National Institute for Public Health and the Environment
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FRONT OFFICE FOOD AND PRODUCT SAFETY

Assessment of volatile organic compounds in vegetable oils and fats

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Nederlandse samenvatting

Vluchtige organische stoffen (VOS) worden gebruikt als extractiemiddelen in de productie van levensmiddelen. Alleen VOS die in bijlage I van Richtlijn 2009/32/EG[1] staan, mogen hiervoor worden gebruikt. Recent heeft de Nederlandse Voedsel- en Warenautoriteit (NVWA) meldingen gekregen over de aanwezigheid in plantaardige oliën en vetten van bepaalde VOS die niet in deze bijlage staan. Eerder werden deze stoffen niet aangetroffen. Sinds de ontwikkeling van een nieuwe analysemethode met een lagere kwantificeringsgrens (*limit of quantification*, LOQ) kunnen VOS nu in theorie gemeten worden tussen 0,01 en 0,5 mg/kg.

Bureau Risicobeoordeling & onderzoek (BuRO) van de NVWA vraagt het Front Office (FO) daarom voor zes VOS, namelijk acetonitril, benzeen, ethylbenzeen, dimethylether, tolueen en xyleen 1) wat hun maximale concentratie in plantaardige oliën en vetten mag zijn alvorens er gezondheidseffecten kunnen optreden en 2) wat de maximale consumptiehoeveelheid van plantaardige oliën en vetten zou zijn alvorens er gezondheidseffecten kunnen optreden bij de meetbare concentraties. Beide vragen moeten worden beantwoord voor zowel kinderen van 1-3 jaar als voor volwassenen.

Het FO heeft voor de zes VOS een overzicht gemaakt van de beschikbare toxicologische informatie in de openbare literatuur en gezondheidskundige grenswaardes afgeleid dan wel overgenomen voor acute en chronische blootstelling. Ook heeft het FO berekend hoeveel plantaardige oliën en vetten kinderen (1-3 jaar) en volwassenen (18-79 jaar) eten in Nederland op basis van gegevens uit de Voedselconsumptiepeiling 2019-2021.

De maximale concentratie acetonitril, ethylbenzeen, tolueen en xyleen die aanwezig mag zijn in plantaardige oliën en vetten met een verwaarloosbaar gezondheidsrisico varieert voor beide leeftijdsgroepen van 8,4 tot 864 mg/kg voor acute blootstelling en van 11 tot

[1] Richtlijn 2009/32/EG van het Europees Parlement en de Raad van 23 april 2009 betreffende de onderlinge aanpassing van de wetgevingen der lidstaten inzake het gebruik van extractiemiddelen bij de productie van levensmiddelen en bestanddelen daarvan (Herschikking)

247 mg/kg voor chronische blootstelling. Deze concentraties zijn vele malen hoger dan de LOQ van de analysemethode. Voor benzeen is de maximale concentratie 0,24 mg/kg voor jonge kinderen en 0,78 mg/kg voor volwassenen voor acute blootstelling en respectievelijk 0,05 en 0,19 mg/kg voor chronische blootstelling. Deze concentraties liggen in of net boven de concentraties die met de verlaging van de LOQ nu gemeten zouden kunnen worden, namelijk 0,01 – 0,5 mg/kg. Een verfijning van de berekening voor benzeen met inbegrip van de onzekerheden, toont aan dat de berekende maximale concentraties alvorens er gezondheidseffecten kunnen optreden in de meeste gevallen conservatief zijn, behalve voor chronische blootstelling voor jonge kinderen. Bovendien toont deze berekening aan dat door een verkleining van de onzekerheden, door het verkrijgen van meer toxiciteitsgegevens, de kans aanwezig is dat de maximale concentraties geheel boven de 0,5 mg/kg zullen liggen, behalve weer voor chronische blootstelling voor jonge kinderen. Voor dimethylether kan geen maximale concentratie worden berekend, omdat voor deze stof geen gezondheidskundige grenswaarde beschikbaar is of kan worden afgeleid. Het toepassen van de 'Margin of Safety' (MOS) aanpak laat echter zien dat de MOS groot genoeg is om een gezondheidsrisico uit te sluiten als plantaardige oliën en vetten 0,5 mg/kg dimethylether zouden bevatten. Dit geldt voor beide leeftijdsgroepen en voor zowel de acute als chronische blootstelling.

De maximale consumptiehoeveelheid van plantaardige oliën en vetten alvorens er gezondheidseffecten kunnen optreden bij beide leeftijdsgroepen als er 0,5 mg/kg acetonitril, ethylbenzeen, tolueen en xyleen aangetroffen zou worden, varieert van 704 tot 140.000 g per persoon voor acute blootstelling en van 704 tot 28.000 g per persoon per dag voor chronische blootstelling. Deze hoeveelheden zijn veel hoger dan de huidige consumptie van deze producten: 42 – 81 g per persoon voor acute consumptie (P97.5) en 32 – 57 g per persoon per dag voor chronische consumptie (P95). Voor benzeen is de maximale consumptiehoeveelheid 20 g per persoon voor jonge kinderen en 126 g per persoon voor volwassenen voor acute blootstelling en respectievelijk 3,3 en 21 g per persoon per dag voor chronische blootstelling bij een concentratie van 0,5 mg/kg. Deze hoeveelheden zijn lager dan de huidige consumptiehoeveelheden. Een verfijnde berekening voor benzeen, zoals uitgevoerd voor de maximale concentraties, laat ook zien dat de maximale consumptiehoeveelheid voor chronische blootstelling voor jonge kinderen mogelijk lager is dan de huidige chronische consumptie van plantaardige oliën en vetten. Zoals voor de maximale concentratie kan er ook geen maximale consumptie van plantaardige oliën en vetten worden berekend voor dimethylether, omdat de beschikbare toxiciteitsgegevens daarvoor te beperkt zijn. Er kan ook geen MOS benadering worden toegepast, omdat er geen minimale MOS berekend kan worden die nodig is om de maximale consumptiehoeveelheid te berekenen.

Subject

In recent months, the Netherlands Food and Consumer Product Safety Authority (*Nederlandse Voedsel- en Warenautoriteit,* NVWA) has received a number of notifications about the presence of certain volatile organic compounds (VOCs) (e.g. xylene and toluene) in vegetable oils and fats. According to the Directive 2009/32/EC these VOCs are not authorised to be used as extraction solvent for the production of vegetable oils and fats (not listed in Annex 1)[2]. Recently, a new analytical method for detection of VOCs in food has been developed with lower limits of quantification (LOQs). This means that VOCs that previously could not be detected could in theory now be detected in concentrations between 0.01 mg/kg (new LOQ) and 0.5 mg/kg (previous LOQ).

[2] Richtlijn 2009/32/EG van het Europees Parlement en de Raad van 23 april 2009 betreffende de onderlinge aanpassing van de wetgevingen der lidstaten inzake het gebruik van extractiemiddelen bij de productie van levensmiddelen en bestanddelen daarvan (Herschikking)

Question

The Office for Risk Assessment & Research (BuRO) of the NVWA asks the Front Office (FO) the following questions regarding these findings:

1. Which maximum concentrations of acetonitrile, benzene, dimethyl ether, ethylbenzene, toluene and xylene in vegetable oils and fats are considered to pose a negligible health risk?
2. What would be the maximum amount of vegetable oils and fats that can be consumed without an appreciable health risk considering the measurable concentrations between 0.01 mg/kg and 0.5 mg/kg?

The answers to both questions should address young children (1-3 years) and adults (18-79 years) living in the Netherlands.

Conclusion

1. The maximum concentration of the volatile organic compounds (VOCs) *acetonitrile*, *ethylbenzene, toluene* and *xylene* in vegetable oils and fats below which a health risk is considered negligible for either young children (1 – 3 years) or adults (18 – 79 years) ranges from 8.4 to 864 mg/kg for acute exposure and from 11 to 247 mg/kg for chronic exposure. This is far above the limit of quantification (LOQ) of the analytical method. For *benzene*, the maximum concentration is 0.24 mg/kg for young children and 0.78 mg/kg for adults for acute exposure and 0.05 and 0.19 mg/kg, respectively, for chronic exposure. This is between the previous and current LOQ or slightly above the previous LOQ. A refinement of the assessment for benzene with inclusion of uncertainties in the calculation shows that the calculated maximum concentrations are conservative, except for chronic exposure for young children. Moreover, the refined calculation indicates that it is possible that when uncertainties would be reduced by obtaining more toxicity data, the maximum concentrations may be above the LOQ. This is especially likely for adults and in the case of acute exposure for children. No maximum concentration of *dimethyl ether* could be calculated. However, a margin of safety (MOS) approach showed that when vegetable oils and fats contain 0.5 mg/kg *dimethyl ether*, the MOS are considered adequate to be of no safety concern.

2. The maximum amount of vegetable oils and fats that can be consumed by young children (1 – 3 years) or adults (18 – 79 years) without an appreciable health risk if *acetonitrile, ethylbenzene, toluene* and *xylene* would be present at 0.5 mg/kg (worst case) ranges from 704 g to 140,000 g per person for acute exposure and from 704 g to 28,000 g per person per day for chronic exposure. These amounts are higher than the current consumption levels of vegetable oils and fats of 42 – 81 g per person for acute consumption (P97.5) and 32 – 57 g per person per day for chronic consumption (P95). For *benzene*, the maximum amount is 20 g per person for young children and 126 g per person for adults for acute exposure and 3.3 and 21 g per person per day, respectively, for chronic exposure. These amounts are lower than the current consumption levels. A refinement of the assessment for benzene with inclusion of uncertainties in the calculation shows that it is possible that when uncertainties are reduced by obtaining more data, maximum amounts may be well above the current consumption of vegetable oils and fats. This is especially likely for adults and in cases of acute exposure for children. No maximum amount can be calculated for *dimethyl ether.* The toxicological dataset is too limited to identify a minimal MOS which is needed for such a calculation.

Introduction

Volatile organic compounds (VOCs) are solvents or extraction solvents that are used in an extraction procedure during the production of, for example, oils, fats, coffee, tea and cocoa butter. Also, the compounds can occur as contaminants in raw materials. VOCs are removed from the product as much as possible before the product enters the food chain.

According to the Directive 2009/32/EC only VOCs that are listed in Annex 1[3] are authorised to be used as extraction solvent for the production of food stuffs and food ingredients. For some of these authorised extraction solvents, conditions of use and/or maximum residue limits (MRLs) have been laid down. These MRLs are also implemented in national law via the Dutch Commodities Act Regulation on Extraction Solvents (*Warenwetregeling extractiemiddelen*).[4] VOCs that are not listed in Annex 1 are not authorised to be used as extraction solvent.

In recent months, the Netherlands Food and Consumer Product Safety Authority (*Nederlandse Voedsel- en Warenautoriteit* (NVWA)) has received a number of notifications about the presence of certain VOCs (e.g. xylene and toluene) in vegetable oils and fats. Since new analytical methods to detect VOCs in vegetable oils and fats have been developed with a lower LOQ (from 0.5 mg/kg to 0.01 mg/kg), these VOCs can now be detected at concentrations between 0.01 mg/kg and 0.5 mg/kg. Most VOCs are not listed in Annex I of the EU Directive 2009/32/EC and therefore not authorised to be used in vegetable oils and fats.

