used, this study is less suitable due to bolus application leading to high initial blood levels of S, the ultimate ototoxicant. To be sufficiently conservative histopathological findings may be the endpoint finally selected leading to a SEL of 110 (AFs of EFSA and ECHA) or 550 mg/person/d (AFs of ECETOC).

The SELs derived from developmental effects in rats after inhalation exposure (6 h/d; 7 d/week) are slightly lower than those proposed for ototoxicity in rats by the same exposure route: 92.4 (AFs of EFSA and ECHA) or 462 mg/person/d (AFs of ECETOC).

When findings in exposed workers are taken as the basis for derivation of SELs the following aspects are to be considered:

1. Daily exposure schedule: the normal workplace exposure of 8 h/d (5 d/week) with about 1 h of break closely resembles of the protracted daily food intake of consumers.
2. The appropriate intraspecies AF to extrapolate from workers to the general population: a default AF of 10 is proposed by EFSA and ECHA if there is virtually no indication about variability in humans. Both ECETOC (2010) and ECHA (2012) support the use of a lower AF if human studies are available covering at least some intra-human variability. Taking into account the more detailed guidance of ECETOC (2010) an AF of 9 is proposed as appropriate and sufficiently conservative.
3. Selection of the point of departure: the derivation of a SEL for consumers could be based on the DNEL (long-term workers) that corresponds with many national occupational exposure

Table 6
SELs (in mg/person/d) derived for the different toxicological endpoints applying the AFs for inter- and intraspecies differences of EFSA (2012), ECHA (2012) or ECETOC (2010).

AF	EFSA		ECHA			ECETOC		
Intra × inter	100		100			20		
Intra		10		10	9		9	3
Endpoint: Ototoxicity								
R; inh; Lat; histo	110		110			550		
R; inh; Lat; hear	220		220			1100		
R; inh; Maekitie	264		264			1320		
R; oral; Chen	50		50			250		
W; inh; 20 ppm		43		43	47.8		47.8	143.3
W; inh; 40 ppm		86		86	95.6		95.6	286.7
Endpoint: Developmental toxicity								
R; inh	92.4		92.4			462		
Endpoint: Colour vision								
W; inh		107.5		107.5	119.4		119.4	358.3

Abbreviations: intra = intraspecies variability; inter = interspecies variability; × = multiplication; R = rat; inh = inhalation; Lat = study of Lataye; histo = endpoint histopathology; hear = endpoint hearing ability; Maekitie = study of Maekitie; W = worker; 20/40 ppm = point of departure 20/40 ppm.

Table 7
Summary of the SELs (mg/person/d) given preference.

Endpoint	AFs of EFSA, ECHA	AFs of ECETOC
Ototoxicity, rat	AF 200: 110	AF 40: 550
Developmental toxicity, rat	AF 100: 92.4	AF 20: 462
Ototoxicity, workers	AF 9: 95.6	AF 9: 95.6
Colour vision, workers	AF 9: 119.4	AF 9: 119.4

limits (20 ppm). But with the publication of ECHA (2012) how to use findings in humans, similar in its strategy to ECETOC (2010), this guideline should be followed leading to 40 ppm as point of departure for ototoxicity.

In conclusion, a SEL of 95.6 mg/person/d is proposed, derived from ototoxicity in workers by the guidance both of ECETOC (2010) and ECHA (2012).

The SELs (in mg/person/d) calculated for developmental toxicity in rats (92.4) and colour vision deficiencies in workers (119.4) are in the same range as those selected for ototoxicity in animals (110) and workers (95.6).

6. Conclusion; derivation of a Specific Migration Limit (SML)

The derivation of a SML includes consideration of:

1. The total intake of food (or liquid) in kg food/person/d that may be consumed by different subgroups of the population, e.g. adults, children, pregnant women. For the purpose of this assessment a default value of 1 kg food/person/d for adults will be used as proposed by the Scientific Committee on Food (EC SCF, 2001).
2. The safe exposure level (SEL) that is the daily exposure of a consumer to a substance that will not lead to adverse health effects (in mg substance/kg bw/d or mg substance/person/d).

The SELs given preference under the aspects mentioned above are summarised in Table 7.

As can be seen from Table 7 there is a large difference in SELs derived from animal data based on the default AFs of EFSA and ECHA in comparison to those of ECETOC. Although the AFs of ECETOC are scientifically justified, the approach of EFSA/ECHA is recommended here as these SELs are very similar to those obtained from data in humans. Under this premise all SELs are in the same range (90–120 mg/person/d) for the different endpoints under consideration.

- ototoxicity, rats;
- developmental toxicity, rats;
- ototoxicity, humans;
- colour vision deficiency, humans.

giving strong support for an overall and consistent SEL of about 90 mg/person/d. The most robust database is available for ototoxicity for which the NOAELs/LOAELs have well been established in experiments in animals with supporting evidence from observational studies in workers, both of which lead to very similar SELs.

