Adopted: 31 January 2024

DOI: 10.2903/j.efsa.2024.8640

SCIENTIFIC OPINION

efsa JOURNAL

Risks for animal and human health related to the presence of polychlorinated naphthalenes (PCNs) in feed and food

EFSA Panel name on Contaminants in the Food Chain (CONTAM) | **Dieter Schrenk** | **Margherita Bignami** | **Laurent Bodin** | **James Kevin Chipman** | **Jesús del Mazo** | **Bettina Grasl-Kraupp** | **Christer Hogstrand** | **Laurentius (Ron) Hoogenboom** | **Jean-Charles Leblanc** | **Carlo Stefano Nebbia** | **Evangelia Ntzani** | **Annette Petersen** | **Salomon Sand** | **Tanja Schwerdtle** | **Christiane Vleminckx** | **Heather Wallace** | **Jerzy Falandysz** | **Andrew Hart** | **Martin Rose** | **Maria Anastassiadou** | **Chantra Eskes** | **Petra Gergelova** | **Matteo Innocenti** | **Elena Rovesti** | **Ben Whitty** | **Elsa Nielsen**

Correspondence: contam@efsa.europa.eu

Abstract

EFSA was asked for a scientific opinion on the risks for animal and human health related to the presence of polychlorinated naphthalenes (PCNs) in feed and food. The assessment focused on hexaCNs due to very limited data on other PCN congeners. For hexaCNs in feed, 217 analytical results were used to estimate dietary exposures for food-producing and non-food-producing animals; however, a risk characterisation could not be performed because none of the toxicological studies allowed identification of reference points. The oral repeated dose toxicity studies performed in rats with a hexaCN mixture containing all 10 hexaCNs indicated that the critical target was the haematological system. A $BMDL_{20}$ of 0.05 mg/kg body weight (bw) per day was identified for a considerable decrease in the platelet count. For hexaCNs in food, 2317 analytical results were used to estimate dietary exposures across dietary surveys and age groups. The highest exposure ranged from 0.91 to 29.8 pg/kg bw per day in general population and from 220 to 559 pg/kg bw per day for breast-fed infants with the highest consumption of breast milk. Applying a margin of exposure (MOE) approach, the estimated MOEs for the high dietary exposures ranged from 1,700,000 to 55,000,000 for the general population and from 90,000 to 230,000 for breast-fed infants with the highest consumption of breast milk. These MOEs are far above the minimum MOE of 2000 that does not raise a health concern. Taking account of the uncertainties affecting the assessment, the Panel concluded with at least 99% certainty that dietary exposure to hexaCNs does not raise a health concern for any of the population groups considered. Due to major limitations in the available data, no assessment was possible for genotoxic effects or for health risks of PCNs other than hexaCNs.

KEYWORDS

feed, food, PCNs, Polychlorinated naphthalenes, risk assessment

CONTENTS

SUMMARY

Polychlorinated naphthalenes (PCNs) are members of the class of chlorinated polycyclic aromatic hydrocarbons (Cl-PAHs), based on the naphthalene ring system, where one or more hydrogen atoms have been replaced by chlorine. Most PCNs that were commercially produced exist as mixtures of congeners with melting points ranging from below room temperature to around 200°C.

PCN technical mixtures were used as dielectrics, lubricants, electric cable insulation, preservatives of wood, paper and fabric, cutting and grinding fluids and plasticisers. They were manufactured in several countries between around 1910 and 1980, when the major producers in European and North American countries and Japan voluntarily stopped production.

PCNs are ubiquitous in the environment and are listed as persistent organic pollutants (POPs) under the Stockholm Convention.

Comparisons between studies should be made with care due to the differences in and reliability of methods used for the determination of the PCNs, the individual PCNs measured and the sampling and geographical origin of the samples.

No information on the toxicokinetics of PCNs following oral exposure is available for humans. Limited studies in experimental animals indicated that PCN congeners from the mono- to hexa- homologue groups are well absorbed when administered orally in a range of doses, both as individual congeners or in mixtures, including commercial formulations. The absorption of the two heptaCNs and the octaCN was less efficient.

Absorbed PCNs were readily distributed to organs and tissues. Maternal transfer to amniotic fluid and placenta has been reported and PCNs have also been detected in fetal tissues.

PCNs showed increasing resistance to enzymatic oxidation due to steric hindrance and increase in the number of substituted chlorines. The hexaCNs showed the slowest turnover.

Mono- to tetraCNs were largely and readily metabolised and excreted in the urine in the form of hydroxylated PCNs (chloronaphthols) and phase II metabolites. The higher chlorinated PCNs were metabolised to a lesser extent and excreted in faeces both as the parent compounds and as metabolites.

A number of oral repeated dose toxicity studies have been performed in rats predominantly with hexaCNs. Most studies were performed with a hexaCN mixture containing all 10 hexaCNs as well as a heptaCN (PCN-73) (composition: hexaCNs at 94.1%, i.e. PCN-66/67 at 81.2%, PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4% and PCN-73 at 5.9%). This mixture will be referred to as 'hexaCN mixture' below. The studies have shown that the haematological system, liver and thymus are target organs in rats. Developmental effects were also reported.

Liver effects were reported in three subacute toxicity studies performed with hexaCNs (two studies with the 'hexaCN mixture'; one study with PCN-66 and PCN-67) and in one study performed with a PCN mixture. A study which aimed to investigate the effects of the 'hexaCN mixture' on blood coagulation and fibrinolysis indicated that hexaCNs may disturb both coagulation and fibrinolysis processes, as well as decreasing the platelet count. In a study with PCN-43, no effects were reported.

In a study aimed at investigating the subchronic toxicity of the 'hexaCN mixture' and its effect on selected liver and haematological parameters, female Wistar rats were given doses of 0, 0.03, 0.1 or 0.3 mg/kg bw per day by gavage for 90 days. The absolute and relative liver weights were increased, and the absolute and relative thymus weights were decreased at the highest dose. On microscopic examination, the only change noted was fatty degeneration in the liver at the highest dose. Haematological changes included decreased red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin at the high dose, an increased red cell distribution width at the mid and high dose, a decrease in the platelet (PLT) count at the mid and high dose and an increased mean platelet volume at the high dose. A no observed adverse effect level (NOAEL) of 0.03 mg/kg bw per day was identified based on the decreased PLT count.

In developmental toxicity studies, maternal effects reported included liver effects in a study performed with the 'hexaCN mixture' and in a study performed with a PCN mixture and effects on the haematological system in the study performed with a PCN mixture. Developmental effects included increased intrauterine mortality of embryos and fetuses ('hexaCN mixture', PCN mixture), decreased fetal body weight and length ('hexaCN mixture', PCN mixture) and delayed ossification (PCN-43, 'hexaCN mixture', PCN mixture).

In a study aimed at investigating the subacute neurotoxicity of the 'hexaCN mixture', behavioural tests indicated that spontaneous locomotor and exploratory activities were decreased, long-term memory was impaired, pain sensibility was enhanced and the level of stress-dependent analgesia was decreased.

No reproductive toxicity or chronic toxicity/carcinogenicity studies were retrieved in the literature search.

Four PCN congeners (PCN-1, PCN-2, PCN-27 and PCN-75) tested in four strains of Salmonella Typhimurium with and without metabolic activation were not mutagenic. However, PCN-1 showed to be weakly mutagenic in TA100 and TA97 in the presence of a specific type of metabolic activation system (Syrian hamster S9). The 'hexaCN mixture' investigated in an in vivo micronucleus bone marrow test after oral administration for 90 days did not induce clastogenic effects. The extremely limited information on genotoxicity of PCNs does not allow to conclude on their genotoxic potential.

None of the retrieved human studies were considered relevant for this mandate. Therefore, the human risk assessment was based on data from studies in experimental animals.

Overall, the considerable decrease in the PLT count reported at the mid and high dose (magnitude of changes –28%, –48%, respectively) in the 90-day oral study performed with the 'hexaCN mixture' in rats was considered as the critical effect for the human risk assessment. The NOAEL for the decreased PLT count was 0.03 mg/kg bw per day.

Benchmark dose (BMD) modelling on the decreased PLT count reported in the 90-day study with the 'hexaCN mixture' was performed in agreement with the 2022 EFSA Guidance on the use of the BMD approach in risk assessment. A benchmark response (BMR) of 20% was agreed for the decreased PLT count. Based on the results, a $BMDL_{20}$ of 0.05 mg/kg bw per day was identified for the 'hexaCN mixture'.

The majority of studies on PCNs carried out in food-producing animals have used technical mixtures (e.g. Halowax). While data from such studies are useful for the hazard identification, they are less so for hazard characterisation.

PCN toxicity to food-producing animals was first observed in cattle, where hyperkeratosis is potentially linked to a drastic drop in serum vitamin A concentrations following exposure. Despite the studies being numerous, the reporting does not adhere to current standards and data cannot be used to derive a reference point.

PCN toxicity presents differently in sheep compared with cattle and was primarily not associated with vitamin A deficiency and hyperkeratosis. Early signs of toxicity were excess nasal discharge, weakness, weight loss and loss of appetite. Gross pathology included liver damage, gastrointestinal haemorrhage, as well as pleural and/or peritoneal fluid. Histological examination revealed non-specific effects in multiple organs.

Oral PCN exposure in pigs at lethal doses caused a decrease in plasma vitamin A concentration, but this returned to normal range prior to death following cessation of exposure. Gross lesions included liver damage and thickening of the intestinal wall. Histological examination revealed non-specific effects in multiple organs. Hyperkeratosis is not observed in pigs exposed to PCNs.

Studies in turkeys and chickens showed dose-dependent effects on survival as well as a range of performance parameters of importance for food-producing animals. Necroscopy revealed enlarged fibrous liver, fluid in the body cavity and pericardial oedema. Plasma uric acid concentration was significantly increased and haematocrit significantly decreased in exposed animals. PCNs affect serum vitamin A concentrations in poultry, but there is no obvious association with adversity.

The few studies in fish carried out with different dietary exposures to PCNs showed no adverse effects at any of the doses tested while induction of *cyp1a* gene expression, protein levels or enzymatic activity (7-ethoxyresorufin-O-deethyl ase, EROD) confirmed exposure. For fish, it was possible to identify a NOAEL of 32.8 mg PCN-52/kg feed (dry weight) (the highest dose tested), but risk characterisation for PCN-52 in fish is not possible due to a lack of exposure data.

There are no studies in other food-producing animals or in non-food-producing animals that allow identification of Reference Points for single PCN congeners or for mixtures of congeners with the same number of substitutions (e.g. hexaCN mixtures).

Regarding the mode of action, several PCNs activate the aryl hydrocarbon receptor (AHR) pathway (show dioxin-like activity) with the PCN-70 and PCN-66 being the most potent. Compared with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), they are weak AHR agonists with relative potencies (mostly derived from reporter gene assays) ranging from ~ 10^{-7} to 10^{-3}. Some of these values are higher than those for some dioxin-like polychlorinated biphenyls (PCBs) with established toxic equivalency factors (TEFs). Together with the very low exposure to PCNs, the Panel considered that it is highly unlikely that PCNs contribute substantially to the overall exposure to toxic equivalents (TEQs).

Like other chemicals with dioxin-like toxicity, exposure to PCN-66 and PCN-67 causes thymus atrophy. PCNs may also activate the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) and most of the effects observed in the liver might be explained by activation of AHR, CAR and PXR. This would include induction of metabolic enzymes, increase in malondialdehyde concentrations indicative of lipid peroxidation and oxidative stress and changes in retinoic acid metabolism. Mode of action data are insufficient to explain the effects of PCNs on the haematological system, fetal development and the nervous system.

A total of 9111 PCNs analytical results (371 samples) generated mostly by the gas chromatography (GC)-based methods in food analysed for 71 PCN congeners fulfilled the quality criteria applied and were considered in the assessment. Out of these, 2317 hexaCNs analytical results analysed in 371 samples on food were available in the final cleaned data set. The left-censored data accounted for 47% of the occurrence values. Among the food categories in which hexaCNs contamination could be expected, the highest percentage of quantified data was found in food categories 'Eggs and egg products', 'Fish, seafood, amphibians, reptiles and invertebrates', 'Milk and dairy products' and 'Meat and meat products'. The highest mean concentration was reported for 'Whole eggs' ranging from 0.002 ng/kg for PCN-70 at the lower bound (LB) to 5.18 ng/kg reported for PCN-69 at the upper bound (UB), followed by 'Diadromous fish' ranging from 0.001 ng/kg for PCN-70 at the LB to 1.51 ng/kg reported for PCN-71/72 at the UB. Occurrence data on hexaCNs in European human milk were taken from pooled samples that were collected and analysed as part of the WHO/UNEP field studies in 2016 and 2019. The average levels ranged between < limit of quantification (LOQ) (PCN-65 and PCN-70) and 49.1 ng/kg lipid (PCN-66/67).

The highest mean human dietary exposure to hexaCNs across all congeners was estimated for the young age groups, with the tendency to decrease moving to the older age groups. Regarding the individual (or pairs of) hexaCNs exposure scenarios, the highest mean exposure across the European dietary surveys was estimated for PCN-69 scenario A (exposure scenario including one high PCN-69 concentration of 313 ng/kg measured in egg sample). The mean exposure ranged from 0.03 ('Adolescents' and 'Very elderly') to 5.61 pg/kg bw per day ('Toddlers') for the minimum LB, and the maximum UB, respectively, and the P95 exposure ranged from 0.06 ('Elderly') to 19.4 pg/kg bw per day ('Toddlers') for the minimum LB and the maximum UB, respectively. The food categories contributing mostly to the overall exposure varied across the hexaCNs; consumption of 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Meat and meat products' in the adult population groups, and 'Milk and dairy products', in infants contributed the most to the dietary exposure to PCN-66/67, while for other individual hexaCNs, the food category 'Eggs and egg products' together with 'Fish, seafood, amphibians, reptiles and invertebrates' made the most important contribution.

Regarding the 'mixture scenario', the highest exposure levels across the European dietary surveys were estimated for young population groups for an exposure scenario including one high PCN-69 concentration of 313 ng/kg measured in egg sample (scenario A). The mean exposure ranged from 0.32 ('Elderly') to 11.5 pg/kg bw per day ('Toddlers') for the minimum LB and the maximum UB, respectively. The P95 exposure ranged from 0.91 ('Elderly') to 29.8 pg/kg bw per day ('Toddlers') for the minimum LB and the maximum UB, respectively. The food categories 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Eggs and egg products' in the adult age groups, and 'Milk and dairy products', in infants contributed the most to the dietary exposure in the 'mixture scenario'.

An exposure scenario for high and frequent consumers of fish meat, resulted in 95th percentile dietary exposure to PCN-66/67 (calculated for consumers only) being approximately twofold higher in comparison to total population with the maximum UB exposure level estimated for 'Toddlers' (11.1 pg/kg bw per day).

An exposure scenario for breastfed infants resulted in median daily exposure estimates for average human milk consumption of 226 pg/kg bw per day for PCN-66/67 and 270 pg/kg bw per day for the 'mixture scenario'. When considering high consumption amount of human milk, the median daily exposure was at a level of 340 pg/kg bw per day for PCN-66/67 and 405 pg/kg bw per day for the 'mixture scenario'.

A total of 1467 PCNs analytical results (31 samples) in feed analysed for 70 PCN congeners fulfilled the quality criteria applied and were considered in the assessment. Out of these, 217 hexaCNs analytical results analysed in 31 samples of feed were available in the final cleaned data set. The hexaCNs occurrence data were very limited for single (or pairs of) congeners and across feed categories, in many cases with all left-censored data. Therefore, the occurrence data were merged, and the CONTAM Panel opted for an animal exposure assessment based on results from the hexaCN homologue group. Only three feed categories, including 'Oil seeds, oil fruits, and products derived thereof (feed)', 'Forages and roughage, and products derived thereof (feed)' and 'Compound feed (feed)' could be considered. Out of these, the highest percentage of quantified data was observed for 'Compound feed' and the highest mean hexaCN concentration was measured for dog pet food (LB–UB: 2.19–2.20 ng/kg).

Dietary exposure for food-producing and non-food-producing animals was performed using a scenario based on model diets and feed materials. Forages were also included for ruminants and horses. Exposure was performed using either a mean or a high exposure scenario (using the highest reliable percentile based on the number of samples available). It was not possible to use compound feeds (complete and/or complementary) to assess dietary exposure due to the lack of data. The animal categories with the highest modelled concentrations of hexaCNs in ng/kg complete feed (expressed in 88% dry matter) were rabbits for fattening, followed by turkeys and ducks for fattening, with concentrations of 0.133, 0.073 and 0.064 ng/kg feed, respectively, using high (UB) occurrence. The CONTAM Panel noted that the occurrence data available in the EFSA database relate to ingredients which constitute up to a maximum of 27% of the model diet (for salmon) and as little as 4% of the model diet for dog. For the remainder diet fractions, no information was available on PCN concentrations.

The CONTAM Panel concluded that due to the limitations and uncertainties in the current database on PCNs, the derivation of a health-based guidance value (HBGV) was not appropriate. Instead, the Panel applied a margin of exposure (MOE) approach to assess a possible health concern. In the discussion of the MOE above which no concern for human health would arise, the Panel started by taking into consideration the default assessment factors for interspecies differences with respect to toxicokinetics and toxicodynamics between humans and rats (factor of 10), for the intraspecies differences in humans (factor of 10) and for the shorter duration of the key study (90 days) compared to a lifetime exposure (factor of 2). The analysis of uncertainties related to the hazard identification and characterisation of PCNs indicated that an additional factor was warranted. Considering the results of the uncertainty analysis, the CONTAM Panel agreed that an additional factor of 10 was appropriate. As a result, the Panel considered that MOEs ≥ 2000 are sufficient to conclude that the current dietary exposure to hexaCNs does not raise a health concern.

The MOEs for the 95th percentile of dietary exposures ranged from 1,700,000 in toddlers to 55,000,000 in elderly (after rounding) and are far above the minimal MOE of 2000. Thus, the current high dietary exposure to hexaCNs does not raise a health concern for these population groups. For breast-fed infants, the estimated MOEs for the highest consumption of breast milk ranged from 90,000 to 230,000 (after rounding) and are far above the minimal MOE of 2000. Thus, the current highest consumption of breast milk does not raise a health concern. No LB and UB estimates were done for the mixture of PCNs from human milk because of the very small sample size (7 pooled samples in total). Because the samples are pooled, they do not take into account the variation between individuals. This has been taken into account in the uncertainty analysis.

There are no studies in food-producing animals or in non-food-producing animals that allow identification of Reference Points for single PCN congeners or for mixtures of congeners with the same number of substitutions (e.g. hexaCN mixtures). Thus, it was not possible to carry out risk characterisation or uncertainty analysis.

An uncertainty analysis was performed for human risk assessment. The risk assessment was affected by considerable non-standard uncertainties, including major limitations in the available database for hazard assessment and limited or lacking occurrence data for many important food categories. The impacts of the non-standard uncertainties affecting the exposure and hazard assessments were quantified using expert knowledge elicitation and combined by probability bounds analysis. Additional uncertainties affecting risk characterisation were taken into account when assessing the overall uncertainty. It was concluded that, even though some of the uncertainties were substantial, the MOEs were so large that they would still not raise a health concern when all the uncertainties were taken into account. Based on the results of the uncertainty analysis, the CONTAM Panel concluded with at least 99% certainty that the current dietary exposure to the hexaCNs would not raise a health concern for any of the population groups and surveys considered in the assessment,

including breast-fed infants. The extremely limited information on genotoxicity of PCNs did not allow the CONTAM Panel to conclude on their genotoxic potential or make any useful quantification of its uncertainty.

Recommendations

- To enable a more robust exposure assessment, analytical methods are needed (i) with improved sensitivity to reduce the amounts of left-censored data and (ii) with improved selectivity to separate coeluting PCN congeners.
- Data providers should be encouraged to submit further occurrence data in food and feed, in particular in different fish species and in infant formulae.
- To monitor PCN occurrence in eggs and other edible products from food-producing animals raised on PCN contaminated soil or in the proximity of other PCN sources.
- When submitting PCN occurrence data to EFSA, it is recommended to provide adequate information on the feed samples analysed. This refers to reporting sufficient details on the samples analysed (e.g. target animals for the complete/complementary compound feed).
- Because the current dietary exposure does not raise a health concern for humans, there is no need for additional experimental animal studies to assess the risk in humans.
- There is a need for non-animal studies to support the assessment of adverse effects of PCNs in food-producing and non-food-producing animals.
- It is recommended that EFSA develops a harmonised guidance to allow extrapolation of data from experimental animals to food-producing and non-food-producing animals.
- PCNs are a small subset of a wider group of halogenated polycyclic aromatic hydrocarbons. There is very little information available about this wider class of compounds, and it is recommended that the risk for animal and human health related to the presence of polyhalogenated PAHs other than PCNs in feed and food is assessed. The initial approach to investigating potential risk associated with this wider group may include studies to identify which compounds should be prioritised on the basis of (i) toxicity and (ii) occurrence in food and the environment.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

Background

Polychlorinated naphthalenes (PCNs) are a group of 75 congeners of the class of chlorinated polycyclic aromatic hydrocarbons. They originate through release from older electrical equipment, inadvertent contamination in industrial chemicals and from combustion processes such as incineration.

PCNs have been shown to be highly bio-accumulative and available data in feed and food show widespread occurrence in feed and food.

Toxicological studies indicate that PCNs interact with the Ah receptor. Therefore, it is expected that exposure to PCNs could result in a pattern of biochemical and toxic responses typical for dioxin-like compounds and therefore PCN toxicity is likely to add to the cumulative toxicity of other dioxin-like compounds.

Terms of Reference

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002, the Commission asks EFSA for a scientific opinion on the risks for animal and human health related to the presence of polychlorinated naphthalenes (PCNs) in feed and food.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the request by the European Commission on the risks for animal and human health related to the presence of PCNs in feed and food. The risk assessment focuses on all the 75 theoretically possible chlorinated naphthalenes. This opinion does not consider naphthalene, brominated naphthalenes or chloro/bromo naphthalenes.

1.3 | Supporting information for the assessment

1.3.1 | Physicochemical properties

Polychlorinated naphthalenes (often called chlorinated naphthalenes, or PCNs) are members of the class of chlorinated polycyclic aromatic hydrocarbons (Cl-PAHs), based on the naphthalene ring system, but where one or more hydrogen atoms have been replaced by chlorine. The generic molecular formula is $C_{10}H_{8-n}Cl_n$, where $n = 1–8$. There are 75 possible chlorinated naphthalenes (See Appendix A), and they are usually identified using the numbering system shown below (Figure 1):

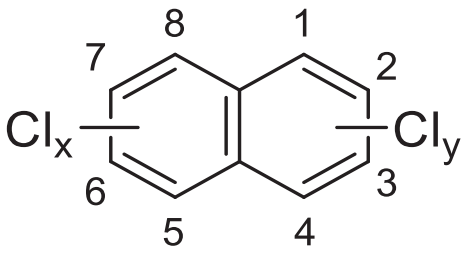


FIGURE 1 Basic structure and congener distribution of PCNs.

Homologue group	No. of individual congeners
Monochloronaphthalenes (monoCNs)	2
Dichloronaphthalenes (diCNs)	10
Trichloronaphthalenes (triCNs)	14
Tetrachloronaphthalenes (tetraCNs)	22
Pentachloronaphthalenes (pentaCNs)	14
Hexachloronaphthalenes (hexaCNs)	10
Heptachloronaphthalenes (heptaCNs)	2
Octachloronaphthalene (octaCN)	1

Most PCNs that were commercially produced exist as mixtures of congeners with melting points ranging from below room temperature to around 200°C. Liquid PCNs are soluble in most organic solvents, whereas the waxy or solid PCNs are soluble in chlorinated solvents or aromatic solvents (Crookes & Howe, 1993). All PCNs are lipophilic and concentrated within fatty tissues and are thus associated with fat in foods. PCNs have low flammability and are of medium to low volatility, volatility decreasing with increasing chlorination (Kover, 1975). Thus, the physicochemical properties of PCNs are comparable to those of other persistent organic pollutants (POPs), e.g. polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and chlorinated paraffins (CPs) (EFSA CONTAM Panel, 2011, 2018, 2020).

Single congeners of PCNs are commercially produced only as analytical standards and for research purposes, with the exception of monoCNs.[1]

Some of the relevant physical and chemical properties of individual PCN congeners and commercial PCN mixtures are listed in Tables A.1 and A.2, respectively (See Appendix A).

Table 1 below presents the PCN congeners tested in the toxicological studies (see Sections 3.1.2 and 3.1.4).

TABLE 1 Physicochemical properties of selected PCN congeners identified as important for this Opinion.

PCN	CAS number	Chlorinated naphthalene	Relative molecular mass (g/mol)	mp (°C)[a]	Log *Ko*/w
PCN-43	31604-28-1	1,3,5,8-tetraCN	266	131	5.5 ± 0.4[b]; 5.8[a]; 6.0[a]
PCN-52	53555-65-0	1,2,3,5,7-pentaCN	300	171	6.3 ± 0.4[b]; 6.9[c]; 8.7[d]
PCN-63	58877-88-6	1,2,3,4,5,6-hexaCN	335	–	6.2 ± 0.5[b]; 10[d]
PCN-64	67922-27-4	1,2,3,4,5,7-hexaCN	335	194	6.3 ± 0.5[b]; 7.6[c]; 9.8[d]
PCN-65	103426-93-3	1,2,3,4,5,8-hexaCN	335	–	6.1 ± 0.6[b]; 10[d]
PCN-66	103426-96-6	1,2,3,4,6,7-hexaCN	335	–	6.4 ± 0.4[b]; 9.7[d]
PCN-67	103426-97-7	1,2,3,5,6,7-hexaCN	335	–	6.4 ± 0.4[b]; 9.7[d]
PCN-68	103426-95-5	1,2,3,5,6,8-hexaCN	335	–	6.1 ± 0.5[b]; 9.8[d]
PCN-69	103426-94-4	1,2,3,5,7,8-hexaCN	335	–	6.4 ± 0.5[b]; 9.8[d]
PCN-70	17062-87-2	1,2,3,6,7,8-hexaCN	335		6.0 ± 0.5[b]
PCN-71	90948-28-0	1,2,4,5,6,8-hexaCN	335	175–177	6.0 ± 0.6[b]; 7.0[e]; 9.9[d]
PCN-72	103426-92-2	1,2,4,5,7,8-hexaCN	335	–	6.1 ± 0.6[b]; 9.9[d]
PCN-73	58863-14-2	1,2,3,4,5,6,7-heptaCN	369	160–162	6.4 ± 0.5[b]; 7.7[e]

[a]WHO (2001).
[b]SciFinder; predicted value for partition co-efficient.
[c]Indicates estimated value.
[d]Harner & Bidleman (1998).
[e]Burreau et al. (1997).

1.3.2 | Production and industrial use

Main sources of PCNs are industrial production, formation as by-products in the manufacturing of other chemicals and industrial processes and formation as unintentionally products in combustion processes (Fernandes et al., 2017). No information is found relating to possible natural sources of PCNs (WHO, 2001).

PCN formulations

PCN manufacture took place in several countries in the past (between 1910 and 1980). In the 1980s, the major producers in European and North American countries and Japan, voluntarily stopped the manufacturing of PCNs (Klimczak et al., 2023).

Precise data on the past manufacture of PCNs are in practice non-existing and largely unavailable regardless of a country or manufacturing company.

The quantity of PCNs manufactured globally has been estimated to be in the range from 150,000 to 400,000 metric tonnes (Falandysz, 1998; Falandysz & Fernandes, 2020). The manufacture of PCNs took place mainly in the countries such as Germany, Great Britain, France, Italy, Poland, USA, Soviet Union and Japan (Klimczak et al., 2023).

In the USA, PCNs were manufactured at least from 1912 and up to 1980. A total of between 50,000 and 150,000 tonnes of PCNs have been manufactured in the US from 1910 to 1960. By 1980 PCNs manufacture in the USA discontinued, but about 15,000 tonnes of PCNs were still imported in 1980-1981. In Germany, PCNs were manufactured until 1983, but around 300 tonnes of PCNs were still used in 1984, mainly as dye intermediates. In the UK, the manufacture stopped in the mid-1960s, although it was reported that in the 1970s, small amounts of PCNs were still being manufactured and were stockpiled for several decades after that date. In Japan, about 4000 tonnes of PCNs were manufactured between 1940 and 1976 and the manufacture and use of PCNs were banned in 1979 (Klimczak et al., 2023)

[1]https://chm.pops.int/Implementation/NationalImplementationPlans/Guidance/NewlyDevelopedGuidance/GuidanceforPCN/tabid/6231/Default.aspx

Production of PCNs, as of PCBs, in the former Soviet Union is classified as state secret. PCN (mono- through tri- to highly chlorinated PCN) and Halowax (composition unknown) were available in the Soviet Union (Klimczak et al., 2023).

Total volume of PCNs manufactured in Poland in the period of 1936–1939 was 47 tonnes of PCN-1 and 36 tonnes of Woskol and estimated total output in the period of 1951–1987 was 555 tonnes for PCN-1 and 333 tonnes for Woskol (Klimczak et al., 2023).

PCNs were produced by catalysed chlorination of naphthalene as technical mixtures commercialised under different names (e.g. Halowax, N-Oil and N-Waxes, Basileum SP-70, Perna wax, Nibren waxes, Naphthalin–Xylamon, Seekay waxes, Clonacire waxes, Hodogaya Amber, Nankai and Tokyo Ohka waxes, Wako-PCN, Monochloronaphthalene, Woskol product and Cerifal Materials) (Klimczak et al., 2023). Each mixtures series (e.g. Halowax series) differed in the chlorination degree of the naphthalene molecule resulting in different congeners present in the mixture (Falandysz et al., 2000, 2008; Falandysz et al., 2006a, 2006b; Noma et al., 2004). Production was voluntary suspended by the companies in the 1980s in most countries (Cui et al., 2018; Fernandes et al., 2017). Some international trade continued as a result of stock piling after production ceased and due to the lack of awareness of some companies of the voluntary suspension (Falandysz et al., 2008; Yamashita et al., 2003). PCN technical mixtures were used as dielectrics, lubricants, electric cable insulation, preservatives of wood, paper and fabric, cutting and grinding fluids and plasticisers (Klimczak et al., 2023).

By-products in other chemicals and industrial processes

Another source of PCNs is their formation as by-products in other industrial chemicals. The presence in commercial PCB mixtures has been reported by several authors (Falandysz, 2007; Haglund et al., 1993; Taniyasu et al., 2005; Yamashita et al., 2000). The total amount released from this source is roughly estimated as < 0.1% of the total global production of PCNs (Klimczak et al., 2023). Other industrial processes that can be sources of PCNs are unintentional emissions associated with the chloro-alkali industry, use of chlorine gas for the manufacture of chloroparaffins, chlorinated methanes, trichloroethene and tetrachloroethene, chlorinated tap water and refining of metals (Fernandes et al., 2017; Klimczak et al., 2023).

Combustion processes

PCNs can be formed in thermal processes, the most important of which seem to be waste incineration, cement kilns co-processing with municipal solid waste incineration, hazardous waste incineration, in forest and savannah fires and when burning firewood and coal in private dwellings (Klimczak et al., 2023; Lee et al., 2005; Wang et al., 2017; Wyrzykowska et al., 2009). They have been detected in fly ash and flue gas of incineration processes, showing significant differences in patterns and concentrations, depending on the conditions of the process (Fernandes et al., 2017). All 75 PCN congeners have been identified in various matrices. Congeners absent or occurring in trace concentrations in technical PCN and PCB mixtures (so called combustion related PCN-13, PCN-26, PCN-29, PCN-44 and PCN-54) have been found in emissions such as fly ash, flue gas and furnace bottom ashes. These are related to waste and coal combustion from industries that use thermal processes including metal ore smelting and refining, secondary metal smelting processes or cement and coke production. They can also be found in environmental samples such as pine needles, lake sediment cores and soil (Hanari et al., 2004; Hanari, Falandysz, Nakano, et al., 2013; Hanari, Falandysz, Petrick, et al., 2013; Horii et al., 2004; Liu et al., 2014; Orlikowska et al., 2009; Wyrzykowska et al., 2007, 2009). The mechanism of PCNs formation during thermal processes was reviewed by Liu et al. (2014) and formation pathways resulting from de novo synthesis during combustion have been documented suggesting that the chlorination of the naphthalene occurs on the active surface of fly-ash (Fernandes et al., 2017). A study of sediment cores from Jiaozhou bay (China) showed that the contribution from the municipal solid waste incineration source of PCNs has been more important in more recent years than other sources (Pan et al., 2012). Unintentional industrial emission of PCNs appears to be the main source in human milk in China (Li et al., 2020).

1.3.3 | Environmental levels and fate

Care should be taken when comparing reports of PCNs because of differences in congeners measured, sensitivity of analysis and treatment of compounds not detected, etc., as for other POPs with multiple congeners of potential interest. This section does not claim to be complete but gives an overview of some aspects relating to the environmental fate and levels of PCNs. Concentrations in liquid or solid materials are generally expressed on a whole (or fresh or wet) weight basis, unless specified as on a dry or fat weight basis. Concentrations in air are generally expressed on a volume basis.

In addition to reporting concentrations for individual (or paired) congeners, or homologue groups, some authors used toxic equivalency factors (TEFs) which are used to estimate the contribution to overall dioxin-like toxicity. This involves multiplying the concentration of an individual congener by a factor (TEF) that relates to the toxicity of that congener relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic congener with a reference value TEF of 1. These are summed to give an overall toxic equivalence (TEQ) for a sample. For PCNs, TEF values have been proposed by several authors and can be taken from e.g. Behnisch et al. (2003), Blankenship et al. (2000), Hanberg et al. (1990), Villeneuve et al. (2000), Villeneuve et al. (2002). The reported ranges of combined in vitro and in silico relative potencies for selected individual PCN congeners as reported from different studies have been tabulated by Fernandes et al. (2017) (see Section 3.1.5). Where authors have estimated toxic equivalents, the overall contribution to dioxin-like toxicity from PCNs is generally much lower when compared to the contribution made by dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) in the same samples.

All 75 PCN congeners from PCN-1 to PCN-75 have been found in some man-made materials including both the intentionally synthesised technical PCN formulations and other chemicals, as well as products of high-temperature thermal processes (fly ash and other emissions from the waste combustion, cement production, metal ore and scrap metal smelting, etc.) (Dong, Zhang, et al., 2022; Hanari, Falandysz, Nakano, et al., 2013; Hanari, Falandysz, Petrick, et al., 2013; Liu et al., 2014). As a consequence of environmental pollution, PCNs are found in environmental matrices such as topsoil, sediments, surface water, vegetation and animals (Helm et al., 1999; Horii et al., 2004; Ieda et al., 2011; Orlikowska et al., 2009), and also in foods and feeds (Wang, Han, Cao, & Yan, 2022; Wang, Zhang, Wang, et al., 2022) all over the globe.

1.3.3.1 | *Degradation*

Little information is reported about the biodegradation of PCNs. Most studies reported biodegradation of low chlorinated congeners, such as PCN-1 and PCN-2, by *Pseudomonas* (WHO, 2001). PCNs were degraded into hydroxylated derivatives (Mori et al., 2009). Biodegradation has also been reported by white-rot fungus *Phlebia lindtneri* (Pilát) Parmasto (current name *Lilaceophlebia tremelloidea* (Bres.) Zmitr.)[2] for PCNs with one to four chlorine atoms in the molecule (Mori et al., 2003, 2009). Different hydroxylated and dihydroxylated metabolites were identified (Mori et al., 2009). Järnberg et al. (1999) did not find any measurable change in the congener composition of tetra- to hexaCNs (Halowax 1014) in a 28-day aerobic degradation experiment.

The PCN-66/67 and PCN-73 congeners have been found to be the most resistant to photolysis, and PCN-52/60 and PCN-66/67 were formed in the field as a result of photodegradation of a higher PCN (Hanari et al., 2020).

1.3.3.2 | *Bioaccumulation*

PCNs are absorbed by biota from food, water and air. Some of them, like tetra- to heptaCN congeners (PCN-42, PCN-52, PCN-58, PCN-60, PCN-61, PCN-64, PCN-66, PCN-67, PCN-68, PCN-69, PCN-71, PCN-72, PCN-73 and PCN-74) show prolonged retention and biomagnification in natural food webs due to the lack of two vicinal hydrogens within their molecular structures. Most of the 75 congeners having from two to six vicinal positions unsubstituted with chlorine, i.e. having vicinal hydrogens, are more easily metabolised and eliminated (Falandysz, 2003; Falandysz et al., 2001). Bioaccumulation and biomagnification of PCNs have been confirmed in studies of wildlife from different trophic levels worldwide (Corsolini et al., 2002; Cui et al., 2018; Falandysz et al., 1998; Falandysz & Rappe, 1996; Falandysz, Strandberg, et al., 1996; Falandysz, Strandberg, Bergqvist, Strandberg, & Rappe, 1997; Falandysz, Strandberg, Strandberg, Bergqvist, & Rappe, 1997; Falandysz, Strandberg, Strandberg, & Rappe, 1997; Lundgren et al., 2002; Nfon et al., 2008). Falandysz & Rappe (1996), Falandysz et al. (1996) and Falandysz, Strandberg, Strandberg, and Rappe (1997) studied accumulation and transfer of PCNs in plankton, blue mussel (Mytilus edulis trossulus), three-spined stickleback (Gasterosteus aculeatus L.), lamprey (Lampetra fluviatilis L.), perch (Perca fluviatilis L.), flounder (Platychthis flesus L.), herring (Clupea harengus membras L.), harbour porpoise (Phocoena phocoena L.) and white-tailed sea-eagle (Haliaeetus albicilla L.) from the Gulf of Gdańsk and Baltic Sea.

The highest bioaccumulation factor for PCNs was found in flounder and mussel for PCN-69, followed by PCN-71/72, PCN-64/68 and PCN-66/67 (Falandysz & Rappe, 1996; Falandysz, Kulp, et al., 1996; Falandysz, Strandberg, Bergqvist, Strandberg, & Rappe, 1997). Some tetraCNs, such as PCN-42, and pentaCNs, such as PCN-52/60, PCN-58, PCN-61 or PCN-57 also showed bioaccumulation potential. Nfon et al. (2008) studied concentrations of PCNs in samples of pelagic (top feeders) and benthic (bottom feeders) species collected in the Baltic Sea within the period 1991–1993. Biomagnification factors for PCNs (from tetra- to hexachlorination degree) were calculated for each predator prey pair of species, as well as food chain magnification factors (FCMFs). FCMFs were lower than 1 for all tetraCNs, suggesting no biomagnification of these compounds. Some pentaCN (PCN-54 and PCN-61) and hexaCN congeners (PCN-68) were biomagnified in the pelagic food chain, whereas only hexaCN (PCN-63, PCN-68, PCN-72) congeners were biomagnified in the benthic food chain. When comparing the FCMFs of PCN congeners to those obtained for PCB congeners, FCMFs of PCN were lower. It has been reported that the higher chlorinated PCNs as well as PCNs with 0–2 vicinal carbon atoms unsubstituted by chlorine atoms are more slowly metabolised than the low chlorinated PCNs and those which do not have three vicinal carbon atoms unsubstituted by chorine, resulting in a greater potential for bioaccumulation (Falandysz, 1998). Cui et al. (2018) studied the concentrations and trophic magnification of PCNs (including monoCNs to octaCN) in marine fish from the Bohai coastal area (China). Results showed that trophic magnification factors (TMF) for the homologues decreased in the order pentaCNs > hexaCNs > triCNs > diCNs > octaCN > monoCNs > heptaCNs > tetraCNs. No trend was found between the TMFs and the decadic logarithm of octanol–water partition coefficient (log K_{ow}) for the homologues.

Rigby et al. (2023) investigated the transfer of several contaminant groups including PCNs, to the milk of dairy cattle as a result of controlled ingestion of recycled industrial and municipal bioresources that can be applied to agricultural land as fertiliser. Over a period of 3–4 weeks dairy cattle were given feed containing 5% of recycled materials that could be used in food production (biosolids, compost-like-output, meat and bonemeal ash, poultry litter ash and paper sludge ash). These materials contained known amounts of PCNs and were found to have transferred to milk. PCN-52, PCN-66/67, PCN-71/72, PCN-73 and PCN-75 were measured and found to reflect the PCN pattern of the ash materials. Summed PCNs were in the

[2]Index Fungorum, 2023; https://www.indexfungorum.org/names/names.asp

range 4.3–7.0 ng/kg fat in milk from the control treatment group fed with added unamended soil, and up to 20 ng/kg fat in milk from dairy cattle ingesting the diet amended with biosolids at 5% of the dry matter intake (Rigby et al., 2023).

PCNs alongside other POPs contained in poultry bedding made from three types of recycled, commercially available materials, showed potential for uptake and accumulation in chickens (muscle tissue, liver and eggs) when housed using conventional husbandry practices. The birds were exposed to the bedding materials from 1-day old to maturity. In the first 3–4 months of laying, an increasing trend in the concentrations of contaminants was observed in the eggs of the hens raised on shredded cardboard, although the increases were not statistically significant (Fernandes et al., 2023).

1.3.3.3 | *Occurrence in the outdoor environment and wildlife*

PCNs are ubiquitous in the environment and are listed as POPs under the Stockholm Convention (see Section 1.3.6). In general, PCNs are present in biota at ng/g lipid levels; however, much higher levels have been reported in some tissues of wildlife species (Jakobsson & Asplund, 2000).

Outdoor air

Bidleman et al. (2010) reviewed PCNs in polar environments. It was found that PCNs are widespread in arctic air with higher levels in the European Arctic. Concentrations were higher during the cold months in arctic Canada and Russia, but no seasonality was noted in subarctic Canada and Greenland. 'Marker' congeners indicative of combustion were evident at some sites. Levels for the sum of PCNs from several different papers measuring a different set of congeners were summarised in the review and were found to be up to 40 pg/m^3. Total toxic equivalents estimated by the authors in air due to PCNs and dioxin-like PCBs were dominated by PCNs in arctic Canada and Russia, but not in subarctic Canada.

Barbaro et al. (2016) investigated the chemical composition of Antarctic aerosol for both natural and anthropogenic sources. For PCNs, highest average concentrations were PCN-53 (0.073 pg/m^3), PCN-46 (0.06 pg/m^3) and PCN-52 (0.04 pg/m^3).

PCNs in ambient air samples were reported by Dat et al. (2018) from three sites in northern Taiwan. The samples were collected using high-volume samplers during winter and summer and concentration, distribution, potential sources and gas/particle partitioning of PCNs were investigated. The average concentration (sum of 73 PCNs from di- to octaCN) observed at industrial site was the highest (172 ± 111 pg/m^3), while PCN levels measured at urban and rural sites were comparable at 45.2 ± 8.20 and 45.9 ± 24.4 pg/m^3, respectively. The PCN concentrations were found to be higher in summer compared with those measured in winter for all three sites. Gas-phase PCNs predominated in ambient air, accounting for 94% ± 6.0% of total concentration, whereas PCNs were associated more with the particulate phase from the industrial site. Based on the ratios of PCN congeners and the fraction of total combustion-related PCNs/total PCNs, thermal processes were identified as the major sources of PCNs at the industrial site.

Dien et al. (2021) conducted an analysis of data from a 16-year monitoring programme (2003–2018) conducted by the Japanese Ministry of Environment to investigate a range of POPs in the atmosphere above Japan. Concentrations of PCNs and some of the other pollutants were influenced by a combination of factors including year, industrial activity (municipal and industrial waste incinerators, cement kilns, steel industry and secondary zinc production), population, temperature and atmospheric boundary layer. Industrial activity showed a strong positive correlation with PCNs. Atmospheric PCNs have a half-life of 8.9 years. Concentrations for the total PCNs ranged from 5.3 to 1600 pg/m^3 with a geometric mean of 115 and an arithmetic mean of 185 pg/m^3.

The median concentration of PCNs in Venice air reported by Gregoris et al. (2014) was 89 pg/m^3 in 2009 and 0.29 pg/m^3 in 2012. In general, samples were dominated by low molecular weight PCNs. In 2012, samples of tetraCNs and pentaCNs accounted together for 81% of total PCNs and the most abundant congener was PCN-52.

Mahmood et al. (2014) determined 39 PCN concentrations in air samples from Pakistan and found concentrations ranging from 1222 to 5052 pg/m^3.

Jin et al. (2020) reported that the Tibetan Plateau plays an important role in the global transportation and distribution of POPs including PCNs. They quantified the congener-specific concentrations of PCNs in lichen, moss, soil and air samples collected on the Tibetan plateau and found that common lichens were effective biomonitors for predicting atmospheric PCNs in the area. The physiochemical properties of the PCNs, the temperatures and the lichen lipid contents were identified as important factors influencing PCN partitioning between lichens and air. Lichen–air partitioning equations were established and used to predict PCN concentrations in air in Southeast Tibet. PCN concentrations in lichens increased with altitude, suggesting that high-mountain cold-trapping influenced the PCN transportation behaviour. Data analysis showed that the major source of PCNs in this region was long-range atmospheric transportation via the Indian monsoon in summer and wind from Southwest Asia in winter.

Mao et al. (2020) reported on the occurrence and sources of PCBs, PCNs and hexachlorobenzene (HCB) in the atmosphere at a regional background site in east China. Concentrations and sources of compounds including 39 PCNs were analysed in air samples collected during the period 2012–2015, and the total PCNs were found to be in the range 6–143 pg/m^3 with an average concentration of 38 ± 27 pg/m^3. These values were slightly lower than others reported from across China (61 ± 6 pg/m^3), but higher than that in Japan (9.5 ± 1.5 pg/m^3) and South Korea (16 ± 2.4 pg/m^3) (Hogarh, Seike, Kobara, Habib, et al., 2012). The congener profile was similar to that observed in other areas such as Japan, South Korea, Ghana, India and Pakistan (Mao et al., 2020; Hogarh et al., 2012), in which triCNs and tetraCNs were the most abundant homologues, making up more than 80% of total PCNs, and it suggested that combustion sources made the biggest contribution to PCNs in the atmosphere.

Surface water, effluents and sludge of municipal waste water treatment plants (WWTPs) and industrial sources

Jakobsson and Asplund (2000) identified that important sources of PCNs in the environment include leakage from landfill containing electrical equipment, leakage of commercially produced PCNs and release of PCNs to the environment through the release of contaminated PCBs. Several industrial processes such as waste incineration and other incineration processes, chloroalkali processes, production of magnesium, copper and aluminium had also been identified as sources of PCNs. PCN patterns found from industrial activities were reported to be considerably different from the patterns of PCNs in commercial PCN products, which might be explained by either degradation of less stable PCN congeners or the formation of PCNs via other mechanisms in these processes.

Hites (2006) reported that PCNs entered the Great Lakes through the production and use of Halowax technical mixtures, as trace contaminants in Aroclor PCB mixtures, and through industrial processes such as chlor-alkali production and waste incineration.

Hu et al. (2019) investigated polychlorinated dibenzo-*p*-dioxin and dibenzofuran, PCN and dioxin-like PCB concentrations in ambient air in an industrial park at the north-eastern edge of the Tibet–Qinghai Plateau, China. PCN concentrations were 21.9–75.1 pg/m^3. These concentrations were higher than those observed at a remote site and were comparable with those found in ambient air in industrial areas in other locations. The combustion-related PCN congener profiles suggested that industrial thermal processes strongly affect PCN concentrations in ambient air at the industrial park. The congeners PCN-29 and PCN-39, PCN-50, PCN-51 and PCN-54 and PCN-66/67 contributed more to their homologue groups in ambient air at the industrial park sampling sites than in ambient air at the background site.

Klimczak et al. (2023) reported on the manufacture and some unintentional production of chloronaphthalenes in Poland. Around 2000 tonnes were known to be produced in total between 1936 and 1987, although data gaps exist for periods around the Second World War. In addition, it was reported that PCBs can contain several hundred mg/kg PCNs as by-product and can also be produced during the manufacture of materials produced with use of chlorine gas, e.g. chloroparaffins, chlorinated methanes, trichloroethene or tetrachloroethene, chlorinated tap water and others including the chloro-alkali industry. They can also be formed during the combustion of organic matrices (municipal and hazardous wastes), fossil fuels and wood, forest and other fires as well as during ore and scrap metal smelting and refining (aluminium, copper, iron, lead, magnesium, zinc), cement production and during the coke production.

A recent monitoring study on mono- to octaCNs in the Arctic atmosphere in samples taken from Ny-Ålesund and London Island, Svalbard from 2011 to 2019, showed on a declining time trend in concentrations after 2013. PCN-1, PCN-2, PCN-24/14, PCN-5/7 and PCN-3 dominated the pattern during the study period, and the mean concentration of the sum of PCNs was 23.5 pg/m^3 (range 4.56–85.2 pg/m^3) (Gebru et al., 2023).

Soil and sediments

A summary of the few measurements that had been made of PCNs in sediments from subarctic regions at the time of the review were reported by Bidleman et al. (2010). No measurements in arctic sediments were identified at that time. Concentrations of total PCNs (4–7 Cl) were typically < 2 ng/g whereas for sediments of lakes and rivers in southern Sweden, the range of concentrations was from 0.6 to 252 ng/g.

Castells et al. (2008) measured PCNs and other chlorinated contaminants in marine sediments from Barcelona (Spain). Concentrations ranging from 0.17 to 3.27 ng/g dry weight (dw) were found for PCNs in the coastal sediments, while for samples collected near to the submarine emissary higher levels ranging from 2.02 to 6.56 ng/g dw were found.

Sediment measurements associated with contamination of the Great Lakes as reported by Hites (2006) indicated that the highest PCN concentrations were in the Detroit River, and congener profiles indicated that Halowax contamination from past inputs was the source.

McGoldrick et al. (2018) measured PCNs in lake trout, walleye, herring gull eggs and sediments from the Great Lakes. Sediments were analysed for the period 2011–2013. Concentrations were highest in suspended sediments from the Detroit River (264 ng/g) and were lower in surficial sediments downstream to the St. Lawrence River (range = 0.440–19.3 ng/g). PCNs declined at all sites from ~ 1980 to 1995.

Dong, Zhang, et al. (2022) investigated PCN contamination of dairy farms within a 10-km distance from iron-smelting plants. It was found that tri- and tetraCNs were the second most common homologues in the plant and soil samples, which contrasted findings for milk and feed (see Section 3.2.3), and excrement. In addition, the proportions of higher chlorinated PCNs were found to be greater in the plants and soil than in the milk, feed and excrement.

Birds

High levels of PCNs have been reported in some wild bird tissues associated with industrial contamination. A diseased adult male white-tailed sea eagle contained 2.4 μg/g lipid in the liver in 1991 and a healthy adult male eagle contained 0.62 μg/g lipid in the liver in 1992, both from an old subpopulation unsuccessful in breeding from the southwestern Baltic Sea in Poland. These birds, as well as some subpopulations in Sweden and Finland, also were heavily contaminated with PCBs, dichloro-diphenyl-trichloroethane (DDT) and sometimes with mercury between the 1960s and 1990s (Falandysz et al., 1994, 2000; Falandysz, Bergqvist, et al., 1996; Henriksson et al., 1966; Jakobsson & Asplund, 2000; Koeman et al., 1972).

The review by Bidleman et al. (2010) reported total PCNs in seabirds from polar regions varied over 100-fold, with higher concentrations in glaucous gull eggs and plasma from Bear Island, and livers of northern fulmar from the eastern Canadian Arctic. Lower concentrations occurred in eggs of glaucous gull from Svalbard and black-backed gull from the Faroe Islands. Concentrations are typically < 10 ng/g but can be as high as 74 ng/g as reported for plasma (whole weight) from glaucous gull (Larus hypoboreus) from Bear Island, Norway.

McGoldrick et al. (2018) measured PCNs in Lake Trout, Walleye, Herring Gull eggs and sediments from the Laurentian Great Lakes. Spatial and temporal trends (1979–2013) of PCN concentrations were studied in herring gull eggs throughout the Great Lakes and St. Lawrence River. Concentrations of PCNs were highest in western Lake Erie at 3020 pg/g whole weight (ww) and declined downstream to St. Lawrence River (range: 34–2370 pg/g ww). In Lake Erie, concentrations of PCNs increased in gulls in the period from 1995 until 2005. PCNs in gull eggs were mostly heptaCNs and the pattern was influenced by differences in not only routes of exposure and differential metabolic ability but also resuspension of PCN contaminated sediments.

Mammals

The review by Bidleman et al. (2010) showed total PCNs in marine mammals from polar regions followed the order: harbour seal ~ pilot whale ≥ polar bear > beluga N ringed seal ~ Weddell seal. Concentrations were typically < 10 ng/g. PCNs accounted for < 1% of total toxic equivalents estimated by the authors in ringed seal, Weddell seal, seabirds and polar bear, but up to 6%–15% in beluga and pilot whale. Total toxic equivalents due to PCNs were generally low in harbour seal, but up to 9% of total toxic equivalents in some animals.

Law (2014) gave an overview of time trends in organic contaminant concentrations in marine mammals between 2008 and 2014. Few studies were reported for PCNs. The two studies that were identified were Rotander et al. (2012) and Ross et al. (2013). Rotander et al. (2012) studied PCNs in marine mammals from the Arctic and sub-Arctic from 1986 to 2009. Species studied were long-finned pilot whales, ringed seal, minke whale, fin whale, harbour porpoise, hooded seal and Atlantic white-sided dolphins. No time trends were found, although, in minke whales off Norway, the lowest concentrations were found in samples from the latest sampling period. Ross et al. (2013) found that PCN concentrations were rapidly declining in harbour seals from the Salish Sea (NW North America) between 1984 and 2009.

Fish and aquatic species

Bidleman et al. (2010) summarised total PCNs in invertebrate animals and fish from polar regions, including the Northern Baltic. Concentrations were mostly < 1 ng/g although concentrations of up to 69 ng/g were observed for Amphipod (*M. Affinis*; a crustacean) from the Bothnian Bay/Sea.