The Office for Risk Assessment & Research (BuRO) of the NVWA asked the following questions to the Front Office (FO):

1. Which maximum concentrations of acetonitrile, benzene, dimethyl ether, ethylbenzene, toluene and xylene[5] in vegetable oils and fats are considered to pose a negligible health risk in young children (1-3 years) and adults (18-79 years) in the Netherlands based on current consumption patterns?
2. What is the maximum amount of vegetable oils and fats that can be consumed by these two consumer groups, without an appreciable health risk based on the measurable concentrations (between 0.01 mg/kg and 0.5 mg/kg)?

To answer these questions, information about the toxicity of the six VOCs and the consumption of vegetable oils and fats in the Netherlands is used. The section 'Toxicology' describes which toxicological reference values are available or can be derived. Background toxicokinetic and toxicological information can be found in Annex 1. Thereafter, the section 'Consumption of vegetable oils and fats' contains an overview of the consumption of vegetable oils and fats for young children and adults. In the section, 'Answer to the questions', both questions are answered and final conclusions are given in the section 'Conclusion'.

Toxicology

Below, for each of the six VOCs is described which (toxicological) evaluations were identified as well as an overview of the available Toxicological Reference Values (TRVs). Next, either the TRV that was used in the current assessment is presented or an oral TRV was derived based on the obtained information. Summaries of the toxicokinetic and toxicological information based on the identified evaluations are given in Annex 1. Due to

[3] Richtlijn 2009/32/EG van het Europees Parlement en de Raad van 23 april 2009 betreffende de onderlinge aanpassing van de wetgevingen der lidstaten inzake het gebruik van extractiemiddelen bij de productie van levensmiddelen en bestanddelen daarvan (Herschikking)

[4] https://wetten.overheid.nl/BWBR0005945/2024-07-01

[5] This is a theoretical calculation. Not all of these VOCs have been detected in vegetable oils and fats.

time constraints no comprehensive literature search was conducted. Since the exposure route is oral, the main focus was on oral toxicokinetic and toxicological data.

Acetonitrile

Acetonitrile has been previously evaluated by the World Health Organisation (WHO, 1993), the U.S. Environmental Protection Agency (U.S. EPA, 1999), the European Chemicals Bureau (ECB, 2002) and, more recent, by Health Canada (2021).

The metabolite of acetonitrile responsible for its main toxicological action is cyanide. Cyanide has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2011) and by the European Food Safety Authority (EFSA) (EFSA, 2016, 2019). Additionally, the Agency for toxic Substances and Disease Registry (ATSDR) has published a draft version of the toxicological profile of cyanide for public comments (ATSDR, 2024a).

Toxicological reference values

Acute

No acute oral TRVs were available for acetonitrile. Therefore, the available data for its metabolite, cyanide, were used. Both JECFA (2011) and EFSA (2016, 2019) derived an Acute Reference Dose (ARfD) for cyanide. Below these are further discussed.

JECFA derived an ARfD for cyanide of 90 µg/kg bw (expressed in cyanide equivalents). This ARfD was based on a prenatal developmental toxicity study where increased skeletal defects were found in hamsters following a single dose of the cyanogenic glycoside linamarin (JECFA, 2011). A $BMDL_{10}$ of 85 mg/kg bw was used as point of departure, together with a 100-fold assessment factor.
EFSA (2016) derived an ARfD of 20 µg/kg bw for cyanide. This ARfD was based on a more recent human study with female volunteers who were given 6.8 mg of cyanide from apricot kernels (i.e. 0.105 mg/kg bw for an average body weight of 65 kg). This exposure resulted in a blood cyanide concentration of about 20 µM which was considered by EFSA to be a threshold for toxicity. The applied intraspecies assessment factors included 1.5 for toxicokinetics and 3.16 for toxicodynamics. In 2019, EFSA extended this ARfD to all types of food containing cyanogenic glycosides, although correction factors for bioavailability are to be applied on the exposure data for different foods; the higher the correction factor, the lower the bioavailability (EFSA, 2019).

For the current assessment of acetonitrile, the ARfD for cyanide derived by EFSA was used because it was based on human data and converted by FO to an ARfD for acetonitrile. Due to a lack of human bioavailability data, a 100% bioavailability of acetonitrile was assumed. Based on their respective molecular weights, the ARfD for acetonitrile would be:

$$\frac{(ARfD\ cyanide \times molecular\ weight\ acetonitrile)}{molecular\ weight\ cyanide} = \frac{(20\ \mu g/kg\ bw \times 41.053\ g/mol)}{26.018\ g/mol} = 32\ \mu g/kg\ bw \text{ (rounded)}$$

Chronic

No chronic oral TRV have been identified for acetonitrile. Therefore, the available data for its metabolite, cyanide, were used. JECFA (2011) derived a Provisional Maximal Tolerable Daily Intake (PMTDI) for cyanide, while EFSA (2019) was of the opinion that the

derivation of a chronic TRV was not possible. ATSDR (2024a) derived an intermediate-duration[6] minimal risk level (ATSDR_MRL)[7]. Below this is further discussed.

For cyanide, JECFA derived a PMTDI of 20 µg/kg bw (expressed in cyanide equivalents). This was based on a $BMDL_{1SD}$[8] of 1.9 mg/kg bw per day from the 13-week NTP study in rats for a decrease in relative cauda epididymis weight and an assessment factor of 100 for inter- and intraspecies differences. No additional assessment factor for the absence of chronic toxicity data was deemed necessary because the toxic effects due to cyanide exposure are mainly acute, and the effect on the cauda epididymis is sensitive, meaning that the type of effect would not change in a longer-term study (JECFA, 2011).

EFSA on the other hand argued that differences in the weight of the cauda epididymis are difficult to measure and comprise a lot of uncertainty. Additionally, EFSA noted that the assessment of spermatogenesis in the NTP study was based on a method not well validated, and the differences in sperm mobility between dose groups were small and not dose-dependent (EFSA, 2019). Therefore, the findings of this study were not used by EFSA as a basis to derive a chronic TRV. Also other long-term animal and human data (e.g. chronic cyanide intoxication due to cassava consumption) were considered by EFSA not to allow identification of a critical effect or point of departure that could be used for derivation of a chronic TRV.

Based on the same NTP results, ATSDR holds the explanation that decreased water intake in the highest dose group and issues in the study setup were responsible for the observed effects on relative cauda epididymis weight (2023, as reported in ATSDR, 2024a). However, the same NTP study also identified a no observed adverse effect level (NOAEL) of 3.96 mg/kg bw per day for increased absolute thyroid weight and decreased serum thyroxine concentrations (2023, as reported in ATSDR, 2024a). Based on this NOAEL and an assessment factor of 100 for inter- and intraspecies extrapolation, ATSDR derived an intermediate-duration ATSDR_MRL of 0.04 mg/kg bw per day (ATSDR, 2024a).

For the derivation of a chronic TRV for acetonitrile, the intermediate-duration oral ATSDR_MRL was used. Since this was a 13-week study, an additional factor of 2 to extrapolate from subchronic data to chronic data was applied (EFSA, 2012; ECHA, 2012). Also, a molecular weight conversion from cyanide to acetonitrile was applied.

$$\frac{(MRL\ cyanide \times molecular\ weight\ acetonitrile)}{molecular\ weight\ cyanide} = \frac{(0.04\ mg/kg\ bw \times 41.053\ g/mol)}{26.018\ g/mol} = 0.063\ mg/kg\ bw \text{ (rounded)}$$

After applying the subchronic to chronic factor, this results in a chronic TRV for acetonitrile to be used in the current assessment of 32 µg/kg bw per day (rounded).

It is noted that the acute and chronic TRV are the same.

<u>Benzene</u>
Benzene has been evaluated by several agencies including RIVM (Baars et al., 2001), U.S. EPA (2003), the International Agency for Research on Cancer (IARC, 2018), the Health Council of the Netherlands (2014), the Committee for Risk Assessment (RAC,

[6] Intermediate-duration according to ATSDR: 15 – 364 days.
[7] A *minimal risk level* is defined by ATSDR as an estimate of the daily human exposure to a hazardous substance that is *likely to be without appreciable risk of adverse non-cancer health effects* over a specified route and duration of exposure (ATSDR, 2024a). They are intended to serve as screening levels.
[8] $BMDL_{1SD}$: Benchmark Dose Lower Confidence Limit at 1 Standard Deviation.

2018) and ATSDR (2024b). We will mainly focus on the toxicological profile of benzene of the ATSDR (2024b), which is the most recent assessment.

Toxicological reference values

Acute
Both the Health Council of the Netherlands (2014) and RAC (2018) did not derive an oral TRV as their main focus is the derivation of occupational exposure limits. ATDSDR (2024b) proposed a provisional acute-duration[9] oral ATSDR_MRL. Below these are further discussed.

ATSDR (2024b) proposed a provisional acute-duration oral ATSDR_MRL of 0.0009 mg/kg bw per day adopted from the provisional intermediate-duration oral ATSDR_MRL. The latter was based on decreased peripheral white blood cell lymphocyte, monocyte and neutrophil counts observed in a 4-week drinking water study (6 days/week) in mice with a duration-adjusted NOAEL of 0.09 mg/kg bw per day (i.e. 0.1 mg/kg bw per day × 6/7) and an assessment factor of 100 for inter- and intraspecies extrapolation. ATSDR reasons that there is strong evidence that haematological effects are also the critical effect for benzene toxicity in humans after acute exposure. However, the only subacute oral study in mice (14 days) observing haematological effects studied a dose of 200 mg/kg bw per day, which was considered not to result in a health-protective ATSDR_MRL as it was two orders of magnitude higher than the LOAELs obtained in the 4-week studies.

For acute exposure, as a conservative approach, the acute-duration oral ATSDR_MRL of 0.0009 mg/kg bw was chosen for the current assessment.

Chronic
U.S. EPA (2003) derived a chronic oral reference dose (RfD) and ATSDR (2024b) a chronic-duration oral ATSDR_MRL. Below these are further discussed.

U.S. EPA (2003) derived a chronic oral RfD of 0.004 mg/kg bw per day based on a BMDL of 1.2 mg/kg bw per day for a decreased lymphocyte count, based on route-to-route extrapolation, and a total assessment factor of 300 (100 for inter- and intraspecies extrapolation, 3 for database deficiencies).

ATSDR (2024b) derived a chronic-duration oral ATSDR_MRL of 0.0003 mg/kg bw per day, which was based on route-to-route extrapolation from the chronic-duration[10] inhalation ATSDR_MRL. According to ATSDR, the results of chronic oral NTP studies (1996) could not be used as a basis for the ATSDR_MRL derivation because the obtained LOAEL of 25 mg/kg bw per day for hematological effects was higher than the LOAEL for the same effect (1 mg/kg bw per day) from the 4-week study (Li et al., 2018, as reported by ATSDR, 2024b). However, since route-to-route extrapolation has a high uncertainty, the duration-adjusted NOAEL of 0.09 mg/kg bw per day from the subacute study will be used in the current report to derive a TRV for benzene. This is considered acceptable as the subacute and chronic studies show the same haematological effect, the most sensitive effect of benzene, and the chronic study possibly did not test lower doses because it was published earlier than the subacute study. Applying assessment factors of 100 for inter- and intraspecies extrapolation and a default factor of 6 for extrapolation from subacute to chronic (ECHA, 2012), resulted in a chronic reference value of 0.00015 mg/kg bw per day.