Using the assumption of a consumption of 1 kg food/d for adult humans a concentration of 90 mg S/kg food can be considered safe. This concentration corresponds to a Specific Migration Limit (SML) of 90 ppm for food packaged in polystyrene food containers. This SML, although above the current regulation for S (i.e. migration of up to 60 ppm into food) is demonstrated to be science based and sufficiently protective of human health.

Derivation of SELs in this publication was based on the application of AFs recently proposed by EFSA (2012), ECHA (2012) and ECETOC (2010). Therefore all considerations are primarily related to the European regulatory environment. Approaches of other regions/countries may be different and the example of the US FDA (2005) for derivation of a HED was briefly mentioned above. If applied to the derivation of a SEL this latter procedure would lead to values that lie between those derived by applying the AFs of ECETOC and EFSA/ECHA.

In summary, a comparison with exposure estimates given in Section 2.1. shows that the SELs and the SML derived from the health-effects data on Styrene are orders of magnitude higher than actual consumer exposures as a consequence of migration of styrene residues from polystyrene-based food containers.

Conflict of Interest

HPG is a private consultant working for the Styrenics Steering Committee of CEFIC and MP for a company that manufactures styrene. EF is the Director General of the Styrenics Steering Committee. MB, EF, and SD are employed by companies that manufacture styrene. 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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References

Beliles, R.P., Butala, J.H., Stack, C.R., Makris, S., 1985. Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. Fund. Appl. Toxicol. 5, 855–868..

Chen, G.D., Chi, L.H., Kostyniak, P.J., Henderson, D., 2007. Styrene induced alterations in biomarkers of exposure and effects in the cochlea: mechanisms of hearing loss. Toxicol. Sci. 98, 167–177.

Chen, G.D., Tanaka, C., Henderson, D., 2008. Relation between outer hair cell loss and hearing loss in rats exposed to styrene. Hear. Res. 243, 28–34.

Chen, G.D., Henderson, D., 2009. Cochlear injuries induced by the combined exposure to noise and styrene. Hear. Res. 254, 25–33.

Cruzan, G., Cushman, J.R., Andrews, L.S., Granville, G.C., Miller, R.R., Hardy, C.J., Coombs, D.W., Mullins, P.A., 1997. Subchronic inhalation studies of styrene in CD rats and CD-1 mice. Fund. Appl. Toxicol. 35, 152–165.

Cruzan, G., Cushman, J.R., Andrews, L.S., Granville, G.C., Johnson, K.A., Hardy, C.J., Coombs, D.W., Mullins, P.A., Brown, W.R., 1998. Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. Toxicol. Sci. 46, 266–281.

Cruzan, G., Cushman, J.R., Andrews, L.S., Granville, G.C., Johnson, K.A., Bevan, C., Hardy, C.J., Coombs, D.W., Mullins, P.A., Brown, W.R., 2001. Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. J. Appl. Toxicol. 21, 185–198.

Cruzan, G., Faber, W.D., Johnson, K.A., Roberts, L.S., Hellwig, J., Carney, E., Yarrington, J.T., Stump, D.G., 2005a. Two generation reproduction study of styrene by inhalation in Crl-CD rats. Birth Def. Res. (Part B) 74, 211–220.

Cruzan, G., Faber, W.D., Johnson, K.A., Roberts, L.S., Hellwig, J., Maurissen, J., Beck, M.J., Radovsky, A., Stump, D.G., 2005b. Developmental neurotoxicity study of styrene by inhalation in Crl-CD rats. Birth Def. Res. (Part B) 74, 221–232.

Duffey, E., Gibney, M.F., 2007. Use of a food-consumption database with packaging information to estimate exposure to food-packaging migrants; epoxidized soybean oil and styrene monomer. Food Additiv. Contam. 24, 216–225.

EC, 1997. European Commission Directive 97/48 of July 1997 Amending for the Second Time Council Directive 82/711/EEC Laying Down the Rules Necessary for Testing Migration of the Constituents of Plastic Materials and Articles Intended to Come into Contact with Foodstuffs.

ECETOC, 2010. Guidance on Assessment Factors to derive a DNEL. Technical Report No. 110. Brussels, October 2010.

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment. Characterisation of Dose [Concentration]–Response for Human Health (Chapter R.8).