In the study by McGoldrick that measured PCNs in gull eggs (see above), Lake trout were also analysed. Concentrations of PCNs were highest in western Lake Erie (7.66 ng/g) and declined downstream to St. Lawrence River (range: 0.034–2.37 ng/g). In Lake Erie, concentrations of PCNs increased in fish between 1995 and 2005. Congener profiles of PCNs in fish differed between Lake Erie and Lake Ontario until post-dredging, where PCN profiles of fish in both lakes became increasingly more similar. Fish had higher concentrations of lower chlorinated PCNs (< hexa) and were influenced by differences in not only routes of exposure and differential metabolic ability but also resuspension of PCN contaminated sediments.

1.3.3.4 | *Occurrence in the indoor environment*

Indoor air and dust

There is very little information reported in the literature about PCNs in indoor air and dust. A study on exposure of PCNs to Pakistani populations via non-dietary sources from neglected e-waste hubs (Waheed et al., 2020). A total of 39 PCN congeners were measured, and the sum of these congeners resulted in concentrations in indoor air and dust samples ranged from 7.0 to 9583 pg/m^3 and from 0.25 to 697 ng/g, respectively. Predominant PCN congeners in indoor air and dust were tri- and tetraCNs.

An investigation into dioxin-like activity in Japanese indoor dusts measured concentrations of several dioxin-like contaminants and found concentrations of PCNs ranging from 1.7 to 26 ng/g in house dust and 1.6–49 ng/g in office dust (Suzuki et al., 2010).

1.3.4 | Sampling and methods of analysis

PCNs are ubiquitous and so precautions must be taken to ensure the integrity of samples and to avoid cross-contamination, both when collecting samples, during transport and storage, and within the laboratory.

Methods of analysis for PCNs in general have commonality with methods for the analysis of similar volatile organic environmental contaminants, such as PCBs and PBDEs. Frequently used extraction methods are hot pressurised liquid extraction, hot liquid extraction and cold liquid extraction. Sample clean-up is usually performed with silica sulfuric acid column, alumina column and carbon column (European Union Reference Laboratory (EURL) for POPs[3]).

[3]https://eurl-pops.eu/analyte#_what-are-pcns-and-where-do-they-come-from

PCNs, like PCBs and PBDEs are amenable to gas chromatography (GC), which can be used for the separation of individual congeners. Historically, GC was mostly used with electron capture detection (ECD), but in recent years, methods have moved towards using the greater sensitivity and specificity offered by mass spectrometry (MS) in particular in recent years using Atmospheric Pressure GC coupled with MS/MS (APGC-MS/MS) and GC-high-resolution MS (GC–HRMS). Recent reports of PCNs in food tend to use highly selective and sensitive measurement techniques such as HRGC-HRMS with stable isotope dilution methods made possible by the use of ^{13}C-labelled PCN analogues of the main congeners of interest.

Detailed descriptions of the methods used for the generation of recent data on the occurrence of PCNs in foods were reviewed by Fernandes et al. (2017).

Several PCN congeners including some reported to be of most toxic and bioaccumulative significance like, PCNs 27, 28, 30, 31, 34, 35, 37, 38, 40, 43, 45 and 48, 55, 59 and 60, 64, 66, 67, 68, 71 and 72, co-elute under routine analysis conditions (HRGC), and determination of their individual concentration in foods and any other materials under routine conditions in practice is not possible (Falandysz et al., 2008; Horii et al., 2004; Tschiggfrei et al., 2023). The development of GC columns for research purposes allows for the separation of PCNs 66 and 67 in Halowax 1014 and in air, but these have not been found to be suitable for the analysis of foods, and data have not been reported for the analysis of foods and biological or environmental samples using such columns (Helm et al., 1999). The closely eluting PCNs 66 and 67 and all other closely eluting congeners were separated in contaminated soil (Ieda et al., 2011) and in Halowax 1014 by using two-dimensional GC/quadrupole mass spectrometric detection, but quantitative results were not reported (Hanari, Falandysz, Nakano, et al., 2013; Hanari, Falandysz, Petrick, et al., 2013).

The dioxin-responsive chemically activated luciferase expression assay (DR-CALUX assay) can be used to measure dioxin-like activity within a sample (Suzuki et al., 2020) and it is used for screening food and feed samples within the EU. In routine analysis, a clean-up with acid silica is applied which makes it more selective to e.g. dioxins and dioxin-like PCBs. PCNs have also been shown to cause a response in the bioassay, although the relative potencies are lower than the lower chlorinated dioxins like TCDD. While it is possible to detect PCNs when present at higher concentrations, the DR CALUX assay is not suitable to use for food control in routine analyses.

1.3.5 | Previous assessments

A few national and international bodies have carried out hazard or risk assessments related to PCNs. These assessments evaluated in a number of cases the environmental risks as well as the risk for human health.

The US-EPA has evaluated chlorinated naphthalene[4] in order to set an ambient water quality criteria. For human health, it was concluded that a quality criterion could not be derived due to the insufficiency of the available data. For aquatic life, acute toxicity data are summarised for fresh water and salt water aquatic life, but a quality criterion is not set (US EPA, 1980).

A 'Concise International Chemical Assessment Document' (CICAD) prepared by the Centre for Ecology & Hydrology in the UK and the Fraunhofer Institute for Toxicology and Aerosol Research in Germany was published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization (WHO, 2001). PCNs, especially dioxin-like PCNs have been detected in adipose tissue, liver, blood and breast milk in the general population (in the ng/kg lipid range). The isomer pattern in the human samples was significantly different from that in commercial chlorinated naphthalene mixtures. The predominant PCNs in most human samples were the two penta- and two hexa-isomers, PCNs 52, 60 and 66, 67, and to a lesser extent some tetra- isomers. Based on the toxicological data, it was concluded that the toxicological profile of PCNs is not well characterised because of the lack of long-term studies, and consequently, a confident risk characterisation could not be performed. Therefore, it was recommended that exposure to PCNs should be minimised as much as possible, because, for example, effects on endocrine functions have been shown to occur at very low doses.

A risk profile on PCNs was prepared in 2002 by an independent consultant for the Dutch Ministry of VROM/DGM for the third meeting of the United Nations Economic Commission for Europe (UNECE) Ad hoc Expert Group on Persistent Organic Pollutants (POPs) (Van de Plassche & Schwegler, 2002). An addendum to this risk profile was prepared in 2005 by the same independent consultant (Belfroid et al., 2005). One of the POP characteristics to be evaluated is toxicity and ecotoxicity. Based on acute toxicity data on cattle and pigs, it was concluded that PCNs are toxic to cattle. The lowest oral dose to cause acute systemic effects in cattle was reported to be 1 mg/kg bw. Subchronic studies on guinea pigs and rats indicated that penta- and hexaCNs are the most toxic congeners with the liver being the critical target organ. The lowest oral dose to cause liver effects was reported to be 2.5 mg/kg bw in guinea pigs exposed to pentaCNs. Relative potencies of individual PCNs related to 2,3,7,8-TCDD from tests in fish, birds and mammals indicate that the potency of several PCNs is in the same range as for some PCB congeners and it was concluded that PCNs have dioxin-like toxicity.

The UK Committee on Toxicity of chemicals in food, consumer products and the environment (UK-COT) published in 2009 a statement on polychlorinated naphthalenes in food (UK-COT, 2009). The Committee chose a 'cumulative approach' for the risk assessment of dioxin-like compounds to be applied for the PCNs in order to be protective of human health, as some PCNs were considered to have shown clear evidence of dioxin-like activity. The Committee applied the criteria published by van den Berg et al. (2006) for inclusion of a dioxin-like substance in the toxic equivalency factor (TEF) concept. It was concluded

[4]Congener not specified.

that it was not possible to establish TEFs for PCNs because of no information regarding persistence and no repeated dose toxicity studies for the PCNs, which are needed for a direct comparison with TCDD. The Committee considered the PCNs to be less persistent than the PCDDs and polychlorinated dibenzofurans (PCDFs). In the absence of other data, the Committee considered that the relative potencies compared to TCDD from in vitro tests could be used as an extremely conservative approach for the cumulative risk assessment for the dioxin-like toxicity of PCNs in food. Worst-case assumptions were applied for the dietary exposure estimations and indicated that the maximum exposure to PCNs for children (4–6 years of age) would be 49% of the tolerable daily intake (TDI) of 2 pg WHO-TEQ/kg bw for dioxins and dioxin-like compounds. For other age groups, the maximum PCNs exposure would be lower. Overall, the Committee concluded that there are no specific toxicological concerns from exposure to PCNs in food despite that the data are insufficient for a robust risk assessment.

The Food Safety Authority of Ireland (FSAI) in collaboration with the Department of Agriculture, Fisheries and Food and the Marine Institute investigated the levels of PCNs in carcass fat, liver, fish, eggs and milk produced in Ireland and a selected number of processed products on the Irish market during 2007–2008 (FSAI, 2010). The following 11 PCNs were analysed: PCN-52, PCN-53, PCN-66/67, PCN-68, PCN-69, PCN-71/72, PCN-73, PCN-74 and PCN-75. The survey showed low levels of the PCNs analysed in the survey in the Irish products investigated in the survey. In order to evaluate a possible health concern from dietary exposure of the PCNs in the investigated products, toxicity equivalent values relative to TCDD were calculated by FSAI based on four studies. It was concluded that the levels observed in the products on the Irish market did not raise concern for human health.

In a report from 2011, RIVM has presented a new methodology for persistence/bioaccumulation (PB) score in order to identify potential POP and persistent, bioaccumulative and toxic (PBT) candidates. For mixtures of commercially available POPs, PB scores were calculated for each individual component in the mixture and the highest score was then selected as representative of the mixture. The PB score for the eight PCNs included varied from 0.67 for PCN-1 to 1.91 for PCN-63. Based on an assumption that commercial mixtures will contain a small fraction of hexaCN congeners, the commercial mixtures can be characterised by the PB score of 1.91 for PCN-63 resulting in a ranking place as number 90 of the 64,721 evaluated substances (RIVM, 2011).

1.3.6 | Legislation

In this Opinion, where reference is made to European legislation, the reference should be understood as relating to the most recent amendment at time of publication of this Opinion, unless otherwise stated.

In order to protect public health, Article 2 of Council Regulation (EEC) No 315/93[5] of 8 February 1993 laying down Community procedures for contaminants in food stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. A number of maximum levels (MLs) are currently laid down in Commission Regulation (EC) No 1881/2006.[6] PCNs are not regulated so far under this Regulation or under any other specific European Union (EU) regulation for food. However, in case of a finding of PCN in food at a level at which the food is no longer assessed to be safe, i.e. potentially be injurious to health, the food cannot be placed on the market in accordance with Article 14 of Regulation (EC) 178/2002.[7]

Council Directive 2002/32/EC[8] regulates undesirable substances in animal feed. PCNs are, so far, not regulated under this Directive or any other specific EU regulation for feed. However, in case of a finding of PCNs in feed at a level at which the feed is no longer assessed to be safe, i.e. potentially causing adverse effects on human or animal health, the feed cannot be placed on the market or fed to any food-producing animal in accordance with Article 15 of Regulation (EC) 178/2002.[7]

In 2015, the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants (POPs) amended Annexes A (Elimination) and C (Unintentional Production, Parts I, II and III) of the Stockholm Convention[9] in order to include PCNs. For chemicals listed in Annex A, countries must take measures to eliminate their production and use. The Council of the EU supported these amendments, as reflected in the Council Decision (EU) 2015/627.[10] The CONTAM Panel noted that the Stockholm Convention considers only PCN congeners with number of chlorines ranging from 2 to 8 on the naphthalene moiety. PCNs 1, 2 are not included in the Stockholm Convention on POPs.

The 75 PCN congeners are listed in Annex I, Part A of Regulation (EU) 2019/1021[11] of the European Parliament and of the Council on POPs. The objective of this Regulation is to protect human health and the environment by prohibiting, phasing out as soon as possible or restricting the manufacturing, placing on the market and use of POPs. Since the substances were included in the POPs Regulation in 2012, their placing on the market and use in the EU are prohibited. Presently, PCNs are listed in Annex IV of this Regulation, on substances subject to waste management provisions set out in Article 7. A concentration limit of 10 mg/kg is applicable for PCNs in waste.

[5]Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–3.

[6]Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364/5, 20.12.2006, p. 5–24.

[7]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L, 31 1.2.2002, p. 1–24.

[8]Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in food. OJ L 140, 30.2.2002, p. 10–22.

[9]https://www.pops.int/TheConvention/Overview/TextoftheConvention/tabid/2232/Default.aspx

[10]Council Decision (EU) 2015/627 of 20 April 2015 on the position to be taken on behalf of the European Union, at the seventh meeting of the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants as regards the proposals for amendments to Annexes A, B and C. OJ L 103, 22.04.2015, p. 8–10.

[11]Regulation (EU) 2019/1021 of 20 June 2019 on persistent organic pollutants. OJ L 169, 25.6.2019, p. 45–77.

No dossiers for PCNs have been submitted under Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation[12]).

According to ANNEX VI of Regulation (EC) No 1272/2008 (Classification, Labelling and Packaging (CLP) Regulation[13]), pentaCN (CAS number: 1321-64-8) are classified Acute Tox. 4 H 302 (Harmful if swallowed), Acute Tox. 4 H 312 (Harmful in contact with skin), Skin Irrit. 2 H 315 (Causes skin irritation), Eye Irrit. 2 H 319 (Causes serious eye irritation), Aquatic Acute 1 H 400 (Very toxic to aquatic life), Aquatic Chronic 1 H 410 (Very toxic to aquatic life with long lasting effects).

2 | DATA AND METHODOLOGIES

2.1 | Supporting information for the assessment

Literature searches were performed for PCNs for years from 1950 onwards in all areas of interest, except for human studies where years from 1900 onwards were considered. Details about the literature searches are given in Appendix B where information is summarised in a narrative way based on expert knowledge and judgement.

The physicochemical properties of PCNs, production and industrial use, environmental fate and levels, analytical methods, previous assessments by national authorities and international bodies and current EU legislation are summarised in Section 1. Studies on the toxicological profile, occurrence and exposure to PCNs are considered in Section 3.

2.2 | Hazard identification and characterisation

2.2.1 | Identification, collection and selection of evidence

Information relevant for the sections under hazard identification and characterisation was identified by a literature search, as described above.

2.2.2 | Appraisal of evidence

The information retrieved, was screened and evaluated by relevant domain experts from the CONTAM Working Group on PCNs in feed and food and was used for the present assessment. The selection of the scientific papers for inclusion or exclusion was based on consideration of the extent to which the study is relevant to the assessment, and on general study quality considerations: sufficient details on the methodology, performance and outcome of the study, on dosing, substance studied and route of administration and on statistical description of the results, irrespective of the results (see Annex A). Major limitations in the information used are documented in this Scientific Opinion and considered in the uncertainty analysis.

Benchmark dose (BMD) analysis was carried out according to the latest EFSA guidance (EFSA Scientific Committee, 2022) and using the EFSA BMD modelling application.

2.3 | Occurrence data submitted to EFSA

2.3.1 | Data collection and validation

Following an European Commission mandate to EFSA, a call for annual collection of chemical contaminant occurrence data in food and feed, including PCNs, was issued by the former EFSA Dietary and Chemical Monitoring Unit (now Integrated Data Unit) in December 2010 with a closing date of 1 October of each year.[14] European national authorities and similar bodies, research institutions, academia, food business operators and other stakeholders were invited to submit analytical data on PCNs in food and feed. The data for the present assessment were provided by organisations from six European countries (including five EU countries and the United Kingdom (UK)[15]).

Analytical data were reported to EFSA as individual PCNs (for more details, see Section 3.2.1).

[12]Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1–849.

[13]Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

[14]https://www.efsa.europa.eu/en/call/call-continuous-collection-chemical-contaminants-occurrence-data-food-and-feed-0

[15]Occurrence data included in the assessment were submitted to EFSA when the UK was a member of the EU.

The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description (SSD) for Food and Feed (EFSA, 2010a). Occurrence data were managed following the EFSA standard operational procedures (SOPs) on 'Data collection and validation' and on 'Analysis of data from the S-DWH for the assessment of dietary exposure'.

By the October 2022, a total of 13,242 analytical results on PCNs (representing 550 samples) in food and feed were available in the EFSA database. Data received after that date were not included for further evaluation within this opinion.

2.3.2 | Data analysis

Following EFSA's Technical report on handling of occurrence data for dietary exposure assessment (EFSA, 2021) to guarantee an appropriate quality of the data used in the exposure assessment, the initial data set was carefully evaluated by applying several data cleaning and validation steps. Special attention was paid to the identification of duplicates and to the accuracy of different parameters, such as 'Sampling strategy', 'Sampling year', 'Sampling country', 'Analytical methods', 'Result express' (expression of results, e.g. fat weight), 'Reporting unit', 'Limit of detection/quantification' and the codification of analytical results under FoodEx classification (EFSA, 2011a, 2011b, 2015). The outcome of the data analysis is presented in Section 3.2.1 and Annex B, Table B.2.

The left-censored data (LCD) (results below limit of detection (LOD) or below limit of quantification [LOQ]) were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' WHO, 2009). The same method is indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option in the treatment of left-censored data. 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, including contaminants. The LB is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (< LOD) or the LOQ (< LOQ). The UB is obtained by assigning the numerical value of the LOD to values reported as < LOD and LOQ to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory.

2.4 | Consumption data

2.4.1 | Food consumption data

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level and was first built in 2010 (EFSA, 2011c; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used have been published in an EFSA Guidance (EFSA, 2011c).

The latest version of the Comprehensive Database, updated in December 2022, contains results from a total of 59 different dietary surveys carried out in 28 different European countries (24 Member States) covering 103,802 individuals. The assessment of this opinion focused only on surveys carried out in the EU Member States.

Within the dietary studies, subjects are classified in different age classes as follows:

Infants:	< 12 months old
Toddlers:	≥ 12 months to < 36 months old
Other children:	≥ 36 months to < 10 years old.
Adolescents:	≥ 10 years to < 18 years old
Adults:	≥ 18 years to < 65 years old
Elderly:	≥ 65 years to < 75 years old
Very elderly:	≥ 75 years old

Nine surveys provided information on specific population groups: 'Pregnant women' (Austria: ≥ 19 years to ≤ 48 years old, Cyprus: ≥ 17 years to ≤ 43 years old; Latvia: ≥ 15 years to ≤ 45 years old, Romania: ≥ 19 years to ≤ 49 years old, Spain: ≥ 21 years to ≤ 46 years old, Portugal: 17 years old to 46 years old), 'Lactating women' (Greece: ≥ 28 years to ≤ 39 years old, Estonia: 18 years old to 45 years old) and 'Vegetarians' (Romania: ≥ 12 years to ≤ 74 years old).

The food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from 3 to 7 days per subject. When two dietary surveys were available for a country and age class, only the most recent one was used. This resulted in a total of 49 dietary surveys (84,991 subjects) carried out in 22 EU countries, used for the chronic dietary exposure assessment (Annex B, Table B.1). Owing to the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

2.4.2 | Feed consumption data

The feeds consumed (and the feed intake) by the most relevant food-producing and non-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 were: (i) ruminants dairy cows (producing ~40 kg milk/day) for which non-forage feeds accounted for 70% of the diet (on a dry matter basis), beef cattle for which non-forage feeds accounted for 20% of the diet (on a dry matter basis), dairy sheep for which non-forage feeds accounted for 35% of the diet (on a dry matter basis), dairy goats for which non-forage feeds accounted for 75% of the diet (on a dry matter basis), lambs and kids for fattening for which non-forage feeds accounted for 50% and 40% of the diet (on a dry matter basis), respectively; (ii) pigs (weaned piglets, fattening pigs and lactating sows); (iii) poultry (broilers, laying hens, turkeys for fattening and ducks for fattening); (iv) rabbits; (v) farmed fish (salmonids); (vi) companion animals (dogs, cats) and (vii) horses. The default values for average feed intakes and body weights used to calculate animals' exposure to PCN are described in Appendix C. They have been derived from information extensively described by the CONTAM Panel in previous Scientific Opinions on the risks for animal and public health (EFSA CONTAM Panel, 2011, 2012) and modified in May and published guidelines.[16] The default values for feed intakes and body weight are based on published guidelines on nutrition and feeding (NRC, 2007; Leeson and Summers, 2008; EFSA FEEDAP Panel, 2017).

2.5 | Food and feed classification

2.5.1 | Food

Consumption and occurrence data were both codified according to the FoodEx2 classification system. FoodEx was developed by EFSA in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances (EFSA, 2011a). Following its first publication, a testing phase was carried out in order to highlight strengths and weaknesses, and to identify possible issues and needs for refinement. Based on the outcome of the testing phase, EFSA published in 2015 the FoodEx2 revision 2 (EFSA, 2015).

The FoodEx2 catalogue hosts several hierarchies used for different data collections, e.g. 'Reporting hierarchy' for the collection of occurrence data and 'Exposure hierarchy' for the collection of food consumption data. It consists of a large number of individual food items aggregated into food groups and broader food categories in a hierarchical parent–child relationship. It contains 21 main food categories at the first level of the 'Exposure hierarchy', which are further divided into subcategories, resulting in seven levels with more than 4000 items in total. In addition, FoodEx2 allows the further description of food items with facets. Facets are descriptors that provide additional information for a particular aspect of a food and are divided into implicit facets, which are integrated in the catalogue, and explicit facets, which are added by users while coding a food item.

2.5.2 | Feed

Regarding the feed, FoodEx2 has 14 main feed categories integrated with broader feed commodities at lower levels defined according to the Catalogue of feed materials as described in Commission Regulation No 68/2013.[17]

2.6 | Exposure assessment

2.6.1 | Human dietary exposure assessment

The CONTAM Panel considered it appropriate to estimate only chronic exposure to PCNs. As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011d), dietary surveys with only 1 day per subject were not considered for chronic exposure as they are not adequate to assess repeated exposure. Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Not all countries provided consumption information for all age groups, and in some cases the same country provided more than one consumption survey.

For calculating the chronic dietary exposure to PCNs, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. Occurrence data and consumption data were linked at the relevant FoodEx level.

The mean and the high (95th percentile) chronic dietary exposures were calculated by combining mean occurrence values for each food collected in different countries (pooled European occurrence data) with the average daily consumption

[16]The approach to estimate feed consumption and the default values for feed intake and body weight used to estimate the exposure to bromide were endorsed by the EFSA CONTAM Panel in its 133 Plenary meeting (https://www.efsa.europa.eu/sites/default/files/2023-07/Minutes_0.pdf).

[17]Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29, 30.1.2013, p. 1–64.

of each food at individual level in each dietary survey and age class. Consequently, individual average exposures per day and body weight were obtained for all individuals. Based on the distributions of individual exposures, the mean and 95th percentile exposures were calculated per survey and per age class. Dietary exposure was assessed using overall European LB and UB mean occurrence of PCNs. All analyses were run using the SAS Statistical Software (SAS enterprise guide 8.2).

Specific exposure scenarios

In addition, the CONTAM Panel considered that it is of interest to also estimate an exposure for consumers only of specific foods of interest. Therefore, two specific exposure scenarios were developed and calculated as follows:

- *Exposure* via *fish meat consumption for consumers only*. Due to higher concentrations levels of PCNs in fish meat, high and frequent consumers of fish meat might have higher exposure to PCNs than consumers that do not eat fish meat. In order to test such a hypothesis, the 95th percentile exposure from the daily consumption of fish meat among consumers only was retrieved from the Comprehensive Database for surveys where the number of selected participants was at least 60. In total, 35 dietary surveys carried out in 20 European countries were included for this evaluation. For this exercise, the CONTAM Panel considered the PCN-66/67 as the most relevant due to very high contribution of fish meat observed in the exposure assessed for total population.
- *Exposure* via *human milk consumption*. For the exposure assessment of breastfed infants, an age of 3 months was selected, corresponding to a weight of about 6.1 kg, and an estimated average daily consumption of 800 mL and a high consumption of 1200 mL of human milk (EFSA Scientific Committee, 2017a). The occurrence data were taken from European pooled milk samples that were collected and analysed as part of the WHO/United Nations Environment Programme (UNEP)-coordinated human milk studies from 2000 to 2019 (Tschiggfrei et al., 2023). The two different exposure scenarios were calculated, including the exposure assessment via human milk of PCN-66/67 and of the mixture of PCN congeners (the mixture scenario A and B; see Section 3.3.3).

2.6.2 | Animal dietary exposure assessment

Estimated model diets for each animal species and category were used to calculate the exposure to PCNs. Similar to animal feed intakes, the estimated diets have been derived from information described by the CONTAM Panel in previous Scientific Opinions (EFSA CONTAM Panel, 2011, 2012) and modified in May 2023 (see Section 2.4.2). The diets are described in Appendix C.

The amendments introduced in May 2023 were also aimed at allowing a certain flexibility in the use of interchangeable feeding materials in relation to occurrence data availability and levels of contamination. With this scope, feed groups were identified, in line for Commission Regulation 2022/1104,[18] and within each group, feed materials could be exchanged, provided the nutritional needs of the various animal species are met. Groups of feed materials are included in Appendix C.

When occurrence data allowed, different scenarios were proposed for animals: one scenario considering consumption of compound feed and one scenario considering feed materials and model diets. In addition, for ruminants, forages were added to allow the daily ratio at the recommended proportion for each ruminant category.

2.7 | Risk characterisation

The general principles of the risk characterisation for chemicals in food as described by the WHO/IPCS (2009) will be 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

PCN compounds as a group are highly lipophilic. The chemical and physical properties of the individual congeners differ due to differences in chemical structure, spatial configuration and size between the 75 PCNs. These differences affect their toxicokinetic characteristics in humans and animals. PCNs are found as complex mixtures, which provides challenges from both the analytical and toxicological points of view.

[18]Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials (Text with EEA relevance), C/2022/4474, OJ L 177, 4.7.2022, p. 4–74.

Information on the absorption, distribution, metabolism and elimination (ADME) of individual congeners is limited. There is no full range of high-purity standards, and technical limitations of the analytical chemistry do not allow full separation and quantification of all 75 PCNs (see Section 1.3.4).

PCN-1 and PCN-2 have all seven adjacent positions on carbon skeleton available for oxidative metabolism and are therefore more easily degraded and eliminated. As the degree of chlorination increases, it leads to differences in molecular size, physical and chemical properties, and an increase in the spatial barrier (steric hindrance) in the molecule. This introduces variations in the metabolic persistency of the individual congeners (Asplund et al., 1986; Falandysz et al., 2001; Fernandes et al., 2022). Consequently, octaCN seems to be relatively resistant to oxidative metabolism. However, the absorbed hexaCNs, PCN-66 and PCN-67, were the most resistant for metabolic degradation in studies with rats (Asplund et al., 1986, 1994).

The main results of the toxicokinetic studies of PCNs are described below.

3.1.1.1 | *Humans*

No data were retrieved on absorption in humans. Some indirect information on oral, inhalatory and dermal absorption is available on occupational and accidental exposure incidents and from application of PCN technical formulations (largely of the Halowax series) on the skin of volunteers, and from more recent reports on accumulation of PCNs in humans (Falandysz, 2003; Fromme et al., 2015; Haglund et al., 1995; Hambrick Jr, 1957; Jin et al., 2019; Kawano et al., 2000; Li et al., 2021; Shelley & Kligman, 1957; Wang, Zhang, Wang, et al., 2022; Weistrand & Norén, 1998; Witt & Niessen, 2000). Inhalation and skin contact have been the main routes of PCN exposure in the workplace (see Section 3.1.3). Occupational poisonings and fatalities indicate that PCN vapours are well absorbed when inhaled. Fatal poisoning occurred after accidental ingestion of Nibren wax via a contaminated meal (Herzberg, 1947), indicating that PCNs are absorbed by humans via oral exposure.

Rice oil accidentally contaminated with PCNs (2.6 mg/kg), PCDDs, PCDFs and PCBs from the Kanechlor KC-400 (PCNs at 635 mg/kg), a PCB formulation, was consumed in the 1968 Yusho incident and in the 1979 Yu Cheng incident and resulted in PCNs appearing in blood, milk and adipose tissues, as well as in fetuses (Haglund et al., 1995; Kuratsune, 1989; Masuda, 1994). No quantitative data on bioavailability were available from these studies. PCNs retained in adipose tissue of the Yusho patients were PCN-28/43, PCN-33/34/37, PCN-35, PCN-38/40, PCN-46, PCN-52/60 and PCN-66/67, while PCN-24, some tetraCNs, pentaCNs and hexaCNs and both heptaCNs – PCN-73 and PCN-74 determined in Yusho rice oil were not detected in exposed individuals (Haglund et al., 1995).

Studies of PCNs in the general population worldwide have reported their detection in human blood, liver, adipose tissue and milk (Fromme et al., 2015; Jin et al., 2019; Li et al., 2021; Lundén & Norén, 1998; Wang, Zhang, Wang, et al., 2022; Weistrand & Norén, 1998) (see Section 3.2.5). Placental transfer of PCNs to fetuses was shown by their occurrence in the aforementioned Yusho rice oil poisoning accident (Kuratsune, 1989) (see Section 3.2.5). These observations suggest that PCNs are well absorbed by humans from foodstuffs. All the monoCN to hexaCN congeners present at a low level in food can be well absorbed while, due to the larger size, heptaCNs and especially octaCN are possibly absorbed to a lesser extent (Falandysz & Fernandes, 2020; Fernandes et al., 2010, 2017). The possible effects of major dietary factors such as dietary pH, fat content and intestinal microbiome on PCNs absorption are unknown.

3.1.1.2 | *Laboratory animals*

3.1.1.2.1 | *Studies in rats*

PCN congeners

Absorption

Absorption of PCN-3 administered orally by gavage to male Wistar rats as a single dose of 400 mg/kg bw in corn oil was rapid, and gradually declined over 8 h after administration (Chu, Secours, et al., 1977).

Distribution

Distribution of orally absorbed PCN-3 from blood into organs and tissues of male Wistar rats was rapid, i.e. within the first few hours, and occurrence in tissues was significantly reduced after 8 h and up to 2 days following administration (Chu, Secours, et al., 1977).

PCN-27 (1,2,3,4-tetraCN)-[ring-U-^{3}H] and PCN-49 (1,2,3,4,5-pentaCN)-[ring-U-^{3}H] administered intraperitoneally to male outbred Wistar rats (approx. 200–250 g bw; $n = 6$ or 8 rats per group) at a single dose of 10 mg/kg bw were rapidly distributed into the organs and tissues. The decline of [^{3}H] radioactivity concentration for both compounds in plasma was biphasic. The maximum plasma concentrations were observed at half a day and 1 day after PCN-27 and PCN-49 administration, respectively. The elimination was biphasic with the half-lives for the first phase being 0.54 day (PCN-27) and 1.3 days (PCN-49), respectively. The half-lives for the second phase were 7.2 days for both compounds. The highest [^{3}H] radioactivity concentrations for both compounds were found in the adipose tissue, liver, kidneys and adrenals. The ^{3}H radioactivity was detected in blood (plasma and erythrocytes), selected tissues including abdominal fat, liver, kidneys, spleen, lungs, brain,

adrenals, sciatic nerve and muscles, as well as in urine and faeces after 0.5, 1, 2, 5 and 14 days; the alimentary tract and its contents, skin, bones or tendons were not examined (Kilanowicz et al., 2004).

Radiolabelled PCN-43 (1,3,5,8-tetraCN)-[ring-U-^{3}H] (purity > 95%, other constituents were other tetraCNs at 3.98% and triCNs at 1.02%) were administered orally by gavage to pregnant female Wistar rats at a single dose of 0.3 mg/dam on gestation days (GD) 17, 18 and 19. These PCNs were detected within the first day post-treatment in the maternal blood, liver, kidneys and in fetuses and declined thereafter, whereas the concentration in brain increased with time. The highest concentrations were measured in adipose tissue and liver (1.56 and 1.1 µg/g tissue, respectively). Highly perfused organs (adrenal glands, lungs, spleen and ovaries) also showed high radioactivity concentrations, approximately four- to fivefold higher than that in the blood. ^{3}H-PCN-43 radioactivity was also detected in the uterus. PCN-43 crossed the blood–brain barrier. The concentration in brain reached 0.31 µg/g wet tissue within 3 days. PCN-43 crossed the placental barrier and the concentrations of radioactively labelled PCN-43 in the fetal liver (0.24 µg/g tissue) and brain (0.24 µg/g tissue) were > 2-fold higher than in maternal blood (0.11 µg/g tissue). In the fetuses, the highest radioactivity concentrations were found in the brain and kidneys. Within the first day, 20% of the test item was detected in adipose tissue and 3 days post-treatment, 24% of the administered dose still remained in the body (Kilanowicz, Markowicz-Piasecka, et al., 2019).

PCN-46 (^{14}C, radiochemical purity > 99%), administered orally by gavage in a single dose of 20 µCi/kg bw (740 kBq/kg bw) (400 mg/kg bw) to male Wistar rats, was rapidly absorbed with maximum concentration in blood after the first hour, and within 24 h distributed into the liver, lungs, kidneys, bladder, skin and adipose tissue; after 7 days radioactivity was detected only in the skin and adipose tissue (Chu, Secours, et al., 1977).

^{14}C-labelled tetra-, penta- and hexaCNs administered orally by gavage (in corn oil) to male Sprague–Dawley rats at a single dose of about 30 µmol/kg bw (1 Ci/mol = 37,000 kBq/mol) accumulated mainly in the liver (based on the fresh weight), which 5 days post-treatment showed 10 pmol/mg of the total ^{14}C radioactivity followed by abdominal fat with 9.2 pmol/mg, kidneys with 3.0 pmol/mg and lungs with 1.5 pmol/mg. A large part of the radioactivity in the liver, kidneys and lungs was non-extractable, with 75% in the liver, 69% in the kidneys and 56% in the lungs, but only 1% in the abdominal fat (Jakobsson, 1994; Jakobsson & Asplund, 2000).

HexaCNs (a mixture of equal amounts of PCN-66/67 and an unidentified hexaCN) dissolved in corn oil were administered orally by gavage to female Sprague–Dawley rats at a single dose of 0.053 mg/kg bw. PCN-66 and PCN-67 accumulated in the liver from the first day, while the unidentified hexaCN vanished rapidly. PCN-66 and PCN-67 remained in the liver and adipose tissue for up to 120 days. The liver to adipose tissue ratio of PCN-66/67 was 7.3 after 1 day, 1.1 after 10 days, 0.8 after 35 days and 0.63 after 120 days (fresh weight), and on lipid basis it was 140, 23, 17 and 13, respectively. The half-life of PCN-66/67 was 42 days in adipose tissue and 36 days in the liver (Asplund et al., 1994).

^{14}C-hexaCNs (mixture of isomers 94.1%; PCN-66/67 (^{14}C-1,2,3,4,6,7–/1,2,3,5,6,7-hexaCN) equivalent mixture at 81.2% and other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%), and PCN-73 at 5.9%[19]) dissolved in sunflower oil were administered either intraperitoneally or orally (intragastric) to adult male Wistar rats at a single dose of 0.3 mg per animal. Peak concentrations of PCN-66/67 1 day after intraperitoneal administration were 38% of the dose in liver and 34% in adipose tissue, and after intragastric administration 32% in the liver and 16% in the adipose tissue. Five days post-treatment approximately 30% of the dose was observed in the adipose tissue from either route of exposure. The maximum levels of PCNs radioactivity concentrations in liver, spleen, blood and muscles occurred within the first day, and in other organs within 5 days after administration. After 21 days from *i.p.* administration, the PCNs radioactivity significantly decreased in liver, blood and muscle tissue and minimally in adipose tissue, while in the brain and sciatic nerve, no downward trends were noted (Kilanowicz, Daragó, & Skrzypińska-Gawrysiak, 2012).

^{14}C-hexaCNs (mixture of isomers 94.1%; PCN-66/67 (^{14}C-1,2,3,4,6,7–/1,2,3,5,6,7-hexaCN) equivalent mixture at 81.2% and other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%), and PCN-73 at 5.9%) dissolved in sunflower oil were administered via gavage to pregnant female rats at a single dose of about 0.3 mg per animal on days of pregnancy 17, 18 or 19. Within 1, 2 or 3 days post-treatment (pregnancy day 20), accumulation of PCNs was highest in the maternal liver and followed by adipose tissue. Hepatic retention ranged from 24.5% to 26.7% of the dose over 2 and 3 days. Relatively high concentrations of radioactively labelled PCNs accumulated also in the reproductive and nervous systems, spleen, ovaries, adrenal glands and uterus, as well as in the sciatic nerve, kidneys and fetal brain. These PCNs crossed the placental barrier and about 2% of the initial dose accumulated in amniotic fluid and placenta 3 days post-treatment, and 0.17% was found in the fetus (Stragierowicz et al., 2018).

Maternal transfer

PCN-43 (purity > 95% and 5% accounted for other – tetraCNs at 3.98% and triCNs at 1.02%) crossed the placental barrier as indicated by that radioactively labelled PCN-43 was detected in rat fetal tissues (Kilanowicz, Stragierowicz, et al., 2019).

PCNs (the mixture of tetraCNs at 54%, pentaCNs at 8%, hexaCNs at 23% and heptaCNs at 14%) administered by gavage in rat crossed, based on fetal effects, the placental barrier but no data on ADME were provided (Kilanowicz et al., 2011).

PCN-66 administered by gavage to pregnant rats on days 14, 15 and 16 of pregnancy was transferred from dam to offspring via both the placental and lactational routes. The amount transferred via the lactational route was one order of magnitude greater than that transferred via the placental route (Omura et al., 2000).

[19] Corrected composition of the hexaCNs mixture based on chromatogram in paper by Kilanowicz & Skrzypinska-Gawrysiak (2010).

A hexaCN mixture (hexaCNs at 94.1% containing PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and a heptaCN (PCN-73 at 5.9%); concentration of polychlorinated dioxins and furans < 0.1 pg/100 µg of the investigated sample) administered by gavage for 10 consecutive days during organogenesis (days 6–15 of gestation; single doses ranging from 0.1 to 1.0 mg/kg bw) to Wistar rats crossed, based on effects in the intrauterine development of embryos/fetuses but without maternal toxicity, the placental barrier (Kilanowicz et al., 2015).

In another study with rats, the same hexaCN mixture about 2% of the initial dose accumulated in amniotic fluid and placenta 3 days post-treatment, and 0.17% was found in the fetus (Stragierowicz et al., 2018).

Metabolism

The tetra- to hexaCNs lacking vicinal hydrogens are more slowly metabolised than other tetra- to hexaCNs and the lower chlorinated congeners with two or more vicinal carbon atoms unsubstituted by chorine (Falandysz, 1998). Oxidation of PCNs via epoxidation (formation of 1,2-shifted hydroxylated metabolites via an arene oxide) has been suggested as a mechanism of metabolic degradation while a direct hydroxylation (insertion of a hydroxyl-group) for some congeners could also be possible.

PCN-11 administered orally by gavage to Sprague–Dawley rats at a single dose of 1000 mg/kg bw was metabolised to the glucuronide-conjugated 2-chloro-6-hydroxynaphthalene and 2,6-dichloro-monohydroxynaphthalene (the position of hydroxyl group not known), which were excreted in the urine together with a small amount of the parent compound (Chu et al., 1976).

The main urinary metabolite of PCN-3 (^{14}C, radiochemical purity > 99%), administered orally by gavage to male Wistar rats at a single dose of 400 mg/kg bw, was, according to the authors, the glucuronide of a dihydrodiol of PCN-3 (about 35%) since it was isolated after hydrolysis with beta-glucuronidase (Chu, Secours, et al., 1977).

Male Wistar rats administered PCN-3 orally by gavage at a single dose of 400 mg/kg bw metabolised it to a glucuronic acid conjugate of 1,2-dichloro-5,6-dihydroxy-5,6-dihydronaphthalene (Ib), which was excreted in the urine. Similarly administered, PCN-12 was in part metabolised as conjugated 2-chloro-7-hydroxynaphthalene (IIb), while PCN-11 was metabolised to 2-chloro-6-hydroxynaphthalene (IIIb) and 2,6-dichloro-5-hydroxynaphthalene (IIIc) (see Figure 2) (Chu, Villeneuve, et al., 1977).

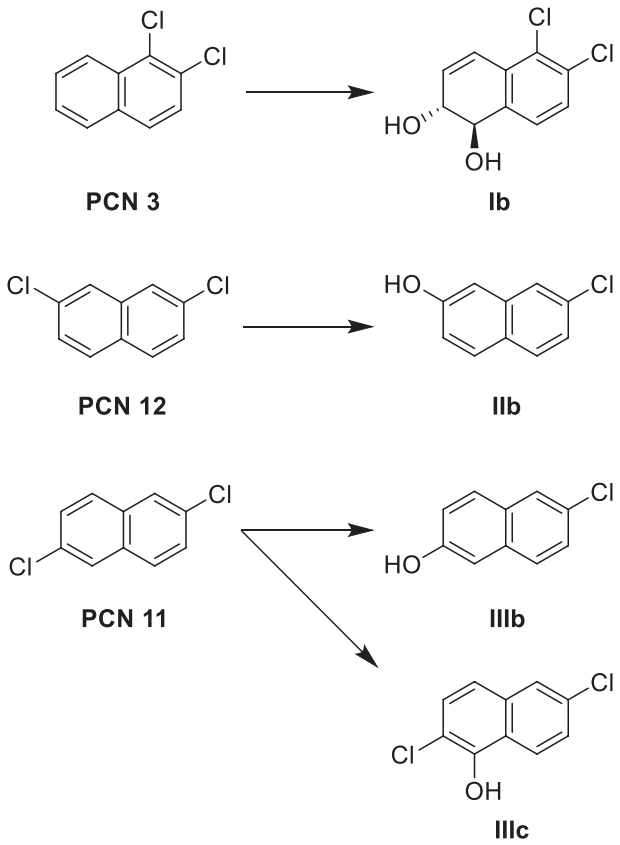


FIGURE 2 Metabolism of PCNs in rat [hydroxylated free compounds and their glucuronide conjugates detected in urine: (I) 1,2-diCN (**PCN-3**) à 1,2-dichloro-5,6-dihydroxy-5,6-dihydronaphthalene (**Ib**) (5,6-dichloro-1,2-dihydroxy-1,2-dihydronaphthalene, IUPAC), (II) 2,7-diCN (**PCN-12**) à 2-chloro-7-hydroxynaphthalene (**IIb**) (7-chloro-2-naphthol, IUPAC) and (III) 2,6-diCN (**PCN-11**) à 2-chloro-6-hydroxynaphthalene (**IIIb**) (6-chloro-2-naphthol, IUPAC) plus 2,6-dichloro-5-hydroxynaphthalene (**IIIc**) (2,6-dichloronaphthol, IUPAC) (adapted, after Chu, Villeneuve, et al., 1977).

Rats administered orally with a radiolabelled PCN mixture (tetra-, penta- and hexaCNs) excreted 99% of the dose during 5 days with 94% in the faeces. In the faeces, 22% of the radioactivity was not extractable compounds and was considered by the authors to be bound to endogenous molecules (Klasson-Wehler et al., 1996), 38% of the radioactivity was bound

to lipids and 5% were water soluble compounds. The remaining 30% of the excreted radioactivity in the faeces corresponded to PCNs according to GC/MS analysis. The faeces contained several metabolites (GC/MS), including hydroxylated tri- to pentaCNs, methylthio-tetra- to hexaCNs, methyl sulfoxide tri- and tetraCNs and a dihydroxy-tetraCN. The metabolites could not be structurally identified due to lack of reference compounds. Trace amounts of a methylthio-tetraCN and a methyl sulfoxide-pentaCN were indicated by GC/MS.

Excretion

Radiolabelled PCN-3 administered orally by gavage to male Wistar rats at a single dose of 400 mg/kg bw was excreted at 35% in the urine and 42% in the faeces over 7 days. For the dose excreted in the faeces, 62% entered the gastrointestinal tract via the bile, indicating biliary excretion and reabsorption of the parent compound or its metabolites, and the faecal radioactivity was corresponding primarily to PCN-3 (Chu, Secours, et al., 1977).

Following a single oral dose of about 30 µmol/kg bw (1 Ci/mol = 37,000 kBq/mol) of ^{14}C-labelled tetra-, penta- and hexaCNs to adult male Sprague–Dawley rats, 13% of the dose was excreted in faeces within the first day, and this could possibly be the non-absorbed parent PCNs. Within 5 days post-treatment excretion rate was 99% of which 94% was via the faeces (Jakobsson, 1994; Klasson-Wehler et al., 1996).

Three days after administration of ^{14}C labelled PCN-43 to pregnant female Wistar rats at a single dose of 0.3 mg/dam on gestational day 17, 18 or 19%, 24% of the dose still remained in the body (Kilanowicz, Stragierowicz, et al., 2019).

PCN-27 (1,2,3,4-tetraCN)-[ring-U-^{3}H] and PCN-49 (1,2,3,4,5-pentaCN)-[ring-U-^{3}H] administered intraperitoneally to male Wistar rats at a single dose of 10 mg/kg bw were excreted in faeces by approximately 70%. According to the authors, PCN-49 was excreted faster (40% excretion) within the first day and up to 65% within 5 days post-treatment, whereas PCN-27 was excreted by 40% within 2 days and up to 70% within 5 days. Only 6% of PCN-27 and 7% of PCN-49 were excreted in urine within 5 days after treatment (Kilanowicz et al., 2004).

Rats administered orally or intraperitoneally with ^{14}C-hexaCNs (mixture of isomers 94.1%; PCN-66/67 (^{14}C-1,2,3,4,6,7–/1,2,3,5,6,7-hexaCN) equivalent mixture at 81.2% and other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 at 5.9%) at a dose of 0.3 mg per animal (body weight 220–240 g) excreted PCNs mainly via faeces. After oral administration, faecal excretion was approximately 34% of the total dose within the first day, and 51% within 5 days. Excretion was slower after intraperitoneal administration with only 5% of the total dose excreted via faeces within the first day and 34% within 5 days. ^{14}C-PCNs excretion via urine was insignificant as < 1% of the total dose was excreted via urine after 5 days regardless of administration route. The decline of ^{14}C-PCNs in plasma was assessed after intraperitoneal administration and it was found to be biphasic. The half-lives for the first phase and the second phase were calculated to be ~ 6 and 350 h, respectively. Overall, hexaCNs (PCN-66/67) had a slow turnover rate in rats, and in the case of repeated exposure, it accumulates in the body (Kilanowicz, Daragó, & Skrzypińska-Gawrysiak, 2012).

Technical PCN formulations

Absorption

No data was retrieved on absorption. Indirect information on absorption comes from studies on appearance of Halowaxes in tissues. In Sprague–Dawley rats administered orally a single dose of 20 mg/kg bw of Halowax 1014 or 1051, the ratios of most of the individual congeners in adipose tissue a day after treatment remained similar to the ratios in the two Halowaxes; the exception was for both heptaCNs and octaCN for which the ratios had decreased. Indirectly, this may mean limited absorption of heptaCNs and octaCN from the intestine. In the liver, almost only the hexaCNs and some heptaCNs were found. The lower concentration of octaCN in adipose tissue was possibly due to the limited absorption from the intestine (Asplund et al., 1986, 1994).

Distribution

Halowax 1014 was administered orally to rats for up to 120 days (animals sacrificed after 1, 10, 30 or 120 days). PCN-66/67 were the dominant PCNs in the liver 1 day after sacrifice, but not in the adipose tissue. With time passing the proportions decreased in favour of the adipose tissue. Apart from a high retention of PCN-66/67 in liver, the adipose tissue seemed to be a reservoir of this compound on long term (Asplund et al., 1986).

Sprague–Dawley rats (body weight 240 g) were administered orally with a single dose of 0.053 mg/kg bw of the mixture of PCN-66, PCN-67 and an unidentified hexaCN in corn oil. Animals were sacrificed at 1, 10, 35 and 120 days after administration. PCN-66 and PCN-67 were found in the liver samples taken at 1 and 35 days after dosing; the unidentified hexaCN was not detected in the liver. The half-lives of PCN-66 and PCN-67 were calculated to be 41 days in the adipose tissue and 36 days in the liver (Asplund et al., 1994).

HexaCN (possibly PCN-66/67) and one heptaCN (possibly PCN-73) but not PCN-75 the major congener in Halowax 1051 (about 90%), were the only PCNs detected in small concentrations in female Spraque-Dawley rats, which were administered orally with Halowax 1051 at a single dose of 20 mg/kg bw and sacrificed at day 30 after administration (Asplund et al., 1986).

For Halowax 1051 (different batches), octaCN (82–93%) and heptaCN (6.2–18.9%) predominate. PCN-74 was 1.2–5.6 times higher than PCN-73, and mono- to hexaCNs were also present at very low concentrations, including PCN-66/67 (Falandysz et al., 2006a; Klimczak et al., 2023).

PCNs (Wako PCN, composed of at least of tri-, tetra- and pentaCNs) administered orally to Wistar rat at a single dose of 20 mg/kg bw were rapidly absorbed and distributed within the tissues and organs. Maximum concentrations in blood, liver, kidneys, spleen and brain were observed at 3 h post-treatment, and PCNs remained in the tissues and organs at least up to 15 days (Takeshita & Yoshida, 1980).

Metabolism/excretion

No studies were retrieved in the literature search.

3.1.1.2.2 | Studies in mice

Absorption

No studies were retrieved in the literature search.

Distribution

PCN-9, PCN-12 and PCN-75, dissolved in olive oil and injected intraperitoneally to JCL:ICR mice at a single dose of 1 mg each, were all accumulated in the tissues and organs. One day after administration, the adipose tissue showed highest accumulation of all three congeners. PCN-75 in adipose tissue increased up to day seven. The half-life of all three compounds was greater in the adipose tissue than in the brain, heart, lungs, liver, spleen, kidneys and testes. PCN-12 had greater half-life than PCN-9 in most tissues, while in adipose tissue, the half-life of PCN congeners followed an order of PCN-75 (3.72 days) > PCN-12 (0.80 days) > PCN-9 (0.27 days) (Oishi & Oishi, 1983).

Metabolism/excretion

No studies were retrieved in the literature search.

3.1.1.3 | *Food-producing animals*

3.1.1.3.1 | *Studies in ruminants*

No relevant studies were retrieved in the literature search.

3.1.1.3.2 | *Studies in pigs*

Absorption

No studies were retrieved in the literature search.

Distribution

PCN-1 and PCN-2 in oil emulsion were administered by injection in the carotid artery into female Yorkshire pigs (ca. 7.5 kg bw) at a dose of 300 mg per animal. After 6 h, when the animals were sacrificed, both compounds had the highest concentrations in the brain and kidneys, while they were also detected in the heart, back and skeletal muscles, lungs and liver. PCN-2 at small concentration occurred also in adipose tissue (Ruzo, Jones, Safe, & Hutzinger, 1976).

Hydroxylated metabolites of PCN-1 and PCN-2 were detected in the pig liver and kidneys (Ruzo, Jones, & Platonow, 1976).

Metabolism

Following administration of PCN-1 and PCN-2 to pigs (ca. 10 kg bw) at a single dose of 30 mg/kg bw (Ruzo et al., 1975) or a single dose (not specified; Ruzo, Jones, Safe, & Hutzinger, 1976) by injection in the carotid artery, hydroxylated metabolites were detected in the urine.

Female Yorkshire pigs, each administered a chloronaphthalene (30 mg/kg bw) by injection in the carotid artery, metabolised PCN-1 to 1-chloro-4-hydroxynaphthalene that was found in blood at 160 min after injection, while PCN-2 was metabolised to 3-chloro-2-hydroxynaphthalene that was found in blood at 200 min later. PCN-3 was metabolised to 1,2-dichloro-4-hydroxynaphthalene, PCN-5 to 1,3-dichloro-4-hydroxynaphthalene, PCN-27 to 1,2,3,4-tetrachloro-5-hydroxynapthalene and 1,2,3,4-tetrachloro-6-hydroxynaphthalene, while PCN-63 was resistant to biotransformation (Ruzo, Jones, & Platonow, 1976).

Excretion

Hydroxylated metabolites of PCN-1 and PCN-2 were excreted by pig through the bile and urine (Ruzo, Jones, & Platonow, 1976).

3.1.1.3.3 | *Studies in solipeds (horses and donkeys)*

No studies were retrieved in the literature search.

3.1.1.3.4 | *Studies in rabbits*

Absorption

PCN-1, diCN, tetraCN, pentaCN, heptaCN and octaCN administered by gavage (a fine suspension of in I % aqueous tragacanth gum) -to male albino rabbits (bw ca 2 kg) at a single dose of 1 g per animal, were absorbed based on excretion of various metabolites, but no quantitative data were provided (Cornish & Block, 1958).

Distribution

No studies were retrieved in the literature search.

Metabolism

PCN-1 and diCN, and to some degree also tetraCN were metabolised by male albino rabbits (ca. 2 kg bw) and excreted in the urine (Cornish & Block, 1958). The PCN-1 metabolites excreted in the urine during the 4-day period after administration were glucuronic acid (54%), mercapturic acid (13%) and ethereal sulfate (10%) derivatives. The diCN metabolites were glucuronic acid (69%), mercapturic acid (18%) and ethereal sulfate (6%) derivatives. The tetraCN metabolites were glucuronic acid (38%), mercapturic acid (3%) and ethereal sulfate (4%) derivatives. No metabolites were found in urine for pentaCN, heptaCN or octaCN. Following oral administration of pentaCN, heptaCN and octaCN, less than 20% of chlorinated (possibly unchanged) compounds could be measured in faeces of rabbits in a 4-day collection period (Cornish & Block, 1958).

PCN-11 administered orally (a fine suspension of PCN-11 in 1% aqueous tragacanth gum) to rabbits (3 animals; average body weight 2.5 kg) at a single dose of 300 mg/kg bw underwent hydroxylation as three monochloronaphthols were detected in the urine (Chu et al., 1976).

Excretion

Orally administered PCN-1 and diCNs were readily excreted with urine and faeces, and tetraCN to a smaller extent, while pentaCN, heptaCN and octaCN were not readily excreted. Following ingestion of penta- and heptaCNs, only a small and variable amount of compounds (as 'chlorinated compounds') were found in urine and faeces and the amount did not exceed 20% of the administered doses (Cornish & Block, 1958).