[9] Acute-duration according to ATSDR: 1 – 14 days
[10] Chronic-duration according to ATSDR: ≥365 days

For chronic exposure, a TRV of 0.00015 mg/kg bw per day is used in the current assessment.

Dimethyl ether

For dimethyl ether two scientific opinions published by EFSA have been identified (EFSA, 2009, 2015), which are the main source of information for this assessment.

Toxicological reference values

No acute or chronic oral TRVs have been identified for dimethyl ether. EFSA (2015) performed route-to-route extrapolation based on inhalation toxicity data and applied a margin of safety (MOS) approach for the evaluation of dimethyl ether as an extraction solvent for the preparation of defatted animal protein products (collagen) and gelatin. Below this is further discussed.

In 2015, EFSA considered it appropriate to perform route-to-route extrapolation based on the inhalation toxicity data in this particular case. This decision was based on the absence of toxicity in tissues of first (possible) contact after inhalation exposure, the limited metabolism, the non-reactive nature of dimethyl ether, the fast distribution and excretion of dimethyl ether and the very large margin between the overall NOAEL and the anticipated maximum exposure. The most critical value derived from the inhalation toxicity data was the lowest no effect level of 1250 ppm (2355 mg/m^3) for foetal toxicity. Based on the assumptions that a rat weighs 0.25 kg, has a breathing rate of 200 mL/min and an inhalation absorption efficiency of 75% for an exposure duration of six hours per day, EFSA estimated the daily internal exposure (for chronic exposure) corresponding to this no effect level to be approximately 518 mg/kg bw per day (i.e. 2355 mg/m^3 × 0.75 × 200 mL/min × 60 min × 6 hour /1,000,000 / 0.25 kg). Subsequently, EFSA applied a MOS approach. Hence, a MOS approach will be applied in the current assessment using 518 mg/kg bw per day as reference value. This can only be conducted for chronic exposure.

Ethylbenzene

Ethylbenzene has been evaluated by U.S. EPA (Becker et al., 1985), IARC (2000), RIVM (Baars et al., 2001), WHO (2003), and ATSDR (2010). The current assessment focuses on the evaluation by ATSDR (2010), being the most recent evaluation of ethylbenzene and including oral studies that were not available when U.S. EPA, WHO and RIVM carried out their evaluations.

Toxicological Reference Values

Acute

ATSDR (2010) derived an oral intermediate-duration ATSDR_MRL. Below this is further discussed.

ATSDR concluded that an acute-duration oral ATSDR_MRL cannot be derived for ethylbenzene, due to the quality and the observed effects in the available acute toxicity studies. Therefore, as a conservative approach, the NOAEL of 75 mg/kg bw per day based on liver toxicity from the 3-month study in rats by Mellert et al. (2007, as reported by ATSDR, 2010) will be used for the current assessment (see for more information Annex 1). ATSDR used this study to derive an intermediate-duration ATSDR_MRL. To this end, ATSDR used a physiologically-based kinetic (PBK) model to derive a human equivalent dose (HED) and a BMDLHED for various liver endpoints. The choices made during this execution could have a marked effect on the outcome of the PBK model, and accordingly for the calculated HED and BMDLHED. As FO was not able to assess these

choices fully, the ATSDR approach was not followed. Instead, an acute TRV of 0.8 mg/kg bw was established based on the NOAEL of 75 mg/kg bw per day from the study of Mellert et al. (2007, as reported by ATSDR, 2010) and applying an assessment factor of 100 for interspecies extrapolation and intraspecies variability (ECHA, 2012).

Chronic
No oral chronic TRVs were identified.

No suitable chronic oral toxicity study with ethylbenzene is available. In a 3-month oral study in rats by Mellert et al. (2007, as reported by ATSDR, 2010) the NOAEL was 75 mg/kg bw per day based on liver toxicity observed at 250 mg/kg bw per day (see for more information Annex 1). ATSDR used this study to derive an intermediate-duration ATSDR_MRL. As described under the derivation of the acute TRV, in the present evaluation it was not possible to assess these choices fully and therefore, it was decided not to follow ATSDR in this approach. Instead, for the present risk assessment a chronic TRV of 0.08 mg/kg bw per day was established, based on the NOAEL of 75 mg/kg bw per day from the study of Mellert et al. and applying an assessment factor of 1000:100 for interspecies extrapolation and intraspecies variability, and 2 for use of a subchronic study and 5 for uncertainties in the database due to lack of oral data on chronic toxicity, reproductive toxicity and developmental toxicity (ECHA, 2012).

Toluene
Toxicological evaluations of toluene by RIVM (Baars et al., 2001; Huiberts, 2023), ECB (2003), Finnish Safety and Chemicals Agency (2013), U.S. EPA (2005), Danish EPA (2016) and ATSDR (2017) were identified. The EU Toluene Risk Assessment Report (ECB, 2003) mainly addresses the toxicity of toluene after exposure through inhalation and the dermal route, and the Finnish Safety and Chemicals Agency (Tukes) report through inhalation. The U.S. EPA (U.S. EPA, 2005), ATSDR (ATSDR, 2017) and RIVM (Huiberts, 2023) published toxicological evaluations of toluene addressing the oral, dermal and inhalation routes. The current assessment for dietary exposure to toluene will be predominantly based on these evaluations.

Toxicological Reference Values

Acute
U.S. EPA (2005) and RIVM (Huiberts, 2023) did not derive an oral acute TRV as this was not the purpose of their evaluations. ATSDR (2017) derived an acute-duration oral ATSDR_MRL. Below this is further discussed.

ATSDR (2017) derived an acute-duration oral ATSDR_MRL of 0.8 mg/kg bw per day based on neurological effects (decreased flash-evoked potential) observed at a LOAEL of 250 mg/kg bw in an acute gavage study in rats. An assessment factor of 300 was applied: 3 for use of a minimally adverse LOAEL, and 100 for interspecies extrapolation and intraspecies variability. In the present report a TRV of 0.8 mg/kg bw will be used for the current assessment.

Chronic
A number of (inter)national authorities has established a (semi-)chronic TRV for toluene, including U.S. EPA (2005), the Danish EPA (2016), ATSDR (2017) and RIVM (Huiberts, 2023). Below these are further discussed.

ATSDR (2017) established a semi-chronic ATSDR_MRL of 0.2 mg/kg bw per day, based on a NOAEL of 22 mg/kg bw per day for immunological endpoints in 28-days studies with

mice and an uncertainty factor of 100 for interspecies extrapolation and intraspecies variability. ATSDR (2017) considered the immunological effects more appropriate than the hepatic and renal effects as a starting point for MRL-derivation since the points of departure (PoDs) for these effects were higher than those for immunological effects. The increased liver and kidney weights were not consistent between studies and were not associated with histopathological changes (see also Annex 1).

Interestingly, U.S. EPA (2005) and the Danish EPA (2016) considered the same endpoints as a basis for a TRV but came to a different conclusion than the ATSDR (2017). U.S. EPA (2005) considered the immunotoxicity data as contradictory and indicated that the data do not provide sufficient certainty to conclude on immunosuppression as a more sensitive endpoint than renal toxicity. The Danish EPA (2016) concluded that the reported effects on neurotransmitter levels and immune response observed in mice after 28 days of oral exposure were uncertain endpoints with regard to risk assessment. Both U.S. EPA (2005) and the Danish EPA (2016) based their TRV on the increased kidney weight as observed in a 90-day NTP study.

RIVM (Huiberts, 2023) noted the considerations from ATSDR (2017), U.S. EPA (2005) and the Danish EPA (2016) about the uncertainties regarding the respective endpoints. RIVM pragmatically chose the NOAEL of 22 mg/kg bw per day for immunotoxicity, as observed in the 28 days studies in mice, as the basis for the TRV, as this is the lowest PoD. The neurological effects observed in repeated dose studies, although clearly adverse, were not chosen as a PoD, as the NOAEL for this effect (625 mg/kg bw per day) is much higher than that for immunotoxic effects. Accordingly, RIVM established an indicative Tolerable Daily Intake (TDI) of 0.037 mg/kg bw per day, based on the NOAEL of 22 mg/kg bw per day and applying a safety factor of 600: 100 for interspecies extrapolation and intraspecies variability, and an additional factor of 6 for extrapolation from sub-acute to chronic exposure (ECHA, 2012).

In the present report, the TRV as established by RIVM (Huiberts, 2023) will be used for the current assessment. As all TRVs in the present report are rounded to one significant figure, a TRV of 0.04 mg/kg bw per day will be used.

Xylene

Toxicological evaluations of xylene by RIVM (Baars et al., 2001), U.S. EPA (2003, 2005) and ATSDR (2007) were identified. The ATSDR evaluation is the most recent and most extensive and addresses the toxicity of xylene after exposure through the oral, inhalation and dermal route. The present assessment will be predominantly based on the ATSDR evaluation.

Toxicological Reference Values

Acute

U.S. EPA (2003, 2005) did not derive an oral acute TRV as not being the purpose of the evaluation. ATSDR (2007) derived an acute-duration oral ATSDR_MRL. Below this is further discussed.

ATSDR (2007) derived an acute-duration oral ATSDR_MRL of 1 mg/kg bw per day based on neurological effects (suppression of visual evoked brain potentials) in an acute gavage study in rats with a NOAEL of 125 mg/kg bw. An assessment factor of 100 for interspecies extrapolation and intraspecies variability) was applied. The ATSDR_MRL applies to mixed xylenes and to individual isomers. In the present report, a TRV of 1 mg/kg bw will be used.

Chronic
RIVM (Baars et al., 2001) derived a TDI, U.S. EPA (2005) a chronic RfD and ATSDR (2007) a chronic-duration oral ATSDR_MRL. Below these are further discussed.

In 2001, RIVM derived a TDI of 0.15 mg/kg bw per day for xylene based on findings of nephropathy in an oral 90-day study in rats with a NOAEL of 150 mg/kg bw per day (Baars et al., 2001).
In 2007, ATSDR re-evaluated the toxicological database for xylene and concluded that the observed nephropathy in various repeated dose studies in rats (increased hyaline droplets in males) is not relevant for human risk assessment. The available animal studies involving chronic-duration oral exposure to xylenes did not clearly identify toxic effects other than mild decreases (5–8%) in body weight gain and observations of somewhat higher unexplained mortality in male F344 rats at 500 mg/kg bw per day, with a NOAEL of 250 mg/kg bw per day. ATSDR (2007) concluded that *"in the absence of data associating health effects in humans with chronic-duration oral exposure to xylenes, the animal bioassays provide a minimal basis for deriving an MRL for humans chronically exposed to xylenes"*. The NOAEL of 250 mg/kg bw per day was selected as the basis of the chronic-duration ATSDR_MRL. The NOAEL was first adjusted for discontinuous exposure (5 days/7 days), resulting in a duration-adjusted NOAEL of 179 mg/kg bw per day. Based on this NOAEL, a chronic-duration oral ATSDR_MRL of 0.2 mg/kg bw per day was derived. A total assessment factor of 1000 (10 for interspecies extrapolation and intraspecies variability, and a modifying factor of 10 to account for the lack of testing for sensitive neurological end points and lack of developmental and multi-generational data) was applied.