EC SCF, 2001. Guidelines of the Scientific Committee on Food for the Presentation of an Application for Safety Assessment of a Substance to be Used in Food Contact

Materials Prior to its Authorisation. <http://ec.europa.eu/food/fs/sc/scf/out82_en.pdf>.
EFSA, 2012. Draft Guidance on Default Assumptions. Guidance on Default Assumptions Used by the EFSA Scientific Panels and Committee, and EFSA Units in the Absence of Actual Measured Data. EFSA Scientific Committee, European Food Safety Authority, 2011. <http://www.efsa.europa.eu/en/efsajournal/pub/2579.htm>.
Engström, J., Bjurström, R., Astrand, I., Övrum, P., 1978. Uptake, distribution and elimination of styrene in man. Concentration in subcutaneous adipose tissue. Scand. J. Work Environ. Health 4, 315–323.
EU, 2011. Commission Regulation (EU) No. 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food.
Gagnaire, F., Langlais, C., 2005. Relative ototoxicity of 21 aromatic solvents. Arch. Toxicol. 79, 346–354.
Health Canada, 1993. Canadian Environmental Protection Act. Priority Substances List. Assessment Report: Styrene. Cat. No.: En40-215/24F.
Holmes, M.J., Hart, A., Northing, P., Oldring, P.K., Castle, L., Stott, D., Smith, G., Wardman, O., 2005. Dietary exposure to chemical migrants from food contact materials: a probabilistic approach. Food Additiv. Contam. 22, 907–919.
Iregren, A., Johnson, A.C., Nylen, P., 2005. Low-level styrene exposure and color vision in Swedish styrene workers. Environ. Toxicol. Pharmacol. 19, 511–516.
IUCLID, 2013. Chemical Safety Report, Styrene. <http://echa.europa.eu/> (accessed 23.09.13).
Johnson, A.C., Morata, T.C., Lindblad, A.C., Nylen, P.R., Svensson, E.B., Krieg, E., Aksentijevic, A., Prasher, D., 2006. Audiological findings in workers exposed to styrene alone or in concert with noise. Noise Health 8, 45–57.
Lataye, R., Campo, P., Loquet, G., Morel, G., 2005. Combined effects of noise and styrene on hearing: comparison between active and sedentary rats. Noise Health 27, 49–64.
Lickly, T.D., Breder, C.V., Rainey, M.L., 1995. A model for estimating the daily dietary intake of a substance from food-contact articles: styrene from polystyrene food-contact polymers. Reg. Toxicol. Pharmacol. 21, 406–417.
Loquet, G., Campo, P., Lataye, R., 1999. Comparison of toluene-induced and styrene-induced hearing losses. Neurotoxicol. Teratol. 21, 689–697.
Maekitie, A., 1997. The Ototoxic Effect of Styrene and its Interaction with Noise. Academic Dissertation for PhD. Medical Faculty of the University of Helsinki, Finland.
Maekitie, A., Pirvola, U., Pyykkoe, I., Sakabira, H., Ruehimaeki, V., Ylikoski, J., 2002. Functional and morphological effects of styrene on the auditory system of the rat. Arch. Toxicol. 76, 40–47.
MAK, 2003. Occupational toxicants; critical data evaluation of MAK values and classification of carcinogens. In: Greim, H. (Ed.), DFG – Deutsche Forschungsgemeinschaft, vol. 20. Wiley-VCH GmbH & Co. KGaA, Styrene, pp. 285–290.
Morata, T.C., Sliwinska-Kowalska, M., Johnson, A.C., Starck, J., Pawlas, K., Zamyslowsa-Szmytke, E., Nylen, P., Toppila, E., Krieg, E., Pawlas, N., Prasher, D., 2011. A multicenter study on the audiometric findings of styrene-exposed workers. Int. J. Audiol. 50, 652–660.
NCI, 1979. Bioassay of styrene for possible carcinogenicity. Carcinogenesis Testing Program, National Cancer Institute, Bethesda, Maryland. Report No. NIH 79-1741.
Neumann, H.G., Thielmann, H.W., Gelbke, J.G., Filser, H.P., Greim, H., Kappus, H., Norpoth, K.H., Reuter, U., Vamvakas, S., Wardenbach, P., Wichmann, H.E., 1997. Proposed changes in the classification of carcinogenic chemicals in the work area. Reg. Toxicol. Pharmacol. 26, 288–295.
O'Brian, A.P., 2001. Measurement of Migration of Substances from Styrene Based Polymers. Pira, Ref. 00A11J0015, April 2001.
Offen, C.P., Cooper, I., Tice, P.A., 1995. Styrene Migration from Small Food Packaging Containers ('Coffee Creamers'). Pira, Ref. J41043, March 1995.
Paramei, G.V., Meyer-Baron, M., Seeber, A., 2004. Impairments of colour vision induced by organic solvents: a meta-analysis study. NeuroToxicology 25, 803–816.