3.1.1.3.5 | *Studies in poultry*

No studies were retrieved in the literature search.

3.1.1.3.6 | *Studies in fish*

Absorption

Diet is major route of POPs in fish and intestinal uptake largely determines the fish body burden of hydrophobic compounds (James & Kleinow, 2014). PCNs, like PCBs, are highly hydrophobic and whereas some uptake via gills is possible (Crookes & Howe, 1993; Sijm et al., 1995), it is minimal under natural conditions (James & Kleinow, 2014). Therefore, branchial uptake of PCNs is not considered in this Opinion.

Absorption efficiency of PCN-52 from feed (benthic worms) by juvenile rainbow trout (*Oncorhynchus mykiss*) as the percentage of total dietary intake of trout at days 14 and 28 has been determined to 60% (Slootweg et al., 2015). Juvenile salmon (*Salmo salar*) exposed to Halowax 1001, 1014 and 1051 (0.1–10 µg/g feed) for 8, 13, 17 and 41 weeks accumulated from triCNs to octaCN. Biomagnification primarily of penta- and hexaCNs was in a dose-dependent manner. PCNs with high biomagnification factor value (from 1.0 to 2.5) were PCN-58, PCN-61, PCN-65, PCN-66/67, PCN-68 and PCN-69 (Åkerblom et al., 2000; Tysklind et al., 1998). Three-spined sticklebacks (G*asterosteus aculeatus*) exposed to feed contaminated with Halowax 1014 (concentrations not specified) accumulated PCNs in body tissues and the uptake efficiency was ~ 20% (Holm et al., 1993).

Distribution

PCNs absorbed by fish from the food across gut are distributed to lipid fraction of the muscle tissue, liver, fat and other organs (Falandysz, 2003; Jansson et al., 1984; Nfon et al., 2008).

Metabolism

The metabolic capacity toward POPs in fish is considered to be higher than that of molluscs, crustaceans or zooplankton, but lower than that of birds or mammals (Falandysz, Bergqvist, et al., 1996). Fish are considered weaker in sequential biotransformation of PCNs than birds of prey (white-tailed sea eagles, *Haliaeetus albicilla*), and this contributes to the retention of a range of PCN congeners in fish compared to adult birds (Falandysz, Bergqvist, et al., 1996; Falandysz, Strandberg, et al., 1996; Falandysz, Strandberg, Bergqvist, Strandberg, & Rappe, 1997; Falandysz, Strandberg, Strandberg, Bergqvist, & Rappe, 1997). In Four horned sculpins (*Myoxocephalus quadricornis*), the hexaCNs contributed to 42% of the total load of PCNs, and in isopods and amphipods that are their food, pentaCNs predominated with 53% contribution (Ishaq et al., 2000). The relative enrichment in higher chlorinated congeners observed with an increase in aquatic food web levels may be due to preferential metabolism of less chlorinated compounds (Falandysz, Kulp, et al., 1996; Hanari et al., 2004; Lundgren et al., 2002).

PCN-52, administered with feed (benthic worms) to juvenile rainbow trout (*Oncorhynchus mykiss*) for 28 days at daily doses of 0.13, 0.27 and 0.55 μg/g fish, induced the first phase of metabolism which was evident from the significant up-regulation of *cyp1a1* expression in the liver, while no changes were seen in *Abcb1a* transcript levels. Biotransformation of PCN-52 could contribute to the 25%–40% of the total PCN-52 intakes which were not recovered in the trout tissue or faeces (Slootweg et al., 2015).

Excretion

PCNs, like PCBs, may be eliminated unchanged from fish, through faecal excretion and diffusion across the gills (James & Kleinow, 2014). According to Slootweg et al. (2015) a relatively small percentage of between 7% and 9% of PCN-52 was recovered in the faeces of juvenile rainbow trout and reflected the compound associated with undigested worms excreted by the fish while the main fraction of diet-bound PCN-52 was bioavailable for uptake.

3.1.1.4 | *Non-food-producing animals*

3.1.1.4.1 | *Studies in dogs*

No relevant studies were retrieved in the literature search.

3.1.1.5 | *Summary on toxicokinetics*

In summary

Human toxicokinetic data have not been retrieved in the literature search. Some indirect information on oral, inhalatory and percutaneous absorption (and redistribution in tissues and organs) is available from the occupational and accidental exposure incidents and from use of PCNs on the skin of volunteers, and from more recent reports on accumulation of PCNs in human. PCNs retained in adipose tissue of the individuals intoxicated due to repeated ingestion of PCN (and PCB) contaminated rice oil (Yusho episode) were PCN-28/43, PCN-33/34/37, PCN-35, PCN-38/40, PCN-46, PCN-52/60 and PCN-66/67, while PCN-24, some tetraCNs, pentaCNs and hexaCNs and both heptaCNs – PCN-73 and PCN-74 determined in contaminated rice oil were not detected in exposed individuals. Placental transfer of PCNs to fetuses was shown by their occurrence in the aforementioned rice oil poisoning accident. These observations imply that all the monoCN to hexaCNs are well absorbed by humans from foodstuffs, which can contain these contaminants because of environmental pollution and transfer through food webs, while due to the larger size, heptaCNs and especially octaCN are possibly absorbed to a lesser extent.

Limited information on toxicokinetics for experimental and food-producing animals were retrieved in the literature search. Data were identified for rats, mice, pigs, rabbits and fish, although the studies covered only some individual compounds (native or radiolabelled congeners such as PCN-1, 2, 3, 5, 9, 11, 12, 27, 43, 49, 52, 63 or 75) and several mixtures. The studies included mixtures composed of the tetraCNs (PCN-43 > 95% and some other tetraCNs and triCNs), the tetra-, penta- and hexaCNs, the hexaCNs (PCN-66/67 and unidentified hexaCN), the hexa- and heptaCNs (PCN-66/67, 64/68, 69, 71/72, 63, 65, 70 and 73) and the technical PCN formulations (Halowax 1014, Halowax 1051, Wako PCN), depending on the animal species and study designs.

Studies in experimental animals indicated that PCN homologues from mono- to hexaCNs administered orally in a range of doses were well absorbed, but the absorption of the two heptaCNs and the octaCN was less efficient. Absorbed PCNs were readily distributed in organs and tissues. The decrease of hexa- and heptaCNs in plasma after intraperitoneal administration in rat was biphasic, with calculated phase I and II half-lives of approximately 6 and 350 h, respectively. Following oral administration (gavage) of tetra-, penta- and hexaCNs (single ^{14}C-labelled dose) in rat, they accumulated mainly in the liver and followed by abdominal fat, kidneys and the lungs. PCN-66 and PCN-67 administered orally by gavage in a single dose accumulated in rat from the first day and persisted in the liver and adipose tissue for up to 120 days. In general, hexaCNs showed slow turnover in rat. Maternal transfer to amniotic fluid and placenta has been reported and PCNs have also been detected in fetal tissues.

PCNs are likely to be metabolised like other similar halogenated compounds and showed increasing resistance to enzymatic oxidation due to steric hindrance and increase in the number of substituted chlorines. Mono- to tetraCNs were largely

and readily metabolised and excreted in the urine in form of hydroxylated PCNs (chloronaphthols) and phase II metabolites. Urinary metabolites of pentaCNs, heptaCNs and octaCN could not be observed in rabbit administered by gavage with a single dose of the parent compounds. Following a single oral dose of tetra-, penta- and hexaCNs in adult male Sprague–Dawley rat excretion rate within 5 days post-treatment was 99% of which 94% was via the faeces. Elimination with faeces dominated excretion of unabsorbed and metabolised (phase II) hydrophobic higher chlorinated naphthalene derivatives.

3.1.1.6 | *Physiologically based kinetic (PBK modelling)*

No studies related to physiologically based kinetic modelling of PCNs were retrieved in the literature search.

3.1.2 | Toxicity in experimental animals

A number of oral toxicity studies have been performed in rats predominantly with hexaCNs. Most studies were performed with a hexaCN mixture containing all 10 hexaCNs as well as a heptaCN (PCN-73) (composition: hexaCNs at 94.1%, i.e. PCN-66/67 at 81.2%, PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4% and PCN-73 at 5.9%). This mixture will be referred to as 'hexaCN mixture' below.

3.1.2.1 | *Acute toxicity studies*

Two acute toxicity studies were retrieved in the literature search, one with the 'hexaCN mixture' and one with a technical mixture. The studies are summarised below, and details are presented in Table 2.

In a study with the aim of investigating the acute toxicity of the 'hexaCN mixture' and its effects on cytochrome P-450 in rats, male Wistar rats were given a single dose of 0, 100 or 250 mg/kg bw by gavage (Kilanowicz & Skrzypinska-Gawrysiak, 2010). The animals were sacrificed at 24, 72 and 240 h after dosing. The relative liver weight was increased at both dose levels. The concentration of malondialdehyde (MDA) in the liver was increased at both doses and the glutathione (GSH) concentration was decreased at the highest dose. The total liver concentration of cytochrome P-450 was increased at both doses (no dose–response relationship) and the CYP1A activity in the liver was strongly induced at both doses.

In another study with the aim of investigating the acute toxicity of a PCN mixture and its effect on cytochrome P-450 in rats, male Wistar rats were given a single dose of 0, 250, 500 or 1000 mg/kg bw by gavage (Galoch et al., 2006). The animals were sacrificed at 24, 72 and 240 h after dosing. The relative liver weight was increased in the mid- and high-dose groups. The concentration of MDA in the liver was increased at the highest dose and the serum activity of sorbitol dehydrogenase (SDH) was increased at all doses (no dose–response relationship). The total liver concentration of cytochrome P-450 was increased at all doses (no dose–response relationship) and the CYP1A activity in the liver was strongly induced at all doses.

In summary, liver effects were reported in the two acute toxicity studies performed with rats.

3.1.2.2 | *Subacute toxicity studies*

Six subacute toxicity studies were retrieved in the literature search, one with PCN-43, one with PCN-66, one with PCN-67, two with the 'hexaCN mixture' and one with a technical mixture. The studies are summarised below, and details are presented in Table 2.

Male Wistar rats were given a dose of PCN-43 (1,3,5,8-tetraCN) of 0 or 10 mg/kg bw per day by gavage (Kilanowicz and Skrzypińska-Gawrysiak, 2010). The animals were sacrificed after 7, 14 or 28 days of dosing. No effects were reported.

In a study with the aim of assessing the relative toxicity and potency of two hexaCN congeners to TCDD, female Sprague–Dawley rats were given PCN-66 (purity 99.9%) or PCN-67 (purity 99.78%) at doses of 0, 0.0005, 0.0015, 0.005, 0.05 or 0.5 mg/kg bw by gavage in sunflower oil 5 days/week for a total of 12 doses (Hooth et al., 2012). The body weight was decreased at the highest dose for both congeners. Decreased absolute and relative thymus weights, as well as thymus atrophy was reported at the highest dose for both congeners. In the liver, histopathological changes included hepatocellular hypertrophy at the highest dose (both congeners), fatty change at the highest (both congeners) and next highest dose (PCN-66), necrosis and inflammation at the highest dose (PCN-67), hepatocellular necrosis associated with acute inflammation at the highest dose (PCN-67) and a reduction in the incidence of hepatic haematopoietic cell proliferation at the highest dose (both congeners). A significant and dose-related induction of hepatic CYP1A1 activity was observed at all doses (PCN-66) and at all doses except the lowest one (PCN-67). Induction of hepatic CYP1A2 activity was observed at two highest doses (both congeners). The relative potency to TCDD was evaluated by dose–response modelling using a Hill function for thymic atrophy and for the cytochrome P450 data (CYP 1A1 and CYP 1A2). The relative potency to TCDD for PCN-66 was 0.0015–0.0072 and for PCN-67 0.00029–0.00067.

In a study with the aim to investigate the subacute toxicity of the 'hexaCN mixture' and its effect on cytochrome P-450 in rats, male Wistar rats were given doses of 0, 1 or 10 mg/kg bw per day by gavage (Kilanowicz & Skrzypinska-Gawrysiak, 2010). The animals were sacrificed at 7, 14 or 28 days, or 7 weeks after dosing. The relative liver weight was increased at both doses (no dose–response relationship), the relative kidney weight was increased at the high dose and the relative spleen weight was decreased at the high dose. The MDA concentration in the liver was increased at both doses. The GSH concentration in the liver was decreased in the high-dose group and increased in the kidney at both doses. The total liver concentration

of cytochrome P-450 was increased at both doses (no dose–response relationship) and the CYP1A activity in the liver was strongly induced at both doses.

In a study with the aim to investigate the subacute toxicity of the 'hexaCN mixture' and its effect on selected haemostasis parameters, female Wistar rats were given doses of 0 or 0.3 mg/kg bw per day by gavage (Kilanowicz, Markowicz-Piasecka, et al., 2019). One group (group I) was first given the vehicle for 14 days and then the hexaCN mixture for 14 days; the other group (group II) was given the hexaCN mixture for 28 days. No general toxicity was observed in both groups. Decreased platelet count and increased mean platelet volume were observed in group II. Kinetic parameters of clot formation and fibrinolysis were decreased in both groups. The blood coagulation parameters activated partial thromboplastin time (APTT) and the APTT ratio were prolonged in group I and the fibrinogen concentration was decreased in both groups. The red blood cell (RBC) lysis was increased in group I.

In a study with the aim of investigating the subacute toxicity of a PCN mixture and its effect on cytochrome P-450 in rats, male Wistar rats were given doses of 0, 1, 10 or 100 mg/kg bw per day by gavage (Kilanowicz et al., 2009). The animals were sacrificed after 7 and 14 (all doses) or 21 doses (only the low- and mid-dose groups). The body weight was decreased in the mid- and high-dose groups. The relative liver weight was increased at all doses with no dose–response relationship after 14 and 21 days. The concentration of MDA in the liver was increased at all doses and the GSH concentration was decreased in the mid- and high-dose groups. The serum activity of SDH was increased in the mid- and high-dose groups. The total liver concentration of cytochrome P-450 was increased at all doses (no dose–response relationship) and the CYP1A activity in the liver was strongly induced at all doses.

In summary, liver effects were reported in three subacute toxicity studies performed with hexaCNs in rats and in one study performed with a PCN mixture in rats. A study with the aim to investigate the effects of hexaCNs on blood coagulation and fibrinolysis indicated that hexaCNs may disturb both coagulation and fibrinolysis processes, as well as may decrease the platelet count. In a study with PCN-43 in rats, no effects were reported.

3.1.2.3 | *Subchronic toxicity studies*

One subchronic toxicity study with the 'hexaCN mixture' was retrieved in the literature search. The study is summarised below, and details are presented in Table 2.

In a study with the aim of investigating the subchronic toxicity of the 'hexaCN mixture' and its effect on selected liver and haematological parameters, female Wistar rats were given doses of 0, 0.03, 0.1 or 0.3 mg/kg bw per day by gavage for 90 days (Klimczak et al., 2018). The absolute and relative liver and adrenal weights were increased at the highest dose and the absolute and relative thymus weights were decreased at the highest dose. At the microscopic examination, the only change noted was fatty degeneration in the liver at the highest dose. The MDA concentration in liver was increased at the mid and high dose and the serum total antioxidant status (TAS) was decreased at the high dose. The total liver concentration of cytochrome P-450 was increased at the low and mid dose (no dose–response relationship), but decreased at the high dose. The CYP1A activity in the liver and kidney was strongly induced at all doses and the CYP2B activity in liver and kidney was also induced at all doses. Haematological changes included decreased red blood cell (RBC) count, haemoglobin concentration (HGB), haematocrit (HTC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) at the high dose, an increased red cell distribution width (RDW) at the mid and high dose, a decrease in the platelet (PLT) count at the mid and high dose and an increased mean platelet volume (MPV) at the high dose. Changes in parameters related to the haem biosynthesis included decreased activity of aminolaevulinic acid dehydratase (ALA-D) and uroporphyrinogen decarboxylase (URO-D) in the liver at the high dose and there was also an impact on the concentration of porphyrins in liver and urine. An increased concentration of aminolaevulinic acid (ALA) was observed at the mid and high dose.

3.1.2.4 | *Chronic toxicity and carcinogenicity studies*

No chronic toxicity/carcinogenicity studies were retrieved in the literature search.

3.1.2.5 | *Developmental and reproductive toxicity studies*

Four studies regarding developmental toxicity were retrieved in the literature search, one with PCN-43, one with PCN-66, one with the 'hexaCN mixture' and one with a technical mixture. The studies are summarised below, and details are presented in Table 2. No reproductive toxicity studies were retrieved in the literature search.

In a study with the aim to investigate the prenatal toxicity of PCN-43 (1,3,5,8-tetraCN) during organogenesis, female Wistar rats were given doses of 0, 0.3, 1.0 or 3.0 mg/kg bw per day by gavage during GD 6–15 (Kilanowicz, Stragierowicz, et al., 2019). No maternal effects and no embryotoxic or teratogenic effects were observed. The only effect reported was delayed ossification in the mid- and high-dose groups.

In a study with the aim of assessing the effects of PCN-66 on the reproductive system of male offspring at various phases of sexual maturation, female Wistar rats were given doses of 0 or 0.001 mg/kg bw per day by gavage during GD 14–16 (Omura et al., 2000). No maternal effects and no embryotoxic or teratogenic effects were observed. An increase in the testicular spermatid count was observed on postnatal day (PND) 48 and an increase in the percentage of postmeiotic tubules was observed on PND 31. The CONTAM Panel noted that the suggested acceleration effect of PCN-66 on the first round of spermatogenesis in the offspring of exposed rats, was without any consequences on the spermatogenesis of the animals

when they reached the reproductive age and therefore, not an adverse effect. An increased in the sperm count in cauda epididymitis was observed on PND 62. Increased serum concentration of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) was observed from PND 31 to PND 48.

In a study with the aim to investigate the prenatal toxicity of the 'hexaCN mixture' during organogenesis, female Wistar rats were given doses of 0, 0.1, 0.3 or 1.0 mg/kg bw per day by gavage during GD 6–15 (Kilanowicz et al., 2015). The maternal body weight was decreased from GD 15 in the high-dose group. The relative weight of the liver, kidneys, adrenal glands, spleen and brain was increased at the high dose. At the microscopic examination, the only change noted was fatty changes in the liver at the highest dose. The MDA concentration in liver was increased at the mid and high dose. The intrauterine mortality of embryos and fetuses was increased at all doses, the fetal body weight was decreased in the mid- and high-dose groups, the fetal length was decreased at all doses and delayed ossification was observed at the high-dose. A strong induction of the CYP1A activity was observed in maternal liver and the enzyme was also induced in the placenta and in the fetal liver.

In a study with the aim of investigating the maternal toxicity and embryotoxic, fetotoxic and teratogenic effects of a PCN mixture, female Wistar rats were given doses of 0, 0.3, 1, 3 or 9 mg/kg bw per day by gavage during GD 6–15 (Kilanowicz et al., 2011). The maternal body weight was decreased from GD 10 at the two highest doses. The haematocrit in maternal blood was decreased at the highest dose. The absolute weight of the liver, ovaries and spleen was decreased at the highest dose and the relative weight of the liver, kidneys, adrenal glands, ovaries and brain was increased from 1 mg/kg bw per day, and in the spleen from 3 mg/kg bw per day. The intrauterine mortality of embryos and fetuses was increased at all doses, the fetal body weight and length were decreased at all doses, delayed ossification was observed at all doses, retarded development of internal organs (brain, kidneys) was observed at all doses and hydronephrosis was found in one female fetus at 0.3 mg/kg bw per day and in two male fetuses at 1.0 mg/kg bw per day.

In summary, maternal effects included liver effects in the study performed with the 'hexaCN' mixture in rats (increased liver weight and fatty changes) and in the study performed with a PCN mixture in rats (increased liver weight) and effects on the haematological system in the study performed with a PCN mixture in rats (decreased haematocrit). Developmental effects included increased intrauterine mortality of embryos and fetuses ('hexaCN mixture', PCN mixture), decreased fetal body weight and fetal length ('hexaCN mixture', PCN mixture) and delayed ossification (PCN-43, 'hexaCN mixture', PCN mixture).

3.1.2.6 | *Neurotoxicity studies*

One subacute neurotoxicity study with the 'hexaCN mixture' was retrieved in the literature search. The study is summarised below, and details are presented in Table 2.

In a study with the aim to investigate the subacute neurotoxicity of the 'hexaCN mixture', male Wistar rats (6 weeks old) were given doses of 0, 0.3 or 1.0 mg/kg bw per day by gavage for 28 days (Kilanowicz, Wiaderna, et al., 2012). The body weight was decreased in the high-dose group. Behavioural tests included assessment of motor behaviour on day 21 after exposure termination, assessment of long-term memory on day 28 after exposure termination, assessment of pain sensitivity and the magnitude of the stress-induced analgesia on day 35 after exposure termination and assessment of auditory function and sensorimotor gating on day 42 after exposure termination. Spontaneous locomotor and exploratory activities were decreased at the high-dose, long-term memory was impaired at both doses and enhanced pain sensibility and a decrease in the level of stress-dependent analgesia were observed at both doses.

3.1.2.7 | *Immunotoxicity studies*

No immunotoxicity studies were retrieved in the literature search.

TABLE 2 Experimental animal studies.

PCN(s)/composition	Study design	Parameters examined	Findings[a]	Reference
Acute toxicity studies				
'HexaCN mixture' The hexaCN mixture (hexaCNs at 94.1%) contained PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 (a heptaCN at 5.9%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Male Wistar rats (5/group) Single dose of 0, 100, 250 mg/kg bw by gavage (in sunflower oil) Sacrifice 24, 72, 240 h after dosing	Body weight (bw) Relative liver, kidney, spleen weights Glutathione (GSH) and malondialdehyde (MDA) concentration in liver and kidneys Activity of alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) in serum Total concentration of cytochrome P-450 in liver and P-450 CYP1A activity in the liver (7-ethoxyresorufin-O-deethylase, EROD)	Increased relative liver weight at both doses (+18%–48%) after 72 and 240 h Increased MDA concentration in the liver at both doses after 240 h Decreased GSH concentration in the liver at the highest dose after 72 and 240 h Increased total concentration of P-450 at both doses and all time points – no dose–response relationship (DR) Strong induction of CYP1A activity at both doses and all time points	Kilanowicz and Skrzypinska-Gawrysiak (2010)
PCN mixture The PCN mixture contained tetraCNs (54%), pentaCNs (8%), hexaCNs (23%) and heptaCNs (14%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Male Wistar rats (5/group) Single dose of 0, 250, 500, 1000 mg/kg bw by gavage (in sunflower oil) Sacrifice 24, 72, 240 h after dosing	Body weight Relative liver weight GSH and MDA concentration in liver Activity of ALT and SDH in serum Total concentration of cytochrome P-450 in liver and P-450 CYP1A activity in the liver (EROD)	Decreased body weight at all doses and all time points – statistically significant according to the text, but not according to Table 1 in the paper Increased relative liver weight in mid- and high-dose groups at 72 h (+27%, +54%, respectively) Increased MDA concentration in the high-dose group at all time points Increased SDH activity at all doses at 72 and 240 h – no DR Increased total concentration of P-450 at all doses and all time points–no DR Strong induction of CYP1A activity at all doses and all time points	Galoch et al. (2006)
Subacute toxicity studies				
PCN-43 1,3,5,8-tetraCN Purity greater than 95% The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Male Wistar rats (5/group) Dose of 0, 10 mg/kg bw per day by gavage (in sunflower oil) Sacrifice 7, 14, 28 days after dosing	Body weight (bw) Relative liver, kidney, spleen weights GSH and MDA concentration in liver and kidneys Activity of ALT and SDH in serum Total concentration of cytochrome P-450 in liver and P-450 CYP1A activity in the liver (EROD)	No effects reported	Kilanowicz and Skrzypinska-Gawrysiak (2010)
PCN-66 1,2,3,4,6,7-hexaCN Purity 99.9%	Female Sprague–Dawley rats (5/group) Doses of 0, 500, 1500, 5000, 50,000, 500,000 ng/kg bw per day (0, 0.0005, 0.0015, 0.005, 0.05, 0.5 mg/kg bw per day) by gavage (in corn oil) 5 days/week for a total of 12 doses	Thymus, right kidney, heart and lungs were weighed Histopathology performed on liver, lung, thyroid and thymus Cytochrome P-450 activities: CYP1A1 (EROD) and CYP1A2 (acetanilide-4-hydroxylase, A4H) activity in liver Dose–response modelling for relative potencies (Hill function) for thymic atrophy, CYP1A1 and CYP1A2 activities	Decreased bw at the highest dose Decreased absolute and relative thymus weight at the highest dose Histopathology liver: increased incidence of diffuse hepatocellular hypertrophy at the highest dose, diffuse micro- and macrovesicular fatty change at the highest and next highest doses; reduced incidence of hepatic haematopoietic cell proliferation at the highest dose Histopathology thymus: atrophy at the highest dose Significant induction of CYP1A1 (EROD) activity all doses Induction of CYP1A2 (A4H) activity at the two highest doses Relative potency to TCDD: 0.0015–0.0072	Hooth et al. (2012)

(Continues)

TABLE 2 (Continued)

PCN(s)/composition	Study design	Parameters examined	Findings[a]	Reference
PCN-67 1,2,3,5,6,7-hexaCN Purity 99.78%	Female Sprague–Dawley rats (5/group) Doses of 0, 500, 1500, 5000, 50,000, 500,000 ng/kg bw per day (0, 0.0005, 0.0015, 0.005, 0.05, 0.5 mg/kg bw per day) by gavage (in corn oil) 5 days/week for a total of 12 doses	Thymus, right kidney, heart and lungs were weighed Histopathology performed on liver, lung, thyroid and thymus Cytochrome P-450 activities: CYP1A1 (EROD) and CYP1A2 (A4H) activity in liver Dose–response modelling for relative potencies (Hill function) for thymic atrophy, CYP1A1 and CYP1A2 activities	Decreased bw at the highest dose Decreased absolute and relative thymus weight at the highest dose Histopathology liver: increased incidence of diffuse hepatocellular hypertrophy at the highest dose, diffuse micro- and macrovesicular fatty change at the highest dose, focal to multifocal hepatocellular necrosis associated with acute inflammation at the highest dose; reduced incidence of hepatic haematopoietic cell proliferation at the highest dose Histopathology thymus: atrophy at the highest dose Significant induction of CYP1A1 (EROD) activity all doses except for the lowest dose Induction of CYP1A2 (A4H) activity at the two highest doses Relative potency to TCDD: 0.00029–0.00067	Hooth et al. (2012)
'HexaCN mixture' The hexaCN mixture (hexaCNs at 94.1%) contained PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 (a heptaCN at 5.9%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Male Wistar rats (5/group) Doses of 0, 1, 10 mg/kg bw per day by gavage (in sunflower oil) Sacrifice 7, 14, 28 days and 7 weeks after dosing	Body weight Relative liver, kidney, spleen weights GSH and MDA concentration in liver and kidneys Activity of ALT and SDH in serum Total concentration of cytochrome P-450 in liver and P-450 CYP1A activity in the liver (EROD)	Decreased bw at both doses after 14 days and at the highest dose after 28 days Increased relative liver weight at both doses after 14 days (no DR) and at the low dose after 28 days Increased relative kidney weight at the high dose after 14 days Decreased relative spleen weight at the high dose after 14 and 28 days Increased MDA concentration in the liver at the high dose after 7 days and at both doses after 14 and 28 days Decreased GSH concentration in the liver at the high-dose after 28 days Increased GSH concentration in the kidney at the high-dose after 7 and 14 days and at the low dose after 28 days Increased total concentration of P-450 at both doses and all time points–no DR Strong induction of CYP1A activity at both doses and all time points	Kilanowicz and Skrzypinska-Gawrysiak (2010)
'HexaCN mixture' The hexaCN mixture (hexaCNs at 94.1%) contained PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 (a heptaCN at 5.9%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Female Wistar rats (5/group) Doses of 0, 0,3 mg/kg bw per day by gavage (in sunflower oil) Group I: vehicle for 14 days and then the hexaCN mixture for 14 days Group II: the hexaCN mixture for 28 days	Body weight Absolute and relative liver, brain, kidney, lung, heart weights Haematological parameters: Red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), RBC distribution width (RDW), RDW standard deviation (RDW-SD), white blood cell count (WBC), platelet count (PLT) and mean platelet volume (MPV) Test of clot formation and lysis (CL test): allows the overall potential of clot formation and fibrinolysis to be established, as well as their kinetic parameters: overall potential of clot formation and fibrinolysis (CL_{AUC}), thrombotin time (Tt), maximum clotting (Fmax), plasma clotting time (Tf), initial plasma clotting velocity (Fvo), clot stabilisation time (Tc), maximum lysis (Lmax), fibrinolysis time (Tl), initial clot fibrinolysis velocity (Lvo) Coagulation tests: activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen Stability of RBCs membrane: RBC lysis assay	No general toxicity observed (bw, organ weights) Decreased PLT and increased MPV in group II Decreased kinetic parameters of clot formation and fibrinolysis: Fmax, Fvo, Lvo, Lmax and CL_{AUC} in both groups Prolongation of APTT and APTT ratio in group I Decreased fibrinogen concentration in both groups Increased RBC lysis in group I	Kilanowicz, Markowicz-Piasecka, et al. (2019)

TABLE 2 (Continued)

PCN(s)/composition	Study design	Parameters examined	Findings[a]	Reference
PCN mixture The PCN mixture contained tetraCNs (54%), pentaCNs (8%), hexaCNs (23%) and heptaCNs (14%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Male Wistar rats (5/group) Doses of 0, 1, 10, 100 mg/kg bw per day by gavage (in sunflower oil) Sacrifice after 7, 14, 21 doses (21 doses: 0, 1, 10 mg/kg bw per day)	Body weight Relative liver weight GSH and MDA concentration in liver Activity of ALT and SDH in serum Total concentration of cytochrome P-450 in liver and P-450 CYP1A activity in the liver (EROD)	Decreased bw at the highest dose after 7 days, at the mid and highest dose after 14 days, and at the mid dose after 21 days Increased relative liver weight at all doses after 7 days, at the low and high dose after 14 days – no DR, and at the low and mid dose after 21 days–no DR Increased MDA concentration at the mid and high dose after 7 and 14 days, and at the low and mid dose after 21 days Decreased GSH concentration at the mid and high doses after 14 days, and at the mid dose after 21 days Increased SDH activity at the high dose after 14 days, and at the mid dose after 21 days Increased total concentration of P-450 at all doses and all time points–no DR Strong induction of CYP1A activity at all doses and all time points	Kilanowicz et al. (2009)
Sub-chronic toxicity studies				
'HexaCN mixture' The hexaCN mixture (hexaCNs at 94.1%) contained PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 (a heptaCN at 5.9%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Female Wistar rats (10/group) Doses of 0, 0.03, 0.1, 0.3 mg/kg bw per day by gavage (in sunflower oil) 90 days	Body weight Liver, kidneys, lungs, adrenals, heart, thymus, spleen, ovaries, uterus weights and microscopy GSH and MDA concentration in liver and kidneys Serum total antioxidant status (TAS) Total concentration of cytochrome P-450 in liver, and P-450 CYP1A (EROD) and CYP2B (pentoxyresorufin O-dealkylation, PROD) activities in liver and kidney <u>Parameters for haem biosynthesis</u>: Activity of aminolevulinic acid synthase (ALA-S) and of aminolevulinic acid dehydratase (ALA-D) in liver URO-D activity in liver and kidney Porphyrins concentration in liver and urine ALA concentration in urine <u>Haematological parameters</u>: Red blood cell (RBC) count, haemoglobin concentration (HGB), haematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), RBC distribution width (RDW), white blood cell count (WBC), platelet count (PLT) and mean platelet volume (MPV)	Increased absolute and relative liver (+54% and +58%) and adrenal weights liver (+32% and +35%) at the high dose Decreased absolute and relative thymus weight (−56% and −50%) at the high dose Liver fatty degeneration (steatosis) at the high dose Increased MDA concentration in liver at the mid and high dose Decreased TAS at the high dose Increased total concentration of P-450 at low and mid dose–no DR, but decreased at the high dose Strong induction of CYP1A activity in liver and kidney at all doses Induction of CYP2B activity in liver and kidney at all doses Decreased activity of ALA-D and URO-D in liver at the high dose Impact on concentration of porphyrins in liver and urine Increased ALA concentration at mid and high dose Decreased RBC count (−5%), HGB (−24%), HTC (−17%), MCV (−7%), MCH (−8%) at the high dose Increased RDW at the mid and high dose (+9%, +15%) Decreased PLT at the mid and high dose (−28%, −48%) Increased MPV at the high dose (+37%)	Klimczak et al. (2018)
Sub-acute neurotoxicity study				
'HexaCN mixture' The hexaCN mixture (hexaCNs at 94.1%) contained PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 (a heptaCN at 5.9%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Male Wistar rats (5/group) Doses of 0, 0.3, 1 mg/kg bw per day by gavage (in sunflower oil) 28 days	Body weight <u>Behavioural tests</u>: Open-field test (assessment of motor behaviour) on day 21 after exposure termination Passive avoidance test (assessment of long-term memory) on day 28 after exposure termination Hot-plate test (assessment of pain sensitivity and the magnitude of the stress-induced analgesia) on day 35 after exposure termination Acoustic startle response and startle prepulse inhibition (assessment of auditory function and sensorimotor gating) on day 42 after exposure termination	Decreased bw at the high-dose Piloerection and changes in the body posture at the high dose Open-field test: Spontaneous locomotor and exploratory activities decreased at the high dose Passive avoidance test: Long-term memory impaired at both doses Hot-plate test: Enhanced pain sensibility and a decrease in the level of stress-dependent analgesia at both doses	Kilanowicz, Daragó, and Skrzypińska-Gawrysiak (2012)

(Continues)

TABLE 2 (Continued)

PCN(s)/composition	Study design	Parameters examined	Findings[a]	Reference
Developmental toxicity studies				
PCN-43 1,3,5,8-tetraCN Purity greater than 95% 5% accounted for other tetraCNs (3.98%) and triCNs (1.02%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Female Wistar rats (9-12/group) Doses of 0, 0.3, 1, 3 mg/kg bw per day by gavage (in sunflower oil) During gestation days (GD) 6–15	Body weight Maternal liver, kidney, adrenals, ovaries, spleen, brain, gravid uterine weights GSH and MDA concentration in liver P-450 CYP1A activity in the liver (EROD) Number of live/dead fetuses, early and late resorptions, live fetuses bw and length External, skeletal, visceral malformations	No maternal effects No embryotoxic or teratogenic effects Delayed ossification at the mid and high dose	Kilanowicz, Stragierowicz, et al. (2019)
PCN-66 1,2,3,4,6,7-hexaCN Purity not given	Female Wistar rats (7/group) Doses of 0, 0.001 mg/kg bw per day by gavage (in corn oil) During GD 14–16	PND 0: offspring counted, sexed, examined for gross malformations, litters randomly reduced to five males and five females Body weight and anogenital distance (AGD) recorded on PNDs 1, 4 and 21 Offspring examined for eye opening on PNDs 14–18 Offspring weaned on PND 21 and housed by sex, based on litters On PNDs 31, 48, 62, and 89, one male offspring from each litter was chosen at random and killed; blood was collected Testes, epididymides, ventral prostate, seminal vesicles weighed Spermatids from testes counted Sperm from the cauda epididymides counted Sperm motility examined Testes microscopic examination Serum LH and FSH determined	No maternal effects No embryotoxic or teratogenic effects Increased testicular spermatid count (approximately +60%) on PND 48 Increased percentage of postmeiotic tubules (approximately +90%) on PND 31 Increased sperm count in cauda epididymidis (approximately +80%) on PND 62 Increased serum concentration of LH and FSH from PND 31 to PND 48	Omura et al. (2000)
'HexaCN mixture' The hexaCN mixture (hexaCNs at 94.1%) contained PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 (a heptaCN at 5.9%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Female Wistar rats (17-19/group) Doses of 0, 0.1, 0.3, 1 mg/kg bw per day by gavage (in sunflower oil) During GD 6–15	Body weight Maternal liver, kidney, adrenals, ovaries, spleen, brain, gravid uterine weights GSH and MDA concentration in liver Activity of ALT and SDH in serum Morphological examination of maternal/fetal liver and placenta Number of live/dead fetuses, early and late resorptions, live fetuses body weight and length External, skeletal, visceral malformations P-450 CYP1A expression in the maternal / fetal liver and placenta by reverse-transferase – polymerase chain reaction (RT-PCR), Western blotting, immunohistochemistry	Decreased maternal bw gain from GD15 at the high dose Increased relative weight liver, kidneys, adrenal glands, spleen, brain at the high dose Increased MDA concentration at the mid and high dose Fatty changes in the liver at the high dose Strong induction of CYP1A activity maternal liver, also induction in placenta and fetal liver Increased intrauterine mortality of embryos and fetuses at all doses Decreased fetal body weight at the mid and high dose Decreased length at all doses Delayed ossification at the high dose	Kilanowicz et al. (2015)

TABLE 2 (Continued)

PCN(s)/composition	Study design	Parameters examined	Findings[a]	Reference
PCN mixture The PCN mixture contained tetraCNs (54%), pentaCNs (8%), hexaCNs (23%) and heptaCNs (14%) The concentration of polychlorinated dioxins and furans was < 0.1 pg/100 μg of the investigated sample	Female rats (11–16/group) Doses of 0, 0.3, 1, 3, 9 mg/kg bw per day by gavage (in sunflower oil) During GD 6–15	Body weight Maternal liver, kidneys, adrenals, ovaries, spleen, brain, gravid uterine weights Haematocrit maternal blood Number of live/dead fetuses, early and late resorptions, live fetuses body weight and length External, skeletal, visceral malformations	Decreased maternal body weight from GD 10 at the two highest doses Decreased haematocrit at the highest dose Decreased absolute weights of liver, ovaries, spleen at the highest dose Increased relative weight of liver, kidneys, adrenal glands, ovaries, brain from 1 mg/kg bw per day and spleen from 3 mg/kg bw per day Increased intrauterine mortality of embryos and fetuses at all doses Decreased fetal body weight and length at all doses Delayed ossification at all doses Retarded development internal organs (brain, kidneys) at all doses Hydronephrosis in one female fetus at 0.3 mg/kg bw per day and in two male fetuses at 1.0 mg/kg bw per day	Kilanowicz et al. (2011)

[a]Only statistically significant results are described.

3.1.2.8 | *Genotoxicity studies*

In vitro

Four PCN congeners, i.e. PCN-1, PCN-2, PCN-27 and PCN-75, have been tested in four strains of Salmonella Typhimurium with and without the metabolic activation provided by S9 mix prepared from Aroclor1254-induced male Sprague–Dawley rat or Syrian hamster liver. All these compounds were tested using a pre-incubation protocol in strains TA100, TA1535, TA98, TA 97, with the exception of PCN-27 where TA1537 substituted the TA97 strain (details in Table 3). PCN-1 was the only congener that provided a positive response in two strains of S. Typhimurium (TA100 and TA97). The mutagenic effect was weak (although reproduced in repeated experiments) and observed only in trials conducted in the presence of Syrian hamster liver S9 mix. Negative results were obtained in the other S. Typhimurium TA1535, TA98 strains (NTP No. report 771958); Zeiger et al., 1992). Using the same experimental conditions, consistently negative results were reported for PCN-2 (Zeiger et al., 1992), PCN-27 (NTP study No. 648470); Haworth et al., 1983) and PCN-75 (NTP study No. A21226). Although none of the studies have been performed according to the present OECD test guidelines, these studies are well performed. The CONTAM Panel noted that because there is no TA102 strain or *Escherichia coli* WP then negative responses in these Ames tests would be scored as of limited relevance (EFSA Scientific Committee, 2011).

In vivo

A mixture of hexaCNs (hexaCNs at 94.1%, contained PCN-66/67 [equivalent mixture at 81.2%], other hexaCNs [PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%] and PCN-73 [a heptaCN at 5.9%]) has been investigated in an in vivo micronucleus bone marrow test in female Wistar rats after oral administration of 0, 0.03, 0.1 or 0.3 mg/kg bw per day by gavage for 90 days (Klimczak et al., 2018) (see Section 3.1.2.3). The presence of micronuclei was determined by scoring 4000 polychromatic erythrocytes per animal. The ratio of polychromatic erythrocytes and normochromatic erythrocytes (PCEs/NCEs) was determined by counting at least 2000 erythrocytes from each bone marrow sample. Similar frequencies of micronucleated PCEs were observed in the treated and control groups. No apparent toxicity to the bone marrow is suggested by the lack of alteration in the PCEs/NCEs ratios. However, systemic effects in form of haematological changes and hepatotoxicity in subacute studies and in this 90-day study are lines of evidence of systemic bioavailability and can be considered as a line of evidence of bone marrow exposure (EFSA Scientific Committee, 2017b). Under the conditions of this study, exposure to a mixture of these hexaCNs for 90 days did not induce clastogenic effects in female rats. The CONTAM Panel noted that the 90-day duration of the study differs from the OECD TG 474, on the micronucleus assay.

Summary of genotoxicity studies

Overall, the information on PCN-1, PCN-2, PCN-27 and PCN-75 is limited to Salmonella Typhimurium strains TA100, TA1535, TA98, TA97 and TA1537, in both absence and presence of metabolic activation. PCN-2, PCN-27 and PCN-75 were negative. PCN-1 showed to be weakly mutagenic in TA100 and TA97 in the presence of a specific type of metabolic activation system (Syrian hamster S9). The 'hexaCN mixture' tested in an in vivo micronucleus test with female rats did not induce clastogenic effects. The extremely limited information on genotoxicity of PCNs does not allow to conclude on their genotoxic potential.

TABLE 3 In vitro genotoxicity studies on PCNs.

Compound	Type of test	Experimental system	Exposure conditions	Result	Comments	Reference
PCN-1	Reverse mutation assay	Salmonella Typhimurium TA97, TA98, TA100, TA1535 +/– 10–30% rat and hamster liver S9	Up to 200 μg/plate	**Positive in TA100 and TA97** (> two-fold increase at 100 and 200 μg/plate in the presence of hamster liver S9 (3 experiments for TA97 and 2 experiments for TA100) **Negative in TA1535, TA98**	**Toxicity**: none in TA97 and TA100 + 30% hamster liver S9	Reported in the NTP study No. 771958 and Zeiger et al. (1992)
PCN-2	Reverse mutation assay	Salmonella Typhimurium TA97, TA98, TA100, TA1535 +/– 10–30% rat and hamster liver S9	Up to 500 μg/plate (TA98, TA100) Up to 333 μg/plate (TA97, TA1535)	**Negative** (sporadic equivocal results in TA100 and TA97)	**Toxicity:** higher in the absence of rat or Syrian hamster S9 Lower toxicity provided by the presence of Syrian hamster rather than rat liver S9; excessive toxicity in the range 100–333 μg/plate	Reported in NTP study No. 328159 and Zeiger et al. (1992)
PCN-27	Reverse mutation assay	Salmonella Typhimurium TA1537, TA98, TA100, TA1535 +/– 10% rat and hamster liver S9	Up to 10,000 μg/plate	**Negative**	**Toxicity:** none (either with or without S9 from rat and hamster liver) **Precipitation**: from 333 to 1000 μg/plate +/– S9 in all strains	Reported in the NTP study No. 648470 and Haworth et al. (1983)
PCN-75	Reverse mutation assay	Salmonella Typhimurium TA97, TA98, TA100, TA1535 +/– 10–30% rat and hamster liver S9	Up to 10,000 μg/plate	**Negative**	**Toxicity:** none (either with or without S9 from rat and hamster liver) **Precipitation**: from 3333 μg/plate +/– S9 in all strains	Reported in the NTP study No. A21226 and Mortelmans and Zeiger (2000)

3.1.3 | Observations in humans

None of the retrieved studies were considered relevant for this mandate. Dougherty et al. (1981) evaluated sperm density distributions obtained from 132 college-aged men that were compared with toxic substance burden data. The technique used for assessment of body burden was negative chemical ionisation (NCI) mass spectrometry. The NCI screening showed the presence of several polychlorinated substances including PCNs. All the remaining studies were related to exposure of workers, i.e. inhalation and dermal exposure, as well as volunteer studies with dermal application.

3.1.4 | Adverse effects in food-producing animals

The majority of studies on PCNs carried out in food-producing animals have used technical mixtures (e.g. Halowax). While data from such studies are useful for the hazard identification they are less so for hazard characterisation.

3.1.4.1 | *Studies in ruminants*

A chronic disease of unknown origin was observed in cattle herds in western New York State and Maryland starting in 1942. The condition was characterised by depression, emaciation, anorexia and thick, dry, wrinkled skin (Olafson, 1947). Animals or specimens from eight herds were received and examined in 1947. Two affected herds were found in western New York, four were scattered throughout central New York, two occurred in the Hudson Valley, three were in Northern Pennsylvania and one was in Maryland. Affected herds were on a variety of feed and the disease appeared in late winter or spring. Olafson (1947) who originally reported on these outbreaks in cattle named the condition 'X disease' because of its unknown aetiology at the time. The thickening of the skin characteristic of 'X disease' was later found to be caused by PCN exposure. Lesions included papillary proliferations of the mucosa of the mouth and oesophagus, papillary proliferations of the lining of large bile ducts and the gall bladder, proliferations and accompanying fibroses of the small bile ducts, liver fibrosis, thickening of the intestinal mucosa and cystic dilatation of the collecting tubules in the renal cortex. Severe mastitis was common in females that died from the disease. Affected animals had blood plasma vitamin A levels in the range of 4.5–11 μg of vitamin A per 100 mL blood, with 25–85 μg of vitamin A per 100 mL being considered normal (Smith et al., 2020).

The original observations were followed by case studies of accidental exposure primarily of cattle to PCNs causing what was later diagnosed as hyperkeratosis (Haughey & Cooper, 1953). Because of these initial findings, early experimental studies on effects of PCNs on farm animals focussed on hyperkeratosis and vitamin A deficiency (Bell, 1952; Copenhaver & Bell, 1954; Hansel et al., 1953; McEntee, 1951; Sikes & Bridges, 1952). A link between hyperkeratosis and vitamin A deficiency was proposed because PCN exposure causes vitamin A deficiency, which by itself leads to overproduction of keratin and development of hyperkeratosis (Marsh et al., 1956). Other notable effects recorded in these early experimental studies included retarded horn growth, loss of appetite, liver pathologies, renal nephropathy, ascites, cardiovascular 'injury' and mortality (Brock et al., 1957; Copenhaver & Bell, 1954; Hansel et al., 1953; Haughey & Cooper, 1953; Sikes & Bridges, 1952). There is also a notable study on reversible reproductive impairment in cattle. In an experiment carried out by Vlahos et al. (1955), a yearling Holstein bull with normal semen was given a single administration of 1.8 g of a mixture of penta and hexaCN. Plasma vitamin A levels were reduced fourfold and semen quality decreased with no motility for 6 months after the exposure. Semen quality returned to normal after about 10 months and the bull was then fertile. The temporary fall in male fertility may be due to or associated with vitamin A deficiency as described in classic reports (Griswold et al., 1989). All of the above studies lack in different aspects of experimental design and few provide information to allow identification of a no effect level, but they can be considered useful in providing qualitative supporting information for the hazard identification.

The influence of degree of PCN chlorination on potency to cause hyperkeratosis in calves (~ 2–4 months of age; 40–122 kg) studied by Bell (1953). The test materials used were: diCN (35.8% Cl), triCN (47.8% Cl), tetraCN (53.5% Cl), pentaCN (59.2% Cl), hexaCN (64.3% Cl), heptaCN (68.5% Cl), octaCN (70.0% Cl). PCN samples were purified to contain compounds of equal degrees of chlorination. Each fraction, however, contained several isomers differing in the positions of Cl atoms in the molecule, as well as other congeners as impurities (e.g. triCNs and pentaCNs could be present as impurities in the tetraCNs). Beyond the degree of chlorination, identity of the PCNs present as well as their relative content was not stated.

The symptoms and lesions produced by various purified chlorinated naphthalenes were characteristic of bovine hyperkeratosis. Prolonged effects were observed in only one case.

- DiCN and triCN cause no adverse effects at a dose of 31 mg/kg bw per day for 7 days and 5.4 mg/kg bw per day for 10 days, respectively.
- TetraCN (3.4 or 5.4 mg/kg bw per day) caused transient adverse effects in two calves (lacrimation, nasal discharge, depression) but a third calf orally exposed to 9.1 mg/kg bw per day for 13 days showed no symptoms.
- PentaCN (2.4–4.5 mg/kg bw per day), hexaCN 1.1–4.5 mg/kg bw per day), and heptaCN (1.1–3.4 mg/kg bw per day) exposures caused severe toxicity with lacrimation, nasal discharge, salivation, reddening of gums and oral mucosa, depression being the main symptoms. Gross pathology on autopsy revealed among other symptoms swelling of kidneys, mottling of liver and distension of the gall bladder.

- PCN-75 given in suspension did not have a toxic effect, but 2.3 mg/kg bw per day given in solution (in mineral oil U.S.P.) for 5 days produced hyperkeratosis.

Although each calf was given a different dose and duration of exposure, it appears that toxicity increased from diCN to hexa- and heptaCN, with octaCN toxicity depending on vehicle.

Marsh et al. (1956) investigated the relationship between PCN induced hyperkeratosis and skin collagen content in calves and pregnant cows. Also reported were plasma levels of vitamin A, carotene and total ascorbic acid. Calves (6 months old; unspecified breed) were given daily oral doses of 5.55 mg/kg bw (2.52 mg/lb) for 6 days of a commercial product containing penta- and hexaCNs (62% chlorine) and were observed for 30 days. Ten calves aged 1–8 months old were used as controls. It appears that oral exposure of calves to 5.55 mg/kg bw per day of the penta- and hexaCN mixture led to reduced collagen in the skin (exposed: 4.33 ± 3.38 g/100 g skin, mean ± SEM, $N = 3$; controls: 19.38 ± 4.14 g/100 g skin, mean ± SEM, $N = 8$) although this was not statistically significant and two of the control calves had extremely low concentrations of collagen in the skin (1 g/100 g skin) according to the analyses. Although no statistical analysis was provided, vitamin A concentrations in exposed calves dropped precipitously (60%–90% decrease) during the 6-day exposure and only partially recovered during the observation period.

Five pregnant cows (4–5 years old; unspecified breed) were exposed orally with a single dose of 0 ($N = 1$), 1.39 ($N = 1$), 2.75 ($N = 1$) and 11.0 ($N = 2$) mg/kg bw (0, 0.63, 1.25, and 5 mg/lb) of 'a purified preparation' (unknown purity) of pentaCN and observed over 6 days. All four animals treated with 'pure pentaCN' showed marked 30%–70% decrease in plasma levels of vitamin A, but without a clear dose–response. This study indicates that the pathological changes in epithelial and connective tissues in bovine hyperkeratosis are associated with reduced plasma vitamin A concentrations after PCN exposure.

The toxicity of Halowax 1014 in sheep was studied by Brock et al. (1957). Ewes (age and breed not identified) were orally exposed to Halowax 1014, which was incorporated into gelatine capsules administered at doses of 1.1, 11 or 27.5 mg/kg bw per day (reported as 0.5, 5 or 12.5 mg/lb per day) until death or termination of the experiment at 135 days. There was no concurrent control group, but results reported from untreated sheep were taken from previous studies (without further information). Blood samples were collected from each sheep before treatment and at 5- to 15-day intervals during treatment for vitamin A analysis of the plasma.

All doses caused mortality with only one animal at the lowest dose surviving until the end of the experiment. The mean time to death at the different doses was 106, 27 and 20 days, respectively. Early signs of toxicity were excess nasal discharge, weakness, weight loss and inappetence and was observed in animals of all dose groups. Plasma vitamin A was significantly decreased after at least 5 days treatment with 11 or 27.5 mg/kg bw per day, but no avitaminosis A was observed with the lowest concentration measured being 17.9 µg/100 mL before death in one animal. In the control group, the mean plasma vitamin A concentration was about 30 µg/100 mL.

Gross pathology found at necropsy of animals from all groups were liver damage, gastrointestinal haemorrhage, as well as pleural and/or peritoneal fluid. Histological examination revealed effects in liver (congestion, haemorrhage and degeneration), gall bladder (congestion), bile duct (coagulation necrosis of the mucous membrane), kidneys (congestion of blood vessels and brown granule deposits), uterus (congestion of the endometrium, haemorrhage), lungs (haemorrhage), heart (swelling, hyperaemia of the myocardium, haemorrhage), spleen (hyperaemia, haemorrhage) and intestinal tract (congestion, haemorrhage, degeneration). These histopathological observations were made in animals from all treatment groups but were less severe in the group treated with the lowest dose (0.23 mg/kg bw per day). This study provides evidence that PCN toxicity presents somewhat differently in sheep compared with cattle and is primarily not associated with vitamin A deficiency and hyperkeratosis.

Overall, exposure of PCN mixtures to cattle causes an acute drop in plasma vitamin A concentrations. Avitaminosis A seems to be causative of the hyperkeratosis characteristic of PCN poisoning in cattle. Other pathologies include papillary proliferations of the epithelia lining the mouth, oesophagus, bile ducts and gall bladder. Fibrosis can occur in bile ducts and in the liver. Cystic dilatation of the collecting tubules in the renal cortex is another common lesion, as is mastitis in cows. The data available suggest that the potency of PCNs in causing these effects increases from diCN to heptaCN. Based on limited information on exposure of sheep to Halowax 1014, PCN toxicity presents somewhat differently in sheep compared with cattle. Vitamin A deficiency is only transient and there is little or no hyperkeratosis. However, effects on internal organs appear to be similar to those observed in cattle. The studies available do not allow identification of a no observed adverse effect level (NOAEL) or other Reference Points for hazard characterisation for ruminants.

3.1.4.2 | *Studies in pigs*

Link et al. (1958) investigated the toxic effects of hexaCNs to pigs after oral administration either daily or on alternate days for eight to 10 days to reach intended cumulative doses. The experiment was terminated after 52 to 64 (Trial 2) days when all animals remaining alive were killed. The experiment was split into three trials, each with a concurrent control. The weights of the pigs in the first trial were different from those in the other two trials with initial weights being 72–82 kg in Trial 1, 15–25 kg in Trial 2, and 15–29 kg in Trial 3. Plasma vitamin A was analysed before the first dose and after 14 days and in Trials 2 and 3 also at a subsequent time point, typically when animals were moribund.