It is noted that U.S. EPA (2005) derived a chronic RfD of 0.2 mg/kg bw per day, based on the same endpoints from the chronic study in rats and applying the same safety factor of 1000.

In the present report a TRV of 0.2 mg/kg bw per day will be used.

Overview of toxicological reference values
Table 1 provides an overview of the reference values that will be used to answer the two questions.

Table 1. Overview of toxicological reference values for acetonitrile, dimethyl ether, benzene, ethylbenzene, toluene and xylene that will be used to answer the two questions.

Compound	**Acute (mg/kg bw)**	**Chronic (mg/kg bw per day)**
Acetonitrile	0.032	0.032
Benzene	0.0009	0.00015
Dimethyl ether*	NA	NA
Ethylbenzene	0.8	0.08
Toluene	0.8	0.04
Xylene	1	0.2

NA = not applicable
* For dimethyl ether, no reference value could be determined. Instead, a margin of safety approach will be applied.

Consumption of vegetable oils and fats in the Netherlands

Dutch National Food Consumption Survey

To answer the first question about the maximum concentration of the six VOCs that can be present in vegetable oils and fats without posing a health risk, the consumption of vegetable oils and fats was calculated for children aged 1-3 years and adults aged 18-79 years in the Netherlands. For this, food consumption data of the most recent Dutch National Food Consumption Survey (DNFCS) of 2019-2021 were used. In this DNFCS, 3570 individuals of 1-79 years reported what and how much they had consumed during two non-consecutive days (van Rossum et al., 2023). Table 2 gives an overview of the number of individuals and the mean body weight of these two age groups.

Table 2. Number of individuals and mean body weight (kg) of children aged 1-3 years and adults aged 18-79 years in the DNFCS of 2019-2021 for which the consumption of vegetable oils and fats was calculated.

Age group	Number of individuals[1]	Mean body weight (kg)
1-3 years	703	14
18-79 years	1747	81

DNFCS: Dutch National Food Consumption Survey

[1] The DNFCS of 2019-2021 contains amounts of foods and beverages consumed per individual per day that were reported on two non-consecutive days. For children of 1-3 years, 1406 consumption days were available to calculate the consumption of vegetable oils and fats, and for adults 3494 days.

Conversion model Primary Agricultural Products (CPAP)

Vegetable oils and fats can be consumed as such, but are more often consumed as an ingredient in a food, such as fine bakery wares, margarines and composite dishes. Only the consumption of vegetable oils and fats as such is recorded in the DNFCS. To also include the consumption of vegetable oils and fats when used as an ingredient in the calculations, the Conversion model Primary Agricultural Products (CPAP) was used (van Dooren, 1995). This model converts foods and beverages reported in the DNFCS to their raw ingredients (primary agricultural products), including weight percentages. For example, cake is converted to 21% egg, 14% milk, 25% sugar, 28% wheat and 21% vegetable oils and fats. This model can thus be used to convert the amounts of foods consumed, such as cake, to amounts of the raw ingredients consumed, such as vegetable oils and fats.

CPAP contains weight percentages of the generic ingredient 'vegetable oils and fats' and/or of specific vegetable fats and oils, such as sunflower oil, palm oil and rapeseed oil, in a food. For the calculation of the consumption of vegetable oils and fats, the weight percentages for the generic ingredient 'vegetable oils and fats' and for individual vegetable oils and fats were summed per food to obtain the total percentage of vegetable oils and fats per food. Weight percentages of these two ingredients within one food do not overlap. No consumption amounts per individual oil and/or fat were calculated (see below).

Calculation of consumption of vegetable oils and fats

The TRVs of the six VOCs are based on both acute and chronic toxicity (see Table 1). Therefore, acute and chronic consumption of vegetable oils and fats was calculated, expressed in gram per kg bw per day, for the two age groups. For this, firstly the total percentage of vegetable oils and fats per food (from CPAP) was linked to the consumed

amount of that food per day as reported in DNFCS 2019-2021 and summed across foods. The resulting total amounts of vegetable oils and fats consumed per individual and per day (in gram per day) were divided by the individual's body weight resulting in a distribution of daily consumed amounts of vegetable oils and fats expressed in gram per kg bw per day. For the acute consumption, only the days were included at which the consumption of foods containing vegetable oils and fats was actually reported. The reason for this is that a risk assessment of acute health effects from a compound in food is often based on an acute (= high, one-time) consumption of a product on an arbitrary day.[11] For chronic consumption, this calculation was based on all consumption days in the DNFCS, so including days at which the consumption of vegetable oils and fats was reported and days at which no consumption was reported.

The distribution of daily consumption amounts of vegetable oils and fats per age group was used as input for the calculation model Monte Carlo Risk Assessment (MCRA), version 10.1, to calculate the acute and chronic consumption using the Observed Individual Means (OIM) mode. This is a model for calculating chronic consumption by averaging the consumption over two days per person (Boon & van der Voet, 2015). Based on the distribution of average consumption amounts per person, the P95 chronic consumption was calculated for the two age groups, as an estimate of a high chronic consumption amount. The acute consumption was calculated as the 97.5th percentile (P97.5) of the distribution of daily amounts of vegetable oils and fats expressed in g/kg bw per day.

The acute and chronic consumption amounts were both weighed in the calculation model for age, gender, region, education, level of urbanisation, day of the week and season using weighing factors (van Rossum et al., 2023). In this way, the consumption amounts are representative for all children of 1-3 years and adults of 18-79 years in the Netherlands and for the whole year.

To quantify the uncertainty in the estimated acute and chronic consumption amounts, the bootstrap methodology was used (this was needed for an APROBA-Plus analysis, see below). The bootstrap algorithm resamples the food consumption data a 100 times. Each of the resulting 100 data sets may be considered as an alternative food consumption data set. For each data set, the acute and chronic statistics were calculated again as described above resulting in 100 estimates for each statistic. Median of these statistics and the 95th uncertainty interval around these statistics are reported (Efron, 1979).

Results

Table 3 shows the acute and chronic consumption of vegetable oils and fats per age group. This includes all days in the DNFCS of 2019-2021 and all food products containing these oils and fats for the chronic consumption and only the days in the DNFCS with the consumption of foods containing vegetable oils and fats for acute consumption.

[11] Please note that in a previous FO report the acute consumption of different types of meat was calculated only based on the days at which the consumption of foods containing at least 75% of the meat type as ingredient were reported (RIVM/WFSR, 2020; 2024). In this way, the acute consumption was based on high consumption levels of these meats. This approach is not suitable to obtain a high, acute consumption level of vegetable oils and fats, because foods with at least 75% of these oils and fats as ingredient, such as margarines and olive oil, are not consumed in large amounts. Foods containing less vegetable oils and fats as ingredient, such as biscuits, crisps, and spreads, are consumed in larger amounts resulting in higher consumption levels of vegetable oils and fats. Therefore, all foods with these oils and fats as ingredient were included to estimate the acute consumption.

Table 3. Acute and chronic consumption of vegetable oils and fats[1]

	Consumed amount (g/kg bw per day)		Consumed amount (g per day)[5]	
	1-3 years	18-79 years	1-3 years	18-79 years
Acute[2,3]				
P97.5	3.0 (2.8 - 3.2)	1.0 (0.9 - 1.0)	42 (39 - 45)	81 (73 - 81)
Chronic[3,4]				
P95	2.3 (2.2 – 2.6)	0.70 (0.67 - 0.74)	32 (31 - 36)	57 (54 - 60)

DNFCS: Dutch National Food Consumption Survey; kg: kilogram; P95: 95th percentile; P97.5: 97.5th percentile; values between brackets indicate the 95% confidence interval

[1] Includes both the generic ingredient 'vegetable oils and fats' as specific oils and fats, including canola oil, coconut fat, linseed oil, maize germ oil, maize/corn oil, olive oil, palm oil, palm kernel oil peanut oil, rapeseed oil, rice oil, safflower seed oil, sesame oil, shea butter soya bean oil and sunflower oil, consumed as such or as an ingredient in food.

[2] Lower and upper limit of the 95% confidence interval around the best estimate of consumption as calculated using the bootstrap methodology (see text for more details).

[3] Acute consumption is a high consumption of a product or ingredient on an arbitrary day, and was calculated based on days in the DNFCS of 2019-2021 on which the consumption of foods containing vegetable oils and fats was reported: 1404 days for children aged 1 to 3 years and 3468 days for adults aged 18 to 79 years.

[4] Chronic consumption is the usual consumption of a product or ingredient over a longer period, ranging from months to a lifetime, and was calculated based on all days in the DNCFS of 2019-2021 (1406 days for children aged 1 to 3 years and 3494 days for adults aged 18 to 79 years) and all food products containing vegetable oils and fats.

[5] Calculated using the mean body weights per age group (see Table 2).

Answer to the questions

1. Which maximum concentrations of acetonitrile, benzene, dimethyl ether, ethylbenzene, toluene and xylene in vegetable oils and fats are considered to pose a negligible health risk?

The maximum concentrations of acetonitrile, benzene, ethylbenzene, toluene and xylene that may be present in vegetable oils and fats below which a health risk is considered negligible for either young children (1 – 3 years) or adults (18 – 79 years) after acute or chronic exposure are presented in Table 4. To calculate these maximum concentrations the following formula was applied:

$$maximum\ concentration\ (mg/kg) = \frac{TRV\ (mg/kg\ bw\ (per\ day^{12})) \times body\ weight\ (kg)}{consumed\ amount\ (kg)}$$

where,

TRV = the acute or chronic TRV per VOC;

body weight = the average body weight for the respective age group (11 kg for 1 – 3 years (te Biesebeek et al., 2014), 70 kg for 18 – 79 years (EFSA, 2012))[13];

consumed amount = the best estimates for P97.5 (for acute) or P95 (for chronic) consumption for the respective age group.

[12] In case of chronic TRV

[13] For this calculation the default values for body weight have been applied as a worst case since the calculated maximum concentration/amount should be protective for the whole population.

Table 4. Maximum concentrations (mg/kg) of acetonitrile, benzene, ethylbenzene, toluene and xylene that can be present in vegetable oils and fats below which a health risk to young children (1 – 3 years) or adults (18 – 79 years) is considered negligible. Based on consumption data of vegetable oils and fats taken from DNFCS 2019-2021 (see Table 3).