Quast, J.F., Humiston, C.G., Kalnins, R.V., Olson, K.J., McCollister, S.B., Wade, C.E., Beyer, J.E., Schwetz, B.A., 1979. Results of a Toxicity Study of Monomeric Styrene Administered to Beagle Dogs by Oral Intubation for 19 Months. DOW Toxicology Laboratory, Health and Environmental Sciences, USA, Midland, MI 48640. Report of July 31, 1979.
RAC, 2012. Opinion Proposing Harmonised Classification and Labelling at EU Level of Styrene (adopted 28.11.12).
Ramsey, J.C., Young, J.D., 1978. Pharmacokinetics of inhaled styrene in rats and humans. Scand. J. Work Environ. Health 4 (Suppl. 2), 84–91.
RAR, 2008. European Union Risk Assessment Report, Styrene. Draft for Publication, June 2008. United Kingdom.
RIVM, 2001. Re-evaluation of Human-Toxicological Maximum Permissible Risk Levels. Report 711701 025.
Seeber, A., Bruckner, T., Triebig, G., 2009. Occupational styrene exposure, colour vision and contrast sensitivity: a cohort study with repeated measurements. Int. Arch. Occup. Environ. Health 82, 757–770.
Sliwinska-Kowalska, M., Zamyslowska-Szmytke, E., Szymczak, W., Kotylo, P., Fiszer, M., Wesolowski, W., Pawlaczyk-Luszczynska, M., 2005. Exacerbation of noise-induced hearing loss by co-exposure to workplace chemicals. Environ. Toxicol. Pharmacol. 19, 547–553.
SSC, 2011. Response of the Styrene Producers Association to the CLH Proposal (September 2011) for the Classification of Styrene as a Cat. 1B Reproductive Toxicant (Developmental Effects) According to Regulation (EC) No. 1272/2008 (CLP); November 14, 2011. ECHA Reference Number 13ea058d-c7bb-456c-9a6e-eedd25012f61.
Steele, D.H., Thornburg, M.J., Stanley, J.S., Miller, R.R., Brooke, R., Cushman, J.R., Cruzan, G., 1994. Determination of styrene in selected foods. J. Agric. Food Chem. 42, 1661–1665.
Tang, W., Hemm, I., Eisenbrand, G., 2000. Estimation of human exposure to styrene and ethylbenzene. Toxicology 144, 39–50.
Triebig, G., Bruckner, T., Seeber, A., 2009. Occupational styrene exposure and hearing loss: a cohort study with repeated measurements. Int. Arch. Occup. Environ. Health 82, 463–480.
UK MAFF, 1994. Survey of Styrene in Food. Food Surveillance Information Sheet, Number 38, October 1994.
UK MAFF, 1999. Total Diet Study: Styrene. Food Surveillance Information Sheet, Number 189, November 1999.
US EPA, 1990. Integrated Risk Information System. Styrene. <http://www.epa.gov/iris/>.
US FDA, 2005. Guidance for Industry; Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). <http://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf>.
Vitrac, O., Leblanc, J.C., 2007. Consumer exposure to substances in plastic packaging. I. Assessment of the contribution of styrene from yogurt pots. Food Additiv. Contam. 24, 194–215.
Vyskocyl, A., El Majidi, N., Thuot, R., Beaudry, C., Charest-Tardif, G., Tardif, R., Gagnon, F., Ska, B., Turcot, A., Drolet, D., Aliyeva, E., Viau, C., 2012. Effects of Concentration Peaks on Styrene Neurotoxicity in the Fibreglass Reinforced Plastics Industry, Phase II. Chemical Substances and Biological Agents, Studies and Research Projects. Institut de recherché Robert-Sauvé en santé et en sécurité du travail (IRSST), Report R-728.
Wang, Y.P., Saito, T., Hosokawa, T., Kurasaki, M., Saito, K., 2001. Changes in middle latency auditory-evoked potentials of the rat exposed to styrene. J. Health Sci. 47, 175–183.
WHO, 2003. Styrene in Drinking Water; Background Document for Development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WHS/03.04/27.
Yang, W.P., Hu, B.H., Chen, G.D., Bielefeld, E.C., Henderson, D., 2009. Protective effect of N-acetyl-L-cysteine (L-NAC) against styrene-induced cochlear injuries. Acta Oto-Laryngol. 129, 1036–1043.
Young, J.D., Ramsey, J.C., Blau, G.E., Karbowski, R.J., Nitschke, K.D., Slauter, R.W., Braun, W.H., 1979. Pharmacokinetics of inhaled or intraperitoneally administered styrene in rats. Dev. Toxicol. Environ. Sci. 4, 297–310.
Zamyslowska-Szmytke, E., Fuente, A., Niebudek-Bogusz, E., Sliwinska-Kowalska, M., 2009. Temporal processing disorder associated with styrene exposure. Audiol. Neurotoxicol. 14, 296–302.