Animals in Trial 1 (dose: 11 mg/kg bw per day; weight: 72–82 kg) showed little or no changes in weight gain, plasma vitamin A concentration or gross pathology and histopathology of liver and kidney.

In Trial 2, pigs with an initial weight of 15–25 kg were orally exposed to hexaCNs at doses of 16.5–22 mg/kg bw per day. Four out of the five exposed pigs were moribund and killed before the end of the experiment. These animals showed

decreased plasma vitamin A concentrations, but vitamin A concentrations recovered after Day 14 and were only marginally depressed at time of death.

In Trial 3 (dose: 22 mg/kg bw per day; weight 15–29 kg), all but one animal died or became moribund and were culled before the end of the experiment at Day 40. Like in Trial 2, exposure to hexaCNs resulted in severely depressed vitamin A concentrations which rebounded subsequently. Depression and anorexia preceded death but there was no hyperkeratosis.

Gross lesions observed in moribund pigs were swelling and haemorrhage in the liver, mild gastritis, slight thickening of the intestinal wall, oedema of the eyelids in some animals and swelling of the epididymis. Some animals showed swollen and yellow discoloured liver without evidence of haemorrhage.

Thus, in this study (Link et al., 1958), doses of 16.5–22 mg/kg bw per day (Trials 2 and 3) were lethal, but the slightly lower dose of 11 mg/kg bw per day (Trial 1) caused no mortalities or any other observed effect. In addition to the lower dose in Trial 1, the differences in effects between trials might be related to the size of the experimental animals as they were much larger in the first trial than in the subsequent two.

Effects of hexaCN on vitamin A and survival in pigs were investigated in a study by Huber and Link (1962). Six pigs aged 6 weeks (16–20 kg) received 22 mg/kg bw per day of hexaCN dissolved in corn oil and administered in gelatine capsules orally for 8 (one animal) or 9 consecutive days. Three pigs (21–24 kg) were kept under the same conditions as a control group. The animals were fed ad libitum with a complete balanced feed to which 800 U/kg feed of vitamin A was added. Body weights and plasma vitamin A levels were recorded at study start, 14 days into the experiment, and at necropsy. The control animals were sacrificed on Day 52. All exposed pigs either died or were moribund and culled 30–40 days after the start of the experiment.

Plasma vitamin A concentrations decreased from 25.4 µg/100 mL at the start of the experiment to 6.6 µg/100 mL on Day 14 ($p < 0.001$, paired *t*-test). At that time, the plasma vitamin A was also significantly lower than that of the control group (25.1 µg/100 mL, $p < 0.001$). At necropsy of exposed pigs (Days 30–40), the average vitamin A concentration was 23.8 µg/100 mL, which was not statistically different from the value at the start of the experiment or to the control group (on Day 52). The authors noted that, the complete recovery of plasma vitamin A concentrations after the exposure period might have been aided by the additional vitamin A supplementation of the feed on top of the normal requirement of pigs. Treated animals showed depression, anorexia and ataxia, and they did not gain any weight during the 30–40 days they remained alive. In contrast, control animals more than doubled in weight during the experimental period. Apart from these symptoms and the initial decline in vitamin A concentrations, other characteristic signs occurring in cattle following PCN exposure were missing.

Gross lesions included slight swelling and slight pale brown discoloration of the liver and slightly thickened intestinal wall. Histopathology revealed degenerative lesions in liver (swollen and granular parenchymal cells, distended sinusoids) and kidneys (tubular dilatation, swollen and granular parenchymal cells, amorphous proteinaceous casts in the tubules). Vaginal tissue exhibited hyperplasia of the stratified squamous epithelium and keratinisation of mucosal surface. Subacute interstitial duodenitis was also evident.

As in cattle, oral PCN exposure in pigs at doses that might eventually kill the animals causes a decrease in plasma vitamin A concentrations, but in pigs, the vitamin A concentrations recover following cessation of exposure even in animals that subsequently die. Typical clinical symptoms of PCN poisoning in cattle, including hyperkeratosis are also absent in pigs. The studies available do not allow identification of NOAEL or other Reference Points for hazard characterisation for pigs.

3.1.4.3 | *Studies in solipeds (horses and donkeys)*

No studies were retrieved in the literature search.

3.1.4.4 | *Studies in rabbits*

No studies were retrieved in the literature search.

3.1.4.5 | *Studies in poultry*

Barnett and Bierer (1960) fed adult as well as young turkeys with Halowax 1014 to assess the potential of this formulation to produce keratoconjunctivitis based on prior observations that the condition was in at least one instance associated with avitaminosis A. There are some issues with this study which reduces its value for hazard characterisation. This includes changes of dosing regimen of the adult birds during the experiment, different groups kept at different facilities, and an initial weight loss in all groups of poults including the control. However, it may be concluded that Halowax 1014 was caused no abnormalities in adult turkeys at doses up to 50 mg/kg feed in diet for 2 months. The dose of 200 mg/kg feed (exposure for 1 month) caused mortality and hepatotoxicity. At 200 mg/kg feed, there was one mortality at 7 days and one at 11 days post exposure. Necropsy of surviving animals of the 200 mg/kg feed group showed: enteritis, swollen and either jaundiced or greenish livers, and swollen jaundiced kidneys. Poults were much more sensitive with a dose-dependent decrease in weight gain during a 4-week post-exposure period evident at all doses (lowest dose = 2 mg/kg feed). Necropsy of poults revealed no notable pathologies at any of the doses up to 20 mg/kg feed. However, the group of poults exposed to 20 mg/kg developed dull feathering lacking in black pigmentation. Although no vitamin A analyses were carried out, the authors stated that there were no signs of vitamin A deficiency.

Pudelkiewicz et al. (1958) investigated effects of Halowax 1014 on Broad Breasted Bronze turkey poults. One-day-old turkey poults of both sexes were exposed for 40 days via the diet at doses of 0, 5, 10, 20, 50 and 100 mg/kg feed ($N = 16$, 8 of each sex). Mortality was observed in all treatment groups and was dose dependent. At the lowest dose (5 mg/kg feed), the mortality was 6.5% and no animals survived beyond 33 days at the highest dose. Mortality was consistently higher in males than in females. There was also a dose-dependent decrease in body weight gain with a 33% reduction at the lowest dose. Feather pigmentation was not affected. Necroscopy revealed fluid accumulation in the body cavity and enlarged liver, which was darker in colour than normal.

Pudelkiewicz et al. (1958) also described an experiment with Broad Breasted Bronze turkey poults exposed to Halowax 1051. One-day-old poults were divided into two groups with 10 poults (5 of each sex). One group was given feed containing 125 mg/kg of Halowax 1051 and the other received the control diet. After 35 days, the average weight gain of the exposed poults was 526 g compared with 486 g for the control. The authors speculated that the increased weight gain of treated poults could have been due to biological variation due to small numbers of animals in the experiment. There were no differences observed in general appearance of poults in the two groups.

Pathological and physiological responses of chickens to Halowax 1014 in the diet were investigated in a study involving three separate experiments all starting with 1-day-old New Hampshire chicks (Pudelkiewicz et al., 1959).

Experiment 1 was designed to investigate the role of vitamin A deficiency in the development of toxicity from Halowax 1014 treatment. Animals were first given a vitamin A-deficient diet for 12 days. Vitamin A (2000 IU) was then added to the diet together with Halowax 1014 at 0, 4, 20, 100 or 2500 mg/kg feed. Alternatively, animals previously depleted of A received 8000 IU vitamin A along with 500 mg/kg feed of Halowax 1014. The experiment was terminated after 40 days. At sacrifice, blood and liver samples were taken for vitamin A analysis. No effects were observed in chicken exposed to Halowax 1014 at 4 or 20 mg/kg feed. Chicken exposed to 100 mg/kg showed an 8% reduction in body weight gain and there was a dose-dependent decrease of vitamin A in plasma and liver. The highest dose (2500 mg/kg feed) caused 100% mortality within 14 days. Birds in this group had a dull feather pigmentation and necroscopy revealed enlarged fibrous liver, fluid in the body cavity and pericardial oedema. In the group with 8000 IU supplemented vitamin A, there was no drop in plasma or liver vitamin A concentrations.

In Experiment 2, the effects of Halowax 1014 on blood and clinical chemistry parameters were investigated. Blood samples were analysed for vitamin A, sodium, potassium, uric acid, creatinine, cholesterol, haematocrit. Chicks of both sexes were divided into two groups with 12 birds fed a diet containing 100 mg/kg of Halowax 1014 and another 10 were kept as a control group. Blood samples were collected for analysis on Day 35. Uric acid concentration was significantly increased by 33% and haematocrit significantly decreased from 27% to 23% in exposed chicken. Sodium, potassium, creatinine and cholesterol concentrations were unchanged. There were no differences between males and females in this experiment.

Experiment 3 was set up to investigate the effect of Halowax 1014 on egg production and hatchability in New Hampshire pullets. The same blood chemistry parameters as in Experiment 2 were also measured here. Four groups of 1-day-old chickens were given diets containing Halowax 1014 at 0, 4, 20 or 100 mg/kg feed for 28 weeks. Females and males were transferred into individual cages at sexual maturity and transferred to a breeder diet with Halowax 1014 added at the same concentrations as before. Egg production was recorded after 1-week acclimation period. Semen was collected once per week and used to inseminate pullets of the same treatment group. Eggs were collected daily. Viability of eggs was determined on Day 19 of incubation, the stage at which the yolk sac begins to enter the body cavity and 2 days before hatching would normally occur. There was a dose-dependent decrease in egg production with no eggs being laid in the group exposed to 100 mg/kg feed. Hatchability was also decreased with only 16% of the eggs from pullets receiving 20 mg/kg feed, compared with 89% in the control. Blood chemistry parameters were mostly unchanged in the 20 mg/kg group, except a significant increase in uric acid. The 100 mg/kg group showed reduced haematocrit (females only), and decreased plasma sodium and potassium concentrations, increased uric acid concentrations and an increase in cholesterol.

Overall, studies in turkeys and chickens show dose-dependent effects on survival as well as a range of performance parameters of importance for food-producing animals. These studies have used Halowax 1014 or 1051 as test substance and toxicity data on single congeners are lacking. It was therefore not possible to identify a Reference Point for PCNs for poultry.

3.1.4.6 | *Studies in fish*

A few studies have investigated the effects of waterborne PCNs to fish and QSAR models have also been developed for acute toxicity of PCN congeners to fish (Gu et al., 2021; Nath et al., 2023; Sişman & Geyikoğlu, 2008; Ward et al., 1981). Because of the high octanol–water partition coefficient (K_{ow}) of PCNs, it is likely that water is not an important route of exposure (Pärt, 1989). Furthermore, in freshwater fish intestinal absorption of waterborne contaminants is very low and branchial uptake has a completely different TK profile from chemicals absorbed through the dietary route. As these studies on waterborne PCNs do not inform the risk assessment for dietary PCNs, they will not be further discussed. Similarly, studies on Halowax 1014 injected into the yolk of rainbow trout larvae was not considered relevant for the present assessment (Norrgren et al., 1993; Pesonen et al., 2000).

Holm et al. (1993) studied effects of PCNs in female three-spined sticklebacks (body weight: 0.9 ± 0.1 g) delivered by feeding a diet containing chironomids which were first freeze dried, mixed with Halowax 1014 and added to feed to obtain two different concentrations of the test substance in addition to a control. The concentrations of PCNs in the three experimental diets were not measured, but after feeding the fish with the diets at 2% of the body weight per day for 3.5 months the concentrations of Halowax 1014 in the bodies of fish in the control, low-dose and high-dose groups were:

'not detected', 65 and 97 mg/kg wet weight, respectively. There were no effects on behaviour, the number of eggs laid or spawning success. Lipid accumulation was the most frequent histological observation in hepatocytes of Halowax 1014 exposed fish, but there was no information on differences in occurrence between the low- and high-dose groups. Hepatic EROD activity was markedly and dose-dependently increased.

Effects of dietary exposure to a mixture of equal parts (1:1:1) of Halowax 1001, 1014 and 1051 was studied in Baltic salmon (*Salmo salar*) fry (0.3 g) (Åkerblom et al., 2000). The fish were fed a commercial salmon feed spiked with different concentrations of the Halowax mixture; 0, 0.1, 1, 2 or 10 mg/kg feed) for up to 41 weeks at a ration of 2% of body weight per day. There were no consistent or dose-related effects on survival and body weight. The specific liver weight (liver somatic index) did also not show a consistent trend over the course of the experiment, but a statistically significant small dose-dependent reduction was observed at week 41. Qualitative histological examination of the gonads were suggestive of delayed ovary maturation. However, there were no effects on plasma vitellogenin concentrations indicating low oestrogenic potential of PCNs. EROD activity in liver was induced at all doses suggesting aryl hydrocarbon receptor (AHR)-mediated upregulation of *cyp1a* expression.

Effects of PCN-52 to fish was studied by feeding PCN-52-exposed *Lumbricus variegatus* worms to rainbow trout fry (0.4–0.5 g) at doses of 0, 0.12, 0.25 or 0.50 mg/kg body wet weight per day (0, 8.2, 16.4 or 32.8 mg/kg feed [dry weight]) for up to 28 days (Slootweg et al., 2015). At the end of the 28 days, the whole body concentrations of PCN-52 in the groups were 0.1, 38.6, 89.2 and 175.5 mg/kg lipid. Survival, body weight, body length, specific liver weight (liver specific index) and food conversion rate were unaffected by the treatment. Expression of *cyp1a* was dose-dependently increased in liver, brain and intestine, indicative of AHR activation. No changes were seen in ATP-binding cassette, sub-family B member 1A (*abcb1a*) transcript levels in the three organs. This gene encodes a p-glycoprotein, also known as Multidrug resistance 3 (Mdr3), which extrudes various hydrophobic amphipathic chemicals. Since no adverse effects were observed, the highest concentration of PCN-52 in feed, 32.8 mg/kg dry weight, was considered a no effect concentration.

No effects have been recorded in fish following exposure to PCNs, except than upregulation of genes and proteins involved in xenobiotic metabolism and in particular Cyp1a. In the study by Slootweg et al. (2015), survival, body weight, body length, specific liver weight (liver specific index) and food conversion rate in rainbow trout were unaffected by exposure to 32.8 mg PCN-52/kg feed (dry weight). This concentration in the feed was taken as a NOAEL for PCN-52 in fish. It was not possible to identify Reference Points for other PCN congeners.

3.1.4.7 | *Summary of studies in food-producing animals*

The majority of studies on PCNs carried out in food-producing animals have used technical mixtures (e.g. Halowax). While data from such studies are useful for the hazard identification, they are less so for hazard characterisation.

PCN toxicity to food-producing animals was first observed in cattle, where hyperkeratosis is potentially linked to a drastic drop in serum vitamin A concentrations following exposure. Despite the studies being numerous, the reporting does not adhere to current standards and data cannot be used to derive a Reference Point.

PCN toxicity presents differently in sheep compared with cattle and is primarily not associated with vitamin A deficiency and hyperkeratosis. The studies available for sheep also do not allow identification of a Reference Point.

Oral PCN exposure in pigs at lethal doses causes a decrease in plasma vitamin A concentrations, but in pigs, these recover following cessation of exposure even in animals that subsequently die. Typical clinical symptoms of PCN poisoning in cattle, including hyperkeratosis are also absent in pigs. The studies available do not allow identification of a Reference Point for pigs.

Early but thorough and relatively well-described studies in poultry showed again that PCNs affect serum vitamin A concentrations but similarly to pigs and sheep, this does not appear to be directly associated with adversity. Studies in turkeys and chickens show dose-dependent effects on survival as well as a range of performance parameters of importance for food-producing animals.

The few studies in fish carried out with different dietary exposures to PCNs showed no adverse effects at any of the doses tested while induction of *cyp1a* gene expression, protein levels or enzymatic activity (EROD) confirmed exposure. For fish, it was possible to identify a NOAEL of 32.8 mg PCN-52/kg feed (dry weight).

There are no studies in other food-producing animals that allow identification of Reference Points for single PCN congeners.

3.1.5 | Adverse effects in non-food-producing animals

3.1.5.1 | *Studies in dogs*

No relevant studies were retrieved in the literature search.

3.1.6 | Mode of action

In this section, only mode of actions for relevant effects reported in experimental animals (Section 3.1.2) and in food-producing animals (Section 3.1.4) are addressed.

3.1.6.1 | *AHR pathway*

Numerous animal and in vitro studies from the 1980s onwards have reported that PCNs and commercial PCN mixtures have dioxin-like effects and are able to activate the Aryl Hydrocarbon Receptor (AHR) (reviewed by Safe, 2005; Falandysz et al., 2001; Falandysz, 2003; Falandysz et al., 2014). Such studies have included rodents, birds and fish (see Sections 3.1.4 and 3.1.5), and the effects in these groups are qualitatively similar. Their ability to activate AHR and induce expression of the related target biotransformation enzymes depends on their degree of chlorine substitution and the lateral (ß) position of the chlorine atoms being particularly important. Some studies report that higher doses of PCNs can inhibit CYP1A-associated ethoxyresorufin *O*-deethylation (EROD) activity (e.g. Brunström et al., 1995). At least in rainbow trout (*Oncorhynchus mykiss*) expression of *cyp1a* mRNA shows a monotonic dose–response during exposure to Halowax 1014 (Pesonen et al., 2000). The authors of this study suggested that PCNs in Halowax 1014 may be CYP1A substrates and act as competitive inhibitors of CYP1A EROD activity. Effects on AHR signalling can also plausibly explain the decrease in vitamin A serum concentrations, an indicator of PCN effects in e.g. cattle, pigs, chickens and other animals (see Section 3.1.4), as AHR has a role in controlling retinol and retinoic acid concentrations (Esteban et al., 2021).

In chicken embryos, Halowax 1014 was found to be 1000-fold less potent in inducing CYP1A-associated hepatic 7-ethoxyresorufin *O*-deethylase (EROD) activity (mainly attributed to AHR induced CYP1A2) than PCB-126 at the same dose (by weight) (Brunström et al., 1995). The highest EROD activity in livers of chick embryos treated with Halowax 1014 (100 nM) was about 15% of the maximal EROD activity caused by TCDD (0.1 nM). Co-administration of Halowax 1014 (10^{-6} M) and TCDD (10^{-10} M) resulted in lower EROD induction than that caused by TCDD (10^{-10} M) alone. A mixture of 50% PCN-66 and 50% PCN-67 was three times more potent than Halowax 1014 in inducing EROD activity while PCN-73 had lower EROD-inducing potencies than Halowax 1014. PCN-73 did also not induce EROD in liver of eider duck (*Somateria mollissima*) embryos.

A mixture of tetra- (54%), penta- (8%), hexa- (23%) and heptaCN (14%) increased EROD activity in the liver of Wistar rats when given by gavage, but without no clear dose dependency in the response (Kilanowicz et al., 2009). Daily repeated doses were 0, 1, 10 and 100 mg/kg bw for 7, 14 and 21 days with the testing material containing < 0.1 pg dioxins/100 µg sample.

Daily administration of PCN-43 at 1 or 10 mg/kg bw per day (by gavage) in rat increased EROD in the liver after 1 week but treatment with 10 mg/kg bw per day of PCN-43 did not (Kilanowicz & Skrzypinska-Gawrysiak, 2010).

In a study conducted for the National Toxicology Program, Hooth et al. (2012) assessed the relative potency of PCN-66 and PCN-67 relative to TCDD in liver of female Harlan Sprague–Dawley rats. Dose response modelling for relative potencies was based on thymic atrophy and on CYP1A1 and CYP1A2 activities. The relative potency of PCN-66 was in the range 1.5×10^{-3}–7.2×10^{-3} and for PCN-67 in the range of 2.9×10^{-4}–6.7×10^{-4} in comparison to TCDD (the reference i.e. a relative potency of 1).

A reanalysis by Safe (2005) of previous data on rats exposed to different PCNs led them to the conclusion that the PCNs most effective in inducing aryl hydrocarbon hydroxylase (AHH) activity were hexa-, hepta- and octaCNs substituted in three of four of the lateral 2, 3, 6 or 7 positions. Reviewing available literature at the time Falandysz et al. (2014) concluded that PCNs 66, 67 and 70 have an order of magnitude greater dioxin-like potency compared to octachloro- and octabromo dibenzo-*p*-dioxin and -dibenzofuran and an order of magnitude more potent than two dioxin-like non-ortho chloro- and bromobiphenyls that are substituted at positions 3,3,4,4- and 3,4,4,5- (i.e. chlorobiphenyls and bromobiphenyls #77 and #81), respectively. The most potent EROD and AHH inducers are PCN-67, PCN-68, PCN-69 and PCN-73 (Hanberg et al., 1990). Congeners with up to four chlorine atoms such as, PCN-1, PCN-2, PCN-6, PCN-9, PCN-12 and PCN-27 are weak inducers of rat liver microsomal AHH (Campbell et al., 1983).

The ability of PCNs to activate AHR is mainly attributed to the planar structure of these molecules (Falandysz, 1998). Suzuki et al. (2020) assessed the TCDD-like activities of 42 PCNs and 6 technical Halowax formulations using the DR-CALUX (dioxin-responsive chemically activated luciferase expression) assay with rat hepatoma luciferase-expressing H4IIE cells. Of the 42 PCNs examined, 31 showed dioxin-like activities with a mass-based potency (REP- EC_{5TCDD}[20]) in the range of 1.2×10^{-7} to 5.1×10^{-3} with PCN-70 and PCN-66 being the strongest AHR agonists. The potency to activate the AHR obtained experimentally for the Halowax formulations decreased in the order: Halowax 1051 > Halowax 1014 > Halowax 1013 > Halowax 1099 > Halowax 1001 > Halowax 1031. Similarly, Villeneuve et al. (2001) used the H4IIE reporter gene assay to determine the potency of Halowaxes in activation of the AHR. The Halowaxes were estimated to have relative potencies of approximately 10^{-8} - 10^{-6} relative to TCDD. The H4IIE-luc luciferase bioassay and H4IIE-EROD bioassay were used to characterise the dioxin-like potency of 18 individual PCN congeners and 1 PCN metabolite (Villeneuve et al., 2000). HexaCNs were the most potent congeners tested with relative potencies around 10^{-3} (relative to TCDD) and pentaCNs were also potent, with relative potencies ranging between 10^{-7} and 10^{-3}. Tetra-, tri-, di- and monoCNs were less active. The REP values of the hydroxy metabolite of PCN 4 were estimated to be 5.5×10^{-8} - 7.6×10^{-9} (H4IIE-EROD) or 1.9×10^{-7} (H4IIE-luc).

In summary, several PCNs activate AHR (show dioxin-like activity) with the PCN-70 and PCN-66 being the most potent. Compared with TCDD they are weak AHR agonists with relative potencies (mostly derived from reporter gene assays) ranging from approximately 10^{-7} to 10^{-3}. However, some of these values are higher than those for some PCBs with established TEFs (van den Berg et al., 2006).

[20]Potency relative to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin based on 5% effective concentration determined from the dose–response curve for the 2,3,7,8-TCDD.

3.1.6.2 | *Effects on the liver*

The effects on the liver observed in rats exposed to PCNs, e.g. increased relative liver weight accompanied by changes in different biochemical parameters (increased concentration of microsomal cytochrome P-450, increased activity of CYP 1A, increased concentration of malondialdehyde and decreased concentration of glutathione (see Section 3.1.2) and of vitamin A) can be related to PCN activation of AHR (see above).

There is, however, evidence that PCNs may also activate the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) as shown in the study by Klimczak et al. (2018) who observed increased expression of CYP2B in rat liver.

Most of the effects observed in the liver might thus be explained by activation of AHR, CAR and PXR. This would include induction of metabolic enzymes, increase in malondialdehyde concentrations indicative of lipid peroxidation and oxidative stress, changes in retinoic acid metabolism (see Sections 3.1.2 and 3.1.4).

3.1.6.3 | *Effects on the thymus*

Effects of PCN-66 and PCN-67 on the thymus of female rats included decreased absolute and relative weight (Hooth et al., 2012; Klimczak et al., 2018) and atrophy (Hooth et al., 2012) (see Section 3.1.2). Thymus atrophy is a hallmark consequence of AHR activation by xenobiotics indicating dioxin-like toxicity of PCN-66 and PCN-67.

3.1.6.4 | *Effects on the haematological system*

Effects on haematological parameters reported in female rats in a subacute study (Kilanowicz, Markowicz-Piasecka, et al., 2019) and in a subchronic study (Klimczak et al., 2018) with a hexaCN mixture were primarily related to blood-clotting (decreased platelet [PLT] count and increased mean platelet volume [MPV], decreased fibrinogen concentration, prolonged activated partial thromboplastin time), but also effects on red blood cell parameters indicating a slight anaemia were reported.

The effects on red blood cells are probably due to haemolysis as increased red blood cell lysis was reported in the subacute study (Kilanowicz, Markowicz-Piasecka, et al., 2019).

The decrease in the PLT count could be due to an increased PLT turnover as indicated by the increase in MPV (Klimczak et al., 2018).

The effect on the blood-clotting time/potential could be due to a decrease in important kinetic parameters of clot formation and fibrinolysis as reported by Kilanowicz, Markowicz-Piasecka, et al. (2019) (see Section 3.1.2).

In summary, the changes on haematological parameters are considered as being relevant for humans as the information on mode of action (MoA) is insufficient to conclude on a species-specific MoA.

3.1.6.5 | *Effects on fetal development*

Pre-natal death (intra uterine mortality), delayed development (delayed ossification, retarded development of internal organs) and decreased body weight and length were reported in two developmental toxicity studies in rat (Kilanowicz et al., 2011; Kilanowicz et al., 2015). No specific studies were retrieved in the literature search regarding MoAs behind these effects.

3.1.6.6 | *Effects on the nervous system*

Effects on the nervous system reported in male rats in a subacute study (Kilanowicz, Wiaderna, et al., 2012) included the motor division, the sensory division as well as cognitive functions. Some hypotheses were presented in the article. No specific studies were retrieved in the literature search regarding MoAs behind these effects.

3.1.7 | Considerations of critical effects and dose–response analysis for human risk assessment

3.1.7.1 | *Consideration of critical effects*

There were no relevant studies on observations in humans related to the risk assessment of PCNs available. Therefore, the human risk assessment was based on data from studies in experimental animals.

A number of repeated dose toxicity studies have been performed in rats with a hexaCN mixture (also including PCN-73 (a heptaCN; see composition in Table 4 below) and two studies have been performed with PCN-66 and PCN-67 (Section 3.1.2). The hexaCN mixture is referred to as 'hexaCN mixture' below. These studies have shown that the haematological system, liver and thymus are target organs in rats. Also developmental effects were reported in rats. The effects observed are considered relevant to humans (see Section 3.1.6 and Table 2).

Haematological changes reported in the 90-day oral gavage study performed with the 'hexaCN mixture' in rats (Klimczak et al., 2018) included decreased red blood cell parameters at the highest dose (0.3 mg/kg bw per day), decreased PLT count at the mid and high dose (0.1, 0.3 mg/kg bw per day; magnitude of changes –28%, –48%, respectively) and an increased MPV at the highest dose (0.3 mg/kg bw per day). Effects on the haematological system were also reported in the 28-day oral gavage

study performed with the 'hexaCN mixture' in rats (only one dose: 0.3 mg/kg bw per day) (Kilanowicz, Markowicz-Piasecka, et al., 2019) and included decreased PLT count and increased MPV. This 28-day study performed after the 90-day study also indicated that hexaCNs may disturb both coagulation and fibrinolysis processes, as well as increased degree of RBC lysis.

Liver effects reported in most of the repeated dose toxicity studies included increased liver weight, increased MDA concentration, decreased GSH concentration, increased total concentration of cytochrome P-450 and strong induction of CYP activities. Histopathological changes reported in the 90-day oral gavage study performed with the 'hexaCN mixture' in rats (Klimczak et al., 2018) included liver fatty degeneration (steatosis) at the highest dose (0.3 mg/kg bw per day). Histopathological changes were also reported after 12 doses of either PCN-66 or PCN-67 by oral gavage (Hooth et al., 2012) and included increased incidence of diffuse hepatocellular hypertrophy at the highest dose (PCN-66 and PCN-67; 0.5 mg/kg bw per day), diffuse micro- and macrovesicular fatty changes at the highest dose (PCN-66 and PCN-67; 0.5 mg/kg bw per day) and next highest dose (PCN-66; 0.05 mg/kg bw per day) and focal to multifocal hepatocellular necrosis associated with acute inflammation at the highest dose (PCN-67; 0.5 mg/kg bw per day).

Effects on the thymus reported in the 90-day oral gavage study performed with the 'hexaCN mixture' in rats (Klimczak et al., 2018) included decreased thymus weight at the highest dose (0.3 mg/kg bw per day). Effect on the thymus was also reported after 12 doses of either PCN-66 or PCN-67 by oral gavage (Hooth et al., 2012) and included decreased thymus weight and atrophy at the highest dose (PCN-66 and PCN-67; 0.5 mg/kg bw per day).

Developmental effects included increased intrauterine mortality of embryos and fetuses ('hexaCN mixture', from 0.1 mg/kg bw per day; PCN mixture, from 0.3 mg/kg bw per day), decreased fetal bw and fetal length ('hexaCN mixture', from 0.1 mg/kg bw per day; PCN mixture, from 0.3 mg/kg bw per day), delayed ossification (PCN-43, from 1 mg/kg bw per day; 'hexaCN mixture', at 1 mg/kg bw per day; PCN mixture, from 0.3 mg/kg bw per day) and retarded development of internal organs (brain, kidneys) (PCN mixture, from 0.3 mg/kg bw per day).

Overall, the considerable decrease in the PLT count reported in the 90-day oral gavage study performed with the 'hexaCN mixture' in rats at the mid and high dose (0.1, 0.3 mg/kg bw per day; magnitude of changes –28%, –48%, respectively) (Klimczak et al., 2018) is considered as the critical effect for human risk assessment. The NOAEL for the decrease in the PLT count was 0.03 mg/kg bw per day.

TABLE 4 Overview of critical effects and no-effect/lowest effect level (NOAEL/LOAEL).

PCN(s)	Liver NOAEL/LOAEL mg/kg bw per day	Thymus NOAEL/LOAEL mg/kg bw per day	Haematological system NOAEL/LOAEL mg/kg bw per day
PCN-66 Total of 12 doses	Liver changes 0.005/0.05	Thymus atrophy 0.05/0.5	Not examined
PCN-67 Total of 12 doses	Liver changes 0.05/0.5	Thymus atrophy 0.05/0.5	Not examined
'HexaCN mixture'[a] 28 days One dose	0.3/–	Not examined	Decreased PLT –/0.3
'HexaCN mixture' 90 days	Liver fatty degeneration (steatosis) 0.1/0.3	Not examined	Decreased PLT count (–28%, –48%) 0.03/0.1
'HexaCN mixture' 28 days Focus nervous system	LOAEL: 0.3 mg/kg bw per day for behavioural effects–lowest dose tested		
'HexaCN mixture' Developmental toxicity Gestation days 6–15	LOAEL: 0.1 mg/kg bw per day for developmental effects–lowest dose tested		

[a]HexaCNs at 94.1% contained PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 (a heptaCN at 5.9%).

3.1.7.2 | *Dose–response analysis (including BMD modelling)*

The benchmark dose (BMD) modelling was performed in agreement with the 2022 EFSA Guidance on the use of the BMD approach in risk assessment (EFSA Scientific Committee, 2022).

The results of the BMD modelling on decreased PLT count in rats after 90 days of repeated oral exposure to the 'hexaCN mixture' (Klimczak et al., 2018) are summarised in the table below. Further details of the BMD analyses and the individual reports of the modelling are shown in Appendix E.

The EFSA guidance on BMD (EFSA Scientific Committee, 2022) recommends estimating the 90% credible interval by using a benchmark response (BMR) that takes into account biological relevance. For the decreased PLT count, the CONTAM Panel agreed to use a BMR of 20% based on the consideration in the JMPR guidance document that a decrease within 20% of the number of PLT indicates that the effect might be non-adverse (JMPR, 2015).

The BMD model averaging on the data from Klimczak et al. (2018) for the decreased PLT count resulted in a benchmark dose lower and upper bound (BMDL-BMDU) of 0.05–0.105 mg/kg bw per day. Based on the results, a **$BMDL_{20}$ of 0.05 mg/kg bw per day** was identified for the hexaCN mixture (see composition in the table).

TABLE 5 $BMDL_{20}$ and $BMDU_{20}$ calculated from the decrease in the PLT count observed after oral administration of the hexaCN mixture in rats (see composition in the table) (Klimczak et al., 2018).

Congener	Reference	Animals/dosing	BMD BMDL–BMDU (mg/kg bw [per day])	Acceptance criteria satisfied[a]
PCNs				
'hexaCN mixture' (hexaCNs at 94.1%) contained PCN-66/67 (equivalent mixture at 81.2%), other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) and PCN-73 (a heptaCN at 5.9%)	Klimczak et al. (2018)	10 female rats (SD) per dose group Oral exposure for 90 days 0, 0.03, 0.1, 0.3 mg/kg bw per day	Platelet (PLT) count in blood: BMR 20%: BMD: 0.075 **BMDL**-BMDU: **0.05**–0.11	Yes

[a]Criteria to judge the width of the BMD credible interval (EFSA Scientific Committee, 2022).

3.1.7.3 | *Derivation of a HBGV/margin of exposure approach*

The CONTAM Panel concluded that due to the limitations and uncertainties in the current database on PCNs, the derivation of a health-based guidance value (HBGV) was not appropriate. Instead, the Panel applied a margin of exposure (MOE) approach to assess a possible health concern.

In the discussion of the MOE above which no concern for human health would arise, the CONTAM Panel started by taking into consideration the default assessment factors (EFSA Scientific Committee, 2012) for interspecies differences with respect to toxicokinetics and toxicodynamics between humans and rats (factor of 10), for the intraspecies differences in humans (factor of 10) and for the shorter duration of the key study (90 days) compared to a lifetime exposure (factor of 2). The default factor of 2 is based on data supporting that a chronic rat NOAEL can be predicted by dividing the NOAEL from a subchronic rat study by 1.7, as well as of statistical evidence (see EFSA Scientific Committee, 2012). According to the EFSA Scientific Committee Guidance on selected default values (EFSA Scientific Committee, 2012), an additional factor can be considered in case of deficiencies in the database on a case-by-case basis. However, a default value has not been proposed, as it will be directly dependent on the data set available. The analysis of uncertainties related to the hazard identification and characterisation of PCNs indicated that an additional factor was warranted for deficiencies in the database (see uncertainties described in Section 3.5.2). Major deficiencies in the database included lack of studies on reproductive toxicity, carcinogenicity, developmental neurotoxicity and immunotoxicity, extremely limited information on genotoxicity, limitations in the key study from which the Reference Point has been derived (only females and lack of a number of standard parameters) and lack of sufficient data on bioaccumulation. Considering the results of the uncertainty analysis, the CONTAM Panel agreed that an additional factor of 10 was appropriate. As a result, the CONTAM Panel considered that MOEs ≥ 2000 ($10 \times 10 \times 2 \times 10$) are sufficient to conclude that the current dietary exposure to hexaCNs does not raise a health concern (see Section 3.5).

3.1.8 | Considerations of critical effects and dose–response analysis for animal risk assessment

3.1.8.1 | *Consideration of critical effects for animal risk assessment*

The CONTAM Panel reviewed the available studies in order to derive Reference Points for food-producing and non-food-producing animals. It was concluded in Section 3.1.4.7 above that for fish it was possible to identify a NOAEL of 32.8 mg PCN-52/kg feed (dry weight). There are no studies in other food-producing animals or in non-food-producing animals that allow identification of Reference Points for single PCN congeners or for mixtures of congeners with the same number of substitutions (e.g. hexaCN mixtures).

3.2 | Occurrence data

An initial number of 13,242 analytical results (550 samples) on PCNs in food ($n = 11{,}775$) and feed ($n = 1467$) were available in the EFSA database. Data were reported by six European countries. The major contributor of data on PCNs in terms of number of results was France that reported 60% of the data, followed by Germany (30%) and Ireland (9%). Other European countries, namely the United Kingdom, the Czech Republic and Malta contributed with < 1% of data; however, these data did not fulfil the inclusion criteria and were not further considered. In terms of number of samples, Germany provided 41% of all data. All food samples were taken from the EU market regardless of their country of origin. Among those, some samples were from food originating from non-EU countries (e.g. non-EU European countries, South America, Africa and Asia). The analytical results

were obtained between 2001 and 2022. The raw occurrence data set on PCNs in feed and food as extracted from the EFSA data warehouse is available at the EFSA Knowledge Junction community in Zenodo at: https://doi.org/10.5281/zenodo.10641396.

The current data were not systematically checked for possible duplicate occurrence with the data reported in Section 3.2.3. This might have resulted in a partial overlap between the data reported in the scientific literature and the data reported to EFSA and used in the current exposure assessment.

The occurrence data were carefully evaluated, and a list of validation steps was applied before being used to estimate dietary exposure (see Annex B, Table B.2 for further details).

Data providers were contacted to clarify inconsistencies identified during the data check. The following modifications were made to the initial data set based on the feedback received and/or expert judgement:

- Out of 11,775 analytical data on food, 22% of the PCN concentrations were expressed on fat weight basis. This referred to three food categories: 'Eggs and egg products' (36% of data reported on fat weight), 'Fish meat and products thereof' (65% of data reported on fat weight) and 'Mammals and birds meat and products thereof' (11% of data reported on fat weight). For consistency and to be able to link the occurrence data to consumption amounts for exposure assessment, the analytical results expressed on fat weight basis were converted to whole weight basis using information on fat content reported.
- The product description of several records allowed a more accurate FoodEx2 classification. In these cases, the samples were reclassified to a more specific FoodEx2 lower level.
- On request for clarification to the data providers while checking for inconsistency between LOQ and the PCN concentration, it was confirmed that the unit of measurement of LOQs was wrongly reported, and that the data reported as below LOD shall be re-classified as below LOQ instead. All corrections were implemented in the data set. A small part of the quantified data resulting to have the PCNs concentrations lower that the LOQ were converted to left-censored (below LOQ).
- A special attention was given to analytical methods used for PCNs measurements. A part of the data (13%) did not provide information on analytical method used. Data analysis showed that the reported PCN concentrations were consistent with those measured by valid methods of analysis and, based on this, the CONTAM Panel decided to consider the data for which no information on the analytical method was provided in the assessment.
- It was not possible to base the assessment only on recent data due to considerable lack of analytical results for certain foods (e.g. seafood). The CONTAM Panel decided to consider the occurrence data sampled since 2007 onwards in order to cover all representative food categories on one side, but at the same time to rely on the most recent data possible.
- The data reported as 'binary' gives only qualitative information (e.g. presence/absence of PCNs) and were excluded from further analysis.
- A part of analytical data were classified as non-specified 'Naphthalenes' and were reported with extremely high LOQ levels. The CONTAM Panel considered these data as not reliable and were excluded.

Based on the data cleaning (see Annex B, Table B.2), 2664 analytical results on food were excluded. All feed data were valid and considered in the assessment.

The resulting final data set included a total of 9111 analytical results (371 samples) on food and a total of 1467 analytical results (31 samples) on feed. In total, food occurrence data were reported for 71 PCNs and feed occurrence data were reported for 70 PCNs. Four PCNs were not reported in food (PCN-1, PCN-2, PCN-9 and PCN-35) and five were not reported for feed (PCN-1, PCN-2, PCN-9, PCN-18 and PCN-35).

Approximately 39% of the data were obtained from samples collected within official monitoring programmes, 51% from surveys, 8% from industry/private programmes and 2% from a combination of programmes.

In the final data set, 58% of the data were reported as 'Objective sampling', 29% as 'Selective sampling', while the remaining 11% were reported as 'Convenient sampling' and 2% as other undefined sampling. It was decided to retain all samples regardless of the sampling strategy.

Most results were obtained by GC-based methods (85%), in particular GC–HRMS. For the remaining data, no information on analytical methods was reported.

The CONTAM Panel decided to focus only on the occurrence data for hexaCNs as a risk characterisation could only be performed for the hexaCNs because a representative repeated dose toxicological study was only available for a mixture of hexaCNs (Section 3.1.2).

For hexaCNs, the final cleaned data set available for the exposure assessment contained a total of 2317 analytical results analysed in 371 samples on food and 217 analytical results analysed in 31 samples on feed.

For all other individual PCN congeners, an overview of the number of data points, the proportion of left-censored data (LCD) as a percentage and statistical description according up to FoodEx2 Level 4 are reported in Annex B, Tables B.3–B.6.

3.2.1 | Occurrence data of hexaCNs in food submitted to EFSA

In total, 2317 hexaCNs analytical results analysed in 371 samples on food were available in the final cleaned data set.

The CONTAM Panel did not apply any LOQ cut-off value to the hexaCNs analytical data used in the present assessment following the principles in the EFSA Technical report on use of cut-off values on the limits of quantification reported in data sets used to estimate dietary exposure to chemical contaminants (EFSA, 2018a).

The following hexaCNs were assessed as pairs due to the fact that they co-elute (see Section 1.3.4): PCN-64/68, PCN-66/67 and PCN-71/72.

The remaining congeners were considered individually (see Table 6).

In consequence, a total of 1938 analytical results (371 samples) in the final data set were considered for the human dietary exposure assessment of different hexaCNs. As shown in Figure 3, these data were reported by three European countries, most of them by Germany (58% of data), followed by France (22% of data) and Ireland (21% of data). Most of the data (79%) were very recent–sampled after 2020–while substantially less data were available for 2007 (Figure 4).

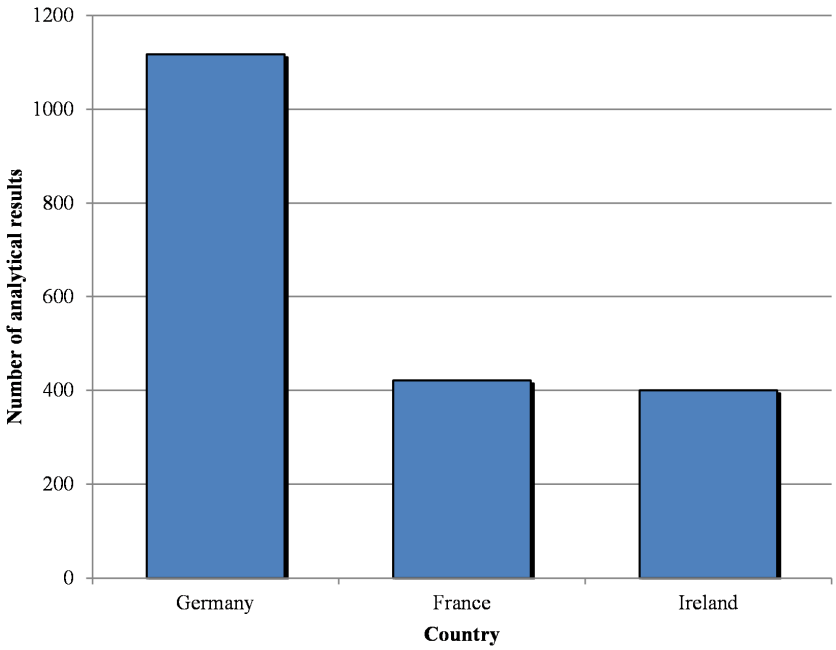


FIGURE 3 Distribution of analytical results reported for hexaCNs across different European countries (final cleaned data set).

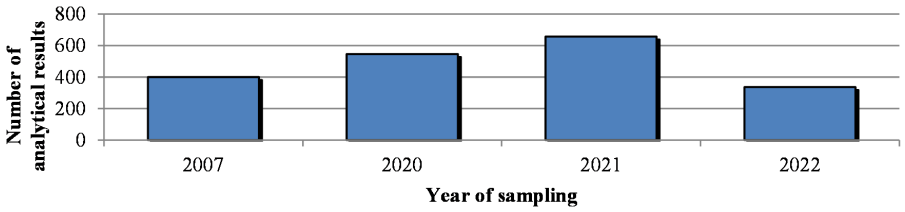


FIGURE 4 Distribution of analytical results reported for hexaCNs by year (final cleaned data set).

Overall, the data set included analytical results for PCN-63, PCN-64/68, PCN-65, PCN-66/67, PCN-69, PCN-70 and PCN-71/72 with different data availability ranging from $n = 181$ reported for PCN-63 to $n = 371$ reported for PCN-69. The proportion of LCD across congeners was very variable and ranged from 8% for PCN-66/67 to 94% for PCN-70. The summary of the hexaCNs is reported in Table 6.

TABLE 6 List of hexaCNs for which data were reported (final cleaned data set).

HexaCNs	PCN number	*N*	%LCD
1,2,3,4,5,6-hexaCN	PCN-63	181	39%
1,2,3,4,5,7-hexaCN/1,2,3,5,6,8-hexaCN	PCN-64/68	282	28%
1,2,3,4,5,8-hexaCN	PCN-65	271	57%
1,2,3,4,6,7-hexaCN/1,2,3,5,6,7-hexaCN	PCN-66/67	281	8%
1,2,3,5,7,8-hexaCN	PCN-69	371	52%
1,2,3,6,7,8-hexaCN	PCN-70	271	94%
1,2,4,5,6,8-hexaCN/1,2,4,5,7,8-hexaCN	PCN-71/72	281	47%
Total		1938	47%

Abbreviations: *N*, number of analytical results; %LCD, proportion of left-censored data.

Overall, the LCD accounted for 47% of the analytical results. All LCD provided information on LOQ and both LOQ and LOD were provided for 93% of LCD. Out of the food categories in which hexaCNs contamination could be expected, the

highest percentage of quantified data was found in food categories 'Eggs and egg products' with proportion of LCD at level of 3% and 6% for PCN-66/67 and PCN-64/68, respectively, 'Fish, seafood, amphibians, reptiles and invertebrates' with proportion of LCD at level of 4% and 7% for PCN-66/67 and PCN-64/68, respectively, 'Milk and dairy products' with proportion of LCD at level of 4% for PCN-66/67 and 'Meat and meat products' with proportion of LCD at level of 5% for PCN-66/67.

Based on the FoodEx2 classification, 10 food categories at FoodEx2 Level 1 were represented (Figure 5). 'Fish, seafood, amphibians, reptiles and invertebrates' was the most represented food group with 875 occurrence values (134 samples) reported, followed by 'Meat and meat products' with 482 occurrence values (110 samples), 'Eggs and egg products' with 316 occurrence values (75 samples) and 'Milk and dairy products' with 113 occurrence values (23 samples). Proportions of non-detected, non-quantified and quantified analytical results by FoodEx2 Level 1 food category considering all merged hexaCNs are presented in Figure 5.

Proportions of non-detected, non-quantified and quantified analytical results by congener regardless the food category are presented in Figure 6.

More details with regard to LCD by FoodEx2 Level 1 food category and individual hexaCNs are presented in Annex B, Table B.7.

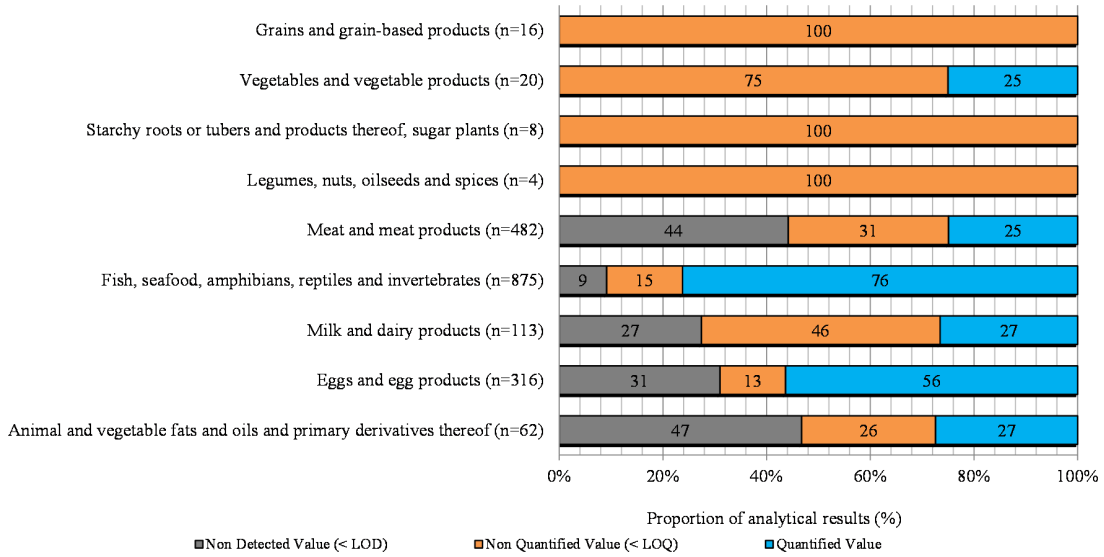


FIGURE 5 Percentage of analytical results below LOD, below LOQ and quantified values in the final data set across the different food categories (FoodEx2 Level 1).

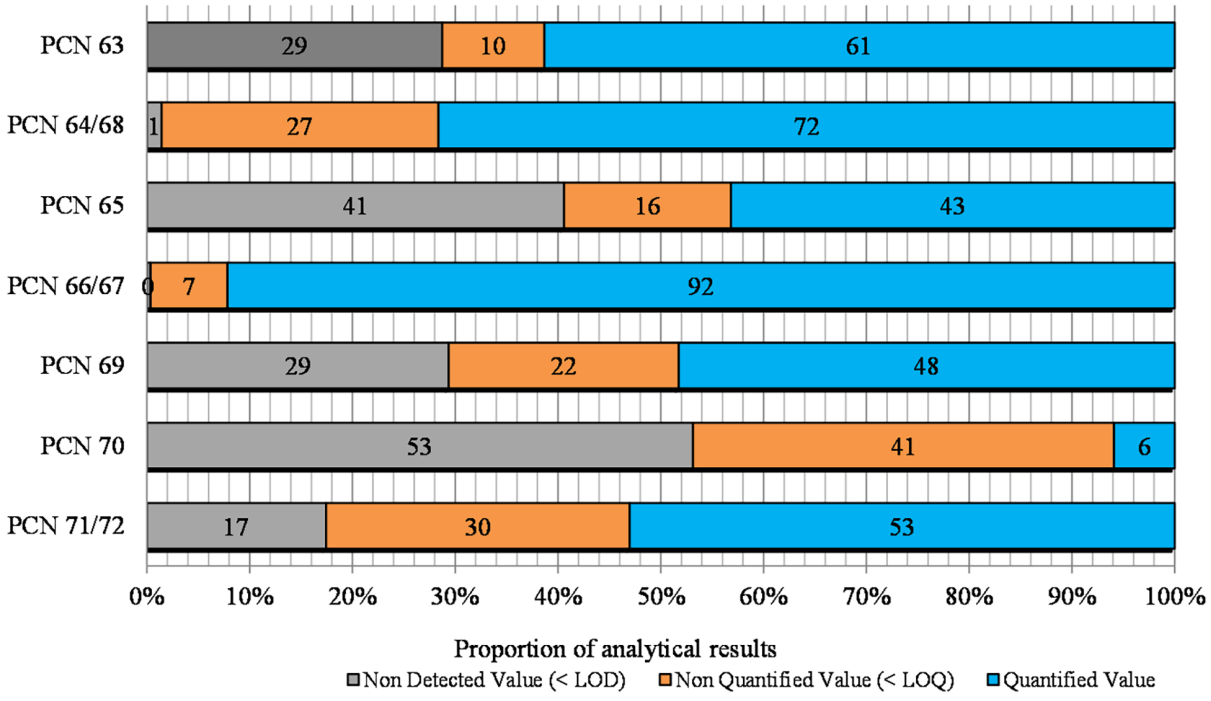


FIGURE 6 Proportion of non-detected, non-quantified and quantified analytical results across the hexaCNs considered (PCN-63, PCN-64/68, PCN-65, PCN-66/67, PCN-69, PCN-70 and PCN-71/72).
The sum of percentages for non-detected, non-quantified and quantified values is not always 100% due to rounding.

The CONTAM Panel decided not to run the exposure assessment for food categories for which the fraction of left-censored data for all hexaCNs was 100%. As a result, the exposure assessment could not be calculated for the following FoodEx2 Level 1 food categories: 'Grains and grain-based products', 'Legumes, nuts, oilseeds and spices' and 'Starchy roots or tubers and products thereof, sugar plants'. In addition, for some congeners, this was also not possible for other food categories (see Annex B, Table B.7). The total number of analytical results used for calculating the mean level in food and further used in the exposure assessment varied according to different scenarios and ranged from 139 analytical results used for assessment of PCN-63 to 2249 analytical results used for the assessment of the group of selected PCN congeners (see Section 3.3.1).

At the most detailed relevant level, the food category was retained if more than six analytical results with quantified values were available. If fewer than six analytical results were available (e.g. for PCN 66/67 the 'poultry liver' with three analytical results at FoodEx2 Level 3) or data were 100% left-censored (e.g. for PCN-66/67 'oysters' with five analytical results at FoodEx2 Level 3), the food category was taken into account at the next upper FoodEx2 level (in this case 'molluscs' at FoodEx2 Level 2).

After matching occurrence and consumption data via FoodEx2 classification system, the number of distinct food categories considered for the exposure assessment by congener were as follows: four for PCN-63, 11 for PCN-64/68, three for PCN-65, 11 for PCN-66/67, eight for PCN-69, two for PCN-70, nine for PCN-71/72 and 11 for the group of selected PCN congeners. The detailed list of the food categories considered across all hexaCNs as well as group of selected PCN congeners including the number of data, LCD and LB and UB mean values as used for the exposure is shown in the Annex C (Tables C.1–C.10).

