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SCIENTIFIC OPINION

Update of the risk assessment on dioxins and dioxin-like PCBs in feed and food

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

The European Commission asked EFSA to update its 2018 risk assessment on polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in feed and food, based on the 2022 WHO Toxic Equivalency Factors (WHO_{2022}-TEFs). A decrease in sperm concentrations as a consequence of early-life exposure was still considered the critical effect in rodents and humans. This can only be prevented by ensuring sufficiently low exposure to PCDD/Fs and DL-PCBs of future mothers. Using the WHO_{2022}-TEFs, the critical human study in the 2018 Opinion still showed a significant inverse association between PCDD/Fs and sperm concentration, but not when including DL-PCBs. Therefore, this study was not used to derive a Tolerable Weekly intake (TWI). Instead, a developmental study with TCDD in rats was used to derive a TWI of 0.6 pg. WHO_{2022}-toxic equivalents (TEQ)/kg bw per week, based on decreased sperm production. Human studies were used as supporting evidence, including an evaluation of necessary uncertainty factors. Dietary exposure assessment based on occurrence data in food from the last decade, showed that using the new TEFs resulted in 27%–35% lower total-TEQ exposure. Mean exposure (lower to upper bound) to PCDD/Fs and DL-PCBs for European adult populations varied between 1.87 and 6.10 pg. WHO_{2022}-TEQ/kg bw per week, and at the P95 between 4.15 and 12.0 pg. WHO_{2022}-TEQ/kg bw per week. The CONTAM Panel concluded that exposure for European women of childbearing age raises a health concern for their sons at the mean (80%–90% certainty) and P95 (95%–99% certainty) exposure. Including uncertainties regarding the TEFs for DL-PCBs decreased this certainty. Human milk data confirmed the exceedance of the TWI but to a lesser extent than dietary exposure. The change in TEFs does not affect the transfer rates of individual congeners but it could impact the transfer rates when using Total-TEQ levels.

KEYWORDS

dioxins, DL-PCBs, feed, food, PCDD/fs, risk assessment, TEFs, transfer

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SUMMARY

In 2018, EFSA published an Opinion on the risks of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) present in food and feed for human and animal health risk (referred to as the 2018 Opinion). For humans, a Tolerable Weekly Intake (TWI) of 2 pg. toxic equivalents (TEQ)/kg bw per week was derived based on the association between blood levels of PCDD/F-WHO_{2005}-TEQ at the age of 8–9 years in boys in the Russian Children's Study and lower sperm concentrations at later age. This effect had previously been shown in rats and in human studies following up the Seveso incident. In these latter studies, the focus was on TCDD only. In the critical study with Russian boys, no significant association was observed for DL-PCB-TEQ or Total-TEQ, but this was in agreement with in vitro studies with human cells, showing that the DL-PCB contributing most to the TEQ level, i.e. PCB-126, is much less potent in humans than in rats or rat-derived cell systems. Therefore, the TWI was based on the dose–response for PCDD/F-WHO_{2005}-TEQ only, but EFSA recommended to reevaluate the Toxic Equivalency Factors (TEFs).

In 2022 an Expert meeting was organised by the World Health Organisation (WHO) in which the WHO_{2005}-TEFs for chlorinated dioxin-like compounds were reevaluated. In this evaluation 'Best-Estimate' TEFs were derived based on an update to the 2006 relative potency database, a consensus-based weighting scheme, a Bayesian dose response modelling and meta-analysis. The 'Best-Estimate' TEF derived from the model was used to assign 2022 WHO-TEFs (WHO_{2022}-TEFs). They were no longer rounded based on a half-log scale but directly based on the observed relative potencies. TEFs for most PCDD/Fs and DL-PCBs were changed. Much more weight was given to in vivo studies and toxicological endpoints in experimental animals and as a result, WHO-TEFs are still dominated by rat studies. For mono-*ortho* PCBs, WHO decided to retain their 2005 WHO-TEFs because of the limited and heterogenous data available for these congeners.

EFSA was subsequently asked by the European Commission to update its 2018 Opinion considering the potential impact of the change of the TEFs to WHO_{2022}-TEFs. The change in TEFs could affect both the exposure assessment and the derivation of the TWI, also considering the need to take into account the updated TEFs for all 29 congeners and as such the sum of PCDD/F- and DL-PCB-TEQ.

EFSA was also asked to update the information on exposure of food-producing animals and the transfer of PCDD/Fs and DL-PCBs from these animals to animal-derived food products.

The draft Scientific Opinion underwent a public consultation from 27 November 2025 to 26 January 2026. The comments received were taken into account when finalising the Scientific Opinion and are presented and addressed in Annex I.

Hazard identification and characterisation

A systematic approach was followed to retrieve relevant new evidence available since the 2018 Opinion.

Regarding toxicokinetic studies, no new studies were identified that add essential information not already provided in the 2018 Opinion.

Based on pooled human milk samples from the WHO/United Nations Environment Programme (UNEP) monitoring program and individual human milk samples from Germany, the application of the new WHO_{2022}-TEFs resulted in consistently lower Total-TEQ concentrations (average decrease of 40%) compared to the Total-TEQ concentrations based on the WHO_{2005}-TEFs. The congeners contributing most to the TEQ concentrations in human milk are PCB-126 followed by PeCDD, TCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDD, and HpCDD. Due to the change in the TEF values, there was a strong increase in the contribution for HpCDD and 1,2,3,4,7,8-HxCDF to the TEQ concentration.

Regarding studies in experimental animals, the new eligible studies since the 2018 Opinion reported effects that were considered not to be adverse and/or not to be suitable to derive a Reference Point. As was the case in the 2018 Opinion, a decrease in sperm production in male offspring of rats upon exposure to TCDD via the dams was the most sensitive endpoint.

Although numerous new eligible epidemiological studies were identified, the number of studies per endpoint was limited, with considerable heterogeneity being observed, and few studies were prospective. Since the 2018 Opinion, no new prospective developmental male reproductive studies were identified. Therefore, the conclusions from the 2018 Opinion that impaired semen quality is likely to be a causal effect of exposure to PCDD/Fs was still considered valid. Regarding DL-PCBs there was no conclusive evidence based on human studies. For all other assessed endpoints, the newly available evidence did also not change the previous conclusions.

In terms of mode of action, the adverse effects on male reproductive development upon maternal exposure to TCDD were predominantly, if not exclusively, dependent on activation of the aryl hydrocarbon receptor (AHR). Both canonical and non-canonical pathways may be involved. The most plausible target causing these effects on male reproductive development is the hypothalamic–pituitary-gonadal axis.

The critical endpoint was identified by integrating lines of evidence from both human and experimental animals considering the respective level of confidence and according to a weight of evidence approach. Based on the available evidence, the CONTAM Panel concluded that developmental effects of TCDD on the male reproductive system occurred at the lowest exposure in experimental animals compared to other effects and were therefore considered as critical. Decreased sperm production in male offspring was the most sensitive among the endpoints assessed in experimental animals.

Consistent with this, lower sperm concentrations were observed in three different human cohorts with men exposed at young age:

- Two Seveso studies showed a statistically significant inverse association between serum levels of TCDD and sperm concentrations, TCDD being the only congener measured in these studies.
- The Russian Children's Study showed a statistically significant inverse association between serum levels of TCDD, PCDD-TEQ, PCDD/F-TEQ and sperm concentrations, but not for PCDF-TEQ, DL-PCB-TEQ or Total-TEQ, expressed using the WHO_{2022}-TEFs.

Considering that the WHO_{2022}-TEFs should be applied in the current risk assessment, with all 29 congeners included, the studies in humans could no longer serve as the primary basis for establishing the TWI. In the Russian Children's Study there was no statistically significant association for Total-TEQ. In the two Seveso studies there was a lack of information on the exposure to other congeners than TCDD in both the exposed and the control groups.

Thus, the CONTAM Panel decided to use the study in rats as the critical study, while the human studies were used as supporting evidence in the weight of evidence approach, including the decision on which uncertainty factors should be applied in the derivation of the TWI.

In the critical rat study, dams were exposed to TCDD and male pups showed a reduced sperm production at the age of 70 and 170 days. At the lowest dose the effect was near-maximal. Benchmark dose (BMD) modelling was performed on this study applying the 2022 EFSA Guidance on the use of the BMD approach in risk assessment. A maternal body burden of 1.7 ng TCDD/kg bw was identified as Reference Point, based on a benchmark dose lower credible limit (BMDL) using a benchmark response (BMR) of 15% decreased sperm production (one standard deviation of the response in the control group).

Although the Reference Point is based on findings on TCDD, the CONTAM Panel concluded that it should also apply to the sum of PCDD/Fs and DL-PCBs based on the TEQ concept.

To derive a Health-Based Guidance Value (HBGV), the CONTAM Panel considered which uncertainty factors should be applied to the Reference Point. The default uncertainty factor for interspecies toxicokinetic variability was not applied because the body burden was the starting point of the assessment. Moreover, there is evidence demonstrating that humans are not more sensitive than rats, which also conforms to the results from three human studies showing an association with lower sperm concentrations. Therefore, the default uncertainty factors for inter- and intraspecies variability in toxicodynamics were not applied. On the other hand, some within-human variability in toxicokinetics of these compounds was deemed appropriate to account for, such as variations in both maternal body fat content and the amount of human milk consumed by the infants, while interindividual differences in metabolism are limited and the absorption is almost complete. To account for this possible variability in the kinetics in women of childbearing age and in their infants, the default uncertainty factor of 3.16 for intraspecies variability in toxicokinetics was applied.

The Reference Point of 1.7 ng WHO_{2022}-TEQ/kg bw corresponded to a lipid-based level of 6.8 ng WHO_{2022}-TEQ/kg body fat (based on 25% body fat). Using the concentration- and age-dependent physiologically based kinetic (PBK) model (the CADM model), this lipid-based level would be reached after chronic exposure to 0.29 pg. WHO_{2022}-TEQ/kg bw per day. Applying the default uncertainty factor of 3.16 for intraspecies variability in toxicokinetics, this resulted in a daily intake of 0.09 pg. WHO_{2022}-TEQ/kg bw per day. Expressed on a weekly basis, this corresponded to an intake of 0.63 pg. WHO_{2022}-TEQ/kg bw per week.

On this basis, a TWI of 0.6 pg. WHO_{2022}-TEQ/kg bw per week was established for the sum of PCDD/Fs and DL-PCBs.

PBK modelling of chronic exposure to PCDD/Fs and DL-PCBs at the TWI resulted in a body burden in women at age 35 of 2.2 ng WHO_{2022}-TEQ/kg fat. This is the highest body burden in women not raising a health concern for their sons. The fat-based level in human milk would be similar to the body burden.

The TWI is considered protective for the general population and all endpoints. It prevents women of childbearing age from reaching body burdens (i.e. levels in their bodies) that could lead to in utero and lactational exposures associated with health concerns in the offspring.

Reduction in sperm production or concentrations was also the critical effect behind the HBGVs established by the former Scientific Committee on Food (SCF) in 2001, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002, the United States Environmental Protection Agency (US-EPA) in 2012, and EFSA in 2018. The CONTAM Panel identified the major reasons for differences in HBGVs, i.e. selection of critical studies, application of benchmark dose modelling, toxicokinetic modelling and application of a correction factor for dosing regimen.

Occurrence and dietary exposure assessment for the European population

Occurrence data of PCDD/Fs and DL-PCBs in food were extracted from the EFSA Occurrence database. After data validation, cleaning procedures and application of the performance criteria according to Commission Regulation (EU) No 2017/644, a total of 54,177 food samples fulfilled the criteria. The food samples included in the final dataset were submitted by 24 Member States plus Norway and Iceland, and were sampled between the years 2013 and 2023. 92% of the samples covered foods of animal origin such as Meat and meat products, Fish and seafood, Milk and dairy products and Eggs and egg products while only a limited number of samples was available for other food categories. Food consumption data from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) were used for the dietary exposure assessments.

When applying the WHO_{2022}-TEFs, the following dietary exposure estimates for the 29 PCDD/Fs and DL-PCBs across surveys were obtained (expressed on a daily basis):

- The mean lower bound (LB) dietary exposure ranged from 0.24 pg. WHO_{2022}-TEQ/kg bw per day in 'Very elderly', to 1.57 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers'.
- The mean upper bound (UB) dietary exposure ranged from 0.41 pg. WHO_{2022}-TEQ/kg bw per day in 'Very elderly', to 2.35 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers'.
- The P95 LB dietary exposure ranged from 0.57 pg. WHO_{2022}-TEQ/kg bw per day in 'Elderly and 'Very elderly', to 3.30 pg WHO_{2022}-TEQ/kg bw per day in 'Toddlers'.
- The P95 UB dietary exposure ranged from 0.86 pg. WHO_{2022}-TEQ/kg bw per day in 'Elderly', to 4.72 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers'.

The exposure estimated with the WHO_{2022}-TEFs is between 27% and 35% lower than that obtained using WHO_{2005}-TEFs across age groups. This is based on the median ratios between the mean LB dietary exposure estimates.

The non-*ortho* DL-PCBs showed the highest contribution (41%) to the total WHO_{2022}-TEQ exposure, followed by PCDFs (27%), PCDDs (24%) and mono-*ortho* PCBs (9%).

Regarding individual congeners, PCB-126 contributed most to the exposure to Total-TEQ (38%), followed by HpCDD (10%), 1,2,3,4,7,8-HxCDF (9%) and PCB-118 (7%). TCDD, PeCDD, TCDF and 2,3,4,7,8-PeCDF each contributed 5%. Compared to the use of WHO_{2005}-TEFs, the contribution of PCB-126 is 13% lower while the contributions of HpCDD and 1,2,3,4,7,8-HxCDF are 8.4% and 7.1% higher, respectively.

'Milk and dairy products' and 'Fish and fish products' are the food categories that contributed more than 10% to the total mean LB dietary exposure in the highest number of surveys with percentage up to 43% in 'Other children' for 'Milk and milk products' and up to 67% in the 'Elderly' for 'Fish and fish products'. 'Vegetables and vegetable products' and 'Meat and meat products' also contributed more than 10% to the total mean LB dietary exposure in a large number of surveys.

Human risk characterisation

The TWI is derived to prevent women of childbearing age from having body burdens (i.e. levels in their bodies) that could lead to in utero and lactational exposures associated with health concerns in the offspring. The CONTAM Panel recognises that infants have higher exposure per kg bw during breastfeeding than the TWI, resulting in a higher body burden. However, this was already taken into account when setting the TWI. Therefore, the TWI is not applicable for infants, and it is not appropriate to compare it to the exposure of infants.

When comparing the mean current dietary exposure to the 29 PCDD/Fs and DL-PCBs expressed on a weekly basis of 'Adolescents', 'Adults', 'Elderly' and 'Very Elderly' using the WHO_{2022}-TEQs, a 3- to 12-fold exceedance of the TWI was observed (lowest LB-highest UB). At the P95, exceedances ranged from 6- to 30-fold (lowest LB-highest UB). For 'Toddlers' and 'Other Children', the mean dietary exposures exceeded the TWI by 6- to 27-fold. At the P95 this ranged from 11- to 55-fold.

The CONTAM Panel noted that for 'Toddlers' and 'Other Children', the observed exceedance of the TWI raises a health concern for developmental effects. For adult age groups, effects other than developmental endpoints might occur at body burdens higher than those presently observed in Europe. However, the available data did not allow to estimate in a quantitative manner at which exposure levels there is a health concern for such effects, noting that at the current exceedance of the TWI there is no evidence for such effects.

Current levels of PCDD/Fs and DL-PCBs in human milk were compared to the level of 2.2 ng WHO_{2022}-TEQ/kg fat, estimated to result from chronic exposure at the TWI using PBK modelling. Two data sets on human milk levels showed that current levels of PCDD/Fs and DL-PCBs are on average within a factor 1.2 of this level. This exceedance is lower than the 3- to 12-fold exceedance of the TWI by 'Adolescents', 'Adults', 'Elderly' and 'Very Elderly' based on the estimated dietary exposures. There could be a number of reasons for this apparent discrepancy, such as: (i) uncertainties associated with the toxicokinetic model applied to link the body burden to a chronic exposure, including the assumption that other PCDD/F and DL-PCB congeners behave similar to TCDD, (ii) overestimation of the current dietary exposure, and (iii) the human milk samples covering only a few countries.

Occurrence and dietary exposure assessment for food-producing animals

Occurrence data of PCDD/Fs and DL-PCBs in feed were extracted from the EFSA Occurrence database. After data validation, cleaning procedures and application of the performance criteria according to Commission Regulation (EU) No 2017/644, a total of 4550 samples were included in the final feed occurrence dataset sampled between 2013 and 2023. The feed samples were submitted by 15 Member States plus Norway and the United Kingdom, with approximately 55% of the samples submitted by one country and covering all 14 Feed Level 1 categories.

The highest mean concentrations of the 29 PCDD/Fs and DL-PCBs in the feed materials that were used in the exposure assessment were reported in 'Fish oil', 'Fish meal' and 'Vegetable oil and fat'. Regarding compound feeds, the highest mean concentrations were reported in 'Complete feed' for 'Fish'.

Dietary exposure was calculated for all food-producing animal species, except for veal calves (receiving milk replacer) due to the lack of suitable occurrence data.

The compound feed exposure scenario was estimated using complete feed for piglets, pigs for fattening, lactating sows, chickens for fattening, laying hens, turkeys for fattening, salmonids, and rabbits for fattening, and with complementary feed plus forages for horses. For the remaining animal species/categories, dietary exposure by compound feed could not be estimated due to insufficient occurrence data.

The estimated mean concentrations of the 29 PCDD/Fs and DL-PCBs were expressed as pg. WHO_{2022}-TEQ/kg feed dry matter (DM), rather than ng WHO_{2022}-TEQ/kg 88% DM, in the daily diets of food-producing animals.

- Based on a model diet exposure scenario, the LB–UB ranges were as follows (expressed as pg. WHO_{2022}-TEQ/kg feed DM): piglets (20–51), pigs for fattening (20–53), lactating sows (23–55), chickens for fattening (27–62), laying hens (22–56), turkeys for fattening (27–67), cattle for fattening (24–68), dairy cows (24–59), lambs for fattening (24–67), salmonids (215–264), rabbits for fattening (22–76), and horses (34–107).
- Based on a compound feed exposure scenario, the estimates were: piglets (14–41), pigs for fattening (11–36), lactating sows (22–38), chickens for fattening (17–43), laying hens (12–41), turkeys for fattening (42–68), salmonids (284–318), rabbits for fattening (31–58), and horses (35–115).

Ratios of WHO_{2022}-TEQ vs. WHO_{2005}-TEQ concentration estimates for the 29 PCDD/Fs and DL-PCBs ranged from 0.8 to 1.2 for most food-producing animal species and categories considered. The variation in this ratio is largely driven by the congener pattern in the feed material or compound feed.

The updated occurrence data and methodological refinements in the current Opinion resulted in generally lower or comparable estimated concentrations of the 29 PCDD/Fs and DL-PCBs in the daily diets of food-producing animals compared to the 2018 Opinion based on WHO_{2005}-TEFs.

Transfer of PCDD/Fs and DL-PCBs from feed to food of animal origin

New studies on the transfer of PCDD/Fs and DL-PCBs in dairy cows, laying hens and pigs were used to update the information on transfer rates of PCDD/Fs and DL-PCBs and contributed to a better understanding of the transfer to milk and eggs, and accumulation in liver, fat and meat of food producing animals. The new data did not provide consistent information on the ratio of the fat-based levels in meat and adipose tissue. The change in TEFs does not affect the transfer rates of individual congeners but the CONTAM Panel noted that it could impact the transfer rates when using Total-TEQ levels. The extent of the impact of the new WHO_{2022}-TEFs on the transfer rate of the Total-TEQ may be evaluated based on a case-by-case basis and ideally using congener-specific toxicokinetic models that are available for some species.

Uncertainty analysis

Sources of uncertainty affecting different parts of the assessment were identified systematically, and their impact on the major conclusions was quantified by expert judgement.

The human risk assessment was affected by a number of substantial uncertainties including the derivation of the Reference Point from the critical study, the absorption/distribution/metabolism/excretion (ADME) and the toxicokinetic model, the relevance for humans of the WHO_{2022}-TEFs for DL-PCBs, the derivation of the TWI including the applicable uncertainty factors, left-censored occurrence data and occurrence in foods of plant origin.

The CONTAM Panel concluded with about 95% certainty that, if the uncertainties were resolved, the TWI would be equal to or higher than the assessed value of 0.6 pg. WHO_{2022}-TEQ/kg bw per week.

The CONTAM Panel concluded with 99%–100% certainty that, if the identified uncertainties affecting the exposure assessment were resolved, the mean exposure of adults would be higher than the TWI of 0.6 pg. WHO_{2022}-TEQ/kg bw per week for all the dietary surveys that were considered.

Taking into account the discrepancy between the exceedances of the TWI obtained for dietary exposure estimates and measured human milk levels, the CONTAM Panel judged with 80%–90% certainty that mean exposure would still raise a health concern based on the WHO_{2022}-TEFs. For P95 exposure, the level of certainty for a health concern was 95%–99%. When uncertainties related to the WHO_{2022}-TEFs for DL-PCBs were also taken into account, the estimated probability for a health concern at the mean and P95 reduced to 33%–66% (about as likely as not) and to 70%–80%, respectively.

The uncertainties affecting the occurrence data in feed and the exposure assessment of food-producing animals were identified. The methodology for the exposure assessment aimed at providing a conservative estimate, and the use of different exposure scenarios tends towards reduction of uncertainty.

Recommendations

To reduce the uncertainties in the human risk assessment, the CONTAM Panel made recommendations. Further development of approaches to compare animal- and human-based data is needed to derive TEFs for PCDD/Fs and DL-PCBs that are more relevant for humans. This is also important for evaluating associations between TEQ levels and specific effects in human studies. To better estimate the actual contribution of plant-derived products to the dietary exposure, more data on occurrence levels in such foods are needed. Further improvement of toxicokinetic models is needed, including parameters related to pregnancy and breastfeeding, and the inclusion of congeners other than TCDD is required. The use of in vitro models for further refinement should be considered. Biomonitoring data, especially individual data on occurrence of PCDD/Fs and DL-PCBs in human milk and covering more European populations, are needed. Development of analytical methods that allow a lower sample volume is needed to determine PCDD/Fs and DL-PCBs in human blood. Considering the differences between levels in meat and fat tissue, the impact of lipid composition on lipid-based PCDD/F and DL-PCB levels in food and biological matrices, including blood and human milk, should be investigated. Better understanding is needed of the involvement of the AHR in the regulation of sperm production and how it is disrupted by PCDD/Fs and DL-PCBs. This would contribute to development of an AOP for the AHR pathway in relation to sperm production and other effects related to reproduction and development. Further investigation on the role of soil ingestion in the contamination of animal-derived food is needed.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the European Commission

Background

The European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM Panel) adopted in 2018 a scientific opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food.[1] The CONTAM Panel recommended, among other, that the 2005 WHO-TEFs should be re-evaluated in order to take into account new in vivo and in vitro data. In particular, more insight into the relative potency of PCB-126 in humans is required.

On 17 to 21 October 2022 the World Health Organization (WHO) held an ad-hoc expert consultation in Lisbon, Portugal, during which the 2005 WHO toxic equivalency factors (TEFs) for dioxin-like compounds, including some polychlorinated biphenyls (PCBs), were re-evaluated.[2] These 2022 WHO-TEF vsalues together with the corresponding scientific background were published in January 2024.[3]

These new TEF values require an update of the scientific opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. Therefore, the European Commission requests EFSA to provide an update of the scientific opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food.

This update should relate to all aspects of the risk assessment where the change in TEF values requires a change. This is of particular relevance for the occurrence data and exposure assessment. In the opinion, the occurrence data on dioxins and dioxin-like PCBs in feed and food from the last 10 years (sampling years 2014–2023 - data submitted to EFSA in the period 2015–2024) should be used, highlighting the main changes following the use of the 2022 WHO-TEF values compared to the use of the 2005 WHO-TEF values in occurrence and relative contribution of the dioxin-like PCBs to the total TEQ level for the different feed and food categories as well as the main changes in contributors to the exposure of animals and humans. A detailed overview of the occurrence data of dioxins and dioxin-like PCBs in feed and food in toxic equivalent values (TEQ) on the basis of the new 2022 WHO-TEF values with information on the main changes compared to the occurrence data based on the 2005 WHO-TEF values should be made available to the Commission before the finalisation of the opinion with a view to initiate discussions with the Member States on the future change of EU legislation on dioxins and PCBs in feed and food, once the opinion is available.

Terms of Reference

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002, the Commission asks EFSA for an update of the scientific opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food on the basis of the new 2022 WHO-TEF values.

The update of the scientific Opinion should relate to all aspects of the risk assessment where the change in TEF values requires a change. The available occurrence data on dioxins and dioxin-like PCBs in feed and food from the last 10 years should be used for the human and animal exposure assessment and the opinion should highlight the main changes in occurrence and exposure in toxic equivalent values (TEQ) following the use of the 2022 WHO-TEF values compared to the use of the 2005 WHO-TEF values. EFSA is requested to provide the Commission before the finalisation of the opinion a detailed, preliminary overview of the occurrence data in feed and food used for the opinion.

1.2 | Interpretation of the Terms of Reference

According to the Terms of Reference, the update of the 2018 EFSA CONTAM Panel Opinion (referred to as the 2018 Opinion) should relate to all aspects of the risk assessment where the new WHO_{2022}-TEFs would have an impact. As mentioned in the protocol for the risk assessment (see Annex A), this is of particular relevance for the human hazard identification and characterisation, and for the occurrence data used to estimate the dietary exposure via food or feed (for food-producing animals), and transfer from feed to food of animal origin.

Thus, sections in the 2018 Opinion related to 'Sources, characteristics and environmental fate', 'Sampling and methods of analysis', 'Previously reported occurrence data in the open literature', 'Food and feed processing', 'Previously reported dietary exposure assessments', will not be subject to an update, because the new WHO_{2022}-TEFs are not expected to have an impact there.

The section of 'Non-dietary exposure' will not be subjected to an update because of the low impact of this source to total human exposure to PCDD/Fs and DL-PCBs.

[1]EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2018. Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal, 16(11), 5333. https://doi.org/10.2903/j.efsa.2018.5333.

[2]https://www.who.int/news/item/15-03-2024-who-expert-consultation-on-updating-the-2005-toxic-equivalency-factors-for-dioxin-like-compounds-including-some-polychlorinated-biphenyls.

[3]Regulatory Toxicology and Pharmacology, Volume 146, January 2024, 105525 https://doi.org/10.1016/j.yrtph.2023.105525.

An update of the effects on the health of food-producing and non-food producing animals is out of the scope of the TORs.

1.3 | Supporting information for the assessment

The sources, characteristics and environmental fate of PCDD/Fs and DL-PCBs, as well as the analytical methods for their determination were described in the 2018 Opinion (EFSA CONTAM Panel, 2018) and are not subject to an update in the present Opinion (see **Section 1.2**). The recent developments in the establishment of WHO-TEFs, assessments performed since the 2018 Opinion and changes in the Legislation at European level are reviewed.

1.3.1 | Previous assessments

In 2018, the EFSA CONTAM Panel published an Opinion on the health risks for humans and animals related to the presence of PCDD/Fs and DL-PCBs in food and feed (EFSA CONTAM Panel, 2018). A Tolerable Weekly Intake (TWI) of 2 pg. WHO_{2005}-TEQ/kg bw per week was derived for humans, which was 7-fold lower than the previous TWI established by the former Scientific Committee on Food (SCF, 2001).

A brief summary of the risk assessment and evaluation of PCDD/Fs and DL-PCBs published before 2018 is provided below, and further details can be found in EFSA CONTAM Panel (2018).

The TWI of 14 pg. WHO_{1998}-TEQ/kg bw per week established by the SCF (2001) was based on reduced sperm production observed in offspring of rat dams that were weekly injected subcutaneously (s.c.) with TCDD (Faqi et al., 1998a). The body burden in the dams was estimated and directly translated into a body burden in humans and corresponding long-term intake, using one-compartment modelling and including factors for absorption and the elimination half-life reported in humans. A daily exposure was calculated that would result in a no-effect body burden level, then multiplied by 7 to derive a TWI of 14 pg. WHO_{1998}-TEQ/kg bw per week. Uncertainty factors applied included a factor of 3 to translate the lowest-observed-adverse effect level (LOAEL) (lowest dose tested, showing a near maximal effect) to a no-observed-adverse effect level (NOAEL), and a factor of 3.2 for intraspecies variability in toxicokinetics in humans. No additional uncertainty factors were applied. The SCF stated: "*With regard to the potential differences in toxicodynamics between experimental animals and humans and within the human population, studies of Ah receptor binding affinity and adverse responses directly dependent on Ah receptor activation suggest that humans are less sensitive to 2,3,7,8-TCDD than responsive rodent strains. However, studies of some biochemical or cellular effects, such as CYP1A1 and CYP1A2 induction, suggest a comparable sensitivity. Therefore, for some endpoints it cannot be excluded that the most sensitive humans might be as sensitive to the adverse effects of 2,3,7,8-TCDD as experimental animals. The Committee concluded that no uncertainty factor needs to be applied for differences in toxicodynamics between experimental animals and humans and for interindividual variation among humans*." In addition, based on studies by Hurst, DeVito, and Birnbaum (2000), Hurst, DeVito, Woodrow-Setzer, & Birnbaum (2000) the SCF argued that the doses applied once a week would cause a higher fetal exposure than the same doses spread over 7 days. To correct for this, the body burden was increased from 25 to 40 ng/kg bw, i.e. by a factor of 1.6 (SCF, 2001).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) used a similar approach to derive a Provisional Tolerable Monthly Intake (PTMI) of 70 pg/kg bw per month, again based on reduced sperm production in rats in the same critical study as SCF, i.e. Faqi et al. (1998a) (FAO/WHO, 2002).

The United States Environmental Protection Agency (US-EPA, 2012) derived a Reference Dose (RfD) for TCDD of 0.7 pg/kg bw per day based on lower sperm concentration (Mocarelli et al., 2008) and increased serum TSH levels (Baccarelli et al., 2008) reported in epidemiological studies from people affected by the Seveso incident. A toxicokinetic model (Emond et al., 2006) was applied to translate the serum levels into a daily dose. For mixtures of PCDD/Fs and DL-PCBs, the use of the WHO_{2005}-TEFs is recommended.

The EFSA CONTAM Panel (2018) based its assessment on an epidemiological study by Mínguez-Alarcón et al. (2017) with Russian boys, in which serum levels of 17 PCDD/Fs and 12 DL-PCBs were analysed. The levels in blood, collected at the age of 8–9 years, showed an inverse association between TCDD, PCDD-TEQ, and PCDD/F-TEQ levels and sperm concentrations in samples obtained at 19 years. However, no such association was found for PCDF-TEQ, DL-PCB-TEQ and Total-TEQ levels (all based on WHO_{2005}-TEFs). However, in vitro studies on human cells showed much lower potencies for DL-PCBs than rat cells, in particular for PCB-126, which contributed on average 50% to the Sum-TEQ level in the Russian children. Therefore, the CONTAM Panel decided to base the new TWI on the association with PCDD/F-TEQ levels and to recommend a re-evaluation of the WHO_{2005}-TEF values to take into account new in vivo and in vitro data (see **Section 1.3.1**). For deriving the TWI, a toxicokinetic model (the concentration- and age-dependent model, CADM) developed by Carrier et al. (1995) and improved by Aylward et al. (2005) and Ruiz et al. (2014), was applied. Because rat studies and two Seveso cohorts suggested that early life is the critical period of exposure (Mocarelli et al., 2008, 2011), serum levels in the boys at 9 years of age were back-calculated, using the CADM model, into the exposure occurring at young age via human milk. The first of the two Seveso studies showed significantly lower sperm concentrations in men that were between 1 and 9 years old at the time of the incident, as compared to those aged 10–19 years (Mocarelli et al., 2008). The second study also showed lower sperm concentrations in men born to exposed mothers as compared to non-exposed, but only among those who were breastfed and not formula-fed (Mocarelli et al., 2011).

Using the CADM model, the CONTAM Panel translated the NOAEL serum concentration for 9-year-old boys into a human milk concentration and a chronic dietary intake that would not result in a serum concentration exceeding this NOAEL, even if boys were breastfed for 12 months. This resulted in a human milk concentration of 5.9 ng TEQ/kg fat at the maternal age of 35 years and an estimated daily intake over time of 0.25 pg. TEQ/kg bw. Multiplying this by 7 and rounding resulted in a TWI of 2 pg. WHO_{2005}-TEQ/kg bw per week. The estimated exposure of much of the EU population exceeded this TWI, thus raising a health concern. However, a major uncertainty in the exposure assessment was the application of the WHO_{2005}-TEFs, and in particular the TEF for PCB-126 which contributed on average more than 50% to the estimated exposure (EFSA CONTAM Panel, 2018).

1.3.2 | Toxic equivalency factors (TEFs)

The relevant 17 PCDD/Fs and 12 DL-PCBs are known to persist in the environment, to accumulate in the human body and to cause similar effects by activating the aryl hydrocarbon receptor (AHR[4]) pathway. However, the congeners have very different potencies. To deal with this difference in potencies, so-called Toxic Equivalency Factors (TEFs) have been introduced, meaning that levels of each congener are multiplied by the assigned TEF and subsequently summed up to a PCDD/F-TEQ, DL-PCB-TEQ or sum-TEQ level. TEFs are based on the available scientific evidence and regularly reviewed, in 1998 and 2005 under the auspices of the World Health Organisation (WHO) (Van den Berg et al., 1998, 2006; EFSA CONTAM Panel, 2018).

Following the recommendation by the EFSA CONTAM Panel (2018) to review the TEFs, WHO organised an expert meeting in 2022. In preparation for this meeting, the database of 2005 with suitable studies was updated (Fitch et al., 2024). This database contained studies showing effects associated with activation of the AHR pathway, a critical step in adverse effects caused by PCDD/Fs and dioxin-like compounds. Suitable studies should compare effects of a congener with those caused by 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), or PCB-126, the most toxic PCDD/F and DL-PCB congeners, respectively. Other requirements for inclusion in the database were a structural relationship with PCDD/Fs, binding to the AHR, eliciting AHR-mediated biochemical and toxic responses, and persistency and accumulation in the food chain. The database contained both in vivo and in vitro data, covering toxicological and biochemical effects. However, in vivo studies and toxicological effects were given a higher weight (Wikoff et al., 2023). As a result, the outcome was dominated by studies on toxicological effects in rodents. Potential species differences in AHR activation and resulting effects were not taken into account. This was because it was argued that "*in the absence of human in vivo evidence to support or reject this for humans, caution is advised for the use of in vitro human data on CYP induction alone to derive a TEF*" (DeVito et al., 2024).

For each congener, the available data were standardised and a best-estimate TEF (BE-TEF) was derived using a Bayesian approach to model the dose–response curves (Fitch et al., 2024). At the WHO meeting, experts discussed the outcome of the modelling and set the new TEFs (DeVito et al., 2024). For PCDD/Fs and non-*ortho* DL-PCBs, the BE-TEFs were directly translated into the TEF, in contrast to the approach in 2005 when expert judgement was applied and TEFs were established on a half-log scale (1, 0.3, 0.1 etc.) (van den Berg et al., 2006). Because of their very low potency, in particular in human cell models, it was discussed if the mono-*ortho* DL-PCBs should be kept in the TEF scheme. However, it was concluded that '*the limited data used in the Bayesian analysis did not convincingly show a deviation from the 2005 WHO-TEFs*', and it was decided to keep the 0.00003 values derived in 2005. Table 1 shows the WHO_{2022}-TEFs in comparison with those of 2005. For several congeners, the WHO-TEF-values decreased, for some they increased. Overall, the impact of the new WHO_{2022}-TEFs on the total TEQ levels depends on the congener profile. However, it was shown that applying the WHO_{2022}-TEFs on some specific data from the literature resulted in decreased total TEQ levels in food and human milk.

TABLE 1 WHO_{2022}-TEFs in comparison to the WHO_{2005}-TEFs.

PCDD/fs	WHO_{2005}-TEFs	WHO_{2022}-TEFs
2,3,7,8-TCDF	0.1	0.07
1,2,3,7,8-PeCDF	0.03	0.01
2,3,4,7,8-PeCDF	0.3	0.1
1,2,3,4,7,8-HxCDF	0.1	0.3
1,2,3,6,7,8-HxCDF	0.1	0.09
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.2
1,2,3,4,6,7,8-HpCDF	0.01	0.02
1,2,3,4,7,8,9-HpCDF	0.01	0.1
OCDF	0.0003	0.002

[4]The abbreviation AHR will be used in the Opinion as a general term to refer to the Aryl Hydrocarbon Receptor, not distinguishing between genes and proteins, and regardless of human or animal origin.

TABLE 1 (Continued)

PCDD/fs	WHO_{2005}-TEFs	WHO_{2022}-TEFs
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	0.4
1,2,3,4,7,8-HxCDD	0.1	0.09
1,2,3,6,7,8-HxCDD	0.1	0.07
1,2,3,7,8,9-HxCDD	0.1	0.05
1,2,3,4,6,7,8-HpCDD	0.01	0.05
OCDD	0.0003	0.001
Non-*ortho* PCBs	**WHO_{2005}-TEFs**	**WHO_{2022}-TEFs**
PCB-81	0.0003	0.006
PCB-77	0.0001	0.0003
PCB-126	0.1	0.05
PCB-169	0.03	0.005
Mono-*ortho* PCBs	**WHO_{2005}-TEFs**	**WHO_{2022}-TEFs**
PCB-123	0.00003	0.00003
PCB-118	0.00003	0.00003
PCB-114	0.00003	0.00003
PCB-105	0.00003	0.00003
PCB-167	0.00003	0.00003
PCB-156	0.00003	0.00003
PCB-157	0.00003	0.00003
PCB-189	0.00003	0.00003

1.3.3 | Legislation

In this Opinion, references to the European legislation (Regulations, Directives, Recommendations, Decisions), should be understood as relating to the most recent amendment at the time of publication of this Opinion, unless otherwise stated.

Food

In order to protect public health, Article 2 of Council Regulation (EEC) No 315/939 of 8 February 1993, laying down Community procedures for contaminants in food, stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. Subsequently, a number of maximum levels (MLs) for PCDD/Fs and for the sum of PCDD/Fs and DL-PCBs for various foodstuffs, mainly of animal origin, are laid down in Commission Regulation (EU) 2023/915 of 25 April 2023 that repeals Commission Regulation (EC) No 1881/2006 setting MLs for certain contaminants in foodstuffs. The MLs for PCDD/Fs and DL-PCBs are not toxicologically based but were derived from the frequency distribution of the respective food classes following the principle 'strict but feasible' ("ALARA - As Low As Reasonably Achievable"). In general, the MLs were set around the 90–95th percentile of the respective frequency distribution.

The MLs for PCDD/Fs and the sum of PCDD/Fs and DL-PCBs in food are, at the time of publication of this Opinion, expressed as pg. WHO_{2005}-TEQ/g using the WHO-toxic equivalency factors (WHO_{2005}-TEFs) for human risk assessment based on the conclusions of the WHO expert meeting in 2005 (van den Berg et al., 2006).

All MLs for PCDD/Fs and PCBs are set as upper bound (UB) concentrations. These are calculated on the assumption that all values of the different congeners below the limit of quantification (LOQ) are equal to the numerical value of the LOQ. Except for certain fish and fish products, liver of fish and terrestrial animals, and foods for infants and young children, all MLs are given on a fat basis.

The respective foodstuffs have to comply with the MLs for PCDD/Fs and for the sum of PCDD/Fs and DL-PCBs. According to Article 2 of Regulation (EU) No 2023/915, foodstuffs not complying with the MLs shall not be used as food ingredients and foodstuffs complying with the MLs shall not be mixed with foodstuffs that exceed the MLs.

Article 9 of the Regulation stipulates that by way of derogation, Finland, Sweden and Latvia may authorise the placing on their market of wild-caught salmon and products thereof originating in the Baltic region and intended for consumption in their territory with levels of PCDD/Fs and/or DL-PCBs higher than those set out in the Regulation. In addition, by way of derogation, Finland and Sweden may also authorise the placing on their market of wild-caught herring larger than 17 cm, wild-caught char, wild-caught river lamprey and wild-caught trout and products thereof originating in the Baltic region and intended for consumption in their territory with levels of PCDD/Fs and/or DL-PCBs exceeding the respective MLs. These derogations apply only provided that a system is in place to ensure that consumers are fully informed of the dietary recommendations with regard to the restrictions on the consumption of the respective fish species by identified vulnerable sections of the population in order to avoid potential health risks. Because certain fish and fishery products from the Baltic region regularly exceed the respective MLs, Commission Recommendation (EU) 2016/688 lays down provisions for the monitoring and management of the presence of PCDD/Fs and PCBs in fish and fishery products from the Baltic region. For specific Member States around the Baltic, the Recommendation sets minimum numbers of samples of several fish species to be taken and analysed in the years 2016–2018. It also includes inter alia information on how to measure the size of the fish, provisions on different geographical region (ICES zones) where the fish is caught and risk management measures recommended to be taken by the competent authorities to ensure that fish from the Baltic region placed on the market in the EU complies with the MLs established in Regulation (EU) No 2023/915. Before 2016, Finland and Sweden had a derogation since 2002 and were obliged to set up specific monitoring programme on the presence of PCDD/Fs and DL-PCBs in fish and to report yearly to the European Commission the results thereof.

The MLs for PCDD/Fs and the sum of PCDD/Fs and DL-PCBs expressed on a fat weight basis are not applicable for foods containing < 2% fat. These have to be calculated on a product basis, assuming a fat content of 2%. For details, see remarks under point 4.1.1 of Regulation (EU) No 2023/915.

In addition to MLs, the European Commission has set action levels (ALs) for both PCDD/Fs and DL-PCBs in various foods as an early warning tool, as outlined in Commission Recommendation 2013/711/EU. ALs, set at a lower level than the ML, generally at 2/3 of the corresponding ML, are meant as a tool for competent authorities and operators to highlight cases where it is appropriate to identify a source of contamination and implement measures for its reduction or elimination. Because their sources are generally different, separate ALs for PCDD/Fs and DL-PCBs were established. In cases where levels of PCDD/Fs and/or DL-PCBs in excess of the ALs are determined, the food can still be marketed as long as the ML is not exceeded. However, it is recommended that Member States, in co-operation with operators, initiate investigations to identify the source of contamination, and take measures to reduce or eliminate it to reduce the levels of contamination over time.

Feed

The maximum content (also termed MLs) for PCDD/Fs and the sum of PCDD/Fs and DL-PCBs are laid down in Annex I, Section V of Directive 2002/32/EC on undesirable substances in animal feed. These legal limits were also based on existing levels according to the principle 'strict but feasible' ("ALARA": "As Low As Reasonably Achievable"). As for food, all legal limits in feed are UB values expressed as TEQ levels relative to a feed with a moisture content of 12% using, at the time of publication of this Opinion, the WHO-toxic equivalency factors (WHO_{2005}-TEFs).

Article 5 of Directive 2002/32/EC prescribes that products intended for animal feed containing levels of an undesirable substance, such as PCDD/Fs and DL-PCBs, that exceed the respective maximum content may not be mixed for dilution purposes with the same, or other, products intended for animal feed.

In addition to maximum content, also action thresholds (as early warning tools to trigger investigations by Member States) are set separately for PCDD/Fs and DL-PCBs, respectively, in Annex II of Directive 2002/32/EC. The action thresholds also refer to products relative to a moisture content of 12%.

2 | DATA AND METHODOLOGIES

The current update of the 2018 EFSA risk assessments on PCDD/Fs and DL-PCBs in food and feed on the basis of the new WHO_{2022}-TEF values, was developed applying a structured methodological approach, which involved developing a priori the protocol or strategy for the risk assessment and performing each step of the risk assessment in line with the strategy and documenting the process. The protocol in Annex A of this Opinion contains the method that was used for all the steps of the risk assessment process, including an evaluation of the inherent uncertainties in the risk assessment, and any subsequent refinements/changes made.

The CONTAM Panel used its previous risk assessment on PCDD/Fs and DL-PCBs in food and feed (EFSA CONTAM Panel, 2018) as a starting point for drafting the current Opinion.

The draft Scientific Opinion underwent a public consultation from 27 November 2025 to 26 January 2026. The comments received were taken into account when finalising the Scientific Opinion and are presented and addressed in Annex I.

2.1 | Supporting information for the assessment

Information on previous assessments and legislation was gathered from the 2018 EFSA Opinion on PCDD/Fs and DL-PCBs in food and feed (EFSA CONTAM Panel, 2018), assessments by international bodies (by checking the original websites of the relevant organisations), and from current EU legislation. The information was summarised in a narrative way based on expert knowledge and judgement.

2.2 | Hazard identification and characterisation

Information relevant for the sections under hazard identification and characterisation was identified as described in Annex A (Section 3 therein). Literature searches were performed to identify studies published since the 2018 Opinion (from 2017 onwards) as reported in Appendix A. The selection for relevance (inclusion/exclusion criteria) of the scientific papers retrieved are detailed in Annex A (Section 3.3 therein). Risk of bias was performed as detailed in Annex A using a tailored OHAT Risk of Bias Tool (as included in the NTP-OHAT Approach for Systematic Review; Rooney et al., 2014).

Benchmark dose (BMD) analyses were carried out according to the most recent EFSA Scientific Committee Guidance on BMD modelling at the time of this assessment (EFSA Scientific Committee, 2022) and the Bayesian BMD Modelling web-app (https://zenodo.org/record/7334435#.Y5osYXbMLD4) available at the EFSA R4EU platform (https://r4eu.efsa.europa.eu/). This tool, developed and maintained by EFSA, is freely available and extensively documented (Hasselt University, 2022; Verlinden et al., 2024).

The PBK CADM model analysis was carried out using the Berkeley Madonna software program (version 10.1.3, Marcoline et al., 2022).

2.3 | Occurrence data submitted to EFSA

2.3.1 | Data collection and validation

The collection of occurrence data and the requirements of the data submission were performed as described in Annex A (Section 4.1 therein). Data on PCDD/Fs and DL-PCBs in food sampled from 2013 to 2023 were used for the present assessment.

2.3.2 | Data analysis

The occurrence data submitted to EFSA was handled, cleaned and validated as described in Annex A (Section 4.1 therein).

Left-censored data were treated using the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009, updated in 2020). This is the same method as indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010). The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). The LB is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the limit of detection (< LOD) or limit of quantification (< LOQ). The UB is obtained by assigning the numerical value of LOD to values reported as < LOD and LOQ to values reported as < LOQ (maximum possible value).

Means for specific food categories calculated on less than six analytical results, were not used in the dietary exposure assessment as a mean calculated on less than six samples is not considered reliable. However, these analytical results were included in the calculation of averages for categories at higher levels of the FoodEx2 classification where this would allow to calculate a mean concentration on more than six samples. An exception to this rule was made to calculate PCDD/F and DL-PCB concentrations in the food category 'Coffee, cocoa, tea and infusions' to avoid losing categories such as 'Coffee beverages' and 'Tea beverages' for which only 3 and 1 samples were available, respectively.

Specific food subcategories for which there were no occurrence data available were attributed with the concentration of the parent FoodEx2 category, e.g. the mean concentration for 'Leafy Brassica' was attributed to 'Chinese cabbages', subcategory for which there were no specific occurrence data.

Composite foods documented to contain ingredients belonging to the food categories for which data were available (e.g. foods belonging to the 'Fine bakery wares' category) were also included in the dietary exposure assessment of PCDD/Fs and DL-PCBs, with LB and UB mean concentration calculated using the available LB and UB mean concentration of the main ingredient (e.g. fine bakery ware, bread and pasta concentration derived from the concentration in wheat flour).

Dilution factors suggested in EFSA guidelines (EFSA, 2018) were used to calculate LB and UB PCDD/F and DL-PCB concentrations for ready-to-eat foods or ready-to-drink beverages from the available concentrations in the dry ingredients (e.g. for infant and follow on formulas).

For those food categories where maximum levels for PCDD/Fs and DL-PCBs in the legislation are expressed as fat weight (e.g. meat and meat products except liver and derived products thereof, eggs and egg products, raw milk and dairy products) (Commission Regulation (EU) 2023/915), the levels used in the exposure assessment were based on fat weight. If data for those food categories were submitted as whole weight, concentrations were converted to fat weight using the provided sample fat percentage.

For these same categories the amount eaten according to the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was converted to fat amount using the fat percentage reported in the Comprehensive Database table describing the food[5] or the information available in the FoodEx2 classification of the food (e.g. implicit or explicit food descriptors (facets) referring to fat content (see **Section 2.5.1**). In addition, for milk the fat percentages

[5]Data describing the food reported in the Comprehensive Database are submitted by MSs at the time of submission of the dietary survey and are stored in EFSA DWH.

provided by the Regulation (EU) No 1308/2013 were applied when no other fat information was available and the FoodEx2 code specified the relevant category (whole (3.5% fat), semi-skimmed (1.8% fat) and skimmed (0.5% fat) milk).

If no fat information was available in the Consumption database for a food linked to a specific eating event, a value was computed by selecting a random fat percentage from the available values in other eating events for the same FoodEx2 code, as was done in the 2018 Opinion (EFSA CONTAM Panel, 2018).

To reduce the uncertainty linked to the difference between LB and UB estimates, samples were checked for compliance with analytical performance criteria based on Commission Regulation (EU) No 2017/644 and taking into account the existing Maximum Levels (MLs) as laid down in Commission Regulation (EU) No 2023/915, and Action Levels (ALs) as given in Commission Recommendation 2013/711, amended by 2014/663/EU. These were expressed in pg. WHO_{2005}-TEQ/g. Following these criteria, samples with a sum of LOQs higher than one fifth of the corresponding MLs for the analysed PCDD/Fs or higher than one third of the corresponding ALs for the analysed DL-PCBs were excluded from the assessment. For food categories for which an ML is not set in the legislation for the sum of PCDD/Fs, ALs were used instead (e.g. fruits and vegetables) again checking that the sum of LOQs was not higher than one third of the corresponding AL.

To be noted that according to the legislation the ML level expressed on a fat basis is not applicable for foods containing < 2% fat. For these foods, the ML is applicable on a product basis (whole weight basis), calculated from the ML established on a fat basis multiplied by 0.02. If the total LOQ (converted to whole weight based on the reported fat percentage, when necessary) was higher than one fifth of the ML × 0.02, the sample was excluded. In the case of criteria based on ALs for samples with less than 2% fat, this criterion was not applied, as indicated in Commission Recommendation 2014/663/EU.

2.4 | Food and feed consumption data

2.4.1 | Food consumption data

Food consumption data from the EFSA Comprehensive Database were used for the dietary exposure assessment. This database contains national data on food consumption at the individual consumer level, which are the most complete and detailed data currently available in the EU.

The food consumption data gathered in the Comprehensive Database were collected using repeated 24-h or 48-h dietary recalls or dietary records covering 3–9 days per individual. Owing to the differences in the methods used for data collection, direct country-to-country comparisons of the exposure estimates should be avoided.

Details of how the Comprehensive Database is used to assess the dietary exposure to food chemicals are published in a 2011 EFSA Guidance (EFSA, 2011b). The latest version of the Comprehensive Database was published in October 2024 and contains results from 58 dietary surveys carried out in 24 Member States covering 98,014 individuals. Seven surveys provide information on 'Pregnant women' and on 'Lactating women' and three surveys provided information on Vegetarians.

A chronic dietary exposure assessment was deemed relevant for PCDD/Fs and DL-PCBs in the context of the Terms of Reference and the available evidence for critical windows of exposure during development (see **Section 2.6**).

For such assessments, surveys in which food consumption data were collected over only 1 day are not considered appropriate. Exclusion of these surveys resulted in a total of 55 dietary surveys carried out in 23 Member States covering 91,035 individuals available to be used in the chronic dietary exposure assessment. Table 2 provides an overview of the population groups and countries included in the dietary exposure assessment.

TABLE 2 Population groups and countries included in the chronic dietary exposure assessment.

Population group	Age range	Countries with food consumption surveys covering more than 1 day
Infants	> 12 weeks to < 12 months	Bulgaria, Croatia, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Poland, Portugal, Slovenia, Spain
Toddlers	≥ 12 months to < 36 months	Belgium, Bulgaria, Croatia, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Poland, Portugal, Slovenia, Spain
Other children	≥ 36 months to < 10 years	Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Poland, Portugal, Spain, Sweden
Adolescents	≥ 10 years to < 18 years	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Poland, Portugal, Romania, Slovenia, Spain, Sweden
Adults	≥ 18 years to < 65 years	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Poland, Portugal, Romania, Slovenia, Spain, Sweden
Elderly	≥ 65 years to < 75 years	Austria, Belgium, Croatia, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Poland, Portugal, Romania, Slovenia, Spain, Sweden
Very elderly	≥ 75 years	Austria, Belgium, Croatia, Denmark, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Poland, Portugal, Romania, Sweden
Pregnant women		Austria, Cyprus, Latvia, Poland, Portugal, Romania, Spain

According to the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants under 16 weeks of age, the exposure assessment for these infants should be carried out separately from that for older infants, following the procedure described in the guidance (EFSA Scientific Committee, 2017). Based on this guidance, infants under 16 weeks of age should be excluded from the dietary exposure estimation of the infants age group. However, due to uncertainty in the reported individual ages of infants in the Comprehensive Database, the cut-off age was based on a validated existing age group in this database corresponding to 12 weeks of age. Thus, food consumption data of infants between 12 and 16 weeks of age were also included in the exposure assessment. As the number of children within this age range in the database is limited, it is not expected that this will have affected the exposure estimate for infants of 16 weeks up to 12 months of age.

Annex B (Table B.1) provides details on the dietary surveys included in the dietary exposure assessments.

2.4.2 | Feed consumption data

The feeds consumed (and the feed intake) by the most relevant food-producing animals can only be based on estimates, since no comprehensive feed consumption database exists covering the EU. The animal species and categories considered in this Opinion are described in EFSA's Statement on animal dietary exposure in the risk assessment of contaminants in feed (EFSA FEEDAP Panel, 2024), and include: piglets, pigs for fattening, lactating sows, chickens for fattening, laying hens, turkeys for fattening, calves (receiving milk replacer), cattle for fattening, dairy cows, lambs for fattening, fish (salmon or trout), rabbits and horses. The default values for average feed intakes and body weights used to calculate food-producing animals' exposure to PCDD/Fs and DL-PCBs are described in Appendix A of the same EFSA Statement.

2.5 | Food and feed classification

2.5.1 | Food classification

Consumption and occurrence data were codified according to the FoodEx2 classification system (EFSA, 2011a, 2011b). Since 2018, all consumption records in the Comprehensive Database as well as all occurrence data submitted to EFSA have been codified according to the FoodEx2 classification system (EFSA, 2015). The FoodEx2 classification system consists of a large number of standardised basic food items aggregated into broader food categories in a hierarchical parent–child relationship. Additional descriptors, called facets, are used to provide additional information about the codified foods (e.g. information on food processing and packaging material).

2.5.2 | Feed classification

Feed samples were classified according to the Catalogue of feed materials as described in Commission Regulation (EU) No 2022/1104[6] amending Regulation (EU) No 68/2013.

2.6 | Dietary exposure assessment

2.6.1 | Human dietary exposure assessment

For calculating the chronic dietary exposure to PCDD/Fs and DL-PCBs, food consumption and body weight data at the individual level were retrieved from the Comprehensive Database. Occurrence data and consumption data were linked at the relevant FoodEx2 level.

Chronic dietary exposures were calculated by combining mean PCDD/F and DL-PCB occurrence values for food samples collected in different countries (pooled European occurrence data) with the average daily consumption for each food at the individual level in each dietary survey and age class. Consequently, individual average exposures per day and body weight were obtained for all individuals.

The equation that describes the calculation of the dietary exposure can be found in Annex A (Section 4.1 therein). The distributions of individual exposures were then used to calculate the mean and high (95th percentile) exposure per survey and per age class. These exposure estimates were obtained using the LB and UB mean concentration of PCDD/Fs and DL-PCBs.

The number of surveys in which food categories contributed more than 10% to the total dietary exposure to PCDD/Fs and DL-PCBs was used to rank main contributors to the overall exposure and for each age group. The 10% threshold was chosen by expert judgement as helpful to identify main contributors. Contribution of different food categories to the total chronic dietary exposure was calculated over LB exposure estimates to avoid that the high contribution of certain food

[6]Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials. OJ L 177, 4.7.2022, p. 4–74.

groups could be artificially driven by the treatment of the left-censored data. The contribution of different food categories to the UB dietary exposure estimates was also calculated to assess which food categories mostly contributed to the uncertainty linked to left-censored data.

All analyses were run using the SAS Statistical Software (SAS enterprise guide 8.3 Update 5).

2.6.2 | Animal dietary exposure assessment

The exposure of food-producing animals via feed was estimated using **model diets**[7] composed of different feed materials for which occurrence data on PCDD/Fs and DL-PCBs were available. Additionally, if occurrence data in **compound feed**[8] were available, the exposure was also assessed based on the consumption of the compound feeds. The occurrence data on feed materials and compound feeds that were used to calculate the exposure of food-producing animals are reported in **Section 3.2.3**. The outcomes of both scenarios, the compound feed and the model diets, were compared.

The model diets used for each animal species and category are detailed in Appendix C of the EFSA Statement on animal dietary exposure in the risk assessment of contaminants in feed (EFSA FEEDAP Panel, 2024). To allow a certain flexibility in constructing model diets, feed materials could be exchanged within each group, provided the nutritional needs of the various animal species are met in relation to occurrence data availability and levels of contamination. Groups of feed materials are included in Appendix B of the Statement.

In the estimations of the dietary exposure of the food-producing animals, two scenarios were considered:

(i) a mean occurrence scenario, in which the mean lower bound (LB) and upper bound (UB) values for each feeding stuff were used; and
(ii) a high-occurrence scenario, in which the highest reliable percentile (HRP) LB and UB values were used, up to the 95th percentile (P95). To estimate dietary exposure to PCDD/Fs and DL-PCBs for different animal species and categories, the calculated mean and high concentrations of dietary PCDD/Fs and DL-PCBs were combined with the estimated feed intake and body weight (see Appendix A of EFSA's Statement).

Soil and clay ingestion was not taken into account in the exposure assessment of food-producing animals.

2.7 | Risk characterisation

The general principles of the risk characterisation for chemicals in food as described by the WHO/IPCS (2009), updated in 2020) were applied as well as the different EFSA guidance documents relevant to this step of the risk assessment (see Annex A).

3 | ASSESSMENT

3.1 | Hazard identification and characterisation

3.1.1 | Toxicokinetics

3.1.1.1 | *Toxicokinetics in experimental animals and humans*

An overview of the available information regarding the toxicokinetics (absorption, distribution, metabolism and excretion), including half-lives of PCDD/Fs and DL-PCBs in both experimental animals and humans was given in the 2018 Opinion (EFSA CONTAM Panel, 2018).

Briefly, in rodents, PCDD/Fs and DL-PCBs are well absorbed and distributed to various tissues and also transferred to the fetus. They accumulate primarily in adipose tissue and in the liver, with a ratio liver to adipose tissue that increases with the applied dose, due to induction and binding to CYP1A2 in the liver, a process called sequestration. Half-lives are relatively short when compared to humans, in part due to the higher rate of metabolism. Faecal excretion is the more important route of elimination. For details, see EFSA CONTAM Panel (2018, Section 3.1.1.2 therein).

In humans, PCDD/Fs and DL-PCBs are well absorbed and subsequently stored primarily in the liver and adipose tissue. Sequestration in the liver has been described at high exposure, but it seems less relevant at current background exposure. They can be transferred to the developing fetus, but also via human milk. The TEQ levels in blood lipids and also in human milk are similar to those in adipose tissue (Nakamura et al., 2008; Patterson et al., 1988; Schecter et al., 1991, 1992). Compared to laboratory animals, most PCDD/Fs and DL-PCBs show longer half-lives in humans. Half-lives vary between

[7]Based on groups of feed materials and composed as in the EFSA FEEDAP Panel (2024) Statement.

[8]As defined in Regulation (EC) No 767/2009, i.e. a mixture of at least two feed materials, whether or not containing feed additives, for animal feeding in the form of complete or complementary feed. In the context of the current Opinion, the term 'compound feed exposure scenario' refers to either the combination of complementary compound feed and forages for ruminants and horses, or the use of complete compound feed for all other animal species.

different congeners and show interindividual differences depending on the levels, age, BMI and sex. For details, see EFSA CONTAM Panel (2018, Section 3.1.1.3 therein).

Table 3 shows the half-lives for congeners that contribute most to the dietary exposure (see **Section 3.3.1.3**) and human milk levels (see **Section 3.1.1.2**). Aylward et al. (2013) calculated half-lives for a number of PCDDs based on blood levels of 56 former chlorophenol workers. Median values are shown, as well as the P25 and P75, showing the variations in the half-lives between persons. The values indicate that half-lives are very similar for most PCDDs but higher for PeCDD and 1,2,3,4,6,7,8-HxCDD, two relatively important congeners in terms of contribution to the TEQ levels. These half-lives were comparable to those obtained for PCDDs by Flesch-Janys et al. (1996) and Rohde et al. (1999). Concerning PCDFs, it was reported that the half-life of 2,3,4,7,8-PeCDF is rather long in some of the Yusho patients (Matsumoto et al., 2009). Flesch-Janys et al. (1996) and Rohde et al. (1999) showed for PCDFs similar values as for PCDDs, with the longest half-life for 2,3,4,7,8-PeCDF (19.6 and 13.9 years). A review by Milbrath et al. (2009) summarises these half-lives and confirms that those for TCDF and 1,2,3,7,8-PeCDF are very short compared to other congeners. Based on a study by Ogura (2004), the half-life for PCB-126 was shorter than for TCDD (2.7 vs. 6.7 years). PCB-77 and PCB-81 showed even shorter half-lives, whereas those for PCB-169 and several other mono-*ortho* PCBs were much longer.

TABLE 3 Reported elimination half-lives of congeners contributing most to the dietary exposure of the European population (see **Section 3.3.1.3**) and human milk concentrations (WHO/UNEP and CVUA/MEL data sets, see **Section 3.1.1.2**).

Congener	Contribution (%)		Half-life (years)			
	Dietary exposure	Human Milk: UNEP data set/ CVUA data set	Aylward et al. (2013)[a]	Flesch-Janys et al. (1996)[b]	Rohde et al. (1999)[c]	Ogura (2004)[d]
TCDD	5	13/11	6.5 (5.0–8.2)	7.2	8.7	6.3–11
PeCDD	5	14/14	10.7 (8.2–15.9)	15.7	13.9	6.6–11
1,2,3,6,7,8-HxCDD	2	8/5	9.0 (7.0–11.3)	13.1	11.6	24–46
HpCDD	10	8/4	6.7 (5.2–9.0)	3.7	4.3	1.4–5.8
TCDF	5	0	–	–	–	0.2–0.9
2,3,4,7,8-PeCDF	5	9/11	–	19.6	13.9	4.9–9.4
1,2,3,4,7,8-HxCDF	9	8/10	–	6.2	–	3.7–10
PCB-126	38	23/26	–	–	–	1.6–4.5
PCB-118	7	3/3	–	–	–	3.8–6.3

[a]Median half-lives and in brackets the P25 and P75 values ($n = 56$).
[b]$n = 5$ up to 48 subjects.
[c]$n = 6$ subjects.
[d]Different populations as reference, $n = 10$ up to 415 subjects.

For the current update, new studies (i.e. published after the last inclusion date of the EFSA CONTAM Panel Opinion, 2018) to inform the toxicokinetics of PCDD/Fs and DL-PCBs in humans and in experimental animals were retrieved and selected as described in Annex A. The CONTAM Panel noted that studies providing insight into the following aspects of the toxicokinetics of PCDD/Fs and DL-PCBs were relevant:

- Any new study that would inform the half-lives of the different congeners, the ratio adipose tissue-blood lipids, –milk, and/or -liver, and about placental transfer, in humans.
- Any new studies that would inform differences in tissue distribution between rats and mice depending on the route of administration.
- Any study that would provide an improved physiologically based kinetic (PBK) model in humans compared to the CADM model used in the 2018 Opinion, e.g. informing whether congeners other than TCDD behave in the same way as TCDD.

One study was identified informing the effect of obesity on the half-life of TCDD in mice (Emond et al., 2018). In obese mice the half-life was markedly prolonged. Applying PBK modelling and classical pharmacokinetic analysis, the authors were not able to explain this effect. This effect could contribute to the differences in the half-lives between humans as observed by Aylward et al. (2013) and shown in Table 3. The study by Emond et al. (2018) in mice could not provide a basis for changing the toxicokinetic considerations in humans, and, thus, the conclusions from the 2018 Opinion were still considered valid.

3.1.1.2 | *Levels in humans*

The 2018 Opinion provided an overview of the analytical methods to analyse PCDD/Fs and DL-PCBs in human lipid-rich matrices, such as adipose tissue, human milk and blood. It also summarised the levels in human milk, blood and other biological tissues from European countries, including the results from the WHO coordinated (United Nations Environment

Programme, UNEP) human milk studies until 2015 (EFSA CONTAM Panel, 2018, Section 3.2.4 therein). It was concluded that the median PCDD/F and DL-PCB levels in human milk from European mothers were generally below 10 ng WHO_{2005}-TEQ/kg fat. In this programme, human milk samples were collected and pooled per country, for European countries from 2014/2015 showing levels of 2.4–5.7 ng WHO_{2005}-TEQ/kg fat for PCDD/Fs, and 4.8–9.6 ng WHO_{2005}-TEQ/kg fat for the sum of PCDD/Fs and DL-PCBs. This indicated a substantial decline since the first measured samples collected in the early 1980s. It was also noted that available results from the last decade were quite similar, and that future years of monitoring might be needed to conclude whether the decrease in concentrations are indeed levelling off.

Regarding levels in human matrices other than human milk, it was noted that the PCDD/F and DL-PCB levels (lipid adjusted) in different human matrices from background populations within European countries are generally similar. However, in particular higher chlorinated PCDD/Fs showed a substantial enrichment in the liver due to sequestration (EFSA CONTAM Panel, 2018, Section 3.2.4 therein).

Since then, studies on the levels in human milk and other matrices from European countries have been published in the literature. A temporal trend study on human milk from a region in Sweden, which included samples from first-time mothers in the period 1996 to 2017, indicated that the declining time trend of PCDD/Fs and DL-PCBs might be levelling off and that continued monitoring is needed (Gyllenhammar et al., 2021). All the studies identified in the literature search performed for this Opinion applied the former WHO_{2005}-TEFs.

Since 1987, WHO/UNEP has conducted several coordinated surveys on the occurrence of POPs, including PCDD/Fs and PCBs in human milk. These surveys were intended to examine levels within countries over time. Six rounds were performed between 1987 and 2015. The data corresponding to the last rounds and until 2014/2015 were reported in the 2018 Opinion (EFSA CONTAM Panel, 2018). Since then, an additional round was performed in 2019. Annex D (Table D.1) shows the results of the coordinated surveys between 2000 and 2019 calculated with WHO_{2005}-TEFs and recalculated using WHO_{2022}-TEFs (Schächtele A, 2024, data provided on the PCDD/F- and PCB-related results of the UNEP/GEF POPs Global Monitoring Plan project and the Stockholm Convention GMP Data Warehouse performed in European countries in the framework of WHO/UNEP human milk survey, see Documentation provided to EFSA). The ratio PCDD/F-TEQ to Total-TEQ is also shown. Due to the low LOQs of the method applied, there were virtually no differences between the LB and UB levels, and thus only the UB levels are given.