	Maximum concentration (mg/kg)			
	Acute (P97.5)		**Chronic (P95)**	
Compound	1-3 years	18-79 years	1-3 years	18-79 years
Acetonitrile	8.4	28	11	40
Benzene	0.24	0.78	0.05	0.19
Ethylbenzene	210	691	27	99
Toluene	210	691	14	49
Xylene	262	864	68	247

It was noted that the maximum concentrations are based on the assumption that vegetable oils and fats are the only source of exposure to the six VOCs. However, other food products may contain residues of these VOCs and there may also be other routes of exposure, such as inhalation (e.g. via smoking or diesel exhaust). In addition, occupational exposure may contribute. Ideally, an allocation factor is applied to consider these other sources of exposure. However, the size of this factor is at present unknown for these compounds and could therefore not be taken into account in the current assessment. This means that the maximum concentrations will be an overestimation of the "true" maximum concentrations when also these other routes/sources would have been considered. However, as can be observed from Table 4, the maximum concentrations for the VOCs *acetonitrile, ethylbenzene, toluene* and *xylene*, for both acute and chronic exposure, were orders of magnitude higher than the concentration range 0.01 – 0.5 mg/kg before appreciable health effects are to be expected. There is enough room for exposure through other exposure routes/sources before health effects are to be expected for these four compounds at concentrations present in vegetable oils and fats of around the LOQ.

For *dimethyl ether*, a MOS approach was applied since no TRV could be determined, which would be needed to calculate a maximum concentration. For this, it was assumed that vegetable oils and fats contain 0.5 mg/kg *dimethyl ether* (worst-case; highest end of LOQ range) and that the daily consumption of vegetable oils and fats is equal to the P95 estimate. For adults, this resulted in a daily exposure to dimethyl ether of 0.058 or 0.00041 mg/kg bw based on a body weight of 70 kg (EFSA, 2012; ECHA, 2012). As no information on oral bioavailability is known, the approach of EFSA to compare the external exposure to the internal NOAEL of 518 mg/kg bw per day (see Toxicology section) was applied. This resulted in a MOS of 1.28×10^6. For young children, the corresponding MOS was 3.54×10^5. Due to the limited dataset, a minimal MOS could not be calculated as was also not done by EFSA. Nevertheless, the MOS are considered to be of no safety concern if dimethyl ether is detected at a concentration of 0.5 mg/kg and thus also when detected at lower concentrations.

For *benzene*, the maximum concentration is around or, especially for children, between 0.5 and 0.01 mg/kg (current LOQ). In combination with exposure via other routes/sources, such as coffee, the maximum concentration in vegetable oils and fats should be even lower. On the other hand, heating of vegetable oils and fats may result in evaporation of this VOC. Based on the deterministic assessment, adverse health effects

cannot be excluded when benzene is detected in vegetable oils and fats at a concentration above the LOQ of 0.01 mg/kg, especially for young children.

2. What is the maximum amount of vegetable oils and fats that can be consumed without an appreciable health risk considering the measurable concentrations between 0.01 mg/kg and 0.5 mg/kg?

The maximum amount of vegetable oils and fats that can be consumed by young children (1 – 3 years) or adults (18 – 79 years) without an appreciable health risk due to the presence *of acetonitrile, benzene, ethylbenzene, toluene and xylene* is given in Table 5. This was calculated using the following formula:

$$maximum\ amount\ (g) = \frac{TRV\ (mg/kg\ bw\ (per\ day^{14})) \times body\ weight\ (kg)}{measured\ concentration\ of\ compound\ (mg/kg)} \times 1000$$

where,
TRV = the acute or chronic TRV per VOC;
body weight = the average body weight for the respective age group (11 kg for 1 – 3 years (te Biesebeek et al., 2014), 70 kg for 18 – 79 years (EFSA, 2012))[15];
measured concentration of compound = 0.5 mg/kg as a worst case.

Table 5. Maximum amount (g) of vegetable oils and fats that can be consumed by young children (1 – 3 years) or adults (18 – 79 years) without an appreciable health risk due to the presence of acetonitrile, benzene, ethylbenzene, toluene and xylene when these compounds are present at a concentration of 0.5 mg/kg.

	Maximum amount (g)			
	Acute		**Chronic**	
Compound	1-3 years	18-79 years	1-3 years	18-79 years
Acetonitrile	704	4480	704	4480
Benzene	20	126	3.3	21
Ethylbenzene	17,600	112,000	1760	11,200
Toluene	17,600	112,000	880	5600
Xylene	22,000	140,000	4400	28,000

Again, these maximum consumption amounts were calculated assuming that the exposure to the respective VOCs only occurs via the consumption of vegetable oils and fats. For *acetonitrile, ethylbenzene, toluene* and *xylene*, in general, this leads to maximum amounts in the order of thousands of grams, which is far above the amount a consumer will eat (see Table 3). Hence, at concentrations between 0.01 and 0.5 mg/kg and at the current consumption levels there is enough room for exposure to these VOCs through other routes/sources.

[14] In case of chronic TRV

[15] For this calculation the default values for body weight have been applied as a worst case since the calculated maximum concentration/amount should be protective for the whole population.

For *dimethyl ether*, no maximum consumption amount of vegetable oils and fats could be calculated as no minimal MOS could be identified (see Toxicology section).

Based on this deterministic assessment, health effects in terms of effects on white blood cell count cannot be excluded for *benzene* as the maximum amount that can be consumed, when considering a concentration of 0.5 mg/kg, may be lower than what is actually consumed by the two age groups (see Table 3). When other routes/sources would also be considered, such as coffee, even lower amounts of these vegetable oils and fats can be consumed. Further, when benzene would be present at a concentration of 0.01 mg/kg in vegetable oils and fats, the maximum amount that can be consumed would be 990 g for young children and 6300 g for adults for acute exposure and 65 g and 1050 g, respectively, for chronic exposure. These amounts are much higher than the current consumption levels and would not be expected to lead to adverse health effects. However, exposure via other routes/sources should first be considered to draw definite conclusions on the maximum amount that in theory can be consumed.

Refined assessment for benzene

As adverse health effects cannot be excluded when benzene is detected in vegetable oils and fats at a concentration between 0.01 and 0.5 mg/kg, especially for young children, the analysis of benzene was refined by performing a (semi)probabilistic assessment. This section describes this assessment.

The assessment was refined by quantifying the fraction of the population which is protected against adverse health effects when exposed below the TRV, and the magnitude (i.e. severity) of the effect. In addition, the uncertainties for all inputs regarding hazard and exposure were visualized and propagated to the overall uncertainty in the assessment. To this end, APROBA-Plus was used, which is an Excel-based tool that facilitates probabilistic risk assessment and includes uncertainty analysis by combining hazard and exposure data graphically. For more details on APROBA-Plus regarding methodology and case studies, refer to Bokkers et al. (2017).

To perform an APROBA-Plus analysis, one has to characterize the fraction of the population which needs to be protected and the extrapolation steps needed to derive a human dose below which no adverse effects are expected from experimental (animal) data. Since the actual extrapolation factor for each step is unknown, these factors are characterized by an uncertainty distribution based on historical information (IPCS, 2017). One extrapolation step in the refined hazard calculations of benzene is the extrapolation from a NOAEL corresponding to an unknown effect size to a dose corresponding to a quantified change in response. Here, the NOAEL is extrapolated to a dose corresponding to a 5% change in white blood cell count, defined as the Benchmark dose at 5% response (BMD_{05}). For the extrapolation of the NOAEL to a 5% effect dose, a factor was used which is readily available from IPCS (2017). Changes in white blood cell count below 20% are considered negligibly small, i.e. they pose no appreciable health effects because normal human homeostasis is capable to balance such a small change in white blood cell counts (Chen et al., in prep). As a result, the effect dose is somewhat conservative. Further refinement is possible by replacing the NOAEL with a dose corresponding to a 20% change in response ($BMDL_{20}$). However, for such a refinement it is needed to perform BMD analyses, which is out of the scope of the current report. Other extrapolation steps are covering inter- and intraspecies differences (differences in sensitivity) and duration extrapolation. To quantify each extrapolation factor including its uncertainty, default values as proposed by IPCS (2017) were used. Regarding the refined exposure calculations, the uncertainty in the selected exposure percentiles (P97.5 for acute and P95 for chronic exposure) due to uncertainties in consumption data was

included (see Table 3), as well as variability in benzene concentration in vegetable oils and fats. The uncertainty in the exposure percentiles was generated via the bootstrap methodology (see section 'Consumption of vegetable oils and fats'). For the variability in the benzene concentration in vegetable oils and fats, the range of 0.01 to 0.5 mg/kg was taken. An overview of all the input data for the APROBA-Plus analysis can be found in Annex 2, Table A1.

Figure 1 shows the resulting APROBA-Plus visualizations for the refined calculations for decreased white blood cell counts due to benzene exposure via consumption of vegetable oils and fats. In each plot the $\log_{10}$ human dose (in mg) below which no adverse effects are expected is plotted on the y-axis and the $\log_{10}$ exposure (in mg) is plotted on the x-axis. The calculations are visualized for young children (7A) and adults (7B) after acute exposure and for young children (7C) and adults aged (7D) after chronic exposure. The points (blue diamonds) in the plots correspond to the deterministic calculation where uncertainties are covered by default assessment factors. They indicate whether the chosen exposure percentile exceeds the dose causing a change in white blood cell count in a sensitive human. However, as usual in traditional risk assessment, the magnitude of the change in cell count and the fraction of the population considered as sensitive subpopulation which needs to be protected remains unquantified. When the input data are entered in such a way that the points lay in the green part of the graph, no adverse health effects of benzene via exposure via consumption of vegetable oils and fats are expected, whereas adverse health effects can be expected when the resulting points lay in the red part of the graph. The ellipses represent the calculations where the magnitude of effect, the fraction of the sensitive population and the uncertainties of each extrapolation step are quantified. The uncertainty in the exposure is represented by the width of the ellipses and the uncertainty in the hazard is represented by the height of the ellipses. All ellipses indicate whether the chosen exposure percentiles exceed the dose causing a 5% change in response in the 1% most sensitive subpopulation. Or in other words, when the ellipse is fully located in the green area, the selected exposure results in a negligible, or acceptable, change in response (of <5%) in 99% of the population.

As can be seen in Figure 1B, acute exposure of adults to benzene of 0.01-0.5 mg per kg vegetable oils and fats is unlikely to cause adverse health effects at the current P97.5 percentile of oil and fat consumption since the full ellipse and the point are within the green area. For young children, however, a change in white blood cell count exceeding 5% may occur in more than 1% of the young children after acute exposure to benzene since a small part of the ellipse is within the red area (Figure 1A). Also the point estimate indicates a risk. The same conclusions can be drawn for the chronic exposure of adults (Figure 1D). In case of chronic exposure of children (Fig. 1C), it turns out that using worst case assumptions indicate a risk of health effects since the points are within the red area. Also, being located in the red area for a large part, the ellipse indicates that it is likely that a change in white blood cell count exceeding 5% may occur in more than 1% of the young children. However, it might still be that the set protection goals, protecting 99% of the population against a 5% (or more) decrease in white blood cells, are met since a fraction of the ellipse is located within the green area of the plots.