3.2.1.1 | *Occurrence data of hexaCNs considered for dietary exposure assessment*

As presented in Figure 5, the majority of the data were reported for the food category 'Fish, seafood, amphibians, reptiles and invertebrates' with 76% quantified values. The average of occurrence values for all hexaCNs reported for this category is shown at the first, second and third levels of the FoodEx2 in Table 7. Occurrence values for the food category 'Diadromous fish' (FoodEx2 Level 3) represented only by 'Salmons, trouts, smelts' were higher compared to the other food categories. Comparing the occurrence values across congeners, the highest were reported for PCN-71/72 followed by PCN-66/67 and the lowest for PCN-70 for this food category. For the FoodEx2 Level 3 food categories other than 'Diadromous fish' the availability of the reported data was rather low. Bearing in mind this limitation, the highest hexaCNs mean concentration levels were observed for PCN-66/67 in 'Abalones, winkles, conchs', 'Mussels' and 'Scallops, pectens' (all belonging to 'Molluscs' at Foodex2 Level 2).

The second most reported food category was 'Meat and meat products'. The average of occurrence values reported for this category is shown for all hexaCNs at the first, second and third levels of the FoodEx2 in Table 7. The highest occurrence values aggregated at FoodEx2 Level 2, were reported for PCN-66/67 in 'Animal fresh fat tissues', in particular in sheep and bovine fat tissue. High PCN-66/67 mean concentration level was found also for sheep liver, but only limited number of data ($n = 3$) were available.

Regarding the food category 'Eggs and egg products' very high mean concentration levels were reported for PCN-69. The average of occurrence values for all hexaCNs reported for this category is shown at the first, second and third level of the FoodEx2 in Table 8. The CONTAM Panel noted that one particularly high PCN-69 concentration of 313 ng/kg was reported for hen eggs that had a considerable impact on the mean concentration level of PCN-69 for the food category 'Eggs and egg products'. After having verified with the laboratory who confirmed the analytical result as valid, the datapoint was kept in the data set for exposure assessment. However, the CONTAM Panel recognised that this might have a considerable impact on the exposure outcome, and therefore performed two exposure scenarios for PCN-69, one with (PCN 69 scenario A) and one without (PCN-69 scenario B) this analytical result included (see Section 3.3.1). The second highest mean hexaCNs concentration level for 'Eggs and egg products' was reported for PCN-65.

A more detailed overview of the number of data points, the proportion of LCD as a percentage and statistical description according to FoodEx2 levels (up to Foodex2 level 4) for food categories 'Fish, seafood, amphibians, reptiles and invertebrates', 'Meat and meat products' and 'Eggs and egg products' are reported in Annex B, Tables B.7–B.10.

Among the remaining food categories aggregated according to FoodEx2 Level 1, PCN-66/67 and PCN 64/68 were measured in samples of 'Animal and vegetable fats and oils and primary derivatives thereof' (in particular in vegetable fats and oils and butter). PCN-65 was measured in samples of 'Milk and dairy products' (in particular in fermented milk or cream, but only very limited data were available).

The mean hexaCN concentrations for the remaining two FoodEx2 Level 1 food categories, namely 'Food products for young population' and 'Vegetables and vegetable products' were very low for all hexaCNs.

Regarding the infant formulae, only one sample on follow-on formulae (powder) was reported, and within this sample, the quantified results were measured only for PCN-64/68 (0.01 ng/kg) and PCN-66/67 (0.02 ng/kg).

An overview of the number of data points, the proportion of LCD as a percentage and statistical description according to FoodEx 2 levels (up to Foodex2 level 4) for food categories of 'Animal and vegetable fats and oils and primary derivatives thereof', 'Milk and dairy products', 'Food products for young population' and 'Vegetables and vegetable products' are reported in Annex B, Tables B.7–B.10.

TABLE 7 Lower bound (LB) and upper bound (UB) mean values in ng/kg for 'Fish, seafood, amphibians, reptiles and invertebrates' at first three levels of FoodEx2 for all hexaCNs.

		PCN-63		PCN-64/68		PCN-65		PCN-66/67		PCN-69		PCN-70		PCN-71/72	
		LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Fish, seafood, amphibians, reptiles and invertebrates (FoodEx2 Level 1)		0.10	0.11	0.43	0.43	0.19	0.20	0.77	0.77	0.57	0.58	< 0.01	0.01	1.20	1.21
FoodEx2 Level 2	FoodEx2 Level 3														
Fish (meat)		0.12	0.12	0.49	0.50	0.22	0.23	0.84	0.84	0.67	0.68	< 0.01	0.01	1.42	1.43
	Diadromous fish	0.12	0.12	0.52	0.52	0.23	0.24	0.88	0.88	0.73	0.73	< 0.01	0.01	1.51	1.51
	Marine fish	0	0.03	0.17	0.19	0	0.03	0.24	0.24	0.06	0.15	0	0.02	0.11	0.19
	Freshwater fish	–	–	–	–	0	0.05	–	–	0.04	0.09	0	0.05	–	–
Molluscs		0.02	0.03	0.01	0.02	0.07	0.08	0.52	0.52	0.02	0.02	0	0.03	0.01	0.01
	Abalones, winkles, conchs	0	0.02	0	0.01	0.09	0.09	2.60	2.60	0	0.02	0	0.02	0	0.01
	Scallops, pectens	0	0.02	0.01	0.01	0.09	0.10	0.71	0.71	0	0.01	0	0.04	0	0.01
	Oysters	–	–	0	0.01	–	–	0	0.01	0.01	0.02	–	–	< 0.01	0.01
	Mussels	0.05	0.06	0.04	0.04	0.03	0.05	0.57	0.57	0.06	0.06	0	0.02	0.03	0.03
Crustaceans		0	0.01	0.03	0.03	0	0.01	0.06	0.06	0	0.02	0	0.01	0	0.01
	Shrimps and prawns	0	0.01	0.03	0.03	0	0.01	0.06	0.06	0	0.02	0	0.01	0	0.01
Fish and seafood processed		0.03	0.06	0.02	0.02	0	0.06	0.30	0.30	0.08	0.12	0	0.04	0.04	0.05
	Processed or preserved fish (including processed offal)	0.03	0.06	0.20	0.20	0	0.06	0.30	0.30	0.08	0.12	0	0.04	0.04	0.05

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

Note: The values are rounded to two decimal places; Values lower than 0.01 are indicated as < 0.01. Summary statistics for the P95 percentile are available in Annex B, Tables B.7–B.10.

TABLE 8 Lower bound (LB) and upper bound (UB) mean values in ng/kg for 'Meat and meat products' at first three levels of FoodEx2 for all hexaCNs.

		PCN-63		PCN-64/68		PCN-65		PCN-66/67		PCN-69		PCN-70		PCN-71/72	
		LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Meat and meat products (FoodEx2 Level 1)		< 0.01	0.02	0.06	0.07	< 0.01	0.04	0.42	0.42	0.03	0.07	0	0.04	0.03	0.05
FoodEx2 Level 2	FoodEx2 Level 3														
Animal fresh fat tissues		–	–	0.12	0.16	–	–	0.91	0.91	0.11	0.18	–	–	0.08	0.13
	Birds fat tissue	–	–	0.41	0.41	–	–	0.51	0.51	0.38	0.38	–	–	0.27	0.27
	Mammals fat tissue	–	–	< 0.01	0.06	–	–	1.06	1.06	0	0.09	–	–	0.01	0.08
Animal liver		0	0.01	0.01	0.01	0	0.04	0.31	0.31	< 0.01	0.03	0	0.04	< 0.01	0.01
	Mammals liver	0	0.01	< 0.01	0.01	0	0.04	0.37	0.37	< 0.01	0.03	0	0.04	< 0.01	0.01
	Poultry liver	–	–	0.02	0.02	–	–	0.02	0.02	0	0.01	–	–	0	0.01
Mammals and birds meat		< 0.01	0.02	0.04	0.04	< 0.01	0.04	0.10	0.10	0.02	0.06	0	0.04	0.01	0.02
	Mammals meat	< 0.01	0.02	0.03	0.04	< 0.01	0.04	0.11	0.11	0.02	0.06	0	0.04	0.01	0.01
	Birds meat	0	0.02	0.07	0.07	0	0.04	0.09	0.09	0.05	0.08	0	0.02	0	0.02
Processed whole meat products		0	0.02	0.01	0.03	0	0.05	0.33	0.33	0	0.02	0	0.02	0	0.01
	Raw cured (or seasoned) meat	0	0.02	0.01	0.04	0	0.05	0.05	0.05	0	0.02	0	0.02	0	0.01
	Cooked cured (or seasoned) meat	–	–	0	0.01	–	–	0	0.01	0	0.01	–	–	0	0.01
Preserved/processed fat tissues		0	0.06	0	0.03	0	0.06	0.12	0.12	0	0.06	0	0.06	0	0.03
	Cured pork fat	0	0.06	0	0.03	0	0.06	0.12	0.12	0	0.06	0	0.06	0	0.03
Sausages		0	0.04	0.05	0.05	0	0.08	0.15	0.15	0	0.04	0	0.04	0	0.02
	Fresh raw sausages	0	0.04	0.05	0.05	0	0.08	0.15	0.15	0	0.04	0	0.04	0	0.02

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

Note: The values are rounded to two decimal places; Values lower than 0.01 are indicated as < 0.01. Summary statistics for the P95 percentile are available in Annex B, Tables B.7–B.10.

TABLE 9 Lower bound (LB) and upper bound (UB) mean values in ng/kg for 'Eggs and egg products' at first three levels of FoodEx2 for all hexaCNs.

		PCN-63		PCN-64/68		PCN-65		PCN-66/67		PCN-69		PCN-70		PCN-71/72	
		LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Eggs and egg products (FoodEx2 Level 1)[a]		0.10	0.10	0.12	0.12	0.24	0.28	0.19	0.19	4.95	4.97	< 0.01	0.05	0.09	0.09
Eggs and egg products (FoodEx2 Level 1)[b]		0.10	0.10	0.12	0.12	0.24	0.28	0.19	0.19	0.79	0.81	< 0.01	0.05	0.09	0.09
FoodEx2 Level 2	FoodEx2 Level 3														
Unprocessed eggs[a]		0.10	0.10	0.12	0.12	0.24	0.28	0.19	0.19	4.95	4.97	< 0.01	0.05	0.09	0.09
Unprocessed eggs[b]		0.10	0.10	0.12	0.12	0.24	0.28	0.19	0.19	0.79	0.81	< 0.01	0.05	0.09	0.09
	Whole eggs[a]	0.11	0.11	0.13	0.13	0.25	0.29	0.20	0.20	5.16	5.18	< 0.01	0.05	0.10	0.10
	Liquid egg products[a]	0	0.02	0.05	0.05	< 0.01	0.08	0.06	0.06	0	0.02	0	0.02	0	0.01
	Whole eggs[b]	0.11	0.11	0.13	0.13	0.25	0.29	0.20	0.20	0.82	0.84	< 0.01	0.05	0.10	0.10
	Liquid egg products[b]	0	0.02	0.05	0.05	< 0.01	0.08	0.06	0.06	0	0.02	0	0.02	0	0.01

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

Note: The values are rounded to two decimal places; Values lower than 0.01 are indicated as < 0.01. Summary statistics for the P95 percentile are available in Annex B, Tables B.7–B.10.

[a]The egg sample with PCN-69 concentration of 313 ng/kg included.

[b]The egg sample with PCN-69 concentration of 313 ng/kg not included.

3.2.2 | Occurrence data of hexaCNs in feed submitted to EFSA

A total of 217 hexaCNs analytical results (31 samples) on feed were available in the final data set and considered for the animal dietary exposure. The occurrence values were reported by two European countries, France ($n = 119$) and Germany ($n = 98$) and were sampled in 2021 and 2022. The data set included 31 analytical results for each of the following congeners: PCN-63, PCN-64/68, PCN-65, PCN-66/67, PCN-69, PCN-70 and PCN-71/72.

All analytical results were expressed on 88% dry matter basis, thus no conversion had to be applied.

The hexaCNs occurrence data were very limited for single PCN congeners (from 2 to 15 analytical results per feed category at FoodEx Level 1, in many cases with all LCD; see Annex B, Table B.7–B.10). Because of this limitation, an animal dietary exposure per congener could not be done. Therefore, the CONTAM Panel opted for an exposure assessment based on results from the hexaCN homologue group.

The CONTAM Panel decided not to run the exposure assessment for feed categories for which the fraction of left-censored data for all hexaCNs was 100%. When considering the Foodex2 Level 1 feed categories, that was of the case for 'Fermentation (by-)products from microorganisms the cells of which have been inactivated or killed (feed)'. Consequently, the exposure assessment was limited to the remaining three feed categories, including 'Oil seeds, oil fruits, and products derived thereof (feed)', 'Forages and roughage, and products derived thereof (feed)' and 'Compound feed (feed)' were available. A high proportion of LCD was observed for, 'Oil seeds, oil fruits and products derived thereof (feed)' (88%), while for the remaining two feed categories the proportion of LCD was lower (65% for 'Forages and roughage, and products derived thereof (feed)' and 41% for 'Compound feed').

The most frequently analysed feed category was 'Oil seeds, oil fruits and products derived thereof (feed)' with 98 analytical results reported. The feed categories 'Forages and roughage, and products derived thereof (feed)' and 'Compound feed (feed)' were less represented with 49 and 56 analytical results available, respectively.

The highest hexaCNs mean concentrations were measured for the feed category 'Compound feed (feed)', in particular for dog pet food. Lower mean hexaCNs concentration levels were measured for feed commodities belonging to the feed category 'Forages and roughage, and products derived thereof (feed)', for which only occurrence data for forage meal/grass meal/green meal were available. Very low concentrations of hexaCNs were observed for 'Oil seeds, oil fruits and products derived thereof (feed)' comprising the data on rape seed – expeller, sunflower seed expeller, linseeds, vegetable fats and oils, edible and rape seed meal feed.

An overview of the number of data points, the proportion of LCD as a percentage, the mean concentration values of an appropriate FoodEx2 food category is presented in Table 10. More details on statistical description and according to lower FoodEx2 levels are reported in Annex B, Tables B.11–B.13.

TABLE 10 Summary of the hexaCNs occurrence data by feed category (ng/kg).

				Mean	
		N	**%LCD**	LB	UB
Oil seeds, oil fruits and products derived thereof (FoodEx2 Level 1)		98	88	0.02	0.04
FoodEx2 Level 2	FoodEx2 Level 3				
Rape seed – expeller (feed)		7	86	< 0.01	0.00
Sunflower seed expeller (feed)		7	71	< 0.01	0.01
Linseeds (feed)		42	90	0.01	0.01
Vegetable fats and oils, edible (feed)		35	86	0.04	0.07
Rape seed meal feed (feed)		7	100	0	0.11
Forages and roughage, and products derived thereof (FoodEx2 Level 1)		49	65	0.17	0.36
Forage meal; [Grass meal]; [Green meal]		49	65	0.17	0.36
Fermentation (by-)products from microorganisms the cells of which have been inactivated or killed (FoodEx2 Level 1)		14	100	0	0.12
Yeasts, inactivated [brewers' yeast, inactivated, if appropriate]		14	100	0	0.12
Compound feed (FoodEx2 Level 1)		56	41	0.50	0.53
Complete feed		21	67	0.10	0.16
	Fattening cattle/Complete feed	7	0	0.30	0.30
	Poultry (starter diets)/Complete feed	14	100	0	0.09
Complementary feed (incomplete diet)		35	26	0.74	0.76
	Pet food, dogs/Complementary feed	7	14	2.19	2.20
	Unspecified Complementary feed	28	29	0.38	0.40

Abbreviations: LB, lower bound; % LCD, proportion of left-censored data; *N*, number of analytical results; P95, 95th percentile; UB, upper bound.

Note: The values are rounded to two decimal places; Values lower than 0.01 are indicated as < 0.01. Summary statistics for the P95 percentile are available in Annex B, Tables B.11–B.13.

3.2.3 | Previously reported occurrence data in the open literature

Several studies were identified in the open literature reporting the levels of PCNs in individual food and feed categories, and these are summarised below. No studies were identified where PCNs had been measured as part of duplicate diet or total diet studies. Most data reported in the scientific literature is for fish. Almost all of the data was generated using methods with quantification by GC–MS.

In addition to reporting concentrations for individual (or paired) congeners, or homologue groups, some authors used TEFs which are used to estimate the contribution to overall dioxin-like toxicity, with TEF values taken from e.g. Behnisch et al. (2003); Blankenship et al. (2000), Hanberg et al. (1990), Villeneuve et al. (2000). The reported ranges of combined in vitro and in silico relative potencies for selected individual PCN congeners as reported from different studies have been tabulated by Fernandes et al. (2017) (see Section 3.1.5). Where authors have estimated toxic equivalents, the overall contribution to dioxin-like toxicity is generally much lower when compared to the contribution made by PCDD/Fs and PCBs in the same samples.

Comparisons between studies should be done carefully due to the differences in and reliability of methods used for the determination of the PCNs, the individual PCNs measured and the sampling and geographical origin of the samples. These differences combined with the relatively small amount of data make it very difficult to identify any general time trends in the data, but some of the specific studies below do report time trends associated with contamination in specific locations. There was no obvious differences in levels of PCNs in food and feed in Europe compared to the rest of the world. High levels were generally associated with identified industrial contamination sites.

Occurrence data from the open literature is summarised below and is collated in Tables D.1, D.2 in Appendix D.

3.2.3.1 | *Food*

European countries

Llobet et al. (2007) measured PCN homologue group totals in 42 samples of 14 edible marine species (3 for each species including sardine, tuna, anchovy, mackerel, swordfish, salmon, hake, red mullet, sole, cuttlefish, squid, clam, mussel and shrimp). The samples were collected between March and April 2005 from local markets in Catalonia (Spain). The highest levels of the total PCNs (wet weight), were found in salmon (227 ng/kg) followed by mackerel (95 ng/kg) and red mullet (68 ng/kg), while the lowest levels of total PCNs corresponded to shrimp (4.9 ng/kg) and cuttlefish (2.7 ng/kg). Other contaminant classes including heavy metals, PCBs, PBDEs, PCDD/Fs and polychlorinated diphenyl ethers (PCDEs) were also measured in these samples and were reported by Martí-Cid et al. (2007).

Concentrations of PCNs and PCDEs were determined in samples of foodstuffs widely consumed by the population of Catalonia, Spain by Martí-Cid et al. (2008). A total of 104 food samples were collected in different cities of Catalonia (Spain) during the period March–June 2006. The first group included meat of beef, pork, chicken, lamb, vegetables, tubers fruits and eggs. The second group included cow's milk and dairy products; cereals, pulses, oils, fats, meat products (boiled ham, frankfurters and salami) and bakery products. The highest total PCNs was found in fish and seafood (47.1 ng/kg), followed by oils and fats (21.5 ng/kg), bakery products (15.3 ng/kg) and dairy products (11.7 ng/kg).

Rose et al. (2015) examined the changing habits of anglers and consumers and characterised a range of legacy and emerging contaminants in freshwater fish species with a view to determining occurrence and possible risk from consumption. Twelve PCN congeners were measured and values for the total of these congeners were also reported. Some samples contained very low levels while others contained close to 1200 ng/kg total PCN.

Zacs et al. (2021) reported on PCNs in a wide variety of food products on sale in Latvia. Samples were collected as part of the official monitoring programme over the years 2019 and 2020, and included 10 samples of milk and dairy products, 9 of eggs, 11 meat samples, 15 fish samples, 1 cod liver, 6 bread and cereal products, 4 vegetable oils, 4 samples of fish oil as a food supplement (purified for POPs), and 4 baby food samples. The highest mean PCN concentrations were observed in fish products which had mean and median total PCN concentrations of 45.7 and 27.1 ng/kg, respectively, while the highest individual sum of PCN concentration of 286 ng/kg was found in the cod liver sample.

In a study by Isosaari et al. (2006), a total of 156 fish composite samples were collected from five areas of the Baltic Sea and from three lakes in Finland. They were analysed for PCNs. Most of the fish were sampled in 2001–2002, but some supplementary sampling was carried out in 2003. Species included burbot (*Lota lota*), Baltic herring (*Clupea harengus membras*), sprat (*Sprattus sprattus*), pike-perch (*Stizostedion lucioperca*), salmon (*Salmo salar*), perch (*Perca fluviatilis*), pike (*Esox lucius*), flounder (*Platichthys flesus*), vendace (*Coregonus albula*) and whitefish (*Coregonus lavaretus*). A cyclostome river lamprey (*Lampetra fluviatilis*) was collected from its spawning site, River Kalajoki. Lakes were also sampled for smelt (*Osmerus eperlanus*) and bream (*Abramis brama*). The number of analysed composite samples was 111 from the Baltic Sea and 45 from the lakes and the river. Salmon from the Archipelago Sea had the highest PCN concentration, 453 ng/kg. If salmon, river lamprey and herring are not taken into account, the 90th percentile in the Baltic Sea fish was 85 ng/kg and 47 ng/kg in the lake fish. PCN concentrations in fish taken from lakes was on average only 43% of the concentration in the same species caught from the Baltic Sea.

Fernandes et al. (2018) investigated the occurrence and spatial distribution of chemical contaminants including PCNs in a subset of 75 samples taken from a total of 182 samples of edible marine fish species collected for a larger study on contaminants in fish from UK and proximate marine waters. Concentrations were reported as the sum of 12 measured

congeners and congener pairs, and concentrations ranged from 0.7 ng/kg for a turbot sample to 265 ng/kg for a sample of sprats. The highest concentrations were recorded for sprats and mackerel with mean concentrations of 67 ng/kg and 68 ng/kg, respectively. The highest PCN concentrations were recorded for samples from the Irish sea, although locations across the southern/eastern UK coasts and northern France had a majority of the higher concentrations.

In another study, Fernandes et al. (2010) measured the same PCNs in Irish foods. PCNs were found in all 44 food samples collected during 2007 including meat, milk, fish, dairy and meat products, eggs, poultry, vegetables, fruits, etc. The most frequently detected congeners were PCN-52, PCN-66/67, and PCN-73. Highest concentrations were observed in fish where a maximum 37 ng/kg was found for the sum of the measured congeners.

Falandysz, Strandberg, Bergqvist, Strandberg, and Rappe (1997) evaluated the PCN concentration and distribution in marine species from Poland. Samples of mussel (*Mytilus trossulus*), Perch (*Perca fluviatilis*), flounder (*Platychthis flesus*) and lamprey (*Lampetra fluviatilis*) were collected in the Gulf of Gdansk in 1992. Total PCN concentrations ranged from 0.63 to 0.110 ng/kg fat.

In another study, Falandysz et al. (1998) measured PCNs in 30 male and female adult three-spined stickleback (*Gasterosteus aculeatus*) from the Gulf of Gdansk, collected in 1992. Total PCN concentrations ranged from 0.035 to 0.130 ng/kg fat.

Kannan et al. (2002) measured different POPs including PCNs in samples of fish and birds including bluefin tuna, swordfish, cormorants and barn swallows collected between 1995 and 1999 from different regions in Italy. Highest and lowest concentrations in fish were found for tuna where the range was 0.007 ng/kg in muscle to 0.552 ng/kg in liver, although a cormorant liver sample exceeded this range with 0.795 ng/kg.

Domingo et al. (2003) measured PCNs in foods collected in Catalonia, Spain. The highest concentration of total PCNs was found in oils and fats (447 ng/kg), followed by cereals (71 ng/kg), fish and shellfish (39 png/kg), and dairy products (36 ng/kg). Lowest concentrations of total PCNs were found in milk (0.4 ng/kg) and fruits (0.7 ng/kg) followed by tubers (3 ng/kg) and vegetables (4 ng/kg). In general, tetraCNs were the predominant homologue in all food groups except for fruits and pulses, which had greater proportions of hexaCNs.

In a study by Godéré et al. (2022) on the health risk assessment from PCNs to French sea food consumers, a total of 37 samples were collected between January and April 2005 from four French coastal cities. Concentrations (sum of 69 PCNs, 2–8 Cl) ranged from 1.9 to 440 ng/kg (medium-bound values), with mean and median levels, respectively, of 100 and 34 ng/kg.

Falandysz and Fernandes (2020) measured PCN) congeners in edible cod liver products from 1972 to 2017. PCNs were found in all samples apart from the most recent sample of cod liver oil which was assumed to be highly purified because cod livers from the same period and location showed appreciable amounts of PCNs. Congeners that are dominant in legacy technical PCN mixtures were only found in low concentrations or were absent, raising the possibility that congeners arising from combustion-related sources may be acquiring a greater significance following the decline and elimination of PCN production and use. The increase in the relative amounts of PCN-70 in the most recent decades supported this hypothesis. The concentrations of sum of PCNs in both medicinal grade and other cod liver oils were in the range 2050–13,400 ng/kg in 1972–2001, while in canned cod liver foods, the sum of PCNs ranged from 1670 to 2240 ng/kg fat (730–1050 ng/kg whole weight [ww] in 2017). The highly purified cod liver oil produced in 2017 showed negligible amounts of PCNs (2.56 ng/kg). Cod liver oil sourced from the Baltic Sea showed the highest levels of contamination.

Another paper by Falandysz et al. (2019) also described an investigation into the occurrence of PCNs and other contaminants in cod liver products (1972–2017). The authors estimated the contribution of the different contaminants to the daily adult toxic equivalence intake and found that PCNs made a relatively small contribution when compared to other contaminants such as dioxins.

Non-European countries

Pagano and Garner (2019) reported on an assessment of legacy organic contaminants and trends in lake trout from Cayuga Lake, New York between 2011 and 2017. This was a 7-year assessment of lake trout contaminants using ~ 10 individual lake trout per year (total $N = 56$). The authors concluded that over the period of the study, the overall contaminant decrease was 34.6% for total PCNs.

In a second study, Pagano and Garner (2020) investigated concentrations, toxic equivalence and age-corrected trends of legacy organic contaminants including PCNs in Lake Champlain lake trout between 2012 and 2018. Whole-fish lake trout (*Salvelinus namaycush*) were collected yearly from 2012 to 2018, excluding 2014. PCN concentrations averaged 635 pg/g with a range of 439–967 pg/g. The major congeners reported were mainly tetra- (43.5%), penta- (34.8%), and hexaCNs (14.6%) comprising approximately 93% of the average total PCNs measured. Two congeners alone (PCN-42 and PCN-52/60) contributed over 46% of the overall PCN total, 23.5% and 22.6%, respectively. A model used by the authors found a decrease of 21% in the total PCNs content of the whole fish during the period of the study.

A third study by Pagano and Garner (2021) reported on PCNs in 123 samples of Lake Trout and Walleye from the Great Lakes and examined trends over the period from 2004 to 2018. Each sample was analysed for 41 PCNs. Highest concentration for total PCNs was found to be over 5700 ng/kg for Walleye in Lake Erie. The authors determined that the overall lake trout and walleye PCN congener distribution significantly shifted to a lower chlorinated composition in the Great Lakes (5.33–4.48 Cl/CN) and resulted in a substantial 59.1% reduction of the overall total PCN TEQ burden over the period.

Cui et al. (2018) reported concentrations of PCNs in a study on trophic magnification in marine fish from the Bohai coastal area, China. PCNs were analysed in a total of 122 fish samples including 17 different species of fish with various dietary habits. Total PCN concentrations were from 7.3 to 214 ng/kg, with the highest concentration in ditrema (*Ditrema*

temminckii) and the lowest in Pacific Saury (*Cololabis saira*). The predominant congeners were triCNs, which contributed 32% ± 14% of the total PCN concentration. The next most abundant congeners, in decreasing order, were the diCNs, pentaCNs and tetraCNs, which contributed 23% ± 11%, 20% ± 17% and 15% ± 7.2%, respectively, of the PCN concentration. The other homologues (the monoCNs, hexaCNs, heptaCNs and octaCN) contributed only small proportions (6.2% ± 2.8%, 1.9% ± 1.1%, 0.75% ± 0.53 and 0.64% ± 0.62%, respectively) to the total PCN concentration.

A study by Zhang et al. (2016) evaluated the potential for dietary exposure to several organic contaminants including PCNs via paired fillet and eggs of fish from a tributary to Lake Ontario, one of the North American Great Lakes. Eleven pairs of skinless fillets and eggs of different fish species like, Chinook salmon, Coho salmon, brown trout and rainbow trout ranging in size were collected in late 2007. The total concentrations of 14 PCNs ranged in the egg samples from 0.28 to 1.67 ng/g (mean/median = 0.92/0.95 ng/g) while in the fillet samples, concentrations were lower and ranged from 50 to 660 ng/kg (mean/median = 340/340 ng/kg).

Kim et al. (2018) measured PCNs in 33 seafood species (5 samples each) including fish, molluscs and crustaceans purchased from local markets in five South Korean cities between 2012 and 2013. Generally, higher PCN levels were found in the samples with higher lipid content. For example, concentrations of PCNs in fatty fish such as salmon and herring were higher than those of lean fish such as pollack (1.2 ng/kg) and cod (0.83 ng/kg). PCNs were not detected in all tuna and fleshy prawn samples possibly due to their low fat content (0.1%). Low PCN levels were detected in other species such as anglerfish (0.19 pg/g), skate (0.24 ng/kg), octopus (0.57 ng/kg) and small octopus (0.66 ng/kg) (fat content of 0.1%–0.5%). Among the PCN homologues, pentaCN was the most predominant homologue, followed by tetra-, hexa- and heptaCN homologues in fish, while tetraCN was the most dominant homologue in molluscs and crustaceans. The authors stated that salmon, herring, tuna, squid, oyster, mackerel, anchovy and eel showed similar levels to the values reported by previous studies (Domingo et al., 2003; Hanari et al., 2004; Kannan et al., 2000).

A study to investigate the occurrence and distribution of 41 congeners of PCNs in major tissues of crucian carp (muscle, gonad, liver and blood) was reported by Kim et al. (2019). Ten crucian carp were collected from each upstream and midstream position in three major rivers, the Namhan, Nakdong and Yeongsan, all in South Korea, giving a total of 60 fish. The ranges of total PCN concentrations were 1.46–3.08 ng/kg in muscle, 20.6–31.6 ng/kg in liver, 14.8–40.7 ng/kg in gonads and 7.57–18.0 pg/mL in the whole blood of the fish. PCN-28/43, PCN-33/34/37, PCN-42, PCN-52/60, PCN-53/55 and PCN-66/67 congeners were detected most frequently and had the highest concentration among all crucian carp tissues. The authors found positive correlations between the PCN concentration in gonads and the total length and weight of fish, suggesting that PCN accumulation in crucian carp gonads increases with growth.

Kannan et al. (2000) reported on PCNs in fishes from Michigan waters including the Great Lakes sampled in 1996–1997. Concentrations of total PCNs ranged from 19 to 31,400 pg/g, and varied depending on sampling location and species.

Jiang et al. (2007) determined the concentrations of several organochlorine contaminants in common seafood in two Chinese coastal cities (Guangzhou and Zhoushan). Seafood samples (mainly harvested locally) were purchased from local markets in Guangzhou and Zhoushan between 2003 and 2004. Four species of fish, two species of crab, two species of cephalopods, two species of shrimp and three species of bivalves were obtained from each city. Mean concentrations for PCNs ranged between 94 and 1300 ng/kg lipid weight.

Gewurtz et al. (2009) examined factors influencing trends of PCNs and other dioxin-like compounds in archived lake trout (*Salvelinus namaycush*) from Lake Ontario, North America between 1979 and 2004 (4–5 samples each year). Concentrations ranged from over 100,000 ng/kg fat in the oldest samples to less than 10,000 ng/kg fat in the most recent samples.

In another study, Gewurtz et al. (2018) investigated levels, patterns, trends and significance of PCNs in a total 470 fillet samples of 18 fish species collected from the Canadian waters of the Great Lakes between 2006 and 2013. Overall, the total PCNs and PCN-66/67 ranged from 6 to 6700 and < 1–500 ng/kg, respectively. On a lipid basis, the total PCNs and PCN-66/67 ranged from 150 to 190,000 and 10–42,000 ng/kg lipid, respectively.

McGoldrick et al. (2018) investigated spatial and temporal trends of PCN concentrations in whole fish and herring gull eggs throughout the Canadian Great Lakes and St. Lawrence River. Lake Trout (*Salvelinus namycush*) or Walleye (*Sander vitreus*) were collected from 11 locations in the Great Lakes. Between 5 and 10 individual fish between the ages of 4 and 6 years were selected for analysis of PCN concentrations from stations in one lake per year for the period spanning 2010–15. Across the Great Lakes and St. Lawrence River, the mean sum of all measured PCN congeners (total PCNs) ranged from 171 to 7660 ng/kg in Lake Trout and Walleye. The results were compared with data generated for samples collected between 1979 and 2013 and showed a decreasing trend.

Dong, Zhang, et al. (2022) investigated PCN contamination of dairy farms within a 10-km distance from iron smelting plants in an industrial region of China. PCN concentrations in the milk samples from all four farms were in the range from 470 to 797 ng/kg fat with mean and median levels, respectively, of 552 and 519 ng/kg fat.

Wang, Zhang, Wang, et al. (2022) conducted a study to investigate concentrations and risks related to PCNs in green tea from different locations in China. The PCN concentrations in all the green tea samples were 3.62–175 ng/kg (mean 36.1 ng/kg). The lower chlorinated PCN homologues were the dominant PCNs in the green tea samples with diCNs and tetraCNs being the dominant contributors to the total PCN concentrations.

In another study, Wang, Zhang, Fan, et al. (2022) investigated PCNs in farmed mitten crabs in China. Crab compound feed and sediments were collected from Anhui Province and Shanghai in China between September and October 2019. For the 36 farmed crab samples, the concentrations of the individual PCNs were in the range of 5.46–43.8 ng/kg (mean: 21.3 ng/kg). The total concentrations of PCNs were in the range of 11.2–42.2 ng/kg (mean: 24.9 ng/kg) in the Anhui crab samples and 5.46–43.8 ng/kg (mean: 21.3 ng/kg) in the Shanghai specimens.

In a study with a focus on analytical method development, Wang, Han, Cao, and Yan (2022) PCNs were analysed in shrimp. Concentrations were only reported for five samples, and only for PCN-1, PCN-9 and PCN-27.

Dong, Li, et al. (2022) measured total PCNs in raw cow milk ($n = 82$) collected during 2019 and 2020 from three different regions in North China with differences in PCNs emission sources. The total concentrations of mono- to octaCNs in raw cow milk samples were in the range of 89.6–2050 pg/g fat (median: 218 ng/kg fat; mean: 303 ng/kg fat). The lipid concentrations in the raw cow milk samples ranged from 2.1% to 4.9% (median: 3.7%; mean: 3.6%). For the individual PCN congeners, PCNs 24/14, PCN-5/7 and PCN-13 were predominant in the raw cow milk, making up approximately 30% of the total PCNs.

Pan et al. (2013) investigated altitudinal distributions of contaminants including PCNs in soil and yak samples from a region of Tibet, China, where Yak meat is widely consumed. The total concentrations of PCNs in the yak muscle and fatty tissue were 123 and 224 ng/kg fat, respectively. PentaCNs were dominant in yak muscle (31%), followed by tetraCNs (29%) and triCNs (19%).

Han et al. (2018) measured the concentrations and distribution of PCNs in the whole blood of animals used for meat including chicken, duck, rabbit, pig, cattle, sheep, horse and donkey. The total concentrations of PCNs were lowest in pig blood. The monoCNs were the predominant homologue group, followed by the diCNs and triCNs. Of the PCN congeners, PCN-1, PCN-5/7, PCN-24/14, PCN-27/30, PCN-52/60, PCN-66/67, and PCN-73 were the most abundant. The highest concentrations were observed in cattle, followed by chicken. The authors concluded that chicken, beef, and pork resulted in higher exposure to PCNs when compared to the meat from other animals.

In a study aiming to estimate the dietary exposure and potential risk to human health from dietary sources containing PCNs in China, Li et al. (2022) analysed 75 PCN congeners in composite samples of fish, meat, eggs, milk, legumes, vegetables, cereals and potatoes. In total, 192 samples (17,280 subsamples) from 24 Chinese provinces were collected for analysis. The concentrations for the sum of the 75 PCN congeners ranged from 7.6 ng/kg in cereals to 92 ng/kg in meat samples. Milk samples had the highest total PCN concentrations on a fat basis (292 to 1730 ng/kg fat). DiCNs and triCNs were the most abundant congeners in all types of food analysed. In fish samples, in addition to diCNs and triCNs, the contribution of tetraCNs and pentaCNs was also significant. The authors estimated TEQs and the total daily toxic equivalence PCN intake in China which ranged from 0.011 to 0.074 pg TEQ/kg bw per day. This suggests that PCNs have low contribution when compared with other contaminants (e.g. dioxins).

3.2.3.2 | *Feed*

European countries

No data was identified in literature.

Non-European countries

Guruge et al. (2004) reported PCNs in Japanese animal related samples such as feed ingredients, mixed feed and animal fat. Mean concentrations of total PCNs (61 congeners) in feed ingredients ranged from 500 to 1500 ng/kg fat with highest concentrations found in fish meal. Concentrations were similar among mixed feeds, which ranged from 98 to 110 ng/kg fat.

Dong et al. (2018) reported concentrations and patterns of 75 PCN congeners in feed raw materials of animal and plant origin. A total of 22 feed raw samples were collected in 2016 from major feed factories in China, including six types of animal-based and three types of feed raw materials of plant origin. It was concluded that the highest concentrations of PCNs were found in fish meal, while the lowest levels were found in whey powder. DiCNs and triCNs were the most abundant homologues, while PCN-5/7 and PCN-24/14 were the predominant PCN congeners. Homologue and congener profiles of PCNs indicated that PCNs in the feed raw materials might originate from industrial PCN mixtures or secondary nonferrous metallurgies. The highest TEQ concentrations of PCNs were detected in porcine plasma protein powder, followed by meat and bone meal. The hexaCNs and heptaCNs were the most abundant contributors to the total TEQ of PCNs in the feed materials.

In the study by Dong, Zhang, et al. (2022) discussed above that investigated PCN contamination of dairy farms within a 10-km distance from iron smelting plants, some feed ingredients were measured in addition to the milk. The PCN concentrations in the 33 feed ingredients ranged from 22.9 to 314 ng/kg (mean: 79.6 pg/g; median: 55.8 ng/kg) and the mean concentrations of PCNs in feed samples from four dairy farms were 91.5, 66.8, 107 and 52.8 ng/kg.

In the mitten crab study, by Wang, Zhang, Fan, et al. (2022) on PCNs in farmed mitten crabs in China, 22 feed samples were collected from the Anhui Province and Shanghai crab farms. Concentrations of PCNs were in the range of 20.1–758 ng/kg (mean: 89.5 ng/kg).

3.2.4 | Food processing

There are very limited data in the scientific literature about the effects of cooking and processing on PCNs in food. However, the CONTAM Panel considers it reasonable to assume that PCNs behave in a similar way to other lipophilic POPs, i.e. that PCNs associate with lipids and amounts in food may be reduced if fats that come from the food during cooking or processing are discarded; on the other hand if moisture is lost during the heating of foods but no fat is discarded, then the total amount will remain unchanged but the concentrations may increase.

There is one study reported in the literature by Wang, Zhang, Wang, et al. (2022), who conducted a study to investigate concentrations and risks related to PCNs in green tea from different locations in China. The PCN concentrations in all the green tea samples were 3.62–175 ng/kg (mean 36.1 ng/kg). The lower chlorinated PCN homologues were the dominant PCNs in the green tea samples with diCNs and tetraCNs being the dominant contributors to the total PCN concentrations. PCN concentrations in tea leaves were found to be higher after brewing. The authors reported that leaves 'stretched' with increasing brewing time and their volume became bigger after freeze-drying, which could help the PCNs release from the tea samples during the accelerate solvent extraction procedures for PCN analysis. Thus, the PCN concentration in brewed tea leaves were found to be higher than the concentration in the unbrewed tea leaves. Lower chlorinated PCN homologues showed higher volatility and aqueous solubility than higher chlorinated PCN homologues, and they dominated PCNs in green tea.

Dong et al. (2013) described the formation and distribution of PCNs in oil heated in the presence of sucralose. Although PCNs were detected in the oil fumes, no PCNs were found in the heated oils. The total concentrations of the PCNs found in peanut and olive oil fumes were 490 and 240 pg/g, respectively. PCDD/Fs and PCBs were also formed during this process.

3.2.5 | Levels in humans

3.2.5.1 | *Human milk*

Since 1987, the WHO in cooperation with the UNEP has conducted several coordinated surveys on the occurrence of various POPs in human milk. These surveys were not primarily intended to compare levels of POPs among countries, but rather to examine levels within countries over time. Therefore, strict protocols had to be followed with respect inter alia to selection of donors, location and time of sampling, storage and pooling of samples and shipping of the samples to the laboratory (WHO, 2007). To ensure consistency in the analytical measurements, from the third survey onwards except for PFASs all samples were analysed by one laboratory, i.e. the EURL for Halogenated POPs in Feed and Food.

PCNs were included in this study for the period 2000–2019 (Tschiggfrei et al., 2023). There were five rounds of this study conducted over the period with the number of participating countries as follows: 2000–2003 (26); 2004–2007 (13); 2008–2011 (45); 2012–2015 (17) and 2016–2019 (42). All samples from 2016 to 2019 were analysed as pools ($n = 50$), and so data on individual variation are not available. Samples were analysed for 26 individual or pairs of congeners. Concentrations in samples from Europe were consistently higher than in samples from other regions. The results for the most recent round that are used for exposure calculations in 3.3.3 are summarised below.

TABLE 11 PCN concentrations for the sum of 26 PCNs (ng/g lipid) based on the 2016–2019 survey on human milk.

Regions	*N*	Median	Min	Max
Africa	14	51.3	30.2	66.4
Asia-Pacific	10	50.4	27.3	114
Latin America and Caribbean	9	41.0	27.3	98.2
Europe	7	152.3	85.5	170

Abbreviation: *N*, number of samples.

The most abundant congeners found in samples from Europe were the pairs of pentaCNs (PCN-52/60) and hexaCNs (PCN-66/67) which each accounted for about 40% of the total. Table 11 represents regional data, but it is important to note that there are some anomalies, e.g. concentrations in human milk from Salomon Islands and Jamaica are more in line with those values from Europe.

A study by Parizek et al. (2023) investigated organohalogenated pollutants in human milk from the Czech Republic, including 15 PCN congeners. PCN-20, PCN-52 and PCN-66 were reported in < 1% of the samples (note that although the concentration was reported for PCN-66, it was not clear that this had been resolved from PCN-67). Individual concentrations of these congeners ranged between 250 and 832 ng/kg lipid weight. The authors concluded that the presence of PCNs was probably caused by accidental contamination from the environment, as there is no obvious source of these compounds in the Czech Republic.

In a study by Lundén and Norén (1998), the most abundant congener in breast milk was PCN-52, followed by PCN-66/67 and two tetraCN (unidentified), which constituted ~ 51%, 19%, 19% and 9%, respectively, of the total PCN amount. The PCN content in the Swedish human milk samples collected between 1972 and 1992 showed a decrease to 16% over this period, indicating a half-life of 8 years (Noren & Meironyte, 2000).

Li et al. (2020) reported occurrence from monoCN to octaCN congeners in 100 pooled human milk samples derived from a total of 4480 individual specimens collected from the nineteen provinces of China in 2017–2020. Concentrations ranged from 211 to 2500 ng/kg lipid. Dominant compounds were PCN-66/67 > PCN-52/60 > PCN-51 and > PCN-37/33/34. These authors concluded that main sources for PCNs in human milk in the samples from China were the unintentional emission of the PCN from an industry that used thermal processes as well as PCN impurities in the technical polychlorinated biphenyl (PCB) formulations in China (Li et al., 2020).

A second study by Li et al. (2021) examined the burden and risk of PCNs in Chinese human milk and made a comparison of exposure from China, Sweden and Ireland. The hazard index calculated for infants in Sweden indicated a strong impact of historical (legacy pollution) residues that nonetheless decreased over time, and a comparison of the hazard indices calculated for China and Ireland suggested that unintentional formation and release of PCNs from current industrial processes was a significant contributor to overall burden.

3.2.5.2 | *Blood*

There are a number of studies reported in the literature on PCNs in human blood. Some of these relate to specific exposure events and others are studies on general background levels, and yet others do not report the levels, so care must be taken when making comparisons or drawing conclusions.

Weistrand et al. (1997) detected PCN-52, PCN-66/67, PCN-71, PCN-73 and PCN-75 in human blood in a study on exposed workers in the Swedish metal reclamation plant.

The most predominant of PCNs in human plasma in Germany were PCN-73, PCN-66/67 and PCN-51 (they accounted for 71% of PCNs). Other congeners including PCN-42, PCN-33/34/37, PCN-44/47, PCN-36/45, PCN-28/43, PCN-38/40, PCN-52/60, PCN-50, PCN-51, PCN-66/67, PCN-73 and PCN-75 were also found, while PCN-29, PCN-30/27, PCN-39, PCN-32, PCN-48/35, PCN-58, PCN-61, PCN-57, PCN-54, PCN-62, PCN-53/55, PCN-59, PCN-49, PCN-56, PCN-64/68, PCN-69, PCN-71/72, PCN-63, PCN-65, PCN-70 and PCN-74 were not detected (Fromme et al., 2015).

Human blood samples collected during the Yusho (1968) and Yucheng (1979) incidents in order to investigate exposure to PCB mixtures, contained also a range of PCNs for which half-lives were estimated between 1.5 and 2.4 years (EFSA CONTAM Panel, 2018; Ryan & Masuda, 1994).

In a pilot study by Horii et al. (2010), blood plasma samples ($n = 43$) from New York State employees and National Guard personnel that had been working in the vicinity of the World Trade Center (WTC) during the week after the collapse of the buildings were collected and analysed for pollutants, including PCNs. Concentrations of PCNs in plasma ranged from < LOD to 38,500 ng/kg lipid with a mean value of 2740 ng/kg lipid. Overall, tetra- to hexaCNs were the most abundant congeners in the plasma samples analysed in this study.

Park et al. (2010) measured PCN concentrations in the blood plasma of 61 healthy human volunteers from Seoul (South Korea). They were 36 residents from near a municipal solid waste incinerator, 14 municipal solid waste workers and 11 members of the general population residing over 10 km away from the municipal solid waste incinerator. The mean total tetra- to octaCN homologue groups concentration for all subjects was 2170 ng/kg lipid. The mean total PCNs concentration for residents, workers and general population was 2110, 2070 and 2480 ng/kg lipid, respectively. The predominant serum PCN congeners in all subjects were tetra- and pentaCN homologues, and the most predominant individual congener was heptaCN (PCN-73), which contributed 17.5% of the total serum PCN concentration.

In utero exposure for PCN was confirmed by their detection in blood of 10 American newborn babies in 2004 (Zeliger, 2011).

Kim et al. (2015) detected PCN-36/45 and PCN-52/60 at the highest median concentrations in all samples of the cord blood from the pregnant mothers and neonates in South Korea, and compounds less frequently detected were PCN-36/45 followed by PCN-52/60, PCN-73 and PCN-30/27. Concentrations of PCN-66/67 were not reported.

Jin et al. (2019) measured PCN levels in human serum samples from an industrial city in Eastern China. Blood donors ($n = 240$ per sex) were in the age range of 18–54 years (mean: 35 years). Serum samples collected were pooled into 8 groups per sex based on their age (< 20, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, ≥ 50). The mean for the sum of the mono- to octaCNs (sum of PCNs) was 31,400 ± 9650 ng/kg lipid in males and 26,000 ± 10,400 ng/kg lipid in females. The concentration range of the sum of PCNs for both sexes was 14,300 ng/kg lipid (females, ≥ 50 years group) to 50,700 ng/kg lipid (males 20–25 years group). PCN-3, PCN-5/7 and PCN-4 were detected in all pooled samples. PCN-5/7 was the most predominant congener pair in serum samples and accounted for 21.6%–51.1% of the total PCN concentration.

In a more recent study, Waheed et al. (2020) investigated the concentrations and health risks of 39 PCNs in indoor dust, air and blood associated with major e-waste recycling hubs in Pakistan. Samples of serum ($n = 250$) were collected from workers, residents and children from five major e-waste hubs and their vicinity. The sum of concentrations of 39 PCN congeners in serum samples ranged from 0.15 to 401 ng/kg lipid weight (worker > resident > children). Predominant PCN congeners in human serum samples were tetra- and pentaCNs.

A study on PCN concentrations and temporal trends in serum from the general Chinese adult population (Chen et al., 2023) found that in samples from 32 adults collected annually from 2012 to 2016, concentrations did not decrease and for some congeners, e.g. PCN-20, there was an increase in concentration. Different PCN concentrations were observed in serum from males and females where PCN-75 was found in significantly higher concentrations in serum from females than males.

3.2.5.3 | *Other human studies*

Adipose tissue

The Yusho and Yucheng incidents described above also produced data on adipose tissue (Hsu et al., 1985; Kuratsune et al., 1972). The main PCN congeners found in adipose tissue of the Yusho patients were PCN-28/43, PCN-33/34/37, PCN-35, PCN-38/40, PCN-46, PCN-52/60 and PCN-66/67, while PCN-24, some tetraCN, pentaCN, hexaCN, PCN-73 and PCN-74 were determined in the Yusho rice oil but were not detected in the victims of the poisoning (Haglund et al., 1995).

PCNs (tetra- to hexa- congeners) were measured in autopsy samples of adipose tissue and liver from seven Swedish adults (two women and five men who had suffered sudden death). The profile was dominated by PCN-66/67 and PCN-52/60 and to a lesser extent PCN-42, PCN-33/34/37, PCN-47, PCN-28/43, PCN-35, PCN-38/40, PCN-46, PCN-53/55, PCN-57, PCN-61, PCN-62 and PCN-68 (Weistrand & Norén, 1998).

DiCNs, triCNs, pentaCNs and hexaCNs were detected and quantified in adipose tissue of Italians to establish background levels in the Italian population. HeptaCNs were detected but below the limit of quantification, and tetraCNs were not detected. The profile was dominated by PCN-66/67 (56%), followed by PCN-50/51 (14%) and PCN-52/60 (10%), and triCNs at 12% (Schiavone et al., 2010).

A study by Kunisue et al. (2009) was performed as a first investigation to measure background concentrations of PCNs in human adipose tissue samples from the USA. Samples were collected in New York City from 2003 to 2005. Adipose tissue samples ($n = 43$) were collected from liposuction procedures performed on male patients and female patients in New York City during October 2003 through November 2005. Concentrations of PCNs ranged from 61 to 2500 ng/kg lipid wt. (median: 410 ng/kg, mean: 590 ng/kg) in males and 21–910 ng/kg lipid wt. (median: 190 ng/kg, mean: 270 ng/kg) in females. Concentrations of PCNs in male donors were significantly higher than concentrations in female donors. There was no apparent age-dependent accumulation pattern for PCNs. Study authors also noted that median PCN concentrations in human adipose tissues were two to three orders of magnitude lower than the respective concentrations of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) previously reported. The contribution of PCNs to toxic equivalence in human adipose tissues was estimated to be < 1% of the polychlorinated dibenzo-*p*-dioxin/dibenzofuran (PCDD/F)-TEQs where TEQs were calculated using DR-CALUX-REPs reported by Behnisch et al. (2003).

3.2.5.4 | *Summary of the levels in human samples*

There are only a few studies in the literature reporting concentrations of PCNs in human tissues. Many of these have been conducted in association with contamination or occupational exposure incidents, but there are some that have examined background levels within the population.

Concentrations of PCNs in human milk were found to be two to three times higher in European samples when compared to other global regions in a study conducted by the UNEP/WHO (notable exceptions were noted in Salomon Islands and Jamaica) (Tschiggfrei et al., 2023).

3.3 | Dietary exposure assessment

3.3.1 | Current dietary exposure assessment for humans

The CONTAM Panel assessed the dietary exposure to individual hexaCNs (PCN-63, PCN-64/68, PCN-65, PCN-66/67, PCN-69, PCN-70 and PCN-71/72) following the methodology described in Section 2.6. For the PCN-69, two exposure scenarios were calculated; one with considering the high PCN-69 concentration of 313 ng/kg measured in egg sample (PCN-69 scenario A) and one without considering this high concentration level (PCN-69 scenario B).

A representative repeated dose toxicity study (90-day study) was only available for a hexaCN mixture that contained hexaCNs at 94.1% PCN-66/67 (equivalent mixture at 81.2%) and the eight other hexaCNs (PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%) as well as a heptaCN (PCN-73 at 5.9%). Therefore, in addition to the exposures to individual PCNs, the CONTAM Panel also calculated the exposure to the hexaCN mixture based on a sum of all hexaCNs and PCN-73 in a separate scenario (hereinafter referred to as 'mixture scenario'); with and without the high PCN-69 concentration of 313 ng/kg in egg sample included referred as mixture scenario A and mixture scenario B, respectively. In practice, within each reported sample, the concentrations of the individual hexaCNs (PCN-63, PCN-64/68, PCN-65, PCN-66/67, PCN-69, PCN-70 and PCN-71/72) and PCN-73 were summed (assuming equipotency) and considered as one concentration value. In case the individual PCN congener within the sample was not reported, the missing value was either imputed by the mean of the other data available for the same food category and PCN congener, or by zero when no data were available at all.

The mean and 95th percentile chronic dietary exposure to PCNs (pg/kg bw per day) was estimated separately for each consumption survey using data recorded at the individual level from the Comprehensive Database (see Section 2.6). Due to the methodological differences among the surveys, chronic dietary exposure was estimated separately for each of them.

A summary of the PCN occurrence data including the number of results, percentage of LCD and mean concentrations for the food categories at the FoodEx2 level as used for exposure assessment for different scenarios is presented in Annex C, Tables C1–C.10.

The contribution (%) of each food category to the total mean exposure was calculated for each age group and dietary survey. Estimations of exposure using the LB approach, which is considered to be less influenced by the value of the LOD/LOQ, were used to present the contribution of the different food categories. The contribution of individual food categories to the mean LB chronic dietary exposure varied between the dietary surveys. This is explained by the specific food consumption patterns in the individual European countries and even in different regions within a country.