Figure 1 and Annex D (Table D2) show the ratio WHO_{2022}-TEF versus WHO_{2005}-TEFs. As shown by the slope coefficients, being much lower than 1, the TEQ-levels decrease with the new WHO_{2022}-TEFs. For the Total-TEQ levels, the new WHO_{2022}-TEFs resulted in a 38% decrease compared to the WHO_{2005}-TEFs. The corresponding decrease for PCDD/Fs and DL-PCBs was 30% and 49%, respectively.

Figure 2 shows the time trend based on these data. The number of pooled samples ($n=16$) from the period 2014–2019 is limited, but the graph indicates a clear decrease in the levels compared to those in the period 2001–2010. Average levels for the samples from the period 2014–2019 were 2.3, 1.4 and 3.7 ng WHO_{2022}-TEQ/kg fat for PCDD/Fs, DL-PCBs and Total-TEQ, respectively, as compared to 5.7, 3.0 and 8.7 ng WHO_{2022}-TEQ/kg fat in the period 2001–2010. For those countries that provided samples over the whole period, several but not all observed a corresponding decrease over time.

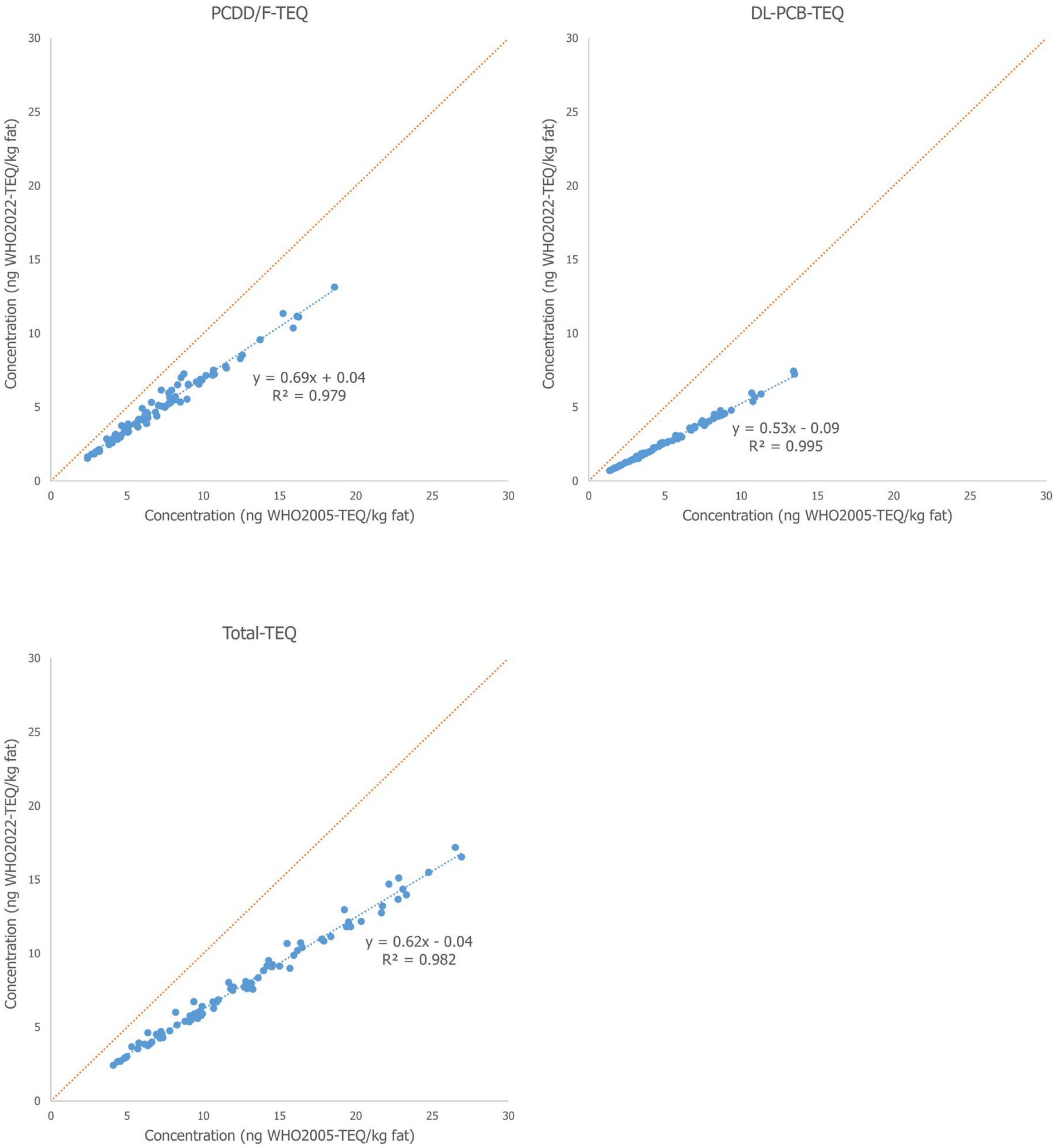

FIGURE 1 Levels of the 17 PCDD/Fs, 12 DL-PCBs and the sum of 29 PCDD/Fs and DL-PCBs in pooled samples of human milk from various European countries as analysed in the frame of WHO/UNEP coordinated studies, expressed as ng WHO_{2005}-TEQ/kg fat and ng WHO_{2022}-TEQ/kg fat (upper bound, UB) (based on Schächtele A, 2024, data provided on the PCDD/F- and PCB-related results of the UNEP/GEF POPs Global Monitoring Plan project and the Stockholm Convention GMP Data Warehouse performed in European countries in the framework of WHO/UNEP human milk survey, see Documentation provided to EFSA).

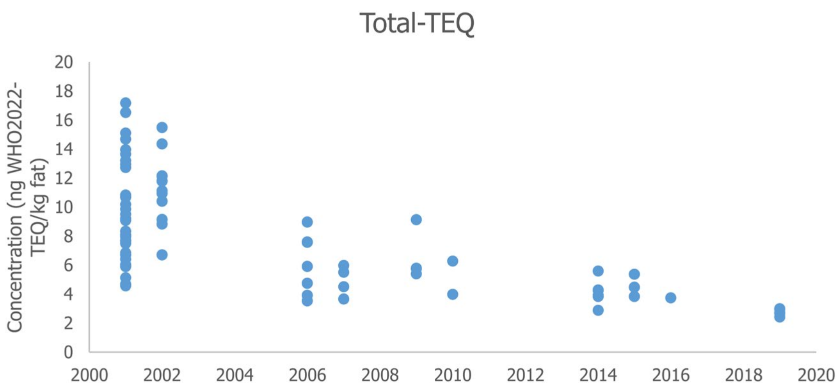

FIGURE 2 Time trend in the levels of Total-TEQ (17 PCDD/Fs plus 12 DL-PCBs) in pooled samples of human milk from various European countries as analysed within the framework of WHO/UNEP coordinated studies, expressed as ng WHO_{2022}-TEQ/kg fat (upper bound, UB) (based on Schächtele A, 2024, data provided on the PCDD/F- and PCB-related results of the UNEP/GEF POPs Global Monitoring Plan project and the Stockholm Convention GMP Data Warehouse performed in European countries in the framework of WHO/UNEP human milk survey, see Documentation provided to EFSA).

A second data set was evaluated provided by the Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe (CVUA-MEL) based on human milk samples from individual women in North-Rhine Westphalia in Germany from the period 2009–2024 (Bernsmann T, 2025, see Documentation provided to EFSA). The conversion to ng WHO_{2022}-TEQ/kg fat leads to a 35%, 50% and 41% reduction in the TEQ-levels for PCDDs, DL-PCBs and the sum, as compared to applying the WHO_{2005}-TEFs. This is in line with the data for the WHO/UNEP programme.

Figure 3 shows the total-TEQ levels in the German data over the whole period and demonstrates a clear decrease over time. The average Total WHO_{2022}-TEQ/kg fat during 2020–2024 was 2.3 ng (n = 43), as compared to 4.2 ng (n = 51) during 2009–2019.

Figure 4 shows the average relative contribution of the different congeners to the TEQ levels for all samples and all years in both datasets and the impact of the change in WHO-TEFs. The contribution of individual congeners is similar for the two data sets, showing the highest contribution by PCB-126, followed by PeCDD, 2,3,4,7,8-PeCDF and TCDD. The reason for the larger contribution of TCDD is the relative decrease in TEFs of several other congeners like PeCDD, 2,3,4,7,8-PeCDF, PCB-126 and PCB-169. However, PCB-126 is still the congener with the highest contribution. The contribution of HpCDD and 1,2,3,4,7,8-HxCDF strongly increased with the new TEFs.

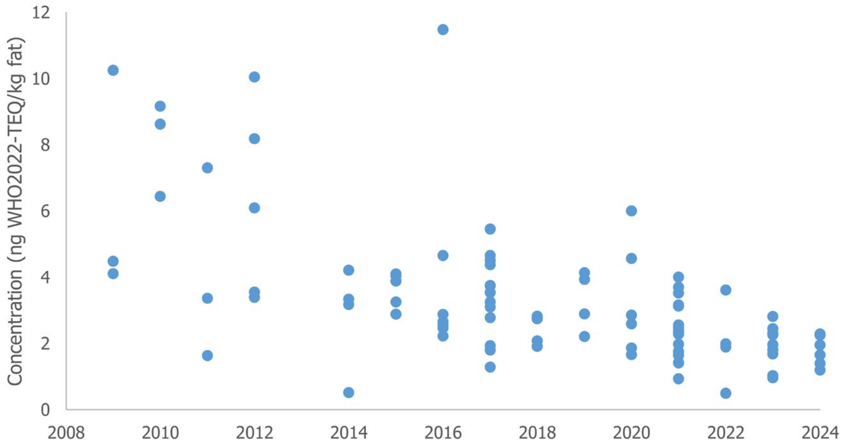

FIGURE 3 Time trend in the levels of the sum of 29 PCDD/Fs and DL-PCBs in individual samples of human milk from North-Rhine Westphalia (Germany), expressed as ng WHO_{2022}-TEQ/kg fat (lower bound, LB) (based on Bernsmann T, 2025, see Documentation provided to EFSA).

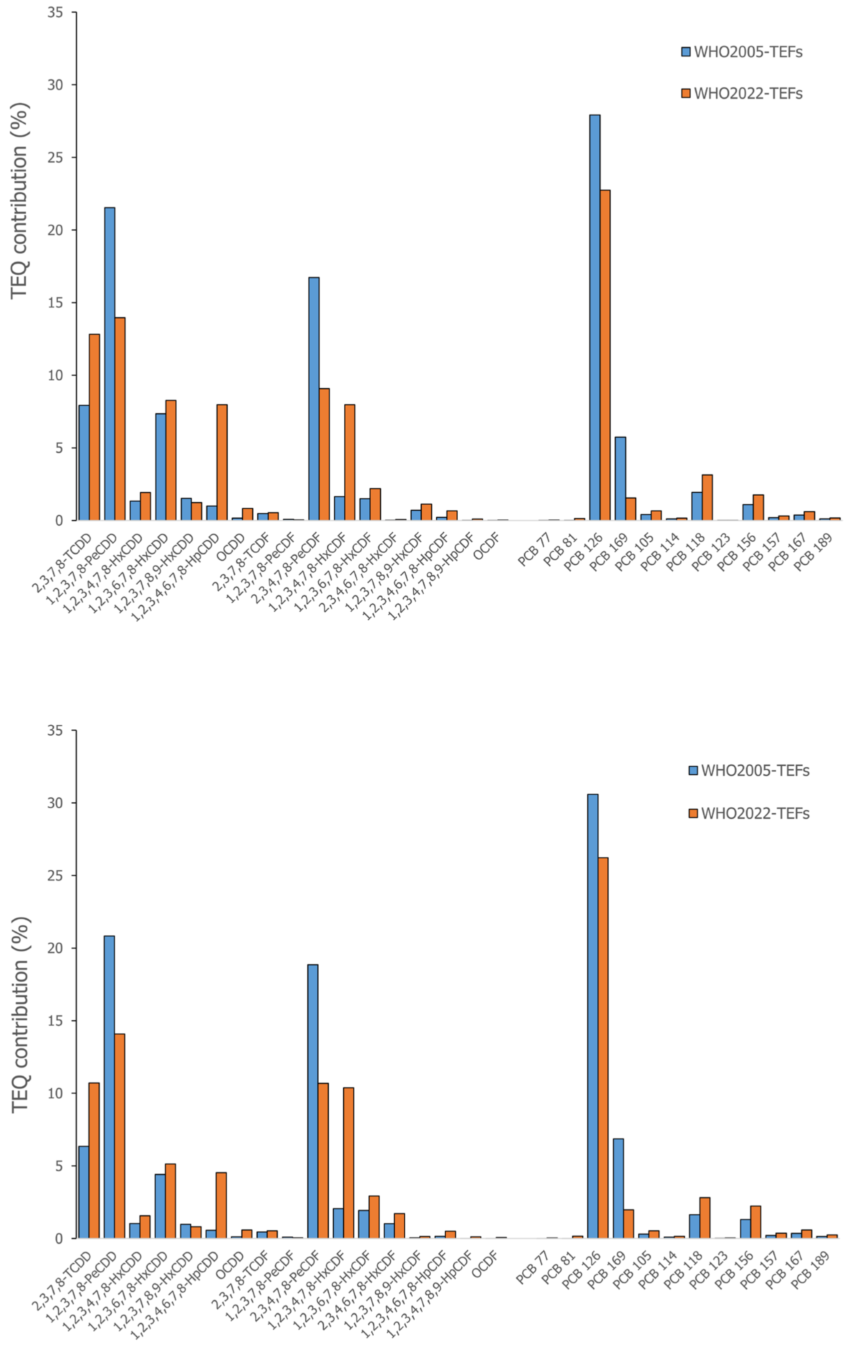

FIGURE 4 Average contribution of PCDD/F and DL-PCB congeners to the sum-TEQ level based on the WHO_{2005}- and WHO_{2022}-TEF values for all pooled or individual human milk samples in the WHO/UNEP (upper) and CVUA-MEL (lower) data sets, respectively (based on Schächtele A, 2024; data provided on the PCDD/F- and PCB-related results of the UNEP/GEF POPs Global Monitoring Plan project and the Stockholm Convention GMP Data Warehouse performed in European countries in the framework of WHO/UNEP human milk survey, and Bernsmann T, 2025, see Documentation provided to EFSA).

3.1.1.3 | *Toxicokinetic modelling*

The 2018 Opinion provided an overview of the available PBK models in humans and in rodents (EFSA CONTAM Panel, 2018, Section 3.1.1.5 therein). In that Opinion, the PBK CADM model developed by Carrier et al. (1995) and improved by Aylward et al. (2005) and Ruiz et al. (2014), was selected. This CADM model was developed for TCDD and estimates the levels in body fat (in all lipid-containing tissues/organs) and liver, taking into account the increased accumulation in the liver at higher exposure (due to sequestration). At low exposure liver sequestration is less relevant and the adipose tissue is the most important tissue for storage. Lipid-based levels in blood and human milk are similar to those in body fat. Biotransformation plays a very minor role in the elimination but a daily loss in the faeces via body lipids is included. A detailed description of this model is given in EFSA CONTAM Panel (2018).

The model was used to translate the serum levels in the boys at 9 years of age from the Russian Children's Study (Mínguez-Alarcón et al., 2017) into an exposure at young age via in utero exposure and through human milk. This resulted in a NOAEC for the body burden in the mothers and corresponding blood level around birth. Subsequently, the model was used to calculate the chronic intake that would result in such a body burden in the mother, and the exposure of the boys in utero and via breastfeeding. Breastfeeding was assumed to last for 12 months at an intake rate of 800 mL per day. This human milk volume represents a worst-case for 12 months of breast-feeding, as milk volumes decline when other food is introduced. Furthermore, a period of 12 months breastfeeding represents a 'worst-case' for most European countries that have in general shorter breast-feeding duration habits. However, such duration is common in some European countries (Theurich et al., 2019).

Since the publication of the 2018 Opinion, one study was identified reporting on a PBK model for mixtures (Liu et al., 2022). The model is based on the one developed by Emond et al. (2006, 2018) which was reviewed by the CONTAM Panel in the 2018 Opinion. This model raised some concern regarding the difference between the lipid-based levels in blood compared to adipose tissue and the rather low exposure resulting in a higher liver to adipose tissue ratio (based on sequestration). Therefore, the CADM model for TCDD was preferred. A further evaluation and improvement of the new models by Liu et al. (2022) would be needed for evaluation of health risk from mixtures, as indicated by the authors.

The CONTAM Panel decided to use the same CADM model as in the 2018 Opinion. The model was evaluated as described by WHO/IPCS (2010) (see Appendix B).

3.1.1.4 | *Transfer from feed to food of animal origin*

In this section, a brief summary of the studies reported in the 2018 Opinion is given, plus studies published since then, retrieved and selected for relevance as described in Annex A.

The same terminology as in the 2018 Opinion to describe the kinetics of PCDD/Fs and DL-PCBs in the food-producing animals was used (EFSA CONTAM Panel, 2018). Briefly, "*the kinetics of contaminants in the animal may be described by factors like the transfer rate (TR, formerly called carry-over rate or COR), describing the percentage of the daily dose excreted in milk or eggs. An alternative is the bioconcentration factor (BCF), describing the ratio between the level in tissues, milk or eggs, and that in the feed. BCFs are more suitable for tissues, since it is more difficult to obtain the information on the total weight of, e.g. muscle and adipose tissues in the animal required to calculate the TRs. Occasionally also the term biotransfer factor (BTF) is used, describing the ratio between the level in the edible product and the daily intake. In fish, another parameter used is the accumulation efficiency, which is the net effect of dietary absorption and elimination, calculated as the concentration in fish, divided by the concentration in feed multiplied by the feeding rate (amount per day) and duration of feeding (in days). For a number of animal species, toxicokinetic models have been developed, describing the time- and dose-related levels in meat, milk, eggs or body fat*" (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion new toxicokinetic models have been developed (Lerch et al., 2025; Oltramare et al., 2024; Savvateeva et al., 2020) or been improved (Notenboom et al., 2023). Initially such models focussed on the Total-TEQ levels like for dairy cows (Derks et al., 1994; Hoogenboom et al., 2010), pigs (Hoogenboom et al., 2007) and laying hens (Van Eijkeren et al., 2006), making them susceptible to a change in the TEFs. Notenboom et al. (2023) described the congener-specific modelling of the transfer of PCDD/Fs and DL-PCBs in laying hens, where first the transfer of each congener is modelled and subsequently the levels in tissues or eggs converted to TEQ levels using the relevant TEFs. Some of these kinetic models are now available via websites like feedfoodtransfer.nl (Notenboom et al., 2023) and contrans.bfr.bund.de (Savvateeva et al., 2020). They can be used to predict the levels in animal-derived food products based on the exposure of the animals. As an example, Figure 5 shows the modelled increase in the levels in milk and body fat of a dairy cow following repeated intake of contaminated feed followed by a decrease in the levels following a switch to clean feed. Based on this model, a steady-state for Total-TEQ in dairy cows would be reached after at least 4 months, assuming no change in the physiology. This will differ for each specific congener and as such steady-state for the Total-TEQ level will also depend on the congener profile. An additional example from the contrans.bfr.bund.de website is shown in Annex E (Figure E5).

As an example, **Figure 6** shows the increase and decrease of levels in eggs and fat of laying hens for a similar scenario. In this case the model predicts that steady-state for Total-TEQ is reached after 1 year of continuous exposure.

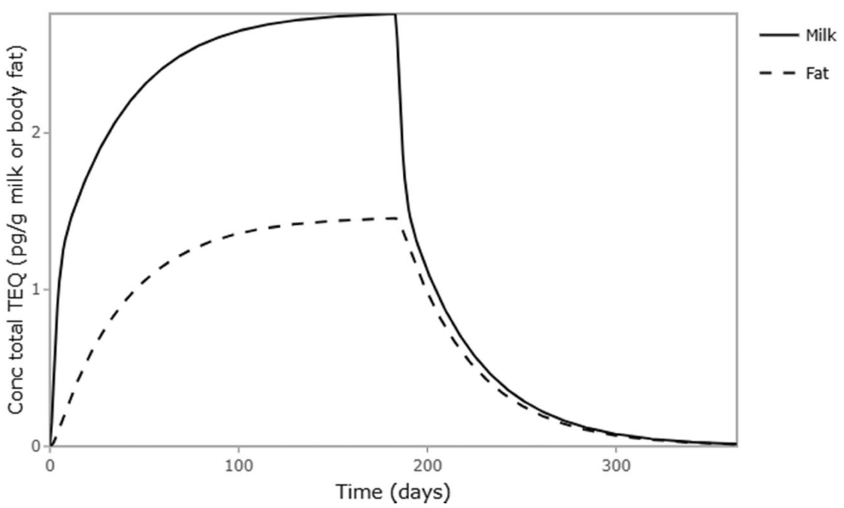

FIGURE 5 Transfer of PCDD/Fs and DL-PCBs from feed to milk and body fat in dairy cows eating 15 kg contaminated grass (88% DM, 0.75 ng TEQ/kg) per day for 6 months followed by 6 months on clean grass (modelled data for WHO_{2005}-TEQ in www.feedfoodtransfer.nl).

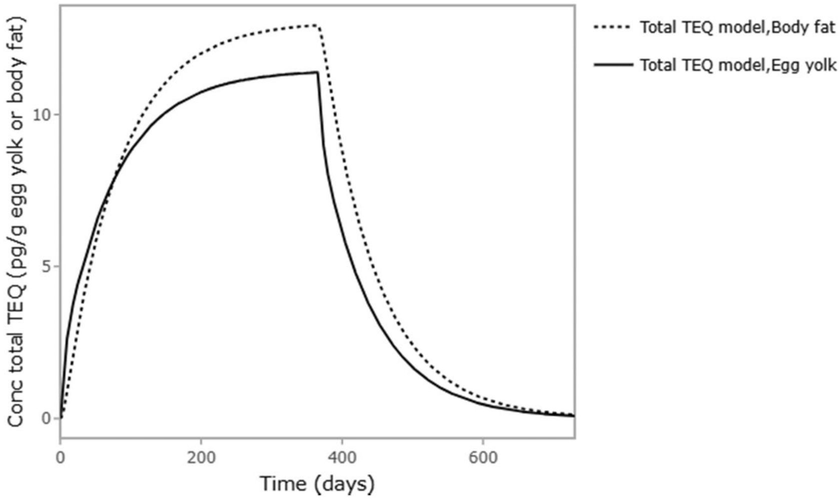

FIGURE 6 Transfer of PCDD/Fs and DL-PCBs from feed to eggs and body fat in laying hens - eating 0.11 kg contaminated feed (88% DM, 0.75 ng TEQ/kg) per day for 1 year followed by 1 year on clean feed (modelled data for WHO_{2005}-TEQ in www.feedfoodtransfer.nl).

3.1.1.4.1 | *Studies considered in the previous EFSA assessment*

Due to their lipophilic properties and poor metabolism, the 29 PCDD/Fs and DL-PCBs under consideration accumulate in fatty edible tissues and products from food producing animals and are excreted via eggs and milk. Various studies have investigated the relationship between the levels in feed and those in edible products, including the decrease after termination of the exposure (EFSA CONTAM Panel, 2018). The ratios between levels in feed and those in milk and eggs were expressed in Transfer Rates (TRs), while those between feed and meat, liver and tissues in Bioconcentration Factors (BCFs). The TR is the fraction of the daily intake that is transferred to milk or eggs collected on that day. The BCF is the ratio between the level in the animal-derived product (e.g. adipose tissue or meat) and that in the total feed ration (mixture of various feed ingredients) of the animal. Ideally these factors are derived after reaching a steady-state situation, but this may require a long period of exposure (see Figures 5 and 6) and in practice treatment is often much shorter, both in experimental studies and during incidents with contaminated feed. In growing animals, the continuous change in body weight and the fraction of body fat prevents reaching steady-state. This is effectively also the case when the body fat content changes over time, like in dairy cows.

EFSA CONTAM Panel (2018) described the TRs and BCFs for individual PCDD/F and DL-PCB congeners. These values are not affected by the change in TEFs. However, the TRs and BCFs for the Total-TEQ levels may change due to the change in relative contribution of the various congeners to the TEQ levels. The impact requires a careful evaluation, based on e.g. the most common congener patterns observed in food and feed. Especially for PCDD/Fs, the various sources show clear differences in the relative contributions to the TEQ level (Hoogenboom et al., 2020), and the impact of the new TEFs can be different for each pattern. Thus far, most toxicokinetic models were developed to model the Total-TEQ levels based on specific animal studies. These models may also be impacted by the change in TEFs, as observed when the TEFs of 1998 were updated to those of 2005 (Notenboom et al., 2023). Congener-specific models are more flexible, since they model the individual congeners and subsequently the predicted levels in food are converted to TEQ levels using the updated TEFs. These models are also more suitable for incidents where the congener patterns are clearly different, as e.g. in the case of pentachlorophenol with a high contribution of higher chlorinated PCDD/Fs, with toxicokinetic behaviour that clearly differs from those of the more common lower chlorinated congeners. In addition, some of the PCDD/Fs, like TCDF, appear to be rapidly metabolised in certain species, and as a result the impact of the change in TEF values on contamination levels in edible products is less relevant.

Several new studies were identified which are briefly described below. The estimated TRs and BCFs are added to the tables that were presented in EFSA CONTAM Panel (2018) (see Annex E) and a summary is reported below.

3.1.1.4.2 | *Studies published since the previous EFSA assessment*

Although case studies may be informative, controlled animal studies are preferred and give a more accurate estimation of the TRs or BCFs. So, in case of sufficient suitable experimental studies describing these factors for individual congeners, results of case studies were regarded as supporting evidence.

3.1.1.4.2.1 | *Studies in ruminants*

Dairy cows

In the 2018 Opinion, TRs could be obtained from nine studies with dairy cows (EFSA CONTAM Panel, 2018). Since then, additional studies have been identified.

Lorenzi et al. (2020) treated four dairy cows (Italian Holstein-Friesian) with a standard mixture of PCDD/Fs and DL-PCBs dissolved in corn oil and mixed daily within 1 kg of the total mixed ration (TMR) for 7 weeks followed by 6 weeks depuration period. After providing the contaminated TMR, cows ate on average 22 kg TMR per day (88% DM) during the exposure period, implying concentrations in the total diet of 0.33, 0.90 and 1.23 ng WHO_{2005}-TEQ/kg TMR (88% DM) for PCDD/Fs, DL-PCBs and the sum (i.e. below the MLs for feed). Four other cows served as control; they received the TMR without the supplemented mixture of PCDD/Fs and DL-PCBs but containing 0.18, 0.14 and 0.33 ng WHO_{2005}-TEQ/kg TMR (88% DM) for PCDD/Fs, DL-PCBs and the sum (UB levels and the actual levels may be much lower, as also suggested by the 8-fold lower sum-TEQ levels in milk). As observed in other studies, milk levels showed a rapid increase during the first week and near maximal levels after 3 weeks. Milk levels exceeded the ML for the sum-TEQ within 1 week, despite the fact that the feed level did not. When switched to non-supplemented feed, the levels rapidly declined in the first week, followed by a much slower decline. Data were extrapolated to steady-state and TRs calculated based on the steady-state levels (see Annex E, Table E.1).

Driesen, Lerch, et al. (2022); Driesen, Zennegg, et al. (2022) fed eight pregnant dairy cows (Simmental) with grass silage mixed with 2.5% (DM base) soil from a polluted area for a period starting 109 days prepartum and lasting up to 288 days after calving. Half of the cows received uncontaminated grass after 164 days in lactation. Each group of 4 animals consisted of 2 primiparous and 2 multiparous cows. Calves were separated from the dams but kept receiving their milk. The level in the grass/soil mixture was 1.9, 0.6 and 2.6 ng WHO_{2005}-TEQ/kg dm for PCDD/Fs, DL-PCBs and the sum, respectively. This resulted in rather stable milk levels throughout the exposure period and with minor differences between primi- and multiparous cows, on day 164 being 4.3, 3.8 and 8.1 ng WHO_{2005}-TEQ/kg fat for PCDD/Fs, DL-PCBs and the sum, respectively. Annex E (Table E.1) shows the TRs on day 164, being relatively low compared to other studies, which may be related to the low daily milk fat production. TRs for primiparous cows were somewhat lower than for multiparous cows, probably due to the lower milk fat excretion. Levels in samples of subcutaneous adipose tissue, taken on day 164, were also similar for the two groups of cows, being on average 3.7, 3.4 and 7.0 ng WHO_{2005}-TEQ/kg fat for PCDD/Fs, DL-PCBs and the sum, respectively, and thus quite similar to the levels in milk. It is noted that the contribution of the DL-PCBs to Total-TEQ levels in milk and adipose tissue is relatively high compared to that in the feed. Milk production showed a decrease over the lactation period from around 13 and 21 L/day on day 25, to 10 and 14 L/day on day 164 and 6 and 9 L/day on day 280, for primi- and multiparous cows, respectively. On day 164, adipose tissue levels of PCDD/Fs, DL-PCBs and the sum in calves were almost twice the levels in the cows. The higher levels in calves compared to the mother cows were also observed for two cases by Bogdal et al. (2017).

Krause et al. (2023) investigated the transfer of PCDD/Fs and DL-PCBs in five high-yielding German Holstein cows at the beginning and end of the lactation period. Cows were treated for 28 days in each period by supplying capsules, followed by a withdrawal period of 100 days. The daily dose was 119 to 154 pg. WHO_{2005}-TEQ for PCDD/Fs, 42 to 56 pg. WHO_{2005}-TEQ for DL-PCBs and 161 to 209 pg. WHO_{2005}-TEQ for the sum. It was shown that the TRs were 10 to 20% higher at the beginning when the cows are in a negative energy balance, as compared to the later period when being in a positive energy balance (see Annex E, Table E.1).

Hao et al. (2023) treated four dairy cows (Chinese Holstein, secondiparous, first 100 days of lactation) with feed mixed with contaminated fly ash at a PCDD/F level of 0.61 ng WHO_{2005}-TEQ/kg for 6 weeks followed by a depuration period of 20 weeks. Milk levels showed an initial rapid increase followed by a much slower increase to an average plateau of 3.4 ng WHO_{2005}-TEQ/kg fat. At the end of the exposure period, two animals were slaughtered. Levels in various types of meat were between 2.0 and 2.8 ng WHO_{2005}-TEQ/kg fat, similar to those in adipose tissue and on average slightly lower than those in milk. The level in liver was 7.5 ng WHO_{2005}-TEQ/kg ww. TRs were only shown for some of the HxCDD/Fs (6%–16%), HpCDD (2%–3%) and OCDD (< 1%). When switched to uncontaminated feed, there was a rapid decrease followed by a much slower phase, with levels below the ML in the EU within 40 days. Levels in adipose tissue of these cows were 3- to 5-fold lower than those in the cows slaughtered directly after the treatment. However, in meat samples the levels were in general higher and in some tissues similar to the levels in cows slaughtered directly after the treatment.

Rigby et al. (2023) investigated the transfer to milk from dairy cows of various contaminants from recycled materials applied to agricultural land. As one of the treatments, biosolids were mixed at up to 5% DM into the feed provided to four Holstein cows for 4 weeks. Levels in the feed were 0.25, 0.07 and 0.32 ng WHO_{2005}-TEQ/kg DM for PCDD/Fs, DL-PCBs and the sum, respectively. This resulted in milk levels at 4 weeks of 0.8, 0.5 and 1.3 ng WHO_{2005}-TEQ/kg fat for PCDD/Fs, DL-PCBs and the sum, respectively. TRs are shown in Annex E (Table E.1).

An important common observation across studies is that milk levels of PCDD/Fs and DL-PCBs increase rapidly after the start of the contamination and reach levels close to those at steady-state within a few weeks. The opposite occurs when the exposure is ended, i.e. an initial rapid decline followed by a much slower decrease. Annex E (Table E.1) includes the minimum and maximum TRs for the different congeners. There are now much more data on the DL-PCBs than in 2018, in particular for the mono-*ortho* PCBs. In general, for PCDD/Fs the TRs show a similar tendency, i.e. a higher transfer of the lower chlorinated congeners with the exception of TCDF and 1,2,3,7,8-PeCDF. For the DL-PCBs, high TRs were observed, with the exception of PCB-77 and -81. The variation between congeners has a clear impact on the TEQ levels in milk resulting from a certain feed level, but in most situations the congeners with high TRs dominate the TEQ levels (Hoogenboom et al., 2021). Annex E (Table E.1) also shows that for a given congener there is quite some variation in the TRs, possibly related to the nature of the contaminated material, but also to the daily milk fat production, which is affected by factors like breed, type of husbandry, days after calving and related energy status, and parity of the cows. These factors affect the eventual TEQ levels in milk that result from a certain feed TEQ level. In addition, the duration of the increased exposure compared to background plays an important role, since TRs increase over time. Ideally, TRs are determined at steady-state, but this requires a longer period and in most studies TRs are based on the highest levels, observed at the end of the exposure period.

Sheep

In the 2018 Opinion, BCFs could be obtained from one study with sheep (EFSA CONTAM Panel, 2018). Since then, two additional studies have been identified.

Lerch et al. (2020) investigated whether undernutrition in combination with mineral oil would accelerate the depletion of TCDD and PCB-126 from the body. Nine non-lactating Romane ewes were first exposed for 27 days to TCDD (280 pg. per day) and PCB-126 (285 pg. per day) via a concentrate. Subsequently 4 ewes received a control diet for 58 days, the other five the special treatment (undernutrition and mineral oil). At the end of this depuration period, in the 4 control animals the TCDD levels in 3 types of adipose tissue and muscle were very similar (around 20 ng/kg lipid), those in serum were 3-fold lower, those in liver 2.5-fold higher. PCB-126 levels in adipose tissue and muscle of these animals were also around 20 ng/kg lipid, just slightly lower in serum, but 7-fold higher in liver, i.e. a higher sequestration in the liver than for TCDD. Based on the 20% body fat (estimated on weighed tissues at slaughter), the major part of the body burden was in the adipose tissue. The absorption was estimated to be around 50% based on the body burden. Both compounds were detected in the faeces, but the amounts estimated to account for less than 10% over the 57-days depuration period. Wool fat contained 1.25-fold higher levels of TCDD, but almost 3-fold higher levels of PCB-126 compared to adipose tissue. Overall, the estimated loss via wool fat was rather small. The group that underwent undernutrition and mineral oil treatment, intended to increase the depletion of TCDD and PCB-126, showed lower body fat content, but slightly higher levels of TCDD and PCB-126 in adipose tissue and muscle (being significant for TCDD). Levels in faeces were also higher. This suggests a higher excretion, but due to the decrease in body fat, the levels in fat and muscle did not decrease. No BCFs were presented.

In another study, Lerch et al. (2024) fed five suckler ewes (Roux de Valais) for 5 months with two batches of contaminated hay at 2.3 and 2.8 ng WHO_{2005}-TEQ/kg DM, followed by a batch at 12.7 ng WHO_{2005}-TEQ/kg DM for one month (only PCDD/Fs reported). This was followed by a 6-month depuration period on uncontaminated hay, also provided to four control ewes over the whole period. The ewes lambed during the month on high contaminated hay and produced milk for 3 months. Total dry matter intake decreased from around 2.7 to 1.6 kg/day at the start and end of the depuration period, respectively. Body weight of the ewes gradually increased during the depuration period, adipose tissue weight as well, but starting after weaning (from 3.5 to 10.5 kg). Milk and sternal adipose tissue were sampled at several time points. PCDD/F-TEQ levels in adipose tissue decreased from 27 to 2 ng/kg fat, partly due to milk excretion during the first 2 months, but also to the subsequent 3-fold increase in body fat. Milk levels decreased more rapidly than those in adipose tissue. At the end of the depuration period, fat based TEQ levels in adipose tissue were similar between exposed and control animals (below the MLs in the EC), being 2-fold higher than in muscle and 6-fold lower than in liver. BTFs but not BCFs were provided. The CONTAM Panel used the data for the last treatment day to calculate BCFs as shown in Annex E (Table E2). It shows the typical decrease in BCFs with higher chlorination and the low BCFs for TCDF and 1,2,3,7,8-PeCDF.

3.1.1.4.2.2 | *Studies in pigs*

In the 2018 Opinion, BCFs could be obtained from two studies with pigs (EFSA CONTAM Panel, 2018). Since then, one additional study has been identified.

Zhang et al. (2022) treated four Yorkshire pigs (25 kg) for 11 days with compound feed (3% of bw) mixed with contaminated fly ash (250 g per day). The fly ash contained 53 ng WHO_{2005}-TEQ/kg, meaning a daily intake of 14 ng WHO_{2005}-TEQ per day. In the fly ash, PCDFs contributed 66%, PCDDs 34% to the TEQ level, with contributions of 2,3,4,7,8-PeCDF, 2,3,4,6,7,8-HxCDF and PeCDD of 32%, 11% and 11%, respectively. Four other pigs were fed a control feed. Levels in a number of muscle tissues varied between 0.31 to 0.67 ng WHO_{2005}-TEQ/kg, as compared to 0.43 ng WHO_{2005}-TEQ/kg fat in pork fat. In liver, the level was 69 ng WHO_{2005}-TEQ/kg lipid or based on 5% lipid 0.35 ng WHO_{2005}-TEQ/kg wet weight. The BCFs shown in Annex E (Table E2) were calculated by dividing the average level of each congener in meat by the level calculated for the feed, assuming 0.75 kg feed plus 0.25 kg fly ash per day. The BCFs were much lower than those reported by Hoogenboom et al. (2004) and Spitaler et al. (2005) (described in the 2018 Opinion), potentially related to a reduced bioavailability from fly ash. In addition, there was quite some variation between muscle fat and adipose tissue fat, partly due to the low levels close to LOQs.

3.1.1.4.2.3 | *Studies in poultry*

In the 2018 Opinion, BCFs could be obtained from six studies in laying hens, ducks or broilers (EFSA CONTAM Panel, 2018). Since then, two additional studies have been identified.

Wang et al. (2021) treated three groups of 45 Hy-line brown laying hens (32-weeks-old) with control feed or feed contaminated with fly ash resulting in Total-TEQ levels of 1.17 or 5.13 ng WHO_{2005}-TEQ/kg dm. PCDD/Fs contributed most to the TEQ-levels. After 14 days of treatment, the animals were transferred to uncontaminated feed. Total-TEQ levels in eggs collected at the peak around day 14 were 1.6/2.0 and 6.6/6.9 ng WHO_{2005}-TEQ/kg fat (LB/UB). This was followed by the typical initial rapid decrease followed by a much slower decline. TRs for PCDD/Fs were calculated for transfer to eggs, based on the levels at day 14 of treatment and those for the high dose are shown in Annex E (Table E3). It was estimated that the TR for the Total-TEQ would be 17% after prolonged exposure leading to a steady-state, as compared to the 8% observed on day 14. The short duration could partly explain the low TRs observed in this study, as compared to, e.g. Hoogenboom et al. (2006).

Wang et al. (2022) reported on the levels in the tissues of the hens in the study by Wang et al. (2021). Levels increased and then decreased during the period on uncontaminated feed. The levels in adipose tissue showed a peak around 1 and 3 ng WHO_{2005}-TEQ/kg fat in the two exposure groups, respectively, as compared to 4 and 5 ng WHO_{2005}-TEQ/kg fat in breast muscle and 2.5 and 15 ng WHO_{2005}-TEQ/kg fat in liver. The latter suggests sequestration in the liver, even at these low doses. It is noted that these levels do not reflect the fold-difference in the levels in the feed, in particular for the muscle. Furthermore, there was a large difference in the fat-based levels between muscle and adipose tissue, especially at the lower dose. The CONTAM Panel noted that the low fat content in breast muscle combined with the applied method for the fat extraction may have contributed to these differences in levels between breast muscle and adipose tissue. Pirard and De Pauw (2006) observed for breast tissue slightly lower fat-based levels than in abdominal fat. As for eggs, Wang et al. (2022) estimated BCFs based on the levels at day 14 (those for adipose tissue shown in Annex E (Table E3). These BCFs were relatively low compared to those observed in other studies, probably due to the short duration and type of contaminated material.

Fernandes et al. (2023) studied the transfer of contaminants in various bedding materials in laying hens. Two materials with increased levels of PCDD/Fs and DL-PCBs were tested, shredded cardboard and dried paper sludge with Total-TEQ of 9.84 and 10 ng WHO_{2005}-TEQ/kg, respectively. Around 25 hens were placed on each material, starting before laying eggs and finishing after 7.5 months. The feed contained 0.08 ng WHO_{2005}-TEQ/kg. The intake of bedding material was estimated to be 2% of the feed, which could be translated to ration levels of 0.27 and 0.28 ng WHO_{2005}-TEQ/kg for the two materials, respectively. For the first material (i.e. shredded cardboard), this resulted in levels in eggs, muscle, skin and liver of 1.42, 2.21, 2.19 and 2.33 pg. WHO_{2005}-TEQ/g fat, respectively. For the second material (i.e. dried paper sludge), levels in eggs, muscle, skin and liver were 2.07, 2.93, 2.56 and 2.59 pg. WHO_{2005}-TEQ/g fat, respectively. In the controls, the levels in eggs, muscle, skin and liver were 0.56, 1.04, 0.79 and 0.90 pg. WHO_{2005}-TEQ/g fat, respectively. The authors calculated biotransfer factors (BTFs), defined as the fat-based concentration in tissues and eggs divided by the daily intake of contaminants. Those for meat are presented in Annex E (Table E3). Based on a total intake of 0.031 ng WHO_{2005}-TEQ per day (assuming 0.11 kg feed and 0.022 kg bedding material), the CONTAM Panel calculated for muscle meat fat of animals kept on dried paper sludge a BTF of 2.93/0.031 = 95). Based on a level in feed of 0.28 (0.031/0.11) ng WHO_{2005}-TEQ/kg, this corresponds to a BCF for Total-TEQ of 2.93/0.28 = 11. For the shredded cardboard BTFs and BCFs for muscle meat of 74 and 8, respectively, were calculated. No data on individual congeners were provided that would allow such estimations for each of these compounds. For comparison with previous studies, the BTFs were normalised to feed concentrations for derive BCF estimates by dividing by a factor 10. For eggs containing 6 g of fat, a TR of $(6 \times 2.07)/(110 \times 0.28) = 40\%$. These values are in agreement with previous studies, as shown in Annex E (Table E3).

3.1.1.4.2.4 | *Studies in fish*

In the previous Opinion the transfer of PCDD/Fs and DL-PCBs from feed to fish was evaluated (see Annex E, Table E4), leading to the following summary: *"As for terrestrial farm animal species, there are data from experimental- and field studies in fish which give insight into the transfer of PCDD/Fs and DL-PCBs to fish. Studies show that PCDD/Fs and DL-PCBs are accumulated to a greater extent in fillet of oily fish (such as salmon and trout) than leaner fish, the latter having higher concentrations of these compounds in the liver (as is the case for cod). Limited data indicate that metabolism and excretion of PCDD/Fs and DL-PCBs occur, depending on the congener in question. The main source of PCDD/Fs and DL-PCBs in farmed fish is marine feed ingredients in fish feeds including fish oil and fishmeal. In addition to the feed composition, the transfer of PCDD/Fs and DL-PCBs to fillet depends on other factors such as species, and animal growth. Some of the studies allow the calculation of the transfer parameter BCF, mainly in salmonids. Toxicokinetic models have been developed for salmon enabling the prediction of fillet concentrations of PCDD/F and DL-PCBs from known feed concentrations. One of the models has been validated and may be useful for harmonising the regulatory limits for these compounds in feed and fish fillet."*

Since the 2018 Opinion, no new studies describing TRs or BCFs for individual congeners were identified.

3.1.1.4.2.5 | *Studies in insects*

Belghit Liland et al. (2024) described the accumulation of contaminants, including PCDD/Fs and DL-PCBs in black soldier fly larvae grown on aquaculture sludge, but without details on individual congeners.

3.1.1.4.3 | *Overall summary*

The new studies confirm the observations from previous transfer studies, showing differences between the congeners. The new data did not provide consistent information on the ratio of fat-based levels in meat and adipose tissue. The change in TEFs does not affect the transfer rates of individual congeners but the CONTAM Panel noted that it could impact the transfer rates when using Total-TEQ levels. The extent of the impact of the new WHO2022-TEFs on the transfer rate of the

Total-TEQ level may be evaluated based on a case-by-case basis and ideally using congener-specific toxicokinetic models that are available for some species.

3.1.2 | Toxicity in experimental animals

3.1.2.1 | *Selection criteria for low-dose rodent toxicity studies*

Studies in experimental animals were retrieved and selected for relevance and agreement with the eligibility criteria as described in **Section 2.2** and Annex A. The selection of the studies for the identification of a Reference Point was based on the same approach as applied in the 2018 Opinion. In brief, toxicity studies in experimental animals were included if:

- Exposure was to the seventeen 2,3,7,8-substituted PCDD/Fs and/or the 4 non-*ortho* DL-PCBs, administered individually or as mixtures. Studies on mono-*ortho* PCBs alone were excluded; for these congeners there are potential issues with impurities of NDL-PCBs or PCDFs that based on the differences in the TEFs might, even at very low fractions, be responsible for the observed effects. As such, deriving a Reference Point as the basis of the risk assessment for the whole group of PCDD/Fs and DL-PCBs is too uncertain.
- Exposure was validated, i.e. levels measured in tissues or concentrations of the compounds in the administered dosing solutions or feed analysed. Since WHO (1998) the risk assessment approach for PCDD/Fs and DL-PCBs has been based on the body burden in experimental animals that is associated with the adverse effects. This approach reduces the uncertainties related to the large differences in kinetics between animals and humans. Therefore, it is important to obtain information that allows an estimation of the body burden.
- The administration of the target compounds resulted in measured and/or estimated body burdens lower than 100 ng WHO_{2005}-TEQ/kg bw, since only such levels might potentially result in a lower Reference Point than the ones previously identified for studies with experimental animals.
- The endpoints studied were regarded as adverse. Therefore, studies only addressing enzyme induction (e.g. CYP modulation), gene expression, co-administration of pro-carcinogens, or -omics profiles, studies on endpoints such as reactive oxygen species (ROS), DNA methylation, and telomer length, that are not quantitatively related to specific diseases, were not further considered for the identification of a Reference Point. However, these studies were considered when assessing the mode of action (**Section 3.1.4**). Furthermore, information on some of such findings in the studies selected for this Scientific Opinion is provided in Annex F (Table F.1).

3.1.2.2 | *Studies in rodents*

Since the 2018 Opinion, a total of four studies in rodents were identified meeting the eligibility criteria (see **Section 3.1.2.1** above and Annex A), i.e. Doskey et al. (2020), Johnson et al. (2020) and VanEtten et al. (2020, 2021). Details of these studies can be found in Annex F (Table F.1).

The study by Doskey et al. (2020) reported on hepatic effects considered as adaptive (hepatic hypertrophy, metabolic changes) or changes without clear adversity (gonadal adipose tissue weight).

The study by Johnson et al. (2020) reported no effect of TCDD and TCDF on conceptus resorption, number of viable foetuses/litter, sex ratio, GD20 fetal body weights, or gravid uterine weights. A treatment-related increase in the number of foetuses with intestinal haemorrhage at the TCDD dose of 10 μg/kg bw was reported (data not shown or quantified by authors). Furthermore, the study reported on transcriptomic effects in maternal and fetal liver, testis, and pituitary.

VanEtten et al. (2020, 2021) studied effects such as relative telomer length or copy numbers of mtDNA but the findings were not quantitatively related to specific diseases. No information on body burden was provided.

The CONTAM Panel considered the effects reported in these studies not adverse and/or not suitable to derive a Reference Point.

3.1.2.3 | *Studies in primates*

No new studies were identified since the 2018 Opinion.

3.1.2.4 | *Genotoxicity*

In the 2018 Opinion the CONTAM Panel concluded that "*overall, the evidence is robust that PCDD/Fs and DL-PCBs are not directly genotoxic in standard assays*". It also concluded that "*there is no robust evidence that the development of cancer caused by TCDD and other PCDD/Fs in experimental animals is associated with direct genotoxicity*" (EFSA CONTAM Panel, 2018).

No relevant data were identified since the 2018 Opinion, so the above-mentioned conclusion still applies.

3.1.3 | Observations in humans

Studies in humans in which the exposure to PCDD/Fs and DL-PCBs has been evaluated in relation to adverse effects were retrieved and selected for relevance and in agreement with the eligibility criteria as described in **Section 2.2** and Annex A. The CONTAM Panel considered studies to be relevant for hazard identification and characterisation if they either (i) analysed any of the following compounds (expressed in TEQ) in biological samples (e.g. blood, human milk, adipose tissue), and/or (ii) assessed dietary exposure to these compounds:

- 17 PCDD/Fs and 12 DL-PCBs,
- 17 PCDD/Fs plus non-*ortho* PCBs, at least one being PCB-126.
- TCDD or any other congener dominating the TEQ, e.g. due to a contamination incident.
- 17 PCDD/Fs.
- 12 DL-PCBs.

The individual studies resulting from this selection were grouped according to health outcome categories. Details of these new studies identified since the 2018 Opinion can be found in Annex F (Table F.2 to F14) with the following considerations:

- Studies on male and female reproductive effects, birth outcomes and growth, cancer, thyroid disease and thyroid hormones, neurodevelopmental effects in children, and effects on teeth and bones were considered to be more critical in the 2018 Opinion. For these effects, data were extracted in a tabulated manner.
- Studies on other effects, e.g. type 2 diabetes and obesity, effects on the immune system, neurotoxicity effects in adults, developmental effects, cardiovascular effects, hepatic disorders and digestive effects, were reported in a descriptive manner.
- Studies that analysed the set of 17 PCDD/Fs only or the set of 12 DL-PCBs only, without indication that these dominated the total TEQ (e.g. due to a contamination incident), would not be suitable as critical studies for deriving a Reference Point as the basis of the risk assessment for the whole group of PCDD/Fs and DL-PCBs. However, these were considered as supporting evidence and were reported in a descriptive manner.
- Studies on male and female sex hormones that were not addressing other endpoints considered adverse by themselves, were reported in a descriptive manner. In line with the 2018 Opinion, the CONTAM Panel considered that changes in serum sex hormones can contribute to mechanistic explanations for adverse reproductive endpoints, but changes in their levels per se in adults or children were not considered to be an adverse effect by themselves.
- An overview with the main characteristics of the cohorts studied can be found in **Section 3.1.3.1**.

The reliability of the individual studies was appraised by considering their internal validity (risk of bias). The appraisal of the studies was done by two reviewers independently for the potential critical endpoints known from the 2018 Opinion, i.e. male and female reproductive effects, and for studies on birth outcomes and growth (Annex F, Table F15). Each study was allocated to one of the three risk of bias tiers as described in Annex A. The CONTAM Panel considered for the discussion all studies independently of the risk of bias outcome.

The sub-sections below provide a brief summary of the effects and associations as reported in the 2018 Opinion, a summary of the effects and associations reported in the studies identified in the open literature since then, and an overall summary of all the evidence available and conclusions thereof. To avoid repetitions between the text below and the data extraction tables, the levels of PCDD/Fs and DL-PCBs are only reported in a subset of studies with a longitudinal study design addressing endpoints considered to be more critical in the 2018 Opinion and that observed positive associations. The details of the studies considered in the 2018 Opinion can be found in EFSA CONTAM Panel (2018).

The provided conclusions are based on the robustness of the associations under study taking into consideration in a qualitative manner: amount of evidence, replication consistency, protection from bias and actual findings.

The CONTAM Panel noted that the identified studies since the 2018 Opinion expressed the PCDD/F and DL-PCB exposure levels as WHO_{2005}-TEQ or previous TEF schemes, and no studies applied the WHO_{2022}-TEFs.

3.1.3.1 | *Description of cohorts*

Similarly to the 2018 Opinion, the epidemiological studies have been conducted in subjects/cohorts exposed to PCDD/Fs and DL-PCBs at different life stages under different exposure conditions, and the studies identified fall into three categories of main sources of exposure:

(i) Industrial accidents or contamination incidents.
(ii) Occupational exposure.
(iii) Background exposure.

Most of the studies identified since the 2018 Opinion extend the information from cohorts described in EFSA CONTAM Panel (2018). An overview of the main characteristics of the cohorts not covered in the 2018 Opinion is given below.

3.1.3.1.1 | *Cohorts based in industrial accidents or contamination incidents*

An overview of the main characteristics of the cohorts based on industrial incidents, such as the Seveso cohort (in 1976), or contamination incidents, such as the Yusho (in 1968) or Yucheng (in 1979) cohorts was given in EFSA CONTAM Panel (2018).

Since the 2018 Opinion, and regarding the Seveso cohort, studies have been identified following up the Seveso Women's Health Study (SWHS) (Ames, Warner, Brambilla, et al., 2018).

In addition, a series of reports on the Seveso Second Generation Study were identified (Ames et al., 2019; Ames, Warner, Mocarelli, et al., 2018; Eskenazi et al., 2021; Slama et al., 2019; Warner et al., 2019; Warner, Rauch, Ames, Mocarelli, Brambilla, & Eskenazi, 2020; Warner, Rauch, Ames, Mocarelli, Brambilla, Signorini, & Eskenazi, 2020; Warner, Rauch, Brambilla, Signorini, Mocarelli, & Eskenazi, 2020; Ye et al., 2018). The Seveso Second Generation Study was initiated in 2014 to characterise the impact of TCDD exposure on the children of SWHS participants. It comprises a total of 611 children (66.4% of 920 alive and eligible) born to 402 mothers. The children's age ranged from 2 to 39 (average age: 23.7) years, and 51% were female (Eskenazi et al., 2018). Pregnancy occurred years to decades post-explosion. According to the authors, estimation of the maternal serum TCDD levels at the time of pregnancy was performed by extrapolation from the nearest preceding serum TCDD measure (1976, 1996, 2008) using a first-order kinetic model (Warner et al., 2014). For all mothers, the median time between the closest TCDD measure and pregnancy was 4.8 years and 87% had a TCDD measurement within one TCDD half-life (9.0 years) (Eskenazi et al., 2018). Due to blood volume constrain, Total-TEQ estimations were only possible for the 1996 and 2008 sampling (Warner et al., 2014), and was restricted to the subset of women who gave live births between 1994 and 2009. The measured serum levels were extrapolated to the time of the pregnancy (Eskenazi et al., 2018).

Since the 2018 Opinion, also studies following up the Yusho (Fukushi et al., 2019; Kondo et al., 2018) and Yucheng (Su et al., 2019) cohorts have been identified.

3.1.3.1.2 | *Occupational exposure cohorts*

An overview of the main characteristics of the cohort of Vietnam veterans who were exposed to the herbicides sprayed during the Vietnam War (mainly Agent Orange), e.g. the Air Force Health Study (AFHS), was given in EFSA CONTAM Panel (2018).

Since the 2018 Opinion, follow-up studies from Vietnam War veterans have been identified, belonging to the AFHS (Corsaro et al., 2024; Kelsey et al., 2019; Nwanaji-Enwerem et al., 2020; Rytel et al., 2021).

In addition, studies from other occupational cohorts, including chemical workers, not described in the 2018 Opinion have been identified as follows:

- The Health Effects in High-Level Exposure to PCB (HELPcB) study. This is a prospective surveillance program including (former) workers from a German company that recycled PCB-containing transformers and capacitors on a largescale, without adequate occupational hygiene practices in place (Kraus et al., 2012). The surveillance program was initiated in 2010 and comprised the analysis of PCDD/Fs and DL-PCBs in blood. Two studies from the HELPcB cohort have been identified as eligible (Leijs et al., 2021; Leijs, Esser, et al., 2018).
- Cohort of chemical workers in Czech Republic with occupational exposure to TCDD during the production of the herbicide 2,4,5-trichlorphenoxyacetic acid between the years 1965 and 1968 in a chemical factory in Central Bohemia. More than 55 workers developed chloracne and other symptoms of intoxication. The first TCDD blood analysis conducted in 1996 resulted in a median of 305 ng/kg lipid (range 74–760 ng/kg lipid). The back-calculated TCDD plasma level at the time of exposure using a physiologically based kinetic model resulted in levels of about 35,000 and 350,000 ng/kg fat in two workers. The studies identified as eligible examined the last eight survivors of 80 workers (Pelcl et al., 2018; Pelclova et al., 2018).
- Cohort of chemical workers in New Zealand occupationally exposed during the production of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Serum concentrations of PCDD/Fs and DL-PCBs were measured. Two studies have been identified as eligible (McBride et al., 2018; 't Mannetje et al., 2018).
- Cohort of chemical workers in the USA of a herbicide-producing chemical plant, occupationally exposed to dioxins during the past production of chlorinated phenols and chlorophenoxy acids. One study has been identified as eligible (Hryhorczuk et al., 2022).

3.1.3.1.3 | *Cohorts with background exposure*

An overview of the main characteristics of some of these cohorts for which exposure occurred mainly via dietary intake in the general population are described in EFSA CONTAM Panel (2018), including the Duisburg cohort, the Russian Children's Study, the Flemish Environment and Health Study (FLEHS), the Norwegian Mother, Father and Child cohort (MoBa), the Hokkaido Study on Environment and Children's Health, the Amsterdam/Zaandam mother–child Cohort and the Rotterdam and Groningen Cohorts.

Since the 2018 Opinion, follow-up studies have been identified for the Russian Children's Study (Burns et al., 2020; Plaku et al., 2023), the Hokkaido Study (Baba et al., 2018; Miyashita, Ait Bamai, et al., 2018; Miyashita, Araki, et al., 2018; Yamazaki et al., 2022; Yim et al., 2022), and the Amsterdam/Zaandam mother–child Cohort (Leijs, Koppe, et al., 2018).

In addition, studies from other cohorts not described in the 2018 Opinion have been identified, pertaining to various exposure levels, as follows:

- The Anniston Community Health Survey: follow-up and dioxin analysis (ACHS II) is a follow-up study of the cross-sectional ACHS I study conducted in 2005–2007 to explore exposure to PCBs and organochlorine pesticides and health outcomes among residents of Anniston, USA, the site of a production facility that produced PCBs from 1929 to 1971. The ACHS II aimed to determine if the body burden of PCBs decreased over time, and in addition included serum PCDD/F and DL-PCB analysis (Cusack et al., 2020). Three studies from the ACHS II have been identified as eligible (Pavuk et al., 2019, 2023; Pittman et al., 2020).
- The European Prospective Investigation into Cancer and Nutrition (EPIC) Study is a long-term, large-scale collaborative project that investigates the relationships between diet, nutrition, lifestyle, and environmental factors, and the incidence of cancer and other chronic diseases across European populations. It was launched in 1990 and consists of approximately 370,000 women and 150,000 men, aged 35–69, recruited between 1992 and 2000 in 23 research centres across 10 European countries: Denmark, France, Greece, Germany, Italy, Norway, Spain, Sweden, the Netherlands, and the UK. Exposure to PCDD/Fs and DL-PCBs was estimated based on usual dietary intakes of participants assessed by country-specific and validated dietary questionnaires and mean occurrence in food in Europe provided by EFSA for the period 1995–2018. Three studies from the EPIC cohort have been identified as eligible (Fiolet et al., 2022, 2024; Ren et al., 2024).
- The E3N cohort (Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale) was created in 1990 to study risk factors associated with cancer and other chronic diseases in adult women in France. It includes 98,995 women aged 40 to 65, residents in France. Follow-up is based on self-administrated questionnaires sent every 2 or 3 years. Exposure to PCDD/Fs and DL-PCBs via the diet was estimated using the occurrence data from the French Total Diet Study (TDS 2) and food consumption data gathered from the E3N cohort. Two studies from the E3N cohort have been identified as eligible (Chetrit et al., 2024; Lemaitre et al., 2021).
- Cohort of men working in four Vietnam military airbases, three of them formerly contaminated during the herbicides spraying campaign in the Vietnam War (Pham et al., 2024).
- Subjects living around former USA airbases in Vietnam which are considered as hotspots of mainly TCDD or PCDD/F contamination because of the handling of dioxin-contaminated herbicides (mainly Agent Orange) during the Vietnam War. A number of studies in populations around the airbases were identified as eligible (Anh et al., 2017; Van Luong et al., 2018; Boda et al., 2018; Oanh et al., 2018; Ngoc et al., 2019; Trang et al., 2022; Vu, Nishijo, et al., 2021; Vu, Pham, et al., 2021; Vu et al., 2023; Nishijo et al., 2021; Nguyen et al., 2018; Nghiem et al., 2019; Pham, Nishijo, et al., 2020; Pham et al., 2021; Pham, Nishijo, Pham-The, et al., 2022; Pham, Ngoc, et al., 2020; Pham, Nishijo, Pham, et al., 2022; Thao et al., 2023, 2024).

In addition, several papers were eligible, reporting data from single cohorts, of which details are described in relation to the specific outcomes below and in Annex F.

3.1.3.2 | *Male reproductive effects*

The 2018 Opinion concluded that three prospective cohorts (two on the Seveso incident and one from the Russian Children's Study) showed an association between exposure to TCDD, and for the Russian Children's Study also other PCDD/Fs, during infancy/prepuberty and impaired semen quality. Based on weight of evidence, including also experimental animal studies, the associations were considered causal (EFSA CONTAM Panel, 2018): Impaired semen quality was observed in men in Seveso but only in those that were prepubertal at the time of the incident (Mocarelli et al., 2008), while in another study on men born to mothers that were exposed during the Seveso incident, impaired semen quality was observed only in those who had been breastfed (Mocarelli et al., 2011). The CONTAM Panel in 2018 concluded that the evidence from both Seveso studies suggests *"a postnatal period of sensitivity that might expand into puberty"*. In the Russian Children's Study, which included boys exposed to slightly higher[9] environmental background levels than the current European exposure levels, associations of serum TCDD with impaired semen quality were observed (Mínguez-Alarcón et al., 2017). Significant associations with impaired semen quality were observed also for PCDD-TEQ and for the sum of PCDD-TEQ and PCDFs-TEQ, but not for DL-PCB-TEQ or Total-TEQ. The association between TCDD and semen parameters became slightly stronger after adjustment for NDL-PCBs but was not affected by adjustment for serum levels of organochlorine pesticides. Blood was collected at 8 to 9 years of age, but the levels of PCDD/Fs and DL-PCBs also reflect the earlier exposure via human milk. A TWI was derived by the CONTAM Panel from this study (EFSA CONTAM Panel, 2018) (see **Section 3.1.1.3**).

In the 2018 Opinion, there was insufficient evidence to conclude on an association between PCDD/Fs or DL-PCBs exposure and cryptorchidism. Conclusions on causality were not possible for changes in age at pubertal onset or sexual maturity (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, four new studies were identified. Details of these studies are reported in Annex F (Table F.2 therein).

[9]Serum concentrations of PCDD/Fs and DL-PCBs in boys varied between 1.9 and 107 pg. WHO_{2005}-TEQ/g fat, with a median of 22 (Burns et al., 2009). Pooled human milk samples collected as part of the WHO field studies across European countries in 2014/2015 revealed levels 4.8–9.6 pg. WHO_{2005}-TEQ/g fat for the sum of PCDD/Fs and DL-PCBs, respectively (EFSA CONTAM Panel, 2018).

Semen quality

No studies were identified evaluating the association between both the 17 PCDD/Fs and the 12 DL-PCBs and semen quality (i.e. attributes of sperm quantity and quality). Two studies evaluated the association between exposure to the 17 PCDD/Fs (diet) alone ($n=200$, Martinez et al., 2025) or the 12 DL-PCBs (serum) alone ($n=50$, Paul et al., 2017) and sperm quality without indication that these dominated the total TEQ. Both studies were cross-sectional and were conducted in Spain. Subjects in the highest tertile of PCDD/F-TEQ dietary exposure, compared to lowest, showed a higher percentage of sperm head abnormalities [β_{adj} (95% CI): 4.65% [0.10%; 9.24%]; p-trend = 0.037] (Martinez et al., 2025). In cases with low semen quality, mono-*ortho* PCB-TEQ levels in serum were statistically significantly higher than in controls with normal semen quality (defined per WHO criteria) (Paul et al., 2017).

The CONTAM Panel noted that the updated evidence on semen quality, although complemented by only a few studies with methodological limitations (e.g. cross-sectional design, exposure assessment via diet), does not contradict the previous conclusion that impaired semen quality is likely a causal effect of exposure to TCDD, other PCDDs and possibly PCDFs.

Hypospadias

One study assessed the association between the 17 PCDD/Fs plus the 12 DL-PCBs and hypospadias.

Tysman et al. (2023) in a small, nested case–control study with a cross-sectional exposure assessment evaluated the association between the levels of the 17 PCDD/Fs plus the 12 DL-PCBs in human milk and hypospadias in Denmark and Finland (n cases = 33). The median (range) Total-TEQ was 18.08 (5.72–46.24) pg. WHO_{2005}-TEQ/g fat. No statistically significant associations were reported for the estimated PCDD/F-TEQ, DL-PCB-TEQ or Total-TEQ and hypospadias.

In summary, the Panel considered that there was insufficient evidence for an association between PCDD/F or DL-PCB exposure and hypospadias due to the availability of only one small study with methodological limitations.

Pubertal development

One study assessed the association between the 17 PCDD/Fs plus the 12 DL-PCBs and pubertal developmental trajectories. Plaku et al. (2023), reporting on the Russian Children's Study (see above), assessed the association between peripubertal serum levels for PCDDs, PCDFs and DL-PCBs and pubertal developmental trajectories estimated through testicular volume and using Group-Based Trajectory Models from ages 8–19 ($n=489$). Three pubertal developmental trajectories were defined: slower (34% of boys), moderate (48%) and faster (18%). Higher peripubertal (at age 8–9 years) serum WHO_{2005}-TEQ (per 1 log unit increase, excluding mono-*ortho* PCBs, median 21.1 pg. TEQ/g lipid) were associated with higher odds of being in the moderate versus faster trajectory (aOR 1.79; 95% CI 1.01, 3.13) and the slower versus faster trajectory (aOR 1.52; 95% CI 0.82, 2.78).

In summary, the CONTAM Panel considered that the overall body of evidence remains inconclusive as regards the association between PCDD/F or DL-PCB exposure and puberty development. The only new study identified was on pubertal trajectories in boys (Tier 1) in the same cohort for which previous results showed associations with later pubertal onset and sexual maturity. Although these results are in line with the overall findings from this cohort, the evidence was considered inconclusive due to lack of confirmation from other cohorts.

3.1.3.3 | *Female reproductive effects*

The 2018 Opinion concluded that for endometriosis the available evidence was insufficient to be used as a basis for the risk assessment. No association between exposure and pubertal development was found, and the evidence was insufficient for other female reproductive effects (menstrual cycle characteristics, ovarian function, time to pregnancy, uterine leiomyoma, and age at menopause) (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, two reports on the Seveso Women Health Study (SWHS) and Seveso Second Generation Study, evaluated TCDD exposure and age at menarche and fecundability and infertility (Eskenazi et al., 2018; Warner, Rauch, Ames, Mocarelli, Brambilla, & Eskenazi, 2020). Details of these studies are reported in Annex F (Table F3 therein).

Warner, Rauch, Ames, Mocarelli, Brambilla, and Eskenazi (2020) evaluated the association between the in-utero exposure to TCDD with reported age of menarche in girls ($n=287$) born to the participants of the SWHS. In utero TCDD exposure was defined by maternal TCDD serum concentrations extrapolated back to the time of pregnancy (median, interquartile range; 15.5 ng/kg lipid, 6.6–35.2 ng/kg lipid). No statistically significant association was found between in utero TCDD exposure and menarche onset ($HR_{adj}=0.86$; 95% CI = 0.71, 1.04). However, in stratified analyses, in utero TCDD (log10) was associated with later age at menarche among daughters whose mothers were premenarche (hazard ratio (HR) = 0.71; 95% CI = 0.52, 0.97) but not postmenarche (HR = 0.89; 95% CI = 0.71, 1.12) at the time of the explosion leading to exposure ($P_{int}=0.24$).