Table 6 provides an overview of the contributions of all included uncertainties to the overall uncertainty in the hazard calculation as calculated by APROBA-Plus. For acute exposure, the uncertainty in extrapolation from NOAEL to BMD accounts for almost half of the total uncertainty (41%). This uncertainty could be reduced by performing a dose-response analysis and deriving a BMD confidence interval, if the underlying toxicity data are available. A reduction in uncertainty may result in an ellipse positioned fully in the green area of the figure. For chronic exposure, the uncertainty in duration extrapolation

accounts for the largest part of the total uncertainty (42%). This uncertainty could be reduced by performing a chronic toxicity study thereby removing the uncertainty for duration extrapolation, or by predicting from the already performed studies whether the effect worsens over time. This is, however, not practically possible within the scope of the current request.

In conclusion, performing the refined calculations for benzene with APROBA-Plus provided visual insights into the likelihood that the conclusions based on the point estimate answers as provided in the tables 4 and 5 above are accurate (based on the preset protection goals) for the most sensitive young children and adults that are exposed to the highest known exposure. For most of the calculations, the point estimate answers are rather conservative, except for chronic exposure of benzene to young children. Therefore, the APROBA-Plus analyses support the answers to the questions as stated above, although they also show that if a reduction in uncertainty could be obtained (for instance by performing BMD-analyses), chances are that protection goals are met since large parts of the ellipses are already positioned in the green area.

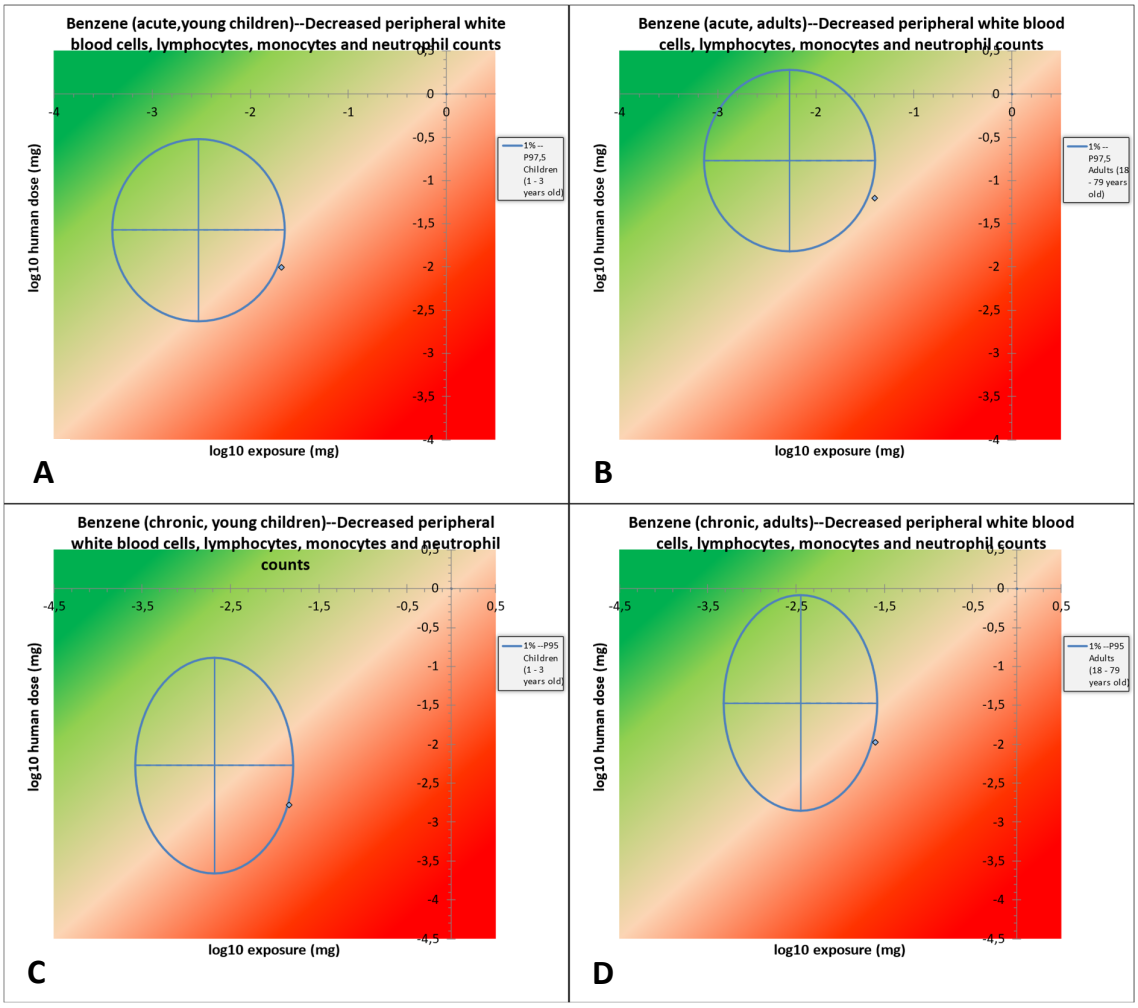

Figure 1. APROBA-Plus visualizations of the calculations for young children (age 1 – 3) and adults (age 18 – 79) about developing decreased peripheral white blood cells, lymphocytes, monocytes and neutrophil counts after acute and chronic exposure to benzene via vegetable oils and fats. The points (blue diamonds) in the plots correspond to the deterministic RfD and exposure whereas the ellipses correspond to the uncertainty in the human dose and the exposure. A) Calculation for young children after acute exposure at P97.5 percentile, B) Calculation for adults after acute

exposure at P97.5 percentile, C) Calculation for young children after chronic exposure at P95 percentile, D) Calculation for adults after chronic exposure at P95 percentile.

Table 6. Contribution of each aspect to the total uncertainty in the hazard calculation for benzene exposure via consumption of vegetable oils and fats.

Aspect	**Acute – adult (18 – 79 years)**	**Acute – young child (1 – 3 years old)**	**Chronic adult (18 – 79 years old)**	**Chronic young child (1 – 3 years old)**
PoD	-	-	-	-
NOAEL to BMD	41%	41%	24%	24%
Interspecies scaling	2%	2%	1%	1%
Interspecies TK/TD	21%	21%	12%	12%
Duration extrapolation	-	-	42%	42%
Intraspecies	36%	36%	21%	21%

BMD: Benchmark dose, NOAEL: No observed adverse effect level, PoD: Point of Departure, TK/TD: Toxicokinetics/Toxicodynamics.

Conclusion

1. The maximum concentration of the volatile organic compounds (VOCs) acetonitrile, ethylbenzene, toluene and xylene in vegetable oils and fats below which a health risk is considered negligible for either young children (1 – 3 years) or adults (18 – 79 years) ranges from 8.4 to 864 mg/kg for acute exposure and from 11 to 247 mg/kg for chronic exposure. This is far above the limit of quantification (LOQ) of the analytical method. For benzene, the maximum concentration is 0.24 mg/kg for young children and 0.78 mg/kg for adults for acute exposure and 0.05 and 0.19 mg/kg, respectively, for chronic exposure. This is between the previous and current LOQ or slightly above the previous LOQ. A refinement of the assessment for benzene with inclusion of uncertainties in the calculation shows that the calculated maximum concentrations are conservative, except for chronic exposure for young children. Moreover, the refined calculation indicates that it is possible that when uncertainties would be reduced by obtaining more toxicity data, the maximum concentrations may be above the LOQ. This is especially likely for adults and in cases of acute exposure for children. No maximum concentration of dimethyl ether could be calculated. However, a margin of safety (MOS) approach showed that when vegetable oils and fats contain 0.5 mg/kg dimethyl ether, the MOS are considered adequate to be of no safety concern.

2. The maximum amount of vegetable oils and fats that can be consumed by young children (1 – 3 years) or adults (18 – 79 years) without an appreciable health risk if acetonitrile, ethylbenzene, toluene and xylene would be present at 0.5 mg/kg (worst case) ranges from 704 g to 140,000 g per person for acute exposure and from 704 g to 28,000 g per person per day for chronic exposure. These amounts are higher than the current consumption levels of vegetable oils and fats of 42 – 81 g per person for acute consumption (P97.5) and 32 – 57 g per person per day for chronic consumption (P95). For benzene, the maximum amount is 20 g per person for young children and 126 g per person for adults for acute exposure and 3.3 and 21 g per person per day, respectively, for chronic exposure. These amounts are lower than the current consumption levels. A refinement of the assessment for benzene with inclusion of uncertainties in the calculation shows that most of the calculated maximum amounts are conservative (except for chronic exposure to young children). Moreover, the refined calculation indicates that it is possible that when uncertainties are reduced by obtaining more data, maximum amounts may be well above the current consumption of vegetable oils and fats. This is especially likely for adults and in cases of acute exposure for children. No

maximum amount can be calculated for dimethyl ether. The toxicological dataset is too limited to identify a minimal MOS which is needed for such a calculation.

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Annex 1 Summary of toxicokinetic and toxicological data

Acetonitrile

Toxicokinetics
There are no oral quantitative human data available for acetonitrile. However, based on human inhalation studies, human poisoning cases, and animal studies, it is indicated that acetonitrile is well absorbed after oral, dermal, and inhalation exposure and that it is distributed throughout the whole human body (U.S. EPA, 1999; ECB, 2002). Once absorbed, acetonitrile is metabolised mostly in the liver by cytochrome P450 (CYP) enzymes into inorganic cyanide, which is responsible for its toxicity. Possibly the enzyme catalase is also involved. The rate of this conversion determines the degree of observed toxicity. Cyanide is further metabolised into thiocyanate, a less toxic compound that can be excreted through urine. This conversion is mediated by a sulphur donor such as the enzyme rhodanese or 3-mercaptopyruvate sulphur transferase. The elimination half-lives are 32 hours for acetonitrile and 20 minutes to 1 hour for cyanide (U.S. EPA, 1999; ECB, 2002; ATSDR, 2024a).

Toxicological information

Acetonitrile
In oral animal studies in mice, rats and guinea pigs, signs and symptoms of acute toxicity with acetonitrile included respiratory issues (e.g. difficulty breathing), neurological symptoms (e.g. fatigue, seizures, convulsions), and, in some cases, death. The dose values resulting in death of 50% of the animals (Lethal Dose $(LD)_{50}$) for mice, rats and guinea pigs after acute oral exposure to acetonitrile ranged from 140 to 6762 mg/kg body weight (bw) with mice and guinea pigs being the most sensitive species (ECB, 2002). In humans, ingestion of 1-2 grams of acetonitrile per kg body weight (bw) can result in death in infants. A dose of 570 mg/kg bw was the highest estimated dose that caused serious health effects without resulting in death (ECB, 2002). Reported health effects in humans include cardiovascular symptoms (e.g. chest pain, tightness in the chest, tachycardia, hypotension), gastrointestinal symptoms (e.g. nausea, emesis), respiratory symptoms (e.g. short and shallow breathing) and neurological symptoms (e.g. headache, seizures).

No oral data on the repeated dose toxicity (subchronic and chronic) of acetonitrile is available. With respect to the reproductive toxicity, there are no oral animal studies specifically investigating fertility effects (ECB, 2002). With respect to developmental toxicity of acetonitrile, adverse effects were mostly observed in rats, rabbits and hamsters at oral doses where maternal toxicity (decreased body weight, death) was also present (ECB, 2002). Adverse effects on foetal development included an increase in resorptions, a decrease in the number of live foetuses/litter and skeletal malformations such as rib fusions and severe axial skeleton - neural tube disorders. The skeletal malformations were observed in a golden hamster study where the presence of maternal toxicity was uncertain (ECB, 2002). In humans, no oral data is available regarding reproductive or developmental toxicity of acetonitrile. Acetonitrile has not been considered mutagenic or carcinogenic (ECB, 2002; Health Canada, 2021).