3.3.1.1 | *Current dietary exposure assessment to PCN-66/67*

Mean and high dietary exposure

Table 12 shows summary statistics for the assessment of chronic dietary exposure to PCN-66/67. Detailed mean and 95th percentile dietary exposure estimates calculated for each dietary survey are presented in Annex C, Table C.11.

TABLE 12 Summary statistics for chronic dietary exposure to PCN-66/67 (pg/kg bw per day) across European countries.

	Minimum		Median		Maximum	
Age group	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Mean dietary exposure in total population (pg/kg bw per day)						
Infants	0.22	0.22	1.10	1.12	2.46	2.51
Toddlers	0.72	0.73	1.11	1.12	1.59	1.61
Other children	0.44	0.45	0.81	0.82	1.38	1.39
Adolescents	0.22	0.22	0.42	0.43	0.74	0.75
Adults	0.18	0.19	0.32	0.33	0.50	0.51
Elderly	0.15	0.15	0.31	0.31	0.46	0.47
Very elderly	0.21	0.21	0.32	0.33	0.48	0.49
95th percentile dietary exposure in total population (pg/kg bw per day)						
Infants[a]	0.70	0.71	3.32	3.39	5.77	5.90
Toddlers[a]	1.78	1.81	2.84	2.85	4.05	4.07
Other children	1.16	1.18	1.87	1.89	3.18	3.18
Adolescents[a]	0.63	0.65	1.04	1.05	1.59	1.60
Adults	0.58	0.59	0.89	0.89	1.34	1.34
Elderly	0.45	0.45	0.88	0.88	1.39	1.39
Very elderly[a]	0.46	0.46	0.97	0.98	1.28	1.28

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

Note: The values are rounded to two decimals.

[a]The 95th percentile estimates obtained on dietary surveys/age groups with fewer than 60 observations may not be statistically robust (EFSA, 2011c) and are therefore not included in this table.

The highest estimated chronic dietary exposure to PCN-66/67 was in the young age groups with the tendency to decrease moving from the younger to the older age groups. Concerning the mean dietary exposure, the highest estimated LB/UB exposure levels were in infants with a maximum exposure of 2.46/2.51 pg/kg bw per day. The highest 95th percentile LB/UB exposure was observed for infants with estimates of 5.77/5.90 pg/kg bw per day.

Dietary exposure in specific groups of the population, namely 'Pregnant women' and 'Lactating women' were within the range of exposure estimates for the adult population while the exposure for 'Vegetarians' was lower than those assessed in adults (only one dietary survey available) (see Annex C, Table C.11).

Contributions of different food groups

Table 13 describes the relative contribution (%) of each food category to the overall mean LB exposure to PCN-66/67 as median and range (minimum and maximum) for all age class across all European dietary surveys.

The detailed contribution to the mean LB chronic dietary PCN-66/67 exposure of the different food categories at FoodEx Level 1 and grouped by age class is shown in Annex C, Table C.12.

Overall, the food categories mainly contributing to the mean LB chronic dietary exposure to PCN-66/67 were 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Meat and meat products' in the adult population groups reaching up to 75% (in very elderly) and 44% (in adults), respectively, and 'Milk and dairy products', in particular for infants and toddlers reaching up to 93%. Due to limited availability of the occurrence data, it was not possible to assess the contribution with regard to specific fish species or meat types. Indicatively, 'diadromous fish', 'marine fish' and 'mammals meat' had the highest contribution among the food subcategories belonging to 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Meat and meat products' and 'milk' had the highest contribution among the food subcategories belonging to 'Milk and dairy products'. It should be noted that considering that the mean PCN-66/67 concentration levels for 'milk' is low (mean of 0.03 ng/kg), its high contribution is likely to be driven by its high consumption. In several surveys, also the food category 'Animal and vegetable fats and oils and primary derivatives thereof' represented only by 'butter' contributed considerably to the overall mean LB chronic dietary exposure to PCN-66/67 reaching up to 48% in very elderly (up to 80% when considering the specific population group of vegetarians).

The food category 'Eggs and egg products' contributed up to 21% in adults and the elderly.

TABLE 13 Relative contribution (%) of the FoodEx2 (Level 1) categories to the overall mean dietary exposure to PCN-66/67 (LB) across different surveys and population groups.

	PCN-66/67		
FoodEx2 level 1	**Min**	**Median**	**Max**
Animal and vegetable fats and oils and primary derivatives thereof	0	10.8	48.1
Eggs and egg products	0	4.87	21.0
Fish, seafood, amphibians, reptiles and invertebrates	2.09	28.3	74.5
Meat and meat products	2.07	22.8	44.1
Milk and dairy products	5.13	21.0	93.3

3.3.1.2 | *Current dietary exposure assessment to other individual hexaCNs*

Mean and high dietary exposure

Tables 14 and 15 show summary statistics for the assessment of chronic dietary exposure to individual hexaCNs other than PCN-66/67. Detailed mean and 95th percentile dietary exposure estimates calculated for each dietary survey are presented in Annex C, Tables C.13–C.19.

The highest mean exposure across the European dietary surveys was estimated for PCN-69 scenario A ranging from 0.03 in adolescents and very elderly to 5.61 pg/kg bw per day in toddlers for the minimum LB and the maximum UB, respectively, with a lowest and highest median of 0.30 (very elderly) and 2.87 pg/kg bw per day (other children) for LB and UB, respectively. The highest P95 exposure was estimated for the same scenario ranging from 0.07 in infants to 19.4 pg/kg bw per day in toddlers for the minimum LB and the maximum UB, respectively, with a lowest and highest median of 1.08 (very elderly) and 11.1 pg/kg bw per day (toddlers) for LB and UB, respectively.

Among individual PCN congeners other than PCN-66/67, the PCN-69 was confirmed to have the highest exposure levels even if the high PCN-69 concentration in egg sample was not considered (PCN-69 scenario B). In this exposure scenario, the mean exposure estimates across European surveys for the age groups ranged from the minimum LB of 0.02 pg/kg bw day in different age groups to the maximum UB of 1.38 pg/kg bw day estimated for toddlers. For the P95 percentile exposure, exposure estimated in this scenario ranged from the minimum LB of 0.06 pg/kg bw day in the elderly to the maximum UB of 3.74 pg/kg bw day in toddlers.

The next highest exposure estimates were calculated for two pairs of co-eluting congeners, namely PCN-64/68 and PCN-71/72. It should be noted that in adult population groups, the maximum mean and P95 percentile exposure levels of PCN-71/72 were higher than those estimated for the PCN-69 scenario B, but this was not the case for the minimum and median exposure levels. This outcome was driven by high concentration of PCN-71/72 measured in diadromous fish, and at the same time large consumption of this type of fish in some European countries.

The exposure levels of the remaining hexaCNs were low or negligible.

Overall, the highest mean exposure to hexaCNs at the LB and UB across all congeners was estimated for the age groups of toddlers and other children with the same tendency to decrease moving from the younger to the older age groups. Depending on hexaCN congener, infants were the least exposed age group for PCN-65, whilst adolescents, adults, the elderly and very elderly were generally the least exposed age groups for other hexaCNs.

Dietary exposure in specific groups of the population, namely 'Pregnant women' and 'Lactating women' were within the range of exposure estimates for the adult population whilst the exposure for 'Vegetarians' was lower than those assessed in adults (only one dietary survey available) (see Annex C, Tables C.13–C.19).

TABLE 14 Mean chronic dietary exposure (pg/kg bw per day) to other individual hexaCNs by age group.

		Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
		LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
PCN-63	**Min**	< 0.01	0.01	0.01	0.04	< 0.01	0.02	< 0.01	0.02	< 0.01	0.02	< 0.01	0.01	< 0.01	0.01
	Median	0.01	0.03	0.07	0.10	0.06	0.09	0.03	0.05	0.02	0.04	0.02	0.03	0.02	0.03
	Max	0.04	0.06	0.12	0.16	0.11	0.15	0.07	0.10	0.04	0.06	0.04	0.05	0.05	0.07
PCN-64/68	**Min**	0.06	0.10	0.16	0.31	0.10	0.19	0.05	0.07	0.05	0.05	0.04	0.05	0.05	0.07
	Median	0.16	0.49	0.32	0.46	0.24	0.34	0.14	0.18	0.12	0.13	0.12	0.13	0.10	0.13
	Max	0.31	0.98	0.72	0.93	0.49	0.67	0.29	0.36	0.21	0.23	0.21	0.23	0.23	0.26
PCN-65	**Min**	< 0.01	0.01	0.01	0.06	< 0.01	0.04	< 0.01	0.02	< 0.01	0.03	< 0.01	0.02	< 0.01	0.02
	Median	0.03	0.07	0.14	0.22	0.14	0.21	0.06	0.11	0.04	0.08	0.04	0.07	0.04	0.07
	Max	0.10	0.14	0.27	0.41	0.25	0.39	0.15	0.23	0.08	0.14	0.08	0.12	0.11	0.15
PCN-69 scenario A[a]	**Min**	0.06	0.08	0.09	0.15	0.05	0.10	0.03	0.05	0.05	0.08	0.04	0.06	0.03	0.07
	Median	0.41	0.44	2.64	2.80	2.76	2.87	1.08	1.13	0.54	0.59	0.38	0.41	0.30	0.35
	Max	1.98	2.01	5.29	5.61	4.35	4.48	2.69	2.75	1.68	1.74	1.43	1.49	1.83	1.90
PCN-69 scenario B[b]	**Min**	0.02	0.04	0.09	0.15	0.05	0.10	0.02	0.04	0.04	0.06	0.02	0.04	0.02	0.06
	Median	0.13	0.20	0.51	0.60	0.51	0.57	0.23	0.29	0.16	0.20	0.15	0.20	0.15	0.20
	Max	0.35	0.37	1.06	1.38	0.87	1.00	0.52	0.63	0.31	0.35	0.28	0.35	0.39	0.46
PCN-70	**Min**	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Median	< 0.01	< 0.01	< 0.01	0.02	< 0.01	0.02	< 0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Max	< 0.01	0.02	< 0.01	0.05	< 0.01	0.04	< 0.01	0.02	< 0.01	0.02	< 0.01	0.01	< 0.01	0.02
PCN-71/72	**Min**	0.01	0.02	0.12	0.14	0.05	0.07	0.03	0.03	0.04	0.04	0.01	0.02	0.03	0.04
	Median	0.10	0.11	0.33	0.39	0.17	0.22	0.11	0.13	0.12	0.14	0.11	0.13	0.09	0.10
	Max	0.27	0.29	0.56	0.74	0.46	0.54	0.28	0.31	0.29	0.30	0.44	0.45	0.52	0.53

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

Note: The values are rounded to two decimals. Values lower than 0.01 are indicated as < 0.01.

[a]The egg sample with PCN-69 concentration of 313 ng/kg included.

[b]The egg sample with PCN-69 concentration of 313 ng/kg not included.

TABLE 15 95th percentile chronic dietary exposure (pg/kg bw per day) to other individual hexaCNs by age group.

		Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
		LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
PCN-63	**Min**	< 0.01	0.05	0.08	0.18	< 0.01	0.06	0.01	0.05	0.01	0.05	0.01	0.03	0.03	0.06
	Median	0.07	0.16	0.28	0.33	0.20	0.25	0.12	0.15	0.11	0.14	0.09	0.11	0.10	0.12
	Max	0.22	0.25	0.45	0.49	0.45	0.50	0.22	0.24	0.15	0.18	0.16	0.17	0.16	0.19
PCN-64/68	**Min**	0.22	0.37	0.51	0.79	0.44	0.55	0.16	0.21	0.20	0.20	0.14	0.16	0.19	0.24
	Median	0.55	1.58	1.05	1.34	0.74	0.92	0.42	0.51	0.40	0.44	0.38	0.45	0.38	0.42
	Max	1.22	2.40	2.03	2.40	1.60	1.78	0.78	0.89	0.66	0.70	0.70	0.72	0.68	0.71
PCN-65	**Min**	< 0.01	0.12	< 0.01	0.24	< 0.01	0.14	< 0.01	0.09	< 0.01	0.09	< 0.01	0.07	< 0.01	0.10
	Median	0.16	0.36	0.63	0.79	0.45	0.58	0.24	0.31	0.23	0.28	0.19	0.26	0.20	0.27
	Max	0.50	0.67	0.97	1.21	1.03	1.20	0.48	0.59	0.32	0.41	0.30	0.34	0.31	0.37
PCN-69 scenario A[a]	**Min**	0.07	0.21	0.31	0.56	0.17	0.33	0.07	0.17	0.16	0.28	0.06	0.10	0.10	0.23
	Median	2.31	2.33	11.0	11.1	9.16	9.26	3.33	3.41	2.36	2.41	1.36	1.41	1.08	1.20
	Max	9.98	10.1	19.3	19.4	15.7	15.8	9.13	9.28	5.84	5.91	4.05	4.12	4.86	4.92
PCN-69 scenario B[b]	**Min**	0.07	0.21	0.31	0.56	0.17	0.33	0.07	0.17	0.09	0.19	0.06	0.10	0.10	0.23
	Median	0.57	0.77	2.09	2.17	1.59	1.72	0.83	0.89	0.77	0.82	0.67	0.72	0.70	0.75
	Max	1.74	1.89	3.33	3.74	3.38	3.56	1.66	1.80	1.06	1.15	0.95	0.98	1.03	1.16

(Continues)

TABLE 15 (Continued)

		Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
		LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
PCN-70	**Min**	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Median	< 0.01	0.02	0.01	0.10	< 0.01	0.08	< 0.01	0.03	< 0.01	0.02	< 0.01	0.01	< 0.01	0.01
	Max	< 0.01	0.09	0.01	0.17	0.01	0.14	< 0.01	0.08	< 0.01	0.05	< 0.01	0.04	< 0.01	0.04
PCN-71/72	**Min**	0.06	0.10	0.27	0.43	0.13	0.19	0.04	0.06	0.05	0.07	0.04	0.06	0.12	0.14
	Median	0.32	0.39	1.35	1.91	0.54	0.80	0.34	0.47	0.59	0.73	0.31	0.50	0.62	0.66
	Max	1.24	1.28	3.23	3.23	3.55	3.57	1.69	1.72	1.54	1.55	1.96	1.98	1.74	1.75

Abbreviations: bw, body weight; LB, lower bound. UB, upper bound.

Note: The values are rounded to two decimals. Values lower than 0.01 are indicated as < 0.01.

[a]The egg sample with PCN-69 concentration of 313 ng/kg included.

[b]The egg sample with PCN-69 concentration of 313 ng/kg not included I): The 95th percentile estimates obtained on dietary surveys/age groups with fewer than 60 observations may not be statistically robust (EFSA, 2011c) and are therefore not included in this table.

Contributions of different food groups

Table 16 describes the relative contribution (%) of each food category to the overall mean LB exposure to individual hexaCNs as median and range (minimum and maximum) for all age class across all European dietary surveys.

The detailed contribution of the different food categories at FoodEx Level 1 to the mean LB chronic dietary exposure to individual hexaCNs grouped by age class is shown in Annex C, Table C.20–C.26.

The highest relative contribution to the mean LB dietary exposure across the individual hexaCNs generally had the food categories 'Eggs and egg products' and 'Fish, seafood, amphibians, reptiles and invertebrates' reaching to 100%. This outcome was highly influenced by a low number of available occurrence data when considering each hexaCN individually (for the majority of the congeners only three food categories could be considered for the exposure assessment).

The highest relative contribution of the mean LB dietary exposure of PCN-63 and PCN-65 was due to the consumption of 'Eggs and egg products' (up to 100% for PCN-65) and 'Fish, seafood, amphibians, reptiles and invertebrates' (up to 99% for both PCN-63 and PCN-65).

The main contributor to the mean LB dietary exposure to PCN-64/68 was the food category 'Fish, seafood, amphibians, reptiles and invertebrates' reaching up to 91% in very elderly. In addition, in infants also 'Milk and dairy products' made an important contribution (up to 73%).

The food category 'Eggs and egg products' contributed the most to the dietary exposure of PCN-69 with a maximum to the overall LB exposure equal to 99% in different age groups observed for the PCN-69 scenario A, and equal to 96% in very elderly in the PCN-69 scenario B. The second mostly contributing food category 'Fish, seafood, amphibians, reptiles and invertebrates'.

Only two food categories, including 'Eggs and egg products' and category 'Fish, seafood, amphibians, reptiles and invertebrates' could be considered for the assessment of PCN-70. Both food categories contributed up to 100% to the mean LB dietary exposure to this congener.

'Fish, seafood, amphibians, reptiles and invertebrates' was the main contributor to the LB exposure of PCN-71/72 (up to 99% in very elderly).

TABLE 16 Relative contribution (%) of the FoodEx2 (Level 1) categories to the overall mean dietary exposure to individual hexaCN (LB) across different surveys and population groups.

	PCN-63			PCN-64/68			PCN-65		
FoodEx2 level 1	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Animal and vegetable fats and oils and primary derivatives thereof	–	–	–	0	3.27	20.1	–	–	–
Eggs and egg products	0	53.0	97.9	0	9.46	51.0	0	67.0	100
Fish, seafood, amphibians, reptiles and invertebrates	0	46.2	99.0	9.81	53.2	91.3	0	32.9	99.4
Meat and meat products	0.20	0.84	30.0	4.58	21.2	43.9	0.05	0.23	8.78
Milk and dairy products	–	–	–	1.07	4.50	73.0	–	–	–

	PCN-69 scenario A			PCN-69 scenario B			PCN-70			PCN-71/72		
FoodEx2 level 1	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Animal and vegetable fats and oils and primary derivatives thereof	–	–	–	–	–	–	–	–	–	–	–	–
Eggs and egg products	0	87.7	99.3	0	53.2	95.9	0	83.0	100	0	7.77	80.7
Fish, seafood, amphibians, reptiles and invertebrates	0.17	9.47	82.1	1.06	37.1	93.3	0	17.0	100	8.97	86.6	99.2
Meat and meat products	0.37	2.01	35.7	2.12	8.12	40.8	–	–	–	0.56	5.49	37.8
Milk and dairy products	–	–	–	–	–	–	–	–	–	–	–	–

3.3.1.3 | *Current dietary exposure assessment to selected group of PCN congeners ('mixture scenario')*

Mean and high dietary exposure

As mentioned above, two different exposure scenarios were calculated for a selected group of PCN congeners (for the list of congeners as grouped within this scenario see above; Section 3.3.1); one with and one without the high PCN-69 concentration of 313 ng/kg in egg sample included, referred as mixture scenario A and mixture scenario B, respectively.

Table 17 shows the summary statistics for the assessment of chronic dietary exposure to mixture scenario A and B. Detailed mean and 95th percentile dietary exposure estimates calculated for all population groups for all dietary surveys are presented in Annex C, Tables C.27, C.28.

Regarding the mixture scenario A, the highest exposure levels across the European dietary surveys were estimated for young population groups. The highest mean LB/UB exposure was observed for toddlers with the levels of the mean exposure estimated at 9.41 and 11.5 pg/kg bw day, respectively. The highest 95th percentile exposure levels were assessed for toddlers with LB/UB of 26.8 and 29.8 pg/kg bw day, respectively.

Similar exposure patterns were observed for the mixture scenario B where the highest exposure levels across the European dietary surveys were estimated for young population groups. The highest mean LB and UB exposure was estimated for infants and toddlers with the levels of the mean exposure estimated up to 8.72 pg/kg bw day. The highest 95th percentile exposure levels were assessed for infants with UB of 20.7 pg/kg bw day.

Overall, dietary exposure in specific groups of the population, namely 'Pregnant women' and 'Lactating women' were within the range of exposure estimates for the adult population while the exposure for 'Vegetarians' was lower than those assessed in adults (only one dietary survey available) (see Annex C, Tables C.27, C.28). However, the CONTAM Panel noted that for the mixture scenario A, the exposure levels were considerably higher in lactating women in one survey reporting high consumption of eggs and egg products.

TABLE 17 Summary statistics for chronic dietary exposure of the mixture scenario A and mixture scenario B (pg/kg bw per day) across European countries.

	Minimum		Median		Maximum	
Age group	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Mixture scenario A						
Mean dietary exposure in total population (pg/kg bw per day)						
Infants	0.56	0.92	2.50	4.43	3.79	8.76
Toddlers	1.31	2.58	4.78	6.12	9.41	11.5
Other children	0.79	1.56	4.71	5.75	7.15	8.41
Adolescents	0.41	0.68	1.94	2.50	4.42	5.28
Adults	0.42	0.61	1.27	1.64	2.52	2.92
Elderly	0.32	0.50	1.24	1.61	2.42	2.90
Very elderly	0.45	0.69	1.35	1.74	3.17	3.69
95th percentile dietary exposure in total population (pg/kg bw per day)						
Infants[a]	2.77	3.32	7.25	14.6	14.3	20.7
Toddlers[a]	3.73	7.34	16.6	18.4	26.8	29.8
Other children	2.22	3.98	13.0	14.4	23.1	25.4
Adolescents[a]	1.18	1.96	6.00	6.97	13.1	14.0
Adults	1.13	1.70	4.65	5.29	7.99	8.64
Elderly	0.91	1.42	4.92	5.53	6.39	7.03
Very elderly[a]	1.09	1.57	4.75	5.02	7.69	8.34
Mixture scenario B						
Mean dietary exposure in total population (pg/kg bw per day)						
Infants	0.49	0.84	1.78	4.11	3.76	8.72
Toddlers	1.31	2.58	2.76	4.19	5.16	7.28
Other children	0.79	1.54	2.29	3.38	3.76	5.55
Adolescents	0.40	0.67	1.22	1.77	2.33	3.28
Adults	0.39	0.58	0.98	1.34	1.48	1.98
Elderly	0.28	0.46	0.94	1.26	1.50	1.85
Very elderly	0.44	0.68	0.96	1.34	1.72	2.25

TABLE 17 (Continued)

	Minimum		Median		Maximum	
Age group	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
95th percentile dietary exposure in total population (pg/kg bw per day)						
Infants[a]	2.19	2.95	5.81	12.5	7.41	20.7
Toddlers[a]	3.73	7.34	8.51	11.3	12.6	16.3
Other children	2.22	3.98	6.10	7.94	12.9	13.8
Adolescents[a]	1.15	1.93	3.53	4.41	6.32	7.34
Adults	1.09	1.67	2.98	3.66	4.64	5.41
Elderly	0.90	1.29	2.64	3.25	5.44	5.67
Very elderly[a]	1.09	1.57	3.27	3.92	5.29	5.60

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

Note: The values are rounded to two decimals.

[a]The 95th percentile estimates obtained on dietary surveys/age groups with fewer than 60 observations may not be statistically robust (EFSA, 2011c) and are therefore not included in this table.

Contributions of different food groups

Table 18 describes the relative contribution (%) of each food category to the overall mean LB exposure of the mixture scenario A and B as median and range (minimum and maximum) for all age class across all European dietary surveys.

The detailed contribution to the mean LB chronic dietary exposure to the mixture scenario A and the mixture scenario B of the different food categories at FoodEx Level 1 and grouped by age class is shown in Annex C, Tables C.29, C.30.

Overall, the food categories having the highest contribution to the mean LB chronic dietary exposure to the selected group of PCN congeners across all age groups were the food categories 'Fish, seafood, amphibians, reptiles and invertebrates', with a contribution reaching up to 84.9% and 87.9% in calculated for very elderly in the scenario A and B, respectively, and 'Eggs and egg products', with a contribution reaching up to 83.7% in very elderly calculated in scenario A. In particular 'diadromous fish', contributed to the exposure from the food category 'Fish, seafood, amphibians, reptiles and invertebrates' most likely due to high lipid content of the major species contributing to this category.

Several other food groups were also important contributors to the mean LB dietary exposure to the selected group of PCN congeners. 'Milk and dairy products' was an important contributor to the exposure particularly for infants contributing up to 85.8% and 88.0% in the mixture scenario A and B, respectively. For other age groups, the contribution of this food category was much lower. 'Meat and meat products' contributed up to 34.5% in adults and adolescents (mixture scenario A) and 37.8% (mixture scenario B) in the elderly, and 'Animal and vegetable fats and oils and primary derivatives thereof' up to up to around 40% in very elderly for both scenarios (72.1% in the specific population group of vegetarians, see Annex C, Table C.30).

TABLE 18 Relative contribution (%) of the FoodEx2 (Level 1) categories to the overall mean dietary exposure of the mixture scenario A and B (LB) across different surveys and population groups.

	Mixture scenario A			Mixture scenario B		
FoodEx2 level 1	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Animal and vegetable fats and oils and primary derivatives thereof	0	3.81	38.1	0	5.69	39.1
Eggs and egg products	0	36.4	83.7	0	15.0	61.3
Fish, seafood, amphibians, reptiles and invertebrates	1.30	25.6	84.9	3.08	38.6	87.9
Meat and meat products	2.51	11.1	34.5	2.88	16.4	37.8
Milk and dairy products	1.61	7.95	85.8	2.44	1.6	88.0

3.3.2 | Exposure of high and frequent consumers of fish meat

In response to concern that high and frequent consumers of fish meat might have elevated PCN-66/67 exposure, the CONTAM Panel considered it of interest to address the issue of PCN-66/67 exposure from fish meat (for more detail see Section 2.6).

Results calculated for the 35 surveys included with the minimum, median and maximum of 95th percentile PCN-66/67 exposure are shown in Table 19. The dietary exposure estimations in high and frequent consumers varied from a minimum LB of 0.91 pg/kg bw day in adults to a maximum UB of 11.1 pg/kg bw day in toddlers.

The PCN-66/67 exposure in high and frequent consumers of fish meat was higher in children age groups than for adult population groups. This is explained by the higher food consumption of children in relation to their body weight.

TABLE 19 Minimum, median and maximum of the 95th percentile dietary exposure to PCN-66/67 among fish meat consumers only by age class (pg/kg bw day).

	Minimum		Median		Maximum	
Age group	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
95th percentile dietary exposure in total population (pg/kg bw per day)						
Infants[a]	1.62	1.62	5.16	5.22	8.63	8.63
Toddlers[a]	2.70	2.71	6.34	6.35	11.1	11.1
Other children	2.56	2.56	4.26	4.27	6.77	6.78
Adolescents[a]	1.51	1.53	2.45	2.46	3.88	3.89
Adults	0.91	0.91	2.09	2.09	3.35	3.35
Elderly	1.02	1.03	2.03	2.04	2.77	2.77
Very elderly[a]	1.58	1.58	2.03	2.03	2.22	2.22

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

Note: The values are rounded to two decimals.

[a]The 95th percentile estimates obtained on dietary surveys/age groups with fewer than 60 observations may not be statistically robust (EFSA, 2011c) and are therefore not included in this table.

The exposure to PCN-66/67 in high and frequent consumers is approximately twofold higher in comparison to total population. However, this finding should be considered as indicative since different assumptions were taken into account; for the exposure assessment of the total population, the PCN-66/67 mean concentrations for diadromous fish and marine fish were considered separately, while in the specific scenario of high and frequent fish meat consumers this could not be possible due to limited number of consumers assessed at 95th percentile. Therefore, the mean concentration level of PCN-66/67 was assigned to all fish meat food categories.

3.3.3 | Exposure of infants through breastfeeding

The occurrence data ($n = 7$) were taken from European pooled milk samples that were collected and analysed as part of the WHO/UNEP study (Tschiggfrei et al., 2023) in 2016 (one sample) and 2019 (six samples) (see Section 2.6.1). The individual concentrations were expressed on fat basis with the fat content ranging from 3.4% to 5.1%. For the purpose of exposure assessment, all data were converted to whole weight basis.

Table 20 shows the minimum, mean, median and maximum concentrations of PCN-66/67 and the mixture of PCN congeners (for the list of congeners as grouped within this scenario see above; Section 3.3.1) in the pooled human milk samples. There was a negligible difference between LB and UB values. As a conservative approach the concentrations are given as UB values.

TABLE 20 Occurrence levels (ng/kg) in pooled human milk samples from European countries collected and analysed in 2016 and 2019 within the WHO/UNEP field study (Tschiggfrei et al., 2023).

	PCN-66/67		Mixture scenario	
	Fat basis	**Whole weight**	**Fat basis**	**Whole weight**
N	7		7	
Minimum	30.1	1.02	32.8	1.11
Mean	41.7	1.67	49.7	1.99
Median	45.8	1.73	53.8	2.06
Maximum	49.1	2.33	65.9	2.84

Abbreviation: *N*, number of samples.

Table 21 shows the minimum, median and maximum exposure levels for the breastfed infants consuming average and high amounts of human milk. The exposure scenario based on average human milk consumption and the median concentrations of the PCN-66/67 and the mixture of the PCN congeners would result in an exposure of 226 pg/kg bw per day and of 270 pg/kg bw per day, respectively. For infants with high human milk consumption, the exposures would be around 50% higher, i.e. median exposure estimates of 340 pg/kg bw per day for the PCN-66/67 and of 405 pg/kg bw per day for the mixture scenario.

Overall, the exposure estimates between calculated for the mixture scenario are approximately 20% higher than the exposure estimates obtained for the individual PCN-66/67 that indicates a major contribution of the PCN-66/67 to the overall exposure of the mixture of the selected PCN congeners.

TABLE 21 Exposure (pg/kg bw per day) for breastfed infants to PCN-66/67 and the mixture of congeners based on the occurrence levels in pooled human milk from Europe collected and analysed in 2016 and 2019.

Exposure estimates (pg/kg bw per day, average consumption)		
	PCN-66/67	**Mixture scenario**
Minimum	134	146
Median	226	270
Maximum	306	372
Exposure estimates (pg/kg bw per day, high consumption)		
	PCN-66/67	**Mixture scenario**
Minimum	201	220
Median	340	405
Maximum	459	559

No data was available for infant formula, and only one sample on follow-on formula was available in the database. Within this sample the concentrations were quantified at very low levels only for PCN-64/68, PCN-66/67 and PCN-73. It was not possible to make an exposure estimate based on these data.

3.3.4 | Non-dietary sources of exposure

As with other POPs, the CONTAM Panel considered that exposure via dust is likely to be an important source of exposure to PCNs after the diet, especially for infants and toddlers who have high hand to mouth contact and relatively low body weight.

As stated in Section 1.3.3, there is very little information reported in the literature about PCNs in dust. A study on exposure of PCNs to Pakistani measured 39 PCN congeners, and the sum of these congeners resulted in concentrations in dust samples from 0.25 to 697 ng/g, respectively. Predominant PCN congeners in indoor air and dust were tri- and tetraCNs (Waheed et al., 2020).

An investigation into dioxin-like activity in Japanese indoor dusts found concentrations ranging from 1.7 to 26 ng/g in house dust and 1.6–49 ng/g in office dust (Suzuki et al., 2010).

No data on PCNs in dust and no data on concentrations in dust from Europe were identified. Given that only two studies reporting concentrations above were identified, it was not possible to make a robust exposure estimate for dust.

3.3.5 | Current dietary exposure assessment for animals

The dietary exposure assessment was assessed considering feed materials and model diets, as described in Section 2.5. In addition, for ruminants and horses, forages were added to allow the daily ratio at the recommended proportion for each ruminant category. Due to the lack of availability of occurrence of PCN in compound feeds (complete and/or complementary), a second exposure scenario considering compound feeds could not be elaborated.

The exposure assessment was performed making use of the flexibility in the composition of the default diets as explained in Section 2.2, and using substitutions of feed materials within the groups detailed in Appendix C.4. In particular, Linseed was used in lieu of rapeseed, rapeseed meal, sunflower meal and soybean meal; while grass meal was used in lieu of Lucerne meal/Alfalfa, as indicated in Appendix C.2. These substitutions were made to maximise the use of available feed occurrence data, within the same feed group.

Suitable occurrence data were only available in feed materials, and not in forages, therefore for ruminants and horses only, these were used in the exposure assessment, contributing to the diet at the 'concentrate: forage' ratio detailed in Appendix C.

The CONTAM Panel noted that the occurrence data available in the EFSA database relate to ingredients which constitute up to a maximum of 27% of the model diet (e.g. 27% for salmon, 26% for piglets (weaned), 25% for dairy cows, 20% for chickens for fattening, 4% for dog (diet with meat), etc.). On the remainder diet fractions, no information was available on PCN concentrations.

3.3.5.1 | *Modelled concentrations in feed*

The modelled concentrations derived using the above approach, expressed in ng/kg feed (88% DM), are included in Table 22 below and tabulated in Appendix C.

TABLE 22 PCN[c] concentration present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds, expressed in ng/kg complete feed (88% DM).

	Modelled concentration ng/kg complete feed[a]			
	Mean		High	
	LB	UB	LB	UB
Pigs				
Piglets (weaned)	0.002	0.005	0.002	0.005
Pigs for fattening	0.001	0.003	0.002	0.004
Sows, lactating	0.002	0.004	0.003	0.005
Poultry				
Chickens for fattening	0.003	0.006	0.008	0.010
Laying hens	0.008	0.017	0.020	0.025
Turkeys for fattening	0.022	0.047	0.063	0.073
Ducks for fattening	0.019	0.041	0.054	0.064
Fish				
Salmonids	0.006	0.012	0.019	0.021
Rabbits				
Rabbits for fattening	0.040	0.085	0.114	0.133
Cattle				
Dairy cows	0.002	0.003	0.001	0.004
Cattle for fattening	0.000	0.001	0.001	0.001
Veal calves	n/a[b]	n/a	n/a	n/a
Small ruminants				
Dairy sheep	0.001	0.001	0.001	0.001
Dairy goat	0.002	0.004	0.002	0.005
Lambs for fattening	0.001	0.002	0.001	0.002
Kids for fattening	0.001	0.002	0.001	0.002
Horses				
All categories	n/a	n/a	n/a	n/a
Dogs				
Dog with meat	0.001	0.003	0.005	0.005
Dogs vegetarian diet	0.002	0.005	0.008	0.009
Cats	< 0.001	0.001	< 0.001	0.001

[a]Expressed as 88% DM.

[b]Exposure could not be estimated for horses and veal calves due to the lack of suitable occurrence data.

[c]Sum of hexaCNs.

Ruminants

Dietary exposure to PCNs (sum of hexaCNs) in ruminants was estimated, for dairy cows, at 0.003 (UB) ng/kg feed using the mean occurrence scenario and 0.004 (UB) ng/kg feed in the high occurrence scenario. For cattle for fattening, it varied between 0.001 ng/kg feed (UB) mean and 0.001 ng/kg feed using the high UB occurrence scenario. Similarly, for dairy sheep, the exposure using both the mean and high UB occurrence scenario was 0.001 ng/kg feed, for and lambs and kids for fattening, the exposure using both the mean and high UB occurrence scenario was 0.002 ng/kg feed. For diary goats, the exposure varied between 0.004 ng/kg feed (UB) mean scenario and 0.005 ng/kg feed using the high UB occurrence scenario.

For veal calf, it was not possible to estimate exposure to PCN (sum of hexaCNs) as no samples of milk replacer were reported for this animal category.

Horses

It was not possible to estimated exposure to PCNs (sum of hexaCNs) for horses as hexaCN concentrations were not reported for the feed materials of the model diet for this animal species.

Pigs

Dietary exposure to PCNs (sum of hexaCNs) in weaned piglets was estimated at 0.005 ng/kg feed for the UB mean and high exposure scenario.

PCN (sum of hexaCNs) dietary exposure in pigs for fattening varied between 0.003 ng/kg feed (UB) in the mean occurrence scenario and 0.004 ng/kg feed (UB) in the high exposure scenario, while for lactating sows, it varied between 0.004 and 0.005 ng/kg feed.

Poultry

Dietary exposure to PCNs (sum of hexaCNs) in chickens for fattening varies between 0.006 and 0.010 ng/kg feed using the mean (UB) and high (UB) occurrence scenario, respectively.

PCN (sum of hexaCNs) dietary exposure in laying hens varies between 0.017 and 0.025 ng/kg feed in the mean and high occurrence scenario. For turkeys and ducks for fattening, the exposure from the mean and high (UB) scenarios varies between 0.047 and 0.073 ng/kg feed and between 0.041 and 0.064 ng/kg feed, respectively.

Rabbits

Dietary exposure to PCNs (sum of hexaCNs) in rabbits for fattening varies between 0.085 ng/kg feed in the mean (UB) occurrence scenario and 0.133 ng/kg feed in the high (UB) occurrence scenario.

Fish

In salmon, dietary exposure to PCNs (sum of hexaCNs) varies between 0.012 ng/kg feed in the (UB) mean occurrence scenario and 0.021 ng/kg feed in the (UB) high occurrence scenario.

Non-food-producing animals

Dietary exposure to PCNs (sum of hexaCNs) in cats was estimated at 0.001 ng/kg feed using both the mean (UB) and high (UB) occurrence scenario.

In dogs (meat diet), dietary exposure to PCN (sum of hexaCNs) varies between 0.005 ng/kg feed in the (UB) mean occurrence scenario and 0.009 ng/kg feed in the (UB) high occurrence scenario. For the dog with vegetarian diet, the exposure varies between 0.003 ng/kg feed and 0.005 ng/kg feed in the (UB) mean occurrence scenario and the (UB) high occurrence scenario, respectively.

3.3.5.2 | *Estimation of animal dietary exposure*

The estimated dietary exposure levels, obtained from the modelled concentrations and the default body weight values for each animal species (see Appendix C.1), are reported in Appendix C.4. Of note that the animal exposure levels are expressed in pg/kg bw per day to allow better visualisation of the figures.

3.4 | Risk characterisation

3.4.1 | Human health risk assessment

The risk characterisation could only be performed for hexaCNs because a representative repeated dose toxicity study was only available for a mixture of hexaCNs (Section 3.1.2).

The CONTAM Panel concluded that due to the limitations and uncertainties in the current database on PCNs, the derivation of a health-based guidance value (HBGV) was not appropriate. Instead, the Panel applied a margin of exposure (MOE) approach to assess a possible health concern.

The CONTAM Panel considered that MOEs ≥ 2000 are sufficient to conclude that the current dietary exposure to hexaCNs does not raise a health concern (Section 3.1.7.3).

The MOEs calculated for dietary exposures across age groups for the high dietary exposure of the hexaCNs based on mixture scenario A (Section 3.3.1) are presented in Table 23. MOEs for the different age categories were calculated using the Reference Point of 0.05 mg/kg bw per day.

The exposure assessment was based on the same congeners as, but in proportions different to, the composition of the test item in the toxicity study, using the occurrence data submitted to EFSA. The impact of this on the risk characterisation was taken into account in the uncertainty analysis (see Section 3.5).

The MOEs in Table 23 ranged from 1,700,000 in toddlers to 55,000,000 in elderly (after rounding) and are far above the minimal MOE of 2000. Thus, the current high dietary exposure to hexaCNs does not raise a health concern for these population groups.

For breast-fed infants, the estimated MOEs for the highest consumption of breast milk ranged from 89,000 to 230,000 (after rounding) and are far above the minimal MOE of 2000. Thus, the current highest consumption of breast milk does not

raise a health concern. No LB and UB estimates were done for the mixture of PCNs from human milk because of the very small sample size (7 pooled samples in total). Because the samples are pooled they do not take into account the variation between individuals. This has been taken into account in the uncertainty analysis (Section 3.5).

TABLE 23 Margins of Exposure (MOEs) across age groups for the high dietary exposure of the hexaCN mixture based on Scenario A.

	Minimum		Median		Maximum	
Age group	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
Infants	18,000,000	15,000,000	6,900,000	3,400,000	3,500,000	2,400,000
Toddlers	13,000,000	6,800,000	3,000,000	2,700,000	1,900,000	1,700,000
Other children	23,000,000	13,000,000	3,800,000	3,500,000	2,200,000	2,000,000
Adolescents	42,000,000	26,000,000	8,300,000	7,200,000	3,800,000	3,600,000
Adults	44,000,000	29,000,000	11,000,000	9,500,000	6,300,000	5,800,000
Elderly	55,000,000	35,000,000	10,000,000	9,000,000	7,800,000	7,100,000
Very elderly	46,000,000	32,000,000	11,000,000	10,000,000	6,500,000	6,000,000

A risk characterisation could not be performed for other PCNs than the hexaCNs. In view of the very large MOEs for the hexaCN mixture presented in Table 23 and that available data indicate that PCN-66/67 and PCN-70 are the most potent congeners (accounting for around 83% of the total PCN content in the mixture), the Panel considers that additional experimental studies are not needed for other PCNs because it is judged that they would give a similar risk characterisation outcome as for the hexaCNs.

3.4.2 | Food-producing and non-food-producing animals health risk assessment

With the exception for PCN-52 in fish, it was not possible to carry out any hazard characterisation because toxicity studies have only been carried out with mixtures of PCNs. For fish it was possible to identify a NOAEL of 32.8 mg PCN-52/kg feed (dry weight) (the highest dose tested). However, risk characterisation for PCN-52 in fish is not possible due to a lack of exposure data. Thus, it was not possible to carry out risk characterisation of PCNs for any food-producing animal.

3.5 | Uncertainty analysis

The aim of the uncertainty analysis was to identify and quantify uncertainties affecting the risk assessment for PCNs in food and assess the overall certainty of the main conclusions, as recommended in the EFSA Guidance on uncertainty analysis (EFSA Scientific Committee, 2018a). As the 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, the uncertainty analysis followed the approach for a standardised assessment (Section 3 of the Guidance).

The combined impact of the identified uncertainties was quantified in a tiered approach, starting with simpler methods (using probability bounds, see Section 3.5.4.1 below). This proved sufficient to reach a clear conclusion for the present assessment, so more refined methods were not needed.

The protocol and detailed results of expert knowledge elicitations (EKEs) that were performed to quantify uncertainty are documented in Annex D.

3.5.1 | Identification of sources of uncertainty

Sources of uncertainty related to the exposure assessment, hazard assessment and risk characterisation for PCNs were listed and discussed (see Appendix F). It was then considered which of these were non-standard[21] sources of uncertainty and which would have most impact on the outcome of the exposure and hazard assessments. Standard sources of uncertainty[22] were not considered further in the uncertainty analysis, as explained in Section 3 of the EFSA Guidance on uncertainty analysis (EFSA Scientific Committee, 2018a).

[21]Non-standard uncertainties are defined by EFSA (2018) as "Any deviations from a standardised procedure or standardised assessment element that lead to uncertainty regarding the result of the procedure. For example, studies that deviate from the standard guidelines or are poorly reported, cases where there is doubt about the applicability of default values, or the use of non-standard or 'higher tier' studies that are not part of the standard procedure."

[22]Standard uncertainties are defined by EFSA (2018) as "Sources of uncertainty that are considered (implicitly or explicitly) to be addressed by the provisions of a standardised procedure or standardised assessment element. For example, uncertainties due to within and between species differences in toxicity are often addressed by a default factor of 100 in chemical risk assessment."

3.5.2 | Exposure assessment

Uncertainties affecting the exposure assessment are described and prioritised in Table F.2 (Appendix F). Important non-standard sources of uncertainty included limited or lack of occurrence data for many important food categories (e.g. various fish species, types of meat, etc.); occurrence data were available only from France, Germany and Ireland which do not geographically reflect the whole EU; the impact of the contribution of one egg sample with high PCN69 occurrence; and use of a default value rather than data for consumption of human milk by breast-fed infants (see Table F.2 for full list).

Uncertainty about the impact of all the identified non-standard uncertainties on the exposure assessment was quantified by expert judgement, using a semi-formal structured method of Expert Knowledge Elicitation (semi-formal EKE, Annex B.8 of EFSA Scientific Committee, 2018b). Three exposure experts provided judgements and reasoning for the following EKE question: 'What is your upper 95% limit[23] for what the "true"[24] value of the highest 95th percentile chronic dietary exposure for the mixture of PCNs would be, among all the population groups and surveys considered in the assessment, including breast-fed infants, if all the identified non–standard uncertainties were resolved (e.g. by obtaining more or better data)?' Because the estimated exposures were markedly higher for breast-fed infants than other groups (Section 3.3), the experts also made judgements on a separate question where breast-fed infants were excluded. The upper 95% limits provided by the experts were 43, 60 and 70 pg/kg bw per day for the EKE question excluding breast-fed infants and 500, 800 and 900 pg/kg bw per day for the EKE question including breast-fed infants; the EKE procedure and experts' reasoning are documented in Annex D. To take account of the scientific uncertainty implied by the differences between experts, the subsequent calculations were repeated with each judgement (Section 3.5.4).

3.5.3 | Hazard assessment

3.5.3.1 | *Genotoxicity*

The extremely limited information on genotoxicity of PCNs did not allow the CONTAM Panel to conclude on their genotoxic potential (see Section 3.1.2.8 and Table 3). It was furthermore considered that the available data were too limited to support any useful quantification of uncertainty.[25]

3.5.3.2 | *Other toxic effects*

Uncertainties affecting the assessment of other toxic effects are described and prioritised in Table F.1. Important non-standard sources of uncertainty included lack of studies on chronic toxicity, reproductive toxicity, carcinogenicity, developmental neurotoxicity and immunotoxicity. The critical study used for the derivation of the Reference Point was conducted only with females, and did not include a number of standard parameters. Furthermore, there was a lack of sufficient data on bioaccumulation to support a body burden approach to risk characterisation (see Table F.1 for full list).

The CONTAM Panel decided to apply a default factor of 2 to address extrapolation from the subchronic critical toxicity study to chronic exposure duration, following EFSA's guidance on default factors (EFSA Scientific Committee, 2012). The default factor of 2 is a standardised assessment element (EFSA Scientific Committee, 2018a) addressing the uncertainty of predicting a chronic rat NOAEL based on a NOAEL from a subchronic rat study. This element of uncertainty was therefore not included in the quantification of the non-standard uncertainties.

Uncertainty about the impact of all the identified non-standard uncertainties on the hazard assessment was quantified by expert judgement, using a semi-formal structured method of Expert Knowledge Elicitation (semi-formal EKE, Annex B.8 of EFSA Scientific Committee, 2018b). In view of the important uncertainties noted above, it was decided to frame the EKE question in terms of an additional uncertainty factor for limitations in the database (EFSA Guidance on default factors; EFSA Scientific Committee, 2012), as follows: 'What additional uncertainty factor should be applied to the proposed Reference Point for the mixture tested by Klimczak et al. (2018) for you to judge with at least 90% probability that the Reference Point divided by the uncertainty factor would be protective, i.e. equal to or lower than the 'true Reference Point'[26] for this mixture?' Three toxicology experts provided judgements and reasoning on this question. Their judgements comprised additional uncertainty factors of 2.5, 5 and 10, respectively; the EKE procedure and experts' reasoning are documented in Annex D.

To take account of the scientific uncertainty implied by the different judgements made by the three experts, the subsequent calculations for the uncertainty analysis were repeated with each judgement (Section 3.5.4). In addition, in order to

[23]Upper 95% limit was defined as an upper estimate such that the expert judged with at least 95% probability that the true value would be lower if all the non-standard uncertainties were resolved. This is a probability bound, i.e. an approximate probability for a specified range of values (Section 12.2 in EFSA (2018)).

[24]'True' refers here to what the exposure estimates would be if all the identified non-standard uncertainties were resolved, e.g. by obtaining perfect data on consumption and occurrence.

[25]This implies a judgement that quantification of uncertainty would result in a probability of PCNs being genotoxic that was not close enough to 0% or 100% to be useful for risk management.

[26]'True Reference Point' was defined as the Reference Point for human risk characterisation that would be obtained if all the hazard uncertainties identified by the WG were resolved, e.g. by obtaining perfect data.

provide a single value for the additional uncertainty factor to be applied in risk characterisation (Section 3.4), the experts agreed on a consensus additional uncertainty factor of 10, while noting that two experts judged that a lower value of 2.5 or 5 could be sufficient.

3.5.4 | Risk characterisation

3.5.4.1 | *Probability bounds analysis*

The elicited judgements quantifying uncertainty for the exposure and hazard assessment were combined by probability bounds analysis to quantify the impact of these uncertainties on the MOE. This method for combining uncertain quantities is described in section 14.1 of EFSA Scientific Committee (2018a) and in more detail in Annex B.13 of EFSA Scientific Committee (2018b). The advantage of this method is that it requires only a probability bound for each quantity: It does not require elicitation of complete probability distributions and allows for any degree of dependence of uncertainty between the quantities. It is conservative in the sense that the lower and upper probability bounds are much wider than the corresponding probability interval that would be obtained by a more refined probabilistic assessment, but avoids the need for refined methods when the conservative estimates are sufficient for decision-making (EFSA Scientific Committee, 2018b, p. 180).

The results of the probability bounds calculations are shown in Table 24. There are multiple results because the calculation was repeated with every combination of the judgements provided by the three exposure experts and the three toxicology experts. This was done to enable the uncertainty analysis to take account of the differences between experts, which are part of the overall scientific uncertainty. The calculations were also repeated with the experts' consensus value for the additional uncertainty factor, which was 10, for comparison.

The calculations were performed as follows:

- The WG's Reference Point for effects on platelet counts (0.05 mg/kg per day, converted to pg/kg per day) was divided by the exposure estimate of each exposure expert (for all population groups including breastfed infants, in pg/kg bw per day), producing an MOE for each expert.
- The minimum MOE value that would not raise a health concern was calculated as the default factor of 10×10 (for intra- and interspecies differences) multiplied by the default factor of 2 (for extrapolation from subchronic to chronic exposure) and by the additional uncertainty factor. This was done four times: once for each toxicology expert (as they gave 3 different estimates for the additional uncertainty factor) and once for the experts' consensus value for the additional uncertainty factor (10).
- The MOE for each exposure expert was compared with the minimum that would not raise a health concern for each toxicology expert and the consensus uncertainty factor. This resulted in 12 comparisons, shown in Table 24: 9 for each combination of the exposure and toxicology experts and 3 for each combination of the exposure experts and the consensus uncertainty factor.
- For each of the 12 combinations, the ratio of the calculated MOE to the minimum MOE that would not raise a health concern was calculated. The ratio is shown in brackets alongside each MOE in Table 24.
- Where the MOE is equal to or above the minimum MOE that would not raise a health concern, i.e. the ratio is ≥ 1, the MOE is highlighted in green in Table 24.
- Probability bounds for the ratios of the calculated MOEs to the minimum MOE that would not raise a health concern were calculated using the second of the two simplest methods for probability bounds analysis, described on page 177 of EFSA Scientific Committee (2018b). The exposure estimates were upper ≥ 95% probability bounds[27] and the hazard judgements were upper ≥ 90% probability bounds. Therefore, the ratios of the calculated MOE to the minimum MOE that would not raise a health concern in Table 24 are lower ≥ 85% bounds, i.e. there is ≥ 85% probability that each ratio would be *higher* if the exposure and hazard uncertainties were resolved.[28]

The MOEs for all 12 combinations in Table 24 exceed the corresponding minimum MOE that would not raise a health concern. Therefore, every one of the 12 combinations indicates with at least 85% probability that the MOEs would not raise a health concern if all of the identified non-standard uncertainties affecting the exposure and hazard assessments were resolved.

[27]A probability bound is a probability or approximate probability for a specified range of values; an approximate probability is a range or bound for a probability (EFSA Scientific Committee, 2018a). For example, an upper ≥ 95% probability bound for the highest P95 exposure is an estimate such that the expert judges there is at least 95% probability that the true value is lower.

[28]The simpler method is applicable because calculation of the ratio can be arranged to be monotonic increasing with respect to each input (see page 177 of EFSA Scientific Committee, 2018b). The reciprocal of the ratio of the calculated MOE to the minimum MOE that would not raise a health concern increases monotonically with the additional UF and the estimated exposure. Consequently, the 'lost' probability for the *upper* bound for the reciprocal of the ratio (which is the *lower* bound for the ratio) is the sum of the lost probabilities for the upper bounds for the additional UF and estimated exposure. The additional UF and exposure estimate are upper ≥ 90% and ≥ 95% bounds, respectively, so the lost probability is ≤ 10% (100% minus ≥ 90%) for the additional UF and ≤ 5% (100% minus ≥ 95%) for the exposure. Summing these gives a lost probability of ≤ 15% for the lower bound of the ratio; so ratios in Table 24 are ≥ 85% lower bounds.

TABLE 24 Calculated MOEs and their ratios to the minimum MOE that would not raise a health concern.[a]

Each cell in the table shows the **calculated MOE** *followed by the* **ratio (in brackets)** *of the calculated MOE to the minimum MOE that does not raise a health concern*			**Exposure experts' judgements of highest P95 exposure (pg/kg bw per day)**			
			900	**800**	**500**	
			Exposure expert A	**Exposure expert B**	**Exposure expert C**	**Minimum MOE**
Hazard experts' judgements for additional Uncertainty factor:	**5**	**Hazard expert A**	55,556 (ratio: 56)	62,500 (ratio: 63)	100,000 (ratio: 100)	1000
	10	**Hazard expert B**	55,556 (ratio: 28)	62,500 (ratio: 31)	100,000 (ratio: 50)	2000
	2.5	**Hazard expert C**	55,556 (ratio: 111)	62,500 (ratio: 125)	100,000 (ratio: 200)	500
Consensus additional uncertainty factor:	**10**	**Hazard–consensus**	55,556 (ratio: 28)	62,500 (ratio: 31)	100,000 (ratio: 50)	2000

[a]Calculated MOEs and their ratios to the minimum MOE that would not raise a health concern for all combinations of the judgements of 3 exposure experts and 3 hazard experts, and for the consensus judgement on the hazard uncertainties. For each ratio, there is at least 85% probability that the calculated value would be equal or higher if all the non-standard uncertainties affecting the exposure and hazard assessments were resolved. Calculated MOEs and ratios are highlighted green if the calculated MOE is equal to or exceeds the corresponding minimum MOE that would not raise a health concern (ratio ≥ 1).

3.5.4.2 | *Assessment of overall uncertainty*

The results in Table 24 take account of all the non-standard uncertainties that were identified for the hazard and exposure assessment, as listed in Tables F.1, F.2 in Appendix F. However, they do not take account of the identified non-standard uncertainties affecting the risk characterisation, described and prioritised in Table F.3, which therefore need to be considered when assessing overall uncertainty for the risk characterisation. The most important of these resulted from the difference between the mixture tested in the critical study by Klimczak et al. (2018) and the proportions of the same congeners in human exposures (Table F.3).