Eskenazi et al. (2021) evaluated the association between exposure to TCDD with fecundability and infertility among the SWHS participants ($n=446$) and their daughters ($n=66$). The median initial 1976 TCDD concentration of SWHS women was 61.4 ng/kg lipid. The median 1976 TCDD serum concentration for the mothers of the daughters under study was 52.8 ng/kg lipid and the maternal TCDD at pregnancy was estimated at 35.8 ng/kg lipid. A 10-fold increase in initial 1976 TCDD was associated with higher risk of infertility in the mothers (RR_{adj} 1.35; 95% CI 1.01–1.79) with consistent, yet not statistically significant, results for TCDD exposure estimated for pregnancy (RR_{adj} 1.27; 95% CI 0.85–1.89). Among the limited number of SWHS daughters, maternal initial 1976 TCDD (log10) concentration was not statistically significantly associated with reduced fecundability (fOR_{adj} 0.59; 95% CI 0.31–1.12; Table II); neither was the TCDD concentration in utero (fOR_{adj} 0.59; 95% CI 0.28–1.23), although the effect direction and magnitude for both outcomes in the SWHS daughters were congruent to those of the mothers.

In summary, based on the overall body of evidence, complemented by a few new studies identified, the CONTAM Panel considered that there was still insufficient evidence for an association between PCDD/F or DL-PCB exposure and female pubertal development since the number of available studies remains limited, with different endpoints and without replication across studies. The CONTAM Panel noted that the available evidence on infertility is based on the investigations after the TCDD incident in Seveso and considered that there was insufficient evidence for an association between PCDD/F or DL-PCB exposure and infertility.

3.1.3.4 | *Changes in the levels of sex hormones*

Eleven studies were identified reporting on changes in sex hormone levels alone, not addressing endpoints considered adverse. Details of these studies are reported in Annex F (Table F4 therein).

Of these, only one evaluated associations between exposure to the 17 PCDD/Fs plus the 12 DL-PCBs (Miyashita, Araki, et al. (2018) measured in maternal blood and progesterone, oestradiol (E2), testosterone (T), androstenedione, dehydroepiandrosterone (DHEA), cortisol, cortisone, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, inhibin B, and insulin-like factor-3 (INSL3) concentrations in cord blood. Maternal blood Total-TEQ, mono-*ortho* PCB-TEQ and non-*ortho* PCB-TEQ were associated with changes in SHBG and DHEA in males but with varying direction of the associations. Non-*ortho* PCB-TEQ was also associated with lower T/E2 ratio and higher adrenal androgen/glucocorticoid ratio in males. In females, mono-*ortho* PCB-TEQ was associated with higher cortisol and cortisone and lower adrenal androgen/glucocorticoid ratio. In the male offspring blood, Total-TEQ, PCDD-TEQ, PCDF-TEQ, non-*ortho* PCB-TEQ, and mono-*ortho* PCB-TEQ were associated with lower inhibin B.

The other ten studies, not reporting on the full set of 17 PCDD/Fs plus the 12 DL-PCBs, were conducted under various exposure settings and most were cross-sectional and included children; the findings varied as regards to the direction and magnitude of the associations observed.

In summary, based on the overall body of evidence, the observed associations between exposure to PCDD/Fs and DL-PCBs and sex hormone levels varied in direction and magnitude. As mentioned above, the CONTAM Panel considered that changes in serum sex hormones can contribute to mechanistic explanations for adverse reproductive endpoints, but changes in their levels in adults or children were not considered to be an adverse effect by themselves.

3.1.3.5 | *Birth outcomes and growth*

In the 2018 Opinion, it was noted that a relationship between high TCDD exposure in fathers and lower sex ratio in offspring (lower number of boys relative to girls), had been consistently observed across three different cohorts, and was considered likely to be causal. The studies on other birth outcomes (birth weight, preterm birth, fetal Yusho disease and anogenital distance) were inconclusive (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, three new eligible studies were identified. Details of these studies are reported in Annex F (Table F5 therein). One of them was longitudinal and reported on the Russian Boys' Study (Burns et al., 2020). The remaining two were cross-sectional (Kaneko et al., 2024; Mahfouz et al., 2024). Exposure was assessed via the 17 PCDD/Fs plus 10 DL-PCBs (Burns et al., 2020), or the 17 PCDD/Fs only (Kaneko et al., 2024; Mahfouz et al., 2024, the latter with no indication that these dominated the total TEQ). Sex ratio (one study), newborn anthropometric characteristics (one study) and pubertal growth attributes (one study) were the endpoints under assessment.

Burns et al. (2020), reporting on the Russian Children's Study, assessed the association between peripubertal (8–9 years) serum levels for PCDDs, PCDFs and NDL-PCBs and adolescent growth, body composition, and near adult height (NAH) ($n = 473$) yearly up to age 19. The median of serum Total-TEQ was 21.1 pg. WHO_{2005}-TEQ/g lipid (PCDD- and PCDF-TEQ were not reported) and the median follow up was 9 years (IQR; 8–10 years). In the adjusted multivariable analyses, higher serum concentrations of peri-pubertal ∑TEQ levels were associated with significantly lower BMI Z-score, FMi, and FFMi.

Kaneko et al. (2024), in a cross-sectional study, evaluated the association between maternal PCDD/F-TEQ in human milk and the sex ratio at birth from three birth cohorts in Vietnam located in two Agent Orange sprayed areas and one Agent Orange unsprayed area ($n = 576$). In the unsprayed areas, all participants had PCDD/F-TEQ levels below 8.5 pg. TEQ/g fat. For TCDD, across all areas and using the low-TCDD group as reference, the adjusted ORs (95% CI) of female birth were 2.11 (1.05, 4.38) for the moderate-TCDD group and 2.77 (1.40, 5.49) for the high-TCDD group. For PCDD/F-TEQ, no statistically significant associations were observed for birth ratio neither overall nor in the sprayed areas. The Panel noted that studies referred to in the 2018 Opinion found associations between paternal exposure and altered sex ratio at birth.

One study reporting on the 17 PCDD/Fs only, without indication that these dominated the total TEQ, was also identified. Mahfouz et al. (2024) assessed the association between the 17 PCDD/Fs in human milk and cord serum and newborn anthropometric indices ($n = 49$). The maternal serum PCDD/F-TEQ level was 3.0 ± 2.0 ng/kg lipid. No statistically significant associations were observed for PCDD/F-, PCDD- or PCDF-TEQ and newborn anthropometric indices.

In summary, the CONTAM Panel considered that, based on the overall body of evidence complemented by only one new study per endpoint and the relevant methodological limitations, the available evidence remains inconclusive for the associations between maternal exposure to PCDD/Fs or DL-PCBs and sex ratio, birth weight or other birth outcomes. The same applies for the association between exposure to PCDD/Fs or DL-PCBs and growth indices.

3.1.3.6 | *Cancer*

The 2018 Opinion concluded that there was no clear link to any specific cancer site despite several studies showed a positive association with all cancers combined. There was no clear dose–response relationship between exposure and cancer development (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, five additional eligible studies were identified, two of which reported on the 12 DL-PCBs only without indication that these dominated the total TEQ. Details of these studies are reported in Annex F (Table F6 therein).

Koual et al. (2019) in a cross-sectional study among breast cancer cases in France evaluated the association between the concentrations of 49 POPs, including 17 PCDD/Fs plus 12 DL-PCBs, in adipose tissue and breast cancer metastasis ($n = 49$) vs. no metastasis. The median (IQR) Total-TEQ (pg WHO_{2005}-TEQ/g fat) was 24.1 (16.1–35.2). No statistically significant associations were observed for PCDD/F-TEQ or DL-PCB-TEQ, neither when measured in serum nor in adipose tissue.

Fiolet et al. (2022), reporting on the EPIC study (*n* cases = 13,241; median follow-up: 14.9 years), evaluated the associations between dietary intake of 17 PCDD/Fs and 35 PCBs and breast cancer. Median (IQR) dietary intakes for PCDD/F-TEQ, DL-PCB-TEQ, Total-TEQ and NDL-PCBs were 19 (13.9–25.5) pg. TEQ/day, 40.1 (28.6–56.4) pg. TEQ/day, 60.1 (44.1–82.0) pg. TEQ/day, and 572 (319–732) ng/day, respectively. No statistically significant association was observed for Total-TEQ, PCDD/F-TEQ or DL-PCB-TEQ.

Hryhorczuk et al. (2022) assessed the association between serum levels of the 17 PCDD/Fs plus 4 DL-PCBs and t(14;18) chromosomal translocation frequencies in blood in 218 former chemical plant workers and 150 participants from the general population. The workers had significantly higher geometric mean serum levels of Total-TEQ than the general population participants (73.8 vs. 17.7 pg. WHO_{2005}-TEQ/g lipid). No statistically significant association was observed with t(14;18) chromosomal translocation frequencies.

In the two studies which reported on the 12 DL-PCBs only without indication that these dominated the total TEQ, no association was observed between the estimated dietary exposure to DL-PCBs and non-Hodgkin's Lymphoma (E3N study, France, n cases = 457) (Lemaitre et al., 2021). A statistically significant higher HR was reported in a prospective case-cohort study within the Korean National Cancer Center Community Cohort (*n* incident cases = 118) between DL-PCB-TEQ in serum and lung cancer (Park et al., 2020).

In summary, the CONTAM Panel noted that based on the overall body of evidence, complemented by the new studies identified, does not support a clear link to any specific cancer site and that, due to the lack of clear dose–response relationship and multiple co-exposures, the CONTAM Panel does not consider these studies suitable for the risk assessment.

3.1.3.7 | *Thyroid disease and thyroid hormones*

In the 2018 Opinion it was concluded that in adults, the epidemiological studies provided insufficient support for an association between TCDD, other PCDDs, PCDFs or DL-PCBs exposure and thyroid disease or thyroid function. As indicated in the 2018 Opinion, "*a causal association between relatively high TCDD exposure and increased TSH in newborns in Seveso is likely*". However, studies with low to moderate exposure to TCDD, other PCDDs, PCDFs or DL-PCBs did not suggest any adverse effects on the thyroid function in children (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, seven new eligible studies were identified. Three studies investigated European populations (Denmark, Germany, Italy), three assessed the thyroid hormone profile in children and, in another three, both PCDD/F, DL-PCBs and thyroid hormones were measured in human milk. None of the new studies assessed thyroid disease status as a study endpoint. Details of these studies are reported in Annex F (Table F7 therein).

Warner, Rauch, Ames, Mocarelli, Brambilla, Signorini, and Eskenazi (2020) evaluated the association of prenatal exposure to TCDD with thyroid hormone concentrations in the prospective Seveso Second Generation Study (children and adolescents, 27%; adults, 73%; $n = 570$). The maternal initial (1976) serum TCDD level was 60.2 (median; IQR: 28.4, 156.0; ng/kg lipid) and the estimated maternal TCDD at pregnancy was 14.4 (median; IQR: 6.4, 33.3; ng/kg lipid; range: 0.1, 1786 ng/kg lipid). Compared to the lowest quartile, maternal initial serum TCDD was associated (β_{adj}, 95% CI) with lower FT3 (Q2: −0.13, −0.26 to 0.00; Q3: −0.22, −0.35 to −0.09; Q4: −0.14, −0.28 to 0.00; *p*-trend = 0.02).

Baba et al. (2018) reporting on the Hokkaido Study on Environment and Children's Health, evaluated the association between maternal serum PCDD/F or DL-PCB levels (23–41 weeks of gestation; median Total-TEQ: 13.8 pg. TEQ/g lipid) and maternal (median, ten weeks of gestation; $n = 386$) and neonatal ($n = 410$) thyroid hormone levels. Increased Total-TEQ was associated with increased neonatal FT4 ($\beta = 0.224$; 95% CI, 0.016–0.433).

Li et al. (2018) in a cross-sectional study, nested within a nested case–control study on cryptorchidism, evaluated the association between 14 PCDD/Fs plus 12 DL-PCBs and thyroid function profile (T4, T3, rT3) both measured in placental samples in Denmark ($n = 58$). No statistically significant association was observed for Total-TEQ.

Muzembo et al. (2019) in a cross-sectional study in Japan ($n = 490$) assessed the association between the serum levels of the 17 PCDD/Fs plus 12 DL-PCBs and various biomarkers including T3 and T4 in human milk. No statistically significant association was observed for Total-TEQ.

Li et al. (2019) in a cross-sectional study within the LUPE cohort in Germany evaluated the association between the levels of the 17 PCDD/Fs plus the 12 DL-PCBs in human milk and total thyroxine (TT4), total triiodothyronine (TT3), and total reverse T3 (TrT3) (also measured in human milk, n = 99). No significant associations were reported.

In a cross-sectional study in Uganda, Matovu et al. (2021) evaluated the association between the levels of the 17 PCDD/Fs plus 12 DL-PCBs and TT4, TT3, TrT3, all measured in human milk (n = 30). The median levels of PCDD/Fs and DL-PCBs in the samples were 0.4 pg. TEQ/g fat and 1.16 pg. TEQ/g fat, respectively. For T3, a statistically significant inverse association was observed for DL-PCB-TEQ (β, 95% CI; −0.170, −0.325 to −0.015) and for PCDD/F-TEQ (β, 95% CI; −0.107, −0.213 to 0.000) in the adjusted analysis.

Pham et al. (2024) evaluated in a cross-sectional study the impact of PCDD/F exposure on thyroid hormone levels in military men working in contaminated sites in central and southern Vietnam and the Sao Vang airbase in northern Vietnam (n = 136). The mean serum PCDD/F-TEQ concentration was 30.2 pg. TEQ/g lipid. For higher FT4 levels, statistically significant associations were observed for PCDD-TEQ (β, 95% CI; 0.26; 0.09–0.44) and for PCDD/F-TEQ (β, 95% CI; 0.26; 0.09–0.43). For higher FT3 levels, a statistically significant association was observed for PCDD/F-TEQ (β, 95% CI; 0.18, 0.001–0.35).

In summary, incorporating the few new mainly small studies into the accumulated evidence that is characterised by mostly cross-sectional data and bearing inconsistencies in the direction of associations, the CONTAM Panel considered that the epidemiological studies provided insufficient support for an association between Total-TEQ, PCDD/F-TEQ, and DL-PCB-TEQ and thyroid function.

3.1.3.8 | *Effects on the nervous system*

The 2018 Opinion noted that only few neurodevelopmental outcomes had been studied in children in several cohorts and/or at similar age, and that the evidence was not sufficient to form a basis for the risk assessment (EFSA CONTAM Panel, 2018).

For effects on the nervous system after exposure in adult life, the studies focused on cohorts occupationally exposed to high levels of TCDD, that showed associations between exposure and different neurophysiological and neuropsychological outcomes. However, estimations of the original exposures were uncertain and some studies were limited by a low number of participants (EFSA CONTAM Panel, 2018).

Neurodevelopment in children

Since the 2018 Opinion, sixteen eligible new publications (including twelve primary and four follow-up publications) were identified. Details of these studies are reported in Annex F (Table F8 therein).

Two studies investigated European populations (Italy, Spain) and five were prospective in design. Neurodevelopment in infants using the age-appropriate Bayley Scales of Infant Development (BSID) was assessed in four studies and the other endpoints assessed neurodevelopment in older children via event-related (one study), Wisconsin Card Sort (one study), Raven's Progressive Matrices (one study), Connor's Continuous Performance (one study), Rey's Auditory Verbal Learning (one study), Kaufman Assessment Battery for Children (KABC-II, one study), Movement Assessment Battery for Children (MABC-2, one study), C-SHARP Aggression Scale (one study), Attention-Deficit/Hyperactivity Disorder (ADHD) or learning disability attributes (two studies), Autism Spectrum Rating Scale (one study), gaze behaviour (two studies), brain potential recording, mirror neuron activity or electroencephalogram (EEG) characteristics (four studies), eating behaviour (one study), and behavioural sexual dimorphism (one study).

Kou et al. (2024) reporting on the ECLIPSES study in Spain assessed the association between maternal dietary exposure to PCDD/F or DL-PCB from fish consumption and neurodevelopment at 40 days of age (Bayley Scales of Infant Development - 3rd Edition (BSID-III); n = 460). No statistically significant association was observed for Total-TEQ, PCDD/F-TEQ or DL-PCB-TEQ and BSID-III scores.

Yim et al. (2022) reporting on the prospective Hokkaido birth cohort study in Japan assessed the association between maternal serum PCDD/F or DL-PCB levels and neurodevelopment at 6 months of age (BSID-II; n = 259) using a multipollutant approach. No statistically significant association was observed for Total-TEQ.

Yamazaki et al. (2022) also reporting on the same study assessed cognitive function in older children (11–14 years old; n = 55). The median Total-TEQ in maternal serum was 17.2 ng/kg lipid. No statistically significant association was observed for Total-TEQ.

Ames et al. (2019) reporting on the Seveso Second Generation Health Study evaluated the association between TCDD in serum and neurodevelopment in children aged 7–17 years old (n = 161) using a battery of tests (Wisconsin Card Sort, Raven's Progressive Matrices, Connor's Continuous Performance, Rey's Auditory Verbal Learning). Overall, maternal serum TCDD was not adversely associated with the assessed endpoints.

Pham TN et al. (2019) assessed the association between exposure to 17 PCDD/Fs and 4 DL-PCBs (PCB-77, −81, −126, −169) in human milk and neurodevelopment at 2 years of age (BSID-III) in Vietnam. Three birth cohort studies were recruited and followed-up for 2 years; two from the contaminated areas (n = 210 pairs, 2012; n = 78 pairs, 2015) and one (n = 120 pairs, 2014) from an unexposed area. Across the various analyses performed, statistically significant differences in means were observed for TCDD and gross motor and composite motor scores, for PCDD/F/non-*ortho* PCB-TEQ and gross motor in boys, and for PCDD-TEQ and the total Bayley scores (association estimates not provided).

Trang et al. (2022) reported on the 5-year follow-up assessment of cognitive (KABC-II) and motor (MABC-2) development of these studies (n = 150 boys, 139 girls). In the adjusted analyses, lower triangles score of KABC-II, and lower balance and lower total score of MABC-2 were associated with PCDD-TEQ, PCDF-TEQ and PCDD/F-TEQ in boys. Lower manual dexterity scores were also associated with PCDF-TEQ. In girls, associations were observed between lower hand movement scores and PCDD-TEQ, PCDF-TEQ and PCDD/F-TEQ, while lower balance scores in the MABC-2 were associated with PCDF-TEQ.

Nishijo et al. (2021) assessed the association between perinatal dioxin exposure related to Agent Orange in Vietnam and aggressive behaviour (using the C-SHARP Aggression Scale). Two birth cohort studies were recruited (159 mother-infant pairs, 2008; 82 mother-infant pairs, 2009) and followed up for 8 years. A statistically significant association was observed for TCDD and covert aggression subscale scores.[10] For PCDD/F-TEQ, no statistically significant associations were observed.

Pham, Nishijo, Pham-The, et al. (2022); Pham, Nishijo, Pham, et al. (2022) reporting on the same studies assessed ADHD symptoms at 5 and 8 years of age. In boys, TCDD was statistically significantly associated with hyperactivity and ADHD scores at 5 years of age (low vs. high exposure). In girls and for PCDD/F-TEQ, a statistically significant association was observed for hyperactivity at 8 years of age. In girls, ADHD Rating Scale (ASRS) unusual behaviour scores were significantly higher with greater TCDD exposure.

Vu, Nishijo, et al. (2021), reporting on the same studies, assessed mirror neuron activity at 9 years of age. For TCDD, in both brain hemispheres, the log power ratio in the theta band was significantly higher during observation of hand movements in girls in the high exposure group. For PCDF-TEQ, again in both brain hemispheres, a statistically significant association was observed between the log power ratios during hand movement observation for the theta wave characteristics and exposure levels (high vs. low exposure groups) in boys. In contrast, for PCDD/F-TEQ, no statistically significant associations were observed.

In the remaining seven studies (all performed in Vietnam) which reported either on the 17 PCDD/Fs only or the 12 DL-PCBs only without indication that these dominated the total TEQ, no association was observed between the estimated dietary DL-PCBs and gaze behaviour or autistic behaviour or overall BSID-III scores (n cases = 50) (Thao et al., 2023), while statistically significant associations were observed in the remaining studies for EEG characteristics (two studies), gaze behaviour (one study), eating behaviour (one study), learning disability attributes (one study), and behavioural sexual dimorphism (one study).

In summary, although a considerable number of new publications complemented the cumulative evidence on the association between Total-TEQ, PCDD/F-TEQ, and DL-PCB-TEQ and neurodevelopment, the number of available studies per endpoint remained small. Moreover, many studies did not implement a prospective study design (among other methodological limitations), and the observed statistically significant associations were not replicated across studies. Based on the above, the CONTAM Panel concluded that the available epidemiological evidence does not provide sufficient support for an association between Total-TEQ, PCDD/F-TEQ, and DL-PCB-TEQ and neurodevelopment.

Effects on the nervous system after exposure in adult life

Since the 2018 Opinion, five newly eligible studies (seven publications) were identified. Details of these are reported in Annex F (Table F9 therein).

Three of these studies were conducted in European countries (France, Czech Republic and Italy). Across all seven publications, the sample size ranged from 8 to 1288 participants. No study assessed exposure to all 29 PCDD/Fs and DL-PCBs in background exposure settings. One study assessed exposure to the 17 PCDD/Fs plus 4 DL-PCBs under background exposure conditions; another examined the 17 PCDD/Fs in occupational settings, one study assessed exposure to 2,3,4,7,8-PeCDF (Yusho), one assessed exposure to TCDD (Seveso), and three studies examined the 17 PCDD/Fs only without indicating whether these congeners dominated the total TEQ. The endpoints studied included brain structural and functional characteristics via EEG and/or MRI assessment (one study population; 3 publications), neurocognitive function (one study), neurophysiological measurements (one study), physical functioning (one study), sleep disorders (one study), and dementia (one study).

Statistically significant associations were identified in the three publications from the small study conducted in Vietnam, across multiple analyses on different brain structural and functional characteristics (Thao et al., 2024; Vu et al., 2023; Vu, Pham, et al., 2021). Moreover, Kondo et al. (2018), in a study including Yusho patients ($n = 140$) and among the numerous analyses performed, reported that the prevalence of moderate to severe difficulty of initiating sleep was high in patients with a fourth quartile 2,3,4,7,8-PeCDF[11] blood concentration (53.3%, $p = 0.02$), and that, after adjustment for age, sex, and BMI, the odds ratio for Pittsburgh Sleep Quality Index Global Score (PSQI GS) ≥ 8 was 4.84 (95%CI, 1.10–21.25). No significant association was found between 2,3,4,7,8-PeCDF blood concentration and PSQI GS ≥ 6.

In summary, based on the overall body of evidence, complemented by the limited number of newly identified studies per endpoint, and taking into consideration the limited prospective data, and the lack of consistency between studies, the CONTAM Panel concluded that the available evidence does not provide sufficient support for an association between exposure to PCDD/Fs-TEQ or DL-PCB-TEQ and neurological outcomes in adults.

[10]The C-SHARP (Children's Scale of Hostility and Aggression: Reactive/Proactive) includes a covert aggression subscale, which assesses behaviours like sneering, making faces, and other subtle forms of aggression that may not be immediately obvious as aggressive.

[11]Corresponding to ≥ 7.2 pg. WHO_{2022}-TEQ/g lipid calculated using a WHO_{2022}-TEF of 0.1.

3.1.3.9 | *Effects on teeth and bone*

The 2018 Opinion concluded that "*childhood exposure to TCDD and/or other PCDD/Fs was dose-relatedly associated with tooth enamel hypomineralisation or enamel defects*". Hypomineralisation of permanent teeth was "*likely to be causally related to exposure and is likely to be a postnatal effect*". It was also concluded that the limited evidence from a single cohort suggested associations between PCDD/F and DL-PCB exposure and some changes in bone parameters (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, no new studies were identified on effects on teeth and bone.

3.1.3.10 | *Type 2 diabetes and obesity*

The 2018 Opinion concluded that the available studies on diabetes and obesity were inconclusive and could not be used as a basis for the risk assessment (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, ten new eligible studies were identified. Details of these studies are reported in Annex F (Table F10 therein).

Sample sizes ranged from 8 to 318,416 participants and four of these studies were longitudinal. Five of the studies were conducted in European countries (France, Czech Republic, Italy). Six studies assessed exposure to the 29 PCDD/Fs and 12 DL-PCBs in background exposure conditions, one study assessed exposure to the 17 PCDD/Fs plus 9 DL-PCBs in a background exposure setting, one study assessed exposure to TCDD in an occupational setting, three focused on TCDD exposure (related to the Seveso incident), and four studies examined the 12 DL-PCBs alone, without indicating whether these congeners dominated the total TEQ. The endpoints studied included type-2 diabetes, gestational diabetes, glucose homeostasis, BMI, obesity/overweight, and metabolic syndrome.

Overall, statistically significant associations were observed in four studies that assessed: gestational diabetes (one study), type 2 diabetes (three studies), BMI (two studies), weight gain (one study), overweight (one study) and obesity (one study) (Chetrit et al., 2024; Muzembo et al., 2019; Pavuk et al., 2023; Warner et al., 2019). Among these studies two were longitudinal and reported statistically significant associations of an opposite direction. One of these is pertaining to dietary Total-TEQ and the risk of weight gain [HR (CI 95%): 1.07 (1.05–1.10)], overweight [1.03 (1.01–1.05)] and obesity [1.08 (1.04–1.12)] [Chetrit et al., 2024]. The other two studies related to BMI where a 10-fold increase in initial maternal TCDD concentration was inversely associated with BMI in the daughters of the Seveso study participants (βadj, 95% CI; children ≥ 18 years old, −0.99 kg/m^2, −1.86 to −0.12; children 2–17 years old, −0.59 kg/m^2, −1.12 to −0.06) and a 10-fold increase in TCDD estimated at pregnancy was inversely associated with insulin (β = −1.24 μIU/mL, 95% CI: −2.38, −0.09) and HOMA2-B (adj-β = −10.17%, 95% CI: −17.76, −1.88) (Warner et al., 2019). Of note, Warner et al. (2019) reported in the same study that in the Seveso sons, initial maternal TCDD was associated with increased risk of metabolic syndrome (RR = 2.09, 95% CI 1.09, 4.02).

In summary, based on the overall body of evidence, complemented by the limited number of newly identified studies per endpoint, and taking into consideration the lack of prospective data and the lack of consistency between studies, the CONTAM Panel considered that the available epidemiological evidence does not provide sufficient support for an association between Total-TEQ, PCDD/F-TEQ, and DL-PCB-TEQ and increased risk of diabetes and obesity/overweight.

3.1.3.11 | *Effects on the immune system*

The 2018 Opinion concluded that some studies suggested adverse effects on the immune system at background exposure during development, but the available studies did not provide sufficient evidence for an association between exposure to PCDD/Fs or DL-PCBs and the functionality of the immune system.

Since the 2018 Opinion, three new eligible studies were identified. Details of these studies are reported in Annex F (Table F11 therein).

One of these studies was conducted in Europe (Italy). Across all studies, the sample size ranged from 53 to 438 participants. One study assessed exposure to the 17 PCDD/Fs and 12 DL-PCBs in background exposure conditions, one study assessed exposure to the 17 PCDD/Fs plus the 12 DL-PCBs in an occupational setting, and one assessed exposure to TCDD (Seveso). The endpoints studied included T-lymphocyte subpopulations and immunoglobulin levels (two studies), wheezing (one study), and atopic conditions (eczema, asthma, hay fever, one study).

Statistically significant associations were observed for DL-PCB-TEQ and CD3+, CD4+/CD8+ ratio, IgE, IgG, and IgM levels in adults in one study (Kim et al., 2018). Miyashita, Ait Bamai, et al. (2018), reporting on the Hokkaido study (n = 327) and among the multiple analyses performed, observed a statistically significant protective association between maternal serum Total-TEQ and frequency of wheezing at age 3.5 years (OR, 95% CI; 0.03, 0.00–0.94) and an opposite statistically significant association between maternal serum total TEQ and frequency of wheezing at age 7 years (OR (95% CI): 7.81 (1.42–42.9)). Furthermore, an association in boys between maternal Total TEQ and lower cord blood IgE (partial regression coefficient (95% CI); − 0.87 (− 1.68 to −0.06)) was observed. In the Seveso second generation study a 10-fold increase in maternal initial 1976 serum TCDD ($\log_{10}$TCDD) was inversely associated with eczema (RR_{adj} = 0.63; 95% CI: 0.40, 0.99) but not with asthma or hay fever. Furthermore, maternal TCDD estimated at pregnancy was not significantly associated with any of the three atopic outcomes (Ye et al., 2018).

In summary, based on the overall body of evidence, complemented by the limited number of newly identified studies per endpoint, and taking into consideration the limited prospective data, and the lack of consistency between studies, the CONTAM Panel considered that the available evidence does not provide sufficient support for an association between exposure to PCDD/Fs-TEQ or DL-PCB-TEQ during development and the functionality of the immune system.

3.1.3.12 | *Cardiovascular effects*

The 2018 Opinion noted an increased risk of cardiovascular mortality based on an epidemiological study of individuals with very high occupational exposure to TCDD (serum TCDD > 1000 ng/kg fat, Steenland et al., 1999). At lower exposure to TCDD, other PCDDs, PCDFs or DL-PCBs, epidemiological studies provided insufficient support for an association with cardiovascular risk (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, two studies were identified. Details of these studies are reported in Annex F (Table F12 therein).

Pelcl et al. (2018) reported a higher prevalence of hypertension, atherosclerotic plaques in the carotid arteries and eye hypertensive angiopathy in eight subjects occupationally exposed to TCDD during the production of herbicides, compared to male population of similar age. Pavuk et al. (2019) carried out a longitudinal study in the follow up of the Anniston Community Health Survey cohort ($n = 338$) and reported a statistically non-significant association between Total-TEQ and hypertension.

In summary, based on the overall body of evidence, complemented by the limited number of newly identified studies per endpoint, and taking into consideration the limited prospective data and the lack of consistency between studies, the CONTAM Panel considered that the available evidence does not provide sufficient support for an association between exposure to PCDD/Fs-TEQ or DL-PCB-TEQ and cardiovascular effects.

3.1.3.13 | *Hepatic disorders and digestive effects*

The 2018 Opinion concluded that the evidence from accidental or occupational exposure studies was insufficient for a causal association with hepatic or digestive diseases (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, one study was identified reporting on associations between blood PCDD/F and DL-PCBs TEQ and liver function (Muzembo et al., 2019). No statistically significant association was found between blood Total-TEQ and AST, ALT and GGT in Japanese adults with background exposure (for details see Annex F, Table F13 therein).

In summary, based on the overall body of evidence, complemented by only one study, the CONTAM Panel considered that the available evidence does not provide sufficient support for an association between exposure to PCDD/Fs-TEQ or DL-PCB-TEQ and hepatic or digestive diseases.

3.1.3.14 | *Chloracne and other dermal effects*

The 2018 Opinion concluded that chloracne is the most unequivocal toxicity outcome, observed in accidental, occupational and unresolved poisoning cases with PCDD/Fs and DL-PCBs, and with children appearing to be particularly sensitive. It was noted that chloracne only occurs after relatively high exposures and appears to be less relevant for deriving an HBGV (EFSA CONTAM Panel, 2018).

Since the 2018 Opinion, one study was identified. Leijs, Esser, et al. (2018) found no statistically significant correlation between serum PCDD/F-TEQ and acneiform lesions on the skin in workers from a transformer recycling company ($n = 92$; mean (range) serum PCDD/F-TEQ levels: 15.47 (2.70–56.16) pg. WHO_{2005}-TEQ/g lipid) (for details see Annex F, Table F14 therein).

In summary, the new study identified does not change the previous conclusion of the CONTAM Panel.

3.1.3.15 | *Other effects*

Since the 2018 Opinion, several studies have been identified related to the exposure to PCDD/Fs and DL-PCBs and effects other than the ones described in previous sections. Details of these studies are reported in Annex F (Table F14 therein).

Four studies examined the association with mortality or morbidity in cohorts of workers at an agrochemical plant (McBride et al., 2018; 't Mannetje et al., 2018) or in background exposed populations (Fiolet et al., 2024; Ji et al., 2022). The limited available evidence on the associations between exposure to PCDD/Fs and DL-PCBs and all-cause mortality, disease-specific mortality, or morbidity attributes was inconclusive.

Three studies focused on associations with physical function (Yusho patients; Fukushi et al., 2019) and lung related function in occupationally and non-occupationally exposed subjects (Leijs, Esser, et al., 2018; Leijs, Koppe, et al., 2018; Zhang et al., 2020). Serum TEQ levels were negatively associated with functional reach and hand grip strength in men, while statistically significant correlations were found between Total-TEQ or PCDD/F-TEQ and VC MAX and FEV1, but with opposite effect directions in the two studies. The limited available evidence on the associations between the exposure to PCDD/Fs and DL-PCBs and physical and lung function was inconclusive.

One study reported on the Seveso Second Generation Study and evaluated the association between in utero exposure to TCDD and right hand 2D:4D digit ratio ($n = 594$), which is considered an indirect marker of prenatal androgen (particularly testosterone) exposure during the second trimester (Slama et al., 2019). No statistically significant associations were observed either for the maternal initial serum TCDD concentration or as TCDD extrapolated to pregnancy.

One study examined the association of PCDD/Fs and DL-PCBs and gut microbiome (De Filippis et al., 2024). The CONTAM Panel noted that it is still unclear what the clinical implications of changes in gut microbiome are.

Another set of studies examined the associations between PCDD/F and/or DL-PCB exposure and telomer length (Alonso-Pedrero et al., 2022), DNA methylation (Su et al., 2019; Kelsey et al., 2019; Nwanaji-Enwerem et al., 2020; Pittman et al., 2020; Rytel et al., 2021; Mattonet, 2022; Stockinger et al., 2024; Corsaro et al., 2024), DNA oxidative damage (Zhang et al., 2019) and mutation rates (Ton et al., 2018). The CONTAM Panel noted that these effects are not quantitatively related to specific diseases and were not further considered for the identification of a Reference Point (these studies were not described in Annex F).

In summary, the CONTAM Panel considered that the evidence related to these endpoints cannot support any robust conclusions. Therefore, these studies were not further considered for the risk assessment.

3.1.4 | Mode of action

The 2018 Opinion (EFSA CONTAM Panel, 2018) included a comprehensive overview of the mode of action of PCDD/Fs and DL-PCBs. The CONTAM Panel concluded that:

- *Binding to the AHR is the molecular initiating event of the toxicities of PCDD/Fs and DL-PCBs. Toxicity is due to inappropriate (in terms of timing, location and/or degree) and sustained activation of the AHR.*
- *AHR signalling can proceed* via *canonical or alternative pathways. The major toxicities of PCDD/Fs and DL-PCBs appear to be primarily mediated by the canonical pathway, in which the AHR acts as a ligand-activated transcription factor.*
- *In animal models, structural variations in the ligand-binding or transactivation domain of the AHR are associated with non-selective or selective differences, respectively, in sensitivity to the manifestations and patterns of TCDD toxicity.*
- *The human AHR has a lower binding affinity to TCDD when compared to rats and most mouse strains.*
- *PCDD/Fs and DL-PCBs affect the expression of a large number of genes and these seem to be species- and congener-dependent, indicating additional modes of action.*

Since the 2018 Opinion (EFSA CONTAM Panel, 2018) several new studies on the mechanism of action have become available. The CONTAM Panel noted that the new information identified does not challenge the previous conclusions but adds to the knowledge on the mode of action connected to the canonical pathway. The CONTAM Panel noted that non-canonical pathways have been described for adverse effects of PCDD/Fs and DL-PCBs (as reviewed by Sondermann et al., 2023) and may also contribute to the effects on the male reproduction system. Also, direct and indirect interactions between ligand activated AHR and steroid receptors (AR, ER) resulting in alteration of steroid receptor levels have been documented (Ohtake et al., 2007; Tijet et al., 2006; Yoshida et al., 2020).

A hypothetical AOP for dioxin-induced rat male reproductive toxicity was published by Johnson et al. (2020). Earlier published AOPs connected AHR binding of PCDD/Fs and DL-PCBs to liver tumour promotion in rodents (Becker et al., 2015; Budinsky et al., 2014; EFSA CONTAM Panel, 2018). None of these have been endorsed by the OECD so far.

The section below summarises the knowledge on the mode of action of TCDD, focusing on male reproduction, i.e. the critical effect identified from experimental animals and epidemiological studies (see **Section 3.1.4.1**). The Panel acknowledges that also female reproduction can be affected, as reviewed by Aldeli et al. (2024), but also note that such effects occur with high variability and at higher exposure body burden than the male reproductive effects. In addition, difference in sensitivity towards TCDD in rodents and humans are discussed (see **Section 3.1.4.2**).

Lower male to female sex ratio at birth, developmental effects on mineralisation of teeth, and higher TSH levels in newborns were identified in the 2018 Opinion as possible critical effects (see **Section 3.1.5**). However, since no new studies that changed the previous conclusions were identified related to these outcomes, their modes of action are not further discussed in the present Opinion.

3.1.4.1 | *Male reproductive toxicity*

This chapter is focused both on new information and on data considered/available in the 2018 Opinion relevant for the mode of action of adverse effects of TCDD (and dioxin-like compounds if tested) on the male reproductive system. TCDD has been mostly studied and there is little knowledge on male reproductive effects of other congeners. A study with a single dose of either PCB-77 or PCB-126 applied at GD15, with a TEQ-dose equivalent to toxic TCDD doses failed to reproduce similar effects on sperm count decrease to those detected by exposure to TCDD, but detected a decrease in ventral prostate weight and anogenital distance (Appendix E, Faqi et al., 1998b). This can be considered a biomarker of risk of male subfertility related to a deregulation of androgen/oestrogen equilibrium (Priskorn et al., 2018). The text below is structured following the lines of evidence for the presence and role of the AHR, the effects of TCDD on biochemical, structural and functional parameters including effects on sperm production, puberty and sexual behaviour with a special focus on the effects of exposure during early stages in life, i.e. during gestation and after birth. It is not clear to what extent transcriptional and post-transcriptional effects, including interaction with other receptors, contribute to these effects. No dose–response considerations are included since many studies used one dose level only.

3.1.4.1.1 | *Expression of the AHR in the male reproductive system and the hypothalamus-pituitary-gonadal axis*

In mice spermatozoa, the AHR is mainly located in the mature sperm flagella and acrosome (Hansen et al., 2014). However, in rats AHR is also expressed in the primary pachytene spermatocytes during stages VII–XI and round spermatids during stages II–XIV of the spermatogenic cycle of the seminiferous epithelium. In adult human testis, the AHR is expressed in all cell types, including interstitial and tubular cells (Schultz et al., 2003). However, in human developing testis, between 7 and 20 weeks of gestation, AHR expression is only detected in developing germ cells (not in other testicular cells). This expression increases with gestation, with the AHR expressed in all germ cells in the first trimester but only in a subpopulation in the second trimester (Gaskell et al., 2004).

Furthermore, the *A*HR is not only expressed in cell types of the gonads but in the entire rat hypothalamus-pituitary-gonadal axis. The effects of PCDD/Fs and DL-PCBs on reproductive organs and their regulatory systems depend on the timing of exposure, whether it occurs during development or at adult age. In neonatal rat brain, the *AHR* is expressed in virtually all GABA/glutamatergic neurons also containing oestrogen receptors within the anteroventral periventricular nucleus of the hypothalamus (Hays et al., 2002). These neurons are there important for regulation of the gonadotropin-releasing hormone neurons and thus for puberty onset in both genders as well as for ovulation in the female (Clarkson & Herbison, 2006). In the pituitary, TCDD strongly induced the levels of cytochrome P4501A1 (*CYP1A1*) mRNA and protein (Huang et al., 2002) indicating that the pituitary is a putative target for dioxins.

The AHR plays a key role in the effects of TCDD on reproduction, including germ cell development and function, as was shown in studies in *AHR*-knockout mice (Huang et al., 2016). In male mice, a constitutively active AHR (no activating ligand required) resulted in decreased weights of testis and ventral prostate and reduced epididymal sperm reserve (Brunnberg et al., 2011), effects also seen in *A*HR-wild-type mice which had been exposed to TCDD during development (see below).

3.1.4.1.2 | *TCDD, the AHR and adverse effects on male reproduction*

Effects of TCDD on expression of genes involved in the development of the male reproductive system have been studied in various experimental models. These might have been mediated directly by the AHR and/or indirectly, e.g. via non-canonical pathways.

The CONTAM Panel noted that several of the studies on adverse effects of TCDD were carried out at one (or two) dose levels only. In several instances, these levels were relatively high, e.g. at levels above 10–100 μg/kg bw per day, limiting their interpretation with respect to (a) relevant mode(s) of action. Nevertheless, such studies were not automatically excluded if they were considered informative.

In adult rats, TCDD (10 μg/kg bw per day over 56 days) caused a significant downregulation of the expression of steroidogenic enzymes: *17β-HSD, 3β-HSD, StAR, CYP11A1 and CYP17A1* (Tahir et al., 2024). *17β-HSD* is primarily expressed in the testes and plays a role as a rate-limiting enzyme for the testosterone biosynthesis process.

TCDD interferes with the regulation of the expression of genes involved in the reproductive pathway in experimental animals, as reviewed by Faiad et al. (2022). These include genes known to be regulated upon TCDD treatment, such as 3-hydroxysteroid dehydrogenase (*3β-HSD*) and steroidogenic acute regulatory protein (*StAR*) (Baba et al., 2008), prostatic secretory protein 94 (*PSP94*) and major androgen-dependent secretory protein (*MP25*) (Fritz et al., 2005; Lin et al., 2002), and those detected after exposure of rat Leydig cells to TCDD, such as cytochrome P450 side chain cleavage (*P450scc*) (Lai et al., 2005). In pubertal mice, treatment with 25 μg/kg bw resulted in histopathological changes in the testicular germinal epithelium and interstitial tissue, a reduction in serum testosterone and reduced sperm count (Faiad et al., 2023).

In addition, in Leydig cells, the oestrogen receptor *α* (ERα) as well as two ER-related proteins: Esrra and Esrrg exhibit AHR-dependent expression in male mice exposed by gavage to a single high dose of 1000 μg/kg bw TCDD in a study comparing *AHR (−/−)* (*AHR*-knockout) to *AHR (+/+) (wild type)* mice (Tijet et al., 2006). This study suggests TCDD may also interfere (at least at high dose) with oestrogen signalling by ERα itself and two of its closely related proteins, Esrra and Esrrg.

Exposure of seminiferous tubular segments from rat testis to TCDD in vitro resulted in increased apoptosis in seminiferous tubule cells (Schultz et al., 2003). In both rats and humans, the AHR level in epididymis is higher than in the testis (Wajda et al., 2017). The downregulation of AHR after TCDD exposure has been detected in spermatogenic cells at different stages as well as in epithelial cells of the epididymis, but not in Sertoli and Leydig cells (Wajda et al., 2017).

In the study by Baba et al. (2008), testosterone levels and Leydig cell numbers were determined in both wild-type and *AHR* (−/−) adult mice. While testosterone levels were clearly lower in *AHR* (−/−) mice, the Leydig cell number was similar in *AHR* (+/+) and *AHR* (−/−), suggesting that the low level of serum testosterone is not due to a decrease in Leydig cell number but rather to the reduced ability to produce testosterone, probably conditioned by the low expression levels of *3ß-HSD* and *StAR* in the mouse *AHR* (−/−) testis, key elements in testosterone synthesis.

The AHR also mediates toxicological processes involving oxidative stress in adult animals. TCDD significantly decreased the activities of SOD, CAT, GPx, and GSR, whereas it increased the level of malondialdehyde (MDA) and reactive oxygen species (ROS) in the testis of 45-day old male Wistar rats administered orally for 45 days with TCDD (Latchoumycadane et al., 2003). The process that includes the reduction in antioxidant enzymes as well as an increase in lipid peroxidation (al-Bayati et al., 1988) and ROS formation indicates the build-up of oxidative stress due to TCDD (at 10 μg/kg bw per day) and a disproportion between pro-oxidants and antioxidative enzymes in testicular tissues (Tahir et al., 2024). Changes of this type were also observed in the testes of adult mice which had been treated for 4 days with 1 μg TCDD/kg bw per day (Jin et al., 2008).

3.1.4.1.3 | *The role of the hypothalamus-pituitary-gonadal axis in male development*

As outlined in EFSA CONTAM Panel (2018), the pituitary gland, that plays a key regulatory role in sexual development, is increasingly recognised as a target for TCDD. Alterations in the production and/or release of gonadotropins in the pituitary gland have been shown to result in an impairment of sexual functions and in sexually dimorphic behaviours after the offspring reaches maturity.

AHR ligands and AHR activation have been suggested to dysregulate fetal germ cell development by induction of apoptosis in developing germ cells (Coutts et al., 2007), potentially generating a reduction in the number of spermatozoa in adults. Exposure to TCDD of adult rats increased the testicular expression of caspase 3 (*Casp3)* and *Bax* (markers of apoptosis), while decreasing *Bcl-2* expression (inhibitor of apoptosis) (Ijaz et al., 2023).

When exposed to TCDD prenatally (on GD15 or earlier), rat pups showed reduced serum LH and FSH levels due to diminished synthesis of the mRNA for their b-subunits in the pituitary (Mutoh et al., 2006; Takeda et al., 2009). The lowered pituitary gonadotropin gene expression is specific for the fetus and does not occur in adult rats after either oral or intracerebroventricular injection of TCDD. Furthermore, it imprints persistent defects in sexual behaviour (discernible at adulthood) as well as in the maturation of gonadal tissues (Takeda et al., 2009). Its primary reasons appear to be perpetually declined gonadotropin-releasing hormone (GnRH) synthesis in the hypothalamus and induction of histone acetylases in the pituitary (Takeda et al., 2012; Takeda et al., 2014). Taketoh et al. (2007) indicated that the reduction in *Cyp17a1* expression in foetuses upon treatment of pregnant Wistar rats on GD15 with TCDD seemed to be specific for fetal stages, because 7day-old pups born from TCDD-treated dams did not exhibit any reduction in *Cyp17a1*. In addition, TCDD failed to reduce *Cyp17a1* expression in cultured fetal testis. The direct injection of LH into foetuses restored the altered *Cyp17a1* expression. The results suggest that maternal exposure to TCDD impairs the expression of testicular CYP17 in a fetal stage-specific manner.

Attenuation of the pituitary expression of gonadotropins, leading to reduced testicular steroidogenesis during the fetal stage was demonstrated to be AHR dependent, i.e. to be absent in AHR-knockout rats (Hattori et al., 2018). These results indicate an impairment of sexual functions and in sexually dimorphic behaviours after the offspring reaches maturity.

In C57/Bl6 mice, maternal exposure to TCDD at GD12 attenuated fetal pituitary gonadotropin mRNA expression gonadotropin-regulated steroidogenesis and GH expression on GD18 leading to the impairment of pup development and sexual immaturity. In general, AHR activation during the late fetal and early postnatal stages was required for these defects (Takeda et al., 2014) since DBA/2J mice bearing a low-affinity AHR required 20 to 100-fold higher doses than C57/Bl6 mice to exhibit similar effects. For some parameters, even a 100-fold higher dose did not result in a statistically significant effect in DBA/2J mice.

Other functions of the pituitary playing a role in sexual development are the production/release of prolactin and growth hormone (GH). Takeda et al. (2020) reported that TCDD treatment of pregnant/nursing rats suppressed the proliferation of prolactin-producing cells in the pituitary. The effect was not seen in AHR-knockout dams. TCDD treatment of rat dams attenuated proliferating GH-positive cells and their cyclin mRNA expression in the fetal pituitary gland. AHR-knockout foetuses were insensitive to TCDD treatment, indicating that the TCDD-induced reduction in growth hormone (GH) and in *Dapl1* (death associated protein-like 1) (a cell cycle regulator) expression was due to AHR activation (Hattori et al., 2021).

During postnatal development, a transient hypothalamic–pituitary-gonadal axis (HPG) activation (termed mini-puberty) occurs. In humans, the postnatal period of mini-puberty is usually early months after birth with a peak at 2–3 months in males and could be a little longer in females. It is characterised by an increase in gonadotropins such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone concentrations, both in males and females (Kuiri-Hänninen et al., 2014). In males, a deficient fetal HPG axis activation may lead to, e.g. a micropenis and/or cryptorchidism. The resulting testicular immaturity (Busch et al., 2023) includes a low number of Sertoli cells, which are crucial for future reproductive capacity (Rohayem et al., 2024). This may compromise the proliferation of both germ and somatic cells and the maturation of germ cells (Hadziselimovic et al., 2005). In addition, disrupted minipuberty may be associated with altered linear growth and neurobehavioural development, possibly due to perturbed early sex hormone signalling (Lucaccioni et al., 2021). The observations by Mocarelli et al. (2008) on the Seveso cohort of an increase of total sperm counts upon exposure of adolescents, was explained by the authors as an impact of TCDD on the specific hormonal regulation (FSH and 17β-estradiol) on Sertoli cells around puberty, resulting in increased Sertoli cell proliferation and hence increase of sperm concentration in adults (Johnston et al., 2004; Sharpe et al., 2003).

Another line of evidence points to the pineal gland, since TCDD reduced the serum levels of melatonin in rats (Linden et al., 1991). Pohjanvirta et al. (1996) suggested that this effect is due to enhanced extrahepatic metabolic degradation of melatonin. In fact, melatonin is a substrate of CYP1A1 and 1A2, e.g. in humans (Mokkawes et al., 2023). Melatonin directly regulates testosterone secretion by binding to its specific receptors on Leydig cells and has been reported as a protector of spermatogonia against oxidative damage (Heidarizadi et al., 2022). Furthermore, maternal melatonin is necessary for a normal somatic growth and postnatal development of reproductive organs of the offspring in rats (Díaz et al., 1999).

3.1.4.1.4 | *Multigenerational and transgenerational effects, epigenetic alterations*

As indicated in the 2018 Opinion epigenetic mechanisms may be altered by TCDD and related compounds mediated by AHR activation or affecting the *AHR* gene regulation (EFSA CONTAM Panel, 2018). Epigenetic changes induced by toxicants that affect the reproductive system, such as TCDD, can be transmitted by affecting the germ cells (sperm or/and eggs) to subsequent unexposed generations to toxicants, altering gene expression. The concept of generational toxicological processes is being assessed as a relevant mode of action to be considered. This includes TCDD-mediated epigenetic modifications in both germlines. For example, evaluating dose-related effects of TCDD in different studies in rodents such as rats exposed via the diet to 0.001, 0.01 or 0.1 μg/kg bw per day. No significant toxicity was noted in the F0 rats of either sex during the 90 days of TCDD ingestion prior to mating. Significant decreases in fertility and neonatal survival were observed in the F0 generation rats receiving 0.1 μg TCDD/kg per day; these effects precluded continuation of this high dose level in subsequent generations. At 0.01 μg TCDD/kg per day, fertility was significantly decreased in the F1 and F2 but not in F0 generation (Murray et al., 1979). But a re-examination of the original Murray et al. data found no statistically significant treatment-related changes in postnatal day 1 sex ratio in any generation of treated animals (Rowlands et al., 2006). Effects of toxicants observed in the F1 or F2 generations are considered multigenerational. The F3 generation is the first generation with no direct exposure to the toxicant, so this exposure would be considered ancestral, and effects would be considered transgenerational (Brehm & Flaws, 2019; Skinner, 2008).

Exposure of gestating F0 generation female (Sprague Dawley) rats to TCDD (100 ng/kg bw per day) by daily intraperitoneal injections from gestational day 8 to 14 was evaluated in the F1 to F3 generation. Kidney disease in males, pubertal abnormalities in females, ovarian primordial follicle loss and polycystic ovary disease were increased in F3 generation. Epigenomic analysis of F3 generation showed 50 statistically significant differentially DNA methylated regions (DMR) in promoters. However, no alteration in sperm numbers or sperm motility in F1 and F3 generation were detected in this study (Manikkam et al., 2012).

In mice, reduced fertility and an increased incidence of premature birth in F1 mice exposed in utero to TCDD as well as in three subsequent generations was reported (Bruner-Tran & Osteen, 2011).

In a study of male and female mice, Hall et al. (2023) found that TCDD caused differential expression of 433 microRNAs (miRNAs) in the testes and 426 miRNAs in ovaries. The mice were dosed at 10 μg/kg bw of TCDD by single intraperitoneal injections and then immediately paired to analyse the offspring. Some of these miRNAs expressed dioxin response elements (DRE) in their 3′ UTR and the induction of such miRNAs occur through a DRE-dependent pathway (Singh et al., 2020). MiRNAs also participate in epigenetic modifications that can be transgenerationally transmitted showing deregulation of key miRNAs involved in germ cell differentiation, without modifications in DNA methylation (Brieño-Enríquez et al., 2015). The interplay between TCDD-activated AHR signalling and epigenetic mechanisms, including potential pathways implicated in these effects, was reviewed by Patrizi and Siciliani de Cumis (2018) and Disner et al. (2021).

3.1.4.1.5 | *Cross-fostering studies*

A few studies addressed the question of whether adverse effects on reproductive development occur upon exposure via lactation only or if prenatal exposure is required. From studies with 'lactation only' exposure, as well as from cross-fostering studies, it became clear that postnatal exposure to TCDD via lactation can also lead to adverse effects on reproductive development including effects on thyroid function (Table 4). The pattern of effects is somewhat different from the pattern upon prenatal exposure. No relevant studies on exposure shortly after weaning were identified.

Usually, markers of puberty are defined by gross anatomic changes, such as preputial separation in males, but other hormonal and histological changes may be considered and is a continuous developmental process, and the accuracy of specific developmental stages in different species and strains is complex. In male mice, initial onset of puberty (spermatozoa in the testes) can occur as early as PND11-12 (Hoffmann, 2018), but seminiferous tubules with evidence of complete spermatogenesis is about PND35 (Picut et al., 2017). In male rats, puberty, considered as first spermiogenesis in tubules, occurs during juvenile period (PND21–32) and a burst of Sertoli cells and spermatogonia proliferation occurs from PND 5–15 (Picut et al., 2017). Peripubertal phase of development is considered about PND30 (Ojeda and Skinner, 2006).

In adult animals, only relatively high dose levels (50 μg TCDD/kg bw and above) exerted such effects.

TABLE 4 Studies in experimental animals on effects of 'lactation only'-exposure to TCDD on developmental effects in the offspring.

Animals	Dose levels	Parameters tested/outcome	References
Rats (dams) were treated on GD15 or untreated. Pups of untreated dams were cross-fostered to TCDD treated dams upon birth and vice versa. The four groups were: controls, animals exposed in utero (IU) only, exposed via lactation only (L) and exposed both in utero and via lactation (IU + L).	1 μg/kg bw	Outcomes in male offspring, relative to controls: IU + L exposure: – later preputial separation – lower relative prostate weight (PND63) – lower relative seminal vesicles weight (PND63) – lower daily sperm production (PND63) – lower epidydimal sperm reserves (PND63) – feminised sexual behaviour – decreased body weight IU exposure: – later preputial separation – lower plasma testosterone (PND63) – lower relative prostate weight (PND63) – lower relative seminal vesicles weight (PND63) – lower daily sperm production (PND63) – lower epidydimal sperm reserves (PND63) L exposure: – lower plasma testosterone (PND63) – lower relative prostate weight (PND63) – lower relative seminal vesicles weight (PND63) – lower epidydimal sperm reserves (PND63) – feminised sexual behaviour	Bjerke and Peterson (1994)

(Continues)

TABLE 4 (Continued)

Animals	Dose levels	Parameters tested/outcome	References
Rats (dams) were treated on GD15 and pups were cross-fostered with dams treated at PND1	1 μg/kg bw	Exposure via lactation but not prenatally led to a decrease in T4 and an increase in TSH in the offspring; no information on body weight	Nishimura et al. (2005)
C57 mice (lactating dams) were treated with TCDD on PND4	1 μg/kg bw	M offspring exerted decreased anogenital distance, body weight and testicular testosterone	Jin et al. (2010)
C57 mice (pregnant) were treated with TCDD on GD15	1 μg/kg bw	M offspring exerted decreased anogenital distance, testis weight, no effects on body weight	Jin et al. (2008)

Abbreviations: GD, gestational day; IU, in utero; L, lactation; PND, postnatal day; T4, thyroxine; TSH, thyroid-stimulating Hormone.

3.1.4.1.6 | *Overall summary of the mode of action of male reproductive effects*

In summary, exposure of adult male rodents can result in various changes in gene expression related to sex steroid hormone homeostasis, and may cause apoptosis and oxidative stress in reproductive organs. These effects are seen at dose levels of 10 μg/kg bw and above.

Maternal exposure to TCDD in rodents, both pre- and postnatally, at substantially lower dose levels (0.01–1 μg/kg bw) can result in adverse effects on male reproductive organs and functions in the offspring. These include deficits in size, structure and function of male reproductive organs, including decreased production of testosterone, changes in sperm production and quality, and a decrease in fertility. Furthermore, effects on sex-specific behaviour and on the onset of puberty have been observed. Most of the studies have been carried out with TCDD. TEQ-equivalent doses of PCB-77 or PCB-126 administered as single dose on GD15 failed to reproduce similar effects as TCDD, but the male offspring of the dams treated with PCB-126 showed a decreased ventral prostate weight and anogenital distance.

The relative sensitivity of different developmental stages in males towards TCDD has not been explicitly studied in a comparative manner. However, given the central role of the pituitary-gonadal axis during early development, it is suggested that the pre- and postnatal stages are more sensitive to TCDD than adulthood stages. Studies in AHR-knockout animals and in mouse strains bearing a low- vs. a high affinity receptor provide evidence that most if not all adverse effects of TCDD on the development of male reproduction are mediated by the AHR.

Although there is good evidence for a crucial role of the activated AHR, the exact molecular mechanism of action of TCDD on the hypothalamic–pituitary-gonadal axis has not been clarified so far. Nevertheless, the biochemical consequences including a disruption of the endocrine hormone production and/or release of hypothalamic/pituitary signalling (including steroid hormones) have been established.

Multigenerational male reproductive toxicity, affecting testosterone biosynthesis and spermatogenesis, has also been reported.

The effects on male reproduction are predominantly, if not exclusively, mediated via activation of the AHR which is present in various cell types of the male reproductive system and of the superior regulatory centres in the hypothalamus and/or pituitary. Both canonical and non-canonical pathways may be involved. Although direct adverse effects of TCDD on male reproductive organs and functions have been observed in adult animals, a disruption of the endocrine regulation of male development during fetal and postnatal periods, including an AHR-dependent interference with the normal function of the hypothalamic–pituitary axis, emerges as the most likely sensitive candidate for a mode of action underlying these effects. It needs to be determined to which extent direct changes in gene expression and/or epigenetic mechanisms contribute to this outcome.

3.1.4.2 | *Interspecies differences in sensitivity towards dioxins*

Sensitivity to the toxicity of TCDD, other PCDD/Fs and DL-PCBs varies considerably between species and strains of experimental mammalians which has been investigated in detail by several authors (reviewed in Birnbaum & Tuomisto, 2000; Denison et al., 2011). In the context of this Opinion, the relative sensitivity of humans in comparison to experimental animals is of utmost interest.

In comparison to cells from sensitive rodent strains, human cells in culture consistently exhibit an about 10-fold lower sensitivity towards TCDD in terms of drug metabolism induction (Budinsky et al., 2010; Connor & Aylward, 2006). A major reason for this difference seems to be the substantially lower affinity of the human AHR to TCDD when compared to rodent (reviewed in Eaton et al., 2024). Furthermore, in human cells, for other PCDD/Fs and DL-PCBs much lower potencies were reported (Larsson et al., 2015) than reflected by the WHO_{2022}-TEFs. Most notably, the inducing potency of PCB-126 is lower in human cells by several orders of magnitude when compared to rodent cells (Silkworth et al., 2005).

When compared to a large number of other genes, those encoding for drug-metabolising enzymes were the most sensitive towards TCDD in human hepatocytes in culture (Black et al., 2012). Interestingly, other effects of TCDD beyond induction of drug metabolism, have also been shown to be less sensitive in human cells in culture when compared to rodent cells. Among 1591 orthologous genes responding to TCDD both in human and rat hepatocytes, most genes were much less sensitive in human hepatocytes. Benchmark dose analysis of gene expression changes showed an average 18-fold cross-species difference in potency among differentially expressed orthologs with the rat being more sensitive than the human (Black et al., 2012).

In a study by Moriguchi et al. (2003), TCDD-sensitive C57BL6J, TCDD-resistant DBA2 mice, and C57BL6J mice expressing the human AHR (h*AHR*) instead of the mouse (*mAhr*) were treated with TCDD. Mice homozygous for *hAHR* exhibited weaker induction of AHR target genes such as *Cyp1a1* and *Cyp1a2* after TCDD treatment than both wild-type C57BL6J and DBA2 mice. Induction was analysed in diverse organs including liver, lung, kidney, intestine, and thymus (Moriguchi et al., 2003). Treatment of pregnant mice (40 μg/kg bw) led to increased incidences of hydronephrosis in the offspring of the three types of mice with a lower severity score in *hAHR* mice. Occurrence of cleft palate was seen in C57BL6J and DBA2 mice but not in *hAHR* mice.

In a few studies, the sensitivity towards induction of drug metabolism by dioxins was investigated in humans. One of these studies used data from a case of poisoning of two women in Vienna (Geusau et al., 2001). One patient with a TCDD blood level of 144,000 ng/kg blood lipid developed severe generalised chloracne while a second patient (26,000 ng/kg blood lipid) exhibited mild facial acne lesions. In the highly intoxicated patient, a moderate increase in blood lipids, leukocytosis, anaemia, and secondary amenorrhoea were observed, while in the second patient all clinical laboratory parameters were normal. In a metabolic breath test and from the analysis of urinary caffeine metabolites, an 8- to 10-fold higher CYP1A2 activity compared to non-smokers was found in the intoxicated women (Abraham et al., 2002). In (other) moderately exposed individuals (up to 1000 ng/kg blood lipid) the authors could not find any increase in CYP1A2 activity.

Connor and Aylward (2006) concluded that no relationship was found between moderately and highly increased TCDD body burdens in humans and TCDD body burdens up to 250 ng TEQ/kg bw, whereas marked elevations in enzyme activity were observed in persons with body burdens above 750 ng TEQ/kg. In contrast, the more sensitive laboratory rodent strains and species exposed to TCDD exhibit significant enzyme induction at body burdens below 50 ng/kg.

Studies on the affinity of the human AHR towards TCDD revealed an about 10-fold lower binding affinity in comparison to the AHR of C57/Bl6 mice, one of the most TCDD-sensitive mouse strains (Flaveny et al., 2010). Genetic polymorphisms of the AHR, often related to different binding affinities to TCDD, exist in humans. However, no substantial impact on effects elicited by AHR ligands has been identified so far (Okey et al., 2005).

Taken together, there is strong evidence from in vitro data, in vivo observations in homozygous transgenic mice bearing the human *AHR*, and in intoxicated individuals, that humans are less sensitive than sensitive rodent strains by at least one order of magnitude towards most, if not all, canonical effects of TCDD. The relative sensitivity towards a few selected congeners, most notably PCB-126, appears to be even much lower.

3.1.5 | Overall weight of evidence, consideration of critical effects and dose–response analysis

The relevant 17 PCDD/Fs and 12 DL-PCBs accumulate in animals and humans, implying that even a low daily exposure may eventually lead to elevated levels in the body (body burden). For contaminants with such properties, application of a body burden approach is appropriate, focussing on levels in the body associated with adverse effects, rather than a daily applied dose.

In the 2018 Opinion (EFSA CONTAM Panel, 2018), concerning studies in experimental animals at low body burdens, the adverse effects of TCDD in rats were reduced sperm production (LOAEL body burden 25 ng/kg bw), delayed puberty development (LOAEL body burden 42–50 ng TCDD/kg bw), altered bone parameters (NOAEL body burden 28 ng/kg bw) and hepatopathy (NOAEL body burden 85 ng TCDD/kg bw). In mice, the lowest extrapolated body burden at the NOAEL was 9 ng/kg bw, based on embryo loss. Studies conducted in primates treated monthly during gestation and lactation (until PND90) showed dental effects and effects on sperm concentration at a total dose of 405–420 ng TCDD/kg bw.

Likewise, in the 2018 Opinion (EFSA CONTAM Panel, 2018), and concerning the adverse associations in epidemiological studies, the causality of reported effects was evaluated. This was based on findings at low doses in experimental animal studies in combination with an evaluation of the body of evidence in the human studies:

- Associations were reported for developmental male reproductive endpoints such as impaired semen quality, for which it was concluded in the 2018 Opinion that it was likely to be a causal effect of exposure to TCDD, other PCDDs and possibly PCDFs, but with no conclusive evidence for DL-PCBs based on human studies.
- A later onset of puberty in boys was observed, for which it was noted in the 2018 Opinion that there was insufficient information to conclude on causal associations.
- Other developmental outcomes were reported, such as a lower sex ratio (boys to girls) after paternal exposure. It was concluded in the 2018 Opinion that it is likely a causal effect after exposure to PCDD/Fs, but for DL-PCBs no studies were identified.
- Increased TSH levels in newborns after in utero exposure were observed, for which it was noted in the 2018 Opinion that a causal association between relatively high TCDD exposure and increased TSH levels in newborns is likely, although most studies with low to moderate exposure to PCDD/Fs and DL-PCBs resulting from background exposure in newborns do not suggest any adverse effects on thyroid function.
- Impaired teeth development measured as enamel defects in boys and girls after perinatal exposure to PCDD/Fs was also observed, which was noted in the 2018 Opinion to be likely a causal effect.
- Increased exposure was also associated with all cancers combined but not specific tumours, and there was no evidence that PCDD/Fs or DL-PCBs are genotoxic substances which interact with DNA.

– Regarding chloracne, it is considered a specific adverse effect in humans but occurring at levels of PCDD/Fs far higher than those associated with other effects.

Among all endpoints identified, in the 2018 Opinion it was noted that the developmental effects on semen quality were found consistently in experimental animals (in studies with TCDD) and in epidemiological studies (associations with serum levels of TCDD, but also PCDD-TEQ and PCDD/F-TEQ), and that this endpoint provided the lowest Reference Point. The developmental enamel defects on teeth appeared to occur at only slightly higher exposure than the developmental effects on semen quality, while other effects were reported to occur at higher exposure levels (EFSA CONTAM Panel, 2018).

3.1.5.1 | *Critical effects and dose–response analysis in epidemiological studies*

Since the 2018 Opinion, a number of epidemiological studies have been published (see **Section 3.1.3**). The CONTAM Panel concluded that the updated evidence remained largely consistent with the previous conclusions. None of the new studies were deemed appropriate for the derivation of an HBGV due to limitations as described above. Consistent with the 2018 Opinion, the lower sperm concentration was considered the most sensitive endpoint. Thus, the three epidemiological studies considered as pivotal in the 2018 Opinion (Mínguez-Alarcón et al., 2017; Mocarelli et al., 2008, 2011) were re-evaluated as outlined below, taking into consideration the new WHO_{2022}-TEFs.