Cyanide
For cyanide, acute oral exposure resulted in various effects in animals, including renal and pancreatic damage, developmental issues, altered glucose homeostasis, respiratory problems, cardiovascular effects, hepatic effects, neurological effects, haematological

effects, thyroid effects, male reproductive effects, effects on body weight and death (ATSDR, 2024a). Symptoms of cyanide intoxication after oral exposure in humans include local irritation (i.e. burning throat), respiratory symptoms (e.g. dyspnoea, hyperpnoea), neurological symptoms (e.g. loss of consciousness, tremors and convulsions) and cardiovascular failure (ATSDR, 2024a). In some cases, neurological effects due to acute cyanide intoxication in humans may also persist and become chronic in the form of parkinsonism-like signs (e.g. slow movement, mild intention tremor and stiffness) or memory impairments (ATSDR, 2024a). For cyanide, the lethal oral dose in humans has been reported to be between 0.5 and 3.5 mg/kg bw (JECFA, 2011).

After intermediate to chronic oral cyanide exposure in animals, haematological effects, renal effects, thyroid effects, male reproductive effects, decreased body weight gain effects and neurological effects were found (ATSDR, 2024a). In humans, neurological effects (e.g. spastic paraparesis (konzo), tropical ataxic neuropathy and ankle clonus) and thyroid effects (e.g. endemic goiter) have been described after chronic exposure. These symptoms were found in human populations consuming cassava containing cyanide at sublethal concentrations (ATSDR, 2024a). According to JECFA and ATSDR, the relationship between cyanide exposure from cassava and the occurrence of toxic effects is consistent (JECFA, 2011). However, these human populations also have a low intake of sulphur-containing amino acids, and cassava may contain other compounds that exert some of the observed effects (e.g. mycotoxins or scopoletin). Therefore, according to EFSA and ATSDR, the causal relationship between chronic cyanide intake via cassava and the neurological and thyroid effects cannot be definitively established, as it may be confounded by nutritional deficiencies and other compounds (EFSA, 2019; ATSDR, 2024a). EFSA notes that the available data do not indicate that cyanide is genotoxic (EFSA, 2019).

Cyanide was observed to lead to developmental neurotoxicity and skeletal malformations in rats, hamsters and goats (EFSA, 2019). In a 13-week National Toxicology Program (NTP) drinking water study from 1993 in rats with sodium cyanide, effects on the male reproductive system (decrease in relative cauda epididymis weight) were observed (JECFA, 2011). A later 13-week drinking water study in rats tried to correct for the decreased water consumption as observed in the NTP-study by using a water-restricted control group to match measured water consumption of the high dose group. In this repeated dose study, no effects on the male reproductive system were found (Tyner and Greeley (2023) as cited by ATSDR, 2024a). In studies where cyanide was administered via a bolus, effects on the male reproductive system, such as alterations in serum reproductive hormones and sperm and morphological changes in the testis were observed (ATSDR, 2024a). In humans, no oral data is available regarding reproductive or developmental toxicity of cyanide.

Benzene

Toxicokinetics
In animals, it was shown that benzene is readily absorbed after oral exposure, distributed in the body and can accumulate in fatty tissues due to its lipophilic nature. ATSDR states that it is reasonable to assume that the absorption from water solutions is nearly 100%. Metabolism of benzene takes place in the liver and other tissues including the bone marrow. Several reactive metabolites that are believed to contribute to the toxicity of benzene are formed. Briefly, in phase I metabolism benzene is oxidised, mediated by CYP2E1, to benzene oxide, which is a reactive intermediate that can be further metabolised through several pathways. The predominant pathway is a non-enzymatic rearrangement to phenol. Phenol can be oxidised by cytochrome P450 (CYP) 2E1 to

hydroquinone and catechol that can both be further metabolised by myeloperoxidase (MPO) to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively. During phase II metabolism these metabolites can be conjugated. These conjugated substances can be excreted in urine. After a high dose, benzene's metabolic pathways can become saturated, resulting in the possible excretion of benzene via exhaled air (ATSDR, 2024b).

Toxicological information
Due to the several reactive metabolites of benzene and its wide distribution through the body, benzene exposure is associated with several adverse effects. The primary and most sensitive targets are the hematopoietic and immune systems (ATSDR, 2024b). The most sensitive effect in acute oral animal studies is decreased peripheral white blood cell counts with a lowest observed adverse effect level (LOAEL) of 200 mg/kg bw per day, which was the only dose tested. The most sensitive effect after subacute oral exposure was decreased peripheral white blood cell counts at a LOAEL of 1 mg/kg bw per day (Li et al. (2018) as reported by ATSDR (2024b)), and in chronic NTP (1996) studies in mice and rats this effect was observed at a LOAEL of 25 mg/kg bw per day, which was the lowest dose tested. Other health effects of oral exposure to benzene include gastrointestinal, endocrine, immunological, developmental and neurological effects and cancer.

Benzene has been classified by IARC as Group 1 carcinogen ('carcinogenic to humans'). Epidemiological evidence indicates a causal relationship between benzene exposure and acute myeloid leukaemia. In addition, positive associations have been observed for non-Hodgkin lymphoma, chronic lymphoid leukaemia, multiple myeloma, chronic myeloid leukaemia, acute myeloid leukaemia in children, and lung cancer (IARC, 2018)[16]. Benzene is also genotoxic as evidenced by several *in vitro* and *in vivo* studies and in humans. Electrophilic metabolites are formed, oxidative and associated DNA damage stress is induced, and oxidative DNA damage and chromosomal changes are induced (IARC, 2018). Both the Health Council of the Netherlands (2014) and RAC (2018) are of the opinion that the genotoxicity of benzene is subject to a threshold mode-of-action. They reason that the leading genotoxic effects of benzene, i.e. aneugenicity and clastogenicity, are early lesions that contribute to the carcinogenic effect, and are "unlikely to result from primary DNA reactivity of the compound or its metabolites" (RAC, 2018) and therefore are assumed to follow a threshold mechanism. Also, antioxidant defence mechanisms that protect against oxidative DNA damage may be involved at low levels (RAC, 2018). Even though both institutes did not have the most recent IARC assessment at hand at the time of their assessment, it is assumed that RAC had the same information available as IARC as both assessments were published in 2018. Consequently, a TRV can be derived for benzene. As an additional note, the route of exposure does not influence the genotoxic mode-of-action of a compound.

Dimethyl ether

Toxicokinetics
There are no oral data available for dimethyl ether. Upon inhalation, dimethyl ether is rapidly taken up and distributed to various tissues and organs, reaching a steady-state concentration 30 minutes after exposure. Excretion of the compound occurs largely unchanged via exhaled air within a short time, and within 90 minutes after exposure has ceased, dimethyl ether levels return to background levels (EFSA, 2009, 2015).

[16] IARC (2018) notes that a small minority of the Working Group considered that benzene also causes non-Hodgkin lymphoma. A separate small minority considered that a positive association was not observed for cancer of the lung.

Toxicological data
Only toxicological data after inhalation was found, which will be addressed here in absence of oral data. In humans, inhalation of dimethyl ether may lead to effects of sore throat, cough, confusion, drowsiness and unconsciousness (EFSA, 2009).

Dimethyl ether has not shown genotoxic potential when tested in several *Salmonella typhimurium* strains, *Drosophila melanogaster*, *Escherichia coli*, primary rat liver cells and human peripheral lymphocytes. Repeated inhalation studies (4- or 13-week exposure) carried out in rats and hamsters did not show consistent toxic effects. A two-year rat inhalation study showed a slight non-significant decrease in animal survival. Based on this, a no effect level of 2000 ppm (equivalent to 3768 mg/m^3), the lowest concentration tested, was proposed. In one inhalation prenatal developmental toxicity study in rats, foetal toxicity was observed (decreased foetal body weight and increased incidence on skeletal variation) at the intermediate concentration tested of 5000 ppm (9421 mg/m^3) and a no effect level of 1250 ppm (2355 mg/m^3) was proposed (EFSA, 2009, 2015).

Ethylbenzene

Toxicokinetics
Ethylbenzene is quickly absorbed after oral exposure in animals, but no distribution studies were available. After inhalation ethylbenzene accumulates into adipose tissue due to its lipophilic nature. It is metabolised in the liver by two CYP enzymes (isoforms CYP2E1 and CYP2B6), and oxidised to phenylethanols (based on a study with microsomes prepared from human liver). Phenylethanols can be further oxidised and conjugated during phase II metabolism. Conjugated metabolites and mandelic acid and phenylglyoxylic acid are excreted in urine (ATSDR, 2010).

Toxicological information
The oral toxicity of ethylbenzene has been tested in rats in a limited number of acute, subacute, sub-chronic and neurotoxicity studies. In acute and subacute (14 days) oral studies, ototoxicity (complete loss of outer hair cells in cochlea) appeared to be the most sensitive effect. In a 2-weeks gavage study in rats complete loss of outer hair cells in cochlea was observed at a dose of 900 mg/kg bw per day (Gagnaire & Langlais (2005) as reported in ATSDR, 2010). Ototoxicity was also observed after subacute inhalation exposure to ethylbenzene. In other oral subacute and short-term toxicity studies (2 weeks-3 months) ototoxicity was not investigated. No other neurotoxic effects of ethylbenzene were observed in the oral subacute and short-term studies (ATSDR, 2010). In a poorly reported rat-study, decreased levels of hormones, including luteinizing hormone, progesterone, and 17 β-estradiol, were described following acute oral exposure to ethylbenzene at 500 or 1,000 mg/kg bw (Ungvary study). ATSDR (2010) noted that no dose response was observed and the statistical analysis of the data was lacking and considered the study of Ungvary unreliable.

In a 28-days and a 3-month oral study (Mellert et al., 2007, as reported in ATSDR, 2010) and a 6-month study (Wolf et al., 1956, as reported in ATSDR, 2010) liver toxicity (increased serum liver enzyme activity, absolute and relative liver weights, dose-related increased incidence of centrilobular hepatocyte hypertrophy) was the most sensitive effect. In the 3-month study (Mellert et al., 2007, as reported by ATSDR, 2010), the NOAEL was 75 mg/kg bw per day based on liver toxicity observed at 250 mg/kg bw per day. In the 6-month study (Wolf et al., 1956, as reported by ATSDR, 2010), the NOAELs were 136 and 408 mg/kg bw per day based on respectively liver and renal toxicity,

although as ethylbenzene was administered for 5 days a week instead of 7, the NOAEL was adjusted to 97.1 mg/kg bw per day. This study was considered by ATSDR to be poorly reported and therefore less reliable. Renal toxicity (increase in relative kidney weight and hyaline droplet nephropathy) observed in the 28-days and 3-month studies was considered by ATSDR to be secondary to increases of α2μ-globulin accumulation, which is considered a rat-specific effect and not relevant to humans.