In addition, it is necessary to take account of any additional sources of uncertainty affecting the assessment. In the present case, one source of additional uncertainty was the range of judgements between experts, as shown in Table 24.

The combined impact of all the identified uncertainties was assessed by expert judgements on the following EKE question: 'Considering the results from the MOE calculations (in Table 24), what is your % probability that, if all of the identified non-standard uncertainties affecting the risk characterisation were resolved, the mixture of PCNs considered would not raise a health concern for non-genotoxic effects for all of the population groups and surveys considered in the assessment, including breast-fed infants?'

Six experts (3 toxicology experts and 3 exposure experts) provided judgements and reasoning on this question. Their individual judgement of the required probability ranged from ≥ 85% to > 99%; individual values and reasoning are documented in Annex D together with details of the EKE procedure.

The most important non-standard source of uncertainty affecting the risk characterisation results from the difference between the mixture tested in the critical study by Klimczak et al. (2018) and the proportions of the same congeners in the dietary exposure. However, the mixture tested by Klimczak et al. (2018), contained about 80% PCN-66/67, which is considered likely to be more potent than other congeners which are present in higher proportions in the dietary exposure. The experts therefore expected that resolving this source of uncertainty would increase the calculated MOEs and ratios in Table 24. The experts considered that resolving all the other additional uncertainties could either increase or decrease the ratios, but if they decreased they would do so by less than a factor of 28 (the smallest ratio in Table 24), and probably much less.

Taking these considerations together, the experts judged that, if all the risk characterisation uncertainties and additional uncertainties were resolved, their combined impact was likely to increase the ratios shown in Table 24 but that, if they decreased, they would remain close to those in Table 24, which range between 28 and 200 (Table 24). However, there remains a ≥ 85% probability that these ratios would be higher if the uncertainties affecting the hazard and exposure assessments were resolved, based on the calculation in Section 3.5.4.1. The probability bounds that were assessed for the hazard and exposure uncertainties do not provide a more precise probability for the ratio being ≥ 1, because they specify a maximum probability for larger values of the additional uncertainty factor and highest exposure estimate than those judged by the experts, but did not assess what those larger values could be. However, considering the large magnitude of the calculated ratios (28–200), the experts judged that the lower bound for their probability of the ratio being ≥ 1 would be much higher than 85%.

After discussing these considerations, the six experts agreed on a consensus judgement of ≥ 99% probability that dietary exposure to the hexaCNs for all the surveys and population groups considered, including breast-fed infants, would not raise a health concern if all the non-standard uncertainties were resolved, while noting that one expert judged that > 95% probability would be a more appropriate consensus. This outcome is also consistent with the very high MOEs reported in Section 3.4.1.

3.5.5 | Summary of the uncertainty analysis

The extremely limited information on genotoxicity of PCNs did not allow the CONTAM Panel to conclude on their genotoxic potential or make any useful quantification of its uncertainty.

The risk assessment for other effects was affected by considerable non-standard uncertainties, including major limitations in the available database for hazard assessment and limited or lack of occurrence data for many important food categories.

The impacts of the non-standard uncertainties affecting the exposure and hazard assessments were quantified using expert knowledge elicitation and combined by probability bounds analysis. Additional uncertainties affecting risk characterisation were taken into account when assessing the overall uncertainty. It was concluded that, even though some of the uncertainties were substantial, the MOEs were so large that they would still not raise a health concern when all the uncertainties were taken into account.

Based on the results of the uncertainty analysis, the CONTAM Panel concluded with at least 99% certainty[29] that the current dietary exposure to the hexaCNs would not raise a health concern for any of the population groups and surveys considered in the assessment, including breast-fed infants.

4 | CONCLUSIONS

Polychlorinated naphthalenes (PCNs) are members of the class of chlorinated polycyclic aromatic hydrocarbons (Cl-PAHs), based on the naphthalene ring system, where one or more hydrogen atoms have been replaced by chlorine. Most PCNs that were commercially produced exist as mixtures of congeners with melting points ranging from below room temperature to around 200°C.

PCN technical mixtures were used as dielectrics, lubricants, electric cable insulation, preservatives of wood, paper and fabric, cutting and grinding fluids and plasticisers. They were manufactured in several countries between around 1910 and 1980, when the major producers in European and North American countries and Japan voluntarily stopped production.

PCNs are ubiquitous in the environment and are listed as POPs under the Stockholm Convention.

Comparisons between studies should be made with care due to the differences in, and reliability of, methods used for the determination of the PCNs, the individual PCNs measured and the sampling and geographical origin of the samples.

4.1 | Hazard identification and characterisation

4.1.1 | Toxicokinetics

- There is no information on the toxicokinetics of PCNs following oral exposure in humans.
- Limited studies in experimental animals indicated that PCN congeners from the mono- to hexa- homologue groups are well absorbed when administered orally in a range of doses, both as individual congeners and as mixtures including commercial formulations. The absorption of the two heptaCNs and the octaCN was less efficient.
- Absorbed PCNs were readily distributed to organs and tissues.
- Maternal transfer to amniotic fluid and placenta has been reported and PCNs have also been detected in fetal tissues.
- PCNs showed increasing resistance to enzymatic oxidation due to steric hindrance and increase in the number of substituted chlorines. The hexaCNs showed the slowest turnover.
- Mono- to tetraCNs were largely and readily metabolised and excreted in the urine in form of hydroxylated PCNs (chloronaphthols) and phase II metabolites. The higher chlorinated PCNs were metabolised to a lesser extent and excreted in faeces both as the parent compounds and as metabolites.

4.1.2 | Adverse effects in experimental animals

- All oral repeated dose toxicity studies were performed in rats.
- Most studies were performed with hexaCNs, predominantly with a hexaCN mixture (hexaCNs at 94.1%, composition: PCN-66/67 at 81.2%, PCN-64/68 at 5.4%, PCN-69 at 2.2%, PCN-71/72 at < 0.1%, PCN-63 at 3.2%, PCN-65 at 0.6% and PCN-70 at 1.4%, and PCN-73 (a heptaCN) at 5.9%). Below, this mixture is referred to as 'hexaCN mixture'.
- The haematological system, liver and thymus were identified as targets. Developmental effects were also reported.
- In a 90-day study with the 'hexaCN mixture', liver effects and changes in red blood cell parameters were reported at the highest dose. A decreased platelet (PLT) count was reported at the mid and high dose. A NOAEL of 0.03 mg/kg bw per day was identified based on the decreased PLT count.

[29]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).

- Developmental effects included increased intrauterine mortality of embryos and fetuses ('hexaCN mixture', PCN mixture), decreased fetal body weight and length ('hexaCN mixture', PCN mixture) and delayed ossification (PCN-43, 'hexaCN mixture', PCN mixture).
- A subacute neurotoxicity of the 'hexaCN mixture' indicated that behavioural performance could be impaired.
- No reproductive toxicity or chronic toxicity/carcinogenicity studies were available for the hazard identification and characterisation.
- Four PCN congeners (PCN-1, PCN-2, PCN-27 and PCN-75) tested in four strains of Salmonella Typhimurium with and without metabolic activation did not show a mutagenic potential in vitro. The 'hexaCN mixture' investigated in an in vivo micronucleus bone marrow test after oral administration for 90 days did not induce clastogenic effects. The extremely limited information on genotoxicity of PCNs does not allow to conclude on their genotoxic potential.

4.1.3 | Observations in humans

- None of the retrieved human studies were considered relevant for this mandate.

4.1.4 | Adverse effects in food-producing animals

- The vast majority of studies on PCNs carried out in food-producing animals have used technical mixtures (e.g. Halowax).
- With the exception for PCN-52 in fish, there are no studies in food-producing animals allowing Reference Points to be identified for single congeners.
- Toxicity to PCNs was first observed in cattle and originally named condition 'X disease'. It presented with symptoms including severe vitamin A deficiency, hyperkeratosis, papillary proliferations of the mucosa of the mouth and oesophagus and decrease of semen quality.
- PCN toxicity presents differently in sheep compared with cattle and was primarily not associated with vitamin A deficiency and hyperkeratosis. Early signs of toxicity were excess nasal discharge, weakness, weight loss and loss of appetite. Gross pathology included liver damage, gastrointestinal haemorrhage, as well as pleural and/or peritoneal fluid. Histological examination revealed non-specific effects in multiple organs.
- Oral PCN exposure in pigs at lethal doses caused a decrease in plasma vitamin A concentration, but this returned to normal range prior to death following cessation of exposure. Gross lesions included liver damage and thickening of the intestinal wall. Histological examination revealed non-specific effects in multiple organs. Hyperkeratosis is not observed in pigs exposed to PCNs.
- Studies in turkeys and chickens showed dose-dependent effects on survival as well as a range of performance parameters of importance for food-producing animals. Necroscopy revealed enlarged fibrous liver, fluid in the body cavity and pericardial oedema. Plasma uric acid concentration was significantly increased and haematocrit significantly decreased in exposed animals. PCNs affect serum vitamin A concentrations in poultry, but there is no obvious association with adversity.
- The few studies in fish carried out with different dietary exposures to PCNs showed no adverse effects at any of the doses tested. It was possible to identify a NOAEL of 32.8 mg PCN-52/kg feed (dry weight) (the highest dose tested) but not for other congeners.
- There are no studies in other food-producing animals that allow identification of Reference Points for single PCN congeners.

4.1.5 | Adverse effects in non-food-producing animals

- There are no studies allowing hazard identification and characterisation of PCNs in non-food-producing animals.

4.1.6 | Mode of action

- Many PCNs are weak AHR agonists (show dioxin-like activity) with relative potencies (mostly derived from reporter gene assays) ranging from approximately 10^{-7} to 10^{-3} compared with TCDD.
- Among PCN congeners, PCN-70 (1,2,3,6,7,8-hexaCN) and PCN-66 (1,2,3,4,6,7-hexaCN) are the most potent AHR agonists.
- PCNs may also activate the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) in liver.
- Changes on haematological parameters are considered as being relevant for humans, but data are insufficient to conclude on a species-specific MoA.
- MoA data are lacking to explain observed effects on fetal development.
- MoA data are lacking to explain observed effects on the nervous system.

4.1.7 | Critical endpoints and dose–response analysis for human risk assessment

- The considerable decrease in the PLT count reported at the mid and high dose (magnitude of changes –28%, –48%, respectively) in the 90-day oral study performed with the 'hexaCN mixture' in rats was considered as the critical effect.
- Benchmark dose (BMD) modelling on the decreased PLT count with a BMR of 20% resulted in a $BMDL_{20}$ of 0.05 mg/kg bw per day for the 'hexaCN mixture'.

4.1.8 | Critical endpoints and dose–response analysis for animal risk assessment

- For fish, it was possible to identify a NOAEL of 32.8 mg PCN-52/kg feed (dry weight).
- There are no studies in other food-producing animals or in non-food-producing animals that allow identification of Reference Points for single PCN congeners or for mixtures of congeners with the same number of substitutions (e.g. hexaCN mixtures).

4.2 | Occurrence data

4.2.1 | Occurrence in food

- A total of 9111 PCNs analytical results (371 samples) generated mostly by the GC-based methods in food analysed for 71 PCN congeners fulfilled the quality criteria applied and were considered in the assessment. Out of these, 2317 hexaCNs analytical results analysed in 371 samples on food were available in the final cleaned data set.
- The left-censored data (LCD) accounted for 47% of the occurrence values. All LCD provided information on LOQ and both LOQ and LOD were provided for 93% of LCD.
- Among the food categories in which hexaCNs contamination could be expected, the highest percentage of quantified data was found in food categories 'Eggs and egg products', 'Fish, seafood, amphibians, reptiles and invertebrates', 'Milk and dairy products' and 'Meat and meat products'. The highest mean concentration was reported for 'Whole eggs' ranging from 0.002 ng/kg for PCN-70 at the LB to 5.18 ng/kg reported for PCN-69 at the UB, followed by 'Diadromous fish' ranging from 0.001 ng/kg for PCN-70 at the LB to 1.51 ng/kg reported for PCN-71/72 at the UB.
- For infant formula only one sample reporting hexaCNs concentration was submitted to EFSA with the results being below LOQ for almost all congeners. Occurrence data on hexaCNs in European human milk were taken from pooled samples that were collected and analysed as part of the WHO/UNEP field studies in 2016 and 2019. The average levels ranged between <LOQ (PCN-65 and PCN-70) and 49.1 ng/kg lipid (PCN-66/67).

4.2.2 | Occurrence in feed

- A total of 1467 PCNs analytical results (31 samples) in feed analysed for 70 PCN congeners fulfilled the quality criteria applied and were considered in the assessment. Out of these, 217 hexaCNs analytical results analysed in 31 samples on feed were available in the final cleaned data set.
- The hexaCNs occurrence data were very limited for single (or pairs of) congeners and across feed categories, in many cases with all LCD. Therefore, the occurrence data were merged, and the CONTAM Panel opted for an animal exposure assessment based on results from the hexaCN homologue group.
- Only three feed categories, including 'Oil seeds, oil fruits and products derived thereof (feed)', 'Forages and roughage, and products derived thereof (feed)' and 'Compound feed (feed)' could be considered. Out of these, the highest percentage of quantified data was observed for 'Compound feed' and the highest mean hexaCN concentration was measured for dog pet food (LB–UB: 2.19–2.20 ng/kg).

4.2.3 | Occurrence data reported in the scientific literature

- There were no obvious differences in levels of PCNs in food and feed in Europe compared to the rest of the world.
- High levels were generally associated with identified industrial contamination sites.

4.2.4 | Food processing

- There are very few data in the scientific literature about the effects of cooking and processing on PCNs in food.

4.2.5 | Levels in humans

- There are only a few studies in the literature reporting concentrations of PCNs in human tissues and many of these have been conducted in association with contamination or occupational exposure incidents.
- Concentrations of PCNs in human milk were found to be two to three times higher in European samples when compared to other global regions in a study conducted by the WHO/UNEP. The most abundant congeners were the pairs of pentaCNs (PCN-52/60) and hexaCNs (PCN-66/67) which each accounted for about 40% of the total.

4.3 | Dietary exposure assessment

4.3.1 | Human dietary exposure assessment

- The highest mean exposure to the individual (or pairs of) hexaCN congeners and the mixture of hexaCNs across all congeners was estimated for the young age groups, with the tendency to decrease moving to the older age groups.

4.3.1.1 | *Human dietary exposure to the individual (or pairs of) congeners*

- The highest mean exposure across the European dietary surveys was estimated for PCN-69 scenario A[30] followed by PCN-66/67.
- For PCN-69 scenario A, the mean exposure ranged from 0.03 ('Adolescents' and 'Very elderly') to 5.61 pg/kg bw per day ('Toddlers') for the minimum LB and the maximum UB, respectively. The P95 exposure ranged from 0.06 ('Elderly') to 19.4 pg/kg bw per day ('Toddlers') for the minimum LB and the maximum UB, respectively.
- For PCN-66/67, the mean exposure ranged from 0.15 ('Elderly') to 2.51 pg/kg bw per day ('Infants') for the minimum LB and the maximum UB, respectively. The P95 exposure ranged from 0.45 ('Elderly') to 5.90 pg/kg bw per day ('Infants') for the minimum LB and the maximum UB, respectively.
- The food categories contributing mostly to the overall exposure varied across the hexaCNs; consumption of 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Meat and meat products' in the adult population groups, and 'Milk and dairy products', in infants contributed the most to the dietary exposure to PCN-66/67, while for other individual hexaCNs, the food category 'Eggs and egg products' together with 'Fish, seafood, amphibians, reptiles and invertebrates' made the most important contribution.

4.3.1.2 | *Human dietary exposure to the mixture of hexaCNs*

- The highest exposure levels across the European dietary surveys were estimated for toddlers in 'mixture scenario' A.[31]
- For 'mixture scenario' A, the mean exposure ranged from 0.32 ('Elderly') to 11.5 pg/kg bw per day ('Toddlers') for the minimum LB and the maximum UB, respectively. The P95 exposure ranged from 0.91 ('Elderly') to 29.8 pg/kg bw per day ('Toddlers') for the minimum LB and the maximum UB, respectively.
- The food categories 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Eggs and egg products' in the adult age groups, and 'Milk and dairy products', in infants contributed the most to the dietary exposure in the 'mixture scenario'.

4.3.1.3 | *Specific exposure scenarios*

- An exposure scenario for high and frequent consumers of fish meat, resulted in 95th percentile dietary exposure to PCN-66/67 (calculated for consumers only) being approximately twofold higher in comparison to total population with the maximum UB exposure level estimated for 'Toddlers' (11.1 pg/kg bw per day).
- An exposure scenario for breastfed infants, resulted in median daily exposure estimates for average human milk consumption of 226 pg/kg bw per day for PCN-66/67 and 270 pg/kg bw per day for the 'mixture scenario'. When considering high consumption amount of human milk, the median daily exposure was at level of 340 pg/kg bw per day for PCN-66/67 and 405 pg/kg bw per day for the 'mixture scenario'.

4.3.2 | Animal dietary exposure assessment

- Exposure was calculated for all animal species, with the exception for horses and veal calves.
- Exposure was calculated for the hexaCN homologue group using model diets and feed materials. Forages were also included for ruminants and horses. Compound feed (complete and/or complementary) could not be used due to the lack of occurrence data.

[30]Exposure scenario including one high PCN-69 concentration of 313 ng/kg measured in egg sample.

[31]Exposure scenario including one high PCN-69 concentration of 313 ng/kg measured in egg sample.

- Exposure was calculated using either a mean or a high exposure scenario. The high scenario was based on the 95th percentile or the highest reliable percentile of occurrence level, depending on the number of occurrence data.
- The animal categories with the highest modelled concentrations of hexaCNs in ng/kg complete feed (expressed in 88% DM) were rabbits for fattening, followed by turkeys and ducks for fattening, with concentrations of 0.133, 0.073 and 0.064 ng/kg feed, respectively, in the high (UB) occurrence scenario.
- The CONTAM Panel noted that the occurrence data available in the EFSA's database relate to ingredients which constitute up to a maximum of 27% of the model diet, and as low as 4% for dogs (diet with meat). On the remainder diet fractions, no information was available on PCN concentrations. Therefore, the uncertainty related to the animal dietary exposure is high.

4.4 | Risk characterisation

4.4.1 | Risk characterisation for humans

- None of the retrieved human studies were considered relevant for this mandate. Therefore, human risk assessment was based on data from studies in experimental animals.
- Due to the limitations and uncertainties in the current database on PCNs, the derivation of a health-based guidance value (HBGV) was not appropriate. Instead, the CONTAM Panel applied a margin of exposure (MOE) approach to assess a possible health concern.
- The MOE takes into account interspecies differences in toxicokinetics and toxicodynamics (factor of 10), intraspecies differences in humans (factor of 10) and the shorter duration of the key study (90 days) compared to a lifetime exposure (factor of 2).
- The analysis of uncertainties related to the hazard identification and characterisation of PCNs indicated that an additional factor was warranted. Considering the results of the uncertainty analysis, the CONTAM Panel agreed that an additional factor of 10 was appropriate.
- MOEs ≥ 2000 are sufficient to conclude that the current dietary exposure to hexaCNs does not raise a health concern.
- The MOEs ranged from 1,700,000 in toddlers to 55,000,000 in elderly (after rounding) and thus, the high dietary exposure to hexaCNs does not raise a health concern.
- For breast-fed infants, the estimated MOEs for the highest consumption of breast milk ranged from 90,000 to 230,000 (after rounding) and thus, the highest consumption of breast milk does not raise a health concern.

4.4.2 | Risk characterisation for food-producing and non-food-producing animals

- It was not possible to carry out risk characterisation of PCNs for any food-producing or non-food-producing animals because no Reference Points were identified for single PCN congeners or for mixtures of congeners with the same number of substitutions (e.g. hexaCN mixtures).

4.4.3 | Uncertainty analysis

- The risk assessment was affected by considerable non-standard uncertainties, including major limitations in the available database for hazard assessment and limited or lacking occurrence data for many important food categories.
- The impacts of the non-standard uncertainties affecting the exposure and hazard assessments for hexaCNs were quantified using expert knowledge elicitation and combined by probability bounds analysis. Additional uncertainties affecting risk characterisation were taken into account when assessing the overall uncertainty. It was concluded that, even though some of the uncertainties were substantial, the MOEs were so large that they would still not raise a health concern when all the uncertainties were taken into account.
- Based on the results of the uncertainty analysis, the CONTAM Panel concluded with at least 99% certainty[32] that the current dietary exposure to the hexaCNs would not raise a health concern for any of the population groups and surveys considered in the assessment, including breast-fed infants.
- The extremely limited information on genotoxicity of PCNs did not allow the CONTAM Panel to conclude on their genotoxic potential or make any useful quantification of its uncertainty.

[32]Probabilities assessed by the uncertainty analysis are reported in conclusions as % certainty for the more probable outcome, following EFSA's guidance on communication of uncertainty (EFSA, 2019).

5 | RECOMMENDATIONS

- To enable a more robust exposure assessment, analytical methods are needed (i) with improved sensitivity to reduce the amounts of left-censored data and (ii) with improved selectivity to separate coeluting PCN congeners.
- Data providers should be encouraged to submit further occurrence data in food and feed, in particular in different fish species and in infant formulae.
- To monitor PCN occurrence in eggs and other edible products from food-producing animals raised on PCN contaminated soil or in the proximity of other PCN sources.
- When submitting PCN occurrence data to EFSA, it is recommended to provide adequate information on the feed samples analysed. This refers to reporting sufficient details on the samples analysed (e.g. target animals for the complete/complementary compound feed).
- Because the current dietary exposure does not raise a health concern for humans, there is no need for additional experimental animal studies to assess the risk in humans.
- There is a need for non-animal studies to support the assessment of adverse effects of PCNs in food-producing and non-food-producing animals.
- It is recommended that EFSA develops a harmonised guidance to allow extrapolation of data from experimental animals to food-producing and non-food-producing animals.
- PCNs are a small subset of a wider group of halogenated polycyclic aromatic hydrocarbons. There is very little information available about this wider class of compounds, and it is recommended that the risk for animal and human health related to the presence of polyhalogenated PAHs other than PCNs in feed and food is assessed. The initial approach to investigating potential risk associated with this wider group may include studies to identify which compounds should be prioritised on the basis of (i) toxicity and (ii) occurrence in food and the environment.

ABBREVIATIONS

A4H	acetanilide-4-hydroxylase
ADME	absorption, distribution, metabolism, and elimination
AGD	anogenital distance
AHH	aryl hydrocarbon hydroxylase
AHR	aryl hydrocarbon receptor
ALA	aminolaevulinic acid
ALA-D	aminolaevulinic acid dehydratase
ALA-S	aminolaevulinic acid synthase
ALT	aminotransferase
APGC-MS/MS	atmospheric pressure gas chromatography coupled with mass spectrometry/mass spectrometry
APTT	activated partial thromboplastin time
ATP	adenosine triphosphate
AUC	area under the curve
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
bw	body weight
CAR	constitutive androstane receptor
CAS	Chemical Abstracts Service
CF	complete feed
CICAD	Concise International Chemical Assessment Document
Cl	chlorine
CL	clot formation and lysis
Cl-PAH	chlorinated polycyclic aromatic hydrocarbons
CONTAM	The EFSA Panel on Contaminants in the Food Chain
CP	chlorinated paraffins
DDT	dichloro-diphenyl-trichloroethane
DM	dry matter
DR	dose–response relationship
DR-CALUX	dioxin-responsive chemically activated luciferase expression
ECD	electron capture detection
EFSA	European Food Safety Authority
EKE	expert knowledge elicitation
EPA	Environmental Protection Agency
EROD	7-ethoxyresorufin-O-deethylase
EURL	European Union Reference Laboratories
FCMF	food chain magnification factor

FEEDAP	The EFSA Panel on Additives and Products or Substances used in Animal Feed
F_{max}	Maximum clotting
FSAI	Food Safety Authority of Ireland
FSH	follicle-stimulating hormone
Fvo	initial plasma clotting velocity
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GC–HRMS	gas chromatography–high resolution mass spectrometry
GD	gestation day
GSH	glutathione
HBGV	health-based guidance value
HCB	hexachlorobenzene
HGB	haemoglobin concentration
HTC	haematocrit
i.p	intraperitoneal administration
ILO	International Labour Organization
IPCS	International Programme on Chemical Safety
K_{ow}	octanol–water partition coefficient
LB	lower bound
LCD	left-censored data
Log K_{ow}	decadic logarithm of octanol–water partition coefficient
LH	luteinizing hormone
Lmax	maximum lysis
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
Lvo	initial clot fibrinolysis velocity
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDA	malondialdehyde
Mdr3	multidrug resistance 3
ML	maximum level
MoA	mode of action
MOE	margin of exposure
MPV	mean platelet volume
MS	mass spectrometry
NCE	normochromatic erythrocytes
NCI	negative chemical ionisation
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
NW	northwest
OECD	Organisation for Economic Co-operation and Development
PB	persistence/bioaccumulation
PBDE	polybrominated diphenyl ethers
PBT	persistent, bioaccumulative and toxic
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-p-dioxins and dibenzofuran
PCDE	polychlorinated diphenyl ether
PCDF	polychlorinated dibenzofuran
PCE	polychromatic erythrocyte
PCN	polychlorinated naphthalene
PFAS	per- and polyfluoroalkyl substances
PLT	platelet
PND	postnatal day
POP	persistent organic pollutant
PT	prothrombin time
PROD	pentoxyresorufin O-dealkylation
PXR	pregnane X receptor
RBC	red blood cell count
RDW	red blood cell distribution width
RDW-SD	red blood cell distribution width – standard deviation

REPs	relative potencies
RIVM	National Institute for Public Health and the Environment
RP	reference point
RT-PCR	reverse-transferase – polymerase chain reaction
SD	standard deviation
SDH	sorbitol dehydrogenase
SEM	standard error mean
SOP	standard operating procedure
SSD	standard sample description
TAS	serum total antioxidant status
Tc	clot stabilisation time
TCDD	tetrachlorodibenzo-*p*-dioxin
TDI	tolerable daily intake
TEF	toxic equivalency factor
TEQ	toxic equivalents
Tf	plasma clotting time
TG	test guidelines
Tl	fibrinolysis time
TMF	trophic magnification factors
Tt	thrombotin time
U.S.P	United States Pharmacopoeia
UB	upper bound
UF	uncertainty factor
UK-COT	The United Kingdom Committee on Toxicity of chemicals in food, consumer products and the environment
UNECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
URO-D	uroporphyrinogen decarboxylase
VROM/DGM	Ministry of Housing, Spatial Planning, and the Environment/Directorate General for the Environment
WBC	white blood cell count
WHO	World Health Organisation
WoS	web of science
WTC	world trade centre
WW	whole weight
WWTP	wastewater treatment plant

ACKNOWLEDGEMENTS

The EFSA Panel on Contaminants in the food chain (CONTAM Panel) wishes to thank Federico Cruciani, Jordi Diaz Ferrero (Working group member until 22 August 2022), Kristine L. Witt and the hearing expert Theresa Zwickel for the support provided to this scientific output. The CONTAM Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2020-00663

PANEL MEMBERS

EFSA Panel on Contaminants in the Food Chain (CONTAM), Margherita Bignami, Laurent Bodin, James Kevin Chipman, Jesús del Mazo, Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Jean-Charles Leblanc, Carlo Stefano Nebbia, Elsa Nielsen, Evangelia Ntzani, Annette Petersen, Salomon Sand, Dieter Schrenk, Tanja Schwerdtle, Christiane Vleminckx and Heather Wallace.

REFERENCES

Åkerblom, N., Olsson, K., Berg, A. H., Andersson, P. L., Tysklind, M., Forlin, L., & Norrgren, L. (2000). Impact of polychlorinated naphthalenes (PCNs) in juvenile Baltic salmon, *Salmo salar*: Evaluation of estrogenic effects, development, and CYP1A induction. *Archives of Environmental Contamination and Toxicology*, *38*, 225–233. https://doi.org/10.1007/s002449910030

Asplund, L., Jakobsson, E., Haglund, P., & Bergman, A. (1994). 1,2,3,5,6,7-Hexachloronaphthalene and 1,2,3,4,6,7-hexachloronaphthalene – Selective retention in rat liver and appearance in wildlife. *Chemosphere*, *28*, 2075–2086. https://doi.org/10.1016/0045-6535(94)90177-5

Asplund, L., Jansson, B., Sundstrom, G., Brandt, I., & Brinkman, U. A. T. (1986). Characterization of a strongly bioaccumulating hexachloronaphthalene. *Chemosphere*, *15*, 619–628. https://doi.org/10.1016/0045-6535(86)90010-x

Barbaro, E., Zangrando, R., Kirchgeorg, T., Bazzano, A., Illuminati, S., Annibaldi, A., Rella, S., Truzzi, C., Grotti, M., Ceccarini, A., Malitesta, C., Scarponi, G., & Gambaro, A. (2016). An integrated study of the chemical composition of Antarctic aerosol to investigate natural and anthropogenic sources. *Environmental Chemistry*, *13*, 867–876. https://doi.org/10.1071/en16056

Barnett, B. D., & Bierer, B. W. (1960). Failure of chlorinated naphthalenes to produce keratoconjunctivitis in turkeys. *Avian Diseases*, *4*, 38–41. https://doi.org/10.2307/1587465

Behnisch, P. A., Hosoe, K., & Sakai, S. (2003). Brominated dioxin-like compounds: In vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. *Environment International*, *29*, 861–877. https://doi.org/10.1016/s0160-4120(03)00105-3

Belfroid, A., van der Aa, E., & Balk, F. (2005). *Addendum to the risk profile of polychlorinated Naphthalenes*. Haskoning Nederland BE Environment.

Bell, W. B. (1952). Further studies on the production of bovine hyperkeratosis by the administration of a lubricant. *Virginia Journal of Science*, *3*, 169–177.

Bell, W. B. (1953). The relative toxicity of the chlorinated naphthalenes in experimentally produced bovine hyperkeratosis (X-disease). *Veterinary Medicine*, *48*, 135–140.

Bidleman, T. F., Helm, P. A., Braune, B. M., & Gabrielsen, G. W. (2010). Polychlorinated naphthalenes in polar environments – A review. *Science of the Total Environment*, *408*, 2919–2935. https://doi.org/10.1016/j.scitotenv.2009.09.013

Blankenship, A. L., Kannan, K., Villalobos, S. A., Villeneuve, D. L., Falandysz, J., Imagawa, T., Jakobsson, E., & Giesy, J. P. (2000). Relative potencies of individual polychlorinated naphthalenes and Halowax mixtures to induce ah receptor-mediated responses. *Environmental Science & Technology*, *34*, 3153–3158. https://doi.org/10.1021/es9914339

Brock, W. E., Jones, E. W., Macvicar, R., & Pope, L. S. (1957). Chlorinated naphthalene intoxication in sheep. *American Journal of Veterinary Research*, *18*, 625–630.

Brinkman, U. A. T., & De Kok, A. (1980). Production, properties and usage. In R. D. Kimbrough (Ed.), *Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products* (Vol. *26*). Elsevier/North-Holland Biomedical Press.

Brunström, B., Engwall, M., Hjelm, K., Lindqvist, L., & Zebuhr, Y. (1995). EROD induction in cultured chick-embryo liver – A sensitive bioassay for dioxin-like environmental pollutants. *Environmental Toxicology and Chemistry*, *14*, 837–842. https://doi.org/10.1002/etc.5620140514

Budavari, S., O'Neil, M. J., Smith, A., Heckelman, P. E., & Kinneary, J. F. (1996). *The Merck index: An encyclopedia of chemicals, drugs and biologicals* (12th ed.). Merck & Co.

Burreau, S., Axelman, J., Broman, D., & Jakobsson, E. (1997). Dietary uptake in pike (*Esox lucius*) of some polychlorinated biphenyls, polychlorinated naphthalenes and polybrominated diphenyl ethers administered in natural diet. *Environmental Toxicology and Chemistry*, *16*(12), 2508–2513.

Campbell, M. A., Bandiera, S., Robertson, L., Parkinson, A., & Safe, S. (1983). Hepta-, hexa-, tetra- and dichloronaphthalene congeners as inducers of hepatic microsomal drug-metabolizing enzymes. *Toxicology*, *26*, 193–205. https://doi.org/10.1016/0300-483x(83)90081-1

Castells, P., Parera, J., Santos, F. J., & Galceran, M. T. (2008). Occurrence of polychlorinated naphthalenes, polychlorinated biphenyls and short-chain chlorinated paraffins in marine sediments from Barcelona (Spain). *Chemosphere*, *70*, 1552–1562. https://doi.org/10.1016/j.chemosphere.2007.08.034

Chen, D., Lou, X., Bao, J., Wang, Y., Fan, Y., Chen, Q., Jin, J., & Chen, L. (2023). Polychlorinated naphthalene concentrations and temporal trends in serum from the general Chinese adult population and effects of polychlorinated naphthalenes on thyroid function. *Environmental Research*, *232*, 116309. https://doi.org/10.1016/j.envres.2023.116309

Chu, I., Secours, V., & Viau, A. (1976). Metabolites of chloronaphthalene. *Chemosphere*, *6*, 439–444. https://doi.org/10.1016/0045-6535(76)90043-6

Chu, I., Secours, V., Villeneuve, D. C., & Viau, A. (1977). Metabolism and tissue distribution of (1,4,5,8-14C)-1,2-dichloronaphthalene in rats. *Bulletin of Environmental Contamination and Toxicology*, *18*, 177–183. https://doi.org/10.1007/BF01686064

Chu, I., Villeneuve, D. C., Secours, V., & Viau, A. (1977). Metabolism of chloronaphthalenes. *Journal of Agricultural and Food Chemistry*, *25*, 881–883. https://doi.org/10.1021/jf60212a004

Copenhaver, J. S., & Bell, W. B. (1954). The production of bovine hyperkeratosis (X disease) with an experimentally made pellet feed. *Veterinary Medicine*, *49*, 96–106.

Cornish, H. H., & Block, W. D. (1958). Metabolism of chlorinated naphthalenes. *Journal of Biological Chemistry*, *231*, 583–588.

Corsolini, S., Kannan, K., Imagawa, T., Focardi, S., & Giesy, J. P. (2002). Polychloronaphthalenes and other dioxin-like compounds in Arctic and Antarctic marine food webs. *Environmental Science and Technology*, *36*, 3490–3496. https://doi.org/10.1021/es025511v

Crookes, M. J., & Howe, P. D. (1993). *Environmental hazard assessment: Halogenated naphthalenes*. Building Research Establishment.

Cui, L., Liu, W., Wang, S., Gao, L., Huang, H., Xia, D., & Qiao, L. (2018). Concentrations and trophic magnification of polychlorinated naphthalenes (PCNs) in marine fish from the Bohai coastal area, China. *Environmental Pollution*, *234*, 876–884.

Dat, N. D., Chang, K. S., & Chang, M. B. (2018). Characteristics of atmospheric polychlorinated naphthalenes (PCNs) collected at different sites in northern Taiwan. *Environmental Pollution*, *237*, 186–195. https://doi.org/10.1016/j.envpol.2018.02.041

Dien, N. T., Hirai, Y., Koshiba, J., & Sakai, S. I. (2021). Factors affecting multiple persistent organic pollutant concentrations in the air above Japan: A panel data analysis. *Chemosphere*, *277*, 130356.

Domingo, J. L., Falcó, G., Llobet, J. M., Casas, C., Teixidó, A., & Müller, L. (2003). Polychlorinated naphthalenes in foods: Estimated dietary intake by the population of Catalonia, Spain. *Environmental Science & Technology*, *37*, 2332–2335. https://doi.org/10.1021/es030009b

Dong, S., Li, J., Zheng, B., Zhang, L., Zhang, S., Zou, Y., Wang, Y., Fan, M., Wang, R., Li, Y., & Wu, Y. (2022). Occurrence and source analysis of polychlorinated naphthalenes in raw cow milk. *Food Chemistry*, *381*, 132247.

Dong, S., Li, X., Wang, P., & Su, X. (2018). Polychlorinated naphthalene concentrations and distribution in feed raw materials. *Chemosphere*, *211*, 912–917. https://doi.org/10.1016/j.chemosphere.2018.08.037

Dong, S., Liu, G., Zhang, B., Gan, L., & Zheng, M. (2013). Formation of polychlorinated naphthalenes during the heating of cooking oil in the presence of high amounts of sucralose. *Food Control*, *32*, 1–5. https://doi.org/10.1016/j.foodcont.2012.11.001

Dong, S., Zhang, S., Li, T., Zou, Y., Cheng, J., & Wang, P. (2022). Occurrence of polychlorinated naphthalenes in dairy cow farms in proximity to an iron smelting plant in China: A preliminary study. *Environmental Research*, *215*, 114361.

Dougherty, R. C., Whitaker, M. J., Tang, S. Y., Bottcher, R., Keller, M., & Kuehl, D. W. (1981). Sperm density and toxic substances: A potential key to environmental health hazards. In J. D. McKinney (Ed.), *Environmental health chemistry: The chemistry of environmental agents of potential human hazards* (pp. 263–278). Ann Arbor Science Publishers.

EFSA (European Food Safety Authority). (2010a). Standard sample description for food and feed. *EFSA Journal*, *8*(1), 1457. https://doi.org/10.2903/j.efsa.2010.1457

EFSA (European Food Safety Authority). (2010b). Management of left-censored data in dietary exposure assessment of chemical substances. *EFSA Journal*, *8*(3), 1557. https://doi.org/10.2903/j.efsa.2010.1557
EFSA (European Food Safety Authority). (2011a). Report on the development of a food classification and description system for exposure assessment and guidance on its implementation and use. *EFSA Journal*, *9*(12), 2489. https://doi.org/10.2903/j.efsa.2011.2489
EFSA (European Food Safety Authority). (2011b). The food classification and description system FoodEx 2 (draft-revision 1). *EFSA Supporting Publications*, *8*(12), 215E. https://doi.org/10.2903/sp.efsa.2011.EN-215
EFSA (European Food Safety Authority). (2011c). Use of the EFSA comprehensive European food consumption database in exposure assessment. *EFSA Journal*, *9*(3), 2097. https://doi.org/10.2903/j.efsa.2011.2097
EFSA (European Food Safety Authority). (2011d). Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. *EFSA Journal*, *9*(12), 2490. https://doi.org/10.2903/j.efsa.2011.2490
EFSA (European Food Safety Authority). (2015). The food classification and description system FoodEx2 (revision 2). *EFSA supporting publication*, *12*(5), EN-804. https://doi.org/10.2903/j.efsa.2015.en-804
EFSA (European Food Safety Authority), Arcella, D., Gómez Ruiz, J. Á., Innocenti, M. L., & Roldán, R. (2017). Scientific report on human and animal dietary exposure to ergot alkaloids. *EFSA Journal*, *15*(7), 4902. https://doi.org/10.2903/j.efsa.2017.4902
EFSA (European Food Safety Authority), Arcella, D., & Gómez Ruiz, J. A. (2018). Technical report on use of cut-off values on the limits of quantification reported in datasets used to estimate dietary exposure to chemical contaminants. *EFSA Supporting Publication*, *15*(7), EN-1452. https://doi.org/10.2903/sp.efsa.2018.EN-1452
EFSA (European Food Safety Authority), Hart, A., Maxim, L., Siegrist, M., Von Goetz, N., da Cruz, C., Merten, C., Mosbach-Schulz, O., Lahaniatis, M., Smith, A., & Hardy, A. (2019). Guidance on Communication of Uncertainty in Scientific Assessments. *EFSA Journal*, *17*(1), 5520. https://doi.org/10.2903/j.efsa.2019.5520
EFSA (European Food Safety Authority), Arcella, D., Cascio, C., Dujardin, B., Gergelová, P., Gómez Ruiz, J. A., Horváth, Z. S., Ioannidou, S., Medina Pastor, P., & Riolo, F. (2021). Technical report on handling occurrence data for dietary exposure assessments. *EFSA Supporting Publication*, *18*(12), EN-7082. https://doi.org/10.2903/sp.efsa.2021.EN-7082
EFSA CONTAM Panel (Panel on Contaminants in the Food Chain). (2011). Scientific opinion on Polybrominated diphenyl ethers (PBDEs) in food. *EFSA Journal*, *9*(5), 2156. https://doi.org/10.2903/j.efsa.2011.2156
EFSA CONTAM Panel (Panel on Contaminants in the Food Chain). (2012). Scientific opinion on ergot alkaloids in food and feed. *EFSA Journal*, *10*(7), 2798. https://doi.org/10.2903/j.efsa.2012.2798
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen, H. K., Alexander, J., Barregard, L., Bignami, M., Bruschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Nebbia, C. S., Oswald, I. P., Petersen, A., Rose, M., Roudot, A.-C., Schwerdtle, T., Vleminckx, C., … Hoogenboom, L. R. (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
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. R., Leblanc, J.-C., Nebbia, C. S., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Wallace, H., Bruschweiler, B., Leonards, P., … Nielsen, E. (2020). Scientific opinion – Risk assessment of chlorinated paraffins in feed and food. *EFSA Journal*, *18*(3), 5991. https://doi.org/10.2903/j.efsa.2020.5991
EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2017). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, *15*(10), 5021. https://doi.org/10.2903/j.efsa.2017.5021
EFSA Scientific Committee. (2011). Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA Journal*, *9*(9), 2379. https://doi.org/10.2903/j.efsa.2011.2379
EFSA Scientific Committee. (2012). Guidance on selected default values to be used by the EFSA scientific committee, scientific panels and units in the absence of actual measured data. *EFSA Journal*, *10*(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579
EFSA Scientific Committee, Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., Silano, V., Solecki, R., Turck, D., Younes, M., Craig, P., Hart, A., Von Goetz, N., … Hardy, A. (2018a). Scientific opinion on the principles and methods behind EFSA's guidance on uncertainty analysis in scientific assessment. *EFSA Journal*, *16*(1), 5122. https://doi.org/10.2903/j.efsa.2018.5122
EFSA Scientific Committee, Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., Silano, V., Solecki, R., Turck, D., Younes, M., Craig, P., Hart, A., Von Goetz, N., … Hardy, A. (2018b). Guidance on uncertainty analysis in scientific assessments. *EFSA Journal*, *16*(1), 5123. https://doi.org/10.2903/j.efsa.2018.5123
EFSA Scientific Committee, Hardy, A., Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., Silano, V., Solecki, R., Turck, D., Bresson, J.-L., Dusemund, B., Gundert-Remy, U., … Dorne, A. (2017a). Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. *EFSA Journal*, *15*(5), 4849. https://doi.org/10.2903/j.efsa.2017.4849
EFSA Scientific Committee, Hardy, A., Benford, D., Halldorsson, T., Jeger, M., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Silano, V., Solecki, R., Turck, D., Younes, M., Aquilina, G., Crebelli, R., Gurtler, R., … Schlatter, J. (2017b). Scientific opinion on the clarification of some aspects related to genotoxicity assessment. *EFSA Journal*, *15*(12), 5113. https://doi.org/10.2903/j.efsa.2017.5113
EFSA Scientific Committee, More, S. J., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T. I., Hernandez-Jerez, A. F., Bennekou, S. H., Koutsoumanis, K., Lambre, C., Machera, K., Mennes, W., Mullins, E., Nielsen, S. S., Schrenk, D., Turck, D., Younes, M., Aerts, M., Edler, L., … Schlatter, J. (2022). Guidance on the use of the benchmark dose approach in risk assessment. *EFSA Journal*, *20*(10), 7584. https://doi.org/10.2903/j.efsa.2022.7584
Esteban, J., Sánchez-Pérez, I., Hamscher, G., Miettinen, H. M., Korkalainen, M., Viluksela, M., Pohjanvirta, R., & Håkansson, H. (2021). Role of aryl hydrocarbon receptor (AHR) in overall retinoid metabolism: Response comparisons to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) exposure between wild-type and AHR knockout mice. *Reproductive Toxicology*, *101*, 33–49.
Falandysz, J. (1998). Polychlorinated naphthalenes: An environmental update. *Environmental Pollution*, *101*, 77–90. https://doi.org/10.1016/s0269-7491(98)00023-2
Falandysz, J. (2003). Chloronaphthalenes as food-chain contaminants: A review. *Food Additives and Contaminants*, *20*, 995–1014. https://doi.org/10.1080/02652030310001615195
Falandysz, J. (2007). Dioxin-like compound load in bulk of Chlorofen - a technical chlorobiphenyl formulation from Poland. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering*, *42*, 1959–1968. https://doi.org/10.1080/10934520701627025
Falandysz, J., Bergqvist, P.-A., Kulp, S. E., Strandberg, L., Strandberg, B., & Rappe, C. (1996). Polychlorinated naphthalenes in white-tailed sea eagles breeding in Poland. *Bromatologia i Chemia Toksykologiczna*, *29*, 401–406.
Falandysz, J., Chudzyński, K., Takekuma, M., Yamamoto, T., Noma, Y., Hanari, N., & Yamashita, N. (2008). Multivariate analysis of identity of imported technical PCN formulation. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*, *43*, 1381–1390. https://doi.org/10.1080/10934520802232022

Falandysz, J., Fernandes, A., Gregoraszczuk, E., & Rose, M. (2014). The toxicological effects of halogenated naphthalenes: A review of aryl hydrocarbon receptor-mediated (dioxin-like) relative potency factors. *Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology Reviews*, *32*, 239–272. https://doi.org/10.1080/10590501.2014.938945

Falandysz, J., & Fernandes, A. R. (2020). Compositional profiles, persistency and toxicity of polychlorinated naphthalene (PCN) congeners in edible cod liver products from 1972 to 2017. *Environmental Pollution*, *260*, 114035. https://doi.org/10.1016/j.envpol.2020.114035

Falandysz, J., Kawano, M., Ueda, M., Matsuda, M., Kannan, K., Giesy, J. P., & Wakimoto, T. (2000). Composition of chloronaphthalene congeners in technical chloronaphthalene formulations of the Halowax series. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering*, *35*, 281–298. https://doi.org/10.1080/10934520009376971

Falandysz, J., Kulp, S.-E., Strandberg, B., Strandberg, L., Bergqvist, P.-A., & Rappe, C. (1996). Polychlorinated naphthalenes in Baltic plankton. *Bromatologia i Chemia Toksykologiczna*, *29*, 261–266.

Falandysz, J., Nose, K., Ishikawa, Y., Lukaszewicz, E., Yamashita, N., & Noma, Y. (2006a). Chloronaphthalenes composition of several batches of Halowax 1051. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*, *41*, 291–301. https://doi.org/10.1080/10934520500423147

Falandysz, J., Nose, K., Ishikawa, Y., Lukaszewicz, E., Yamashita, N., & Noma, Y. (2006b). HRGC/HRMS analysis of chloronaphthalenes in several batches of Halowax 1000, 1001, 1013, 1014 and 1099. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*, *41*, 2237–2255. https://doi.org/10.1080/10934520600872748

Falandysz, J., Puzyn, T., Szymanowska, B., Kawano, M., Markuszewski, M., Kaliszan, R., Skurski, P., Blazejowski, J., & Wakimoto, T. (2001). Thermodynamic and physico-chemical descriptors of chloronaphthalenes: An attempt to select features explaining environmental behaviour and specific toxic effects of these compounds. *Polish Journal of Environmental Studies*, *10*, 217–235.

Falandysz, J., & Rappe, C. (1996). Spatial distribution in plankton and bioaccumulation features of polychlorinated naphthalenes in a pelagic food chain in southern part of the Baltic proper. *Environmental Science & Technology*, *30*, 3362–3370.

Falandysz, J., Smith, F., Panton, S., & Fernandes, A. R. (2019). A retrospective investigation into the occurrence and human exposure to polychlorinated naphthalenes (PCNs), dibenzo-p-dioxins and furans (PCDD/Fs) and PCBs through cod liver products (1972–2017). *Chemosphere*, *231*, 240–248.

Falandysz, J., Strandberg, B., Strandberg, L., Bergqvist, P. A., & Rappe, C. (1997). Concentrations and biomagnification of polychlorinated naphthalenes in black cormorants *Phalacrocorax carbo* sinensis from the Gulf of Gdansk, Baltic Sea. *Science of the Total Environment*, *204*, 97–106. https://doi.org/10.1016/s0048-9697(97)00159-9

Falandysz, J., Strandberg, L., Bergqvist, P. A., Kulp, S. E., Strandberg, B., & Rappe, C. (1996). Polychlorinated naphthalenes in sediment and biota from the Gdansk Basin, Baltic Sea. *Environmental Science & Technology*, *30*, 3266–3274.

Falandysz, J., Strandberg, L., Bergqvist, P. A., Strandberg, B., & Rappe, C. (1997). Spatial distribution and bioaccumulation of polychlorinated naphthalenes (PCNs) in mussel and fish from the Gulf of Gdańsk, Baltic Sea. *Science of Total Environment*, *203*, 93–104.

Falandysz, J., Strandberg, L., Strandberg, B., & Rappe, C. (1997). Polychlorinated naphthalenes in mussel *Mytilus trosulus* and flounder *Platichthys flesus* from the Gulf of Gdansk. *Bromatologia i Chemia Toksykologiczna*, *30*, 183–191.

Falandysz, J., Strandberg, L., Strandberg, B., & Rappe, C. (1998). Polychlorinated naphthalenes in three-spined stickleback *Gasterosteus aculeatus* from the Gulf of Gdansk. *Chemosphere*, *37*, 2473–2487. https://doi.org/10.1016/s0045-6535(97)10241-7

Falandysz, J., Yamashita, N., Tanabe, S., Tatsukawa, R., Rucińska, L., Mizera, T., & Jakuczun, B. (1994). Congener-specific analysis of polychlorinated biphenyls in white-tailed sea eagles *Haliaeetus albicilla* collected in Poland. *Archives of Environmental Contamination and Toxicology*, *26*, 13–22.

Fernandes, A., Mortimer, D., Gem, M., Smith, F., Rose, M., Panton, S., & Carr, M. (2010). Polychlorinated naphthalenes (PCNs): Congener specific analysis, occurrence in food, and dietary exposure in the UK. *Environmental Science & Technology*, *4*(4), 3533–3538. https://doi.org/10.1021/es903502g

Fernandes, A. R., Kilanowicz, A., Stragierowicz, J., Klimczak, M., & Falandysz, J. (2022). The toxicological profile of polychlorinated naphthalenes (PCNs). *Science of the Total Environment*, *837*, 155764.

Fernandes, A. R., Lake, I. R., Dowding, A., Rose, M., Jones, N. R., Smith, F., & Panton, S. (2023). The transfer of environmental contaminants (brominated and chlorinated dioxins and biphenyls, PBDEs, HBCDDs, PCNs and PFAS) from recycled materials used for bedding to the eggs and tissues of chickens. *Science of the Total Environment*, *892*, 164441.

Fernandes, A. R., Mortimer, D., Holmes, M., Rose, M., Zhihua, L., Huang, X., Smith, F., Panton, S., & Marshall, L. (2018). Occurrence and spatial distribution of chemical contaminants in edible fish species collected from UK and proximate marine waters. *Environment International*, *11*(4), 219–230. https://doi.org/10.1016/j.envint.2018.02.047

Fernandes, A. R., Rose, M., & Falandysz, J. (2017). Polychlorinated naphthalenes (PCNs) in food and humans. *Environment International*, *104*, 1–13. https://doi.org/10.1016/j.envint.2017.02.015

Fernandes, A. R., Tlustos, C., Rose, M., Smith, F., Carr, M., & Panton, S. (2011). Polychlorinated naphthalenes (PCNs) in Irish foods: Occurrence and human dietary exposure. *Chemosphere*, *85*(3), 322–328.

Fromme, H., Cequier, E., Kim, J. T., Hanssen, L., Hilger, B., Thomsen, C., Chang, Y. S., & Völkel, W. (2015). Persistent and emerging pollutants in the blood of German adults: Occurrence of dechloranes, polychlorinated naphthalenes, and siloxanes. *Environment International*, *85*, 292–298. https://doi.org/10.1016/j.envint.2015.09.002

FSAI (The Food Safety Authority of Ireland). (2010). *Investigation into levels of polychlorinated Naphthalenes (PCNs) in carcass fat*. Monitoring & Surveillance Series.

Galoch, A., Sapota, A., Skrzypinska-Gawrysiak, M., & Kilanowicz, A. (2006). Acute toxicity of polychlorinated naphthalenes and their effect on cytochrome P450. *Human & Experimental Toxicology*, *25*, 85–92. https://doi.org/10.1191/0960327106ht574oa

Gebru, T. B., Li, Y., Dong, C., Yang, Y., Yang, R., Pei, Z., Zhang, Q., & Jiang, G. (2023). Spatial and temporal trends of polychlorinated naphthalenes in the Arctic atmosphere at Ny-Ålesund and London Island. *Science of The Total Environment*, *878*, 163023.

Gewurtz, S. B., Gandhi, N., Drouillard, K. G., Kolic, T., MacPherson, K., Reiner, E. J., & Bhavsar, S. P. (2018). Levels, patterns, trends and significance of polychlorinated naphthalenes (PCNs) in Great Lakes fish. *Science Total Environment*, *62*(4), 499–508. https://doi.org/10.1016/j.scitotenv.2017.11.332

Gewurtz, S. B., Lega, R., Crozier, P. W., Whittle, D. M., Fayez, L., Reiner, E. J., Helm, P. A., Marvin, C. H., & Tomy, G. T. (2009). Factors influencing trends of polychlorinated naphthalenes and other dioxin-like compounds in lake trout (*Salvelinus namaycush*) from Lake Ontario, North America (1979–2004). *Environmental Toxicology and Chemistry*, *28*, 921–930. https://doi.org/10.1897/08-307.1

Godéré, M., Marchand, P., Vénisseau, A., Guiffard, I., Leblanc, J. C., Le Bizec, B., & Dervilly, G. (2022). Health risk assessment to polychlorinated naphthalenes dioxin-like compounds in French sea food consumers. *Chemosphere*, *308*(Pt 3), 136563. https://doi.org/10.1016/j.chemosphere.2022.136563

Gregoris, E., Zambon, S., Piazza, R., Barbante, C., Gambaro, A., Argiriadis, E., De, P. S., Vecchiato, M., Donateo, A., & Contini, D. (2014). Gas-particle distributions, sources and health effects of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs) in Venice aerosols. *Science Total Environment*, *476*(7), 393–405.