In the 2018 Opinion, the CONTAM Panel selected as critical study an epidemiological study with Russian boys, in which the serum levels of all 17 PCDD/Fs and 12 DL-PCBs were analysed (Mínguez-Alarcón et al., 2017). Details on this study are given by the EFSA CONTAM Panel (2018). Blood levels measured at the age of 8–9 years showed an inverse association with sperm concentrations in samples obtained at 19 years for TCDD, PCDD-TEQ, and PCDD/F-TEQ. No significant association was observed for PCDF-TEQ, DL-PCB-TEQ or the sum of PCDD/F- and DL-PCB-TEQ (Total-TEQ). The TEQ levels were based on WHO_{2005}-TEFs. The 2018 Opinion decided to base the TWI on the association with PCDD/F-TEQ levels, with a recommendation to evaluate the TEFs. This was based on the fact that in vitro studies with human cells show consistently that the most relevant DL-PCB, PCB-126 has a much lower relative potency than suggested by its assigned TEF of 0.1 from 2005 (EFSA CONTAM Panel, 2018).

For the current update, the multivariable-adjusted mean semen parameters stratified by quartiles of serum PCDD, PCDF and DL-PCB concentrations, expressed in WHO_{2022}-TEQ, were obtained from the study authors (Mínguez-Alarcón L, Hauser R, 2024–2025; see Documentation provided to EFSA). The results are shown in Table 5. The CONTAM Panel noted that the observed statistically significant inverse associations between quartiles of TCDD, PCDD-TEQ and PCDD/F-TEQ and sperm concentration were maintained when using WHO_{2022}-TEFs. For PCDD/Fs, a NOAEC of 6.65 ng WHO_{2022}-TEQ/kg lipid was identified for the lowest exposure quartile, which is slightly lower than the 7.0 ng WHO_{2005}-TEQ/kg lipid reported in the 2018 Opinion. In the other three quartiles, sperm concentration was approximately 40% lower than in Q1. However, as in the 2018 Opinion using WHO_{2005}-TEFs, no significant association was observed for Total-TEQ when applying the new WHO_{2022}-TEFs.

Figure 7 shows the TEQ contribution of PCDD/Fs and DL-PCBs for the median serum level using either WHO_{2005}- or WHO_{2022}-TEFs. The relative contribution of a number of congeners decreased but for some others it actually increased. This may explain the relatively small decrease in the NOAEC for PCDD/Fs of the study. Furthermore, PCB-126 was still the most relevant congener and also the relative contribution of PCB-118 increased.

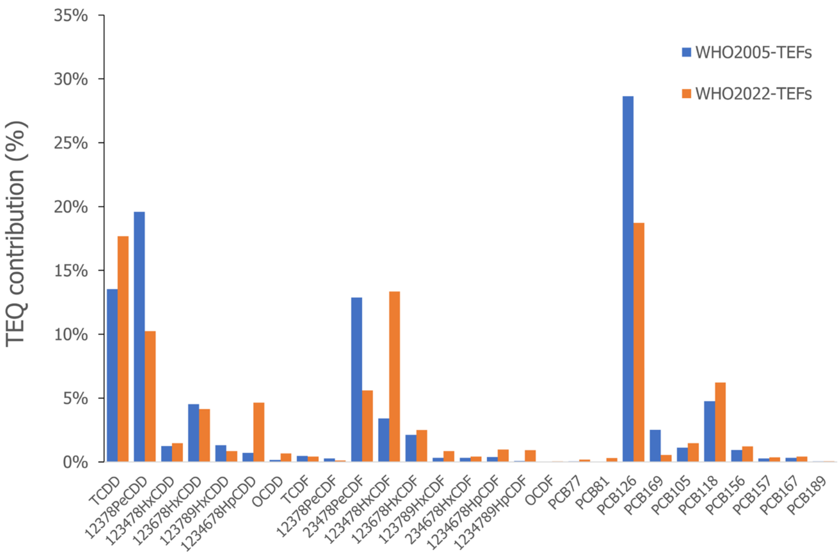

FIGURE 7 Relative contribution of PCDD/Fs and DL-PCBs to the median level in serum of Russian boys based on WHO_{2005}- or WHO_{2022}-TEFs (Burns et al., 2009). At this median level the Total-TEQ concentration decreased from 21.4 ng WHO_{2005}-TEQ/kg lipid to 16.4 ng WHO_{2022}-TEQ/kg lipid.

The study authors of Mínguez-Alarcón et al. (2017) provided EFSA with a spline-based regression model for sperm concentration versus blood levels, calculated with WHO_{2022}-TEFs, based on the adjusted individual observations ($n=256$; for most of the 133 boys two semen samples were available) (Figure 8A for Total-TEQ and Figure 8B for PCDD/F-TEQ) (Mínguez-Alarcón L, Hauser R, 2024–2025; see Documentation provided to EFSA). The CONTAM Panel noted that few observations were present at higher blood levels (e.g. above about 30 ng WHO_{2022}-TEQ/kg lipid, as visualised in the rug plot), making the relationship less reliable at higher concentrations, as illustrated by the corresponding wide confidence interval. Of note, also in the fourth quartile of PCDD/F-TEQ levels (> 16.5 ng WHO_{2022}-TEQ/kg lipid), there was a statistically significantly lower sperm concentration than in the first quartile (Table 5). The CONTAM Panel noted that in Mocarelli et al. (2008) lower sperm concentrations were observed in men in the Seveso cohort exposed between 1 and9 years as compared to the non-exposed group, also at substantially higher serum concentrations of TCDD (e.g. average in Q4 of 733 pg/g lipid) than in the study of the Russian boys. Although the spline curves for PCDD/F-TEQ and Total-TEQ (PCDD/Fs plus DL-PCBs) look quite similar in the Russian boys, no significant decrease across quartiles was observed for Total-TEQ, contrary to that for PCDD/F-TEQ (Table 5). In the 2018 Opinion the TWI was based on the association in the Russian Children's Study by Mínguez-Alarcón et al. (2017) for PCDD/F-TEQs alone, based on the body of evidence that in human in vitro models the DL-PCBs are less potent than reflected by the WHO_{2005}-TEFs. This approach is no longer appropriate following the update of the WHO-TEFs (the uncertainties of the WHO_{2022}-TEFs for human risk assessment will be discussed in **Section 3.5**).

Therefore, based on the above, in the continued absence of a statistically significant association between sperm concentrations and Total-TEQ after applying the WHO_{2022}-TEFs, the CONTAM Panel considered that the data from Mínguez-Alarcón et al. (2017) could no longer serve as the primary basis for deriving an HBGV as in the 2018 Opinion, but considered that the data were important as supporting evidence (see **Section 3.1.5.3**).

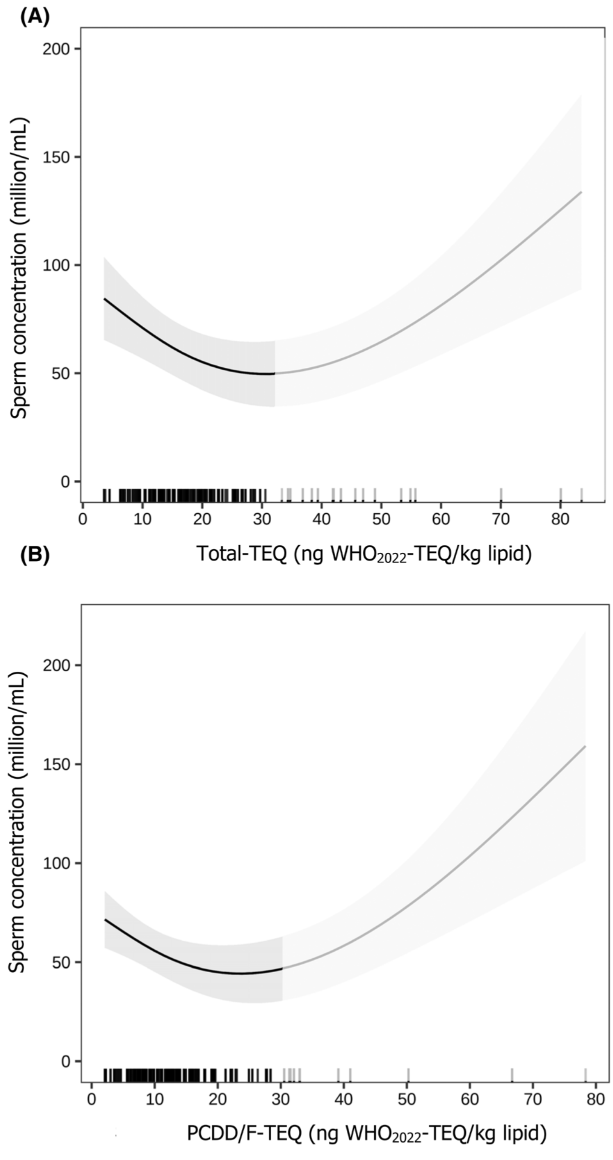

FIGURE 8 Spline regression model for sperm concentration at 19 years and rug plot based on individual observations for serum levels at 8–9 years of: (A) Total-TEQ and (B) PCDD/F-TEQ (expressed in WHO_{2022}-TEQ), sperm concentrations adjusted for BMI, smoking status, alcohol drinker, season, and abstinence time at the mean level of continuous covariates and adjusted for frequency of categorical measures. The figures were submitted to EFSA by the study authors (Mínguez-Alarcón L, Hauser R, 2024–2025; see Documentation provided to EFSA). Shading on right hand side of the figures and axis were added by EFSA.

TABLE 5 Multivariable adjusted mean semen parameters by quartiles (Q) of serum PCDDs, PCDFs and DL-PCBs (**expressed in WHO_{2022}-TEQ**) among 133 young men in the Russian Children's Study contributing 256 semen samples. The table was submitted to EFSA by the study authors (Mínguez-Alarcón L, Hauser R, 2024–2025, see Documentation provided to EFSA).

	Median	Sperm concentration (million/mL)	Total sperm count (million)	Motile sperm (%)	Total motile sperm count (million)
WHO_{2022}-TEQ (ng TEQ/kg lipid)					
PCDD-TEQ[a]					
Q1 [0.92–4.39]	3.29	67.1 (55.2, 81.5)	167 (130, 214)	55.1 (52.4, 57.8)	90.6 (68.1, 121)
Q2 [4.46–6.77]	5.33	39.3 (29.7, 51.9)*	92.6 (65.7, 131)*	52.6 (49.3, 55.9)	47.4 (32.0, 70.3)*
Q3 [6.78–9.43]	7.98	38.6 (29.3, 51.0)*	81.3 (58.1, 114)*	53.7 (50.4, 57.0)	42.8 (29.1, 63.0)*
Q4 [9.45–25.9]	13.9	39.2 (28.6, 53.7)*	110 (79.9, 154)*	54.0 (50.4, 57.6)	58.3 (39.9, 85.1)
p, trend		0.007	0.04	0.78	0.07
PCDF-TEQ[a]					
Q1 [0.73–3.08]	2.62	45.7 (33.8, 61.9)	109 (77.8, 154)	54.9 (52.1, 57.8)	59.4 (40.7, 86.5)
Q2 [3.10–4.68]	3.53	45.6 (34.6, 60.2)	92.9 (64.6, 133)	51.7 (48.7, 54.8)	46.8 (30.8, 70.9)
Q3 [4.69–7.44]	5.68	38.7 (29.4, 51.0)	104 (74.5, 146)	51.7 (48.1, 55.4)	52.6 (35.3, 78.5)
Q4 [7.54–70.7]	10.1	49.2 (38.4, 63.1)	130 (102, 166)	56.8 (53.5, 60.1)	72.8 (55.0, 96.4)
p, trend		0.90	0.34	0.43	0.33
PCDD/F-TEQ[a]					
Q1 [2.08–8.08]	6.65	63.6 (52.0, 77.8)	145 (110, 190)	54.9 (52.3, 57.4)	78.5 (58.2, 106)
Q2 [8.11–12.0]	9.63	37.4 (27.7, 50.5)*	89.6 (62.6, 128)*	53.3 (49.9, 56.7)	46.3 (30.7, 70.0)*
Q3 [12.2–16.4]	13.7	40.9 (31.2, 53.8)*	90.7 (65.3, 126)*	52.6 (49.5, 55.7)	46.8 (32.1, 68.2)*
Q4 [16.5–78.4]	23.9	41.0 (30.2, 55.6)*	117 (85.8, 160)	54.6 (50.7, 58.4)	62.0 (43.1, 89.2)
p, trend		0.04	0.36	0.84	0.38
Non-*ortho* DL-PCB-TEQ[a,b]					
Q1 [0.31–2.21]	1.84	53.0 (40.8, 68.9)	121 (89.7, 165)	54.7 (51.9, 57.5)	65.7 (47.0, 91.8)
Q2 [2.23–3.30]	2.75	40.6 (28.9, 57.2)	102 (68.8, 151)	53.0 (49.9, 56.1)	52.8 (33.7, 82.6)
Q3 [3.32–4.89]	3.88	36.1 (27.2, 47.9)	88.9 (62.9, 126)	54.3 (50.7, 57.9)	47.0 (31.2, 70.7)
Q4 [4.94–33.2]	6.49	51.4 (40.1, 65.8)	126 (95.2, 168)	53.4 (49.6, 57.3)	65.8 (47.0, 92.0)
p, trend		0.78	0.97	0.74	0.93
Total-TEQ[a]					
Q1 [3.55–12.1]	9.56	56.9 (42.1, 76.9)	131 (94.9, 183)	53.9 (50.9, 57.0)	69.8 (48.1, 101)
Q2 [12.7–16.9]	14.5	39.3 (28.9, 53.5)	81.1 (55.2, 119)*	53.5 (50.4, 56.6)	42.5 (27.8, 65.2)
Q3 [17.1–24.1]	20.0	38.4 (30.8, 47.8)	109 (81.7, 144)	53.2 (50.3, 56.1)	56.7 (40.8, 78.7)
Q4 [25.1–83.5]	34.7	44.7 (32.7, 61.1)	110 (80.3, 151)	54.1 (49.9, 58.3)	57.7 (39.7, 83.7)
p, trend		0.61	0.75	0.99	0.76

[a]Data are presented as predicted estimates (95% CI) adjusted for BMI, smoking status, alcohol drinker, season, and abstinence time at the mean level of continuous covariates and adjusted for frequency of categorical measures. Motile sperm and total motile sperm count models were further adjusted for time to start semen analysis.

[b]PCB-77, −81, −126 and −169.

*$p \leq 0.05$.

Besides Mínguez-Alarcón et al. (2017), the 2018 Opinion considered two studies from the Seveso cohort as pivotal studies, i.e. Mocarelli et al. (2008, 2011). Details and considerations about these Seveso studies can be found in EFSA CONTAM Panel (2018).

Briefly, Mocarelli et al. (2008) observed lower sperm concentrations (53.6 vs. 72.5 million/mL; $p = 0.025$) in boys who were between 1 and 9 years during the incident where TCDD was released into the environment ($n = 71$, 34% of the relevant population eligible in 1976), compared to controls [healthy blood donors, $n = 82$; not living in TCDD-contaminated areas (i.e. they were not exposed to TCDD by the Seveso explosion)]. The exposed boys (1–9 years at the time of the exposure) showed an average TCDD serum level of 210 ng/kg lipid. It was assumed that serum TCDD concentrations for the comparison groups were ≤ 15 ng/kg lipid in 1976–1977, on the basis of serum results for residents of uncontaminated areas around Seveso. In addition, 16 participants with serum TCDD levels above 2000 ng/kg lipid were excluded without particular reason given by the authors. When categorised into quartiles with median TCDD levels of 68, 142, 345 and 733 ng/kg lipid, no clear dose–response relationship was observed.

Mocarelli et al. (2011) studied 39 out of the 78 (50%) men born between 1977 and 1984 to mothers exposed to TCDD during the Seveso incident in 1976, and 58 comparison men (matched by age and socioeconomic status) born to mothers exposed only to background levels. The TCDD levels in the mothers at the actual time of conception were estimated from the original levels in 1976–1977, and the median was reported to be 26 ng/kg lipid. In the group of exposed mothers of breastfed sons, the median level was 19 ng/kg lipid and in the group that was formula fed it was 28 ng/kg lipid. The results showed that breastfed sons of exposed mothers ($n = 21$) had lower sperm concentrations than the background exposed breastfed comparisons ($n = 36$), while there was no difference between formula-fed exposed and control boys. Considering the exposed men only, the sperm concentration of those that were breastfed ($n = 21$) was on average 40% lower than those receiving formulas ($n = 18$), but this did not reach statistical significance.

After stratification of the breastfed exposed group into low ($n = 12$, median 13 ng/kg lipid) and high ($n = 9$, median 60 ng/kg lipid) maternal TCDD levels, lower sperm concentrations were observed in the latter group. Moreover, the lower serum TCDD group still had significantly lower sperm concentrations than the controls, resulting in a potential LOAEL for TCDD of 13 ng/kg lipid. The Panel noted that the difference in serum levels of other PCDD/Fs and DL-PCBs as compared to the background levels in this subgroup, was likely minimal. The limited number of participants was also acknowledged.

In both studies from Seveso, blood samples were collected from exposed boys and mothers, respectively, within a timeframe ranging from several weeks to up to 1 year after the Seveso incident in 1976. At a later stage, these were analysed for levels of TCDD, but not for other PCDD/Fs and DL-PCBs. Since the Seveso incident occurred in a time period when the environmental exposure to PCDD/Fs and DL-PCBs was around its historical peak, the contribution of congeners other than TCDD to the TEQ levels in both the exposed and the reference groups complicates the interpretation of the study results. Unfortunately, analysis of PCDD/Fs and DL-PCBs in human samples started at a much later stage and there are few studies that can be used to estimate the background levels in 1976. To investigate the existing TEQ levels at the time of the explosion, Eskenazi et al. (2004) determined serum levels of PCDD/Fs and DL-PCBs in a number of pooled samples from women of various age classes from the non-ABR zone, collected just after the incident. Pools were based on 20–21 people per sample and data were initially expressed in WHO_{1998}-TEFs. Warner et al. (2014) compared the TEQ levels in these nine pools, based on WHO_{1998}- and WHO_{2005}-TEFs and also presented the median levels and range for individual PCDD/F and DL-PCB congeners. Table 6 shows the median levels using the WHO_{1998}-, WHO_{2005}- and WHO_{2022}-TEFs, as well as the relative contribution of TCDD, other PCDDs, PCDFs and DL-PCBs to the total TEQ. **Figure 9** shows the relative contributions of individual congeners to the PCDD/F-TEQ levels, based on WHO_{2005}- and WHO_{2022}-TEFs. Minimum, median and maximum blood levels were evaluated.

TABLE 6 Median serum TEQ levels of TCDD, other PCDDs, PCDFs, DL-PCBs and their sum using WHO_{1998}-, WHO_{2005}- and WHO_{2022}-TEFs. Data on individual congeners in pooled serum samples from women living outside the ABR zone in Seveso were obtained from Warner et al. (2014).

	Median serum level (ng WHO-TEQ/kg lipid)			Relative WHO-TEQ contribution (%)		
	WHO_{1998}-TEFs	WHO_{2005}-TEFs	WHO_{2022}-TEFs	WHO_{1998}-TEFs	WHO_{2005}-TEFs	WHO_{2022}-TEFs
TCDD	17.1	17.1	17.1	17	24	29
Other PCDDs	12.3	12.6	15.0	12	17	25
PCDFs	17.0	11.6	9.4	17	16	16
DL-PCBs	52.8	31.0	18.2	53	43	30
Total	99.2	72.3	59.6	100	100	100

Abbreviations: DL-PCBs, Dioxin-like polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; TEF, toxic equivalency factor; TEQ, toxic equivalent; WHO, World Health Organisation.

Based on the serum concentrations from women living outside the Seveso area and comparing the WHO_{2022}- and WHO_{2005}-TEFs it is evident that:

- TEQ for the PCDDs increased using WHO_{2022}-TEFs compared to WHO_{2005}-TEFs, despite a reduction of the TEF for PeCDD, was caused by the higher TEF for HpCDD.

- TEQ for the PCDFs was only slightly reduced following the reduction to a third of the TEF value for 2,3,4,7,8-PeCDF, mainly compensated by a 3-fold increase of the TEF for 1,2,3,4,7,8-HxCDF.
- TEQ for the DL-PCBs was markedly decreased, primarily due to the 50% reduction of the TEF for PCB-126, the congener contributing around 80 and 70% to the PCB-TEQ level for WHO_{2005}- and WHO_{2022}-TEFs, respectively.
- Overall, the contribution of TCDD to the Total-TEQ level increased only slightly for WHO_{2022}-TEFs as compared to WHO_{2005}-TEFs, from 24 to 29%, which clearly demonstrates the importance of the other PCDD/Fs and DL-PCBs to the total TEQ-levels in the Seveso studies. The contribution of TCDD was, however, slightly higher at higher TEQ-values based on both the WHO_{2005}- and WHO_{2022}-TEFs, corresponding to 37% and 43%, respectively (see **Figure 9**). In addition, samples collected shortly after the incident in 1976 showed that TCDD levels in the boys from the first study by Mocarelli et al. (2008) were much higher (median of 210 ng/kg lipid) than those measured in the blood collected in 1976 from mothers from the second study by Mocarelli et al. (2011) (median 52 ng/kg lipid, extrapolated to 26 ng/kg lipid at the time of conception between 1976 and 1983). Consequently, the contribution of other congeners to the Total-TEQ levels may have been lower in the boys compared to the mothers. The transfer of other congeners during breastfeeding likely resulted in higher Total-TEQ levels in breastfed boys than would be indicated by the measured TCDD levels alone.

Based on the complex exposure scenario described above, the CONTAM Panel decided not to use these two Seveso studies as the primary basis to derive the HBGV because the uncertainties were considered to be too high. This includes the absence of data on the serum levels in the control groups. However, both studies are considered important supporting evidence in the derivation of the TWI (see **Section 3.1.6**).

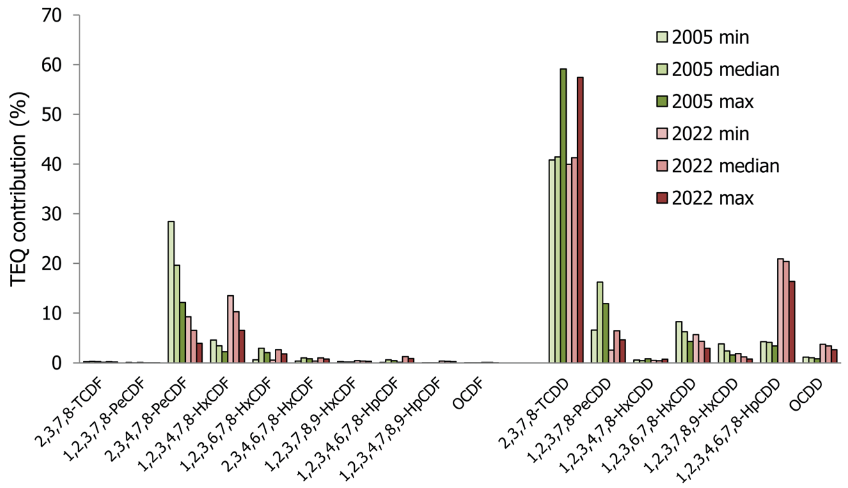

FIGURE 9 Relative contribution of PCDD/F congeners to the PCDD/F-TEQ level using WHO-TEFs of 2005 and 2022, based on minimum, medium and maximum congener levels as reported for the Seveso cohort by Warner et al. (2014).

3.1.5.2 | *Critical effects and dose–response analysis in studies in experimental animals*

The 2018 Opinion described several studies in rodents. Details about these studies, including calculation of the body burdens, can be found in EFSA CONTAM Panel (2018). The data showed the lowest benchmark dose lower credible limits (BMDLs) for the study by Faqi et al. (1998a) on reduced sperm production in rats.

Regarding the studies published since the 2018 Opinion (EFSA CONTAM Panel, 2018), those that complied with the eligibility criteria set in **Section 3.1.2.1** reported on effects that were considered not to be adverse and/or were not suitable to derive a Reference Point.

The study by Faqi et al. (1998a) was described in the 2018 Opinion (EFSA CONTAM Panel, 2018). Briefly, female Wistar rats were treated s.c. with a single dose of 0, 25, 60 or 300 ng ^{14}C-labelled TCDD/kg bw 2 weeks prior to mating (loading dose), followed by weekly injections of 0, 5, 12 and 60 ng/kg bw during mating, pregnancy and lactation as a maintenance dose, aiming at stable body burdens. Three animals from each group were killed on GD21 and levels of the radiolabelled TCDD in liver and adipose tissue of dams, and livers of foetuses were determined. This confirmed that the intended body burdens were obtained by the dosing strategy. Male offspring was killed on either PND70 or PND170 ($n = 20$ per group and time-point). Reduced sperm production, together with an increase in abnormal sperm morphology, were observed. No data on sperm vitality or motility were provided.

This study was also one of the few that measured tissue levels, allowing an estimation of the body burden in relation to the adverse effects (see Appendix C for calculation of the body burden of the dams based on tissue levels). The dosing regimen of one loading and weekly maintenance doses guaranteed a small variation in the body burden during pregnancy and lactation, contrary to most other rat studies that applied only a single dose at GD15 (see Appendix C). Single dose regimen results in a decrease of the maternal body burden over time and consequently also the exposure to the offspring both in utero and during lactation. Since the critical window for the effect on sperm production is unknown it is not possible to estimate the body burden that would correspond to the observed effects in such single dose studies. The fact that the dose is given subcutaneously, as in Faqi et al. (1998a), rather than orally is less important for a compound that is well absorbed and hardly metabolised (Abraham et al., 1998). For contaminants accumulating over time, the body burden, as reflected by the tissue levels, is considered the best dose-metric for risk assessment.

BMD modelling of daily sperm production data from the study by Faqi et al. (1998a) was performed using the latest EFSA BMD guidance (EFSA Scientific Committee, 2022). A rounded BMR of 15% was selected, equivalent to a response of one standard deviation above the mean for the control group[12] in the study at PND170.[13] Figure 10 shows the dose–response curve, including the BMD estimate and its credible interval. A $BMDL_{15}$ of 1.7 ng/kg bw was calculated, being the highest body burden in dams not leading to adverse effects in pups. The detailed BMD report can be found in Annex G.

As shown in Figure 10, at PND170 (the last of the two time-points), a near-maximum response was obtained at the lowest dose, corresponding to a body burden in the dams of 25.1 ng TCDD/kg bw. Despite this uncertainty, the CONTAM Panel still considered deriving a Reference Point from the BMDL to be a more informative and scientifically robust approach than extrapolating from a LOAEL to a NOAEL using an uncertainty factor. The uncertainties arising from this were discussed in **Section 3.5.1.2**.

According to the data suitability test in the Bayesian BMD web tool, the dose–response data provided sufficient information to estimate the BMD. Furthermore, the appropriateness of the BMDL as a Reference Point was assessed in line with the recommendations outlined in the 2022 EFSA BMD Guidance (EFSA Scientific Committee, 2022, p. 32). The CONTAM Panel noted that the distributional assumption of the lognormal models, specifically the assumption of a constant coefficient of variation, was unacceptable albeit borderline (Bartlett test p-value = 0.0485). However, sensitivity analyses showed that this issue had a minor effect on the estimated BMD, BMDL and BMDU values (see Annex G for details), and this was taken into account in the uncertainty analysis (see **Section 3.5**).

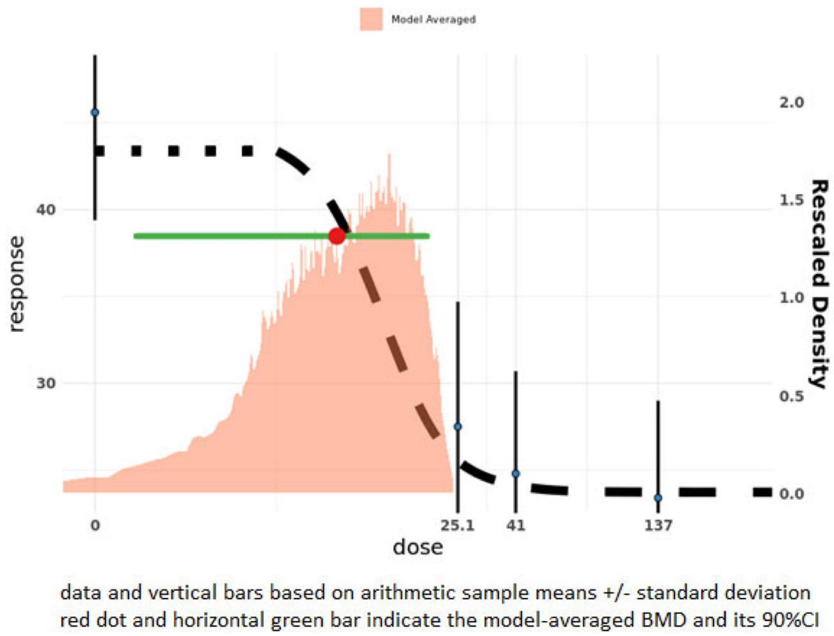

FIGURE 10 Dose–response curve obtained with Bayesian BMD modelling of the daily sperm production in male offspring at PND170 versus the body burdens of 0, 25.1, 41 and 137 ng TCDD/kg bw in the rat dams (Faqi et al., 1998a). A BMR of 15% was applied, resulting in a $BMDL_{15}$ of 1.7 ng/kg bw (BMD: 9.0 ng/kg bw; BMDU: 19.3 ng/kg bw).

The CONTAM Panel also performed BMD modelling on the studies modelled in the 2018 Opinion (see Table 13 in EFSA CONTAM Panel, 2018) using the 2022 BMD Guidance and applying the same BMRs as in the 2018 Opinion for comparison. The results are shown in Appendix D (and the individual BMD reports in Annex G). The study by Faqi et al. (1998a) provided the lowest BMDL.

3.1.5.3 | *Critical effect*

The critical endpoint was identified by integrating evidence from both human and experimental animal lines of evidence considering the respective level of confidence and according to a weight of evidence approach (EFSA Scientific Committee, 2017).

[12]Mean daily sperm production × 10^6: mean + SD = 45.6 + 6.2.

[13]At present there is no Guidance on which BMR to use specifically for a decrease in sperm production. Criteria for the selection of BMR is under discussion by the EFSA Scientific Committee.

PCDD/Fs and DL-PCBs have been reported to cause a number of effects in animals and humans (EFSA CONTAM Panel, 2018) (see **Section 3.1.5** above). Effects on the male reproductive system seem to occur at the lowest exposure and have therefore been selected as critical.

In rodents, there are several studies showing that exposure to TCDD during the fetal or early postnatal stage can adversely affect male development and reproduction, and that the effects are dependent on the agonistic action of TCDD at the AHR pathway. These effects include reduced sperm count, mobility and viability of spermatozoa, reduced weights of male reproductive organs, and changes in anogenital distance and delayed puberty onset (see Appendix E). In one study no effects on sperm count were observed, but there was an effect on balano-preputial separation, a marker of delayed puberty (Bell et al., 2007a; see EFSA CONTAM Panel, 2018). The adverse effects of TCDD on the hypothalamic–pituitary-gonadal axis, a key regulatory network involved in the coordinated development of reproductive functions, are likely to play a central role in these outcomes (Hattori et al., 2018, 2021; Johnson et al., 2020; Takeda et al., 2009, 2012, 2014, 2020). A disruption of the endocrine regulation of male development during early stages of fetal and postnatal periods, including an AHR-dependent interference with the normal function of the hypothalamic–pituitary-gonadal axis, is the most likely candidate for a mode of action underlying these sensitive effects (see **Section 3.1.4**). Since these fundamental regulatory events are crucial and conserved across mammalian species and beyond (McArdle et al., 2020), the CONTAM Panel considered that it is likely that these effects also occur in humans.

In humans, three studies in three different cohorts showed associations with lower sperm concentrations. One was the study by Mocarelli et al. (2008), which showed lower sperm concentrations in adult men exposed to TCDD between the age 1 and 9 years during the Seveso incident, whereas no such effect was observed in adult men exposed at the age of 10–17 years. This was one of the two studies used by the US-EPA (2012) to derive a Reference Dose of 0.7 pg/kg bw per day (see **Section 3.1.3**).

The study by Mínguez-Alarcón et al. (2017) on Russian boys, showed an association between serum levels of PCDD/F-TEQ and reduced sperm concentrations in adulthood. EFSA CONTAM Panel (2018) used this study to set its TWI. In addition, in the same cohort of boys, a delayed pubertal onset, delayed sexual maturity and slower pubertal developmental trajectories were reported in association with PCDD/F-TEQ (Humblet et al., 2011; Korrick et al., 2011; Burns et al., 2016; Plaku et al., 2023).

Mocarelli et al. (2011) reported on breastfed and formula-fed boys born to mothers that were exposed during the Seveso incident. There was a significant lower sperm concentration in adulthood in the 21 breastfed boys from Seveso compared with the 36 breastfed controls. No significant difference was observed between the 18 formula-fed boys from Seveso and the formula-fed controls, implying that besides in utero exposure, lactational exposure is of relevance.

The CONTAM Panel concluded that decreased sperm concentration is the most sensitive among the endpoints assessed. It may also be indicative of other adverse developmental reproductive effects in males, including pubertal development (Jensen et al., 2016).

3.1.5.4 | *Critical window of exposure*

Regarding effects on semen quality, the 2018 Opinion concluded that "*the evidence from the Seveso studies suggests that there may be a postnatal period of sensitivity that might expand into puberty*" (EFSA CONTAM Panel, 2018). Since the 2018 Opinion, there is no new information on this.

Most studies on effects of TCDD on the male reproductive system and its functions in experimental animals have been carried out by treating the dams before (in utero) and/or after birth (during lactation). These studies indicate that the effects occur during these early stages in development. Among these, cross-fostering studies showed that either intrauterine or lactational exposure alone and in combination can elicit a similar pattern of these adverse effects, although some differences were found. However, no data on effects of exposure close to puberty were identified. Studies in adult rodents indicate that these are less sensitive than immature animals towards adverse effects of TCDD on the male reproductive system and functions.

Therefore, the CONTAM Panel considered that the above-mentioned conclusion from the 2018 Opinion still applies.

3.1.5.5 | *Selection of the critical study and reference point*

The CONTAM Panel concluded that the data from the human studies (Mínguez-Alarcón et al., 2017; Mocarelli et al., 2008, 2011) could not form the primary basis for deriving an HBGV, based on the considerations made in **Section 3.1.5.1**.

It was therefore decided to derive a Reference Point from the studies with experimental animals, using the three human studies as supporting evidence.

Among the experimental animal studies, the one by Faqi et al. (1998a) was identified as the critical study and showed a reduced daily sperm production in rat male offspring of dams treated with TCDD. Modelling with Bayesian BMD analysis allowed the derivation of a $BMDL_{15}$ of 1.7 ng/kg bw (expressed as the body burden in the dam), which was the lowest BMDL (see **Section 3.1.5.2**).

3.1.6 | Derivation of the health-based guidance value

As in the 2018 Opinion (EFSA CONTAM Panel, 2018), the CONTAM Panel considered it appropriate to set a health-based guidance value (HBGV), more specifically a Tolerable Weekly Intake (TWI).

The TWI aims to prevent adverse effects in humans during pre- and post-natal development. Since concentrations of PCDD/Fs and DL-PCBs build up in humans over time, this aim can only be achieved by preventing women of childbearing age from having body burdens (i.e. levels in their bodies) that could lead to in utero and lactational exposures associated with health concerns in the offspring.

The highest body burden not associated with adverse effects (i.e. effects exceeding the BMR, see **Section 3.1.5.2**) was derived from the critical rat study (Faqi et al., 1998a). The CONTAM Panel noted that this body burden should not be considered a critical body burden (i.e. a body burden directly associated with adverse effects in the animal) but should prevent such a body burden associated with adverse effects is reached in the pups by maternal transfer.

The CONTAM Panel noted that for the rat pups in the critical study (Faqi et al., 1998a), the critical body burden is difficult to determine, because the body burden varies during exposure in utero and lactation, and the precise timing of the critical window for the developmental effect on sperm production is uncertain (see **Section 3.1.5.4**). Consequently, the rather constant body burden in the dams (see Appendix C) offers a much more solid basis for establishing a TWI.

The situation in humans likely reflects the situation in rats, with a relatively high exposure pre- and especially postnatally (via breastfeeding), leading to higher body burdens in infants than in mothers (Abraham et al., 1996, 1998).

3.1.6.1 | *Application of uncertainty factors (UFs)*

The CONTAM Panel considered which UFs should be applied to the Reference Point of 1.7 ng/kg bw (expressed as the body burden in the rat dam), which is the lowest $BMDL_{15}$ derived from BMD modelling of data from the study by Faqi et al. (1998a) (see **Section 3.1.5.2**). This $BMDL_{15}$ of 1.7 ng/kg bw would correspond to a lipid-based level of 6.8 ng/kg fat based on a body fat content of 25% in women.

Since a body burden approach is applied, the UF of 4 for interspecies variability in toxicokinetics is not required.

The CONTAM Panel reviewed the need to apply the two UFs of 2.5 and 3.16 for inter- and intraspecies variability in toxicodynamics, respectively, in a combined approach. There is evidence that humans are not more sensitive than rats (see **Section 3.1.4.2**). With respect to the relative sensitivity of humans vs. laboratory rodent species, the human AHR exhibits an about ten-fold lower binding affinity for TCDD than the AHR of most rodent strains. Likewise, cultured human cells are less sensitive to the effects of TCDD on expression of the genes regulated by AHR. In transgenic mice bearing the human AHR, a lower sensitivity towards induction of drug metabolism, cleft palate and hydronephrosis was found than in mice bearing the murine wild-type receptor. The sensitivity was lower than in the most sensitive mouse strain.

Considering observations in humans, Connor and Aylward (2006) in their review concluded that no relationship was found between TCDD body burdens up to 250 ng TEQ/kg bw and drug metabolism enzyme activity, whereas marked elevations were observed in persons with body burdens above 750 ng TEQ/kg. In contrast, the more sensitive laboratory rodent strains and species exposed to TCDD exhibit significant enzyme induction at body burdens below 50 ng/kg bw. This review includes a study on two women intoxicated by TCDD, presenting chloracne, showing TCDD blood levels of 26 and 144 ng/g lipid. In the highly intoxicated patient, a moderate and transient increase in blood lipids, leukocytosis, anaemia, and secondary amenorrhoea were observed, while in the second patient all clinical laboratory parameters studied were normal (Geusau et al., 2001). The CONTAM Panel noted that it is not clear whether these findings also are relevant for adverse effects on male reproductive development, such as reduced sperm concentrations.

To address this, the CONTAM Panel explored whether the three pivotal human studies showing reduced sperm concentrations could be used as supporting evidence for assessing the relative sensitivity of humans. In these studies, lipid-based blood levels in a group of boys and/or mothers were used to evaluate the association with the sperm concentrations at later age. It has been shown that such blood levels are quite similar to those in adipose tissue and as such reflect the body burden expressed on a lipid basis (EFSA CONTAM Panel, 2018).

US-EPA (2012) derived its RfD from both the increase in TSH levels and the reduced sperm concentrations from the Seveso studies. For the latter effect, US-EPA used the TCDD serum level in the lowest quartile of the Mocarelli et al. (2008) study (68 ng/kg lipid), showing a similar reduction in sperm concentration as the other three quartiles with higher TCDD blood levels (see **Section 1.3.1**). The serum level in the controls was assumed to be ≤ 15 ng/kg lipid (i.e. the LOQ of the method) but was not measured in samples collected at the time of the incident in 1976. In the absence of a clear dose–response relationship, implying that a maximal effect occurred already in the lowest quartile, the US-EPA applied a UF of 10 to derive a NOAEL for the intake leading to this serum level (and a UF of 3 for intraspecies variability in kinetics between humans).

In the Mocarelli et al. (2011) study with sons born to exposed mothers, the average serum TCDD level in the mothers of breastfed infants was 19 ng/kg lipid, which was considered a LOAEC. The levels were based on those measured in blood taken during the incident and extrapolated to the time of conception. The levels in the control mothers were assumed to be below the LOQ of 10 ng/kg lipid but were not measured. Dividing the LOAEC of 19 ng/kg lipid by a UF of at least 3 would result in a NOAEC of 6.3 ng/kg lipid or less. It is important to note that Mocarelli et al. (2008, 2011) did not analyse other PCDD/Fs and DL-PCBs, and the contribution of these congeners to the total TEQ may have been considerable in the mothers during that period (see **Section 3.1.5.1**). In the boys of the Mocarelli et al. (2008) study, TCDD levels were much higher and, as a result, the relative contribution of other congeners to the total TEQ levels may have been lower.

The study of Mínguez-Alarcón et al. (2017) showed a NOAEC for PCDD/F-TEQ of 6.7 ng/kg body fat and a lower NOAEC for PCDD-TEQ only of 3.3 ng/kg body fat. For PCDFs no significant association was observed. The PCDD-TEQ may be more comparable with the TCDD levels studied in the two Seveso studies and the critical rat study.

As mentioned above, the $BMDL_{15}$ of 1.7 ng/kg bw derived from the Faqi et al. (1998a) study in rats, would correspond to a lipid-based level of 6.8 ng/kg fat based on a body fat content of 25% in women. In humans, at this body burden most of the PCDD/Fs and DL-PCBs will be in the adipose tissue. Table 7 shows the highest body burden not raising a health concern in boys for the three pivotal human studies, for comparison with the value of 6.8 ng/kg body fat derived from the critical rat study (Faqi et al., 1998a). The CONTAM Panel noted that the values derived from the three pivotal human studies (after application of UFs) of 6.8, 6.7 and 6.3 ng/kg lipid (see Table 7) are similar to the highest body burden in rat dams not leading to adverse effects in pups (6.8 ng/kg fat). For the Mocarelli et al. (2008) study, the body burden is based on boys (average age (±SD): 6.2 ± 2.5 years) rather than mothers but, as shown in Figure 11, the level in such boys would be similar to that in the mothers (EFSA CONTAM Panel, 2018).

TABLE 7 Highest body burden not raising a health concern in boys for the three pivotal human studies, for comparison with that of 6.8 ng/kg body fat derived from the critical rat study (Faqi et al., 1998a).

Study	Body burden at LOAEC or NOAEC (ng/kg body fat)	Congeners	Type	UFs	Highest body burden after application of UFs in the human studies (ng/kg body fat)
Mocarelli et al. (2008) Boys	68 (in blood, boys Q1)	**TCDD** (other congeners not analysed)	LOAEC	10 for LOAEC to NOAEC[a]	**6.8**
Mocarelli et al. (2011) Mothers/boys	19 (in blood, mothers)[b]	**TCDD** (other congeners not analysed)	LOAEC	> 3 for LOAEC to NOAEC[c]	**< 6.8**
Mínguez-Alarcón et al. (2017) Boys	3.3 (in blood, boys at 9 years)	**PCDDs**[d]	NOAEC (Q1)yet[e]	None[f]	**3.3**
	6.7 (in blood, boys at 9 years)	**PCDD/Fs**[d]	NOAEC (Q1)[e]	None[f]	**6.7**[g]

Abbreviations: LOAEC, lowest-observed-adverse-effect concentration; NOAEC, no-observed-adverse-effect concentration; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans; PCDDs, polychlorinated dibenzo-*p*-dioxins; PND, postnatal day; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

[a] As applied by US-EPA to derive the RfD.

[b] Stratifying the group of breastfed boys in two quantiles showed a significant difference for the group from mothers with the lower levels (average 13 ng/kg lipid) compared to those from non-exposed mothers; group size was small.

[c] A factor 3 is often applied to derive a NOAEL from a LOAEL; unclear if in this case a factor 3 is sufficient.

[d] In this study there was a significant association with WHO_{2022}-TEQ levels for PCDDs and PCDD/Fs, but not for PCDFs, DL-PCBs or the sum of PCDD/Fs and DL-PCBs. Therefore, also PCDDs only are shown.

[e] Expressed as WHO_{2022}-TEFs.

[f] No UF applied as explained in EFSA CONTAM Panel (2018).

[g] Based on CADM model and underlying assumptions corresponding to 5.6 ng/kg body fat in the mothers.

Based on the above considerations, the CONTAM Panel concluded that applying UFs of 2.5 and 3.16 for inter- and intraspecies variability in toxicodynamics, respectively, would not be scientifically justified and thus too conservative. A similar conclusion was drawn by SCF (2000, 2001) without detailed reasoning (see **Section 1.3.1**). The remaining factor of 3.16 for intraspecies variability in toxicokinetics was applied by SCF (2001) (see **Section 1.3.1**).

Toxicokinetic variability in humans will affect the exposure of the infants. Differences in the half-lives for PCDD/Fs between humans have been described, despite the fact that, e.g. enzymatic degradation probably plays a minor role for the most relevant congeners which show long half-lives (Aylward et al., 2013) (see **Section 3.1.1**). A study by Kobayashi et al. (2013) also suggested that polymorphic differences in a number of AHR-related genes did not result in clear differences in TEQ-levels in blood. More important, and due to the lipophilic characteristics of PCDD/Fs and DL-PCBs, there could be other factors leading to interindividual differences in kinetics. For instance, the human milk concentrations may vary depending both on the maternal body burden and the amount of body fat. Also the infant's human milk consumption will vary. Variations related to these differences were not addressed by the three human studies or by the other uncertainty factors mentioned above. Therefore, it was considered appropriate to apply the default uncertainty factor of 3.16 to account for within human variability in kinetics (EFSA Scientific Committee, 2012). The CONTAM Panel is aware that applying the default UF may be too conservative considering the limited biotransformation (see **Section 3.5**). However, there are insufficient data on these factors to derive a non-default uncertainty factor. Considering that these compounds are highly lipophilic and tend to accumulate in lipid-rich tissues, interindividual differences in distribution may be more relevant than metabolism, further supporting the application of the default toxicokinetic UF of 3.16. This UF should be applied to the daily dose leading to the highest body burden in mothers not raising a health concern for their sons.

3.1.6.2 | *Deriving the TWI*

The CONTAM Panel estimated the long-term daily intake in humans leading to the body burden of 1.7 ng WHO_{2022}-TEQ/kg bw (i.e. the Reference Point) using the PBK CADM model (see **Section 3.1.1.3**). For this, it was noted that the body burden in women of childbearing age of 1.7 ng WHO_{2022}-TEQ/kg bw (i.e. the Reference Point in rat dams) correspond to a lipid-based level of 6.8 ng WHO_{2022}-TEQ/kg body fat, based on 25% body fat in women. Using the CADM model, reaching this lipid-based level at the age of 35 years was shown to require a chronic exposure of 0.29 pg. WHO_{2022}-TEQ/kg bw per day (see Figure 11).

The graph shows that the blood levels reach a steady-state at adolescent age, and that there will not be big differences between girls breastfed for 12 months, 6 months, or not at all by the time they reach childbearing age. Shorter or no breast-feeding would have a limited effect at childbearing age, and probably even less if the higher exposure during childhood on a body weight basis (see **Section 3.3.1**) was taken into account.

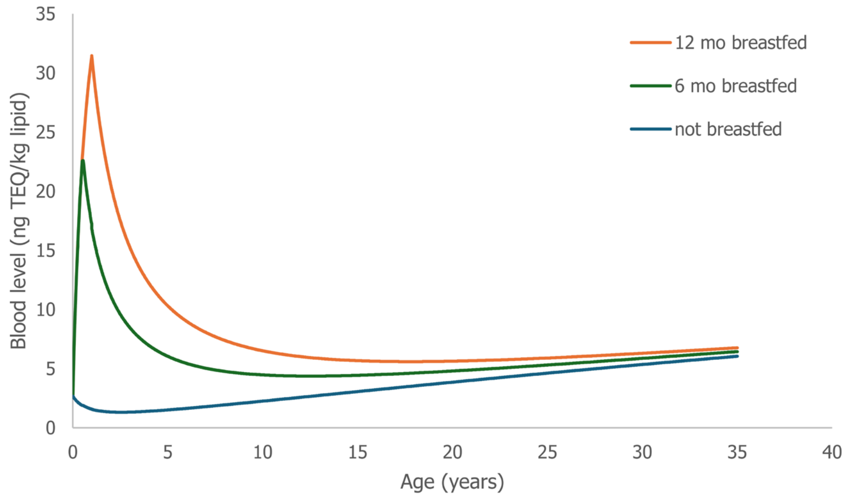

FIGURE 11 Modelling of the lipid-based levels in blood of PCDD/Fs and DL-PCBs in females, using the PBK CADM model. A daily intake of 0.29 pg. WHO_{2022}-TEQ/kg bw per day is applied after the breastfeeding period of 6 or 12 months until 35 years. The increase of PCDD/Fs and DL-PCBs in the blood levels for a woman non-breastfed as an infant is also shown. The modelling results in a serum level of 6.8 ng WHO_{2022}-TEQ/kg fat at the age of 35 years for the woman breastfed as an infant for 12 months, and slightly lower when breastfed for 6 months or not breastfed. The level at 35 years is equal to the highest body burden in women not raising health concern for their sons, based on 25% body fat. The peaks at 6 and 12 months are at the end of the breastfeeding period, assuming a daily human milk consumption of 800 mL with 3.5% fat and a level of 6.8 ng WHO_{2022}-TEQ/kg fat.

Applying the UF of 3.16 for intraspecies toxicokinetic variability resulted in a daily intake of 0.29/3.16 = 0.09 pg. WHO_{2022}-TEQ/kg bw per day.

As in 2018, the CONTAM Panel decided to base the HBGV on a weekly intake since daily variations in the intake are not critical for the body burden. This resulted in a weekly intake of 0.63 pg. WHO_{2022}-TEQ/kg bw (7 × 0.09 pg. WHO_{2022}-TEQ/kg bw). Rounding resulted in a **TWI of 0.6 pg WHO_{2022}-TEQ/kg bw per week**.

In addition, the CADM model was used to derive the body burden in women after chronic exposure to PCDD/Fs and DL-PCBs at the TWI of 0.6 pg. WHO_{2022}-TEQ/kg bw per week. This resulted in a body burden in women at age 35 of 2.2 ng WHO_{2022}-TEQ/kg fat. This is the highest body burden in women not raising a health concern for their sons. The fat-based level in human milk would be similar to the body burden (Nakamura et al., 2008; t Mannetje et al., 2012; Wittsiepe et al., 2007).

In Figure 12, the schematic view of the steps in the derivation of this TWI is shown.

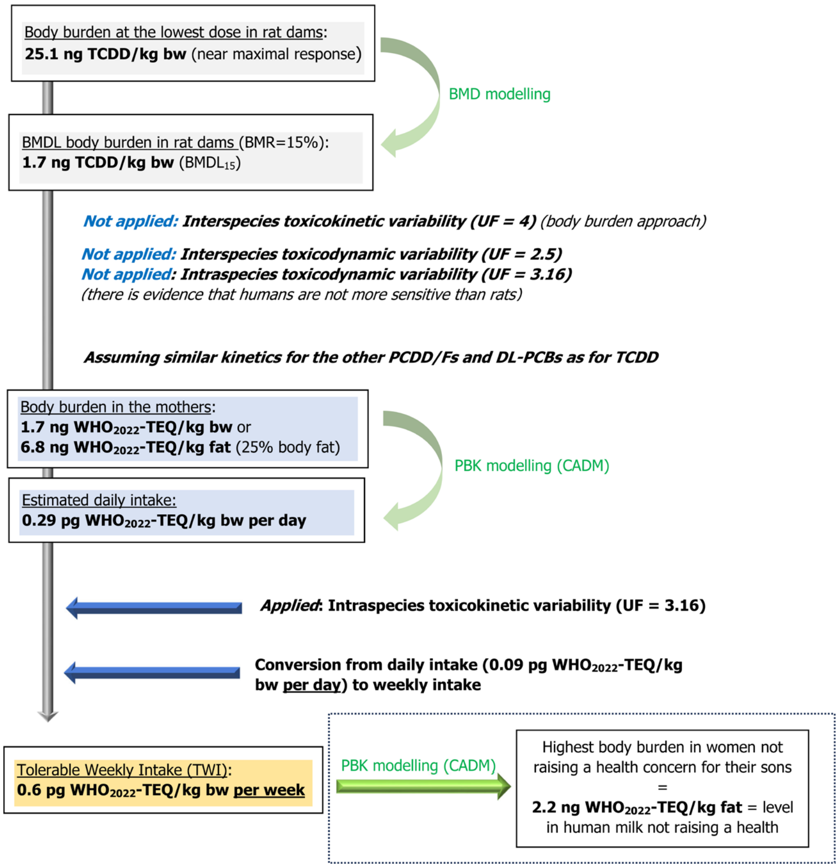

FIGURE 12 Schematic view of the steps for the derivation of the HBGV in this Opinion. Dotted box: To facilitate comparison with the levels in human milk, the CADM model was used to derive the body burden and human milk levels in women after chronic exposure to PCDD/Fs and DL-PCBs at the TWI.

3.1.6.3 | *Applicability of the TWI*

This TWI is considered protective for the general population and prevents women from reaching a concentration in the body that could lead to in utero and lactational exposures associated with health concerns in the offspring. Based on the available data, this TWI is considered to be protective towards all endpoints. These include lower sex ratio, higher TSH levels in newborns and developmental enamel defects on teeth, the latter appearing to occur at only slightly higher exposure than the developmental effects on semen quality, as indicated by the CONTAM Panel in 2018.

Although this TWI is based on findings on TCDD only, the CONTAM Panel concluded that the TWI should apply to the sum of PCDD/Fs and DL-PCBs based on the TEQ concept.

The TWI is derived to prevent women of childbearing age from having body burdens (i.e. levels in their bodies) that could lead to in utero and lactational exposures associated with health concerns in the offspring. The CONTAM Panel recognises that infants have higher exposure per kg bw during breastfeeding than the TWI, resulting in a higher body burden (see Figure 11). However, this was already taken into account when setting the TWI. Therefore, the TWI is not applicable for infants, and it is not appropriate to compare it to the exposure of infants.

3.1.6.4 | *Considerations of the TWI in relation to previous assessments*

An overview of the previous assessments is given in **Section 1.3.1**. In this section, explanations of the considerations made in the current Opinion that led to different TWIs are discussed, even if the current TWI is based on the same critical studies as in previous assessments.

The CONTAM Panel is aware that the TWI of 0.6 pg. WHO_{2022}-TEQ/kg bw per week is 3-fold lower than the TWI derived in its 2018 Opinion. Applying the new WHO_{2022}-TEFs to the Mínguez-Alarcón et al. (2017) study resulted in only a slight decrease in the NOAEC for PCDD/F-TEQ for a lower sperm concentration (see Table 5). It should be noted that there is still substantial evidence that PCB-126, the congener contributing most to the TEQ-levels in the Russian boys, shows a much lower potency in human cells than reflected by its WHO_{2022}-TEF. However, the approach taken by WHO in deriving the TEFs did not allow basing them solely on biochemical effects in human cells (DeVito et al., 2024; Wikoff et al., 2023). Using the WHO_{2022}-TEFs, the study by Mínguez-Alarcón et al. (2017) showed NOAECs of 3.3 and 6.7 ng WHO_{2022}-TEQ/kg lipid for PCDD- and PCDD/F-TEQ levels, respectively, but no significant association for PCDF-, DL-PCB- or Total-TEQ. When ignoring the DL-PCBs, the Reference Point would thus be 6.7 ng WHO_{2022}-TEQ/kg lipid for the PCDD/F-TEQ level in blood of 9-year-old boys. This level is clearly affected by the higher exposure during breastfeeding, since formula-fed boys showed on average lower levels at the age of 8–9 years (Burns et al., 2009). Back-extrapolation to estimate the corresponding levels in human milk, using the CADM model and the same considerations as in the 2018 Opinion, revealed a level of 5.6 ng/kg fat in human milk, and hence the highest body burden in the mothers not raising a health concern for their sons, on a lipid weight basis assuming 25% fat in women. For a 35-year-old woman, such a human milk level and body burden are obtained after chronic exposure to 0.24 pg. WHO_{2022}-TEQ/kg bw per day, or 1.7 pg. WHO_{2022}-TEQ/kg bw per week. However, the continued lack of an association with Total-TEQ despite the revision of the WHO-TEFs prevented the use of this study for deriving the HBGV. The current Opinion therefore used an experimental rat study with TCDD as the critical study. The CONTAM Panel also noted that contrary to the 2018 Opinion, the breastfeeding assumptions in the CADM model did not play a significant role in establishing the TWI in the current update, since it based on the body burden in the mothers and the long-term exposure that would result in such a body burden. The early exposure via human milk results in a minor effect on the maternal body burden at the age of 35 years (see Figure 11).

The CONTAM Panel is aware of the 23-fold lower TWI than the one of 14 pg. WHO_{1998}-TEQ/kg bw per week derived by SCF (2001) which was based on the same critical study in rats. The latter decrease is not linked to the application of the new WHO-TEFs since it is based on a study with TCDD, i.e. the reference compound in the TEF scheme (TEF = 1). In comparison with the assessment by SCF (2001), the estimated highest body burden in dams not leading to adverse effects in pups was much lower. SCF derived for the lowest dose a LOAEL body burden of 40 ng/kg bw and applied a factor 3 for a LOAEL to a NOAEL to derive a NOAEL body burden of 40/3 = 13.3 ng TCDD/kg bw (of note: the UF of 3 was applied on the estimated daily intake leading to the LOAEL body burden). BMD modelling applied in the current Opinion resulted in a 7.8-fold lower value for the highest body burden of 1.7 ng TCDD/kg bw (see **Section 3.1.5.2**). This much lower body burden compared to the lowest applied dose showing a near maximal response, is supported by other endpoints (e.g. CYP1 induction, DeVito, 1996; Johnson et al., 2020) but also reduced epidydimal sperm numbers in the study by Mably et al. (1992). In the latter study, single TCDD doses of 0.064, 0.16, 0.40, 1.0 μg/kg bw were applied on day GD15 by gavage. At PND120 the lowest dose resulted in a 28% decrease of epidydimal sperm concentrations in the pups, compared to 57% at the highest dose. This shows that the slope of the dose–response curve is rather shallow within the dose range from 0.064 to 1 μg/kg bw, i.e. a 16-fold difference to go from a 28% decrease to a 57% decrease (see Appendix E, Figure E.1). It is noted that the doses in this study seem higher than in Faqi et al. (1998a) but due to the short half-life in rats and the lack of knowledge on the critical window, the body burdens in the dams will rapidly decrease after a single dose, a common issue in most of the studies (see Appendix C).

Furthermore, this lower highest body burden of 1.7 ng TCDD/kg bw in rat dams seems more in line with the three human studies (see Table 7). As in 2001, the UFs for interspecies variability in toxicokinetics and those for inter- and intraspecies variability in toxicodynamics were not applied, whereas in both assessments the UF of 3.16 for intraspecies variability in humans was applied. The CONTAM Panel decided to use the calculated body burden for the lowest dose in the current assessment and not to correct for a single high dose (by an implied factor of 1.6, applied by SCF in 2001). This would have increased the highest body burden (for more details see Appendix C). Applying the PBK CADM model instead of one-compartment modelling further reduced the TWI by a factor 3, primarily due to the 2-fold higher absorption factor in the PBK model but also a longer half-life at lower body burden. Overall, this resulted in the $7.8 \times 3 \approx 23$-fold difference.

Compared to the reference dose (RfD) of 0.7 pg. TEQ/kg bw per day derived by US-EPA (2012), the TWI derived in the current assessment is 8-fold lower. This may be partly explained by using a different toxicokinetic model, as shown by the fact that for a similar highest body burden a different estimated human intake was estimated.

3.2 | Occurrence data

3.2.1 | Occurrence data in food and feed submitted to EFSA

A total of 83,031 food samples and 6992 feed samples on PCDD/Fs and DL-PCBs sampled from year 2013 were available in the EFSA database by the 4th of September 2024 (Annex H).

Data providers were contacted to clarify a number of possible inconsistencies which were identified during the data check. The following modifications were made to the initial data set based on the feedback received:

- All results were converted to the unit of measure (pg/g) laid down in Commission Regulation (EU) No 2023/915.
- The matrix description (free text, facets) of a number of records allowed a more accurate FoodEx2 classification. In these cases, the samples were reclassified according to this information.
- Only the samples that were analysed for all 29 congeners were retained for further validation steps while samples that were incomplete (n food samples = 17,767 and n feed samples = 250) were excluded. These were mainly constituted by samples for which only PCB-118 was analysed (*n* = 12,317).
- Samples for which issues on the reported analytical value could not be resolved (e.g. unit of measure, correct parameter, etc.) after data provider consultation (n food samples = 390 and n feed samples = 63) were excluded.
- One sample of potato was excluded, with a concentration for the 29 PCDD/Fs and DL-PCBs of 0.24 pg. WHO_{2022}-TEQ/g whole weight, as it was having what was considered by expert judgement a too high impact on the mean concentration of the limited number of samples available (*n* = 11). The concentration range of the other samples was 0.002–0.006 pg. WHO_{2022}-TEQ/g whole weight. The impact of this exclusion will be discussed in **Section 3.5**.

– Five feed samples (barley grain, soya (bean) expeller, monocalcium phosphate, and two fish meal samples) were excluded based on expert judgement, as their concentrations of the 29 PCDD/Fs and DL-PCBs were 10- to 7719-fold higher than the maximum concentration found in the other samples.
– Samples reported as 'suspect sampling' were excluded (n food samples = 739 and n feed samples = 3).
– Only results of samples reported to be analysed by GC–HRMS or GC–MS/MS (reported by the data providers also as HRGC–HRMS, GC–HRMS (magnetic sector), GC-QqQ-MS–MS or GC–MS) were considered sufficiently reliable to be included in the assessment. Samples with results obtained with other analytical methods or without information on the analytical method were excluded from the assessment (n food samples = 2173).

In addition, due to the uncertainty in the reporting of certain food categories the following steps were taken:

– The PCDD/Fs and DL-PCBs concentrations in liquid formulas and reconstituted cereal based foods for the young population (for which there was also a limited number of samples available) was calculated from the PCDD/Fs and DL-PCBs concentrations in the corresponding powder/non reconstituted form applying the dilution factors provided in EFSA (2018).
– The concentration in fresh aromatic herbs was set to be the same as that of other leafy vegetables.

Expression of results

According to Commission Regulation (EU) No 2023/915,[34] levels of foodstuffs of terrestrial animal origin (except offal), marine oils and vegetable fats and oils are to be given on a fat weight basis. Levels in products of aquatic origin (except marine oil), liver of terrestrial animals and products of plant origin except vegetable fats and oils are expressed on a whole weight basis; levels in feedstuffs have to be expressed on an 88% dry matter basis.

Samples which should have been reported on fat weight or 88% dry matter basis according to legislation but were submitted to EFSA on whole weight basis, and for which transformation was not possible because their fat or moisture content was missing, were excluded from the current assessment (n food samples = 1991 and n feed samples = 824).

Based on data provider feedback, where percentage fat was missing for food categories concerning animal and vegetable fat and oils, a fat percentage of 100% was assumed.

After the data validation steps described above, 23,060 food samples (28%) and 1145 feed samples (16%) were excluded, while 59,971 food samples and 5847 feed samples were available for the next validation step. Annex B (Table B.2) shows a summary of the sample exclusion.

In most cases, the fat content of a sample will be determined gravimetrically after extraction with organic solvents. Fat samples may be analysed after a drying step to remove the water; correction for the loss of water is unlikely the expression per gram fat is probably correct. For some samples like liver, fish, blood but also lean meat, a specific extraction may be required, using more polar solvents. If not applied, the actual lipid content may be underestimated and the levels of PCDD/Fs and DL-PCBs overestimated since these may be efficiently extracted. It is unclear if this is behind the higher average levels reported for meat as compared to animal fats from the same species, and the variable results observed in transfer studies. In the case of blood analysis, a special approach may be needed to correct for all lipids, like the separate determination of the levels of triglycerides, cholesterol esters and phospholipids (Patterson, Furst, et al., 1989; Patterson, Fürst, et al., 1989). In some studies, only the first two are determined and a correction factor is applied for total lipids.

3.2.2 | Occurrence data on food submitted to EFSA

3.2.2.1 | *Application of performance criteria*

In Commission Regulation (EU) No 2017/644, performance criteria for the analytical methods are defined, including the LOQs based on the ML for PCDD/Fs or ALs for DL-PCBs. Some of the current legislative categories for MLs and ALs do not align with the food categories defined by the FoodEx2 system for occurrence data. When information was provided for only a parent group without any further details, the following assumptions were taken into consideration for the application of the performance criterion on the occurrence data:

For the sum of PCDD/Fs and foods with MLs:

- Meat products: In cases of fresh meat, cured or processed meat, and sausages, if the animal source was unspecified, all meat-associated products were assumed to be made with pig meat, and the corresponding ML was applied. If the source of animal was mixed (e.g. pig and bovine), the ML for pig meat was applied, as it was the lowest.
- Bird meat: If no details were provided, the ML for poultry meat was applied.
- Animal fat: If the source was not specified, it was assumed to be pig fat, as it has the lowest ML, and this ML was applied accordingly.
- Liver: If no source was provided, the ML for the liver of the group of bovine, caprine, pig, poultry, or horse was used.

By applying the performance criteria as described in **Section 2.3.2** a total of 5732 food samples were excluded based on the performance criteria applied to the LOQs for the sum of PCDD/Fs and 159 based on the performance criteria applied to the LOQs for the sum of the DL-PCBs.

Overall, 5794 samples were excluded as they failed one or both of the criteria while 54,177 fulfilled the criteria and were included in the final dataset.

3.2.2.2 | *Description of the final dataset of occurrence in food*

The food samples included in the final dataset were submitted by 24 MSs plus Norway and Iceland, and sampled between the years 2013 and 2023 (Table 8). Germany and France submitted 64% of the samples. Sample distribution across the sampling year was comparable among the years.

The number of samples available per FoodEx2 Level 1 are displayed in Table 9. To be noted is the limited availability of data for 'Vegetables and vegetable products', and the lack of information on whether the samples had been washed or peeled before analysis. Specific food subcategories for which there were no occurrence data available were attributed with the concentration of the parent FoodEx2 category. In particular the mean concentration for 'Leafy Brassica' derived mainly from samples of 'Curly kale', was attributed to more diverse vegetables such as bulb vegetables, root and tuber vegetables (excluding starchy- and sugar-) such as carrots, Head brassicas and Legumes with pods that might have a caused overestimation of exposure from vegetables. Table 10 shows which parent category (Source FoodEx2) was used for each of the vegetable food subcategories at the Level 2 of the FoodEx2 classification and the original number of available samples for the subcategory (N samples) and for the parent category (Source N samples). These uncertainties are discussed in **Section 3.5**.

The highest mean LB of the sum of the 29 PCDD/Fs and DL-PCBs was found in bonito fish meat (55 pg. WHO_{2022}-TEQ/g whole weight, see Annex B) and in cod liver (13 pg. WHO_{2022}-TEQ/g whole weight).

The highest P95 LB of the sum of the 29 PCDD/Fs and DL-PCBs was found in fish liver (up to 37 pg. WHO_{2022}-TEQ/g whole weight in cod liver).

For an overview, the mean and P95 LB and UB concentrations calculated using WHO_{2005}-TEQ and WHO_{2022}-TEQ for the sum of the 29 PCDD/Fs and DL-PCBs in selected food groups are shown in Table 11. The concentrations for the individual congeners, the sum of the 29 PCDD/Fs and DL-PCBs and the 17 PCDD/Fs used in the chronic dietary exposure assessment for each FoodEx2 code in the consumption database for which a concentration could be estimated are available in Annex B (Tables 3 and 4).

TABLE 8 Number of food samples per year and country available in the final data set.