In a 2-year oral study in rats (Maltoni et al., 1985 as reported in ATSDR, 2010) an increase in number of malignant tumours was observed in females and in combined male and female groups exposed to 500 mg/kg bw per day (only dose tested). No data on specific tumour type were presented and no information on survival was provided. Also systemic toxicity was not examined in this study.

In a long-term inhalation study with ethylbenzene in mice and rats (NTP, 1999, as reported by ATSDR, 2010) it was concluded that ethylbenzene has carcinogenic properties based on observations of renal tubule neoplasms and testicular adenomas, alveolar/bronchiolar neoplasms and hepatocellular neoplasms (NTP, 1999). No information on the mode of action was provided. Based on this study, ethylbenzene is classified as possibly carcinogenic to humans (Group 2B) by IARC (2000). *In vivo* genotoxicity studies with ethylbenzene were negative. In the majority of *in vitro* genotoxicity studies with ethylbenzene no genotoxic effects were observed. Some *in vitro* studies showed positive findings, which were mostly observed at cytotoxic concentrations.

No specific oral reproductive toxicity studies with ethylbenzene were available. In repeated dose oral studies no effects on reproductive organs were observed. Inhalation studies also provided no clear evidence of a reproductive effect of ethylbenzene. After inhalation exposure to ethylbenzene, developmental toxicity studies in mice and rats showed decreased foetal body weight, and increased incidences of supernumerary and/or extra ribs. RIVM (2001) considered these effects marginally adverse. One developmental toxicity study in rabbits with inhalation exposure showed no clear developmental effects while in another study reduced numbers of live pups per litter due to abortions were reported. RIVM (2001) considered the reliability of this study doubtful in view of the high maternal mortality and incomplete reporting of this study.

Toluene

Toxicokinetic information
Following oral administration, toluene is almost completely absorbed in human and animal studies. In rats the maximum concentration (C_{max}) was reached after 1.5-3 hours. No reliable data on distribution of toluene were available. The main metabolite of toluene in the rat is hippuric acid and the minor metabolites are the glucuronyl conjugate of benzoic acid, sulphate and glucuronide conjugates of ortho- and para-cresol, S-benzylmercapturic acid, and S-p-toluyl mercapturic acid. Following oral administration, toluene is excreted through expiration and toluene metabolites are excreted in urine (ATSDR, 2017; RIVM, 2023).

Toxicological information
The oral toxicity of toluene has been tested in acute, short-term repeated dose, developmental and neurotoxicity studies in mice and rats. In these studies, effects on the heart, liver, kidneys, body weight gain, nervous system and prenatal development were observed. The most critical effects were considered to be increased liver and kidney

weight, neurological effects and immunological effects (U.S. EPA, 2005; ATSDR, 2017; RIVM, 2023). No oral long-term or adequate reproductive toxicity studies with toluene were available. However, the majority of animal studies with inhalation exposure to toluene provide little evidence for reproductive toxicity induced by toluene.

Human and animal studies with inhalation exposure generally do not support a concern for carcinogenicity of toluene. In addition, the majority of *in vitro* and *in vivo* genotoxicity studies with toluene do not show genotoxic effects. IARC (1999) has classified toluene as a Group 3 carcinogen ('not classifiable as to its carcinogenicity to humans'), as evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals.

Xylene

Toxicokinetics
Following oral administration, approximately 90% of xylene is absorbed in rats. Limited data show that at least 34% (ortho-xylene) and 53% (meta-xylene) is absorbed after oral administration in humans. In rats, C_{max} was reached after 20 minutes. Xylene is distributed primarily to fat. In humans and rats, xylene is mainly excreted in the urine as methylhippuric acid (ATSDR, 2007).

Toxicological information
The oral toxicity of xylene has been tested in acute, short-term and chronic repeated dose studies in mice and rats (ATSDR, 2007). Limited or indirect information is available on developmental and reproductive toxicity and carcinogenicity. After oral exposure a large range of clinical signs of neurotoxicity were observed in various studies. Effects on body weight gain were observed at high doses. Increases in liver weights were observed, which are likely the result of enzyme induction. Possible effects on the immune system are suggested by decreased thymus and spleen weights in the absence of histopathological changes, observed after 10 days and 3 months, respectively, in one study. ATSDR (2007) considered the observed renal toxicity (increased hyaline droplets) in a 3-month study male rats not relevant for humans. Although in chronic studies in rats and mice no increases in tumour formation were seen, these studies have limitations prohibiting a definitive conclusion on the carcinogenic potential of xylene. ATSDR (2007) considered neurotoxicity the most critical effect in acute studies and mild effects on body weight and somewhat higher, unexplained mortality the most critical effects in repeated dose studies.

The majority of *in vitro* and *in vivo* genotoxicity studies with xylene do not show genotoxic effects. DNA fragmentation may occur if cells are exposed to levels that cause cytotoxicity. IARC (1999) has classified xylene as a Group 3 carcinogen ('not classifiable as to its carcinogenicity to humans') as the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals.

Annex 2 Input data for APROBA-Plus analysis for benzene

Table A1: Inputs related to study, endpoint, protection goals, adjustment, variability, uncertainty and exposure scenarios for benzene and outputs in APROBA-Plus.

Description	**Benzene acute – adult (18 – 79 years)**	**Benzene acute – young child (1 – 3 years old)**	**Benzene chronic adult (18 – 79 years old)**	**Benzene chronic young child (1 – 3 years old)**	**Common value(s)**
Inputs related to study endpoint and protection goals					
Endpoint	Decreased peripheral white blood cells, lymphocytes, monocytes and neutrophil counts	Decreased peripheral white blood cells, lymphocytes, monocytes and neutrophil counts	Decreased peripheral white blood cells, lymphocytes, monocytes and neutrophil counts	Decreased peripheral white blood cells, lymphocytes, monocytes and neutrophil counts	Case-specific
Data-type	Continuous	Continuous	Continuous	Continuous	Case-specific
Route of exposure	Oral	Oral	Oral	Oral	Case-specific
Study type	Subacute	Subacute	Subacute	Subacute	Case-specific
Test species	Mouse	Mouse	Mouse	Mouse	Case-specific
Body weight test species (kg)	0.020	0.020	0.020	0.020	0.020
Human median body weight (kg)[17]	70	70	70	70	70
Target BMR (= *M*, user input for BMDLs only)	5%	5%	5%	5%	5%
Population incidence goal (= *I*)	1%	1%	1%	1%	5%, 1%, 0.1%, 0.01%
Probabilistic coverage goal	95%	95%	95%	95%	95%
PoD type	NOAEL	NOAEL	NOAEL	NOAEL	Case-specific
PoD value (= NOAEL x body weight[a])	6.30 (0.09 x 70)	0.99 (0.09 x 11)	6.30 (0.09 x 70)	0.99 (0.09 x 11)	Case-specific

[17] Value used for allometric scaling of the average adult mouse to the average adult human. Also, for children an adult body weight is used because for the allometric scaling adult mice were used. The intraspecies AF is considered to cover differences between the average human (adult) and the sensitive human (child).

Description	Benzene acute – adult (18 – 79 years)	Benzene acute – young child (1 – 3 years old)	Benzene chronic adult (18 – 79 years old)	Benzene chronic young child (1 – 3 years old)	Common value(s)
BMDU (User input for BMDL PoDs)	-	-	-	-	Leave blank if PoD is NOAEL
PoD units	mg	mg	mg	mg	mg/kg bw/day
Deterministic overall AF	100	100	600	600	
Deterministic RfD	0.063	0.0099	0.01050	0.00165	
Inputs related to adjustment, variability and uncertainty					
PoD	LCL: 6.30 UCL: 6.30	LCL: 0.99 UCL: 0.99	LCL: 6.30 UCL: 6.30	LCL: 0.99 UCL: 0.99	Calculated from inputs
NOAEL to BMD	LCL: 0.07 UCL: 1.57	LCL: 0.07 UCL: 1.57	LCL: 0.07 UCL: 1.57	LCL: 0.07 UCL: 1.57	LCL: 0.07 UCL: 1.57
Interspecies scaling	LCL: 8.35 UCL: 16.03	LCL: 8.35 UCL: 16.03	LCL: 8.35 UCL: 16.03	LCL: 8.35 UCL: 16.03	LCL: 8.02 UCL: 15.21
Interspecies TK/TD	LCL: 0.33 UCL: 3.00	LCL: 0.33 UCL: 3.00	LCL: 0.33 UCL: 3.00	LCL: 0.33 UCL: 3.00	LCL: 0.33 UCL: 3.00
Duration extrapolation	LCL: 1.00 UCL: 1.00	LCL: 1.00 UCL: 1.00	LCL: 0.62 UCL: 40.00	LCL: 0.62 UCL: 40	LCL: 0.62 (chronic), 1.00 (acute) UCL: 40 (chronic), 1.00 (acute)
Intraspecies	LCL: 2.24 UCL: 41.88	LCL: 2.24 UCL: 41.88	LCL: 2.24 UCL: 41.88	LCL: 2.24 UCL: 41.88	LCL: 2.24 UCL: 41.88
Inputs related to exposure					
P95 exposure[b] (mg)	-	-	LCL: 0.000478 UCL: 0.0263 Det.: 0.0251	LCL: 0.000266 UCL: 0.0161 Det.: 0.0147	-
P97.5 exposure[b] (mg)	LCL: 0.000729 UCL: 0.0405 Det.: 0.0405	LCL: 0.000392 UCL: 0.0224 Det.: 0.0210	-	-	-
Outputs					
Probabilistic RfD (mg)	0.0150	0.0024	0.0014	0.00004	-
Target human dose (HD_M^I) (P05) (mg)	0.0150	0.0024	0.0014	0.00004	-
Target human dose (HD_M^I) (P95) (mg)	1.915	0.301	0.8271	0.026	-

Description	Benzene acute – adult (18 – 79 years)	Benzene acute – young child (1 – 3 years old)	Benzene chronic adult (18 – 79 years old)	Benzene chronic young child (1 – 3 years old)	Common value(s)
Degree of uncertainty	127	127	594	594	
Estimated "Coverage" of deterministic RfD	74.9%	74.9%	72.7%	72.7%	-

[a] Body weight as proposed by te Biesebeek et al (2014): 70 kg for adults (age 18-79) and 11 kg for young children (age 1-3)

[b] Expressed per person (mg) by multiplying the consumed amount (kg oil and fat/kg bw, Table 3) with the concentration (0.01 (LCL) or 0.5 (UCL) mg benzene/kg oil or fat), and with the bodyweight from table 2: 81 kg for adults (18-79) and 14 kg for young children (age 1-3).

AF: Assessment factor, BMD: Benchmark dose, BMDL: Benchmark dose lower limit, BMR: Benchmark response, BMDU: Benchmark dose upper limit, Det.: Deterministic, HD_M^I : Target human dose, I: Incidence, LCL: Lower confidence limit, M: Magnitude, Max: maximum, Min: minimum, NOAEL: No observed adverse effect level, PoD: Point of Departure, RfD: Reference Dose, TK/TD: Toxicokinetics/Toxicodynamics, UCL: Upper confidence limit.