Country/year	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	Total	%by country
Austria		16	31	30	16	30	29	24	30	37	55	**298**	**0.6%**
Belgium							9	249	111			**369**	**0.7%**
Croatia		11	18	19	2		14	3	8	11	15	**101**	**0.2%**
Cyprus	16	15	17	17	19	19	17	17	21	22	22	**202**	**0.4%**
Czechia	13	17	33	33	38	39	44	36	28	33	37	**351**	**0.6%**
Denmark	268	172	335	357	327	398	351	337	322	346	345	**3558**	**6.6%**
Finland	29				25	35	18	9		12		**128**	**0.2%**
France	647	1	2271	2277	2212	2188	2229	2259	1799	1654	1314	**18,851**	**34.8%**
Germany	1016	1648	31	1380	710	1694	1804	1724	1774	1829	2017	**15,627**	**28.8%**
Greece							6	6	12		12	**36**	**0.1%**
Hungary	150	1	112	72	34	37	19	12	14	14	10	**475**	**0.9%**
Iceland											2	**2**	**0.0%**
Ireland	2	8	78	91	113	139	123	39	94	131	148	**966**	**1.8%**
Italy					145	699	613	563	390	1082	694	**4186**	**7.7%**
Latvia			19	20	20	18	19	9	6	22	52	**185**	**0.3%**
Lithuania			24	6			117	2	1	5	41	**196**	**0.4%**
Malta		2										**2**	**0.0%**
Netherlands	2	160	135	105	106	115	102	121	223	238	240	**1547**	**2.9%**
Norway	545	682	242	298	291	681	152	116	129	140	142	**3418**	**6.3%**
Poland	167	171	139	142	137	118	118	121	118	111	115	**1457**	**2.7%**
Portugal		92	126	48	24		24	1	3		3	**321**	**0.6%**
Romania			12		2							**14**	**0.03%**
Slovakia	19											**19**	**0.04%**
Slovenia	47	23	25	17	26	4	2		10	8		**162**	**0.3%**
Spain	45	54	27	47	41	26		32	119	145	394	**930**	**1.7%**
Sweden	60	64	83	72	10	74	71	85	65	65	127	**776**	**1.4%**
Total	**3026**	**3137**	**3758**	**5031**	**4298**	**6314**	**5881**	**5765**	**5277**	**5905**	**5785**	**54,177**	**100%**
% by year	**6%**	**6%**	**7%**	**9%**	**8%**	**12%**	**11%**	**11%**	**10%**	**11%**	**11%**	**100%**	

TABLE 9 Number of food samples (N samples) available per FoodEx2 Level 1 in the final dataset.

FOODEX2_L1	No. food samples
Meat and meat products	22,737
Fish and seafood	13,647
Eggs and egg products	7399
Milk and dairy products	6293
Animal and vegetable fats and oils and primary derivatives thereof	1730
Food products for young population	810
Legumes, nuts, oilseeds and spices	409
Vegetables and vegetable products	326
Products for non-standard diets, food imitates and food supplements	303
Sugar and similar, confectionery and water-based sweet desserts	163
Grains and grain-based products	110
Composite dishes	90
Major isolated ingredients, additives, flavours, baking and processing aids	51
Fruit and fruit products	40
Coffee, cocoa, tea and infusions	20
Amphibians, reptiles, and terrestrial invertebrates	19
Seasoning, sauces and condiments	17
Starchy roots or tubers and products thereof, sugar plants	11
Fruit and vegetable juices and nectars (including concentrates)	1
Water and water-based beverages	1

TABLE 10 Examples of concentration imputation from FoodEx2 parent categories. For those food subcategories for which less than 6 samples or no samples were available, the columns 'Source FoodEx2' and 'Source N samples' indicate the FoodEx2 parent category from which the concentration was obtained and the available number of samples for the parent category.

FOODEX2_L2_ID	N samples	Source FoodEx2	Source N samples
Algae and prokaryotes organisms	3	Vegetables and vegetable products	172
Bulb vegetables	5	Vegetables and vegetable products	172
Flowering brassica	1	Vegetables and vegetable products	172
Flowers used as vegetables	0	Vegetables and vegetable products	172
Fruiting vegetables	28	Fruiting vegetables	28
Fungi, mosses and lichens	9	Fungi, mosses and lichens	9
Herbs and edible flowers[a]	91	Leafy vegetables	99
Leafy vegetables	99	Leafy vegetables	99

(Continues)

TABLE 10 (Continued)

FOODEX2_L2_ID	N samples	Source FoodEx2	Source N samples
Legumes with pod	0	Vegetables and vegetable products	172
Processed or preserved vegetables and similar	2	Vegetables and vegetable products	172
Root and tuber vegetables (excluding starchy- and sugar-)	21	Root and tuber vegetables (excluding starchy- and sugar-)	21
Sprouts, shoots and similar	0	Vegetables and vegetable products	172
Stems/stalks eaten as vegetables	6	Stems/stalks eaten as vegetables	6

[a]Although a large number of samples was available for Herbs and edible flowers the concentration from Leafy vegetables was used for this category due to the uncertainty of data reporting (it is often not clear if reported concentrations for this category refer to fresh or dry herbs).

TABLE 11 Mean and P95 levels for the sum of 29 PCDD/Fs and DL-PCBs in selected food groups.

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g fat weight)					WHO_{2022}-TEQ				WHO_{2005}-TEQ			
					Mean		P95[a]		Mean		P95[a]	
FOODEX2_L1	FOODEX2_L2	FOODEX2	FoodEx2 level	N	LB	UB	LB	UB	LB	UB	LB	UB
Meat and meat products	Mammals and birds meat	Bovine fresh meat	4	1856	0.52	0.65	1.51	1.62	0.83	0.98	2.54	2.58
Meat and meat products	Mammals and birds meat	Pig fresh meat	4	1572	0.07	0.18	0.25	0.36	0.07	0.21	0.28	0.39
Meat and meat products	Mammals and birds meat	Sheep fresh meat	4	552	0.44	0.54	1.27	1.34	0.73	0.84	2.37	2.39
Meat and meat products	Mammals and birds meat	Goat fresh meat	4	47	0.70	0.77			1.19	1.27		
Meat and meat products	Mammals and birds meat	Equine fresh meat	4	127	2.97	3.04	7.95	7.95	4.67	4.73	14.62	14.62
Meat and meat products	Mammals and birds meat	Rabbit fresh meat	4	118	0.15	0.26	0.40	0.46	0.24	0.38	0.68	0.78
Meat and meat products	Mammals and birds meat	Deer fresh meat	4	266	1.36	1.45	3.92	4.05	2.44	2.55	7.27	7.28
Meat and meat products	Mammals and birds meat	Wild boar fresh meat	4	448	2.99	3.15	11.68	11.69	3.83	4.02	15.01	15.49
Meat and meat products	Mammals and birds meat	Hare fresh meat	4	17	0.37	0.50			0.64	0.82		
Meat and meat products	Mammals and birds meat	Poultry fresh meat (muscle meat)	4	3882	0.18	0.31	0.67	0.83	0.25	0.42	0.88	1.09
Meat and meat products	Animal fresh fat tissues	Bovine fat tissue	4	2743	0.35	0.41	0.92	0.98	0.60	0.67	1.63	1.69
Meat and meat products	Animal fresh fat tissues	Pig fat tissue	4	4370	0.05	0.12	0.14	0.22	0.05	0.14	0.15	0.23
Meat and meat products	Animal fresh fat tissues	Sheep fat tissue	4	1455	0.28	0.34	0.77	0.81	0.47	0.55	1.17	1.21
Meat and meat products	Animal fresh fat tissues	Goat fat tissue	4	71	0.27	0.33	0.63	0.67	0.41	0.48	1.02	1.05
Meat and meat products	Animal fresh fat tissues	Equine fat tissue	4	188	2.37	2.39	6.10	6.11	3.88	3.90	10.05	10.06
Meat and meat products	Animal fresh fat tissues	Deer, fresh fat tissue	4	16	1.07	1.11			1.96	1.99		
Meat and meat products	Animal fresh fat tissues	Wild boar, fresh fat tissue	4	15	0.99	1.07			1.44	1.54		
Meat and meat products	Animal fresh fat tissues	Poultry fat tissue	4	272	0.08	0.17	0.31	0.40	0.13	0.23	0.53	0.55
Milk and dairy products	Milk, whey and cream	Cow milk	5	4217	0.37	0.47	0.77	0.88	0.61	0.73	1.27	1.38
Milk and dairy products	Cheese	Cheese	2	923	0.24	0.30	0.49	0.59	0.39	0.47	0.82	0.92
Eggs and egg products	Unprocessed eggs	Hen eggs	4	6846	0.54	0.67	2.06	2.17	0.72	0.87	2.70	2.76
Animal and vegetable fats and oils and primary derivatives thereof	Animal and vegetable fats/oils	Olive oils	4	195	0.03	0.08	0.09	0.16	0.03	0.11	0.14	0.21
Animal and vegetable fats and oils and primary derivatives thereof	Animal and vegetable fats/oils	Pork lard	5	400	0.07	0.12	0.06	0.14	0.26	0.31	0.33	0.36

(Continues)

TABLE 11 (Continued)

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g fat weight)					WHO$_{2022}$-TEQ				WHO$_{2005}$-TEQ			
					Mean		P95[a]		Mean		P95[a]	
FOODEX2_L1	FOODEX2_L2	FOODEX2	FoodEx2 level	N	LB	UB	LB	UB	LB	UB	LB	UB
Animal and vegetable fats and oils and primary derivatives thereof	Animal and vegetable fats/oils	Fish oil	5	81	0.96	1.06	3.03	3.23	1.77	1.90	5.17	5.40
Animal and vegetable fats and oils and primary derivatives thereof	Fat emulsions and blended fats	Butter	3	116	0.20	0.23	0.45	0.47	0.34	0.38	0.77	0.77

29 PCDD/Fs and DL-PCBs (pg TEQ/g whole weight)					WHO$_{2022}$-TEQ				WHO$_{2005}$-TEQ			
					Mean		P95		Mean		P95	
FOODEX2_L1	FOODEX2_L2	FOODEX2	FoodEx2 Level	N	LB	UB	LB	UB	LB	UB	LB	UB
Meat and meat products	Animal liver	Bovine liver	4	522	0.10	0.11	0.24	0.25	0.09	0.11	0.26	0.27
Meat and meat products	Animal liver	Pig liver	4	321	0.26	0.27	0.86	0.87	0.18	0.19	0.70	0.70
Meat and meat products	Animal liver	Sheep liver	4	1461	0.31	0.33	1.08	1.11	0.41	0.43	1.48	1.51
Meat and meat products	Animal liver	Goat liver	4	59	0.13	0.14	0.57	0.58	0.14	0.15	0.44	0.45
Meat and meat products	Animal liver	Equine liver	4	104	0.27	0.28	0.76	0.76	0.38	0.38	0.93	0.94
Meat and meat products	Animal liver	Rabbit liver	4	4	0.44	0.44			0.63	0.63		
Meat and meat products	Animal liver	Deer liver	4	81	1.25	1.25	4.30	4.30	1.77	1.77	5.13	5.13
Meat and meat products	Animal liver	Wild boar liver	4	169	3.69	3.70	21.3	21.3	3.48	3.49	22.2	22.2
Meat and meat products	Animal liver	Chicken liver	4	142	0.01	0.03	0.04	0.05	0.01	0.03	0.03	0.06
Meat and meat products	Animal liver	Turkey liver	4	17	0.01	0.03			0.01	0.03		
Meat and meat products	Animal liver	Duck liver	4	15	0.04	0.04			0.06	0.07		
Meat and meat products	Animal liver	Goose liver	4	14	0.01	0.01			0.02	0.02		

TABLE 11 (Continued)

					WHO$_{2022}$-TEQ				WHO$_{2005}$-TEQ			
29 PCDD/Fs and DL-PCBs (pg TEQ/g whole weight)					**Mean**		**P95**		**Mean**		**P95**	
FOODEX2_L1	**FOODEX2_L2**	**FOODEX2**	**FoodEx2 Level**	**N**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Fish and seafood	Fish (meat)	Freshwater fish	3	928	0.21	0.24	0.77	0.78	0.36	0.40	1.29	1.32
Fish and seafood	Fish (meat)	Diadromous fish	3	3219	0.73	0.78	3.68	3.73	1.34	1.40	6.75	6.84
Fish and seafood	Fish (meat)	Marine fish	3	5590	0.61	0.65	1.94	1.97	1.17	1.21	3.69	3.71
Fish and seafood	Fish and seafood processed	Processed or preserved fish (including processed offal)	3	248	0.56	0.60	3.54	3.60	0.97	1.02	5.37	5.38
Fish and seafood	Fish and seafood processed	Processed or preserved seafood	3	38	0.11	0.17			0.20	0.25		
Fish and seafood	Fish offal	Fish liver	3	836	12.46	12.92	36.76	37.03	22.40	22.91	66.39	66.77
Fish and seafood	Fish offal	Fish roe	3	27	0.21	0.28			0.34	0.44		
Fish and seafood	Fish offal	Other fish offal	3	27	3.81	3.89			6.81	6.89		
Fish and seafood	Molluscs	Abalones, winkles, conchs	3	18	0.05	0.07			0.09	0.12		
Fish and seafood	Molluscs	Clams, cockles, arkshells	3	61	0.04	0.09	0.10	0.44	0.06	0.13	0.16	0.48
Fish and seafood	Molluscs	Mussels	3	767	0.21	0.25	0.64	0.67	0.36	0.41	1.06	1.06
Fish and seafood	Molluscs	Oysters	3	445	0.40	0.41	1.03	1.03	0.75	0.76	1.76	1.76
Fish and seafood	Molluscs	Scallops, pectens	3	443	0.05	0.06	0.21	0.22	0.08	0.09	0.33	0.33
Fish and seafood	Molluscs	Freshwater molluscs	3	15	0.02	0.03			0.02	0.04		
Fish and seafood	Molluscs	Squids, cuttlefishes, octopuses	3	137	0.04	0.08	0.18	0.28	0.07	0.12	0.31	0.46
Fish and seafood	Crustaceans	Freshwater crustaceans	3	16	0.01	0.03			0.02	0.04		
Fish and seafood	Crustaceans	Crabs, sea-spiders	3	162	0.48	0.50	3.59	3.60	0.81	0.83	6.10	6.11
Fish and seafood	Crustaceans	Shrimps and prawns	3	219	0.08	0.11	0.70	0.70	0.13	0.16	1.07	1.07
Fish and seafood	Crustaceans	Lobsters, spiny-rock lobster	3	105	0.11	0.12	0.22	0.24	0.18	0.19	0.38	0.38
Food products for young population	Infant and follow-on formulae	Follow-on formulae, powder	4	193	0.004	0.06	0.02	0.06	0.004	0.06	0.02	0.08

(Continues)

TABLE 11 (Continued)

29 PCDD/Fs and DL-PCBs (pg TEQ/g whole weight)					WHO_{2022}-TEQ				WHO_{2005}-TEQ			
					Mean		P95		Mean		P95	
FOODEX2_L1	FOODEX2_L2	FOODEX2	FoodEx2 Level	N	LB	UB	LB	UB	LB	UB	LB	UB
Food products for young population	Infant and follow-on formulae	Infant formulae, powder	4	245	0.002	0.01	0.01	0.03	0.003	0.01	0.01	0.04
Starchy roots or tubers and products thereof, sugar plants	Starchy roots and tubers	Potatoes and similar-	3	10	0.01	0.02			0.01	0.01		
Fruit and fruit products	Fruit and fruit products	Fruit and fruit products	1	27	0.00	0.02			0.00	0.02		
Grains and grain-based products	Cereals and cereal primary derivatives	Cereals and cereal primary derivatives	2	93	0.004	0.03	0.02	0.12	0.00	0.03	0.02	0.14
Vegetables and vegetable products	Fruiting vegetables	Fruiting vegetables	2	28	0.01	0.02			0.01	0.03		
Vegetables and vegetable products	Leafy vegetables	Leafy vegetables	2	99	0.04	0.05	0.10	0.10	0.05	0.06	0.13	0.14

Abbreviations: LB, lower bound; UB, upper bound.

[a]The 95th percentile estimates obtained with less than 595 observations may not be statistically robust (EFSA, 2011a). Those estimates were not included in this table.

3.2.2.3 | *Occurrence data for special food groups*

A comparison of occurrence for the sum of 29 PCDD/Fs and DL-PCBs, of the 17 PCDD/Fs and of the 12 DL-PCBs within special food groups was performed to understand the impact of some factors such as production method and geographical origin.

The FoodEx2 classification system allows adding specific information about the production method of the sample. Submitting this information is not compulsory but may be added by the data provider. Definitions of possible production methods according to the relevant controlled terminology is shown in Table 12. Multiple values can be provided where relevant (e.g. Outdoor/free-range growing condition and Organic production or Outdoor/free-range growing condition and Conventional (non-organic) production).

TABLE 12 Definitions of production methods according to the FoodEx2 facet 'Production method'.

Production method	Definition
Battery production[a]	Production of animals in cages (applies to poultry, rabbits)
Traditional production	Production of food using traditional/artisan methods
Organic production	A method of production which places the highest emphasis on environmental protection and, with regard to livestock production, animal welfare considerations.
Conventional (non-organic) production	Products produced without use of organic production methods
Farmed domestic or cultivated	Animals produced in captivity (applies also to game and fish), plants produced by cultivation
Outdoor/free-range growing condition	Grown outdoor, in an open environment
Production method unknown	/
Wild or gathered or hunted	An animal or plant products harvested from their natural environment

[a]Battery production of eggs is prohibited since 1 January 2012 (Council Directive 1999/74/EC). What is reported as battery production after this date presumably refers to enriched caging.

3.2.2.3.1 | *Chicken eggs*

Differences between levels in chicken eggs from indoor vs. outdoor, as well organic vs. conventional types of production, were investigated and the occurrence values for the different production methods are shown in **Tables 13–18** for the WHO_{2022}-TEQ and WHO_{2005}-TEQ sum of the 29 PCDD/Fs and DL-PCBs, the 17 PCDD/Fs and the 12 DL-PCBs.

In general, eggs from hens with outdoor (including organic production)/free range access showed higher levels of both PCDD/Fs and DL-PCBs than those kept indoors.

TABLE 13 Sum of 29 PCDD/F and DL-PCB levels expressed in pg. WHO_{2022}-TEQ/g fat and pg. WHO_{2005}-TEQ/g fat weight in chicken eggs between indoor and outdoor production methods.

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g fat weight)				WHO_{2022}-TEQ				WHO_{2005}-TEQ			
				Mean		P95		Mean		P95	
FOODEX2_L1	FOODEX2	Production method	N	LB	UB	LB	UB	LB	UB	LB	UB
Eggs and egg products	Hen eggs	Outdoor/free-range growing condition	2736	0.70	0.82	2.40	2.43	0.94	1.08	3.19	3.21
Eggs and egg products	Hen eggs	Indoor/Battery caged	170	0.14	0.26	0.37	0.49	0.17	0.32	0.49	0.59
Eggs and egg products	Hen eggs	Unspecified	3940	0.45	0.59	1.89	1.98	0.59	0.75	2.41	2.57

TABLE 14 Sum of 17 PCDD/F levels expressed in pg. WHO_{2022}-TEQ/g fat and pg. WHO_{2005}-TEQ/g fat weight in chicken eggs between indoor and outdoor production methods.

17 PCDD/fs (pg WHO-TEQ/g fat weight)				WHO_{2022}-TEQ				WHO_{2005}-TEQ			
				Mean		P95		Mean		P95	
FOODEX2_L1	FOODEX2	Production method	N	LB	UB	LB	UB	LB	UB	LB	UB
Eggs and egg products	Hen eggs	Outdoor/free-range growing condition	2736	0.45	0.56	1.61	1.63	0.46	0.58	1.69	1.72
Eggs and egg products	Hen eggs	Indoor/Battery caged	170	0.10	0.22	0.27	0.43	0.10	0.24	0.32	0.46
Eggs and egg products	Hen eggs	Unspecified	3940	0.29	0.41	1.31	1.39	0.28	0.42	1.36	1.43

TABLE 15 Sum of 12 DL-PCB levels expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2005}-TEQ/g fat weight in chicken eggs between indoor and outdoor production methods.

12 DL-PCBs (pg WHO-TEQ/g fat weight)				WHO_{2022}-TEQ				WHO_{2005}-TEQ			
				Mean		P95		Mean		P95	
FOODEX2_L1	FOODEX2	Production method	N	LB	UB	LB	UB	LB	UB	LB	UB
Eggs and egg products	Hen eggs	Outdoor/free-range growing condition	2736	0.25	0.26	0.75	0.75	0.49	0.50	1.47	1.47
Eggs and egg products	Hen eggs	Indoor/Battery caged	170	0.04	0.04	0.11	0.11	0.07	0.08	0.19	0.19
Eggs and egg products	Hen eggs	Unspecified	3940	0.16	0.18	0.55	0.57	0.31	0.33	1.05	1.06

TABLE 16 Sum of 29 PCDD/F and DL-PCB levels expressed in pg. WHO2022-TEQ/g fat and pg. WHO2005-TEQ/g fat weight in chicken eggs between organic and conventional production methods.

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g fat weight)				WHO_{2022}-TEQ				WHO_{2005}-TEQ			
				Mean		P95		Mean		P95	
FOODEX2_L1	FOODEX2	Production method	N	LB	UB	LB	UB	LB	UB	LB	UB
Eggs and egg products	Hen eggs	Unspecified	5482	0.54	0.67	2.12	2.26	0.70	0.85	2.70	2.76
Eggs and egg products	Hen eggs	Organic production	1123	0.61	0.72	1.87	2.06	0.86	0.99	2.72	2.78
Eggs and egg products	Hen eggs	Conventional (non-organic) production	241	0.45	0.56	1.70	1.72	0.56	0.69	2.26	2.26

TABLE 17 Sum of 17 PCDD/F levels expressed in pg. WHO2022-TEQ/g fat and pg. WHO2005-TEQ/g fat weight in chicken eggs between organic and conventional production methods.

17 PCDD/fs (pg WHO-TEQ/g fat weight)				WHO_{2022}-TEQ				WHO_{2005}-TEQ			
				Mean		P95		Mean		P95	
FOODEX2_L1	FOODEX2	Production method	N	LB	UB	LB	UB	LB	UB	LB	UB
Eggs and egg products	Hen eggs	Unspecified	5482	0.35	0.46	1.40	1.49	0.34	0.47	1.45	1.56
Eggs and egg products	Hen eggs	Organic production	1123	0.37	0.47	1.36	1.43	0.41	0.52	1.48	1.50
Eggs and egg products	Hen eggs	Conventional (non-organic) production	241	0.31	0.40	1.03	1.04	0.30	0.40	1.16	1.17

TABLE 18 Sum of 12 DL-PCB levels expressed in pg. WHO2022-TEQ/g and pg. WHO2005-TEQ/g fat weight in chicken eggs between organic and conventional production methods.

12 DL-PCBs (pg WHO-TEQ/g fat weight)				WHO_{2022}-TEQ				WHO_{2005}-TEQ			
				Mean		P95		Mean		P95	
FOODEX2_L1	FOODEX2	Production method	N	LB	UB	LB	UB	LB	UB	LB	UB
Eggs and egg products	Hen eggs	Unspecified	5482	0.19	0.21	0.63	0.63	0.36	0.38	1.17	1.17
Eggs and egg products	Hen eggs	Organic production	1123	0.24	0.25	0.69	0.69	0.46	0.47	1.31	1.31
Eggs and egg products	Hen eggs	Conventional (non-organic) production	241	0.13	0.16	0.69	0.69	0.26	0.29	1.30	1.30

3.2.2.3.2 | *Wild-caught vs. farmed eel, salmon and trout and other fatty fish species*

In **Tables 19–21** the occurrence values for the sum of 29 PCDD/Fs and DL-PCBs, the 17 PCDD/Fs or the 12 DL-PCBs, respectively, in eel, salmon and trout and other fatty fish according to being farmed or wild-caught as indicated in production method are shown.

Comparing the differences between farmed and wild-caught eel, levels in farmed eel were substantially lower, but the number of samples was low. DL-PCBs contribute much more to the total TEQ levels than PCDD/Fs. In several European countries the catching and/or selling of wild eel is discouraged or in some countries even prohibited.

Salmon and trout samples reported as 'wild or gathered or hunted' had higher levels of all congener groups than those reported as farmed.

Also for fatty fish other than eels, salmons and trout, the data indicate that levels of both PCDD/Fs and DL-PCBs in farmed fatty fish are lower than those in wild-caught fatty fish.

TABLE 19 Sum of 29 PCDD/Fs and DL-PCBs expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2005}-TEQ/g whole weight for farmed and wild-caught eel, salmon and trout and other fatty fish.[14]

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g whole weight)			WHO_{2022}-TEQ Mean		P95[a]		WHO_{2005}-TEQ Mean		P95[a]	
FOODEX2	**Production method**	**N**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
River eels	Farmed	27	0.75	0.77			1.44	1.44		
	Wild-caught	65	2.44	2.47	7.60	7.61	4.37	4.40	12.39	12.39
	Unspecified	217	3.55	3.64	12.09	12.10	6.27	6.37	20.84	20.85
Salmon and trout	Farmed	699	0.18	0.21	0.45	0.47	0.32	0.36	0.83	0.84
	Wild-caught	133	1.68	1.74	4.41	4.44	3.28	3.34	8.62	8.62
	Unspecified	1769	0.36	0.42	2.14	2.16	0.67	0.74	4.15	4.16
Other fatty fish	Farmed	143	0.12	0.17	0.38	0.58	0.20	0.28	0.59	0.89
	Wild-caught	1660	1.02	1.06	2.92	3.02	1.96	2.00	5.69	5.72
	Unspecified	674	0.53	0.57	1.75	1.80	0.97	1.03	3.29	3.31

[a]The 95th percentile estimates obtained with less than 59 observations may not be statistically robust (EFSA, 2011a). Those estimates were not included in this table.

TABLE 20 Sum of 17 PCDD/Fs expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2005}-TEQ/g whole weight for farmed and wild-caught eel, salmon and trout, and other fatty fish.

17 PCDD/fs (pg WHO-TEQ/g whole weight)			WHO_{2022}-TEQ Mean		P95[a]		WHO_{2005}-TEQ Mean		P95[a]	
FOODEX2	**Production method**	**N**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
River eels	Farmed	27	0.15	0.16			0.27	0.28		
	Wild-caught	65	0.62	0.65	2.25	2.26	0.88	0.91	3.76	3.77
	Unspecified	217	0.43	0.51	1.28	1.48	0.63	0.73	1.73	1.88
Salmon and trout	Farmed	699	0.05	0.08	0.14	0.21	0.08	0.11	0.24	0.29
	Wild-caught	133	0.61	0.65	1.67	1.68	1.21	1.24	3.33	3.33
	Unspecified	1769	0.11	0.17	0.78	0.78	0.20	0.27	1.45	1.46
Other fatty fish	Farmed	143	0.04	0.08	0.11	0.22	0.07	0.11	0.19	0.24
	Wild-caught	1660	0.43	0.47	1.47	1.50	0.82	0.86	2.82	2.82
	Unspecified	674	0.17	0.21	0.60	0.62	0.29	0.34	1.07	1.07

[a]The 95th percentile estimates obtained with less than 59 observations may not be statistically robust (EFSA, 2011a). Those estimates were not included in this table.

[14]Smelt, Char, Sardines and sardine-type fishes, Scad, Carps, Sprat, Seerfish, Mackerels, Herrings, Halibut.

TABLE 21 Sum of 12 DL-PCBs expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2005}-TEQ/g whole weight for farmed and wild-caught eel, salmon and trout, and other fatty fish.

12 DL-PCBs (pg WHO-TEQ/g whole weight)			WHO_{2022}-TEQ Mean		P95[a]		WHO_{2005}-TEQ Mean		P95[a]	
FOODEX2	Production method	N	LB	UB	LB	UB	LB	UB	LB	UB
River eels	Farmed	27	0.60	0.60			1.17	1.17		
	Wild-caught	65	1.82	1.82	4.58	4.58	3.49	3.49	8.76	8.76
	Unspecified	217	3.13	3.13	10.75	10.75	5.64	5.65	18.97	18.97
Salmon and trout	Farmed	699	0.13	0.13	0.31	0.31	0.24	0.24	0.59	0.59
	Wild-caught	133	1.07	1.09	2.77	2.77	2.08	2.11	5.46	5.46
	Unspecified	1769	0.25	0.25	1.37	1.37	0.47	0.47	2.64	2.64
Other fatty fish	Farmed	143	0.07	0.09	0.19	0.35	0.13	0.17	0.37	0.67
	Wild-caught	1660	0.59	0.60	1.65	1.65	1.13	1.14	3.16	3.16
	Unspecified	674	0.36	0.36	1.22	1.22	0.68	0.69	2.37	2.37

[a]The 95th percentile estimates obtained with less than 59 observations may not be statistically robust (EFSA, 2011a). Those estimates were not included in this table.

3.2.2.3.3 | *Baltic vs. non-Baltic origin of salmon, trout and herrings*

Salmon and trout and herring samples from fishing areas in the Baltic Sea were also investigated and compared with samples from other regions. The following fishing areas indicated in the sample information were considered to identify fish fished in the Baltic Sea: Belt Sea, FAO Division 27.Iii.D, Southern Central Baltic - West, East Of Gotland Or Gulf Of Riga, Gulf Of Riga, East Of Gotland (Open Sea), Archipelago Sea, Bothnian Sea, Bothnian Bay, West Of Gotland, Southern Central Baltic - East, Baltic West Of Bornholm, Sound, Sound And Belt Sea Or The Transition Area, Gulf Of Finland.

Tables 22–24 show LB and UB mean and P95 levels for the sum of 29 PCDD/Fs and DL-PCBs, the 17 PCDD/Fs or the 12 DL-PCBs, respectively, of salmon and trout and herring samples based on Baltic origin and non-Baltic or unknown origin. Higher levels of PCDD/Fs and DL-PCBs were found in samples of Baltic origin than in those of non-Baltic origin or unknown origin.

TABLE 22 Mean and P95 of the sum of 29 PCDD/Fs and DL-PCBs expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2022}-TEQ/g whole weight in salmon, trout and herring samples from Baltic and non-Baltic fishing areas.

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g whole weight)			WHO_{2022}-TEQ Mean		P95		WHO_{2005}-TEQ Mean		P95	
FOODEX2	Fishing area	N	LB	UB	LB	UB	LB	UB	LB	UB
Salmon and trout	Baltic	77	2.37	2.44	4.43	4.44	4.59	4.62	8.62	8.62
	Non-Baltic	260	0.42	0.44	1.72	1.79	0.80	0.82	3.39	3.42
	Unspecified	2264	0.30	0.36	1.64	1.65	0.57	0.63	3.13	3.13
Herrings	Baltic	232	1.29	1.33	3.51	3.51	2.51	2.55	7.38	7.38
	Non-Baltic	173	0.64	0.70	1.43	1.44	1.23	1.29	2.72	2.75
	Unspecified	341	1.24	1.26	3.25	3.25	2.47	2.49	6.77	6.77

TABLE 23 Mean and P95 of the sum of 17 PCDD/Fs expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2022}-TEQ/g whole weight in salmon, trout and herring samples from Baltic and non-Baltic fishing areas.

17 PCDD/fs (pg WHO-TEQ/g whole weight)			WHO_{2022}-TEQ Mean		P95		WHO_{2005}-TEQ Mean		P95	
FOODEX2	Fishing area	N	LB	UB	LB	UB	LB	UB	LB	UB
Salmon and trout	Baltic	77	0.90	0.94	1.66	1.67	1.73	1.74	3.25	3.28
	Non-Baltic	260	0.10	0.12	0.47	0.49	0.18	0.20	0.86	0.87
	Unspecified	2264	0.10	0.15	0.57	0.57	0.17	0.23	0.98	0.98
Herrings	Baltic	232	0.78	0.81	2.25	2.26	1.52	1.55	4.83	4.83
	Non-Baltic	173	0.27	0.33	0.70	0.70	0.52	0.57	1.27	1.28
	Unspecified	341	0.65	0.66	1.79	1.81	1.31	1.32	3.88	3.89

TABLE 24 Mean and P95 of the sum of 12 DL-PCBs expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2022}-TEQ/g whole weight in salmon, trout and herring samples from Baltic and non-Baltic fishing areas.

12 DL-PCBs (pg WHO-TEQ/g whole weight)			WHO_{2022}-TEQ				WHO_{2005}-TEQ			
			Mean		P95		Mean		P95	
FOODEX2	Fishing area	N	LB	UB	LB	UB	LB	UB	LB	UB
Salmon and trout	Baltic	77	1.47	1.50	2.77	2.77	2.86	2.88	5.42	5.42
	Non-Baltic	260	0.32	0.33	1.35	1.35	0.62	0.62	2.55	2.55
	Unspecified	2264	0.21	0.21	0.93	0.93	0.40	0.40	1.79	1.79
Herrings	Baltic	232	0.51	0.52	1.38	1.38	0.98	1.00	2.70	2.70
	Non-Baltic	173	0.37	0.37	0.90	0.90	0.72	0.72	1.75	1.75
	Unspecified	341	0.59	0.59	1.39	1.44	1.16	1.17	2.74	2.86

3.2.2.3.4 | *Crab meat*

Tables 25–27 show LB and UB mean and P95 levels for the sum of 29 PCDD/Fs and DL-PCBs, the 17 PCDD/Fs or the 12 DL-PCBs, in brown, white and unspecified crab meat. Higher levels of PCDD/Fs and DL-PCBs are found in samples reported as brown crab meat ($n=20$) compared to those reported as white crab meat ($n=2$) or that did not report the type of crab meat ($n=140$). This is consistent with studies in brown crabs (Oehme et al., 1990) and Chinese mitten crabs (Hoogenboom et al., 2015), that show much higher levels in brown meat than in white meat. Since brown crab meat, or mixed brown- and white crab meat is consumed in some European countries, but the information to differentiate between the two types of meat are not available in the Comprehensive Database, all samples for crab meat were included in the calculation of the mean occurrence in crab meat ($n=162$).

TABLE 25 Mean and P95 of the sum of 29 PCDD/Fs and DL-PCBs expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2005}-TEQ/g whole weight in brown, white and unspecified crab meat.

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g whole weight)		WHO_{2022}-TEQ				WHO_{2005}-TEQ			
		Mean		P95		Mean		P95	
Type of crab meat	N	LB	UB	LB	UB	LB	UB	LB	UB
Brown meat	20	1.39	1.46	4.39	4.41	2.33	2.41	7.51	7.52
White meat	2	0.22	0.26	0.27	0.36	0.38	0.42	0.50	0.57
Unspecified	140	0.35	0.36	2.71	2.71	0.60	0.61	4.59	4.60

TABLE 26 Mean and P95 of the sum of 17 PCDD/Fs in pg. WHO_{2022}-TEQ/g and pg. WHO_{2005}-TEQ/g whole weight in brown, white and unspecified crab meat.

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g whole weight)		WHO_{2022}-TEQ				WHO_{2005}-TEQ			
		Mean		P95		Mean		P95	
Type of crab meat	N	LB	UB	LB	UB	LB	UB	LB	UB
Brown meat	20	0.81	0.87	2.63	2.64	1.23	1.31	4.06	4.07
White meat	2	0.15	0.19	0.18	0.26	0.26	0.29	0.32	0.39
Unspecified	140	0.23	0.25	1.88	1.88	0.37	0.38	2.84	2.85

TABLE 27 Mean and P95 of the sum of 12 DL-PCBs expressed in pg. WHO_{2022}-TEQ/g and pg. WHO_{2005}-TEQ/g whole weight in brown, white and unspecified crab meat.

29 PCDD/fs and DL-PCBs (pg WHO-TEQ/g whole weight)		WHO_{2022}-TEQ				WHO_{2005}-TEQ			
		Mean		P95		Mean		P95	
Type of crab meat	N	LB	UB	LB	UB	LB	UB	LB	UB
Brown meat	20	0.58	0.59	2.07	2.07	1.10	1.11	3.84	3.84
White meat	2	0.07	0.07	0.09	0.10	0.13	0.13	0.18	0.18
Unspecified	140	0.12	0.12	0.79	0.79	0.23	0.23	1.56	1.56

3.2.3 | Occurrence data in feed submitted to EFSA

3.2.3.1 | *Application of performance criteria*

The cleaned data set for feed for food- and non-food-producing animals contained 5847 feed samples, in which all 29 PCDD/Fs and DL-PCBs were analysed.

Similar to the food samples (see **Section 3.2.2.1**), these samples were checked for compliance with analytical performance criteria based on Commission Regulation (EU) No 2017/771 and taking into account the existing action thresholds (see **Section 1.3.3**). These were expressed in ng WHO_{2005}-TEQ/kg, based on 88% dry matter.

Samples for which the sum-TEQ of LOQs concerning the 17 PCDD/Fs was higher than one fifth of the corresponding ML were excluded. A total of 1284 feed samples did not comply with the criterion and were therefore excluded.

Samples for which the sum-TEQ of LOQs concerning the 12 DL-PCBs was higher than one third of the AL were excluded. A total of 257 feed samples did not comply with the criterion and were therefore excluded.

Overall, 1297 samples failed one of the two performance criteria, and 4550 samples remained in the final feed data set fulfilling the above criteria, and all the conditions listed in **Section 3.2.1** (see Annex C).

3.2.3.2 | *Description of the final data set of occurrence in feed*

Figure 13 illustrates the distribution of feed samples across the sampling years in the final dataset. Apart from 2017 and 2018, when slightly more samples were collected, sampling was generally evenly distributed across different years.

Samples were collected in 15 European countries. France accounted for approximately 55% ($n = 2488$) of the total, while 27% ($n = 1245$) were collected in Denmark, the Netherlands, and Norway (Figure 14).

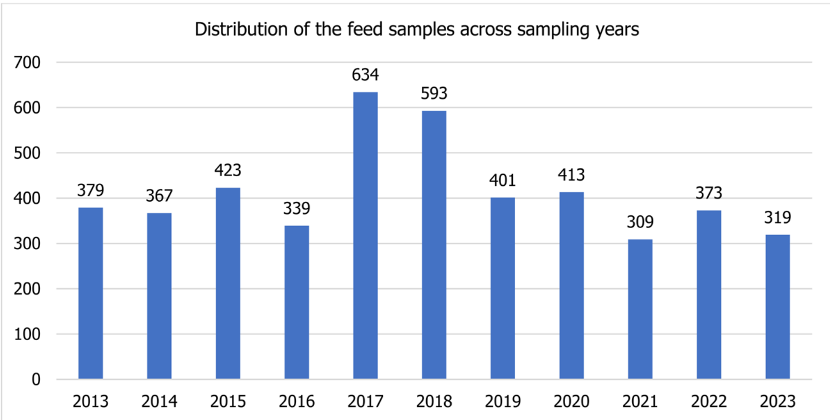

FIGURE 13 Number of feed samples by sampling year in the final dataset ($n = 4550$).

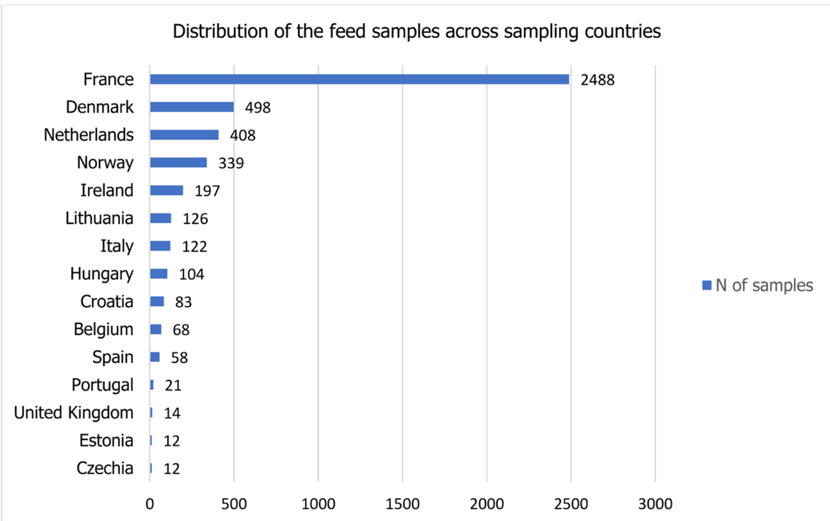

FIGURE 14 Number of feed samples per sampling country in the final dataset ($n = 4550$).

Data were submitted across all 14 Feed Level 1 categories (Table 28). Most data were reported for 'Compound feed' (41%), followed by 'Fish, other aquatic animals and products derived thereof' (16%), and 'Minerals and products derived thereof' (14%). Fewer than 10 samples were available for 'Other plants, algae, fungi and products derived thereof', 'Legume seeds and products derived thereof', and 'Products and co-products obtained by fermentation using microorganisms'. The proportion of left-censored data in the final dataset was 5.2% for 29 PCDD/Fs and DL-PCBs, and 9.3% for 17 PCDD/Fs.

Analytical methods

All feed data on PCDD/Fs and DL-PCBs in the final dataset were analysed by GC–HRMS or GC–MS/MS (see **Section 3.2.1**). Among the samples for which LOQs were available for all 29 congeners, the highest sensitivities for the analysis of the 29 PCDD/Fs and DL-PCBs were reported for the samples of 'Unspecified complete feed', 'Maize' and 'Sunflower seed meal'. In the same subset, the lowest reported total LOQ was 0.08 pg. WHO_{2005}-TEQ/kg (88% DM) in 'Unspecified complete feed'. The mean total LOQ for the sum of 29 PCDD/Fs and DL-PCBs in the final dataset was 0.11 ng WHO_{2005}-TEQ/kg (88% DM), and the median total LOQ was 0.06 ng WHO_{2005}-TEQ/kg (88% DM). However, these summary statistics include a few samples for which LOQs were not reported for all congeners; in such cases, the total LOQ was calculated by summing only the available congener-specific LOQs.

Occurrence data used for exposure estimates

Table 29 summarises the occurrence of the sum of 29 PCDD/Fs and DL-PCBs across the different Feed Level 1 categories, presenting mean and median concentrations along with 75th and 95th percentiles (ng WHO-TEQ/kg DM,[15] LB–UB).

[15]DM refers to 100% dry matter, to which all samples were converted.

TABLE 28 Concentrations of the sum of 29 PCDD/Fs and DL-PCBs in feed samples (Feed Level 1) as per Commission Regulation (EU) 2022/1104; all values expressed as ng WHO-TEQ/kg dry matter (DM).

Occurrence of 29 PCDD/Fs and DL-PCBs (ng WHO-TEQ/kg DM)[a]		WHO_{2022}-TEQ								WHO_{2005}-TEQ							
		Mean		Median		P75		P95		Mean		Median		P75		P95	
	N	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Cereal grains and products derived thereof	240	0.05	0.09	0.01	0.03	0.02	0.08	0.11	0.17	0.05	0.10	0.01	0.04	0.02	0.11	0.13	0.24
Oil seeds, oil fruits, and products derived thereof	401	0.10	0.16	0.01	0.06	0.09	0.19	0.60	0.65	0.10	0.17	0.01	0.08	0.09	0.22	0.41	0.48
Legume seeds and products derived thereof	4	0.02	0.04	–	–	–	–	–	–	0.01	0.04	–	–	–	–	–	–
Tubers, roots, and products derived thereof	20	0.03	0.10	0.01	0.09	0.03	0.14	–	–	0.04	0.12	0.005	0.10	0.03	0.15	–	–
Other seeds and fruits, and products derived thereof	24	0.06	0.07	0.02	0.05	0.06	0.08	–	–	0.08	0.10	0.03	0.07	0.08	0.11	–	–
Forages and roughage, and products derived thereof	257	0.16	0.20	0.03	0.08	0.07	0.12	0.32	0.33	0.23	0.29	0.04	0.10	0.09	0.15	0.30	0.31
Other plants, algae, funghi and products derived thereof	9	0.03	0.06	0.01	0.06	–	–	–	–	0.04	0.08	0.01	0.08	–	–	–	–
Milk products and products derived thereof	120	0.02	0.05	0.01	0.04	0.02	0.06	0.08	0.14	0.03	0.07	0.01	0.05	0.03	0.08	0.11	0.18
Land animal products and products derived thereof	139	0.15	0.20	0.07	0.13	0.14	0.17	0.50	0.52	0.21	0.28	0.07	0.15	0.16	0.21	0.70	0.73
Fish, other aquatic animals and products derived thereof	713	1.15	1.26	0.57	0.69	1.59	1.80	4.19	4.24	2.13	2.26	1.05	1.16	2.99	3.14	7.72	7.75
Minerals and products derived thereof	639	0.13	0.27	0.004	0.18	0.03	0.19	0.22	0.34	0.16	0.33	0.002	0.22	0.04	0.22	0.25	0.39
Products and co-products obtained by fermentation using microorganisms	2	0.002	0.32	–	–	–	–	–	–	0.001	0.45	–	–	–	–	–	–
Miscellaneous	134	0.36	0.84	0.02	0.23	0.16	0.40	1.28	1.92	0.37	1.02	0.01	0.27	0.13	0.45	2.26	2.95
Compound feed	1848	0.11	0.14	0.02	0.05	0.14	0.19	0.45	0.49	0.18	0.22	0.02	0.06	0.26	0.31	0.81	0.85

Abbreviations: DL-PCBs, dioxin-like polychlorinated biphenyls; DM, dry matter; LB, lower bound; P, percentile; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]Percentiles were only included when considered statistically robust ($\alpha = 0.05$), based on the number of analytical results per substance: at least 5 for the median, 11 for the 75th percentile, 29 for the 90th percentile and 59 for the 95th percentile (Meeker et al., 2017).

For exposure estimations, feed materials were grouped into ad hoc feed categories based on feed type and the reported concentrations of PCDD/Fs and DL-PCBs. Categories with fewer than five reported samples were excluded.

Table 29 lists all the feed categories used to estimate dietary exposure to the sum of 29 PCDD/Fs and DL-PCBs for the animal species and categories considered in this Opinion, along with their respective mean and median concentrations and 75th and 95th percentiles (ng WHO-TEQ/kg DM, LB–UB). Among these, the highest concentrations of the 29 PCDD/Fs and DL-PCBs were reported in the following feed materials:

- For 'Fish, other aquatic animals and products derived thereof': 'Fish oil', almost 400 samples were available, with mean concentrations ranging from 1.76 to 1.92 ng WHO_{2022}-TEQ/kg and 95th percentile concentrations (LB–UB, DM) from 4.83 to 4.96 ng WHO_{2022}-TEQ/kg.
- For 'Fish, other aquatic animals and products derived thereof': 'Fish meal' ($n = 299$), the mean and 95th percentile concentrations (LB–UB, DM) varied from 0.35 to 0.39 ng WHO_{2022}-TEQ/kg and from 0.87 to 0.89 ng WHO_{2022}-TEQ/kg, respectively.
- Among plant-based feed materials, 'Oil seeds, oil fruits, and products derived thereof': 'Vegetable oil and fat' ($n = 163$) showed mean concentrations (LB–UB, DM) of 0.22–0.30 ng WHO_{2022}-TEQ/kg and 95th percentile concentrations of 0.76–0.81 ng WHO_{2022}-TEQ/kg.
- Within 'Compound feed', the highest mean and 95th percentile concentrations (LB–UB, DM) were reported for 'Fish/Complete feed' ($n = 520$), with values ranging from 0.28 to 0.32 ng WHO_{2022}-TEQ/kg and from 0.65 to 0.67 ng WHO_{2022}-TEQ/kg, respectively.
- For 'Land animal products and products derived thereof': 'Animal fat' ($n = 119$), the mean and 95th percentile concentrations (LB–UB, DM) were 0.16–0.21 ng WHO_{2022}-TEQ/kg and 0.51–0.55 ng WHO_{2022}-TEQ/kg, respectively.

TABLE 29 Feed samples according to Commission Regulation (EU) 2022/1104 as used for the estimation of dietary exposure to the sum of 29 PCDD/Fs and DL-PCBs (concentrations expressed as ng WHO-TEQ/kg dry matter (DM)).

Occurrence of 29 PCDD/fs and DL-PCBs (ng WHO-TEQ/kg DM)[a]																	
		WHO_{2022}-TEQ								**WHO_{2005}-TEQ**							
		Mean		**Median**		**P75**		**P95**		**Mean**		**Median**		**P75**		**P95**	
	N	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Cereal grains and products derived thereof																	
Maize	169	0.03	0.05	0.01	0.03	0.03	0.08	0.10	0.14	0.03	0.07	0.01	0.04	0.03	0.09	0.10	0.18
Barley	10	0.01	0.05	0.01	0.02	–	–	–	–	0.02	0.07	0.01	0.03	–	–	–	–
Oil seeds, oil fruits, and products derived thereof																	
Soya (beans) (including expeller, meal, extruded, co-product, toasted)[b]	45	0.01	0.03	0.01	0.02	0.02	0.04	–	–	0.01	0.04	0.01	0.03	0.01	0.05	–	–
Vegetable oil and fat	163	0.22	0.30	0.12	0.21	0.26	0.34	0.76	0.81	0.21	0.31	0.13	0.24	0.29	0.38	0.53	0.57
Sunflower (expeller, meal, dehulled meal)[c]	60	0.01	0.02	0.01	0.02	0.01	0.03	0.06	0.10	0.01	0.03	0.004	0.02	0.01	0.03	0.05	0.09
Rape seed (expeller, meal, extruded)[d]	89	0.01	0.05	0.01	0.03	0.01	0.05	0.03	0.14	0.01	0.06	0.004	0.04	0.01	0.06	0.04	0.17
Tubers, roots, and products derived thereof																	
Dried (sugar) beet pulp	5	0.01	0.12	0.01	0.13	–	–	–	–	0.003	0.15	0.003	0.16	–	–	–	–
Forages and roughage, and products derived thereof																	
Lucerne (field dried, high temperature dried, extruded, meal)[e]	8	0.04	0.13	0.02	0.11	–	–	–	–	0.03	0.14	0.02	0.13	–	–	–	–
Maize silage	77	0.04	0.07	0.02	0.06	0.04	0.08	0.11	0.16	0.04	0.08	0.03	0.07	0.05	0.10	0.11	0.18
Other plants, algae, funghi and products derived thereof																	
(Sugar) cane molasses	5	0.01	0.05	0.003	0.06	–	–	–	–	0.01	0.06	0.004	0.08	–	–	–	–
Land animal products and products derived thereof																	
Animal fat	119	0.16	0.21	0.08	0.13	0.16	0.20	0.51	0.55	0.23	0.30	0.08	0.16	0.18	0.23	0.89	0.89
Fish, other aquatic animals and products derived thereof																	
Fish meal	299	0.35	0.39	0.27	0.34	0.50	0.53	0.87	0.89	0.64	0.69	0.52	0.58	0.91	0.93	1.59	1.59
Fish oil	398	1.76	1.92	1.31	1.57	2.61	2.73	4.83	4.96	3.27	3.46	2.46	2.77	4.83	4.90	9.05	9.07
Fish protein, hydrolysed	8	0.14	0.30	0.08	0.26	–	–	–	–	0.22	0.42	0.14	0.35	–	–	–	–
Minerals and products derived thereof																	
Mineral salts[f]	41	0.03	0.05	0.03	0.04	0.04	0.06	–	–	0.03	0.05	0.02	0.04	0.04	0.06	–	–

TABLE 29 (Continued)

Occurrence of 29 PCDD/fs and DL-PCBs (ng WHO-TEQ/kg DM)[a]																	
		WHO$_{2022}$-TEQ								WHO$_{2005}$-TEQ							
		Mean		Median		P75		P95		Mean		Median		P75		P95	
	N	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Compound feed																	
Piglets (weaning diets)/ Complete feed	46	0.01	0.04	0.01	0.03	0.02	0.04	–	–	0.01	0.05	0.01	0.04	0.01	0.05	–	–
Growing/Fattening pigs/ Complete feed	208	0.01	0.04	0.01	0.03	0.01	0.04	0.03	0.10	0.01	0.04	0.01	0.04	0.01	0.05	0.03	0.09
Breeding pigs, Sows/ Complete feed[g]	39	0.02	0.04	0.01	0.02	0.02	0.04	–	–	0.02	0.04	0.01	0.03	0.01	0.04	–	–
Fattening chickens (broilers) and Poultry (starter diets)/Complete feed[h]	222	0.02	0.04	0.01	0.03	0.01	0.04	0.04	0.11	0.01	0.05	0.01	0.03	0.01	0.05	0.04	0.12
Laying hens/Complete feed	87	0.01	0.04	0.01	0.03	0.02	0.05	0.04	0.11	0.02	0.05	0.01	0.04	0.02	0.06	0.05	0.13
Turkeys/Complete feed	5	0.04	0.07	0.02	0.04	–	–	–	–	0.04	0.08	0.03	0.06	–	–	–	–
Fish/Complete feed	520	0.28	0.32	0.23	0.27	0.36	0.39	0.65	0.67	0.51	0.55	0.43	0.47	0.66	0.69	1.19	1.20
Rabbits/Complete feed	39	0.03	0.06	0.02	0.04	0.04	0.07	–	–	0.03	0.07	0.03	0.05	0.04	0.08	–	–
Horses/Complementary feed	23	0.01	0.06	0.01	0.04	0.01	0.07	–	–	0.01	0.08	0.01	0.05	0.01	0.09	–	–

Abbreviations: DL-PCBs, dioxin-like polychlorinated biphenyls; DM, dry matter; LB, lower bound; P, percentile; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]Percentiles were only included when considered statistically robust (α = 0.05), based on the number of analytical results per substance: at least 5 for the median, 11 for the 75th percentile, 29 for the 90th percentile and 59 for the 95th percentile (Meeker et al., 2017).

[b]Ad hoc feed category consisting of the samples of 'Soya (beans)', 'Soya (bean) expeller', 'Soya (bean) meal', 'Soya beans, extruded', 'Co-product from soybean preparation' and 'Toasted soya (beans)'.

[c]Ad hoc feed category consisting of the samples of 'Sunflower seed expeller', 'Sunflower seed meal', 'Sunflower seed meal feed', 'Sunflower seed meal, dehulled' and 'Sunflower seed meal feed, dehulled'.

[d]Ad hoc feed category consisting of the samples of 'Rape seed expeller', 'Rape seed meal', 'Rape seed meal feed' and 'Rape seed, extruded'.

[e]Ad hoc feed category consisting of the samples of 'Lucerne; [Alfalfa]', 'Lucerne meal; [Alfalfa meal]', 'Lucerne, field dried; [Alfalfa field dried]', 'Lucerne, high temperature dried; [Alfalfa, high temperature dried]' and 'Lucerne, extruded; [Alfalfa, extruded]'.

[f]Ad hoc feed category consisting of the samples of (i) 'Monocalcium phosphate; [Calcium tetrahydrogen diorthophosphate]', 'Monodicalcium phosphate, 'Calcium carbonate [Limestone]', 'Calcium salts of organic acids', 'Dicalcium phosphate; [Calcium hydrogen orthophosphate]' and 'Calcareous marine shells', (ii) 'Zinc oxide' and (iii) 'Copper sulphate'. Each of the three groups is given equal weight.

[g]Ad hoc feed category consisting of the samples of 'Complete feed' for 'Breeding pigs' and 'Sows'.

[h]Ad hoc feed category consisting of the samples of 'Complete feed' for 'Fattening chickens (broilers)' and 'Poultry (starter diets)'.

3.3 | Dietary exposure assessment

3.3.1 | Dietary exposure assessment for humans

Chronic dietary exposure was estimated for the 29 PCDD/Fs and DL-PCBs, and for the 17 PCDD/F congeners, following the methodology described in **Section 2.6.1**. A comparison between dietary exposure estimates based on WHO_{2005}-TEFs for the current Opinion and the 2018 Opinion as well as a comparison between dietary exposure estimates based on WHO_{2005}-TEFs and on WHO_{2022}-TEFs for the current Opinion, are also provided.

Detailed summary statistics on the exposure estimates to the 29 PCDD/Fs and DL-PCBs, and to the 17 PCDD/F congeners based on the WHO_{2005}- and WHO_{2022}-TEFs for each dietary survey are presented in Annex B (Table B5).

The impact of uncertainties regarding the WHO_{2022}-TEFs on the dietary exposure assessment was taken into account in the uncertainty analysis (see **Sections 3.5.1.3** and **3.5.1.4**).

3.3.1.1 | *Dietary exposure estimates for the current opinion based on WHO_{2005}-TEFs*

First the exposure based on WHO_{2022}-TEFs using occurrence data for the period 2013–2023 was compared to the exposure estimated based on WHO_{2005}-TEFs in the 2018 Opinion based on occurrence data for the period 2010–2016.

Table 30 shows the LB and UB summary statistics of the estimated chronic dietary exposure to the 29 PCDD/Fs and DL-PCBs based on the WHO_{2005}-TEFs, across surveys for each age group. LB and UB summary statistics of the estimated chronic dietary exposure to only the 17 PCDD/Fs based on the WHO_{2005}-TEFs, across surveys for each age group are provided in Appendix F (Table F.1).

TABLE 30 Mean and P95 dietary exposure (LB and UB) based on WHO_{2005}-TEQ to the 29 PCDD/Fs and DL-PCBs congeners (minimum, median and maximum across surveys) for each age group.

Range of mean dietary exposure (pg WHO-TEQ/kg bw per day) using WHO_{2005}-TEFs							
		Minimum		Median		Maximum	
Age group	**N surveys**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Infants[a]	14	0.34	0.75	0.79	1.53	1.67	2.56
Toddlers	17	0.85	1.62	1.36	2.23	2.43	3.39
Other children	21	0.71	1.37	1.01	1.65	1.67	2.46
Adolescents	23	0.43	0.68	0.55	0.89	1.08	1.47
Adults	23	0.38	0.59	0.54	0.81	0.92	1.21
Elderly	21	0.36	0.57	0.56	0.80	1.15	1.43
Very elderly	16	0.35	0.55	0.55	0.79	1.05	1.33
Range of P95 dietary exposure (pg WHO-TEQ/kg bw per day) using WHO_{2005}-TEFs							
		Minimum		**Median**		**Maximum**	
Age group	***N* surveys**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Infants[a]	13	0.73	1.55	2.47	3.51	4.80	6.17
Toddlers	16	1.81	2.79	3.28	4.50	5.66	7.63
Other children	21	1.39	2.40	2.51	3.47	4.27	5.19
Adolescents	22	0.94	1.56	1.38	1.97	3.61	4.40
Adults	23	0.79	1.21	1.57	1.93	2.93	3.42
Elderly	21	0.76	1.16	1.74	2.07	3.80	4.05
Very elderly	12	0.64	1.17	1.45	1.76	2.44	2.84

[a]Not including intake from human milk.

When applying the WHO_{2005}-TEFs, it was noted that for the sum of 29 PCDD/Fs and DL-PCBs:

- The mean LB dietary exposure to the 29 PCDD/Fs and DL-PCBs ranged from 0.34 pg. WHO_{2005}-TEQ/kg bw per day in 'Infants' to 2.43 pg. WHO_{2005}-TEQ/kg bw per day in 'Toddlers', across surveys.
- The mean UB dietary exposure to the 29 PCDD/Fs and DL-PCBs ranged from 0.55 pg. WHO_{2005}-TEQ/kg bw per day in the 'Very elderly' to 3.39 pg. WHO_{2005}-TEQ/kg bw per day in 'Toddlers', across surveys.
- The P95 LB dietary exposure to the 29 PCDD/Fs and DL-PCBs ranged from 0.64 pg. WHO_{2005}-TEQ/kg bw per day in the 'Very elderly' to 5.66 pg. WHO_{2005}-TEQ/kg bw per day in 'Toddlers', across surveys.
- The P95 UB dietary exposure to the 29 PCDD/Fs and DL-PCBs ranged from 1.16 pg. WHO_{2005}-TEQ/kg bw per day in 'Elderly' to 7.63 pg. WHO_{2005}-TEQ/kg bw per day in 'Toddlers', across surveys.

Table 31 shows the medians and ranges of the ratios between the mean dietary exposure estimates (LB/UB) based on WHO_{2005}-TEQ of the current Opinion and the mean dietary exposure estimates (LB/UB) of the 2018 Opinion for the surveys in common to the two Opinions for each population group.

TABLE 31 Median and ranges of the ratios between the mean dietary exposure estimates (LB/UB) based on WHO_{2005}-TEQ of the current Opinion and the mean dietary exposure estimates (LB/UB) of the 2018 Opinion for the surveys in common to the two Opinions for each population group.

Age group[a]	N	Median ratio LB	Range ratio LB	Median ratio UB	Range ratio UB
Infants[b]	3	0.89	0.58–1.33	1.33	1.08–1.75
Toddlers	4	0.90	0.59–0.99	1.27	0.88–1.32
Other children	5	0.83	0.65–1.28	1.14	0.95–1.45
Adolescents	4	0.83	0.61–1.41	1.19	0.87–1.75
Adults	3	0.97	0.83–1.10	1.20	1.09–1.37
Elderly	4	0.86	0.80–0.98	1.05	0.98–1.20
Very elderly	6	0.86	0.80–0.96	1.08	0.96–1.16

Abbrevition: *N*, number of surveys.

[a]The ratio for one of the two 'Lactating women' surveys was higher (2.10) than for the other age groups. This is at least partly due to the inclusion in the current Opinion of bonito consumption with high concentrations (mean 113 pg. WHO_{2005}-TEQ/g for the 29 PCDD/Fs and DL-PCBs), while this species was assigned the mean fish meat concentration in the 2018 Opinion that was much lower (1.65 pg. WHO_{2005}-TEQ/g for the 29 PCDD/Fs and DL-PCBs). The CONTAM Panel notes a high uncertainty in the exposure from bonito consumption since there is a wide range in the occurrence data (0 to 590 pg. WHO_{2005}-TEQ/g for the 29 PCDD/Fs and DL-PCBs, median: 0.13) and few samples ($n = 9$) and it was consumed by 2 of the 65 subjects in the survey on lactating women.

[b]Not including intake from human milk.

The median ratio of the mean LB dietary exposure to the 29 PCDD/Fs and DL-PCBs congeners based on the WHO_{2005}-TEQ between the current Opinion and the 2018 Opinion on the same surveys ranged between 0.83 and 0.97 for the population groups included in Table 31. While the range of the median ratios across age groups indicates a decrease of the LB exposure of 17% to 3%, for some surveys an increase of the exposure was estimated up to a ratio of 1.4.

The highest LB/UB mean values were found in 'Toddlers' in both Opinions (2.43/3.39 pg. WHO_{2005}-TEQ/kg bw per day in the current Opinion and 2.12/2.57 pg. WHO_{2005}-TEQ/kg bw per day in the 2018 Opinion).

A survey by survey comparison between the result of the dietary exposure assessment for the current Opinion and the 2018 Opinion can be found in Annex B (Table 7).

3.3.1.2 | *Dietary exposure estimates based on WHO_{2022}-TEFs for the current opinion*

Secondly the impact of the change in WHO-TEFs on the exposure was evaluated based on the same updated set of occurrence data.

Table 32 show the LB and UB summary statistics of the estimated chronic dietary exposure to the 29 PCDD/Fs and DL-PCBs based on the WHO_{2022}-TEFs, across surveys for each age group. LB and UB summary statistics of the estimated chronic dietary exposure to only the 17 PCDD/Fs based on the WHO_{2022}-TEFs, across surveys for each age group are provided in Appendix F (Table F.2).

TABLE 32 Mean and P95 dietary exposure (LB and UB) based on WHO_{2022}-TEQ to the 29 PCDD/Fs and DL-PCBs congeners (minimum, median and maximum across surveys) for each age group.

Range of mean dietary exposure (pg WHO-TEQ/kg bw per day) using WHO_{2022}-TEFs							
		Minimum		Median		Maximum	
Age group	N surveys	LB	UB	LB	UB	LB	UB
Infants[a]	14	0.26	0.60	0.57	1.15	1.19	1.87
Toddlers	17	0.62	1.22	0.95	1.68	1.57	2.35
Other children	21	0.51	1.02	0.72	1.25	1.11	1.71
Adolescents	23	0.29	0.48	0.38	0.65	0.66	1.02
Adults	23	0.27	0.43	0.36	0.56	0.56	0.81
Elderly	21	0.25	0.42	0.37	0.55	0.68	0.91
Very elderly	16	0.24	0.41	0.35	0.57	0.63	0.87

(Continues)

TABLE 32 (Continued)

Range of P95 dietary exposure (pg WHO-TEQ/kg bw per day) using WHO_{2022}-TEFs							
		Minimum		Median		Maximum	
Age group	N surveys	LB	UB	LB	UB	LB	UB
Infants[a]	13	0.60	1.23	1.55	2.57	3.13	4.39
Toddlers	16	1.23	1.96	2.04	3.08	3.30	4.72
Other children	21	0.97	1.74	1.52	2.31	2.40	3.28
Adolescents	22	0.61	1.08	0.87	1.33	2.10	2.54
Adults	23	0.59	0.90	0.92	1.25	1.65	2.09
Elderly	21	0.57	0.86	1.03	1.28	1.92	2.11
Very elderly	12	0.57	0.90	0.88	1.17	1.40	1.71

[a]Not including intake from human milk.

When applying the new WHO_{2022}-TEFs, it was noted that for the sum of 29 PCDD/Fs and DL-PCBs:

- The mean LB dietary exposure to the 29 PCDD/Fs and DL-PCBs ranged from 0.24 pg. WHO_{2022}-TEQ/kg bw per day in the 'Very elderly' to 1.57 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers', across surveys.
- The mean UB dietary exposure to the 29 PCDD/Fs and DL-PCBs ranged from 0.41 pg. WHO_{2022}-TEQ/kg bw per day in the 'Very elderly' to 2.35 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers', across surveys.
- The P95 LB dietary exposure to the 29 PCDD/Fs and DL-PCBs ranged from 0.57 pg. WHO_{2022}-TEQ/kg bw per day in 'Elderly' and the 'Very elderly' to 3.30 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers', across surveys.
- The P95 UB dietary exposure to the 29 PCDD/Fs and DL-PCBs ranged from 0.86 pg. WHO_{2022}-TEQ/kg bw per day in 'Elderly' to 4.72 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers', across surveys.

The special population groups 'Pregnant women' and 'Lactating women' resulted in mean and P95 exposure estimates comparable to the adult population group with the exception of surveys where these special population groups had a higher consumption of 'Fish meat' and 'Processed or preserved fish (including processed offal)' than the adult population group (mean LB/UB exposure up to 1.1/1.3 pg. WHO_{2022}-TEQ/kg bw per day, with a ratio with the exposure of the adult population of the same country up to 3).

For two surveys on the special population group 'Vegetarians' (vegetarian adults from Romania and vegetarian children from Poland), mean LB dietary exposure to the sum of 29 PCDD/Fs and DL-PCBs based on WHO_{2022}-TEQ/kg was 25% lower than the mean LB dietary exposure of the corresponding general population in the same country. For the survey on pesco-vegetarian adults from Poland the mean LB dietary exposure to the sum of 29 PCDD/Fs and DL-PCBs was 10% higher mostly due to higher consumption of 'Fish and seafood' followed by higher consumption of 'Milk and dairy products', 'Eggs and egg products' and 'Vegetable and vegetable products', than the general adult population.

Table 33 shows the medians and the ranges of the ratios between the mean dietary exposure (LB/UB) estimates based on WHO_{2022}-TEFs and the mean dietary exposure estimates (LB/UB) based on WHO_{2005}-TEFs for the current Opinion across surveys for each population group.

The median of the ratio between the mean LB dietary exposure to the 29 PCDD/Fs and DL-PCBs congeners based on the

TABLE 33 Ranges and medians of the ratios between mean dietary exposure estimates (LB/UB) based on WHO_{2022}-TEFs and the mean dietary exposure (LB/UB) estimates based on WHO_{2005}-TEFs for the current Opinion across same surveys for each population group.

Age group	*N*	Median LB	Range ratio LB	Median UB	Range ratio UB
Infants[a]	11	0.73	0.68–0.87	0.78	0.72–0.89
Toddlers	14	0.69	0.64–0.77	0.72	0.69–0.83
Other children	20	0.69	0.62–0.79	0.72	0.66–0.79
Adolescents	19	0.69	0.61–0.78	0.72	0.65–0.78
Adults	18	0.66	0.60–0.78	0.7	0.64–0.79
Elderly	19	0.65	0.58–0.78	0.69	0.63–0.79
Very elderly	13	0.66	0.60–0.81	0.7	0.65–0.79

Abbreviation: N, number of surveys.

[a]Not including intake from human milk.

WHO_{2022}-TEFs and the mean LB dietary exposure to the 29 PCDD/Fs and DL-PCBs congeners based on the WHO_{2005}-TEFs across surveys ranged between 0.65 and 0.73 across age groups. The highest LB/UB mean values were found in 'Toddlers' in both estimates (1.57/2.35pg WHO_{2022}-TEQ/kg bw per day and 2.43/3.39 pg. WHO_{2005}-TEQ/kg bw per day). This means that exposure estimated with the new WHO_{2022}-TEFs are between 27% and 35% lower than those obtained using WHO_{2005}-TEFs. Annex B (Table B5) shows the LB and UB ratios for each survey.

3.3.1.3 | *Contribution of the individual congeners and congener families to the total dietary exposure*

The contribution of the individual congeners and congeners family to the total mean LB WHO_{2022}-TEQ dietary exposure to the 29 PCDD/Fs and DL-PCBs are presented in Figure 15 (left graph).

As congeners family, the non-*ortho* PCBs had the highest contribution (41%) followed by the PCDFs (27%) and PCDDs (24%). As individual congeners, PCB-126 contributed most to the exposure (38%), followed by 1,2,3,4,6,7,8-HpCDD (9.7%) and 1,2,3,4,7,8-HxCDF (9.1%). For the latter two congeners, the WHO-TEF value was increased, contrary to many others. The major differences with the contribution of individual congeners to the exposure calculated using WHO_{2005}-TEFs (Figure 15, right graph) is that the contribution of PCB-126 is 13% lower (but it remains high) while the contribution of 1,2,3,4,6,7,8-HpCDD and of 1,2,3,4,7,8-HxCDF are 8.4% and 7.1% higher, respectively.

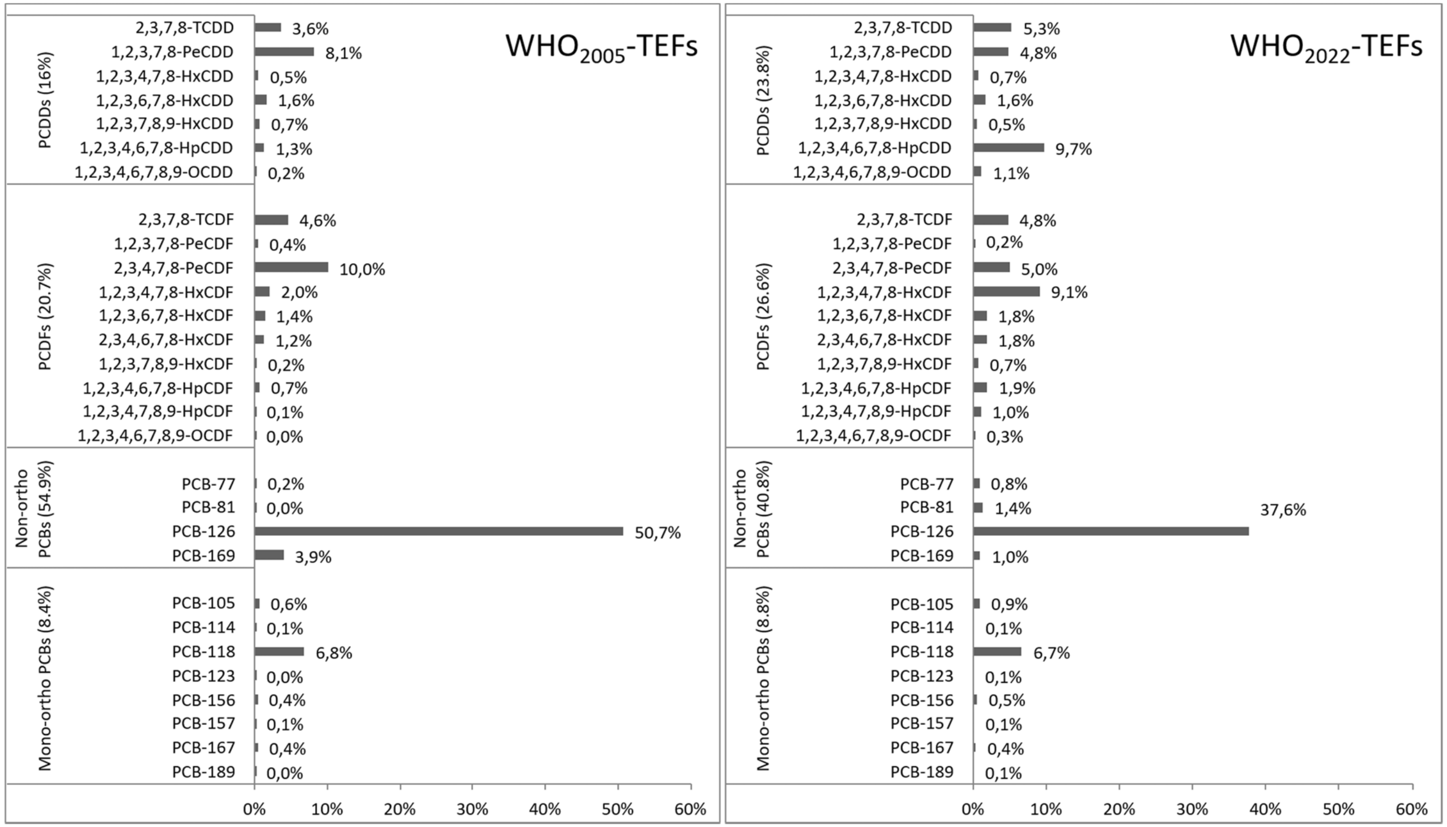

FIGURE 15 Percentage contribution of each congener weighted with WHO_{2005}-TEFs (left graph) and WHO_{2022}-TEFs (right graph) and their corresponding families to the overall LB mean exposure of the 29 PCDD/Fs and DL-PCBs.

3.3.1.4 | *Contribution of different food groups to the total dietary exposure*

Table 34 shows the number of surveys in which food categories at the Level 1 of the FoodEx2 classification have contributed more than 10% to the total mean LB WHO_{2022}-TEQ dietary exposure (see **Section 3.3.1.2**) for each age group.

'Milk and dairy products' and 'Fish and fish products' are the food categories that have contributed more than 10% to the total mean LB dietary exposure in the highest number of surveys with percentage up to 43% in 'Other Children' for 'Milk and milk products' and up to 67% in the 'Elderly' for 'Fish and fish products'.

'Vegetables and vegetable products' and 'Meat and meat products' have contributed more than 10% to the total mean LB dietary exposure also in a large number of surveys up to 41% in 'Infants' for 'Vegetable and vegetable products' and 23% in 'Adults' for 'Meat and meat products'.

TABLE 34 Number of surveys (and in parenthesis the range of percentage contribution to the mean exposure across surveys) in which food categories at the Level 1 of the FoodEx2 classification have contributed more than 10% to the total mean LB WHO_{2022}-TEQ dietary exposure for each age group (in parenthesis in the header the total number of surveys). The parenthesis in the cells show the range of percentage contribution across surveys.

Food	Infants (14)	Toddlers (17)	Other children (21)	Adolescents (23)	Adults (23)	Elderly (21)	Very elderly (16)	*N* surveys
Milk and dairy products	11 (6.6–35.1)	17 (13.6–34)	21 (16.6–43.2)	23 (13.8–31.5)	21 (8.1–20.1)	17 (5.5–17.6)	11 (7.4–20.6)	121
Fish and seafood	8 (2.3–33.1)	14 (5.3–47.4)	18 (3.7–46.9)	22 (5.4–50.1)	22 (8.1–57.2)	21 (11.3–67.3)	15 (5.8–59.7)	120
Vegetables and vegetable products	13 (9.9–41)	15 (6.6–18.7)	15 (2.5–15.8)	15 (6.4–16.3)	17 (2.8–17.6)	11 (2.3–19)	9 (2.6–20.8)	95
Meat and meat products	4 (2.3–18)	10 (6.5–19.3)	16 (6.1–20.7)	21 (9.1–22.3)	21 (7.7–22.6)	11 (5.3–17.9)	9 (6.6–19.7)	92
Animal and vegetable fats and oils	3 (1.9–13.1)	3 (1.7–11.6)	5 (0.9–16.1)	3 (1.6–17.9)	3 (0.8–15.7)	2 (0.9–14.7)	6 (0.9–16.1)	25
Food products for young population	6 (0.6–26.5)	0 (0.1–4.4)	0 (0–0.5)	0 (0–0.1)	0 (0–0)	0 (0–0)	0 (0–0)	6
Coffee, cocoa, tea and infusions	0 (0–1.8)	1 (0–10.7)	2 (0–17.3)	3 (0.1–14.6)	0 (0.2–9.4)	0 (0.2–3.5)	0 (0.2–3.7)	6
Eggs and egg products	0 (0.3–8.7)	1 (1.9–11.1)	0 (0.9–9.6)	1 (2.1–10.1)	0 (2.2–9.8)	1 (0.8–10.5)	1 (1.6–10.5)	4
Starchy roots or tubers, sugar plants	3 (2.1–20.2)	1 (1.9–12.3)	0 (1.8–6.4)	0 (2.3–7.7)	0 (1.9–5.3)	0 (1.2–5.6)	0 (0.9–7.3)	4
Grains and grain-based products	0 (0.5–7.2)	0 (1.4–9.2)	0 (2.6–9.4)	1 (2.6–11.9)	0 (1.8–9.1)	1 (1.4–13.2)	1 (1.7–10.1)	3

The contribution to the total mean LB and UB dietary exposure of each food category at Level 1 and Level 3 of the FoodEx2 classification for the 29 PCDD/Fs and DL-PCBs and the 17 PCDD/Fs in each survey are available in Annex B (Table B8 and Table B9, respectively).

Figure 16 shows the contribution of individual food categories at the Level 1 of the FoodEx2 classification to the total mean LB dietary exposure to the 29 PCDD/Fs and DL-PCBs for each 'Infant', 'Toddler' and 'Adults' survey. The graph that shows the contribution of individual food categories at Level 1 of the FoodEx2 classification to the total mean UB dietary exposure to the 29 PCDD/Fs and DL-PCBs is available in Annex B (Figure B10). Uncertainties linked to the contribution of specific food categories is discussed in **Section 3.5**.

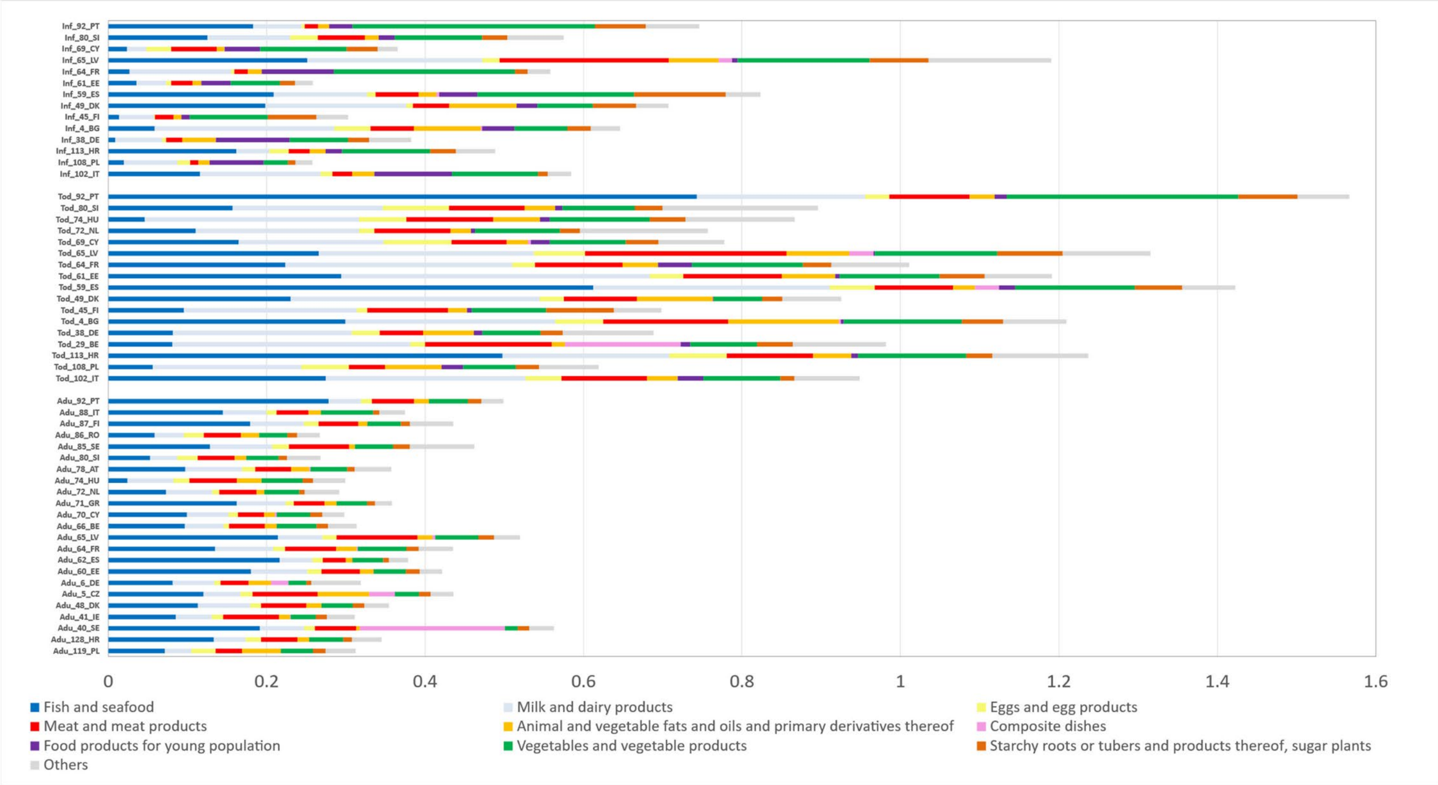

FIGURE 16 Contribution (pg WHO_{2022}-TEQ/kg bw per day) of individual food categories at the Level 1 of the FoodEx2 classification to the total mean LB dietary exposure to the 29 PCDD/Fs and DL-PCBs for each 'Infant', 'Toddler' and 'Adult' survey. The category Others includes: 'Legumes, nuts, oilseeds and spices', 'Fruit and fruit products,' 'Amphibians, reptiles, and terrestrial invertebrates', 'Sugar and similar, confectionery and water-based sweet desserts', 'Coffee, cocoa, tea and infusions', 'Products for non-standard diets, food imitates and food supplements', 'Seasoning, sauces and condiments', 'Major isolated ingredients, additives, flavours, baking and processing aids'.

3.3.2 | Exposure assessment for food-producing animals

The dietary exposure assessment for food-producing animals was conducted taking into account the data on the occurrence of the sum of 29 PCDD/Fs and DL-PCBs and the sum of 17 PCDD/Fs, in compound feeds and feed materials submitted to EFSA (see **Section 3.2.3**).

The data were expressed both as WHO_{2005}-TEQ and WHO_{2022}-TEQ, which allowed the comparison between: (i) dietary exposure estimates based on WHO_{2005}-TEFs and WHO_{2022}-TEFs for the current Opinion, as well as (ii) a comparison between exposure estimates based on the current Opinion and the 2018 Opinion.

Two exposure scenarios were followed: a model diet exposure scenario and a compound feed exposure scenario, as described in **Section 2.6.2**. The outcomes of both scenarios were then compared at both the mean and high-level occurrence.

Regarding the model diet exposure scenario, the exposure assessment was performed making use of the flexibility in the composition of the default diets as explained in **Section 2.6.2** and using substitutions of feed materials within the groups detailed in Appendix B of the 2024 EFSA Statement on animal dietary exposure in the risk assessment of contaminants in feed (EFSA FEEDAP Panel, 2024). Specifically, the following substitutions were made to the example model diets presented in Appendix C of the Statement:

- 'Maize' and 'Barley' represented the category 'Cereal grains and products derived thereof' across all considered animal species and categories.
- The ad hoc group 'Soya (beans) (including expeller, meal, extruded, co-product, toasted)' replaced 'Soybean meal' and 'Soybean hulls' in all relevant diets. Additionally, it replaced 'Beans' in the diets of bovines and ovines, and both 'Peas' and 'Beans' in the diets of salmonids.
- The ad hoc group 'Sunflower (expeller, meal, dehulled meal)' was used in lieu of 'Sunflower meal' in all relevant diets.
- The ad hoc group 'Rape seed (expeller, meal, extruded)' replaced 'Rape seed' and 'Rape seed meal' in all relevant diets.
- '(Sugar) cane molasses' was used in place of 'Molasses' wherever it was included.
- For salmonids, 'Fish protein, hydrolysed' replaced both 'Feather meal' and 'Blood meal'.
- The ad hoc group 'Lucerne (field dried, high temperature dried, extruded, meal)' replaced both 'Alfalfa meal' and 'Lucerne meal' in all applicable diets.

These substitutions were used to build the final model diets, which were then used to estimate the concentrations of PCDD/Fs and DL-PCBs in the daily diets of the considered food-producing animals. Full details of the final model diets are included in Appendix G.

Forages were included in the diets of dairy cows, cattle for fattening, lambs for fattening and horses in the proportions specified in Table C10 of the abovementioned Statement (EFSA FEEDAP Panel, 2024). 'Maize silage' was used for ruminants and 'Lucerne (field dried, high temperature dried, extruded, meal)' for horses. Since only one sample was available for both 'Grass, herbs, legume plants [green forage]' and 'Hay', these feed materials were not incorporated into the model diets. For cattle and lambs for fattening, two exposure assessments are presented, one with high complementary feed and low forage consumption (80:20 DM) and the other with low complementary feed and high forage consumption (20:80 DM).

The compound feed exposure scenario could be made with complete feed for piglets, pigs for fattening, lactating sows, chickens for fattening, laying hens, turkeys for fattening, salmonids, and rabbits for fattening and with complementary feed plus forages for horses. For cattle for fattening, dairy cows, veal calves and lambs for fattening, dietary exposure to PCDD/Fs and DL-PCBs by compound feed could not be established, as there was not enough occurrence data available for calculation.

3.3.2.1 | *Estimated concentrations of 29 PCDD/fs and DL-PCBs in food-producing animal diets*

The estimated concentrations of the 29 PCDD/Fs and DL-PCBs in the daily diets of food-producing animals (expressed in pg. WHO-TEQ/kg feed DM) are summarised below and presented in tabular form in Table 35. Corresponding dietary intake estimates, expressed in pg. WHO-TEQ/day and pg. WHO-TEQ/kg bw per day, are provided in Appendix H. Comparable results for the sum of 17 PCDD/Fs are available in Appendix I.

It should be pointed out that MLs for feed and feed ingredients are expressed in ng rather than pg. per kg, and in addition on 88% DM rather than 100% DM.

Pigs

When using model diets based on individual feed materials and applying WHO_{2022}-TEFs, the estimated concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of piglets ranged from 20 to 51 pg. TEQ/kg feed in the mean occurrence scenario, and from 64 to 103 pg. TEQ/kg feed in the high occurrence scenario. In the diets of pigs for fattening, the corresponding concentrations ranged from 20 to 53 pg. TEQ/kg feed (mean) and from 67 to 107 pg. TEQ/kg feed (high), and in the diets of lactating sows from 23 to 55 pg. TEQ/kg feed (mean) and from 78 to 116 pg. TEQ/kg feed (high).

When applying WHO_{2005}-TEFs and using the same individual feed materials, the concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of piglets ranged from 22 to 62 pg. TEQ/kg feed in the mean occurrence scenario and from 62 to 131 pg. TEQ/kg feed in the high occurrence scenario. In the diets of pigs for fattening, the corresponding concentrations ranged from 23 to 65 pg. TEQ/kg feed (mean) and from 65 to 137 pg. TEQ/kg feed (high), and in the diets of lactating sows from 26 to 66 pg. TEQ/kg feed (mean) and from 73 to 143 pg. TEQ/kg feed (high).

Considering complete feeds and applying WHO_{2022}-TEFs, the concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of piglets, pigs for fattening and lactating sows varied between 11 and 41 pg. TEQ/kg feed in the mean occurrence scenario and between 21 and 111 pg. TEQ/kg feed in the high occurrence scenario. When using WHO_{2005}-TEFs, the corresponding concentrations ranged between 11 and 51 pg. TEQ/kg feed (mean) and between 25 and 118 pg. TEQ/kg feed (high).

Overall, complete feeds showed lower contamination levels than modelled diets, under both mean and high occurrence scenarios.

Poultry

When using model diets based on individual feed materials and applying WHO_{2022}-TEFs, the estimated concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of chickens for fattening ranged from 27 to 62 pg. TEQ/kg feed in the mean occurrence scenario, and from 84 to 123 pg. TEQ/kg feed in the high occurrence scenario. In the diets of turkeys for fattening, the corresponding concentrations ranged from 27 to 67 pg. TEQ/kg feed (mean) and from 77 to 120 pg. TEQ/kg feed (high) and in the diets of laying hens from 22 to 56 pg. TEQ/kg feed (mean) and from 62 to 105 pg. TEQ/kg feed (high).

When applying WHO_{2005}-TEFs and using the same individual feed materials, the concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of chickens for fattening ranged from 29 to 74 pg. TEQ/kg feed in the mean occurrence scenario, and from 74 to 137 pg. TEQ/kg feed in the high occurrence scenario. In the diets of turkeys for fattening, the corresponding concentrations ranged from 28 to 79 pg. TEQ/kg feed (mean) and from 67 to 135 pg. TEQ/kg feed (high), and in the diets of laying hens from 24 to 68 pg. TEQ/kg feed (mean) and from 58 to 126 pg. TEQ/kg feed (high).

Considering complete feeds and applying WHO_{2022}-TEFs, concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of chickens for fattening, turkeys for fattening and laying hens varied between 12 to 68 pg. TEQ/kg feed in the mean occurrence scenario and between 35 to 110 pg. TEQ/kg feed in the high occurrence scenario. When using WHO_{2005}-TEFs, the corresponding concentrations ranged from 14 and 80 pg. TEQ/kg feed (mean) and from 38 and 132 pg. TEQ/kg feed (high).

Overall, complete feeds showed lower or comparable contamination levels than modelled diets based on individual feed materials, except for turkeys for fattening, where the opposite was observed at mean occurrence level.

Bovines

When using model diets based on individual feed materials and applying WHO_{2022}-TEFs, the estimated concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of cattle for fattening ranged from 24 to 68 pg. TEQ/kg feed in the mean occurrence scenario and from 78 to 152 pg. TEQ/kg feed in the high occurrence scenario. In the diets of dairy cows, the respective concentrations ranged from 24 to 59 pg. TEQ/kg feed (mean) and 73 to 122 pg. TEQ/kg feed (high).

When applying WHO_{2005}-TEFs and using the same individual feed materials, the concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of cattle for fattening ranged from 25 to 82 pg. TEQ/kg feed in the mean occurrence scenario and from 72 to 175 pg. TEQ/kg feed in the high occurrence scenario. In the diets of dairy cows, the corresponding concentrations ranged from 26 to 72 pg. TEQ/kg feed (mean) and from 69 to 145 pg. TEQ/kg feed (high).

Dietary exposure to PCDD/Fs and DL-PCBs in **veal calves** could neither be estimated for a model diet, nor for compound feeds as there was not enough occurrence data available for calculation.

Ovines

When using model diets based on individual feed materials and applying WHO_{2022}-TEFs, the estimated concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of lambs for fattening ranged from 24 to 67 pg. TEQ/kg feed in the mean occurrence scenario and from 76 to 149 pg. TEQ/kg feed in the high occurrence scenario. Applying WHO_{2005}-TEFs, the corresponding concentrations ranged from 26 to 81 pg. TEQ/kg feed (mean) and from 71 to 172 pg. TEQ/kg feed (high).

Fish

When using model diets based on individual feed materials and applying WHO_{2022}-TEFs, the estimated concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) of in the daily diets of salmonids ranged from 215 to 264 pg. TEQ/kg feed under the mean occurrence scenario, and from 569 to 615 pg. TEQ/kg feed under the high occurrence scenario. Applying WHO_{2005}-TEFs, the corresponding concentrations from 375 to 435 pg. TEQ/kg feed (mean) and from 975 to 1021 pg. TEQ/kg feed (high).

Considering complete feeds and applying WHO_{2022}-TEFs, the concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of salmonids varied between 284 and 318 pg. TEQ/kg feed in the mean occurrence scenario and between 655 and 673 pg. TEQ/kg feed in the high occurrence scenario. When using WHO_{2005}-TEFs, the corresponding concentrations ranged between 513 and 551 pg. TEQ/kg feed (mean) and between 1194 and 1201 pg. TEQ/kg feed (high).

Overall, complete feeds showed slightly higher contamination levels than modelled diets, under both mean and high occurrence scenarios.

Rabbits

When using model diets based on individual feed materials and applying WHO_{2022}-TEFs, the estimated concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of rabbits for fattening ranged from 22 to 76 pg. TEQ/kg feed under the mean occurrence scenario, and from 49 to 112 pg. TEQ/kg feed under the high occurrence scenario. Applying WHO_{2005}-TEFs, the corresponding concentrations ranged from 21 to 90 pg. TEQ/kg feed (mean) and from 51 to 132 pg. TEQ/kg feed (high).

Considering complete feeds and applying WHO_{2022}-TEFs, the concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of rabbits for fattening varied between 31 and 58 pg. TEQ/kg feed in the mean occurrence scenario and between 57 and 102 pg. TEQ/kg feed in the high occurrence scenario. When using WHO_{2005}-TEFs, the corresponding concentrations ranged between 34 and 70 pg. TEQ/kg feed (mean) and between 55 and 123 pg. TEQ/kg feed (high).

Overall, complete feeds showed similar contamination levels than modelled diets, under both mean and high occurrence scenarios, with narrower LB–UB ranges observed for complete feeds.

Horses

When using model diets based on individual feed materials and applying WHO_{2022}-TEFs, the estimated concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) of in the daily diets of horses ranged from 34 to 107 pg. TEQ/kg feed under the mean occurrence scenario, and from 42 to 115 pg. TEQ/kg feed under the high occurrence scenario. Applying WHO_{2005}-TEFs, the corresponding concentrations ranged from 27 to 118 pg. TEQ/kg feed (mean) and from 33 to 129 pg. TEQ/kg feed (high).

Considering complementary feeds plus forages and applying WHO_{2022}-TEFs, the concentrations of the 29 PCDD/Fs and DL-PCBs (LB–UB, DM) in the daily diets of horses varied between 35 and 115 pg. TEQ/kg feed in the mean occurrence scenario and between 35 and 117 pg. TEQ/kg feed in the high occurrence scenario. When using WHO_{2005}-TEFs, the corresponding concentrations ranged between 27 and 128 pg. TEQ/kg feed (mean) and between 27 and 131 pg. TEQ/kg feed (high).

Overall, complementary feeds plus forages showed similar contamination levels than modelled diets, under both mean and high occurrence scenarios.

TABLE 35 Lower bound (LB) and upper bound (UB) mean and high concentrations (the highest reliable percentile based on the number of samples available, up to 95th percentile) of the 29 PCDD/Fs and DL-PCBs in pg. WHO-TEQ/kg dry matter (DM) in daily diets of considered food-producing animal species and categories.

	Estimated concentrations of the 29 PCDD/fs and DL-PCBs in food-producing animal diets (pg WHO-TEQ/kg DM)							
	WHO_{2022}-TEQ				WHO_{2005}-TEQ			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Estimates derived from model diets								
Pigs								
Piglets	20	51	64	103	22	62	62	131
Pigs for fattening	20	53	67	107	23	65	65	137
Sows, lactating	23	55	78	116	26	66	73	143
Poultry								
Chickens for fattening	27	62	84	123	29	74	74	137
Laying hens	22	56	62	105	24	68	58	126
Turkeys for fattening	27	67	77	120	28	79	67	135
Cattle								
Cattle for fattening (high complementary feed/low forage)[a]	24	62	78	132	25	74	72	158
Cattle for fattening (low complementary feed/high forage)[a]	32	68	105	152	35	82	98	175
Dairy cows[a]	24	59	73	122	26	72	69	145
Small ruminants								
Lambs for fattening (high complementary feed/low forage)[a]	24	58	76	121	26	71	71	145
Lambs for fattening (low complementary feed/high forage)[a]	32	67	105	149	35	81	98	172
Fish								
Salmonids	215	264	569	615	375	435	975	1021
Rabbits								
Rabbits for fattening	22	76	49	112	21	90	51	132
Horses								
All categories[a]	34	107	42	115	27	118	33	129
Estimates derived from compound feed								
Pigs								
Piglets	14	41	21	69	15	51	26	91
Pigs for fattening	11	36	29	97	11	44	30	90
Sows, lactating[b]	22	38	47	111	23	44	25	118

TABLE 35 (Continued)

	Estimated concentrations of the 29 PCDD/fs and DL-PCBs in food-producing animal diets (pg WHO-TEQ/kg DM)							
	WHO_{2022}-TEQ				WHO_{2005}-TEQ			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Poultry								
Chickens for fattening[c]	17	43	35	110	14	47	38	119
Laying hens	12	41	37	105	15	53	46	132
Turkeys for fattening	42	68	42	68	44	80	44	80
Fish								
Salmonids	284	318	655	673	513	551	1194	1201
Rabbits								
Rabbits for fattening	31	58	57	102	34	70	55	123
Horses								
All categories[a]	35	115	35	117	27	128	27	131

Abbreviations: DL-PCBs, dioxin-like polychlorinated biphenyls; DM, dry matter; LB, lower bound; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]For bovines, ovines and horses, complementary feed was complemented with forages.

[b]The estimates are based on ad hoc feed category consisting of the samples of 'Complete feed' for 'Breeding pigs' and 'Sows'.

[c]The estimates are based on ad hoc feed category consisting of the samples of 'Complete feed' for 'Fattening chickens (broilers)' and 'Poultry (starter diets)'.

3.3.2.2 | *Impact of WHO-TEF revision on estimated concentrations of the 29 PCDD/fs and DL-PCBs in food-producing animal diets*

The impact of the updated WHO-TEFs on the estimated concentrations of the 29 PCDD/Fs and DL-PCBs in the daily diets of the considered food-producing animals was assessed by comparing concentration estimates calculated for the current Opinion using both WHO_{2022}-TEFs and WHO_{2005}-TEFs.

Table 36 presents the ratios of WHO_{2022}-TEF concentration estimates to WHO_{2005}-TEF concentration estimates for the 29 PCDD/Fs and DL-PCBs, for each animal species and category, under both mean and high occurrence (LB/UB) scenarios.

For most of the animal species and categories considered, the calculated ratios ranged from 0.8 to 1.2. For salmonids, the ratios were lower (0.5–0.6). In comparison, slightly higher ratios were observed for lactating sows (up to 1.9; high LB), and horses (up to 1.3; mean and high LB). It was noted that the variation in WHO_{2022}/WHO_{2005} TEQ ratios, whether above or below 1, is largely driven by the congener pattern in the feed material or compound feed, as further discussed in Appendix J. In particular the presence of some higher chlorinated dioxin and furan congeners for which the TEFs increased is responsible for only a minor change in the TEQ levels. This is relevant because, as shown in the section on transfer (see **Section 3.1.1.4**), these congeners show lower BCFs and TRs than the lower chlorinated congeners. As a result, they result in relatively lower TEQ levels in animal-derived products.

TABLE 36 Ratios of WHO_{2022}-TEF concentration estimates to WHO_{2005}-TEF concentration estimates for the 29 PCDD/Fs and DL-PCBs in daily diets of food-producing animals.

Concentration ratios	Mean LB	Mean UB	High[a] LB	High[a] UB
Estimates derived from model diets				
Pigs				
Piglets	0.9	0.8	1.0	0.8
Pigs for fattening	0.9	0.8	1.0	0.8
Sows, lactating	0.9	0.8	1.1	0.8
Poultry				
Chickens for fattening	0.9	0.8	1.1	0.9
Laying hens	0.9	0.8	1.1	0.8
Turkeys for fattening	1.0	0.8	1.1	0.9
Cattle				
Cattle for fattening (high complementary feed, low forage)[b]	1.0	0.8	1.1	0.8
Cattle for fattening (low complementary feed, high forage)[b]	0.9	0.8	1.1	0.9
Dairy cows[b]	0.9	0.8	1.1	0.8
Small ruminants				
Lambs for fattening (high complementary feed, low forage)[b]	0.9	0.8	1.1	0.8
Lambs for fattening (low complementary feed, high forage)[b]	0.9	0.8	1.1	0.9
Fish				
Salmonids	0.6	0.6	0.6	0.6
Rabbits				
Rabbits for fattening	1.1	0.8	1.0	0.8
Horses				
All categories[b]	1.3	0.9	1.2	0.9
Estimates derived from compound feeds				
Pigs				
Piglets	0.9	0.8	0.8	0.8
Pigs for fattening	1.0	0.8	1.0	1.1
Sows, lactating	1.0	0.9	1.9	0.9
Poultry				
Chickens for fattening	1.2	0.9	0.9	0.9
Laying hens	0.8	0.8	0.8	0.8
Turkeys for fattening	0.9	0.8	0.9	0.8

TABLE 36 (Continued)

Concentration ratios	Mean LB	Mean UB	High[a] LB	High[a] UB
Fish				
Salmonids	0.6	0.6	0.5	0.6
Rabbits				
Rabbits for fattening	0.9	0.8	1.0	0.8
Horses				
All categories[b]	1.3	0.9	1.3	0.9

Abbreviations: DL-PCBs, dioxin-like polychlorinated biphenyls; LB, lower bound; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEF, Toxic Equivalency Factor; UB, upper bound; WHO, World Health Organization.

[a]The highest reliable percentile based on the number of samples available, up to 95th percentile.

[b]For bovines, ovines and horses, complementary feed was complemented with forages.

3.3.2.3 | *Comparison of the 29 PCDD/F and DL-PCB concentration estimates in food-producing animal diets: EFSA 2018 vs. current opinion*

The impact of updated occurrence data and methodological refinements on the estimated concentrations of the 29 PCDD/Fs and DL-PCBs in the daily diets of food-producing animals (calculated using WHO_{2005}-TEFs) was assessed by comparing values reported in the current Opinion with those presented in the 2018 Opinion (EFSA CONTAM Panel, 2018). The previous assessment was based on occurrence data from 2010–2016, whereas the current Opinion used data from 2013 to 2023.

Table 37 presents the estimated concentrations of the 29 PCDD/Fs and DL-PCBs (pg WHO_{2005}-TEQ/kg feed DM) in the daily diets of each considered food-producing species and categories, under mean and high/P95 occurrence (LB/UB) scenarios as reported in both Opinions. The table also presents the ratios of current estimates to those from 2018.

For estimates derived from a model diet exposure scenario, concentrations of 29 PCDD/Fs and DL-PCBs were consistently lower in the current Opinion for piglets and all poultry categories under the high occurrence scenario (ratios: 0.3–1.0). Under the mean occurrence scenario, estimates were comparable for piglets, chickens for fattening and laying hens, although the LB–UB ranges reported in the 2018 Opinion were narrower (ratios: 0.8–1.4). For turkeys for fattening, current estimates were slightly higher at the mean occurrence level (ratios: 1.4–2.2). For cattle, concentrations were comparable between the two Opinions, except for dairy cows under high occurrence scenario, where current estimates were lower (ratios 0.5–0.9). No comparison was made for lambs for fattening, as this category was not assessed in 2018.

For estimates derived from a compound feed exposure scenario, concentrations of the 29 PCDD/Fs and DL-PCBs were lower or comparable in the current Opinion for pigs for fattening and lactating sows (ratios 0.2–0.9) and slightly lower for salmonids (ratios: 0.8–0.9). For rabbits for fattening and horses, the estimates were comparable across both Opinions, with narrower LB–UB ranges reported in the 2018 Opinion.

There are several methodological differences between the two opinions related to exposure assessments in food-producing animals. These may contribute to differences in the results, in addition to changes in data availability and possible general changes in concentrations in feed. These differences include changes in model diet compositions (including the proportion of forages for cattle for fattening and horses), different ad hoc groupings of feed materials and compound feeds, and changes in default feed intakes and body weights for some animal species and categories. Additionally, whereas the 2018 Opinion derived high-level estimates from the 95th percentile, the current Opinion used the highest reliable percentile (HRP).

TABLE 37 Estimated concentrations of the 29 PCDD/Fs and DL-PCBs (pg WHO_{2005}-TEQ/kg feed dry matter (DM)) in the daily diets of food-producing animals, as reported in the current and the 2018 Opinion, under mean and high/P95[a] occurrence (LB/UB) scenarios, including ratios calculated by dividing current estimates by those from 2018.

	Current opinion				EFSA CONTAM panel 2018 opinion							
	Pg WHO_{2005}-TEQ/kg feed DM				Pg WHO_{2005}-TEQ/kg feed DM				Concentration ratios			
	Mean LB	Mean UB	High LB	High UB	Mean LB	Mean UB	P95 LB	P95 UB	Mean LB	Mean UB	High/ P95 LB	High/ P95 UB
Estimates derived from model diets												
Pigs												
Piglets[b]	22	62	62	131	27	45	172	206	0.8	1.4	0.4	0.7
Poultry												
Chickens for fattening	29	74	74	137	32	52	192	223	0.9	1.4	0.4	0.6
Laying hens	24	68	58	126	30	47	182	208	0.8	1.4	0.3	0.6
Turkeys for fattening	28	79	67	135	21	36	122	145	1.4	2.2	0.5	0.9
Cattle												
Cattle for fattening (high complementary feed/low forage)[c,d]	25	74	72	158	30	43	88	96	0.8	1.7	0.8	1.6
Cattle for fattening (low complementary feed/high forage)[c,e]	35	82	98	175	46	64	110	117	0.8	1.3	0.9	1.5
Dairy cows[c,f]	26	72	69	145	47	65	140	165	0.5	1.1	0.5	0.9
Small ruminants												
Lambs for fattening (high complementary feed/low forage)[c,g]	26	71	71	145	–	–	–	–	–	–	–	–
Lambs for fattening (low complementary feed/high forage)[c,g]	35	81	98	172	–	–	–	–	–	–	–	–
Estimates derived from compound feed												
Pigs												
Pigs for fattening	11	44	30	90	27	49	143	168	0.4	0.9	0.2	0.5
Sows, lactating[h]	23	44	25	118	25	42	–	–	0.9	1.0	–	–
Fish												
Salmonids	513	551	1194	1201	609	660	1345	1349	0.8	0.8	0.9	0.9
Rabbits												
Rabbits for fattening	34	70	55	123	46	60	–	–	0.7	1.2	–	–

TABLE 37 (Continued)

	Current opinion				EFSA CONTAM panel 2018 opinion							
	Pg WHO_{2005}-TEQ/kg feed DM				Pg WHO_{2005}-TEQ/kg feed DM				Concentration ratios			
	Mean LB	Mean UB	High LB	High UB	Mean LB	Mean UB	P95 LB	P95 UB	Mean LB	Mean UB	High/ P95 LB	High/ P95 UB
Horses												
All categories[c]	27	128	27	131	84	100	–	–	0.3	1.3	–	–

Abbreviations: DL-PCBs, dioxin-like polychlorinated biphenyls; DM, dry matter; LB, lower bound; P, percentile; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]In the current Opinion, the highest reliable percentile (based on the number of available samples) is presented up to the 95th percentile, whereas in 2018, only the 95th percentile was reported.

[b]This entry refers to the concentrations of the sum of 29 PCDD/Fs and DL-PCBs in the modelled diet for 'Piglets' in the current Opinion, and for 'Pigs: starter' in the 2018 Opinion.

[c]For bovines, ovines and horses, complementary feed was complemented with forages.

[d]This entry refers to the concentrations of the sum of 29 PCDD/Fs and DL-PCBs in the modelled diet for 'Cattle for fattening (high complementary feed/low forage)' in the current Opinion, and for 'Beef cattle: cereal-based diet' in the 2018 Opinion.

[e]This entry refers to the concentrations of the sum of 29 PCDD/Fs and DL-PCBs in the modelled diet for 'Cattle for fattening (low complementary feed/high forage)' in the current Opinion, and for 'Beef cattle: maize silage-based diet' in the 2018 Opinion.

[f]This entry refers to the concentrations of the sum of 29 PCDD/Fs and DL-PCBs in the modelled diet for 'Dairy cows' in the current Opinion, and for 'Dairy cows: maize silage-based' in the 2018 Opinion.

[g]Dietary exposure for 'Lambs for fattening' was not assessed in EFSA 2018 Opinion.

[h]This entry refers to the concentrations of the sum of 29 PCDD/Fs and DL-PCBs in the estimated diet for 'Breeding pigs' and 'Sows' in the current Opinion, and for 'Lactating sows' in the 2018 Opinion.

3.4 | Risk characterisation

3.4.1 | Risk characterisation based on dietary exposure

The CONTAM Panel evaluated the current exposure using mean levels for the sum of 29 PCDD/Fs and DL-PCBs in various food groups, expressed in WHO_{2022}-TEQs (See **Section 3.3.1.2**). The exposure was subsequently compared with the newly established TWI of 0.6 pg. TEQ/kg bw per week. Since the exposure was estimated on a daily basis, the values were first extrapolated to a weekly basis, simply by multiplication with a factor 7 (Table 38).

The TWI should prevent that the body burden in women of childbearing age will result in exposure of the offspring in utero and during breastfeeding that could raise a health concern. For this reason, an estimation of the exposure of infants was not included in the risk characterisation (see **Section 3.1.6**).

TABLE 38 Weekly intake of the sum of 29 PCDD/Fs and DL-PCBs expressed as WHO_{2022}-TEQs.

Range of mean dietary exposure (pg WHO-TEQ/kg bw per week) using WHO_{2022}-TEFs							
		Minimum		Median		Maximum	
Age group[a]	*N* surveys	LB	UB	LB	UB	LB	UB
Toddlers	17	4.33	8.56	6.64	11.7	11.0	16.4
Other children	21	3.59	7.15	5.01	8.74	7.77	12.0
Adolescents	23	2.03	3.35	2.68	4.53	4.63	7.16
Adults	23	1.87	3.01	2.50	3.90	3.94	5.64
Elderly	21	1.77	2.95	2.57	3.85	4.79	6.35
Very elderly	16	1.69	2.90	2.47	3.96	4.41	6.10
Range of P95 dietary exposure (pg WHO-TEQ/kg bw per week) using WHO_{2022}-TEFs							
		Minimum		Median		Maximum	
Age group[a]	**N surveys**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Toddlers	16	8.58	13.8	14.3	21.5	23.1	33.1
Other children	21	6.77	12.2	10.7	16.2	16.8	22.9
Adolescents	22	4.29	7.55	6.06	9.32	14.7	17.8
Adults	23	4.15	6.30	6.47	8.72	11.5	14.6
Elderly	21	3.96	6.04	7.22	8.98	13.4	14.8
Very elderly	12	3.98	6.27	6.16	8.18	9.77	12.0

Abbreviations: bw, body weight; LB, lower bound; *N* surveys, number of surveys; UB, upper bound; DL-PCBs, dioxin-like polychlorinated biphenyls; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEQ, toxic equivalents.

[a]**Section 2.4.1** describes the age range within each age class.

Concerning the sum of 29 PCDD/Fs and DL-PCBs, for 'Adults' the mean LB and UB exposure varied from 1.87 and 5.64 pg. WHO_{2022}-TEQ/kg bw per week, which exceeds the TWI by a factor 3 to 9. Similar mean exposure is estimated for 'Adolescents', 'Elderly' and 'Very Elderly' (LB–UB range 1.69–7.16 pg. TEQ/kg bw per week). For 'Toddlers' and 'Other Children', the LB–UB exposure ranged from 3.59 to 16.44 pg. WHO_{2022}-TEQ/kg bw per week, being 6- to 27-fold higher than the TWI.

When focussing on the higher end of the exposure (P95), the intake of the sum of 29 PCDD/Fs and DL-PCBs by 'Adolescents', 'Adults', 'Elderly' and 'Very Elderly' ranged from 3.96 to 17.79 pg. WHO_{2022}-TEQ/kg bw per week, being 7- to 30-fold the TWI. Again, 'Toddlers' and 'Other Children' showed a higher intake, being 6.77 to 33.06 pg. WHO_{2022}-TEQ/kg bw per week (11- to 55-fold the TWI).

3.4.1.1 | *Interpretation of the exceedance of the TWI for the different age groups*

'Toddlers' and 'Other children' generally have in general a 2-fold higher intake than older age groups (see Table 38), and their dietary exposure exceeds the TWI. The CONTAM Panel notes that blood levels (reflecting the body burden) reach a peak level during breastfeeding and then decline sharply, as illustrated in Figure 17. An intake higher than the TWI slows down the decrease in the blood levels of PCDD/F- and DL-PCB-TEQ in previously breastfed infants. In non-breastfed infants it would increase the rate of building up the body burden.

The exceedance of the TWI for Toddlers and Other children, i.e. 6- to 27-fold for the mean dietary exposure and 11- to 55-fold at the P95 exposure, means high body burdens and plasma levels during the period that may cover the critical window for male developmental effects. A more than 6-fold exceedance of the TWI by 'Toddlers' and 'Other children' would result in serum levels higher than the NOAEC for PCDD/F-TEQ of 6.7 ng WHO_{2022}-TEQ/kg fat at the age of 8–9 years as observed in the Russian Children's Study (see Table 5 and **Section 3.1.6.4**). Considering the uncertainties in the critical window for the effects on sperm concentrations, the exceedance of the TWI raises a health concern for these two age groups. As discussed in the Uncertainty section (see **Section 3.1.5**), there are strong indications that the actual TEQ-dietary exposure is overestimated, due to differences in kinetics between congeners, the exposure via plant-based food and uncertainties around the TEFs for DL-PCBs.

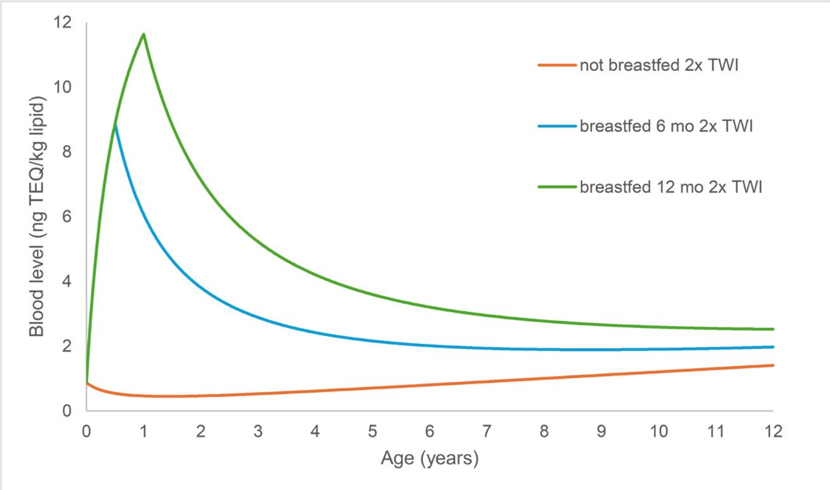

FIGURE 17 Modelling of the lipid-based blood levels (reflecting the body burden) of PCDD/Fs and DL-PCBs in children up to the age of 12 years, using the PBK CADM model. Infants were either not breastfed or breastfed for 6 or 12 months with 800 mL milk per day containing 3.5% fat and a level of 2.2 ng WHO_{2022}-TEQ/kg fat (the level resulting from chronic exposure of the future mothers at the TWI). After the breast-feeding, the children were exposed via food at two-fold the TWI, in accordance with the exposure assessment. The non-breastfed children were exposed via food at two-fold the TWI from birth.

Besides 'Toddlers' and 'Other children', the CONTAM Panel noted that the most relevant part of the population are women of childbearing age in order to protect their future infants. The Panel also noted that not only male infants are sensitive to exposure to PCDD/Fs and DL-PCBs, since developmental tooth enamel defects are relevant for both female and male infants. In the 2018 Opinion it was concluded that exposure to PCDD/Fs via human milk may lead to increased enamel defects in teeth in the children, although the available data were not including DL-PCBs and were not suitable for dose–response assessment and did not allow the derivation of a Reference Point. Enamel defects on teeth are also developmental and appear to occur at slightly higher exposure than the developmental effects on sperm concentration (see EFSA CONTAM Panel, 2018).

'Adolescents', 'Adults', and older age groups, appear to be less sensitive than younger age groups. Exposure to PCDD/Fs and DL-PCBs can cause chloracne, but this occurs at much higher exposure levels than the current exposure via food. Another association considered in the 2018 Opinion to be causal was a change in sex ratio at birth after paternal exposure in adult life. Although not quantified, this effect seems to occur at exposure levels lower than those causing chloracne, but still much higher than those associated with adverse developmental effects and seem thus not relevant at current levels in Europe.

Several other possible adverse effects in adults have been investigated, but none of them could be causally related to exposure to PCDD/Fs and DL-PCBs since the evidence was either insufficient, showed no associations, or was inconclusive (see **Section 3.1.3**).

The 2018 Opinion concluded that in rodents, bone effects were seen at body burdens somewhat higher but still in a similar range than those causing male reproductive effects. In humans, there was limited evidence from one cohort (Seveso Women Health Study) indicating associations between PCDD/F and DL-PCB exposure and some changes in bone parameters. It was indicated that "*observations at a later age might be more sensitive for assessing possible associations between early life TCDD exposure and measures such as bone strength*" (EFSA CONTAM Panel, 2018).

Effects other than developmental endpoints effects might occur at body burdens higher than those presently observed in Europe. However, the available data did not allow the CONTAM Panel to estimate in a quantitative manner at which exposure levels there is a health concern for such effects in adolescents, adults and older age groups. The CONTAM Panel noted that at the current exceedance of the TWI there is no evidence for such effects.

3.4.2 | Risk characterisation based on levels in human milk

Based on the PBK modelling, a TWI of 0.6 pg. WHO_{2022}-TEQ/kg bw per week would lead to a body burden in women (i.e. future mothers) of 2.2 ng WHO_{2022}-TEQ/kg fat, which would be equal to the level in human milk (see **Section 3.1.6.2** and Figure 17) and can thus be compared with reported human milk levels.

The UNEP studies on pooled milk samples collected in the last round in 2019 from, among others, a number of European countries (Czech Republic, Germany (2 pooled samples), Ireland, Slovak Republic and Sweden) showed for the six pooled samples of these countries an average level for PCDD/Fs and DL-PCBs of 2.7 ng WHO_{2022}-TEQ/kg fat (range 2.4–3.0) (see **Section 3.1.1.2** and Annex D). Data on individual milk samples from German women reported by CVUA showed over the period 2021–2024 a mean level for PCDD/Fs and DL-PCBs of 2.3 ng WHO_{2022}-TEQ/kg fat (range 0.5–6.0) (See **Section 3.1.1.1** and Annex D). The data from UNEP and CVUA show that average levels in human milk are close to 2.2 ng WHO_{2022}-TEQ/kg fat. Although based on a small number of women, the data from CVUA indicate that, at the higher end, the level may exceed the critical value by a factor 2.6. The CONTAM Panel noted that this fold-exceedance is lower than the fold-exceedance of the TWI based on the dietary estimated exposures.

There could be a number of reasons for this apparent discrepancy, such as: (i) uncertainties associated with the toxicokinetic model applied to link the body burden to a chronic exposure, including the assumption that other PCDD/F and DL-PCB congeners behave similarly to TCDD, (ii) overestimation of the current dietary exposure, and (iii) the human milk samples covering only a few countries. This is further discussed in **Section 3.5.1.4**.

3.5 | Uncertainty analysis

As indicated in the protocol for the risk assessment (Annex A), an evaluation of the inherent uncertainties in the risk assessment was performed, and it was based on the Guidance on uncertainty analysis in scientific assessments of the EFSA Scientific Committee (EFSA Scientific Committee, 2018) and the guidance on communication of uncertainty in scientific assessments (EFSA, 2019).

The aim of the uncertainty analysis is to identify and quantify uncertainties affecting the risk assessment of the update of the 2018 Opinion on PCDD/Fs and DL-PCBs in food and feed, and assess the overall certainty of the main conclusions (EFSA Scientific Committee, 2018).

The human risk assessment followed the normal approach of the CONTAM Panel, including standardised elements to address some sources of uncertainty, e.g. default uncertainty factors and use of EFSA's Comprehensive Database on consumption. Thus, the uncertainty analysis followed the approach for a standardised assessment (Section 3 of the Guidance).

For animal health, only an update of the exposure estimates for food-producing animals was performed. An update of the effects on the health of food-producing and non-food producing animals, and risk characterisation, were outside of the scope of the TORs (see **Section 1.2**). Therefore, the combined impact of the uncertainties was not quantified, but an evaluation of the most important uncertainties affecting the exposure estimates was performed (see **Section 3.5.2**).

3.5.1 | Uncertainty analysis of the human risk assessment

3.5.1.1 | *Identification of sources of uncertainty in the human risk assessment*

In a first step the sources of uncertainties related to the hazard identification and characterisation and dietary exposure assessment of PCDD/F and DL-PCB exposure via food were listed and discussed (see **Tables 39** and **40**, respectively). The uncertainty analysis focuses on uncertainties, which are specific to the current assessment. For these, it was judged which have most impact on the outcome of the hazard identification and characterisation and the exposure estimations.

TABLE 39 Uncertainties identified and their impact on the outcome of the hazard identification and characterisation.

Description of the uncertainty		Impact of the uncertainty on the hazard identification and characterisation[a]
Chemical composition and analytical methods		
Dosing and chemical composition	–	**2 – Medium impact**. For the critical study, uncertainty on whether the specific activity of the labelled substance is correctly defined.
Hazard identification and characterisation		
Toxicokinetics	ADME	**2 – Medium impact**. – Different absorption of the congeners not taken into account. – Impact of breastfeeding on the exposure of the offspring might be different between rodents and humans. – Different kinetics and in particular half-lives of relevant congeners not taken into account.
	Toxicokinetic model	**2 – Medium impact**. – The CADM model has been calibrated against human data. – The CADM model is based on TCDD, but it is applied to all PCDD/Fs and DL-PCB congeners, some with different toxicokinetics, and resulting half-lives. – Variation and uncertainties in the parameters in the CADM model, e.g. the body fat percentage in women, absorption rate. – The model includes the relevant tissues/compartments (liver and body fat)
	Transfer from feed to the food of animal origin	**0 – Negligible impact**. It does not affect the human hazard identification and characterisation.

TABLE 39 (Continued)

Description of the uncertainty		Impact of the uncertainty on the hazard identification and characterisation[a]
Toxicity studies in experimental animals: critical endpoint and study design	For the eligible studies-Study design	**1 – Low impact**. Limited information on certain exposure time points between weaning and puberty. Different study designs for similar endpoints (e.g. single exposure vs. repeated exposure). Subcutaneous administration (but body burden used for dose–response).
	For the critical study(ies) - Dosing regime/ dose–response	**3 – High impact**. Near maximal effect at the lowest dose in the best available study. The steepness of the dose–response curve is unclear, and around which dose the decrease in sperm production starts. Lack of other studies in the same rat strain with repeated dosing.
Epidemiological studies	Study design	**2 – Medium impact**. – Mainly cross-sectional study designs. – Uncertainty in the relevance of the WHO_{2022}-TEFs for humans. – Lack of data on all 29 PCDD/Fs and DL-PCBs. – Back calculation of exposure. – Background exposure to other contaminants not taken into account. – Lack of feasibility to perform meta-analysis. – Low number of epidemiological studies on the critical endpoint at low exposure.
	Critical study in the 2018 Opinion (Russian Children's Study)	**2 – Medium impact**. – Lack of association for Total WHO-TEQ in the critical study in the 2018 Opinion. – Uncertainty regarding critical window for effect on semen quality outcome. – Impact of sub-group distribution of formula fed infants.
	Other two pivotal studies (Seveso cohort studies)	**2 – Medium impact**. – Back calculation of exposure. – Lack of measurements on PCDD/Fs and DL-PCBs other than TCDD, e.g. in the Seveso cohort studies. – Lack of levels in non-exposed groups used as controls. – Uncertainty regarding critical window for effect on semen quality outcome. – Regarding Mocarelli et al. (2011), uncertainty around the sub-group analyses based on breastfeeding status (breast- versus formulafed).
Genotoxicity	Uncertainty in genotoxicity	**0 – Negligible impact**. No new data; the conclusion of the 2018 Opinion applies.
Mixture approach using TEFs	Uncertainty on the sensitivity of humans towards TCDD	**3 – High impact**. Human cells and mice bearing the hAHR are substantially less sensitive to TCDD than rodent cells and wild-type rodent models. Human cells are substantially less sensitive to TCDD (about 10-fold) than rodent cells.
	Uncertainty in the applicability of the TEFs to the critical endpoint	**2–3 – Medium-High impact**. – The assessment is based on TCDD and extrapolated to other congeners via the TEFs. – Few studies on sperm effects for other congeners. – No TCDD-like developmental effects for PCB-77 and –126 on male reproduction in rats (Faqi et al., 1998b); but no direct comparison with TCDD in these studies.
Mode of action	Relevance of the mode of action to humans	**1 – Low impact**. Most of the data originated from rodent models. There is evidence that the regulatory pathways are similar between rodents and humans.
	Role of AHR in mode of action	**1– Low impact**. Based on the evidence there is little uncertainty that the AHR is the most relevant mediator in the effects on male development. The exact molecular mechanism of action of effects on male reproduction is unknown.
	Role of the non-canonical pathways	**1 – Low impact**. It can occur at high doses and may contribute to the effects on male development.
Selection of Reference Point	BMD modelling	**3 – High impact**. BMD analysis of data showing a near-maximal effect at the lowest dose (see previous entry above). See detailed considerations in **Section 3.5.1.2** below. **1/2 – Low/Medium impact**. The assumption of a constant Coefficient of Variation was marginally rejected at the 5% significance level; however sensitivity analysis showed the impact of this to be minor (see Annex G).
	Selection of BMR	**2 – Medium impact**. Limited information on the relevant BMR for sperm concentration.

(Continues)

TABLE 39 (Continued)

Description of the uncertainty		Impact of the uncertainty on the hazard identification and characterisation[a]
Derivation of the TWI	Inter-species variability not correctly covered by standard UFs	**2 – Medium impact**. The default UF for **inter-species variability in kinetics** not applied because a body burden approach was used. However, differences in the % of body fat content between humans and rats not taken into account. **1 – Low impact**. The default UF for **inter-species variability in dynamics** not applied; there is evidence that humans are not more sensitive than rodents. There is some possibility that humans could be less sensitive. However, this is not supported by the three pivotal human studies.
	Intra-species variability not correctly covered by standard UFs	**2 – Medium impact**. The default UF for **intra-species variability in kinetics** applied. The CONTAM Panel is aware that applying this UF may be too conservative considering the limited biotransformation. However, there are insufficient data to deviate from the default uncertainty factor (see **Section 3.1.6.1**). **1 – Low impact**. The default UF for **intra-species variability in dynamics** not applied; the most sensitive population is covered in the pivotal studies.
	Critical window of exposure for the critical effects	**1 – Low impact**. Limited information on the critical window of exposure, and differences between rodents and humans.

Abbreviations: ADME, absorption, distribution, metabolism, excretion; AHR, aryl hydrocarbon receptor; BMD, benchmark dose; BMR, benchmark response; CADM Model, Concentration and Age Dependent Model; hAHR, human Aryl hydrocarbon Receptor; PCB, polychlorinated biphenyls; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TCDD, tetrachlorodibenzo-*p*-dioxin; TEF, toxic equivalency factor; TEQ, toxic equivalents; TWI, tolerable weekly intake; UF, uncertainty factor; WHO, world health organisation.

[a]0: Uncertainty with negligible impact; 1: Uncertainty with low impact; 2: Uncertainty with medium impact; 3: Uncertainty with high impact. This ordinal scale provides a relative assessment of the impact of the individual uncertainties as an aid to quantifying their combined impact by expert judgement, as described in Section 11 of EFSA Scientific Committee (2018).

TABLE 40 Uncertainties identified and their impact on the outcome of the exposure assessment.

Description of the uncertainty		Impact of the uncertainty on the exposure assessment[a]
Occurrence data		
Analytical measurements	Performance (e.g. specificity for the target compounds) of the analytical method (GC-ECD, GC–MS, etc)	**1 – Low impact**. Only results of samples reported to be analysed by GC–HRMS or GC–MS/MS (reported by the data providers also as HRGC–HRMS, GC–HRMS (magnetic sector), GC-QqQ-MS–MS or GC–MS) were considered sufficiently reliable to be included in the assessment.
	Analytical capability of the method - sensitivity (e.g. LOQ, LOD)	**2 - Medium impact**. The uncertainty linked to left-censored data is handled by the substitution method (WHO/IPCS, 2009, updated in 2020) and it was contained by applying the performance criteria established in the Legislation. Results are expressed as LB and UB estimates, where for the maximum mean exposures, LB estimates are 25%–36% lower than UB estimates.
	Consideration of recovery (e.g. correction carried out or not)	**0 – Negligible impact**. Methods are well established and include recovery correction by the use of isotope-labelled standards.
	Potential errors in reporting the occurrence data (e.g. in the classification of the congeners, food category, unit of measurement, parameter, fat vs. whole weight, etc.) – unidentified errors (not apparent from the data provided)	**1 – Low impact**. The data validation phase and the feedback from Data Providers have brought to light the presence of possible mistakes in data reporting especially on such a complex dataset as the one concerning PCDD/Fs and DL-PCBs. Identified mistakes were addressed. Unidentified reporting errors are not expected to influence the mean occurrence, given the high number of samples in most food categories.
	Missing information in reporting the occurrence data (e.g. analytical method)	**1 – Low impact**. Information relevant to guarantee quality in the data included in the assessment were available in the submitted data or retrieved through data provider feedback. When not available (e.g. information on analytical methods or form of certain food categories), samples were excluded to reduce the uncertainty.
	Unclear whether and what kind of the treatment/processing has been applied prior to the analysis of the sample that is submitted to EFSA	**2 – Medium impact**. Some uncertainty is linked to the fact that some steps in sample preparation, such as peeling and washing of vegetables, might affect PCDD/F and DL-PCB concentrations.

TABLE 40 (Continued)

Description of the uncertainty		Impact of the uncertainty on the exposure assessment[a]
	Composite foods	**0 – Negligible impact**. Composite foods that contain ingredients belonging to the food categories for which data were available, were included in the dietary exposure assessment of PCDD/Fs and DL-PCBs with LB and UB mean concentration calculated using the available LB and UB mean concentration of the main ingredient. For example, fine bakery ware, bread and pasta concentration derived from the concentration in wheat flour. As the contribution of these composite foods is low the impact of this uncertainty is also considered low
	Composite dishes	**2 – Low impact**. Uncertainty in the composition of the composite dishes, and whether the concentration in the composite dishes is representative of the food that is consumed.
Other data, factors and assumptions	Estimation of fat content for the consumed foods in case the chemical occurrence levels are expressed in fat weight	**1–2 – Low-Medium impact**. – Fat content was retrieved from available data in the submitted dataset or retrieved through data providers' feedback. Some mistakes in the reporting of the variable that used to indicate whether the reported concentration refers to fat weight or whole weight and in the reporting of fat content might be present. – Samples reported as animal fat were not used in the calculation of the concentration in meat. Fat samples showed lower lipid-based levels than meat, without a clear explanation. Combining meat and fat data resulted in lower levels. The use of only meat data could have caused an overestimation of the exposure. – The use of fat content from composition tables and/or imputation of the missing information from these values on the consumption side might add to the uncertainty when using lipid-based concentrations in food.
	Use of conversion factors to determine the amounts and/or proportions of the different food components used to prepare the processed food (e.g. yield factors and dilution factors)	**1 – Low impact**. Dilution factors suggested in EFSA guidelines (EFSA, 2018) were used to calculate LB and UB PCDD/F and DL-PCB concentrations for ready-to-eat foods or ready-to-drink beverages from the available concentrations in the dry ingredients, e.g. for infant and follow on formula.
Representativeness of the data	Limited number or lack of analytical results for food categories expected to contribute to the exposure	**1 – Low impact**. – Data availability on some foods of plant origin was very limited overall (starchy tubers, coffee and tea beverages) or for some specific types of vegetables. – One sample with high PCDD/F and DL-PCB concentration in potato was excluded as it was considered an outlier driving the mean concentration of the available 12 samples. – Mean PCDD/F and DL-PCB concentration in coffee and tea beverages was estimated from less than 5 samples and dilution factor was used to calculate concentration in the beverages from dried ingredients. – Fruit and vegetable juices were not included in the assessment (only one sample available and uncertainties in calculating concentration from the Raw Primary Commodity model). – Excluding all food of plant origin from the exposure assessment provides maximum mean LBs that are 8% to 25% lower than the LBs estimated by the Opinion scenario, but this scenario should be considered an underestimation of the exposure linked to food of plant origin as food of plant origin does contribute to the exposure based on the available occurrence data.
	Extrapolation of data from one food category to others	**1 – Low impact**. Specific food subcategories for which there were no occurrence data available were assigned with the concentration of the parent FoodEx2 category. In particular, samples from curly kale were used to calculate higher Level averages such as for 'Leafy vegetables' or the even more general 'Vegetables and vegetable products', and then also used for more diverse vegetables such as bulb vegetables, Flowering brassicas and Legumes with pods, that might have caused an overestimation of exposure from vegetables. The impact was estimated to be low as it would only partially affect the contribution of food categories with a relatively low contribution to the exposure. **2 – Medium impact for Toddlers and Lactating women**. Samples with outlier PCDD/F and DL-PCB concentration for Bonito fish meat were included in the calculation of the average of the parent food category as they were confirmed as being correct by the data provider. Not considering these 2 outlier samples would lower the max mean LB exposure for Toddlers of 20% and of Lactating women of 54.5% (0.1%–6% for the other age groups).

(Continues)

TABLE 40 (Continued)

Description of the uncertainty		Impact of the uncertainty on the exposure assessment[a]
	Sampling strategy not fully random	**1 – Low impact**. Samples reported as suspect samples were excluded from the assessment.
	Uneven distribution of the data per year (e.g. recent years not sufficiently represented)	**1 – Low impact**. Samples from the 10 most recent years were used and were well distributed across the sampling years.
	Uneven distribution of the data per country (e.g. large number of MSs not sufficiently represented)	**1 – Low impact**. The food samples included in the final dataset were submitted by 24 Member States plus Norway and Iceland. Germany and France submitted 64% of the samples. Considering the EU-wide market, no difference is expected in mean concentrations across Member States. The proportion of locally consumed food varies among Member States.
	Impact of production method (e.g. wild vs. farmed, indoor/outdoor husbandry), on the concentration of PCDD/F and DL-PCB	**1 – Low impact**. Samples from hen eggs from production method with outdoor access compared to samples from indoor production methods, and samples of wild-caught eel, salmon and trout versus farmed fishes, had higher concentrations. This difference cannot be matched to the consumption database as the information on production method is not available. These samples were included in the estimation of mean concentrations; the uncertainty generated is estimated to be low, considering that some of these foods are not frequently consumed and sample availability approximately reflects the market share of the different production methods.
	Impact of the fishing area on the concentration of PCDD/F and DL-PCB in fatty fishes	**1 - Low impact**. Samples of fatty fish species from the Baltic Sea had higher concentrations of PCDD/Fs and DL-PCBs than samples of fatty fishes caught in other fishing areas. This difference cannot be matched to the consumption database as the information on the food origin is not available. These samples were included in the estimation of mean concentrations. Excluding samples for which the fishing area indicated a Baltic origin would have decreased the max LB exposure estimates up to 8%.
Consumption data		
Data reporting	Unidentified errors in reporting consumption data, e.g. in the classification of the food, portion size, body weight estimation, memory errors, capacity to report details in dietary surveys. Different dietary survey methodologies (e.g. dietary record vs. 24-h re-call), dietary software, interview options, use of portion-size measurement. 1. Use of national standard recipes and ingredients factors for composite dishes (potentially leading to, e.g. underestimation of minor ingredients, overestimation of standard ingredients). 2. Different sample size and response rate of the dietary surveys. Long-term (chronic) exposure assessed based on few days of consumption per individual.	**1 – Low impact**. Uncertainties and limitations related to the use of the EFSA Comprehensive Food Consumption Database have been described by EFSA (EFSA, 2011a). These uncertainties are common to dietary exposure assessments performed using the Comprehensive Database, and have the potential to cause either an over- or under-estimation of the exposure but, since the Comprehensive Database is accepted as an appropriate tool for routine use in EFSA assessments, they are regarded as standard uncertainties. No non-standard uncertainties affecting the use of the Comprehensive Database for the PCDD/Fs and DL-PCBs exposure assessment were identified.
	Consumption data refers to processed/cooked food, while occurrence data mostly refer to unprocessed/raw food	**2 – Medium impact**. Not taken into consideration. – Concentration measured in the samples might not be representative of the concentration in the consumed foods due to differences in the preparation methods used for the samples and for general consumption. – Changes in the fat content of food commodities during cooking/processing practices may lead to changes of the lipophilic contaminants in the processed food compared to the raw food commodity. – Assuming that analysed samples are not washed and peeled, the dietary exposure linked to vegetables could have been partly overestimated.

TABLE 40 (Continued)

Description of the uncertainty		Impact of the uncertainty on the exposure assessment[a]
Exposure estimates		
Supplements and specific consumption of food with high contamination levels not covered by food surveys	–	**1 – Low impact**. Clay ingestion (e.g. by pregnant women as a food supplement) not taken into account. Considering the limited proportion of the population consuming clay this has a low impact. Similar applies to brown meat of, e.g. crustaceans.
Non-dietary exposure	Sources of exposure other than dietary - how important is dietary exposure to the total	**1 – Low impact**. Dietary exposure to PCDD/Fs and DL-PCBs is believed to represent the main source of PCDD/Fs and DL-PCBs in the general population.

Abbreviations: EU, European Union; GC, gas chromatography; HRMS, high resolution mass spectrometry; LB, lower bound; MS/MS, tandem mass spectrometry; MS, mass spectrometry; PCB, polychlorinated biphenyls; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; QqQ, triple quadrupole; RPC, Raw Primary Commodity; TCDD, tetrachloro dibenzo-*p*-dioxin; UB, upper bound.

[a]0: Uncertainty with negligible impact; 1: Uncertainty with low impact; 2: Uncertainty with medium impact; 3: Uncertainty with high impact. This ordinal scale provides a relative assessment of the impact of the individual uncertainties as an aid to quantifying their combined impact by expert judgement, as described in Section 11 of EFSA Scientific Committee (2018).

3.5.1.2 | *Uncertainty assessment for the hazard assessment*

No uncertainty analysis was performed for genotoxicity, since no relevant new data on genotoxicity were identified, and the conclusion of the Panel's 2018 Opinion (EFSA CONTAM Panel, 2018) was retained.

Non-standard sources of uncertainty affecting other parts of the hazard assessment were identified in Table 39. These include a number of substantial (medium and high impact) uncertainties affecting the critical study, the selection of the Reference Point, absorption/distribution/metabolism/excretion (ADME) and the toxicokinetic model, the mixture approach using TEFs and the derivation of the TWI including the applicable uncertainty factors.

Regarding the uncertainties affecting the critical study, the CONTAM Panel decided to base the derivation of the TWI on the same rat study used by SCF (2000, 2001), i.e. Faqi et al. (1998a). In this study, there is some uncertainty regarding the body burden of the dams. Groups of rat dams were treated s.c. with 3 doses of TCDD from 2 weeks before mating, during gestation and lactation. Body burdens in the dams were calculated based on the TCDD levels in adipose tissue and liver measured only in three dams per dose group on GD21. However, the measured level confirmed exposure as targeted by the authors.

In this study, male offspring were examined on PND70 and PND170, showing a reduced sperm production. At PND70 the sperm production was 81%, 73% and 67% compared to the controls, at PND170 this was 60%, 54% and 51%. The sperm production on PND170 in the treatment groups was similar to that on PND70, and the further relative decrease at PND170 was primarily due to a higher sperm production in the controls but not in the exposed groups. However, this increase in the control group is expected based on the sexual maturity at PND170.

At both time-points in this study, the sperm production at the lowest dose was close to that in the two other dose groups, and a near maximum response was obtained at the lowest dose. EFSA's Guidance on BMD analysis states that, in cases where all exposure doses show responses significantly different from the control, the available information may not be sufficient for estimating the BMD (EFSA Scientific Committee, 2022). Nevertheless, the resulting BMDL-BMDU interval obtained by the CONTAM Panel for the Faqi et al. (1998a) dataset was not unusually large and met the acceptability criteria contained in the 2022 EFSA BMD Guidance.

In the advanced output available from the software (see Annex G, Section G.2.1) comparison between prior and posterior distributions for each parameter in the model showed peaked posteriors for all parameters, and less difference between prior and posterior for the technical parameter d, which influences the shape and slope of the fitted dose–response models. This indicates that the resulting model fits were driven mainly by the prior for this technical parameter d, which is therefore informative for this dataset. Recent simulation studies that include similar datasets have demonstrated that the default prior used for the technical parameter d produces BMD estimates which meet the acceptability criteria and BMDLs with appropriate coverage, i.e. close to the nominal 95% probability that BMDL<true BMD (Aerts et al., 2022).

The construction of the default prior used for the technical parameter d was inspired by the priors used in the US-EPA Bayesian implementation of BMD analysis. It is biologically appropriate because it closely resembles the distribution of median d estimates obtained for data from more than 3000 NTP datasets analysed by Kremer et al. (2026). Furthermore, the mass of the prior for values below 1 is restricted to around 20%, which avoids estimation of the BMDL being implausibly low or on the boundary set by the optimisation algorithm as occurs when using a frequentist analysis, where prior distributions are not involved (Kremer et al., 2026).

In general, therefore, there are reasons to consider that BMDLs obtained using EFSA's default prior for the technical parameter d are appropriate also for datasets such as that from the Faqi et al. (1998a) study, where the exposed doses are all significantly different from the control.

In the specific case of the Faqi et al. (1998a) study, considering the near-maximal effect at the lowest dose and the width of the dose–response interval for other related effects, the current BMDL seems not very low or overconservative (see

Section 3.1.6.4). In addition, the use of the BMDL from the Faqi et al. (1998a) study is supported by the comparison with the human data (see bullet points below).

The BMDL differed markedly between PND70 and PND170 and when covariate analysis was performed, as shown in Table 41 (see also Annex G). Nevertheless, the BMD analysis provided a BMD and BMDL on both days, and the modelling outcome complied with the criteria for using BMDL as a Reference Point, as set out in the 2022 EFSA BMD guidance (EFSA Scientific Committee, 2022; p. 32). For PND170 the $BMDL_{15}$ was 1.7 ng/kg bw, whereas for PND70, a $BMDL_{15}$ of 9.4 ng/kg bw was derived. When using the PND as covariate, a $BMDL_{15}$ for PND170 of 3.7 ng/kg bw was derived. The BMDL is more than 10-fold lower than the lowest dose tested. However, a factor of 10 or more between doses causing low and maximal effects is seen also for other endpoints, e.g. CYP1 induction (DeVito et al., 1996; Johnson et al., 2020) and effects on male reproductive organs and epidydimal sperm numbers (Mably et al., 1992) (see **Section 3.1.6.4**). The CONTAM Panel selected the BMDL of 1.7 ng/kg bw as the Reference Point for deriving the TWI and took account of these issues when quantifying uncertainty (see below).

TABLE 41 BMDL, BMD and BMDU based on a BMR of 15% for reduced sperm production in male offspring of rats on PND70 or PND170, expressed as the body burden in the dams (ng/kg bw).

PND	BMDL	BMD	BMDU	Co-variate analysis
70	9.4	20.5	32.5	No
170	1.7	9.0	19.3	No
70	6.3	14.9	32.6	Yes
170	3.7	11.0	31.5	Yes

Abbreviations: BMD, benchmark dose; BMDL, benchmark dose lower credible limit; BMDU, benchmark dose upper credible limit; BMR, benchmark response; PND, postnatal day.

Another uncertainty is the expression of the body burden, either per kg bw or per kg body fat. In the dams of the study by Faqi et al. (1998a), the levels in adipose tissue would be higher than those estimated for humans for a similar per kg body weight. This difference in TCDD concentrations in adipose tissue between rats and humans is caused by the much lower body fat content in rats (estimated to be 12%, as compared to 25% in women). In addition, it was shown that a relatively large part of the body burden in rats was present in the liver, due to sequestration, which based on the PBK CADM model is less relevant for humans at this body burden. Arguing that a lipid-based body burden would be more relevant than a body burden per kg body weight would lead to a higher critical lipid-based body burden for the rat than observed in humans, implying that humans are more sensitive than rats based on the three pivotal human studies showing a lower sperm concentration. Therefore, it was assumed that a per kg bw body burden is the best dose metric to be applied.

Regarding the uncertainty of TEFs for human risk assessment, the CONTAM Panel in the 2018 Opinion based the TWI on the Russian Children's Study showing an inverse association between WHO_{2005}-TEQ levels in blood for PCDDs and PCDD/Fs and sperm concentrations. Similar associations were not observed for TEQ levels of PCDFs, DL-PCBs or the sum of PCDD/Fs and DL-PCBs. DL-PCBs contributed largely to the TEQ levels in blood, and in particular PCB-126. However, various studies with human cells showed that the most relevant DL-PCB, PCB-126, in terms of contribution to the TEQ levels showed a much lower relative potency than reflected by its WHO_{2005}-TEF. Larsson et al. (2015) summarised the results from the EU-SYSTEQ project and derived a so-called Consensus Toxicity Factor of 0.003 for PCB-126 based on human in vitro studies, as compared to 0.09 based on rat cells, i.e. a factor 30 lower. The outcome of the study with Russian boys thus seemed to confirm that this much lower potency of PCB-126, and as a result the DL-PCB-TEQ, also applies to the reduced sperm concentrations in humans. No other human studies were identified that could support or contradict this conclusion. The studies by Mocarelli et al. (2008, 2011) focused on TCDD only. Based on these considerations, the EFSA CONTAM Panel (2018) ignored the poor association with DL-PCBs and Total-TEQ when setting the TWI based on the Russian Children's Study and recommended to reevaluate the TEFs. This was performed in 2022 in a WHO workshop (DeVito et al., 2024). In vitro studies, including those with human cells, were included in the assessment but much more weight was given to in vivo studies in rodents and to toxicological endpoints. It was decided not to base the TEFs solely on biochemical effects in human cells following, e.g. a parallelogram approach (i.e. comparison of results from in vitro and in vivo studies in rats, followed by comparison of in vitro studies in rats and humans). As a result, the outcome of studies with rats dominated the TEF for PCB-126, which was only lowered by a factor 2 (from 0.1 to 0.05). Overall, TEQ levels based on the WHO_{2022}-TEFs became lower in food and in human milk (see **Sections 3.3.1** and **3.1.1.2**) but PCB-126 is still the most contributing congener with respect to dietary exposure or levels in human milk. This relatively small decrease is also caused by a change in the WHO_{2022}-TEFs of several other relevant PCDD/Fs. The possibility that the new WHO_{2022}-TEFs do not reflect the toxic potencies for humans adds a considerable uncertainty to the risk assessment, including the evaluation of associations between levels and effects in human studies.

The CONTAM Panel quantified the combined impact of all the identified uncertainties collectively, except for the uncertainty regarding the TEFs, which was considered in a subsequent step (see below). It was found sufficient to do this in a single step by expert group judgement rather than more formal methods of expert knowledge elicitation (Section 12.6 of EFSA, 2018),[16] and without first quantifying uncertainty for different parts of the assessment separately (Section 3.2 of EFSA, 2018). The resulting probabilities are reported in the conclusions as % certainty for the more probable outcome in each case, following EFSA's guidance on communication of uncertainty (EFSA, 2019).

The expert group judgement quantified overall uncertainty by addressing the following question:

> What is your % probability that, if all non-standard uncertainties affecting the hazard characterisation were resolved, the TWI that would be derived from an appropriate rodent study would be equal to or higher than 0.6 pg WHO_{2022}-TEQ/kg bw per week?

The consensus probability of the expert group was about 95% (between 93% and 97%). The main points of the reasoning for this consensus probability were as follows:

- the effect on sperm production was observed for a sensitive rat strain.
- there is strong evidence that human cells and mice expressing the human AHR are less sensitive to TCDD, suggesting that a UF smaller than 1 should be applied to derive the TWI; at least it can be concluded that humans are not more sensitive than rats, which is the basis for not using the UFs for intra- and interspecies variability in toxicodynamics.
- on the other hand, the three human studies show a reduced sperm concentration at a (potential) body burdens similar to the one derived for rats in the study by Faqi et al. (1998a), suggesting that humans are not less sensitive than rodents, but similar in sensitivity towards this endpoint.
- a potential explanation could be that the true BMD from the rat study would even be lower, considering that a near-maximal response was observed at the lowest dose in the critical rat study, and also considering that for other endpoints the full dose–response curve covers a wide dose-range (CYP1 induction and effects on male reproductive organs and sperm production).

Although there is uncertainty regarding the WHO_{2022}-TEFs for DL-PCBs, due to the much lower potency of most of the DL-PCBs in human cells, this was excluded from considerations and judgement above because the Terms of Reference requested that this Opinion should apply the WHO_{2022}-TEFs. However, in a separate judgement it was considered that this additional uncertainty would not alter the assessed probability reported above. This is because the TWI was based on the critical study for TCDD, and WHO_{2022}-TEFs for DL-PCBs were involved only in the analysis of the Russian Children's Study (Mínguez-Alarcón et al., 2017), which comprised only part of the supporting evidence that was considered when deriving the TWI.

3.5.1.3 | *Uncertainty assessment for the human dietary exposure assessment*

The non-standard sources of uncertainty affecting the hazard assessment were identified in Table 40. The most important non-standard source of uncertainty affecting the human dietary exposure assessment was estimated to be the one linked to the analytical method sensitivity, and thus to the left-censored data. This uncertainty was handled by the substitution method (WHO/IPCS, 2009, updated in 2020) and it was contained by applying the performance criteria established in the Legislation. Results are expressed as LB and UB estimates, where for the maximum mean exposures, LB estimates are 25%–36% lower than UB estimates (i.e. UB estimates up to 57% higher than LB estimates) (see Table 30).

The exposure assessment was repeated for alternative scenarios (a form of sensitivity analysis) to investigate the potential impact of other uncertainties that were rated as medium in Table 40.

A scenario was performed to investigate the combined impact of uncertainties linked to the contribution of food of plant origin, including uncertainties associated with washing and peeling of vegetables and extrapolations between food categories that involved data from curly kale. This was done by excluding all food of plant origin (with the exception of vegetable fat). The maximum mean LBs of this scenario are 8%–27% lower than the LBs estimated by the Opinion scenario (scenario S2 in Table 42). However, this scenario should be considered an underestimation of the exposure linked to food of plant origin as food of plant origin does contribute to the exposure based on the available occurrence data (see Annex B).

The uncertainty linked to the inclusion of two bonito samples with outlier concentrations after data provider confirmation, was estimated to have a low impact on most population groups besides Toddlers (21% lower mean LB exposure when the bonito samples were excluded) and lactating women (54.5% lower mean LB exposure when the bonito samples were excluded). The greater impact on the lactating women is due to the reporting of bonito consumption by two individuals among the small number of participants ($n = 65$) in one of the two surveys available (scenario S3 in Table 42).

It is well-known that fish from certain areas may contain relatively high levels of PCDD/Fs and DL-PCBs. This applies to eel from contaminated rivers and lakes, but also to various fatty fish species from, e.g. the Baltic Sea. When included in the

[16] However, this and subsequent judgements in the uncertainty analysis met the minimal requirements for a semi-formal elicitation procedure (Annex B.8 in EFSA, 2018), including a predefined question and expert group (the Dioxins Working Group) who received basic training and were asked to consider and express their judgements individually before developing a consensus through a group discussion, supported by an experienced facilitator.

occurrence data this may result in an overestimation of the exposure from such fish in areas where they are not consumed, and vice versa. Fish from the Baltic Sea should be monitored to a higher extent (as described in **Section 1.3.3**) and there may be a bias towards relatively high levels in the data submitted to EFSA. However, when compliant with the MLs (including measurement certainty), this fish can be put on the EU-market and were therefore not excluded from the database. Excluding samples for which the fishing area indicated a Baltic origin had low impact as it decreased the maximum mean LB exposure estimates less than 8% across age groups (scenario S4 in Table 42).

A further scenario was run to assess the combined impact of several of the more important uncertainties, in particular excluding the two outlier bonito samples, excluding kale samples from the calculation of higher level means for vegetables, excluding samples reported as concerning fish fished in the Baltic region and using samples referring to fat fresh tissues in the estimates of the concentration in fresh meat resulted in maximum mean LB exposure estimates up to 22% lower across age groups (scenario S5 in Table 42). Excluding also all food of plant origin from the previous scenario, the maximum mean LB exposure estimates would be up to 34% lower across age groups (scenario S6 in Table 42).

TABLE 42 Comparison of the maximum Mean LB dietary exposure (pg WHO_{2022}-TEQ/kg bw per day) between the Opinion scenario and the five uncertainty scenarios across age groups.

Age group	N surveys	S1	S2	S3	S4	S5	S6	S2/S1	S3/S1	S4/S1	S5/S1	S6/S1
Infants	14	1.19	0.89	1.12	1.11	1.06	0.81	74%	94%	93%	89%	68%
Toddlers	17	1.57	1.15	1.24	1.44	1.22	1.03	73%	79%	92%	78%	66%
Other children	21	1.11	0.86	1.05	1.04	1.04	0.85	77%	95%	94%	94%	76%
Adolescents	23	0.66	0.56	0.62	0.63	0.60	0.51	84%	94%	95%	90%	77%
Adults	23	0.56	0.51	0.56	0.53	0.52	0.48	91%	100%	94%	93%	85%
Elderly	21	0.68	0.63	0.68	0.64	0.64	0.58	92%	99%	94%	93%	85%
Very elderly	16	0.63	0.57	0.63	0.59	0.59	0.53	91%	100%	94%	93%	84%

S1: Opinion scenario.
S2: scenario that excluded food of plant origin.
S3: scenario that excluded 2 samples of bonito with outlier concentration values.
S4: scenario that excluded fish reported as fished in the Baltic region.
S5: scenario that excluded the two outlier bonito samples, kale samples from the calculation of higher level means for vegetables, samples reported as concerning fish fished in the Baltic region and using samples referring to fat fresh tissues in the estimation of the concentration in fresh meat.
S6: Scenarios as S5 but that also excluded all food of plant origin.

The results from the alternative scenarios reported above as well as data on the occurrence of PCDD/Fs and DL-PCBs in fruit and vegetables sampled in Norway (the Norwegian Food Safety Authority, 2025, see Documentation provided to EFSA) were used to inform quantification of overall uncertainty for the exposure assessment. It was found to be sufficient to do this by expert group discussion rather than formal or semi-formal expert knowledge elicitation (Section 12.6 of EFSA, 2018). The resulting probabilities are reported in the conclusions as % certainty for the more probable outcome in each case, following EFSA's guidance on communication of uncertainty (EFSA, 2019).

The expert group judgement quantified overall uncertainty by addressing the following question:

> What is your % probability that if all non-standard uncertainties affecting the exposure assessment were resolved, the resulting mean exposure of adults would be higher than the TWI of 0.6 pg WHO_{2022}-TEQ/kg bw per week in the survey where the true mean is the lowest?

The question was framed in this way to focus on the most relevant age group (adults) and provide an assessment of the probability that their true mean exposure would be above the TWI for all the surveys considered. The TWI was treated as a fixed value for this question, considering only uncertainties in the exposure assessment. This is in line with the current approach to chemical risk assessment, which deals with uncertainty in the hazard characterisation when setting an HBGV and then treats this as a fixed value for the purposes of risk characterisation[17] and risk management.

The consensus probability of the expert group for this question was 99%–100%. The reasoning for this was as follows:

- when all the scenarios considered in the sensitivity analyses reported above were combined, the resulting estimate for the lowest mean LB exposure for adults was 1.29 pg. WHO_{2022}-TEQ/kg bw per week (Table 43),
- considering that this value combined best-case assumptions for most of the larger uncertainties, it was judged to be an underestimation of what the overall exposure would be if all the non-standard uncertainties were resolved.

Uncertainty regarding the WHO_{2022}-TEFs for DL-PCBs was excluded from the considerations and judgement above because the Terms of Reference requested that this Opinion should apply the WHO_{2022}-TEFs. However, to provide an indication

[17]Exposure uncertainty is taken into account when performing risk characterisation, e.g. by consideration of LB and UB estimates and, when helpful, special exposure scenarios (as in the present Opinion). Hazard uncertainties are not normally reconsidered at this stage. For example, when additional uncertainty factors are included in the derivation of the HBGV, the risk characterisation is not repeated with and without those factors.

of the potential impact of this additional uncertainty on the exposure assessment, a separate judgement was performed. This addressed the same question as specified above, but took into account the additional uncertainty regarding the TEFs for DL-PCBs as well as those considered in the judgement above. It was judged that this would reduce the probability reported above to 90–95%. The reasoning for this was based primarily on the observation that, when considered together, the DL-PCBs contribute slightly less than half the total exposure (see **Section 3.3.1.3**), and therefore the exposure estimates would be reduced by approximately 50% in a scenario where their TEFs were reduced to zero (this is an extreme scenario: in practice the reduction would be smaller). As noted above, the lowest mean LB exposure for adults combining best-case assumptions for the most important exposure uncertainties was 1.29 pg. WHO_{2022}-TEQ/kg bw per week (see Table 43). A reduction of 50%, to take into account the uncertainty regarding the DL-PCB TEFs, would bring that exposure estimate closer to, but still slightly above, the TWI of 0.6 pg. WHO_{2022}-TEQ/kg bw per week.

TABLE 43 Minimum Mean LB dietary exposure (pg WHO_{2022}-TEQ/kg bw per week) of the Opinion scenario and the five uncertainty scenarios across age groups.

Age group	N surveys	S1	S2	S3	S4	S5	S6
Infants	14	1.80	0.85	1.66	1.64	1.60	0.80
Toddlers	17	4.33	3.19	4.14	4.11	4.08	3.12
Other children	21	3.59	2.62	3.33	3.32	3.28	2.55
Adolescents	23	2.03	1.36	1.93	1.92	1.88	1.31
Adults	23	1.87	1.34	1.70	1.69	1.66	1.29
Elderly	21	1.77	1.23	1.60	1.60	1.57	1.12
Very elderly	16	1.69	1.15	1.51	1.52	1.49	1.13

S1: Opinion scenario.

S2: Scenario that excluded food of plant origin.

S3: Scenario that excluded 2 samples of bonito with outlier concentration values.

S4: Scenario that excluded fish reported as fished in the Baltic region.

S5: Scenario that excluded the two outlier bonito samples, kale samples from the calculation of higher level means for vegetables, samples reported as concerning fish fished in the Baltic region and using samples referring to fat fresh tissues in the estimation of the concentration in fresh meat.

S6: Scenarios as S5 but that also excluded all food of plant origin.

3.5.1.4 | *Uncertainty assessment for the risk characterisation*

The impact of uncertainties affecting comparison of the human dietary exposure estimates with the TWI was quantified by the uncertainty analysis for the exposure assessment, since this was expressed in terms of probability that mean adult exposures exceed the TWI for all the surveys considered (93%–97% probability, see preceding section).

The current assessment also included a risk characterisation based on levels in human milk (**Section 3.4.2**). The available data showed that average levels in human milk were close to the highest human milk level not leading to adverse effects in sons, and, at the higher end of the reported range, exceeded the critical value by a factor 2.6. The CONTAM Panel noted that this exceedance is lower than that of the TWI based on the estimated dietary exposures. It is uncertain whether the discrepancy is due to: (i) uncertainties associated with the toxicokinetic model applied to link the body burden to a chronic exposure, including the assumption that other PCDD/F and DL-PCB congeners behave similar to TCDD, or (ii) an overestimation of the current dietary exposure, (iii) the human milk samples covering only a few countries, or a combination of these.

Considering the first option, Table 44 compares the estimated contribution of the most relevant PCDD/Fs and DL-PCBs to the current exposure from food (see also Figure 15) and those in human milk from the UNEP and CVUA data sets described in **Section 3.1.1.1**. The data suggest a clear shift in the relative contribution, most likely due to differences in toxicokinetics. This is also reflected in differences in the half-lives for these congeners, PCB-126 showing, e.g. a much shorter half-life than TCDD (see Table 3). TCDF, for example, contributes very little in human milk, as also shown in certain animal species like pigs and cows (see **Section 3.1.1.4**). As a result of the kinetic differences, the relative contribution of TCDD but also of PeCDD to the TEQ levels in human milk is more than two-fold higher, whereas that of the two DL-PCBs (PCB-126 and -118) is lower than the ones estimated for dietary exposure.

Using the PBK CADM model, the median LB exposure estimate for adults of 2.5 pg. WHO_{2022}-TEQ/kg bw per week would result in a level in human milk of 8.2 pg. WHO_{2022}-TEQ/g fat, i.e. a 3- to 3.5-fold higher level than observed in the studies by UNEP and CVUA (see Table 43). Based on the contribution of TCDD to the food exposure of 5.3%, this implies a mean exposure to TCDD of 0.13 pg/kg bw per week. Again, using the PBK CADM model, such an exposure would lead to TCDD human milk levels of 0.50 ng/kg fat. In the two data sets on human milk, TCDD contributed on average 13% and 11% to the levels, i.e. on average 0.35 and 0.25 ng/kg fat. The predicted levels based on the estimated exposure are 143 and 201% of the measured levels. A similar calculation for PCB-126-TEQ would result in an estimated milk level of 3.3 ng WHO_{2022}-TEQ/kg fat, as compared to 0.6 ng WHO_{2022}-TEQ/kg fat for both human milk data sets, i.e. a 5-fold overestimation based on exposure. Table 44 shows similar results as for TCDD for PeCDD, 1,2,3,6,7,8-HxCDD and 2,3,4,7,8-PeCDF, whereas TCDF, 1,2,3,4,7,8-HxCDF and PCB-118 show the much higher overestimation similar to PCB-126.

This suggests that the much larger difference between the dietary estimated exposure for PCDD/Fs and DL-PCBs and the observed human milk levels is at least partly caused by differences in toxicokinetics that are not taken into account in the applied model. Differences in half-lives in humans are unlikely to be considered by the WHO-TEFs, which are primarily based on data from rodents. It should be stressed that calculations are based on LB estimates of the dietary exposure and would even be 1.5-fold larger when using UB estimates.

TABLE 44 Relative contribution (%) of the most relevant PCDD/Fs and DL-PCBs to the Total-WHO$_{2022}$-TEQ levels, as estimated for the mean dietary exposure of adults and the two data sets on human milk levels, the corresponding average exposure and human milk levels to the congeners, and the comparison of the estimated human milk levels based on the exposure using the PBK CADM model with the measured levels. In addition, Total-TEQ based on all congeners is shown for comparison.

Congener	Contribution to dietary exposure (%)	Dietary exposure (pg TEQ/kg bw per week)	Predicted human milk level (ng TEQ/kg fat)	Contribution to predicted human milk level (%)		Average measured congener level (ng TEQ/kg fat)		Fold-overestimation of the predicted level	
				CVUA	UNEP	CVUA	UNEP	CVUA	UNEP
TCDD	5.3	0.13	0.50	10.7	12.8	0.25	0.35	2.0	1.4
PeCDD	4.8	0.12	0.45	14.1	14.0	0.32	0.38	1.4	1.2
1,2,3,6,7,8-HxCDD	1.6	0.04	0.16	5.1	8.3	0.12	0.22	1.4	0.7
HpCDD	9.7	0.24	0.89	4.5	8.0	0.10	0.22	8.6	4.1
TCDF	4.8	0.12	0.45	0.5	0.5	0.01	0.01	39	33
2,3,4,7,8-PeCDF	5.0	0.13	0.47	10.7	9.1	0.25	0.25	1.9	1.9
1,2,3,4,7,8-HxCDF	9.1	0.23	0.84	10.4	8.0	0.24	0.22	3.5	3.9
PCB-126	37.6	0.94	3.30	26.2	22.7	0.60	0.61	5.5	5.4
PCB-118	6.7	0.17	0.62	2.8	3.1	0.06	0.08	9.6	7.4
Total-TEQ	100	2.50	8.18	100	100	2.3	2.7	3.6	3.0

The discrepancy between the exceedances obtained for dietary exposure and those based on human milk levels represents a high impact uncertainty for both approaches to risk characterisation, and for the overall conclusion on risk characterisation. The overall impact of this uncertainty on the conclusions was quantified by expert group judgement, using the same methodology as for the TWI and exposure assessment (see above), by addressing the following question:

> What is your % probability that, if all the non-standard uncertainties affecting the assessments based on dietary exposure and human milk levels were resolved, there would be a health concern related to the mean exposure and human milk levels of women of child-bearing age in the EU population?

This question was framed to refer to a health concern (exceedance of the TWI or the resulting level in human milk) for either dietary exposure or human milk levels (respectively) or both, and to the mean of the EU population as a whole, rather than the population with the lowest exposure (because the dietary exposure estimates and human milk level data refer to different subsets of EU member states). The experts were asked to take account of this when making their judgements.

The consensus probability of the expert group was 80%–90%. This reflects the experts' assessment of two main considerations. First, the relative reliability of the dietary exposure estimates, which indicate an exceedance for all the surveys considered in 23 different EU Member States, but are affected by the modelling issues discussed above. And second, the data on human milk levels, which are not far above the body burden in women, i.e. future mothers, of 2.2 ng WHO$_{2022}$-TEQ/kg fat that would result from chronic exposure at the TWI, but are available for only 5 Member States (see **Section 3.1.1.2**).

Uncertainty regarding the WHO$_{2022}$-TEFs for DL-PCBs was excluded from the considerations and judgement above because the Terms of Reference requested that this Opinion should apply the WHO$_{2022}$-TEFs. However, to provide an indication of the potential impact of this additional uncertainty on the exposure assessment, a separate judgement was performed. This addressed the same question as specified above, but took into account the additional uncertainty regarding the TEFs for DL-PCBs as well as those considered in the judgement above. It was judged that this would reduce the probability reported above to 33%–66%, taking into account that, when considered together, the DL-PCBs contribute slightly less than half the total dietary exposure and about one third of the total levels in human milk (Table 44). It was therefore considered that the exposures and human milk levels would be reduced by proportions close to these contributions if the uncertainties affecting the DL-PCB TEFs were resolved.

In view of these lower probabilities for exceedance at the population mean when taking into account the human milk level data and the uncertainties affecting the DL-PCB TEFs, compared to the assessment based on the dietary exposure estimates alone (see preceding section), the uncertainty analysis also considered the P95 of the EU population. This was done by addressing the following question:

What is your % probability that, if all the non-standard uncertainties affecting the assessments based on dietary exposure and human milk levels were resolved, there would be a health concern related to the P95 exposure and human milk levels of women of childbearing age in the EU population?

The consensus probability of the expert group for this question was 95%–99%. When making their judgements, the experts considered:

- the P95 estimates for dietary exposure in **Tables 38** and **42** (including the scenario combining multiple best-case assumptions) and the modelling issues affecting them (discussed above),
- the range of human milk levels reported for the one EU member state for which individual data were available (CVUA, range 0.5 to 6 ng WHO_{2022}-TEQ/kg fat), and how these might relate to the P95 for the whole EU population, taking into account that the sample size was small (34 women in the period 2021–2024) and that other Member States have higher fish consumption.

Uncertainty regarding the WHO_{2022}-TEFs for DL-PCBs was excluded from the considerations and judgement above because the Terms of Reference requested that this Opinion should apply the WHO_{2022}-TEFs. As an additional step, the experts judged that taking into account also the uncertainty regarding the TEFs for DL-PCBs and their contributions to the dietary exposures and human milk levels (Table 43) would reduce the probability reported above to 70%–80%.

3.5.2 | Identification of sources of uncertainty in the exposure assessment of food-producing animals

The following main uncertainties were identified linked to the occurrence data in feed used to estimate the exposure of food-producing animals:

- Occurrence data were insufficient for several feed materials, including wheat, oat and their derived products, hay and grass, peas and beans, as well as various land-animal products. This lack of data prevented their use in model diet exposure scenarios. Consequently, substitutions with other feed materials were made where nutritionally appropriate. This could potentially lead to under- or overestimation of PCDD/F and DL-PCB levels in model diets.
- Species-specific compound feed data were not available in sufficient quantity for cattle for fattening, dairy cows, and veal calves (receiving milk replacer). As a result, the compound feed exposure scenario could not be carried out for these food-producing animals.
- The number of samples was low for certain feed materials and compound feeds, which can lead to an under- or overestimation of the concentrations of PCDD/Fs and DL-PCBs. For example, fewer than 11 samples were available for barley, dried (sugar) beet pulp, lucerne ad hoc group, (sugar) cane molasses, hydrolysed fish protein and complete feed for turkeys, preventing reliable calculation of the 75th percentile.
- Approximately 55% of samples were collected in one country, creating a geographical imbalance that may bias exposure estimates (under- or overestimation).
- Some EU Member States did not submit occurrence data, reducing the overall representativeness of the dataset and potentially leading to under- or over-estimation of the levels of PCDD/Fs and DL-PCBs in the samples.
- The amount of left-censored data was high for some PCDD/F and DL-PCB congeners. When using the LB approach, levels in the feed samples might have been underestimated while they have been overestimated at the UB approach. At mean occurrence level, UB estimates were up to 13-fold higher than LB estimates in feed materials and compound feeds used for exposure assessment.

Regarding the **exposure estimates:**

- The exposure estimates were affected by all the uncertainties affecting the occurrence data, including the lack of data for certain feed materials (see above).
- The exposure was calculated using model diets, feed intake and default body weights, which are considered as a standardised procedure for the purpose of uncertainty analysis. The methodology could under- or overestimate the actual exposure, nevertheless the approach is aimed at providing a conservative estimate. This, together with the use of different exposure scenarios (e.g. compound feeds) tends towards reduction of uncertainty.
- Soil and clay ingestion was not taken into account.

As no risk characterisation was required in the Terms of Reference for food-producing animals and the exposure estimates are not expected to be used in risk management, the combined impact of the uncertainties was not quantified.

4 | CONCLUSIONS

In this Opinion, the CONTAM Panel re-assessed the risk related to the presence of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in food and feed on the basis of the new World Health Organisation Toxic Equivalency Factors (WHO$_{2022}$-TEF) values. The update of the scientific Opinion related to all aspects of the risk assessment where the new WHO$_{2022}$-TEFs would have an impact, as specified in the Interpretation of the Terms of Reference. The update considered the new scientific developments and information available since the previous EFSA Opinion (referred to as the 2018 Opinion) (EFSA CONTAM Panel, 2018).

4.1 | Hazard identification and characterisation

4.1.1 | Toxicokinetics

- No new studies were identified that add essential information not already provided in the 2018 Opinion.

4.1.2 | Levels in human milk

- Based on pooled human milk samples from the WHO/United Nations Environment Programme (UNEP) monitoring program and individual human milk samples from Germany, the application of the new WHO$_{2022}$-TEFs resulted in consistently lower Total-toxic equivalents (TEQ) concentrations (average decrease of 40%) compared to the Total-TEQ concentrations based on the WHO$_{2005}$-TEFs.

4.1.3 | Transfer from feed to food of animal origin

- New studies on the transfer of PCDD/Fs and DL-PCBs in dairy cows, laying hens and pigs contributed to a better understanding of the transfer to milk and eggs, and accumulation in liver, fat and meat of food-producing animals. The change in TEFs does not affect the transfer rates of individual congeners but the CONTAM Panel noted that it could impact the transfer rates when using Total-TEQ levels.
- The new data did not provide consistent information on the ratio of fat-based levels in meat and adipose tissue.

4.1.4 | Toxicity in experimental animals

- Since the 2018 Opinion, the new eligible studies reported effects that were considered not to be adverse and/or not to be suitable to derive a Reference Point.
- As was the case in the 2018 Opinion, a decrease in sperm production in male offspring of rats upon exposure to TCDD via the dams was the most sensitive endpoint.

4.1.5 | Observations in humans

- Since the 2018 Opinion, no new prospective developmental male reproductive studies were identified. The conclusions from the 2018 Opinion that impaired semen quality is likely to be a causal effect of exposure to PCDD/Fs was still considered valid. Regarding DL-PCBs there was no conclusive evidence based on human studies.
- For all other assessed endpoints, the newly available evidence did also not change the previous conclusions.

4.1.6 | Mode of action of male reproductive effects

- The adverse effects on male reproductive development upon maternal exposure to TCDD, were predominantly, if not exclusively, dependent on activation of the aryl hydrocarbon receptor (AHR). Both canonical and non-canonical pathways may be involved.
- The most plausible target causing effects on male reproductive development is the hypothalamic–pituitary-gonadal axis.

4.1.7 | Critical effect and derivation of the tolerable weekly intake (TWI)

- The critical endpoint was identified by integrating evidence from both human and experimental animal lines of evidence considering the respective level of confidence and according to a weight of evidence approach.

- Developmental effects of TCDD on the male reproductive system occurred at the lowest exposure in experimental animals compared to other effects, and were therefore considered as critical. Decreased sperm production in male offspring was the most sensitive among the endpoints assessed in experimental animals.
- Consistent with this, lower sperm concentrations were observed in three different human cohorts with men exposed at young age:

  – Two Seveso studies showed a statistically significant inverse association between serum levels of TCDD and sperm concentrations, TCDD being the only congener measured in these studies.
  – The Russian Children's Study showed a statistically significant inverse association between serum levels of TCDD, PCDD-TEQ, PCDD/F-TEQ and sperm concentrations, but not for PCDF-TEQ, DL-PCB-TEQ or Total-TEQ, expressed using the WHO_{2022}-TEFs.

- Considering that the WHO_{2022}-TEFs should be applied in the current risk assessment, with all 29 congeners included, the studies in humans could no longer serve as the primary basis for establishing the tolerable weekly intake (TWI). Instead, it was decided to use the study in rats as the critical study, while the human studies were used as supporting evidence in the weight of evidence approach, including the decision on which uncertainty factors should be applied in the derivation of the TWI.
- A maternal body burden of 1.7 ng TCDD/kg bw was identified as Reference Point from the critical study in rats, based on a benchmark dose lower credible limit (BMDL) using a benchmark response (BMR) of 15% decreased sperm production.
- Although the Reference Point is based on findings on TCDD, the CONTAM Panel concluded that it should also apply to the sum of PCDD/Fs and DL-PCBs based on the TEQ concept.
- The default uncertainty factor for interspecies toxicokinetic variability was not applied because the body burden was the starting point of the assessment. The default uncertainty factors for inter- and intraspecies variability in toxicodynamics were not applied based on evidence demonstrating that humans are not more sensitive than rats.
- To account for the possible variability in the kinetics in women of childbearing age and in their infants, the default uncertainty factor of 3.16 for intraspecies variability in toxicokinetics was applied.
- The Reference Point of 1.7 ng WHO_{2022}-TEQ/kg bw corresponded to a lipid-based level of 6.8 ng WHO_{2022}-TEQ/kg body fat (based on a 25% body fat). Using a physiologically based kinetic (PBK) model, this lipid-based level would be reached after chronic exposure to 0.29 pg. WHO_{2022}-TEQ/kg bw per day. Applying the default uncertainty factor of 3.16 for intraspecies variability in toxicokinetics, this resulted in a daily intake of 0.09 pg. WHO_{2022}-TEQ/kg bw per day, i.e. 0.63 pg. WHO_{2022}-TEQ/kg bw per week.
- On this basis, a TWI of 0.6 pg. WHO_{2022}-TEQ/kg bw per week was established for the sum of PCDD/Fs and DL-PCBs.
- PBK modelling of chronic exposure to PCDD/Fs and DL-PCBs at the TWI resulted in a body burden in women at age 35 of 2.2 ng WHO_{2022}-TEQ/kg fat. This is the highest body burden in women not raising a health concern for their sons. The level in human milk would be similar to the body burden.
- The TWI is considered protective for the general population and all endpoints. It prevents women of childbearing age from reaching body burdens (i.e. levels in their bodies) that could lead to in utero and lactational exposures associated with health concerns in the offspring.

4.2 | Occurrence in food and human dietary exposure assessment of the European population

- When applying the WHO_{2022}-TEFs, the following dietary exposure estimates for the 29 PCDD/Fs and DL-PCBs across surveys were obtained (expressed on a daily basis):

  – The mean lower bound (LB) dietary exposure ranged from 0.24 pg. WHO_{2022}-TEQ/kg bw per day in 'Very elderly', to 1.57 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers'.
  – The mean upper bound (UB) dietary exposure ranged from 0.41 pg. WHO_{2022}-TEQ/kg bw per day in 'Very elderly', to 2.35 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers'.
  – The P95 LB dietary exposure ranged from 0.57 pg. WHO_{2022}-TEQ/kg bw per day in 'Elderly' and 'Very elderly', to 3.30 pg. WHO_{2022}-TEQ/kg bw per day in 'Toddlers'.
  – The P95 UB dietary exposure ranged from 0.86 pg. WHO_{2022}-TEQ/kg bw per day in 'Elderly, to 4.72 pg WHO_{2022}-TEQ/kg bw per day in 'Toddlers'.

- The exposure estimated with the WHO_{2022}-TEFs is between 27% and 35% lower than that obtained using WHO_{2005}-TEFs across age groups. This is based on the median ratios between the mean LB dietary exposure estimates.
- The non-*ortho* DL-PCBs showed the highest contribution (41%) to the total WHO_{2022}-TEQ exposure, followed by PCDFs (27%), PCDDs (24%) and mono-*ortho* PCBs (9%).
- Regarding individual congeners, PCB-126 contributed most to the exposure to Total-TEQ (38%), followed by HpCDD (10%), 1,2,3,4,7,8-HxCDF (9%) and PCB-118 (7%). TCDD, PeCDD, TCDF and 2,3,4,7,8-PeCDF each contributed 5%.
- 'Milk and dairy products' and 'Fish and fish products' are the food categories that contributed more than 10% to the total

mean LB dietary exposure in the highest number of surveys with percentage up to 43% in 'Other children' for 'Milk and milk products' and up to 67% in the 'Elderly' for 'Fish and fish products'. 'Vegetables and vegetable products' and 'Meat and meat products' also contributed more than 10% to the total mean LB dietary exposure in a large number of surveys.

4.3 | Occurrence data in feed and dietary exposure assessment in food-producing animals

- The estimated mean concentrations of the 29 PCDD/Fs and DL-PCBs were expressed as pg. WHO_{2022}-TEQ/kg feed dry matter (DM), rather than ng WHO_{2022}-TEQ/kg 88% DM, in the daily diets of food-producing animals.
  - Based on a model diet exposure scenario, the LB–UB ranges were as follows expressed as pg. WHO_{2022}-TEQ/kg feed DM): piglets (20–51), pigs for fattening (20–53), lactating sows (23–55), chickens for fattening (27–62), laying hens (22–56), turkeys for fattening (27–67), cattle for fattening (24–68), dairy cows (24–59), lambs for fattening (24–67), salmonids (215–264), rabbits for fattening (22–76), and horses (34–107).
  - Based on a compound feed exposure scenario, the estimates were: piglets (14–41), pigs for fattening (11–36), lactating sows (22–38), chickens for fattening (17–43), laying hens (12–41), turkeys for fattening (42–68), salmonids (284–318), rabbits for fattening (31–58), and horses (35–115).
- Ratios of WHO_{2022}-TEQ vs. WHO_{2005}-TEQ concentration estimates for the 29 PCDD/Fs and DL-PCBs ranged from 0.8 to 1.2 for most food-producing animal species and categories considered. The variation in this ratio is largely driven by the congener pattern in the feed material or compound feed.
- The updated occurrence data and methodological refinements in the current Opinion resulted in generally lower or comparable estimated concentrations of the 29 PCDD/Fs and DL-PCBs in the daily diets of food-producing animals compared to the 2018 Opinion based on WHO_{2005}-TEFs.

4.4 | Risk characterisation

- The TWI is not applicable for infants, and it is not appropriate to compare it to the exposure of infants.
- When comparing the mean current dietary exposure to the 29 PCDD/Fs and DL-PCBs expressed on a weekly basis of 'Adolescents', 'Adults', 'Elderly' and 'Very Elderly' using the WHO_{2022}-TEQs, a 3- to 12-fold exceedance of the TWI is observed (lowest LB-highest UB). At the P95, this ranged from 6- to 30-fold (lowest LB-highest UB).
- For 'Toddlers' and 'Other Children', the mean dietary exposure exceeded the TWI 6- to 27-fold (lowest LB-highest UB). At the P95 this ranged from 11- to 55-fold (lowest LB-highest UB).
- The CONTAM Panel noted that for 'Toddlers' and 'Other Children', the observed exceedance of the TWI raises a health concern for developmental effects.
- For adult age groups, effects other than developmental endpoints might occur at body burdens higher than those presently observed in Europe. However, the available data did not allow to estimate in a quantitative manner at which exposure levels there is a health concern for such effects, noting that at the current exceedance of the TWI there is no evidence for such effects.
- Current levels of PCDD/Fs and DL-PCBs in human milk were compared to the level of 2.2 ng WHO_{2022}-TEQ/kg fat, estimated to result from chronic exposure at the TWI using PBK modelling. Two data sets on human milk levels showed that current levels of PCDD/Fs and DL-PCBs are on average within a factor 1.2 of this level. This exceedance is lower than the 3- to 12-fold exceedance of the TWI by 'Adolescents', 'Adults', 'Elderly' and 'Very Elderly' based on the estimated dietary exposures.

4.5 | Uncertainty analysis

- The human risk assessment was affected by a number of substantial uncertainties, including the derivation of the Reference Point from the critical study, the absorption/distribution/metabolism/excretion (ADME) and the toxicokinetic model, the relevance for humans of the WHO_{2022}-TEFs for DL-PCBs, the derivation of the TWI including the applicable uncertainty factors, left-censored occurrence data and occurrence in foods of plant origin.
- The CONTAM Panel concluded with about 95% certainty[18] that, if the uncertainties were resolved, the TWI would be equal to or higher than the assessed value of 0.6 pg. WHO_{2022}-TEQ/kg bw per week.
- The CONTAM Panel concluded with 99%–100% certainty that, if uncertainties affecting the exposure assessment were resolved, the mean exposure of adults would be higher than the TWI of 0.6 pg. WHO_{2022}-TEQ/kg bw per week for all the dietary surveys that were considered.

[18]The impact of uncertainties on each part of the risk assessment was quantified using % probabilities assessed by expert judgement, following EFSA's guidance on uncertainty analysis (EFSA Scientific Committee, 2018a). When reporting conclusions, the resulting probabilities are expressed as % certainty for the more probable outcome, following EFSA's guidance on communication of uncertainty (EFSA, 2019).

- Taking into account the discrepancy between the exceedances of the TWI obtained for dietary exposure estimates and measured human milk levels, the CONTAM Panel judged with 80%–90% certainty that mean exposure would still raise a health concern based on the WHO_{2022}-TEFs. For P95 exposure, the level of certainty for a health concern was 95%–99%. When uncertainties related to the WHO_{2022}-TEFs for DL-PCBs in humans were also taken into account, the estimated probability for a health concern at the mean and P95 reduced to 33%–66% (about as likely as not) and to 70%–80%, respectively.
- The uncertainties affecting the occurrence data in feed and the exposure assessment of food-producing animals were identified. The methodology for the exposure assessment aimed at providing a conservative estimate, and the use of different exposure scenarios tends towards reduction of uncertainty.

5 | RECOMMENDATIONS

To reduce the uncertainties in the human risk assessment, the CONTAM Panel recommends that,

- Further development of approaches to compare animal- and human-based data is needed to derive TEFs for PCDD/Fs and DL-PCBs that are more relevant for humans. This is also important for evaluating associations between TEQ levels and specific effects in human studies.
- To better estimate the actual contribution of plant-derived products to the dietary exposure, more data on occurrence levels in such foods are needed.
- Further improvement of toxicokinetic models is needed, including parameters related to pregnancy and breastfeeding, and the inclusion of congeners other than TCDD is required. The use of in vitro models for further refinement should be considered.
- Biomonitoring data, especially individual data on occurrence of PCDD/Fs and DL-PCBs in human milk and covering more European populations, are needed.
- Development of analytical methods that allow a lower sample volume is needed to determine PCDD/Fs and DL-PCBs in human blood.
- Considering the differences between levels in meat and fat tissue, the impact of lipid composition on lipid-based PCDD/F and DL-PCB levels in food and biological matrices, including blood and human milk, should be investigated.
- Better understanding is needed of the involvement of the AHR in the regulation of sperm production and how it is disrupted by PCDD/Fs and DL-PCBs. This would contribute to development of an AOP for the AHR pathway in relation to sperm production and other effects related to reproduction and development.
- Further investigation on the role of soil ingestion in the contamination of animal-derived food is needed.

6 | DOCUMENTATION AS PROVIDED TO EFSA

1. Mínguez-Alarcón L, Hauser R, 2024–2025. Data provided about the Russian Children's Study and the study by Mínguez-Alarcón et al. (2017), used in **Section 3.1.5**.
2. Schächtele A, 2024. Data provided on the PCDD/F- and PCB-related results of the UNEP/GEF POPs Global Monitoring Plan project and the Stockholm Convention GMP Data Warehouse performed in European countries in the framework of WHO/UNEP human milk survey, and used in **Sections 3.1.1.2** and **3.4.2**.
3. Bernsmann T, 2025. Data provided on the PCDD/F- and PCB-related results by the Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe (CVUA-MEL) on human milk samples from individual women in an area of Germany from the period 2009–2024 and used in **Sections 3.1.1.2** and **3.4.2**.
4. The Norwegian Food Safety Authority, 2025. Data provided on the occurrence of PCDD/Fs and DL-PCBs in fruit and vegetables from 2022 and used to inform the uncertainty analysis regarding the exposure estimates in **Section 3.1.5**.

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
AFHS	Air Force Health Study
AHR	aryl hydrocarbon receptor
AL	action level
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCF	bioconcentration factor
BMD	benchmark dose
BMDL	benchmark dose lower credible limit
BMDU	benchmark dose upper credible limit
BMI	body mass index
BMR	benchmark response

BPS	balanopreputial separation
BTF	biotransfer factor
bw	body weight
CADM	concentration- and age-dependent model
CI	confidence interval
CONTAM	EFSA Panel on Contaminants in the Food Chain
CYP	cytochrome P450
DL-PCBs	dioxin-like PCBs
DM	dry matter
DRE	dioxin-responsive element
dw	dry weight
ER	oestrogen receptor
EU-RL	European Reference Laboratory
FAO/WHO	Food and Agriculture Organization of the United Nations/World Health Organization
FLEHS	Flemish Environment and Health Study
FSH	follicle-stimulating hormone
GC	gas chromatography
GD	gestation day
GGT	gamma-glutamyl transferase
HBGV	health-based guidance value
HR	hazard ratio
HRMS	high-resolution mass spectrometry
i.p.	intraperitoneal
i.v.	intravenous
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin
IPCS	International Programme on Chemical Safety
IQR	interquartile range
JECFA	Joint FAO/WHO Committee on Food Additives
LB	lower bound
LD50	lethal dose, median
LDH	lactate dehydrogenase
LH	luteinising hormone
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
ML	maximum level
MoBa	Norwegian Mother, Father and Child Cohort
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NDL-PCBs	non-dioxin-like PCBs
NHANES	National Health and Nutrition Examination Survey
NOAEL	no-observed-adverse-effect level
NTP	US National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
PBPK	physiologically based pharmacokinetic (model)
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-*p*-dioxins
PCDFs	polychlorinated dibenzofurans
PND	postnatal day
RfD	reference dose
ROS	reactive oxygen species
RR	relative risk
s.c.	subcutaneous
SCF	Scientific Committee on Food
SD	Standard deviation
SOD	superoxide dismutase
SWHS	Seveso Women's Health Study
T3	triiodothyronine
T4	thyroxine

TBG	thyroxine-binding globulin
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TDS	Total Diet Study
TEF	toxic equivalency factor
TEQ	toxic equivalents
TR	transfer rate
TSH	thyroid-stimulating hormone
TWI	tolerable weekly intake
UB	upper bound
UF	uncertainty factor
UNEP	United Nations Environment Programme
US-EPA	United States Environmental Protection Agency
WG	Working Group
WHO	World Health Organisation

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Literature searches

TABLE A.1 Details of the literature search*

Main search string: dioxin* OR tetrachlorodibenzodioxin* OR dioxin ADJ like ADJ PCB OR TEQ* OR TCDD* OR PCDD* OR PCDF* OR 1,2,3,7,8 ADJ pentachlorodibenzodioxin OR 1,2,3,7,8 ADJ pentachlorodibenzo*p*dioxin OR 1,2,3,7,8 ADJ pentachlorodibenzo*para*dioxin OR 1,2,3,7,8 ADJ PeCDD OR 2,3,7,8 ADJ tetrachlorodibenzodioxin OR 1,2,3,7,8 ADJ pentachlorodibenzodioxin OR 1,2,3,4,7,8 ADJ hexachlorodibenzodioxin OR 1,2,3,6,7,8 ADJ hexachlorodibenzodioxin OR 1,2,3,7,8,9 ADJ hexachlorodibenzodioxin OR 1,2,3,4,6,7,8 ADJ heptachlorodibenzodioxin OR octachlorodibenzodioxin* OR 2,3,7,8 ADJ tetrachlorodibenzofuran OR 1,2,3,7,8 ADJ pentachlorodibenzofuran OR 2,3,4,7,8 ADJ pentachlorodibenzofuran OR 1,2,3,4,7,8 ADJ hexachlorodibenzofuran OR 1,2,3,6,7,8 ADJ hexachlorodibenzofuran OR 1,2,3,7,8,9 ADJ hexachlorodibenzofuran OR 2,3,4,6,7,8 ADJ hexachlorodibenzofuran OR 1,2,3,4,6,7,8 ADJ heptachlorodibenzofuran OR 1,2,3,4,7,8,9 ADJ heptachlorodibenzofuran OR octochlorodibenzofuran* OR 3,3,4,4 ADJ tetrachlorobiphenyl OR PCB*77 OR 3,4,4,5 ADJ tetrachlorobiphenyl OR PCB*81 OR 3,3,4,4,5 ADJ hexachlorobiphenyl OR PCB*126 OR 3,3,4,4,5,5 ADJ hexachlorobiphenyl OR PCB*169 OR 2,3,3,4,4 ADJ pentachlorobiphenyl OR PCB*105 OR 2,3,4,4,5 ADJ pentachlorobiphenyl OR PCB*114 OR 2,3,4,4,5 ADJ pentachlorobiphenyl OR PCB*118 OR 2,3,4,4,5 ADJ pentachlorobiphenyl OR PCB*123 OR 2,3,3,4,4,5 ADJ hexachlorobiphenyl OR PCB*156 OR 2,3,3,4,4,5 ADJ hexachlorobiphenyl OR PCB*157 OR 2,3,4,4,5,5 ADJ hexachlorobiphenyl OR PCB*167 OR 2,3,3,4,4,5,5 ADJ heptachlorobiphenyl OR PCB*189 Toxicity in experimental animal string: Main search string AND (rat* OR mice OR monkey* OR guinea ADJ pig* OR mini ADJ pig* OR rabbit* OR hamster* OR dog* OR cat* OR mink OR Hare OR Chinchilla* OR vivo OR primate*) AND (toxic* OR combined ADJ effect* OR dose ADJ depend* OR repro* OR immun* OR disrupt* OR oxidative ADJ stress OR antioxidant* OR susceptibil* OR neuro* OR dimorphi*) Humans string: (Tetrachlorodibenzodioxin OR "2,3,7,8-Tetrachlorodibenzo-p-dioxin" OR TCDD OR dioxin* OR "polychlorinated biphenyl*" OR PCB$ OR TEQ OR "total equivalen*" OR coplanar OR PCDD$ OR PCDF$ OR "Polychlorinated dibenzofuran" OR "Polychlorinated dibenzodioxin") AND (epidemiolog* OR "cohort stud*" OR "case control stud*" OR "adverse effect*" OR "observational stud*" OR "case serie*" OR "casereport*" OR "cross sectional stud*" OR urine OR serum OR plasma OR haema* OR hema* OR blood OR sperm OR semen OR hormone* OR reproduct*) AND (human OR women OR men OR child*) Sources: Web of Science (all databases) and PUBMED Language: all Period: 01/01/2017-10/06/2024	10-06-2024	Toxicity in experimental animals only: 5238 results Humans only: 5912 results
String on transfer from feed to food: (TCDD OR dioxins OR *dioxin OR dioxin-like* OR coplanar ORPCB* OR polychlorinatedbiphenyl* OR PCDD* OR PCDF*) AND (Administration OR absorption OR distribution OR "tissue distribution" OR bioavailab* OR metaboli* OR biotransform* OR activat* OR half-li* OR excret* OR Clearance OR eliminat* OR bioconcentrat* OR *kinetic* OR PBPK OR PBK OR transfer OR carry-over OR carryover OR "carryover") The transfer string was restricted to specific animal categories like, pigs, poultry, horses, fishes and ruminants Sources: Web of Science (all databases) and PUBMED Language: all Period: 01/01/2017-24/06/2024	24-06-2024	Transfer feed to food only: 483 results
Same string searches as above Sources: Web of Science (all databases) and PUBMED Language: all Period: 10/06/2024-29/01/2025	29-01-2025	Toxicity in experimental animal only: 280 results Humans only: 579 results
Only main search string applied for general Above mentioned strings applied for transfer from feed to food search Sources: Web of Science (all databases) and PUBMED Exclusion criteria applied: area like material science, engineering, polymer, optics etc. Language: all Period for general search: 29/01/2025-20/08/2025 Period for search on "transfer": 24/06/2024-20/08/2025	20-08-2025	General search: 686 results Transfer feed to food only: 50 results

*This search is based on terms already optimised in previous literature searches developed in 2018. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2018.EN-1137 and https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2018.EN-1136.

APPENDIX B

Evaluation of the PBK CADM model

This analysis respects the key principles and best practices for characterising and applying physiologically based pharmacokinetic (PBPK) models in risk assessment, described by the World Health Organization (WHO) on Characterisation and the Application of Physiologically based Pharmacokinetic Models in Risk Assessment (WHO/IPCS, 2010), a project conducted within the International Programme on Chemical Safety (IPCS).

The CADM model evaluation was performed using Berkeley Madonna (version 10.1.2) based on a critical dose metric of a $BMDL_{15}$ of 1.7 ng/kg bw derived from the Faqi et al. (1998a) study in rats, which would correspond to a lipid-based level of 6.8 ng/kg fat based on a body fat content of 25% in women. Applying an uncertainty factor of 3.16, the input dose was 0.09 pg/kg bw per day, and the human milk level using the CADM model was predicted to be 2.2 ng/kg fat at 35 years. These values (input dose and human milk level) were used for all simulations.

B.1. | Sensitivity analysis of CADM

Sensitivity analysis (SA) provides a quantitative evaluation of how parameters influence the dose metrics (level in fat).

Sensitivity coefficients were determined based on the serum concentrations resulting from a 10% change in the value of each parameter using the forward difference method. Sensitivity coefficients were normalised using the following equation:

$$\text{Sensitivity Coefficient} = ((A - B) / B) / (C - D) / D))$$

where A is the serum concentration resulting from e.g. a 10% change in the parameter value, B is the serum concentration resulting from the initial parameter value, C is the value of parameter change by 10%, and D is the initial parameter value

Results of the sensitivity analysis

In **Figure B.1** and **Table B.1** the results of the sensitivity analysis are shown.

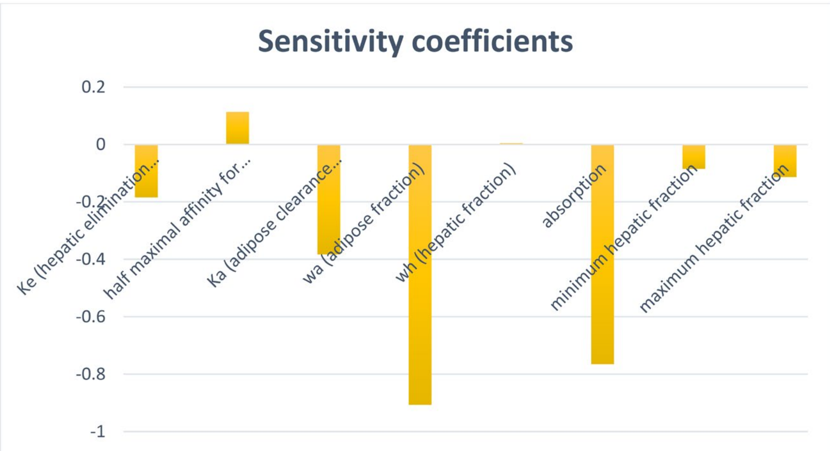

FIGURE B.1 Results of sensitivity analyses of the CADM model, considering an input dose was 0.09 pg/kg bw per day, and a level in body fat and human milk of 2.2 ng/kg fat at 35 years.

According to WHO/IPCS (2010), the sensitivity of the parameter is considered as high (absolute value greater than or equal to 0.5), medium (absolute value greater than or equal to 0.2 but less than 0.5) or low (absolute value greater than or equal to 0.1 but less than 0.2).

TABLE B.1 Results of the sensitivity analysis

	Classification	Parameters
Sensitivity analysis	High	Adipose fraction, oral absorption
	Medium	Adipose clearance factor
	Low	Hepatic elimination rate constant per month Half maximal affinity for liver sequestration Hepatic fraction Minimum hepatic fraction Maximal hepatic fraction

B.2. | Uncertainty analysis of the CADM

The notion of uncertainty encompasses both true uncertainty (i.e. in model parameter value) and variability (i.e. from population variability).

In order to evaluate the impact of parameter variability on the level in fat, parameters were randomly varied between minimal/maximal value (with 100 iterations), with the mean values set to those used during model calibration.

The uncertainty coefficients were calculated using the following equation:

$$\text{Uncertainty coefficients} = \text{Ratio}\,''\text{P95} / \text{P50}'' = (\text{Mean} + 2 \times \text{standard deviation}) / \text{mean}$$

For Ke, the minimal/maximal values of hepatic elimination rate constant per month were from Ruiz et al. (2014). "*The model predicts TCDD serum lipids levels much better using a ke of 0.074 per month. This value is well within the range (0.04–1.00 per year) observed in humans*". Then the corresponding min/max value used for uncertainty analysis were 0.0033 and 0.083 per month.

For K half, the minimal/maximal values of half maximal affinity for liver sequestration, were calculated from a mean of 80.1 ± one SD of 18.9 (value described by Ruiz et al., 2014).

For Ka, a geometric mean of 0.0028 and a geometric standard deviation of 2.307 was reported by Ruiz et al. (2014), then an arbitrary (or default value) of min/max value was calculated on the basis of 50% variability.

For wa, the minimal/maximal values of Adipose fraction were 0.20 and 0.30.

For wh, Fmin and Fmax, no range values were reported. The CONTAM Panel decided to use an arbitrary (or default value) of min/max value, calculated on the basis of 50% variability.

According to WHO/IPCS (2010), uncertainty analysis results are summarised as high uncertainty (value could be a factor of 2 or higher), medium uncertainty (value could be a factor between 0.3 and 2) or low uncertainty (value could be a factor of 0.3 or lower).

Results of the uncertainty analysis

In Table B.2 the results of the uncertainty analysis are shown.

TABLE B.2 Results of the uncertainty analysis based on an input dose of 0.09 pg/kg bw per day and lipid-based human milk level of 2.2 ng/kg fat

	Input parameters			Resulting serum level (ng/kg fat)			
	Initial value	Min	Max	Mean[a]	Mean − 1 SD	Mean + 1 SD	Uncertainty coefficient (P95/P50)[b]
Ke (hepatic elimination rate constant per month	0.05	0.0033	0.083	2.30	2.17	2.43	1.11
Half maximal affinity for liver sequestration	100	60	100	2.24	2.23	2.26	1.01
Ka (adipose clearance factor)	0.0025	0.00125	0.00375	2.29	2.00	2.57	1.25
wa (adipose fraction)	0.25	0.20	0.30	2.31	2.04	2.58	1.24
wh (hepatic fraction)	0.030	0.015	0.045	2.27	2.27	2.27	1.00
Absorption	0.97	0.80	1.00	2.13	2.02	2.24	1.11
Minimum hepatic fraction	0.01	0.005	0.015	2.26	2.20	2.31	1.05
Maximum hepatic fraction	0.7	0.35	1.00	2.27	2.25	2.29	1.02

[a]Mean according to the simulation with uniform distribution between mean and max of each parameter.

[b]Uncertainty coefficients = Ratio "P95/P50" = (Mean + 2 × standard deviation)/mean.

B.3. | Conclusion on the model evaluation

Coupling the results of sensitivity and uncertainty analysis

The outcome of sensitivity and uncertainty analyses might inform the reliability of a model to provide dose metric predictions of use in risk assessment, as illustrated in **Table B.3a** (WHO/IPCS, 2010).

The reliability of the model predictions regarding dose metrics that can be used for risk assessment is based on the level of sensitivity of the predictions to the model parameters and the level of uncertainty of the parameter values. If the highly sensitive parameters are also the ones that are highly uncertain, then the reliability of the model for risk assessment applications would be questionable (WHO/IPCS, 2010).

TABLE B.3 Sensitivity and uncertainty analyses in determining the reliability of PBK model predictions of dose metrics for risk assessment (see WHO/IPCS, 2010). Low reliability (black box); medium reliability (grey boxes); high reliability (white boxes)

		Uncertainty		
		High	**Medium**	**Low**
Sensitivity	High			
	Medium			
	Low			
B3a				

		Uncertainty		
		High	**Medium**	**Low**
Sensitivity	High		Adipose fraction, Absorption	
	Medium		Adipose clearance factor	
	Low		Rest of the parameters	
B3b				

Overall conclusion

In conclusion, according to the IPCS/WHO (2010) guidance, the model can be used with a medium/high reliability.

APPENDIX C

Calculation of the body burden for the critical rat study

Most of the studies on developmental male fertility described in Appendix E applied a single TCDD dose around GD15. As shown by, among others Hurst, DeVito, and Birnbaum (2000) and Yonemoto et al. (2005), most of the dose is after 1 day present in the liver but then redistributes to the adipose tissue. Such a high single dose also results in an increased exposure of the fetus, when compared to the same overall dose applied orally over a longer period (Hurst, DeVito, Woodrow-Setzer, & Birnbaum, 2000). SCF (2001) decided to raise the body burden resulting from the lowest dose (LOAEL) applied in the Faqi et al. (1998a) study to a body burden of 40 ng/kg bw (i.e. a factor of 1.6 higher). The reasoning was that a relatively higher daily exposure is required to obtain the same exposure of the fetus as after a single high dose. The CONTAM Panel reevaluated the need to increase the body burdens of the dams. Contrary to many other developmental studies, Faqi et al. (1998a) did not apply a single high dose around GD15, but actually applied a subcutaneous loading dose 2 weeks before mating and then every week a subcutaneous so-called maintenance dose being 20% of the loading dose, to ensure a steady-state body burden between 80 and 100% of the initial Body Burden. Figure C.1 shows a schematic presentation of the expected body burden over the study period. During lactation the body burden may decrease slightly more quickly due to the excretion of TCDD via the milk. It seems unlikely that this dosing regimen results in a similar peak exposure of the fetus as after a single oral dose at GD15, also regarding the subcutaneous application route which causes a slow release into the body (Abraham et al., 1988).

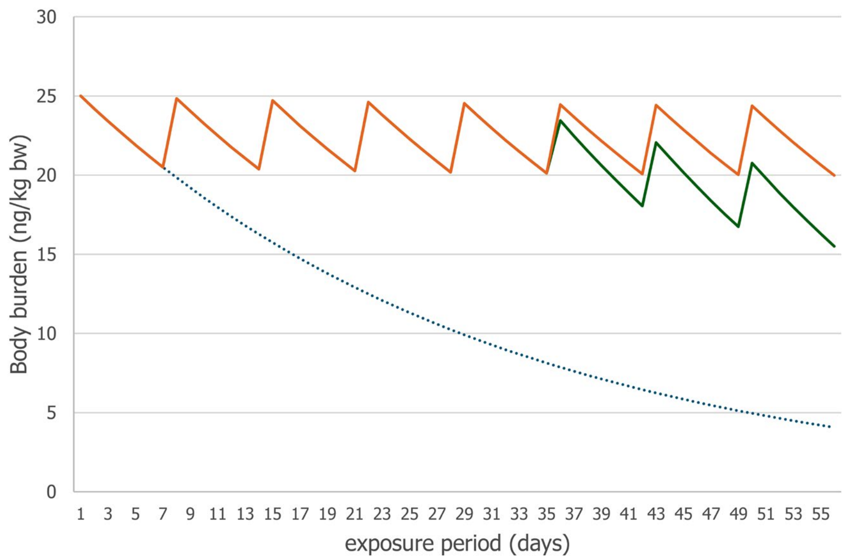

FIGURE C.1 Estimated Body Burden in dams exposed to the lowest dose of TCDD in the study by Faqi et al. (1998a), based on a half-life of 3 weeks. The dotted line shows the Body Burden after a single sc dose of 25 ng/kg bw. The solid orange line shows the Body Burden after the weekly sc maintenance doses, the green solid line includes the effect of lactation, assuming similar lipid-based levels in the body and milk (based on 30 mL milk per day containing 9% fat and 20% body fat in the dams).

Overall, this dosing scheme applied by Faqi et al. (1998a) seems more representative for the background human exposure than a single high dose. It was therefore decided not to increase the body burden and base it on the measured levels in adipose tissue and liver.

In the 2018 Opinion (EFSA CONTAM Panel, 2018, Appendix B therein), it was described how body burdens in animal studies were calculated based on measured levels. The description for the critical study was as follows: *"In the Faqi study, dams were treated subcutaneously before mating and throughout mating, pregnancy and lactation. The animals received an initial loading dose of 25, 60 or 300 ng TCDD/kg bw 2 weeks prior to mating, followed by weekly maintenance doses of 5, 12 or 60 ng/kg bw. Effects on male reproduction were studied on postnatal days (PND) 70 and 170. For this study, no NOAEL was identified and the lowest dose was the LOAEL (*i.e. *loading dose of 25 ng/kg bw, and weekly maintenance dose of 5 ng/kg bw). For this dose scheme, the TCDD concentration determined in maternal liver and adipose tissue at GD21 amounted to respectively 80 and 150 ng/kg tissue (*Figure 1 *of the manuscript). Assuming the female rat body at GD21 to consist of 12% adipose tissue and 4.5% liver tissue, the body burden was calculated to be* $150 \times 0.12 + 80 \times 0.045 = 21.6$ *ng/kg bw. The fraction of the body burden residing in the liver + adipose tissue after the initial loading dose of 25 ng/kg bw is estimated to be* $0.7548 \times \ln(0.025) + 88.77 = 86\%$*. Likewise, at its equivalent repeated dose of* $25/7 = 3.6$ *ng/kg bw per day this fraction would be* $1.934 \times \ln(3.6) + 82.39 = 85\%$*. It was decided not to apply an additional factor to correct for the single maintenance dose applied each week, also because an s.c. dose does not behave similar as a single gavage dose, and is likely to distribute more slowly to other tissues. The chronic maternal GD21 body burden at the 25/5 ng/kg exposure protocol thus is estimated to be* $21.6/0.86 = 25.1$ *ng/kg bw. The other two dose groups showed levels in liver of respectively 140 and 640 ng/kg, and in adipose tissue of 240 and 780 ng/kg. The fraction of TCDD residing in the liver was calculated to be 87% and 88%, meaning that body burdens amount to 41 and 137 ng/kg bw for the medium and high dose".*

APPENDIX D

Overview of the BMD modelling of critical effects from the selected rat and mice studies in EFSA CONTAM Panel (2018) and its current update

TABLE D.1 BMD modelling of critical effects from the selected rat and mice studies in EFSA CONTAM Panel (2018) and its current update

Critical endpoint	Reference	BMDL 2018 opinion[c]	BB at the BMDL (ng/kg bw) 2018 opinion	BMDL current update
Studies in rats				
Decreased sperm production and sperm counts in F1 male Wistar rats	**Faqi et al. (1998a)**	**Based on BB (ng/kg bw)**		
	At PND70	$BMDL_{10} = 0.15$	0.15	Covariate analysis: $BMDL_{10} = 2.5$[d] Standard analysis: $BMDL_{10} = 5.6$
	At PND170	$BMDL_{10} = 0.0014$	0.0014	Covariate analysis: $BMDL_{10} = 2.4$[d] Standard analysis: $BMDL_{10} = 1.4$
Decreased pup weight from PND1 to PND7 in male F1 Wistar (Han) rats	**Bell et al. (2007b)**	**Single dose (ng/kg bw)**		
	Individual data set using litter variability	$BMDL_5 = 68$	78 (GD16)	$BMDL_5 = 212.6$
Delay in BPS in male F1 Wistar (Han) rats	**Bell et al. (2007a)**	**Based on ng/kg bw per day** $BMDL_5 = 3.5$	61 (GD21)	$BMDL_5 = 5.8$[e]
Hepatopathy in female Sprague Dawley rats	**NTP** (2006)	**Based on ng/kg bw per day** $BMDL_{10} =$		$BMDL_{10} =$
	Multinucleated hepatocytes	3.8	97	3.7[e]
	Fatty change	4.3	*Not calculated*	4.8
	Necrosis	8.0	*Not calculated*	13.0[f]
	Oval cell hyperplasia	8.2	*Not calculated*	6.3
	Bile duct hyperplasia	8.1	*Not calculated*	7.4
	Hepatopathy	4.9	*Not calculated*	4.2
Increase in liver weight and AST and increased incidence of histopathological findings in the liver of Long-Evans *(Turku/AB)* rats	**Viluksela et al.** (2000)	Not modelled[a]	–	–

(Continues)

TABLE D.1 (Continued)

Critical endpoint	Reference	BMDL 2018 opinion[c]	BB at the BMDL (ng/kg bw) 2018 opinion	BMDL current update
Decreased tibia length, tibia geometry parameters, tibia ash weight, and increased plasma ALP activity in Long-Evans *(Turku/AB)* rats	**Jämsä et al.** (2001)	**Based on BB in ng/kg bw**		
	Tibial length	$BMDL_5 = 372$	372	499
	Tibial peri circumferences	$BMDL_5 = 104$	104	184
	Tibial endo circumferences	$BMDL_5 = 47.8$	48	93.1
	Tibial CSA	$BMDL_5 = 13.8$	14	122
	Tibial ash weight	$BMDL_5 = 15.9$	16	54.5
	Plasma ALP activity[b]	–	–	$BMDL_{10} = 43.2$[e]
Studies in mice				
Embryonic loss in female NIH mice day 1–3	**Li et al.** (2006)	Based on ng/kg bw per day $BMDL_{10} = 11.4$	53	24.4

[a]No BMD analysis performed for Viluksela et al. (2000) for the following reasons: (i) The histopathological effects in the liver were observed only in the mid and high dose groups with a steep increase from the low dose and control groups (100% response). These type of data sets are not ideal for the dose-response analysis, in particular in studies with low number of animals. (ii) Changes in enzymatic activities and liver weights are not tabled in the publication but are reported only in diagrams of low graphic quality, making the digitization very uncertain.

[b]The results of the modelling show that this is not the most sensitive parameter of the study and therefore no additional actions were taken. The modelling of ALP was not further considered in the Opinion.

[c]The BB at the BMDLs is reported in the 2018 Opinion.

[d]Covariate analysis with PND as a covariate.

[e]According to the Bayesian BMD web tool, none of the models provide an adequate fit to the data.

[f]According to the Bayesian BMD web tool, there is insufficient evidence of a substantial dose-effect.

APPENDIX E

Overview of studies on male reproduction/puberty development in experimental animals in view of their dose-response

TABLE E.1 Overview of studies on male reproduction/puberty development in experimental animals in view of their dose-response

Study	Species, strain, sex	Treatment	Outcome method, site	Dose-response based on body burden possible
TCDD (and mixtures)				
Mably et al. (1992)	Rat, dams Holtzman	GD15, single dose, gavage: 0.064, 0.16, 0.40, 1.0 μg/kg bw	M offspring at day 49, day 63 and day 120: daily sperm production reduced. Fertility not significantly reduced. Sperm count: Cauda epididymis Daily sperm production: Testis	No (no body burden measured)
Johnson et al. (1992)	Rat, M adult Sprague–Dawley	Single i.p.: 12.5, 25.0, 50 μg/kg bw	4-week after treatment: Sperm count: Epididymis Daily sperm production: Testis Daily sperm production was not decreased. Reduced Leydig cell volume.	No (no effect on spermatogenesis)
Gray et al. (1995)	Rat, dams Long-Evans hooded	GD6-15, gavage: 0.5 μg/kg bw per day	Various adverse effects on spermatogenesis, delayed puberty Sperm count: Epididymis, testis Daily sperm production: Testis Ejaculated sperm	No (no body burden measured; one dose level)
Sommer et al. (1996)	Rat, dams Holtzman	GD15, gavage: 1.0 μg/kg bw	PND92-93: reduced sperm production Sperm count: Epididymis, Vas deferens Daily sperm production: Testis Ejaculated sperm	No (no body burden measured; one dose level)
Theobald and Peterson (1997)	Mouse, dams, ICR	GD14, gavage: 0, 15, 30, 60 μg/kg bw	PND65, PND114/128: decreased sperm numbers Sperm count: Epididymis Daily sperm production: Testis	No (no body burden measured)
Gray et al. (1997)	Rat, dams Long-Evans	GD15, gavage: 0.05, 0.2, 0.8 μg/kg bw	M offspring at day 49, day 63 and month 15: reduced sperm counts/epidydimal sperm reserve, delayed puberty Sperm count: Epididymis Daily sperm production: Testis Ejaculated sperm	No (no body burden measured)
Faqi et al. (1998a)	Rat, dams Wistar	2 weeks before mating, sc: 25, 60, 300 ng/kg bw plus 5, 12, 60 ng/kg bw once per week during mating, pregnancy and lactation	M offspring at PND70 and PND170: reduced sperm counts, abnormal sperm increased Sperm count: Cauda epididymis Daily sperm production: Testis Sperm transition rate, abnormal sperm	Yes
El-Sabeawy et al. (1998)	Rat, M Sprague–Dawley	21-day-old M, ip: 0.1, 1.0, 5.0, 10 μg/kg bw	After 90 days: reduced testicular and epididymal sperm counts and motility Daily sperm production: Testis Sperm motility	No (no body burden measured)

(Continues)

TABLE E.1 (Continued)

Study	Species, strain, sex	Treatment	Outcome method, site	Dose-response based on body burden possible
Ohsako et al. (2001)	Rat, dams Holtzman	GD15, gavage: 12.5, 50, 200, 800 ng/kg bw repeating the Mably et al. (1992) protocol	M offspring at day 49 or day 120: no effect on sperm production, but decreased prostate weights and anogenital distance Sperm count: Cauda epididymis Daily sperm production: Testis	No (no body burden measured)
Hamm et al. (2003)	Rat, dams Long Evans	GD15, gavage: defined TEQ mixture: 0.05, 0.2, 0.8, 1.0, 2.0 μg/kg bw	M offspring at PND32, PND49, PND63: reduction in sperm count and in weight of genital organs (prostate, testis, epididymis) Sperm count: Cauda epididymis	No (no body burden measured; complex mixture)
Simanainen et al. (2004)	Rat, dams Line A Line B Line C	GD15, gavage: 0.1–1000 μg/kg bw 0.1–300 μg/kg bw 0.1–30 μg/kg bw	M offspring at PND14, PND21, PND28, PND35, PND49: reduced sperm count, cauda epididymis sperm reserve, reduced prostate weight. Sperm count: Cauda epididymis Daily sperm production: Testis	No (no body burden measured)
Yonemoto et al. (2005)	Rat, dams Long-Evans	GD15, gavage: 12.5, 50, 200, 800 ng/kg bw	M offspring at PND49 and PND63: delayed puberty, seminal vesicle and prostate weight Sperm count: Cauda epididymis Ejaculated sperm, sperm motility	No (unclear numbers of animals)
Bell et al. (2007b)	Rat, dams Han-Wistar (strain is exceptionally resistant to TCDD, 10-100-fold lower than Long-Evans)	GD15, single dose gavage: 50, 200, 1000 ng/kg bw	M offspring at PND70: increased sperm count, more abnormal sperm at PND70, delayed puberty Sperm count: Cauda, epididymis, testis Sperm motility	No (no effect on numeric spermatogenesis)
Bell et al. (2007a)	Rat, dams, Han Wistar	2, 4, 8, 46 ng/kg bw per day over 12 weeks in the diet, during which the rats were mated and allowed to litter; rats were switched to control diet after parturition	M offspring at PND70 and PND120: delayed puberty (BPS)	No (no body burden measured)
Arima et al. (2009)	Rhesus monkey, dams	GD20, sc: 30, 300 ng/kg bw, plus 5% of the initial dose every 30 days	Adult M offspring: ejaculated sperm count, viability, motility reduced, decreased epididymis sperm reserve Sperm count: Ejaculated sperm Sperm viability, motility	No (no body burden measured; only two dose levels)
Rebourcet et al. (2010)	Rat, dams Sprague-Dawley	GD15, gavage: 10, 100, 200 ng/kg bw	M offspring at PND145 or PND200: decreased sperm reserve at PND200. Sperm count: Epididymis (caput, corpus, cauda) Daily sperm production: Testis	No (no body burden measured; only one effective dose level)
PCB-77				
Faqi et al. (1998b)	Rat, dams, Wistar	GD15, gavage: 100 μg/kg bw	Adult M offspring: seminal vesicle weight decreased, no decrease in sperm production or quality Daily sperm production: Testis Sperm quality: Ductus deferens	No (no body burden measured)

TABLE E.1 (Continued)

Study	Species, strain, sex	Treatment	Outcome method, site	Dose-response based on body burden possible
PCB-126				
Faqi et al. (1998b)	Rat, dams, Wistar	GD15, gavage: 10 µg/kg bw	Adult M offspring: Ventral prostate weight decreased, anogenital distance reduced, increased number of mounts with intermissions; other reproductive parameters including sperm production unchanged Daily sperm production: Testis. Sperm quality: Ductus deferens	No (no body burden measured)
PCB-169				
None				

Abbreviations: BPS, Balano-preputial separation; bw, body weight; GD, gestational day; ip, intraperitoneal; M, male; PND, postnatal day; sc, subcutaneous.

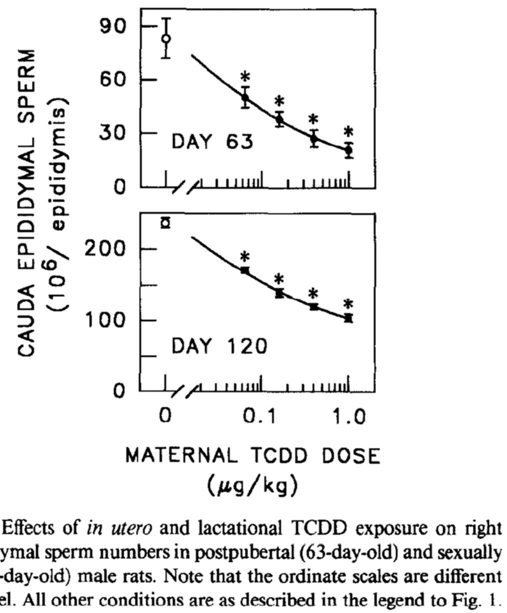

FIGURE E.1 Dose-response of the cauda epidydimal sperm numbers in male rats born to dams exposed by gavage on day GD15 to single TCDD doses of 0.064, 0.16, 0.40, 1.0 µg/kg bw (from Mably et al., 1992, © Elsevier).

(Continues)

APPENDIX F

Dietary exposure to the 17 PCDD/Fs

Chronic dietary exposure to the 17 PCDD/F congeners was estimated following the methodology described in **Section 2.6.1**. **Table F.1** and **F.2** show the LB and UB summary statistics of the estimated chronic dietary exposure to the 17 PCDD/Fs based on the WHO_{2005}-TEFs and the WHO_{2022}-TEFs, respectively, across surveys for each age group.

TABLE F.1 Mean and P95 dietary exposure (LB and UB) based on WHO_{2005}-TEFs to the 17 PCDD/Fs (minimum, median and maximum across surveys) for each age group

Range of mean dietary exposure (pg WHO-TEQ/kg bw per day) using WHO_{2005}-TEFs							
		Minimum		Median		Maximum	
Age group	**N surveys**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Infants[a]	14	0.15	0.53	0.31	0.99	0.68	1.50
Toddlers	17	0.34	1.01	0.52	1.38	0.75	1.62
Other children	21	0.30	0.79	0.40	1.01	0.70	1.39
Adolescents	23	0.15	0.38	0.24	0.52	0.36	0.80
Adults	23	0.15	0.34	0.19	0.43	0.33	0.59
Elderly	21	0.15	0.33	0.19	0.41	0.43	0.69
Very elderly	16	0.14	0.31	0.20	0.44	0.38	0.64
Range of P95 dietary exposure (pg WHO-TEQ/kg bw per day) using WHO_{2005}-TEFs							
		Minimum		**Median**		**Maximum**	
Age group	**N surveys**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Infants[a]	13	0.32	1.12	0.77	2.05	1.65	3.11
Toddlers	16	0.70	1.58	1.07	2.35	1.67	2.93
Other children	21	0.56	1.40	0.86	1.86	1.37	2.39
Adolescents	22	0.33	0.83	0.47	1.00	1.14	1.76
Adults	23	0.35	0.66	0.48	0.84	1.00	1.46
Elderly	21	0.33	0.63	0.49	0.85	1.18	1.60
Very elderly	12	0.28	0.60	0.47	0.82	0.84	1.44

Abbreviations: LB, lower bound; TEF, toxic equivalency factor; TEQ, toxic equivalents; UB, upper bound.

[a]Not including intake from human milk.

When applying the WHO_{2005}-TEFs, it was noted that for the 17 PCDD/Fs:

- The mean LB dietary exposure to the 17 PCDD/Fs ranged from 0.14 pg WHO_{2005}-TEQ/kg bw per day in the Very elderly to 0.75 pg WHO_{2005}-TEQ/kg bw per day in Toddlers, across surveys.
- The mean UB dietary exposure to the 17 PCDD/Fs ranged from 0.31 pg WHO_{2005}-TEQ/kg bw per day in the Very Elderly to 1.62 pg WHO_{2005}-TEQ/kg bw per day in Toddlers, across surveys.
- The P95 LB dietary exposure to the 17 PCDD/Fs congeners ranged from 0.28 pg WHO_{2005}-TEQ/kg bw per day in the Very elderly to 1.67 pg WHO_{2005}-TEQ/kg bw per day in Toddlers, across surveys.
- The P95 UB dietary exposure to the 17 PCDD/Fs congeners ranged from 0.60 pg WHO_{2005}-TEQ/kg bw per day in the Very elderly to 3.11 pg WHO_{2005}-TEQ/kg bw per day in Infants, across surveys.

TABLE F.2 Mean and P95 dietary exposure (LB and UB) based on WHO_{2022}-TEFs to the 17 PCDD/Fs (minimum, median and maximum across surveys) for each age group

Range of mean dietary exposure (pg WHO-TEQ/kg bw day) using WHO_{2022}-TEQ							
		Minimum		Median		Maximum	
Age group	***N* surveys**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Infants[a]	14	0.14	0.49	0.30	0.90	0.67	1.33
Toddlers	17	0.35	0.91	0.48	1.21	0.73	1.43
Other children	21	0.29	0.65	0.38	0.87	0.61	1.17
Adolescents	23	0.15	0.32	0.21	0.45	0.33	0.69
Adults	23	0.13	0.29	0.17	0.37	0.25	0.48
Elderly	21	0.14	0.28	0.17	0.35	0.31	0.51
Very elderly	16	0.13	0.29	0.18	0.38	0.28	0.51
Range of P95 dietary exposure (pg WHO-TEQ/kg bw day) using WHO_{2022}-TEQ							
		Minimum		Median		Maximum	
Age group	***N* surveys**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Infants[a]	13	0.41	1.02	0.69	1.80	1.51	3.07
Toddlers	16	0.62	1.36	0.92	1.92	1.43	2.37
Other children	21	0.54	1.13	0.73	1.47	1.12	2.04
Adolescents	22	0.31	0.68	0.43	0.83	0.75	1.36
Adults	23	0.28	0.52	0.36	0.69	0.64	1.07
Elderly	21	0.28	0.52	0.37	0.66	0.71	1.10
Very elderly	12	0.28	0.50	0.34	0.66	0.54	0.96

Abbreviations: LB, lower bound; TEF, toxic equivalency factor; TEQ, toxic equivalents; UB, upper bound.
[a]Not including intake from human milk.

When applying the new WHO_{2022}-TEFs, it was noted that for the 17 PCDD/Fs:

- The mean LB dietary exposure to the 17 PCDD/Fs ranged from 0.13 pg WHO_{2022}-TEQ/kg bw per day in Adults and the Very elderly to 0.73 pg WHO_{2022}-TEQ/kg bw per day in Toddlers, across surveys.
- The mean UB dietary exposure to the 17 PCDD/Fs ranged from 0.28 pg WHO_{2022}-TEQ/kg bw per day in the Elderly to 1.43 pg WHO_{2022}-TEQ/kg bw per day in Toddlers, across surveys.
- The P95 LB dietary exposure to the 17 PCDD/Fs congeners ranged from 0.28 pg WHO_{2022}-TEQ/kg bw per day in Adults, Elderly and the Very elderly to 1.51 pg WHO_{2022}-TEQ/kg bw per day in Infants, across surveys.
- The P95 UB dietary exposure to the 17 PCDD/Fs congeners ranged from 0.5 pg WHO_{2022}-TEQ/kg bw per day in the Very elderly to 3.07 pg WHO_{2022}-TEQ/kg bw per day in Infants, across surveys.

APPENDIX G

Compositions of model diets used in the food-producing animal exposure assessment

TABLE G.1 Model diet compositions for chickens for fattening, turkeys for fattening and laying hens.

Groups of feed materials	% of diet: Chickens for fattening	% of diet: Turkeys for fattening	% of diet: Laying hens	Feed materials	Model diet composition (%): Chickens for fattening	Model diet composition (%): Turkeys for fattening	Model diet composition (%): Laying hens
Cereal grains and products derived thereof	75	65	65	Maize	39	35	35
				Barley	36	30	30
Oil seeds, oil fruits and products derived thereof	20	20	20	Soya (beans) (including expeller, meal, extruded, co-product, toasted)[a]	15	16	10
				Rape seed (expeller, meal, extruded)[b]	–	–	8
				Vegetable oil and fat	5	4	2
Tubers, roots, and products derived thereof	2	2	3	(Sugar) cane molasses	2	2	3
Forage dehydrated	–	10	2	Lucerne (field dried, high temperature dried, extruded, meal)[c]	–	10	2
Minerals and products derived thereof	2.5	2.5	9.5	Mineral salts[d]	2.5	2.5	9.5
Feed additives	0.5	0.5	0.5	Premix[e]	0.5	0.5	0.5

[a]Ad hoc feed category consisting of the samples of 'Soya (beans)', 'Soya (bean) expeller', 'Soya (bean) meal', 'Soya beans, extruded', 'Co-product from soybean preparation' and 'Toasted soya (beans)'.

[b]Ad hoc feed category consisting of the samples of 'Rape seed expeller', 'Rape seed meal', 'Rape seed meal feed' and 'Rape seed, extruded'.

[c]Ad hoc feed category consisting of the samples of 'Lucerne; [Alfalfa]', 'Lucerne meal; [Alfalfa meal]', 'Lucerne, field dried; [Alfalfa field dried]', 'Lucerne, high temperature dried; [Alfalfa, high temperature dried]' and 'Lucerne, extruded; [Alfalfa, extruded]'.

[d]Ad hoc feed category consisting of the samples of (i) 'Monocalcium phosphate; [Calcium tetrahydrogen diorthophosphate]', 'Monodicalcium phosphate, 'Calcium carbonate [Limestone]', 'Calcium salts of organic acids', 'Dicalcium phosphate; [Calcium hydrogen orthophosphate]' and 'Calcareous marine shells', (ii) 'Zinc oxide' and (iii) 'Copper sulphate'. Each of the three groups is given equal weight.

[e]No sufficient data for the sum of PCDD/Fs and DL-PCBs (29 congeners) and to the sum of PCDD/Fs (17 congeners) were available, and therefore no contribution from these feeds has been assumed.

TABLE G.2 Model diet compositions for piglets, pigs for fattening and lactating sows.

Groups of feed materials	% of diet: Piglets	% of diet: Pigs	% of diet: Lactating sows	Feed materials	Model diet composition (%): Piglets	Model diet composition (%): Pigs	Model diet composition (%): Lactating sows
Cereal grains and products derived thereof	68	77	75	Maize	48	52.5	57
				Barley	20	24.5	18
Oil seeds, oil fruits and products derived thereof	26	16	18	Soya (beans) (including expeller, meal, extruded, co-product, toasted)[a]	22	11	16
				Rape seed (expeller, meal, extruded)[b]	3	4	–
				Vegetable oil and fat	1	1	2
Tubers, roots, and products derived thereof	3	4	4	(Sugar) cane molasses	3	4	4
Minerals and products derived thereof	2.5	2.5	2.5	Mineral salts[c]	2.5	2.5	2.5
Feed additives	0.5	0.5	0.5	Premix[d]	0.5	0.5	0.5

[a]Ad hoc feed category consisting of the samples of 'Soya (beans)', 'Soya (bean) expeller', 'Soya (bean) meal', 'Soya beans, extruded', 'Co-product from soybean preparation' and 'Toasted soya (beans)'.

[b]Ad hoc feed category consisting of the samples of 'Rape seed expeller', 'Rape seed meal', 'Rape seed meal feed' and 'Rape seed, extruded'.

[c]Ad hoc feed category consisting of the samples of (i) 'Monocalcium phosphate; [Calcium tetrahydrogen diorthophosphate]', 'Monodicalcium phosphate, 'Calcium carbonate [Limestone]', 'Calcium salts of organic acids', 'Dicalcium phosphate; [Calcium hydrogen orthophosphate]' and 'Calcareous marine shells', (ii) 'Zinc oxide' and (iii) 'Copper sulphate'. Each of the three groups is given equal weight.

[d]No sufficient data for the sum of PCDD/Fs and DL-PCBs (29 congeners) and to the sum of PCDD/Fs (17 congeners) were available, and therefore no contribution from these feeds has been assumed.

TABLE G.3 Model diet compositions for dairy cows and cattle for fattening.

Groups of feed materials	% of diet		Feed materials	Model diet composition (%)	
	Dairy cows	Cattle for fattening		Dairy cows	Cattle for fattening
Cereal grains and products derived thereof	55	60	Maize	25	45
			Barley	20	5
			Maize protein feed [Maize gluten feed][a]	10	10
Oil seeds, oil fruits and products derived thereof	31	22	Soya (beans) (including expeller, meal, extruded, co-product, toasted)[b]	10	–
			Rape seed (expeller, meal, extruded)[c]	20	20
			Vegetable oil and fat	1	2
Tubers, roots, and products derived thereof	11	15	Dried (sugar) beet pulp	8	12
			(Sugar) cane molasses	3	3
Minerals and products derived thereof	2.5	2.5	Mineral salts[d]	2.5	2.5
Feed additives	0.5	0.5	Premix[a]	0.5	0.5
Daily ration[e]					
Complementary feed: Forages (DM)				60:40	80:20/20:80

[a]No sufficient data for the sum of PCDD/Fs and DL-PCBs (29 congeners) and to the sum of PCDD/Fs (17 congeners) were available, and therefore no contribution from these feeds has been assumed.

[b]Ad hoc feed category consisting of the samples of 'Soya (beans)', 'Soya (bean) expeller', 'Soya (bean) meal', 'Soya beans, extruded', 'Co-product from soybean preparation' and 'Toasted soya (beans)'.

[c]Ad hoc feed category consisting of the samples of 'Rape seed expeller', 'Rape seed meal', 'Rape seed meal feed' and 'Rape seed, extruded'.

[d]Ad hoc feed category consisting of the samples of (i) 'Monocalcium phosphate; [Calcium tetrahydrogen diorthophosphate]', 'Monodicalcium phosphate, 'Calcium carbonate [Limestone]', 'Calcium salts of organic acids', 'Dicalcium phosphate; [Calcium hydrogen orthophosphate]' and 'Calcareous marine shells', (ii) 'Zinc oxide' and (iii) 'Copper sulphate'. Each of the three groups is given equal weight.

[e]Two exposure estimates are presented for cattle for fattening, separated by a slash (/). The first refers to a scenario with high complementary feed and low forage consumption (80:20 DM), while the second represents low complementary feed and high forage consumption (20:80 DM).

TABLE G.4 Model diet composition for lambs for fattening.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Cereal grains and products derived thereof	67	Maize	42
		Barley	25
Oil seeds, oil fruits and products derived thereof	20	Soya (beans) (including expeller, meal, extruded, co-product, toasted)[a]	15
		Rape seed (expeller, meal, extruded)[b]	8
		Vegetable oil and fat	2
Tubers, roots, and products derived thereof	5	Dried (sugar) beet pulp	2
		(Sugar) cane molasses	3
Minerals and products derived thereof	2.5	Mineral salts[c]	2.5
Feed additives	0.5	Premix[d]	0.5
Daily ration[e]			
Complementary feed: Forages (DM)			80:20/20:80

[a]Ad hoc feed category consisting of the samples of 'Soya (beans)', 'Soya (bean) expeller', 'Soya (bean) meal', 'Soya beans, extruded', 'Co-product from soybean preparation' and 'Toasted soya (beans)'.

[b]Ad hoc feed category consisting of the samples of 'Rape seed expeller', 'Rape seed meal', 'Rape seed meal feed' and 'Rape seed, extruded'.

[c]Ad hoc feed category consisting of the samples of (i) 'Monocalcium phosphate; [Calcium tetrahydrogen diorthophosphate]', 'Monodicalcium phosphate, 'Calcium carbonate [Limestone]', 'Calcium salts of organic acids', 'Dicalcium phosphate; [Calcium hydrogen orthophosphate]' and 'Calcareous marine shells', (ii) 'Zinc oxide' and (iii) 'Copper sulphate'. Each of the three groups is given equal weight.

[d]No sufficient data for the sum of PCDD/Fs and DL-PCBs (29 congeners) and to the sum of PCDD/Fs (17 congeners) were available, and therefore no contribution from these feeds has been assumed.

[e]Two exposure estimates are presented for lambs for fattening, separated by a slash (/). The first refers to a scenario with high complementary feed and low forage consumption (80:20 DM), while the second represents low complementary feed and high forage consumption (20:80 DM).

TABLE G.5 Model diet composition for horses.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Cereal grains and products derived thereof	82	Maize	40
		Barley	12
		Wheat feed[a]	30
Legume seeds and products derived thereof	10	Beans[a]	10
Tubers, roots, and products derived thereof	5	(Sugar) cane molasses	5
Minerals and products derived thereof	2.5	Mineral salts[b]	2.5
Feed additives	0.5	Premix[a]	0.5
Daily ration			
Complementary feed: Forages (DM)			25:75

[a]No sufficient data for the sum of PCDD/Fs and DL-PCBs (29 congeners) and to the sum of PCDD/Fs (17 congeners) were available, and therefore no contribution from these feeds has been assumed.

[b]Ad hoc feed category consisting of the samples of (i) 'Monocalcium phosphate; [Calcium tetrahydrogen diorthophosphate]', 'Monodicalcium phosphate, 'Calcium carbonate [Limestone]', 'Calcium salts of organic acids', 'Dicalcium phosphate; [Calcium hydrogen orthophosphate]' and 'Calcareous marine shells', (ii) 'Zinc oxide' and (iii) 'Copper sulphate'. Each of the three groups is given equal weight.

TABLE G.6 Model diet composition for rabbits for fattening.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Cereal grains and products derived thereof	25	Maize	12.5
		Barley	12.5
Oil seeds, oil fruits and products derived thereof	30	Sunflower (expeller, meal, dehulled meal)[a]	20
		Soya (beans) (including expeller, meal, extruded, co-product, toasted)[b]	10
Tubers, roots, and products derived thereof	20	Dried (sugar) beet pulp	18
		(Sugar) cane molasses	2
Forage dehydrated	20	Lucerne (field dried, high temperature dried, extruded, meal)[c]	20
Land animal products and products derived thereof	2	Animal fat	2
Minerals and products derived thereof	2.5	Mineral salts[d]	2.5
Feed additives	0.5	Premix[e]	0.5

[a]Ad hoc feed category consisting of the samples of 'Sunflower seed expeller', 'Sunflower seed meal', 'Sunflower seed meal feed', 'Sunflower seed meal, dehulled' and 'Sunflower seed meal feed, dehulled'.

[b]Ad hoc feed category consisting of the samples of 'Soya (beans)', 'Soya (bean) expeller', 'Soya (bean) meal', 'Soya beans, extruded', 'Co-product from soybean preparation' and 'Toasted soya (beans)'.

[c]Ad hoc feed category consisting of the samples of 'Lucerne; [Alfalfa]', 'Lucerne meal; [Alfalfa meal]', 'Lucerne, field dried; [Alfalfa field dried]', 'Lucerne, high temperature dried; [Alfalfa, high temperature dried]' and 'Lucerne, extruded; [Alfalfa, extruded]'.

[d]Ad hoc feed category consisting of the samples of (i) 'Monocalcium phosphate; [Calcium tetrahydrogen diorthophosphate]', 'Monodicalcium phosphate, 'Calcium carbonate [Limestone]', 'Calcium salts of organic acids', 'Dicalcium phosphate; [Calcium hydrogen orthophosphate]' and 'Calcareous marine shells', (ii) 'Zinc oxide' and (iii) 'Copper sulphate'. Each of the three groups is given equal weight.

[e]No sufficient data for the sum of PCDD/Fs and DL-PCBs (29 congeners) and to the sum of PCDD/Fs (17 congeners) were available, and therefore no contribution from these feeds has been assumed.

TABLE G.7 Model diet composition for salmonids.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Cereal grains and products derived thereof	15	Maize	10
		Maize protein [Maize gluten][a]	5
Oil seeds, oil fruits, and products derived thereof	29	Soya (beans) (including expeller, meal, extruded, co-product, toasted)[b]	14
		Sunflower (expeller, meal, dehulled meal)[c]	5
		Rape seed (expeller, meal, extruded)[d]	3
		Vegetable oil and fat	7
Land animal products and products derived thereof	18	Animal by-products[a]	15
		Animal fat	3

TABLE G.7 (Continued)

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Fish, other aquatic animals and products derived thereof	32	Fish meal	15
		Fish oil	7
		Fish protein, hydrolysed	10
Minerals and products derived thereof	2	Mineral salts[e]	2
Feed additives	4	(mainly amino acids)[a]	4

[a]No sufficient data for the sum of PCDD/Fs and DL-PCBs (29 congeners) and to the sum of PCDD/Fs (17 congeners) were available, and therefore no contribution from these feeds has been assumed.

[b]Ad hoc feed category consisting of the samples of 'Soya (beans)', 'Soya (bean) expeller', 'Soya (bean) meal', 'Soya beans, extruded', 'Co-product from soybean preparation' and 'Toasted soya (beans)'.

[c]Ad hoc feed category consisting of the samples of 'Sunflower seed expeller', 'Sunflower seed meal', 'Sunflower seed meal feed', 'Sunflower seed meal, dehulled' and 'Sunflower seed meal feed, dehulled'.

[d]Ad hoc feed category consisting of the samples of 'Rape seed expeller', 'Rape seed meal', 'Rape seed meal feed' and 'Rape seed, extruded'.

[e]Ad hoc feed category consisting of the samples of (i) 'Monocalcium phosphate; [Calcium tetrahydrogen diorthophosphate]', 'Monodicalcium phosphate, 'Calcium carbonate [Limestone]', 'Calcium salts of organic acids', 'Dicalcium phosphate; [Calcium hydrogen orthophosphate]' and 'Calcareous marine shells', (ii) 'Zinc oxide' and (iii) 'Copper sulphate'. Each of the three groups is given equal weight.

APPENDIX H

Estimated food-producing animal dietary intakes of 29 PCDD/Fs and DL-PCBs

This Appendix provides the estimated dietary intakes of the 29 PCDD/Fs and DL-PCBs for the considered food-producing animal species and categories as pg WHO-TEQ/day and pg WHO-TEQ/kg bw per day. Estimates are based on mean and high LB and UB concentrations in diets composed of individual feed materials or compound feeds (complete feed/complementary feed + forages), as reported in **Table 34** and default feed intakes and body weights reported in Appendix A of EFSA's 2024 Statement on animal dietary exposure in the risk assessment of contaminants in feed (EFSA FEEDAP Panel, 2024).

H.1.1. | Estimated dietary intakes based on model diets

TABLE H.1 Estimated exposure to the 29 PCDD/Fs and DL-PCBs for the considered food-producing animal species and categories using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **model diets** composed of different feed materials.

	WHO_{2022}-TEQ								WHO_{2005}-TEQ							
	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day
Pigs																
Piglets	18	**0.9**	45	**2.2**	57	**2.8**	91	**4.5**	20	**1.0**	54	**2.7**	55	**2.7**	115	**5.8**
Pigs for fattening	45	**0.7**	117	**1.9**	147	**2.5**	236	**3.9**	51	**0.9**	143	**2.4**	142	**2.4**	300	**5.0**
Sows, lactating	123	**0.7**	289	**1.7**	414	**2.4**	610	**3.5**	136	**0.8**	349	**2.0**	385	**2.2**	753	**4.3**
Poultry																
Chickens for fattening	4.2	**2.1**	10	**4.9**	13	**6.7**	19	**9.7**	4.7	**2.3**	12	**5.9**	12	**5.8**	22	**11**
Laying hens	2.3	**1.2**	6.0	**3.0**	6.6	**3.3**	11	**5.6**	2.5	**1.3**	7.2	**3.6**	6.2	**3.1**	13	**6.6**
Turkeys for fattening	4.8	**1.6**	12	**3.9**	14	**4.5**	21	**7.1**	5.0	**1.7**	14	**4.6**	12	**3.9**	24	**7.9**
Cattle																
Cattle for fattening (high complementary feed, low forage)[a]	190	**0.5**	493	**1.2**	625	**1.6**	1060	**2.6**	198	**0.5**	593	**1.5**	576	**1.4**	1263	**3.2**
Cattle for fattening (low complementary feed, high forage)[a]	259	**0.6**	543	**1.4**	844	**2.1**	1212	**3.0**	281	**0.7**	652	**1.6**	783	**2.0**	1400	**3.5**
Dairy cows[a]	470	**0.7**	1180	**1.8**	1455	**2.2**	2450	**3.8**	510	**0.8**	1431	**2.2**	1377	**2.1**	2906	**4.5**
Small ruminants																
Lambs for fattening (high complementary feed, low forage)[a]	21	**1.1**	51	**2.6**	67	**3.4**	106	**5.3**	23	**1.2**	62	**3.1**	63	**3.1**	127	**6.4**
Lambs for fattening (low complementary feed, high forage)[a]	29	**1.4**	59	**3.0**	92	**4.6**	131	**6.5**	31	**1.6**	71	**3.5**	86	**4.3**	151	**7.6**
Fish																
Salmonids	0.5	**3.8**	0.6	**4.6**	1.2	**10.0**	1.3	**11**	0.8	**6.6**	0.9	**7.6**	2.0	**17**	2.1	**18**

(Continues)

TABLE H.1 (Continued)

	WHO2022-TEQ								WHO2005-TEQ							
	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day
Rabbits																
Rabbits for fattening	2.2	**1.1**	7.6	**3.8**	4.9	**2.5**	11	**5.6**	2.1	**1.0**	9.0	**4.5**	5.1	**2.6**	13	**6.6**
Horses																
All categories[a]	274	**0.7**	856	**2.1**	334	**0.8**	921	**2.3**	213	**0.5**	942	**2.4**	268	**0.7**	1034	**2.6**

Abbreviations: bw, body weight; DL-PCBs, dioxin-like polychlorinated biphenyls; LB, lower bound; PCDD/Fs, polychlorinated dibenzo-p-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]For bovines, ovines and horses, complementary feed was complemented with forages.

H.1.2. | Estimated dietary intakes based on compound feeds

TABLE H.2 Estimated exposure to the 29 PCDD/Fs and DL-PCBs for the considered food-producing animal species and categories using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **compound feeds** (complete feed/complementary feed + forages).

	WHO2022-TEQ								WHO2005-TEQ							
	Exposure mean LB		ExposureMean UB		Exposure high LB		Exposure high UB		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day
Pigs																
Piglets	12	**0.6**	36	**1.8**	18	**0.9**	61	**3.0**	13	**0.7**	45	**2.2**	23	**1.2**	80	**4.0**
Pigs for fattening	25	**0.4**	80	**1.3**	64	**1.1**	213	**3.5**	24	**0.4**	98	**1.6**	65	**1.1**	198	**3.3**
Sows, lactating	118	**0.7**	199	**1.1**	250	**1.4**	586	**3.4**	123	**0.7**	233	**1.3**	134	**0.8**	625	**3.6**
Poultry																
Chickens for fattening	2.7	**1.4**	6.8	**3.4**	5.5	**2.8**	17	**8.7**	2.2	**1.1**	7.4	**3.7**	5.9	**3.0**	19	**9.4**
Laying hens	1.3	**0.6**	4.3	**2.2**	3.9	**2.0**	11	**5.6**	1.6	**0.8**	5.7	**2.8**	4.8	**2.4**	14	**7.0**
Turkeys for fattening	7.4	**2.5**	12	**4.0**	7.4	**2.5**	12	**4.0**	7.8	**2.6**	14	**4.7**	7.8	**2.6**	14	**4.7**
Fis																
Salmonids	0.6	**5.0**	0.7	**5.6**	1.4	**11**	1.4	**12**	1.1	**9.0**	1.2	**10**	2.5	**21**	2.5	**21**

TABLE E.1 (Continued)

	WHO_{2022}-TEQ								WHO_{2005}-TEQ							
	Exposure mean LB		ExposureMean UB		Exposure high LB		Exposure high UB		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day
Rabbits																
Rabbits for fattening	3.1	**1.5**	5.8	**2.9**	5.7	**2.9**	10.2	**5.1**	3.4	**1.7**	7.0	**3.5**	5.5	**2.8**	12.3	**6.1**
Horses																
All categories[a]	277	**0.7**	920	**2.3**	278	**0.7**	940	**2.3**	213	**0.5**	1024	**2.6**	215	**0.5**	1050	**2.6**

Abbreviations: bw, body weight; DL-PCBs, dioxin-like polychlorinated biphenyls; LB, lower bound; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]For horses, complementary feed was complemented with forages.

APPENDIX I

Food-producing animal exposure to the 17 PCDD/Fs

This Appendix provides detailed information on the assessment of the exposure of food-producing animals to the sum of the 17 PCDD/Fs. It is divided into two subsections:

- **I.1. Estimated concentrations of the 17 PCDD/Fs in food-producing animal diets**

This subsection presents the estimated mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB and UB concentrations of the 17 PCDD/Fs in the diets of the considered food-producing animal species and categories. The estimates shown in **Table H.1** are based on model diets and concentration data on individual feeds while **Table H.2** includes concentration estimates derived from compound feeds (complete feed/complementary feed + forages). All the estimates are presented as WHO_{2005}-TEQ and WHO_{2022}-TEQ. For bovines, ovines and horses, model diets and/or complementary feeds were combined with forages to meet the animals' nutritional requirements.

- **I.2. Estimated food-producing animal dietary intakes of the 17 PCDD/Fs**

This subsection provides the estimated dietary intakes of the 17 PCDD/Fs for the considered food-producing animal species and categories. Estimates are based on mean and high LB and UB concentrations in diets composed of individual feed materials and compound feeds (complete feed/complementary feed + forages), as reported in **Subsection H.1** and default feed intakes and body weights reported in Appendix A of the 2024 EFSA Statement on animal dietary exposure in the risk assessment of contaminants in feed (EFSA FEEDAP Panel, 2024).

I.1 | Estimated concentrations of the 17 PCDD/Fs in animal diets

I.1.1 | Estimated concentrations in the model diets

TABLE I.1 Lower bound (LB) and upper bound (UB) mean and high concentrations (the highest reliable percentile based on the number of samples available, up to 95th percentile) of the 17 PCDD/Fs in pg WHO-TEQ/kg DM in daily diets of considered food-producing animal species and categories. Estimates are derived from **model diets** based on individual feed materials.

	Occurrence of the 17 PCDD/fs (pg WHO-TEQ/kg DM)							
	WHO_{2022}-TEQ				WHO_{2005}-TEQ			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Pigs								
Piglets	15	42	54	90	12	47	32	101
Pigs for fattening	15	44	56	93	13	49	33	104
Sows, lactating	17	46	67	102	15	51	39	111
Poultry								
Chickens for fattening	20	52	75	111	17	56	45	108
Laying hens	16	46	53	90	14	51	32	96
Turkeys for fattening	21	54	68	105	16	58	40	104
Cattle								
Cattle for fattening (high complementary feed/low forage)[a]	18	51	67	111	14	55	43	118
Cattle for fattening (low complementary feed/high forage)[a]	23	57	89	129	17	60	67	139
Dairy cows[a]	17	48	61	103	13	53	44	111
Small ruminants								
Lambs for fattening (high complementary feed/low forage)[a]	17	48	65	104	14	53	42	111
Lambs for fattening (low complementary feed/high forage)[a]	23	56	88	127	17	60	66	137
Fish								
Salmonids	81	128	249	287	118	174	352	399

TABLE I.1 (Continued)

	Occurrence of the 17 PCDD/fs (pg WHO-TEQ/kg DM)							
	WHO_{2022}-TEQ				WHO_{2005}-TEQ			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Rabbits								
Rabbits for fattening	16	55	33	81	10	59	23	85
Horses								
All categories[a]	27	74	34	81	14	74	17	82

Abbreviations: DM, dry matter; LB, lower bound; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]For bovines, ovines and horses, complementary feed was complemented with forages.

I.1.2. Estimated concentrations in compound feed (complementary or complete feed)

TABLE I.2 Lower bound (LB) and upper bound (UB) mean and high concentrations (the highest reliable percentile based on the number of samples available, up to 95th percentile) of the 17 PCDD/Fs in pg WHO-TEQ/kg fry matter (DM) **compound feeds** (complete feed/complementary feed + forages)

	Occurrence of the 17 PCDD/fs (pg WHO-TEQ/kg DM)							
	WHO_{2022}-TEQ				WHO_{2005}-TEQ			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Pigs								
Piglets	11	37	18	65	9	42	16	82
Pigs for fattening	8	32	26	72	5	36	21	78
Sows, lactating[a]	19	32	34	106	19	35	18	111
Poultry								
Chickens for fattening[b]	14	38	32	96	9	39	25	111
Laying hens	6	34	23	88	5	41	21	114
Turkeys for fattening	35	50	35	50	39	50	39	50
Fish								
Salmonids	82	114	235	246	124	161	389	404
Rabbits								
Rabbits for fattening	21	46	45	92	17	49	33	93
Horses								
All categories[c]	27	80	27	84	14	81	13	87

Abbreviations: DM, dry matter; LB, lower bound; PCDD/Fs, polychlorinated dibenzo-p-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]The estimates are based on ad hoc feed category consisting of the samples of 'Complete feed' for 'Breeding pigs' and 'Sows'.

[b]The estimates are based on ad hoc feed category consisting of the samples of 'Complete feed' for 'Fattening chickens (broilers)' and 'Poultry (starter diets)'.

[c]For horses, complementary feed was complemented with forages.

I.2 | Estimated animal dietary intakes of the 17 PCDD/Fs

I.2.1 | Estimated dietary intakes based on model diets

TABLE I.3 Estimated exposure to the 17 PCDD/Fs for the considered food-producing animal species and categories using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **model diets** composed of different feed materials.

	WHO_{2022}-TEQ								WHO_{2005}-TEQ							
	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day
Pigs																
Piglets	13	**0.6**	37	**1.9**	48	**2.4**	79	**4.0**	11	**0.5**	42	**2.1**	28	**1.4**	89	**4.4**
Pigs for fattening	32	**0.5**	97	**1.6**	123	**2.1**	204	**3.4**	28	**0.5**	109	**1.8**	72	**1.2**	229	**3.8**
Sows, lactating	91	**0.5**	243	**1.4**	355	**2.0**	541	**3.1**	77	**0.4**	269	**1.5**	205	**1.2**	585	**3.3**
Poultry																
Chickens for fattening	3.2	**1.6**	8.1	**4.1**	12	**5.9**	17	**8.7**	2.7	**1.4**	8.9	**4.4**	7.1	**3.5**	17	**8.5**
Laying hens	1.7	**0.9**	4.9	**2.4**	5.6	**2.8**	9.6	**4.8**	1.4	**0.7**	5.4	**2.7**	3.4	**1.7**	10	**5.1**
Turkeys for fattening	3.6	**1.2**	9.5	**3.2**	12	**4.0**	18	**6.2**	2.9	**1.0**	10	**3.4**	7.0	**2.3**	18	**6.1**
Cattle																
Cattle for fattening (high complementary feed, low forage)[a]	142	**0.4**	406	**1.0**	533	**1.3**	891	**2.2**	112	**0.3**	437	**1.1**	340	**0.9**	944	**2.4**
Cattle for fattening (low complementary feed, high forage)[a]	182	**0.5**	453	**1.1**	710	**1.8**	1032	**2.6**	136	**0.3**	481	**1.2**	533	**1.3**	1109	**2.8**
Dairy cows[a]	334	**0.5**	970	**1.5**	1221	**1.9**	2050	**3.2**	255	**0.4**	1050	**1.6**	872	**1.3**	2215	**3.4**
Small ruminants																
Lambs for fattening (high complementary feed, low forage)[a]	15	**0.8**	43	**2.1**	57	**2.8**	91	**4.6**	12	**0.6**	47	**2.3**	37	**1.8**	98	**4.9**

TABLE I.3 (Continued)

	WHO_{2022}-TEQ								WHO_{2005}-TEQ							
	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day
Lambs for fattening (low complementary feed, high forage)[a]	20	**1.0**	49	**2.5**	78	**3.9**	112	**5.6**	15	**0.7**	53	**2.6**	58	**2.9**	120	**6.0**
Fish																
Salmonids	0.2	**1.4**	0.3	**2.2**	0.5	**4.4**	0.6	**5.0**	0.2	**2.1**	0.4	**3.1**	0.7	**6.2**	0.8	**7.0**
Rabbits																
Rabbits for fattening	1.6	**0.8**	5.5	**2.8**	3.3	**1.7**	8.1	**4.0**	1.0	**0.5**	5.9	**2.9**	2.3	**1.1**	8.5	**4.3**
Horses																
All categories[a]	218	**0.5**	590	**1.5**	270	**0.7**	648	**1.6**	113	**0.3**	589	**1.5**	137	**0.3**	659	**1.6**

Abbreviations: bw, body weight; LB, lower bound; PCDD/Fs, polychlorinated dibenzo-p-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]For bovines, ovines and horses, complementary feed was complemented with forages.

I.2.2 | Estimated dietary intakes based on compound feeds

TABLE I.4 Estimated exposure to the 17 PCDD/Fs for the considered food-producing animal species and categories using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **compound feeds** (complete feed/complementary feed + forages)

	WHO$_{2022}$-TEQ								WHO$_{2005}$-TEQ							
	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day	Pg WHO-TEQ/ day	Pg WHO-TEQ/ kg bw per day
Pigs																
Piglets	9.4	**0.5**	32	**1.6**	16	**0.8**	57	**2.8**	7.8	**0.4**	37	**1.8**	15	**0.7**	72	**3.6**
Pigs for fattening	17	**0.3**	70	**1.2**	57	**0.9**	159	**2.6**	10	**0.2**	78	**1.3**	47	**0.8**	171	**2.9**
Sows, lactating	99	**0.6**	169	**1.0**	180	**1.0**	560	**3.2**	100	**0.6**	184	**1.1**	96	**0.5**	584	**3.3**
Poultry																
Chickens for fattening	2.2	**1.1**	6.0	**3.0**	5.0	**2.5**	15	**7.6**	1.4	**0.7**	6.1	**3.1**	3.9	**2.0**	17	**8.7**
Laying hens	0.7	**0.3**	3.6	**1.8**	2.4	**1.2**	9.3	**4.6**	0.5	**0.3**	4.3	**2.2**	2.2	**1.1**	12	**6.0**
Turkeys for fattening	6.2	**2.1**	8.8	**2.9**	6.2	**2.1**	8.8	**2.9**	6.8	**2.3**	8.9	**3.0**	6.8	**2.3**	8.9	**3.0**
Fish																
Salmonids	0.2	**1.4**	0.2	**2.0**	0.5	**4.1**	0.5	**4.3**	0.3	**2.2**	0.3	**2.8**	0.8	**6.8**	0.8	**7.1**
Rabbits																
Rabbits for fattening	2.1	**1.1**	4.6	**2.3**	4.5	**2.2**	9.2	**4.6**	1.7	**0.8**	4.9	**2.4**	3.3	**1.6**	9.3	**4.7**
Horses																
All categories[a]	218	**0.5**	636	**1.6**	219	**0.5**	671	**1.7**	109	**0.3**	645	**1.6**	107	**0.3**	692	**1.7**

Abbreviations: bw, body weight; LB, lower bound; PCDD/Fs, polychlorinated dibenzo-p-dioxins and dibenzofurans; TEQ, toxic equivalents; UB, upper bound; WHO, World Health Organization.

[a]For horses, complementary feed was complemented with forages.

APPENDIX J

Congener profiles in complete feed for food-producing animals

The variation in WHO_{2022}/WHO_{2005}-TEQ ratios, whether above or below 1, is primarily driven by differences in congener profiles, specifically the concentrations of individual congeners and the TEFs assigned to them under the 2005 and 2022 WHO-TEF schemes. Congener contributions at the mean LB level, calculated under both WHO-TEF schemes, are illustrated in **Figure J.1** for complete feed for fish (salmonids), and in **Figure J.2** for chickens for fattening. In complete feed for fish, the mean LB ratio was 0.6, indicating a decrease in TEQ concentrations under the updated TEFs. In contrast, the ratio for complete feed for chickens for fattening was 1.2, indicating an increase.

A decrease in TEQ concentrations under the WHO_{2022}-TEFs is observed when the dominant congeners in the samples are those whose TEFs were reduced in the revision. This is clearly demonstrated in the congener profile of complete feed for fish (**Figures J.1**), where PCB-126 (whose WHO-TEF was reduced from 0.1 to 0.05) dominates the exposure. In contrast, an increase in TEQ may occur when congeners with higher WHO-TEFs in 2022 are present in higher concentrations, and their increased weighting offsets the reductions in other congeners. This can be seen in the congener profile of complete feed for chickens for fattening (**Figure J.2**), where 1,2,3,4,7,8,9-HpCDF (WHO-TEF increased from 0.01 to 0.1), 1,2,3,4,6,7,8-HpCDD (from 0.01 to 0.05), and 1,2,3,4,7,8-HxCDF (from 0.1 to 0.3) became more prominent under the 2022 WHO-TEFs.

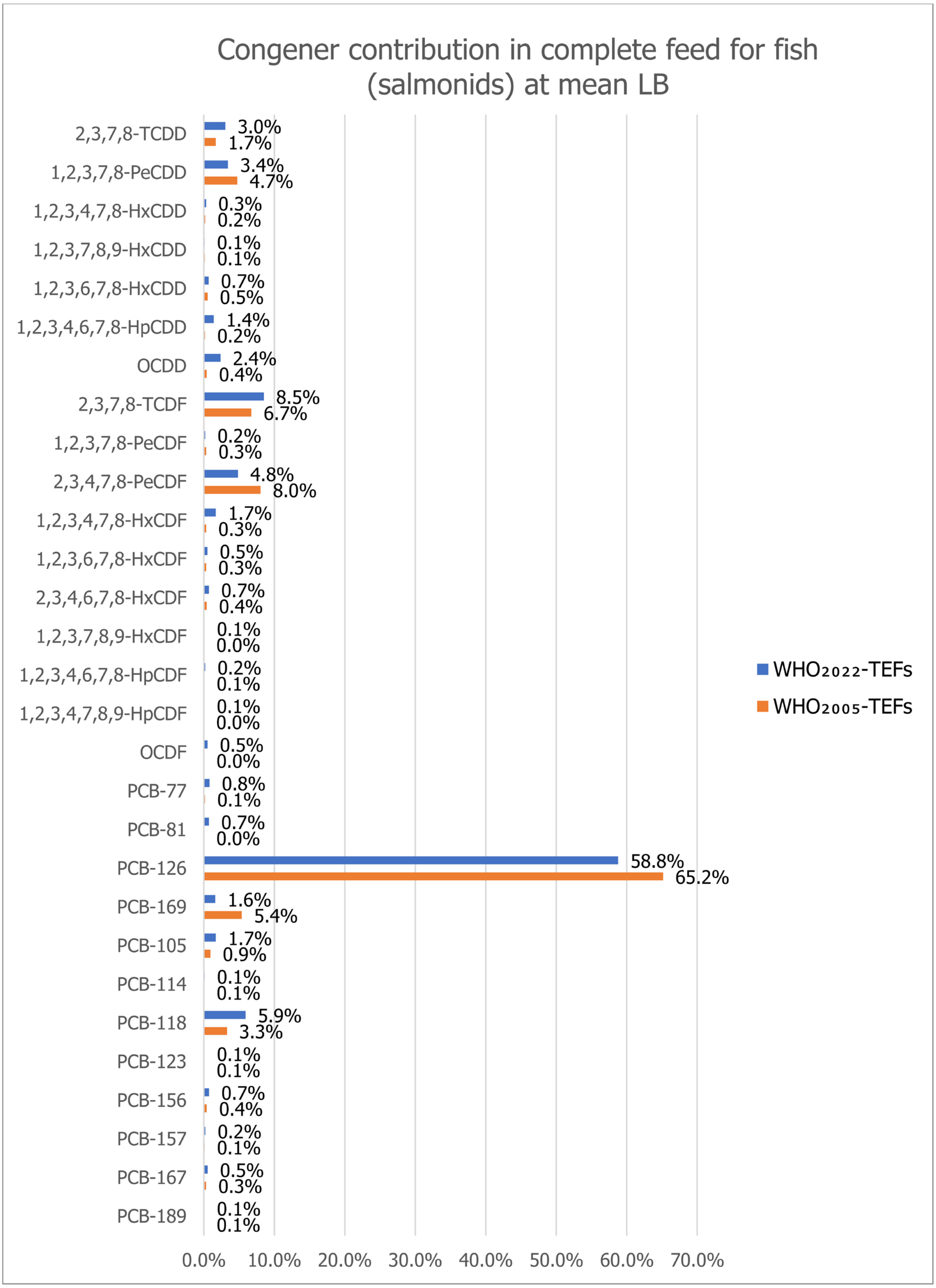

FIGURE J.1 Percentage contribution of each congener, weighted using WHO_{2022} and WHO_{2005}-TEFs, to the overall mean lower-bound (LB) TEQ concentration of complete feed for fish (salmonids), based on the 29 PCDD/Fs and DL-PCBs.

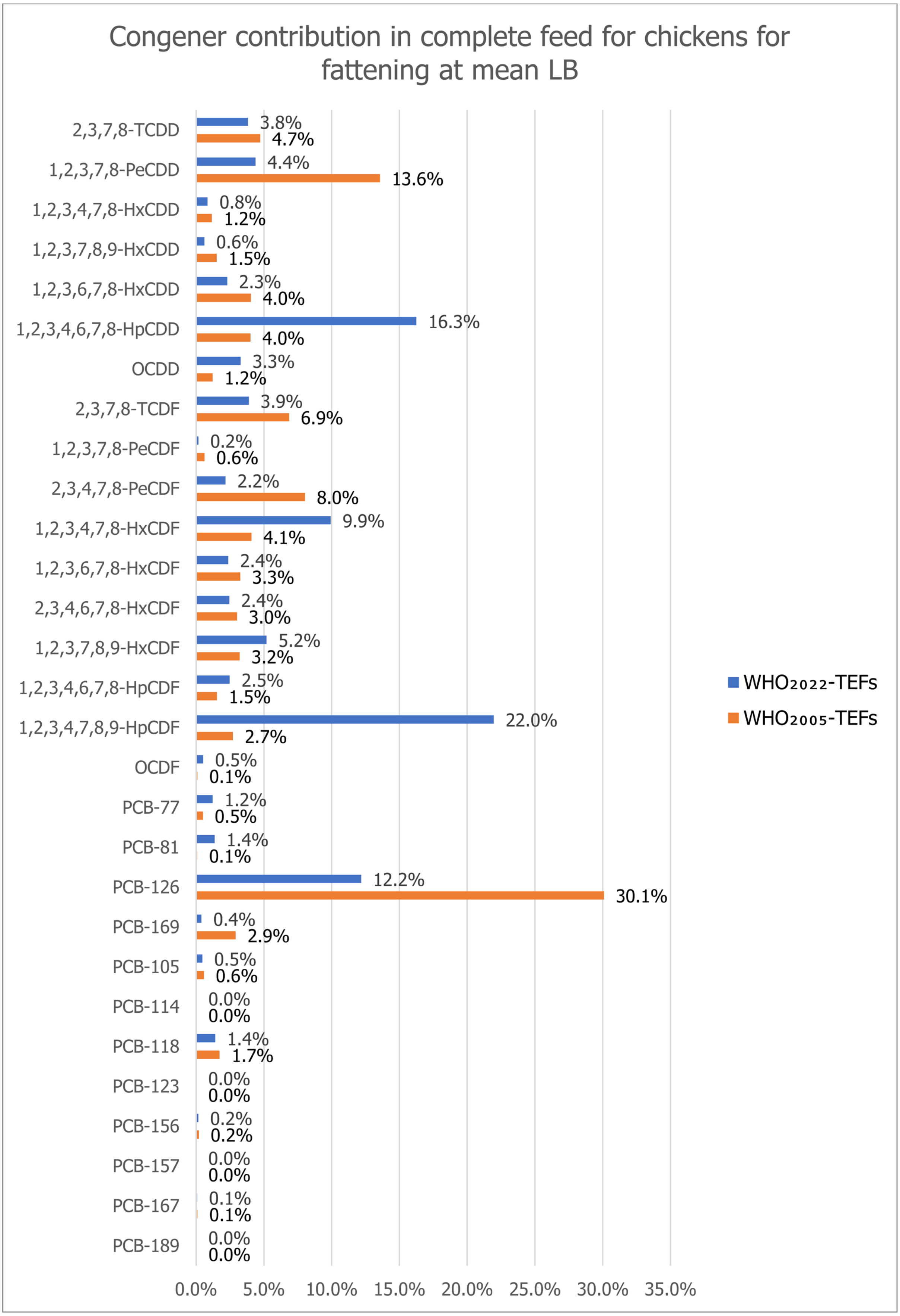

FIGURE J.2 Percentage contribution of each congener, weighted using WHO_{2022} and WHO_{2005}-TEFs, to the overall mean lower-bound (LB) TEQ concentration of complete feed for chickens for fattening based on the 29 PCDD/Fs and DL-PCBs.

ANNEXES

ANNEX A

The protocol for the update of the risk assessment on PCDD/Fs and DL-PCBs in feed and food on the basis of the new 2022 WHO-TEF values

The Annex is provided as a separate pdf file containing the risk assessment protocol applied by the CONTAM Panel to update the previous risk assessments of PCDD/Fs and DL-PCBs in food and feed on the basis of the new 2022 WHO-TEF values, and is available under the Supporting Information section on the online version of the Scientific output.

ANNEX B

Occurrence data on PCDD/Fs and DL-PCBs in food submitted to EFSA, dietary surveys per country and age group, and detailed results of the human chronic dietary exposure assessment to PCDD/Fs and DL-PCBs.

The Annex is provided as a separate excel file, containing the summary statistics of the occurrence data on PCDD/F and DL-PCBs in food submitted to EFSA, and data about the dietary surveys per country and age group available in the EFSA Comprehensive Database used in the dietary exposure assessment, as well as the detailed results of the human chronic dietary exposure assessment to PCDD/Fs and DL-PCBs for the different scenarios, including the contribution of different food groups,' and is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.20084620.

ANNEX C

Occurrence data on PCDD/Fs and DL-PCBs in feed submitted to EFSA

The Annex is provided as a separate excel file, containing the occurrence data on PCDD/Fs and DL-PCBs in feed submitted to EFSA, and the chronic dietary exposure to PCDD/Fs and DL-PCBs for food-producing animals, and is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.20085091.

ANNEX D

Occurrence data in human milk

The Annex is provided as a separate pdf file containing the data of the WHO/UNEP coordinated surveys on the occurrence of PCDD/Fs and PCBs in human milk between 2000 and 2019, and is available under the Supporting Information section on the online version of the Scientific output.

ANNEX E

Transfer of PCDD/Fs and DL-PCBs from feed to food of animal origin

The Annex is provided as a separate excel file, containing the transfer rates and bioconcentrations factors related to the transfer of PCDD/Fs and DL-PCBs from feed to food of animal origin, and is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.20085143.

ANNEX F

Studies in experimental animals and observations in humans since the 2018 Opinion

The Annex is provided as a separate pdf file containing tables with details of the eligible studies in experimental animals and observations in humans, and is available under the Supporting Information section on the online version of the Scientific output.

ANNEX G

BMD modelling

The Annex is provided as a separate pdf file containing the BMD reports of the studies in experimental animals, and is available under the Supporting Information section on the online version of the Scientific output.

ANNEX H

Raw occurrence data on PCDD/Fs and DL-PCBs in food and feed

The raw occurrence data on PCDD/Fs and DL-PCBs in food and feed extracted from EFSA Data Warehouse on the 4th of September 2024 and sampled between years 2013 and 2023 are available on EFSA's Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.20266437

ANNEX I

Outcome of the public consultation

The Annex is provided as a separate pdf file containing the comments received during the public consultation and the replies by the CONTAM Panel, and this is available under the Supporting Information section on the online version of the Scientific output.