Griswold, M. D., Bishop, P. D., Kim, K. H., Ping, R., Siiteri, J. E., & Morales, C. (1989). Function of vitamin a in normal and synchronized seminiferous tubules. *Annals of the New York Academy of Sciences*, *564*, 154–172. https://doi.org/10.1111/j.1749-6632.1989.tb25895.x

Gu, W., Du, M., Li, Q., Li, Y., Li, X., & Ren, Z. (2021). Identification and regulation of ecotoxicity of polychlorinated naphthalenes to aquatic food chain (green algae-*Daphnia magna*-fish). *Aquatic Toxicology*, *233*, 105774.

Guruge, K. S., Seike, N., Yamanaka, N., & Miyazaki, S. (2004). Accumulation of polychlorinated naphthalenes in domestic animal related samples. *Journal of Environmental Monitoring*, *6*, 753–757. https://doi.org/10.1039/b404192p

Haglund, P., Jakobsson, E., Asplund, L., Athanasiadou, M., & Bergman, A. (1993). Determination of polychlorinated naphthalenes in polychlorinated biphenyl products via capillary gas chromatography–mass spectrometry after separation by gel-permeation chromatography. *Journal of Chromatography*, *634*, 79–86. https://doi.org/10.1016/0021-9673(93)80314-x

Haglund, P., Jakobsson, E., & Masuda, Y. (1995). Isomer-specific analysis of polychlorinated naphthalenes in Kanechlor KC 400, Yusho rice oil, and adipose tissue of a Yusho victim. *Organohalogen Compounds*, *26*, 405–410.

Hambrick, G. W., Jr. (1957). The effect of substituted naphthalenes on the pilosebaceous apparatus of rabbit and man. *Journal of Investigative Dermatology*, *28*(2), 89–103.

Han, Y., Li, H., Lei, R., Liu, W., Liu, G., Gao, L., & Su, G. (2018). Distribution of polychlorinated naphthalenes (PCNs) in the whole blood of typical meat animals. *Journal of Environmental Sciences (China)*, *72*, 208–212.

Hanari, N., Falandysz, J., Nakano, T., Petrick, G., & Yamashita, N. (2013). Separation of closely eluting chloronaphthalene congeners by two-dimensional gas chromatography/quadrupole mass spectrometry: An advanced tool in the study and risk analysis of dioxin-like chloronaphthalenes. *Journal of Chromatography A*, *1301*, 209–214. https://doi.org/10.1016/j.chroma.2013.05.070

Hanari, N., Falandysz, J., Petrick, G., Nakano, T., & Yamashita, N. (2013). Preliminary evaluation of environmental photodegradation of chloronaphthalenes by congener-specific determination with comprehensive two-dimensional GC/qMS. *Organohalogen Compounds*, *75*, 5–8.

Hanari, N., Falandysz, J., Yamazaki, E., & Yamashita, N. (2020). Photodegradation of polychlorinated naphthalene in mixtures. *Environmental Pollution*, *263*. https://doi.org/10.1016/j.envpol.2020.114672

Hanari, N., Horii, Y., Okazawa, T., Falandysz, J., Bochentin, I., Orlikowska, A., Puzyn, T., Wyrzykowska, B., & Yamashita, N. (2004). Dioxin-like compounds in pine needles around Tokyo Bay, Japan in 1999. *Journal of Environmental Monitoring*, *6*, 305–312. https://doi.org/10.1039/b311176h

Hanberg, A., Waern, F., Asplund, L., Haglund, E., & Safe, S. (1990). Swedish dioxin survey: Determination of 2, 3, 7, 8-TCDD toxic equivalent factors for some polychlorinated biphenyls and naphthalenes using biological tests. *Chemosphere*, *20*(7–9), 1161–1164.

Hansel, W., Ol, P., & McEntee, K. (1953). Bovine hyperkeratosis: Studies on a German wood preservative. *The Cornell Veterinarian*, *43*, 311–327.

Harner, T., & Bidleman, T. F. (1998). Measurement of octanol–air partition coefficients for polycyclic aromatic hydrocarbons and polychlorinated naphthalenes. *Journal of Chemical & Engineering Data*, *43*, 40–46.

Haughey, K. G., & Cooper, B. S. (1953). Anoutbreak of bovine hyperkeratosis in New Zealand. *New Zealand Veterinary Journal*, *1*, 99–103. https://doi.org/10.1080/00480169.1953.33116

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., & Zeiger, E. (1983). Salmonella mutagenicity test results for 250 chemicals. *Environmental Mutagensis (United States)*, *5*(1).

Helm, P. A., Jantunen, L. M. M., Bidleman, T. F., & Dorman, F. L. (1999). Complete separation of isomeric penta- and hexachloronaphthalenes by capillary gas chromatography. *HRC Journal of High Resolution Chromatography*, *22*, 639–643.

Henriksson, K., Karppanen, E., & Helminen, M. (1966). High residue of mercury in Finnish white-tailed eagles. *Ornis Fennica*, *43*(2), 38–45.

Herzberg, J. J. (1947). Chlorine acne after enjoying chlorinated paraffin. *Dermatologische Wochenschrift*, *119*(7), 425–433.

Hites, R. A. (2006). *Persistent organic pollutants in the Great Lakes*. Springer Science & Business Media.

Hogarh, J. N., Seike, N., Kobara, Y., Habib, A., Nam, J. J., Lee, J. S., Li, Q., Liu, X., Li, J., Zhang, G., & Masunaga, S. (2012). Passive air monitoring of PCBs and PCNs across East Asia: A comprehensive congener evaluation for source characterization. *Chemosphere*, *86*, 718–726. https://doi.org/10.1016/j.chemosphere.2011.10.046

Hogarh, J. N., Seike, N., Kobara, Y., & Masunaga, S. (2012). Atmospheric polychlorinated naphthalenes in Ghana. *Environmental Science & Technology*, *46*, 2600–2606. https://doi.org/10.1021/es2035762

Holm, G., Norrgren, L., Andersson, T., & Thuren, A. (1993). Effects of exposure to food contaminated with PBDE, PCN or PCB on reproduction, liver morphology and cytochrome P450 activity in the three-spined stickleback, *Gasterosteus aculeatus*. *Aquatic Toxicology*, *27*, 33–50. https://doi.org/10.1016/0166-445x(93)90045-3

Hooth, M. J., Nyska, A., Fomby, L. M., Vasconcelos, D. Y., Vallant, M., DeVito, M. J., & Walker, N. J. (2012). Repeated dose toxicity and relative potency of 1, 2,3,4,6,7-hexachloronaphthalene (PCN 66) 1,2,3,5,6,7-hexachloronaphthalene (PCN 67) compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for induction of CYP1A1, CYP1A2 and thymic atrophy in female Harlan Sprague–Dawley rats. *Toxicology*, *301*, 85–93. https://doi.org/10.1016/j.tox.2012.07.005

Horii, Y., Falandysz, J., Hanari, N., Rostkowski, P., Puzyn, T., Okada, M., Amano, K., Naya, T., Taniyasu, S., & Yamashita, N. (2004). Concentrations and fluxes of chloronaphthalenes in sediment from Lake Kitaura in Japan in past 15 centuries. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*, *39*, 587–609. https://doi.org/10.1081/ese-120027727

Horii, Y., Jiang, Q., Hanari, N., Lam, P. K., Yamashita, N., Jansing, R., Aldous, K. M., Mauer, M. P., Eadon, G. A., & Kannan, K. (2010). Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, and naphthalenes in plasma of workers deployed at the world trade center after the collapse. *Environmental Science & Technology*, *44*, 5188–5194. https://doi.org/10.1021/es100282d

Hsu, S. T., Ma, C. I., Hsu, S. K., Wu, S. S., Hsu, N. H., Yeh, C. C., & Wu, S. B. (1985). Discovery and epidemiology of PCB poisoning in Taiwan: A four-year followup. *Environmental Health Perspectives*, *59*, 5–10.

Hu, J., Wu, J., Xu, C., Zha, X., Hua, Y., Yang, L., & Jin, J. (2019). Preliminary investigation of polychlorinated dibenzo-p-dioxin and dibenzofuran, polychlorinated naphthalene, and dioxin-like polychlorinated biphenyl concentrations in ambient air in an industrial park at the northeastern edge of the Tibet- Qinghai plateau, China. *Science Total Environment*, *648*, 935–942. https://doi.org/10.1016/j.scitotenv.2018.08.241

Huber, W. G., & Link, R. P. (1962). Toxic effects of hexachloronaphthalene on swine. *Toxicology and Applied Pharmacology*, *4*, 257–262. https://doi.org/10.1016/0041-008x(62)90065-0

Huybrechts, I., Sioen, I., Boon, P. E., Ruprich, J., Lafay, L., Turrini, A., Amiano, P., Hirvonen, T., De Neve, M., Arcella, D., Moschandreas, J., Westerlund, A., Ribas-Barba, L., Hilbig, A., Papoutsou, S., Christensen, T., Oltarzewski, M., Virtanen, S., Rehurkova, I., … Van Klaveren, J. (2011). Dietary exposure assessments for children in Europe (the EXPOCHI project): Rationale, methods and design. *Archives of Public Health*, *69*, 4. https://doi.org/10.1186/0778-7367-1169-1184

Ieda, T., Ochiai, N., Miyawaki, T., Ohura, T., & Horii, Y. (2011). Environmental analysis of chlorinated and brominated polycyclic aromatic hydrocarbons by comprehensive two-dimensional gas chromatography coupled to high-resolution time-of-flight mass spectrometry. *Journal of Chromatography A*, *1218*, 3224–3232. https://doi.org/10.1016/j.chroma.2011.01.013

Ishaq, R., Karlson, K., & Näf, C. (2000). Tissue distribution of polychlorinated naphthalenes (PCNs) and non-ortho chlorinated biphenyls (non-ortho CBs) in harbour porpoises (*Phocoena phocoena*) from Swedish waters. *Chemosphere*, *41*(12), 1913–1925.

Isosaari, P., Hallikainen, A., Kiviranta, H., Vuorinen, P. J., Parmanne, R., Koistinen, J., & Vartiainen, T. (2006). Polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls, naphthalenes and polybrominated diphenyl ethers in the edible fish caught from the Baltic Sea and lakes in Finland. *Environmental Pollution*, *14*(1), 213–225. https://doi.org/10.1016/j.envpol.2005.08.055

Jakobsson, E. (1994). *Synthesis and analysis of chlorinated naphthalenes biological and environmental implications* [Thesis]. Department of Environmental Chemistry, Stockholm University, Sweden.

Jakobsson, E., & Asplund, L. (2000). Polychlorinated naphthalenes (PCNs). *New Types of Persistent Halogenated Compounds*, *3*, 97–126.

James, M. O., & Kleinow, K. M. (2014). Seasonal influences on PCB retention and biotransformation in fish. *Environmental Science and Pollution Research*, *21*, 6324–6333.

Jansson, B., Asplund, L., & Olsson, M. (1984). Analysis of polychlorinated naphthalenes in environmental samples. *Chemosphere*, *13*, 33–41. https://doi.org/10.1016/0045-6535(84)90006-7

Järnberg, U. G., Asplund, L. T., Egeback, A. L., Jansson, B., Unger, M., & Wideqvist, U. (1999). Polychlorinated naphthalene congener profiles in background sediments compared to a degraded Halowax 1014 technical mixture. *Environmental Science & Technology*, *33*, 1–6. https://doi.org/10.1021/es980360a

Jiang, Q., Hanari, N., Miyake, Y., Okazawa, T., Lau, R. K., Chen, K., Wyrzykowska, B., So, M. K., Yamashita, N., & Lam, P. K. (2007). Health risk assessment for polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans, and polychlorinated naphthalenes in seafood from Guangzhou and Zhoushan, China. *Environmental Pollution*, *14*(8), 31–39. do i: 10.1016/j.envpol.2006.11.002

Jin, J., Wang, S., Hu, J., Wu, J., Li, M., Wang, Y., & Jin, J. (2019). Polychlorinated naphthalenes in human serum samples from an industrial city in eastern China: Levels, sources, and sex differences. *Ecotoxicology and Environmental Safety*, *177*, 86–92. https://doi.org/10.1016/j.ecoenv.2019.04.007

Jin, R., Fu, J., Zheng, M., Yang, L., Habib, A., Li, C., & Liu, G. (2020). Polychlorinated naphthalene congener profiles in common vegetation on the Tibetan plateau as biomonitors of their sources and transportation. *Environmental Science & Technology*, *54*, 2314–2322. https://doi.org/10.1021/acs.est.9b06668

JMPR (Joint FAO/WHO Expert Meeting on Pesticide Residues). (2015). Pesticide residues in food: guidance document for WHO monographers and reviewers. WHO/HSE/FOS/2015.1. World Health Organization.

Kannan, K., Corsolini, S., Imagawa, T., Focardi, S., & Giesy, J. P. (2002). Polychlorinated-naphthalenes, –biphenyls, –dibenzo-*p*-dioxins, –dibenzofurans and p,p'-DDE in bluefin tuna, swordfish, cormorants and barn swallows from Italy. *Ambio*, *31*, 207–211.

Kannan, K., Yamashita, N., Villeneuve, D. L., Hashimoto, S., Miyazaki, A., & Giesy, J. P. (2000). Vertical profile of dioxin-like and estrogenic potencies in a sediment core from Tokyo Bay, Japan. *Central European Journal of Public Health*, *8*(Suppl), 32–33.

Kawano, M., Ueda, M., Falandysz, J., Matsuda, M., & Wakimoto, T. (2000). Polychlorinated naphthalenes (PCNs) in human adipose tissue in Japan. *Organohalogen Compounds*, *47*, 159–162.

Kilanowicz, A., Czekaj, P., Plewka, D., Sapota, A., Skrzypinska-Gawrysiak, M., Bruchajzer, E., Darago, A., Czech, E., Wiaderkiewicz, A., & Sitarek, K. (2015). Developmental toxicity of hexachloronaphthalene in Wistar rats. A role of CYP1A1 expression. *Reproductive Toxicology*, *58*, 93–103.

Kilanowicz, A., Daragó, A., & Skrzypińska-Gawrysiak, M. (2012). The effect of exposure route on the distribution and excretion of hexachloronaphthalene in rats. *International Journal of Occupational Medicine and Environmental Health*, *25*, 185–195. https://doi.org/10.2478/S13382-012-0021-y

Kilanowicz, A., Galoch, A., & Sapota, A. (2004). Tissue distribution and elimination of selected chlorinated naphthalenes. *International Journal of Occupational Medicine and Environmental Health*, *17*, 355–360.

Kilanowicz, A., Markowicz-Piasecka, M., Klimczak, M., Stragierowicz, J., & Sikora, J. (2019). Hexachloronaphthalene as a hemostasis disturbing factor in female Wistar rats–- a pilot study. *Chemosphere*, *228*, 577–585. https://doi.org/10.1016/j.chemosphere.2019.04.147

Kilanowicz, A., Sitarek, K., Skrzypinska-Gawrysiak, M., & Sapota, A. (2011). Prenatal developmental toxicity of polychlorinated naphthalenes (PCNs) in the rat. *Ecotoxicology and Environmental Safety*, *74*, 504–512. https://doi.org/10.1016/j.ecoenv.2010.08.025

Kilanowicz, A., & Skrzypinska-Gawrysiak, M. (2010). Toxicity of hexachloronaphthalene (HxCN) and induction of CYP 1A in rats. *Ecotoxicology and Environmental Safety*, *73*, 196–205. https://doi.org/10.1016/j.ecoenv.2009.08.018

Kilanowicz, A., Skrzypinska-Gawrysiak, M., Sapota, A., Galoch, A., & Daragó, A. (2009). Subacute toxicity of polychlorinated naphthalenes and their effect on cytochrome P-450. *Ecotoxicology and Environmental Safety*, *72*, 650–657. https://doi.org/10.1016/j.ecoenv.2008.07.010

Kilanowicz, A., Stragierowicz, J., Klimczak, M., Bruchajzer, E., & Sitarek, K. (2019). Prenatal toxicity and maternal-fetal distribution of 1,3,5,8-tetrachloronaphthalene (1,3,5,8-TeCN) in Wistar rats. *Chemosphere*, *226*, 75–84.

Kilanowicz, A., Wiaderna, D., Lutz, P., & Szymczak, W. (2012). Behavioral effects following repeated exposure to hexachloronaphthalene in rats. *Neurotoxicology*, *33*(3), 361–369.

Kim, J., Shin, E. S., Choi, S. D., Zhu, J., & Chang, Y. S. (2018). Polychlorinated naphthalenes (PCNs) in seafood: Estimation of dietary intake in Korean population. *Science Total Environment*, *624*, 40–47. https://doi.org/10.1016/j.scitotenv.2017.12.009

Kim, J.-T., Son, M.-H., Lee, D.-H., Seong, W. J., Han, S., & Chang, Y.-S. (2015). Partitioning behavior of heavy metals and persistent organic pollutants among Feto-maternal bloods and tissues. *Environmental Science & Technology*, *49*, 7411–7422. https://doi.org/10.1021/es5051309

Kim, K. W., Choo, G., Cho, H. S., Lee, B. C., Park, K., & Oh, J. E. (2019). The occurrence and distribution of polychlorinated naphthalenes (PCNs), focusing on tissue-specific bioaccumulation in crucian carp in South Korea. *Science Total Environment*, *66*(5), 484–491. https://doi.org/10.1016/j.scitotenv.2019.01.238

Klasson-Wehler, E., Jakobsson, E., & Orn, U. (1996). Metabolism of polychlorinated naphthalenes and a tetrabrominated diphenyl ether. *Organohalogen Compounds*, *28*, 495–499.

Klimczak, M., Darago, A., Bruchajzer, E., Kuzajska, K., Kilanowicz, A., Domeradzka-Gajda, K., & Stepnik, M. (2018). The effects of hexachloronaphthalene on selected parameters of heme biosynthesis and systemic toxicity in female wistar rats after 90-day oral exposure. *Environmental Toxicology*, *33*, 695–705.

Klimczak, M., Liu, G., Fernandes, A. R., Kilanowicz, A., & Falandysz, J. (2023). An updated global overview of the manufacture and unintentional formation of polychlorinated naphthalenes (PCNs). *Journal of Hazardous Materials*, *131*, 786.

Koeman, J. H., Pennings, J. H., De Goeij, J. J., Tjioe, P. S., Olindo, P. M., & Hopcraft, J. (1972). A preliminary survey of the possile contamination of Lake Nakuru in Kenya with some metals and chlorinated hydrocarbon pesticides. *Journal of Applied Ecology*, 411–416.

Koistinen, J., Paasivirta, J., & Vuorinen, P. J. (1989). Dioxins and other planar polychloroaromatic compounds in Baltic, Finnish and Arctic fish samples. *Chemosphere*, *19*(1-6), 527–530.

Kover, F. D. (1975). *US Environmental Protection Agency. Office of Toxic Substances*. Environmental Hazard Assessment Report-Chlorinated Naphthalenes.

Kunisue, T., Johnson-Restrepo, B., Hilker, D. R., Aldous, K. M., & Kannan, K. (2009). Polychlorinated naphthalenes in human adipose tissue from New York, USA. *Environmental Pollution*, *15*(7), 910–915. https://doi.org/10.1016/j.envpol.2008.11.012

Kuratsune, M., Yoshimura, T., Matsuzaka, J., & Yamaguchi, A. (1972). Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. *Environmental Health Perspectives*, *1*, 119–128.

Kuratsune, M. Y., (1989). With reference to Yu-Cheng. Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products. 381–400.

Law, R. J. (2014). An overview of time trends in organic contaminant concentrations in marine mammals: Going up or down? *Marine Pollution Bulletin*, *82*, 7–10. https://doi.org/10.1016/j.marpolbul.2014.03.024

Lee, R. G. M., Coleman, P., Jones, J., Jones, K. C., & Lohmann, R. (2005). Emission factors and importance of PCDD/fs, PCBs, PCNs, PAHs and PM10 from the domestic burning of coal and wood in the U.K. *Environmental Science & Technology*, *39*, 1436–1447. https://doi.org/10.1021/es048745i

Leeson, S., & Summers, J. D. (2008). *Commercial poultry nutrition third edition*. Nottingham UniversityPress. Digitally reprinted in 2008 from: Commercial Poultry Nutrition, Third Edition University Books P.O. Box 1326, Guelph, Ontario.

Lei, Y. D., Wania, F., & Shiu, W. Y. (1999). Vapor pressures of the polychlorinated naphthalenes. *Journal of Chemical & Engineering Data*, *44*(3), 577–582.

Li, C., Li, J., Bing, L., Wu, Y., Yang, L., Zheng, M., Min, Y., Zhang, L., & Liu, G. (2021). Burden and risk of polychlorinated Naphthalenes in Chinese human Milk and a global comparison of human exposure. *Environmental Science & Technology*, *5*(5), 6804–6813. https://doi.org/10.1021/acs.est.1c00605

Li, C., Zhang, L., Li, J., Min, Y., Yang, L., Zheng, M., Wu, Y., Yang, Y., Qin, L., & Liu, G. (2020). Polychlorinated naphthalenes in human milk: Health risk assessment to nursing infants and source analysis. *Environment International*, *13*(6), 105436. https://doi.org/10.1016/j.envint.2019.105436

Li, C., Zhang, L., Yang, Q., Wu, Y., Zheng, M., Yang, L., Lyu, B., Liu, X., Jin, R., Sun, Y., & Chen, C. (2022). Comprehensive evaluation of dietary exposure and health risk of polychlorinated naphthalenes. *Environmental Science & Technology*, *56*(9), 5520–5529.

Link, R. P., Smith, J. C., & Newton, D. I. (1958). Toxic effect of chlorinated naphthalenes in pigs. *Journal of American Veterinary Medical Association*, *133*, 83–85.
Liu, G., Zheng, M., & Cai, Z. (2014). Occurrence, profile and possible sources of PCNs in Hong Kong soils, and a comparison with PCBs, PCDDs and PCDFs. *Environmental Science and Pollution Research International*, *21*, 13656–13663. https://doi.org/10.1007/s11356-014-3258-0
Llobet, J. M., Falcó, G., Bocio, A., & Domingo, J. L. (2007). Human exposure to polychlorinated naphthalenes through the consumption of edible marine species. *Chemosphere*, *66*, 1107–1113. https://doi.org/10.1016/j.chemosphere.2006.06.058
Lundén, A., & Norén, K. (1998). Polychlorinated naphthalenes and other organochlorine contaminants in Swedish human milk, 1972–1992. *Archives of Environmental Contamination and Toxicology*, *34*, 414–423. https://doi.org/10.1007/s002449900338
Lundgren, K., Tysklind, M., Ishaq, R., Broman, D., & van Bavel, B. (2002). Polychlorinated naphthalene levels, distribution, and biomagnification in a benthic food chain in the Baltic Sea. *Environmental Science & Technology*, *3*(6), 5005–5013. https://doi.org/10.1021/es0201146
Mackay, D., Shiu, W. Y., Bobra, A., Billington, J., Chau, E., Yeun, A., & NgC, S. F. (1982). *Volatilisation of organic pollutants from water*. (EPA Report No. 600/3-82-019). US Environmental Protection Agency, Environmental Research Laboratory.
McEntee, K., Hansel, W. I., & Olafson, P. E. (1951). The production of hyperkeratosis (X-disease) by feeding fractions of processed concentrate. *The Cornell Veterinarian*, *41*, 237–239.
Mahmood, A., Malik, R. N., Li, J., Zhang, G., & Jones, K. C. (2014). PCNs (polychlorinated napthalenes): Dietary exposure via cereal crops, distribution and screening-level risk assessment in wheat, rice, soil and air along two tributaries of the river Chenab, Pakistan. *Science of the Total Environment*, *481*, 409–417.
Mao, S., Zhang, G., Li, J., Zhao, S., Cheng, Z., Geng, X., Wang, J., Xu, Y., Li, Q., & Wang, Y. (2020). Occurrence and sources of PCBs, PCNs, and HCB in the atmosphere at a regional background site in east China: Implications for combustion sources. *Environmental Pollution*, *262*, 114267.
Marsh, C. L., Olson, C., & Blore, I. C. (1956). Observations on collagen, vitamin a, and ascorbic acid in bovine hyperkeratosis. *American Journal of Veterinary Research*, *17*, 410–414.
Martí-Cid, R., Bocio, A., Llobet, J. M., & Domingo, J. L. (2007). Intake of chemical contaminants through fish and seafood consumption by children of Catalonia, Spain: Health risks. *Food and Chemical Toxicology*, *45*, 1968–1974. https://doi.org/10.1016/j.fct.2007.04.014
Martí-Cid, R., Llobet, J. M., Castell, V., & Domingo, J. L. (2008). Human exposure to polychlorinated naphthalenes and polychlorinated diphenyl ethers from foods in Catalonia, Spain: Temporal trend. *Environmental Science & Technology*, *42*, 4195–4201. https://doi.org/10.1021/es800064p
Masuda, Y. (1994). The Yusho rice oil poisoning incident. In *InDioxins and health 1994* (pp. 633–659). Springer US.
McGoldrick, D. J., Pelletier, M., de Solla, S. R., Marvin, C. H., & Martin, P. A. (2018). Legacy of legacies: Chlorinated naphthalenes in Lake trout, walleye, herring Gull eggs and sediments from the Laurentian Great Lakes indicate possible resuspension during contaminated sediment remediation. *Science Total Environment*, *634*, 1424–1434. https://doi.org/10.1016/j.scitotenv.2018.04.077
Merten, C., Ferrari, P., Bakker, M., Boss, A., Hearty, A., Leclercq, C., Lindtner, O., Tlustos, C., Verger, P., Volatier, J. L., & Arcella, D. (2011). Methodological characteristics of the national dietary surveys carried out in the European Union as included in the European food safety authority (EFSA) comprehensive European food consumption database. *Food additives & contaminants, Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *28*, 975–995. https://doi.org/10.1080/19440049.2011.576440
Mori, T., Kitano, S., & Kondo, R. (2003). Biodegradation of chloronaphthalenes and polycyclic aromatic hydrocarbons by the white-rot fungus *Phlebia lindtneri*. *Applied Microbiology and Biotechnology*, *61*, 380–383. https://doi.org/10.1007/s00253-003-1253-3
Mori, T., Nakamura, K., & Kondo, R. (2009). Fungal hydroxylation of polychlorinated naphthalenes with chlorine migration by wood rotting fungi. *Chemosphere*, *77*, 1230–1235. https://doi.org/10.1016/j.chemosphere.2009.08.046
Mortelmans, K., & Zeiger, E. (2000). The Ames salmonella/microsome mutagenicity assay. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *455*(1–2), 29–60.
Nath, A., Ojha, P. K., & Roy, K. (2023). Computational modeling of aquatic toxicity of polychlorinated naphthalenes (PCNs) employing 2D-QSAR and chemical read-across. *Aquatic Toxicology*, *257*, 106429.
NRC (National Research Council). (2007). *Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids*. The National Academies Press. https://doi.org/10.17226/11654
Nfon, E., Cousins, I. T., & Broman, D. (2008). Biomagnification of organic pollutants in benthic and pelagic marine food chains from the Baltic Sea. *Science of the Total Environment*, *397*, 190–204. https://doi.org/10.1016/j.scitotenv.2008.02.029
Noma, Y., Ishikawa, Y., Falandysz, J., Jecek, L., Gulkowska, A., Miyaji, K., & Sakai, S. (2004). By-side impurities in chloronaphthalene mixtures of the Halowax series: All 209 chlorobiphenyls. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*, *39*, 2035–2058. https://doi.org/10.1081/ese-120039373
Noren, K., & Meironyte, D. (2000). Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. *Chemosphere*, *40*, 1111–1123. https://doi.org/10.1016/s0045-6535(99)00360-4
Norrgren, L., Andersson, T., & Bjork, M. (1993). Liver morphology and cytochrome P450 activity in fry of rainbow trout after microinjection of lipid-soluble xenobiotics in the yolk-sac embryos. *Aquatic Toxicology*, *26*, 307–316. https://doi.org/10.1016/0166-445x(93)90036-z
NTP Experiment Number: 328159. Test Type: Genetic Toxicology - Bacterial Mutagenicity.
NTP Experiment Number: 648470. Test Type: Genetic Toxicology - Bacterial Mutagenicity.
NTP Experiment Number: 771958. Test Type: Genetic Toxicology - Bacterial Mutagenicity.
NTP Experiment Number: A21226. Test Type: Genetic Toxicology – Bacterial Mutagenicity.
Oishi, H., & Oishi, S. (1983). Tissue distribution and elimination of chlorinated naphthalenes in mice. *Toxicology Letters*, *15*, 119–122. https://doi.org/10.1016/0378-4274(83)90204-7
Olafson, P. (1947). Hyperkeratosis (X disease) of cattle. *The Cornell Veterinarian*, *87*, 279–291.
Omura, M., Masuda, Y., Hirata, M., Tanaka, A., Makita, Y., Ogata, R., & Inoue, N. (2000). Onset of spermatogenesis is accelerated by gestational administration of 1,2,3,4,6,7-hexachlorinated naphthalene in male rat offspring. *Environmental Health Perspectives*, *108*, 539–544. https://doi.org/10.2307/3454616
Opperhuizen, A., van der Velde, E. W., Gobas, F. A. P. C., Liem, D. A. K., van der Steen, J. M., & Hutzinger, O. (1985). Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. *Chemosphere*, *14*, 1871–1896.
Orlikowska, A., Hanari, N., Wyrzykowska, B., Bochentin, I., Horii, Y., Yamashita, N., & Falandysz, J. (2009). Airborne chloronaphthalenes in scots pine needles of Poland. *Chemosphere*, *75*, 1196–1205. https://doi.org/10.1016/j.chemosphere.2009.02.024
Pagano, J. J., & Garner, A. J. (2019). Comprehensive assessment of legacy organic contaminants and trends in lake trout from Cayuga Lake, New York: 2011–2017. *Journal of Great Lakes Research*, *45*, 1290–1298. https://doi.org/10.1016/j.jglr.2019.09.023
Pagano, J. J., & Garner, A. J. (2020). Concentrations, toxic equivalence, and age-corrected trends of legacy organic contaminants in Lake Champlain lake trout: 2012–2018. *Environmental Research*, *18*(4), 109329. https://doi.org/10.1016/j.envres.2020.109329
Pagano, J. J., & Garner, A. J. (2021). Polychlorinated Naphthalenes across the Great Lakes: Lake trout and walleye concentrations, trends, and TEQ Assessment-2004-2018. *Environmental Science and Technology*, *55*, 2411–2421. https://doi.org/10.1021/acs.est.0c07507
Pan, J., Yang, Y., Taniyasu, S., Yeung, L. W., Falandysz, J., & Yamashita, N. (2012). Comparison of historical record of PCDD/fs, dioxin-like PCBs, and PCNs in sediment cores from Jiaozhou Bay and coastal Yellow Sea: Implication of different sources. *Bulletin of Environmental Contamination and Toxicology*, *89*, 1240–1246. https://doi.org/10.1007/s00128-012-0836-z

Pan, J., Yang, Y., Zhu, X., Yeung, L. W., Taniyasu, S., Miyake, Y., Falandysz, J., & Yamashita, N. (2013). Altitudinal distributions of PCDD/fs, dioxin-like PCBs and PCNs in soil and yak samples from Wolong high mountain area, eastern Tibet-Qinghai plateau, China. *Science Total Environment*, *444*, 102–109. https://doi.org/10.1016/j.scitotenv.2012.11.013

Parizek, O., Gramblicka, T., Parizkova, D., Polachova, A., Bechynska, K., Dvorakova, D., Stupak, M., Dusek, J., Pavlikova, J., Topinka, J., & Sram, R. J. (2023). Assessment of organohalogenated pollutants in breast milk from The Czech Republic. *Science of The Total Environment*, *871*, 161938.

Park, H., Kang, J. H., Baek, S. Y., & Chang, Y. S. (2010). Relative importance of polychlorinated naphthalenes compared to dioxins, and polychlorinated biphenyls in human serum from Korea: Contribution to TEQs and potential sources. *Environmental Pollution*, *158*, 1420–1427. https://doi.org/10.1016/j.envpol.2009.12.039

Pärt, P. (1989). Bioavailability and uptake of Xenobiotics in fish. In L. Landner (Ed.), *Chemicals in the Aquatic Environment. Springer series on environmental management*. Springer.

Pesonen, M., Teivainen, P., Lundström, J., Jakobsson, E., & Norrgren, L. (2000). Biochemical responses of fish sac fry and a primary cell culture of fish hepatocytes exposed to polychlorinated naphthalenes. *Archives of Environmental Contamination and Toxicology*, *38*, 52–58. https://doi.org/10.1007/s002449910007

Pudelkiewicz, W. J., Boucher, R. V., Callenbach, E. W., & Miller, R. C. (1958). Some physiological responses of broad breasted bronze poults to chlorinated naphthalene. *Poultry Science*, *37*, 185–187. https://doi.org/10.3382/ps.0370185

Pudelkiewicz, W. J., Boucher, R. V., Callenbach, E. W., & Miller, R. C. (1959). Some physiological responses of New Hampshire chickens to a mixture of penta- and hexachloronaphthalenes. *Poultry Science*, *38*, 424–430. https://doi.org/10.3382/ps.0380424

Rigby, H., Dowding, A., Fernandes, A., Humphries, D., Petch, R. G., Reynolds, C. K., Rose, M., & Smith, S. R. (2023). Transfer of polychlorinated, polybrominated and mixed-halogenated dioxins, furans and biphenyls, polychlorinated naphthalenes and alkanes, polycyclic aromatic hydrocarbons and chlorobenzenes to the milk of dairy cattle from controlled ingestion of industrial and municipal bioresources recycled to agricultural land. *Science of the Total Environment*, *886*, 163546.

RIVM (The National Institute for Public Health and the Environment). (2011). Identifying potential POP and PBT substances. Development of a new Persistence/Bioaccumulation-score. RIVM Report 601356001/2011.

Rose, M., Fernandes, A., Mortimer, D., & Baskaran, C. (2015). Contamination of fish in UK fresh water systems: Risk assessment for human consumption. *Chemosphere*, *122*, 183–189. https://doi.org/10.1016/j.chemosphere.2014.11.046

Ross, P. S., Noel, M., Lambourn, D., Dangerfield, N., Calambokidis, J., & Jeffries, S. (2013). Declining concentrations of persistent PCBs, PBDEs, PCDEs, and PCNs in harbor seals (*Phoca vitulina*) from the Salish Sea. *Progress in Oceanography*, *115*, 160–170. https://doi.org/10.1016/j.pocean.2013.05.027

Rotander, A., van Bavel, B., Rigét, F., Auðunsson, G. A., Polder, A., Gabrielsen, G. W., Víkingsson, G., Mikkelsen, B., & Dam, M. (2012). Polychlorinated naphthalenes (PCNs) in sub-Arctic and Arctic marine mammals, 1986–2009. *Environmental Pollution*, *164*, 118–124. https://doi.org/10.1016/j.envpol.2012.01.035

Ruzo, L. O., Jones, D., & Platonow, N. (1976). Uptake and distribution of chloronaphthalenes and their metabolites in pigs. *Bulletin of Environmental Contamination and Toxicology*, *16*, 233–239. https://doi.org/10.1007/bf01685233

Ruzo, L. O., Jones, D., Safe, S., & Hutzinger, O. (1976). Metabolism of chlorinated naphthalenes. *Journal of Agricultural and Food Chemistry*, *24*, 581–583. https://doi.org/10.1021/jf60205a007

Ruzo, L. O., Safe, S., Hutzinger, O., Platonow, N., & Jones, D. (1975). Hydroxylated metabolites of chloronaphthalenes (Halowax 1031) in pig urine. *Chemosphere*, *4*, 121–123. https://doi.org/10.1016/0045-6535(75)90086-7

Ryan, J. J., & Masuda, Y. (1994). Polychlorinated naphthalenes (PCNs) in the rice oil poisonings. *Organohalogen Compounds*, *21*, 251–254.

Safe, S. H. (2005). Halogenated hydrocarbons, toxicity and environmental impact. *Proceedings*, 135–151.

Schoene, K., Böhmer, W., & Steinhanses, J. (1984). Determination of vapour pressures down to 0.01 Pa by headspace gas-chromatography. *Fresenius Journal of Analytical Chemistry*, *319*, 903–906.

Schiavone, A., Kannan, K., Horii, Y., Focardi, S., & Corsolini, S. (2010). Polybrominated diphenyl ethers, polychlorinated naphthalenes and polycyclic musks in human fat from Italy: Comparison to polychlorinated biphenyls and organochlorine pesticides. *Environmental Pollution*, *158*, 599–606. https://doi.org/10.1016/j.envpol.2009.08.011

Shelley, W. B., & Kligman, A. M. (1957). The experimental production of acne by penta and hexachloronaphthalenes. A.M.A. *Archives of Dermatology*, *75*, 689–695. https://doi.org/10.1001/archderm.1957.01550170057010

Sijm, D. T. H. M., Verberne, M. E., De Jonge, W. J., Paert, P., & Opperhuizen, A. (1995). Allometry in the uptake of hydrophobic chemicals determined in vivo and in isolated perfused gills. *Toxicology and Applied Pharmacology*, *131*, 130–135. https://doi.org/10.1006/taap.1995.1054

Sikes, D., & Bridges, M. E. (1952). Experimental production of hyperkeratosis ("X disease") of cattle with a chlorinated naphthalene. *Science*, *116*, 506–507. https://doi.org/10.1126/science.116.3019.506

Sişman, T., & Geyikoğlu, F. (2008). The teratogenic effects of polychlorinated naphthalenes (PCNs) on early development of the zebrafish (*Danio rerio*). *Environmental Toxicology and Pharmacology*, *25*, 83–88. https://doi.org/10.1016/j.etap.2007.09.001

Slootweg, T., Segner, H., Mayer, P., Smith, K., Igumnova, E., Nikiforov, V., Dömötörová, M., Oehlmann, J., & Liebig, M. (2015). Transfer and effects of 1,2,3,5,7-pentachloronaphthalene in an experimental food chain. *Comparative Biochemistry and Physiology, Part C: Toxicology & Pharmacology*, *169*, 46–54. https://doi.org/10.1016/j.cbpc.2015.01.001

Smith, P. B., Van Metre, D. C., & Pusterla, N. (2020). *Diseases of the nervous system*. Large Animal Internal Medicine, Mosby, E-book.

Stragierowicz, J., Kilanowicz, A., Sitarek, K., & Grobelski, B. (2018). The effect of prenatal exposure on disposition of hexachloronaphthalene in female Wistar rats and fetal compartment. *International Journal of Occupational Medicine and Environmental Health*, *31*, 685–695.

Suzuki, G., Michinaka, C., Matsukami, H., Noma, Y., & Kajiwara, N. (2020). Validity of using a relative potency factor approach for the risk management of dioxin-like polychlorinated naphthalenes. *Chemosphere*, *244*, 125448. https://doi.org/10.1016/j.chemosphere.2019.125448

Suzuki, G., Someya, M., Takahashi, S., Tanabe, S., Sakai, S. I., & Takigami, H. (2010). Dioxin-like activity in Japanese indoor dusts evaluated by means of in vitro bioassay and instrumental analysis: Brominated dibenzofurans are an important contributor. *Environmental Science and Technology*, *44*(21), 8330–8336.

Takeshita, R., & Yoshida, H. (1980). Environmental contamination by polychlorinated naphthalenes. VII. Distribution in rats. *Eisei Kagaku*, *26*, 307–312.

Taniyasu, S., Falandysz, J., Swietojańska, A., Flisak, M., Horii, Y., Hanari, N., & Yamashita, N. (2005). Clophen A60 composition and content of CBs, CNs, CDFs, and CDDs after 2D-HPLC, HRGC/LRMS, and HRGC/HRMS separation and quantification. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances and Environmental Engineering*, *40*, 43–61. https://doi.org/10.1081/ese-200,033,521

Tschiggfrei, K., Schächtele, A., Fernandes, A. R., Falandysz, J., van Duursen, M., van den Berg, M., & Malisch, R. (2023). WHO- and UNEP-coordinated exposure studies 2000–2019: Findings of polychlorinated Naphthalenes. In R. Malisch, P. Fürst, & K. Šebková (Eds.), *Persistent organic pollutants in human Milk*. Springer. https://doi.org/10.1007/978-3-031-34,087-1_11

Tysklind, M., Nystrom, M., Akerblom, N., Andersson, P. L., van Bavel, B., & Norrgren, L. (1998). Determination and modelling of biomagnification factors for polychlorinated naphthalenes (PCNs) in salmon (*salmon Salmo salar*). *Organohalogen Compounds*, *39*, 13–16.

UK-COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment). (2009). First Draft Statement on Polychlorinated Naphthalenes in Food.

US EPA (Environmental Protection Agency). (1980). Ambient Water Quality Criteria For Chlorinated Naphthalene. EPA 440/5–80-031.

Van de Plassche, E., & Schwegler, A. M. (2002). Polychlorinated naphthalenes. In Further assessment of persistent organic pollutants (POPs), compendium of substance-related information, convention on long-range transboundary air pollution, UN, economic commission for Europe, Geneva 2002 Feb 16 (pp. 1–17).

Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., & Peterson, R. E. (2006). The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences*, *93*(2), 223–241.

Villeneuve, D. L., Kannan, K., Khim, J. S., Falandysz, J., Nikiforov, V. A., Blankenship, A. L., & Giesy, J. P. (2000). Relative potencies of individual polychlorinated naphthalenes to induce dioxin-like responses in fish and mammalian in vitro bioassays. *Archives of Environmental Contamination and Toxicology*, *39*, 273–281. https://doi.org/10.1007/s002440010105

Villeneuve, D. L., Khim, J. S., Kannan, K., & Giesy, J. P. (2001). In vitro response of fish and mammalian cells to complex mixtures of polychlorinated naphthalenes, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons. *Aquatic Toxicology*, *54*, 125–141. https://doi.org/10.1016/s0166-445x(00)00171-5

Villeneuve, D. L., Khim, J. S., Kannan, K., & Giesy, J. P. (2002). Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. *Environmental Toxicology: An International Journal*, *17*(2), 128–137.

Vlahos, K., McEntee, K., Olafson, P., & Hansel, W. (1955). Destruction and restoration of spermatogenesis in a bull experimentally poisoned with highly chlorinated naphthalene. *The Cornell Veterinarian*, *45*, 198–209.

Waheed, S., Khan, M. U., Sweetman, A. J., Jones, K. C., Moon, H. B., & Malik, R. N. (2020). Exposure of polychlorinated naphthalenes (PCNs) to Pakistani populations via non-dietary sources from neglected e-waste hubs: A problem of high health concern. *Environmental Pollution*, *259*, 113838.

Wang, X., Han, Y., Cao, J., & Yan, H. (2022). Headspace solid-phase-microextraction using a graphene aerogel for gas chromatography–tandem mass spectrometry quantification of polychlorinated naphthalenes in shrimp. *Journal of Chromatography A*, *1672*, 463012.

Wang, X., Thai, P. K., Mallet, M., Desservettaz, M., Hawker, D. W., Keywood, M., Miljevic, B., Paton-Walsh, C., Gallen, M., & Mueller, J. F. (2017). Emissions of selected Semivolatile organic Chemicals from Forest and Savannah fires. *Environmental Science and Technology*, *51*, 1293–1302. https://doi.org/10.1021/acs.est.6b03503

Wang, Y., Zhang, S., Fan, M., Wang, R., Zou, Y., Wang, P., Cheng, J., & Dong, S. (2022). Polychlorinated naphthalenes in farmed Chinese mitten crabs in China: Concentration, distribution and source analysis. *Environmental Research*, *206*, 112582.

Wang, Y., Zhang, S., Wang, Y., Wu, X., Zou, Y., Zhou, W., Wang, P., Cheng, J., & Dong, S. (2022). Concentration and risk assessment of PCNs in green tea in different locations in China. *Science of The Total Environment*, *847*, 157587.

Ward, G. S., Parrish, P. R., & Rigby, R. A. (1981). Early life stage toxicity tests with a saltwater fish: Effects of eight chemicals on survival growth, and development of sheepshead minnows (*Cyprinodon variegatus*). *Journal of Toxicology and Environmental Health*, *8*, 225–240. https://doi.org/10.1080/15287398109530066

Weistrand, C., & Norén, K. (1998). Polychlorinated naphthalenes and other organochlorine contaminants in human adipose and liver tissue. *Journal of Toxicology and Environmental Health. Part A*, *53*, 293–311. https://doi.org/10.1080/009841098159295

Weistrand, C., Noren, K., & Nilsson, A. (1997). Occupational exposure–- organochlorine compounds in blood plasma from potentially exposed workers. *Environmental Science and Pollution Research*, *4*, 2–9. https://doi.org/10.1007/bf02986256

WHO (World Health Organization). (2001). Howe Paul D, Melber, Christine, Kielhorn, Janet, Mangelsdorf, Inge, World Health Organization. et al. (2001). Chlorinated naphthalenes. https://apps.who.int/iris/handle/10665/42403

WHO (World Health Organization). (2007). Fourth WHO-coordinated survey of human milk for persistent organic pollutants in cooperation with UNEP—Guidelines for developing a National Protocol. Revised 1 October 2007. https://www.who.int/foodsafety/chem/POPprotocol.pdf

WHO (World Health Organization). (2009). Environmental health criteria 240. In *Principles and methods for the risk assessment of chemicals in food*. WHO. https://www.who.int/publications/i/item/9789241572408WHO/IPCS (World Health Organization/International Programme on Chemical Safety). (2009). Principles and Methods for the Risk Assessment of Chemicals in Food, International Programme on Chemical Safety, Environmental Health Criteria 240. Chapter 6: Dietary Exposure Assessment of Chemicals in Food. https://www.who.int/ipcs/food/principles/en/index1.html

Witt, K., & Niessen, K. H. (2000). Toxaphenes and chlorinated naphthalenes in adipose tissue of children. *Journal of Pediatric Gastroenterology and Nutrition*, *30*, 164–169. https://doi.org/10.1097/00005176-200002000-00013

Wyrzykowska, B., Hanari, N., Orlikowska, A., Bochentin, I., Rostkowski, P., Falandysz, J., Taniyasu, S., Horii, Y., Jiang, Q., & Yamashita, N. (2007). Polychlorinated biphenyls and -naphthalenes in pine needles and soil from Poland–- concentrations and patterns in view of long-term environmental monitoring. *Chemosphere*, *67*, 1877–1886. https://doi.org/10.1016/j.chemosphere.2006.05.078

Wyrzykowska, B., Hanari, N., Orlikowska, A., Yamashita, N., & Falandysz, J. (2009). Dioxin-like compound compositional profiles of furnace bottom ashes from household combustion in Poland and their possible associations with contamination status of agricultural soil and pine needles. *Chemosphere*, *76*, 255–263. https://doi.org/10.1016/j.chemosphere.2009.03.019

Yamashita, N., Kannan, K., Imagawa, T., Miyazaki, A., & Giesy, J. P. (2000). Concentrations and profiles of polychlorinated naphthalene congeners in eighteen technical polychlorinated biphenyl preparations. *Environmental Science and Technology*, *34*, 4236–4241. https://doi.org/10.1021/es001122u

Yamashita, N., Taniyasu, S., Hanari, N., Horii, Y., & Falandysz, J. (2003). Polychlorinated naphthalene contamination of some recently manufactured industrial products and commercial goods in Japan. *Journal of Environmental Science and Health Part A, Toxic/Hazardous Substances and Environmental Engineering*, *38*, 1745–1759. https://doi.org/10.1081/ese-120,022,876

Zacs, D., Perkons, I., Pasecnaja, E., & Bartkevics, V. (2021). Polychlorinated naphthalenes (PCNs) in food products in Latvia: Congener-specific analysis, occurrence, and dietary exposure of the general population. *Chemosphere*, *264*, 128460. https://doi.org/10.1016/j.chemosphere.2020.128460

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., & Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environmental and Molecular Mutagenesis*, *19*(S21), 2–141.

Zeliger, H. I. (2011). Human toxicology of chemical mixtures. *In Utero Exposure*. https://doi.org/10.1016/B978-1-4377-3463-8.00006-0

Zhang, X., Gandhi, N., Bhavsar, S. P., & Bhavsar, S. P. (2016). Persistent Organohalogens in paired fish fillet and eggs: Implications for fish consumption advisories. *Journal of Agricultural and Food Chemistry*, *64*, 2832–2840.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: EFSA CONTAM Panel (EFSA Panel name on Contaminants in the Food Chain), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. R., Leblanc, J.-C., Nebbia, C. S., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Wallace, H., Falandysz, J., Hart, A., … Nielsen, E. (2024). Risks for animal and human health related to the presence of polychlorinated naphthalenes (PCNs) in feed and food. *EFSA Journal*, *22*(3), e8640. https://doi.org/10.2903/j.efsa.2024.8640

APPENDIX A

Physicochemical properties of PCNs congeners and mixtures of PC.

TABLE A.1 Physicochemical properties of PCNs congeners based on the WHO (2001), unless otherwise indicated.

Chlorinated naphthalenes	PCN number	CAS number	Relative molecular Mass (g/mol)	Boiling point (°C)	Melting point (°C)	Vapour pressure (KPa)	Aqueous solubility (μg/L)	Log$K_{o/w}$ octanol/water partition coefficient
Monochloro naphthalene (monoCN)		25586-43-0[a]	163					
1-monoCN	1	90-13-1		259	–2.3	2.1×10^{-3}[b] 3.9×10^{-3}[c] 3.8×10^{-3}[d]	2870	4.0 ± 0.2[e]; 3.9[f]
2-monoCN	2	91-58-7		259[g]	60	1.1×10^{-3}[h] 3.8×10^{-3}[d]	924	4.0 ± 0.2
Dichloro naphthalene (diCN)			197					
1,2-diCN	3	2050-69-3		297	36		137	4.6 ± 0.2[e]; 4.4
1,3-diCN	4	2198-75-6		291	62			4.7 ± 0.2[e]
1,4-diCN	5	1825-31-6		288	68	1.7×10^{-4}[b]	314; 309	4.7 ± 0.2[e]; 4.7; 4.9; 6.9[h]
1,5-diCN	6	1825-30-5			107		396	4.7 ± 0.2[e]; 4.7
1,6-diCN	7	2050-72-8			49			4.5 ± 0.3[e]
1,7-diCN	8	2050-73-9		286	64		235	4.8 ± 0.2[e]; 4.6
1,8-diCN	9	2050-74-0			89		590; 309	4.3 ± 0.3[e]; 4.2; 4.4
2,3-diCN	10	2050-75-1			119		862; 85	4.6 ± 0.2[e]; 4.5; 4.7
2,6-diCN	11	2065-70-5		285	141			4.6 ± 0.2[e]
2,7-diCN	12	2198-77-8			113–116		240	4.6 ± 0.2[e]; 4.8
Trichloro naphthalene (triCN)		1321-65-9	232					
1,2,3-triCN	13	50402-52-3			81			5.2 ± 0.2[e]
1,2,4-triCN	14	50402-51-2			92			5.3 ± 0.2[e]; 7.3[i]
1,2,5-triCN	15	55720-33-7			79			5.1 ± 0.3[e]
1,2,6-triCN	16	51570-44-6			92			4.9 ± 0.3[e]
1,2,7-triCN	17	55720-34-8			88			5.3 ± 0.2[e]
1,2,8-triCN	18	55720-35-9			84			4.9 ± 0.4[e]
1,3,5-triCN	19	51570-43-5			103			5.5 ± 0.2[e]; 7.3[i]
1,3,6-triCN	20	55720-36-0			81			5.1 ± 0.3[e]
1,3,7-triCN	21	55720-37-1		274[b]	113	1.3×10^{-4}[b]	64; 65	5.3 ± 0.3[e]; 5.4; 5.6

TABLE A.1 (Continued)

Chlorinated naphthalenes	PCN number	CAS number	Relative molecular Mass (g/mol)	Boiling point (°C)	Melting point (°C)	Vapour pressure (KPa)	Aqueous solubility (μg/L)	Log$K_{o/w}$ octanol/ water partition coefficient
1,3,8-triCN	22	55720-38-2			90			4.7 ± 0.4[e]
1,4,5-triCN	23	2437-55-0			71-72			5.0 ± 0.4[e]
1,4,6-triCN	24	2437-54-9			68			5.2 ± 0.3[e]
1,6,7-triCN	25	55720-39-3			109			5.2 ± 0.3[c]
2,3,6-triCN	26	55720-40-6			91		16.7	5.1 ± 0.3[e]; 5.1
Tetrachloro naphthalene (tetraCN)		1335-88-2	266					
1,2,3,4-tetraCN	27	20020-02-4			199		4.2	5.8 ± 0.33[e]; 5.8; 5.5
1,2,3,5-tetraCN	28	53555-63-8			141		3.7	5.8 ± 0.4[e]; 5.8
1,2,3,6-tetraCN	29	149864-78-8						5.4 ± 0.4[e]
1,2,3,7-tetraCN	30	55720-41-7			115			5.7 ± 0.3[e]
1,2,3,8-tetraCN	31	149864-81-3						5.3 ± 0.4[e]
1,2,4,5-tetraCN	32	6733-54-6						5.3 ± 0.4[e]; 8.6[i]
1,2,4,6-tetraCN	33	51570-45-7			111			5.7 ± 0.4[e]; 8.1[i]
1,2,4,7-tetraCN	34	67922-21-8			144			5.7 ± 0.4[e]; 8.1[i]
1,2,4,8-tetraCN	35	6529-87-9						5.6 ± 0.4[e]; 8.4[i]
1,2,5,6-tetraCN	36	67922-22-9			164			5.4 ± 0.4[e]
1,2,5,7-tetraCN	37	67922-23-0			114			5.8 ± 0.4[e]; 8.1[i]
1,2,5,8-tetraCN	38	149864-80-2						5.4 ± 0.4[e]; 8.4[i]
1,2,6,7-tetraCN	39	149864-79-9						5.5 ± 0.4[e]
1,2,6,8-tetraCN	40	67922-24-1			125–127			5.3 ± 0.4[e]
1,2,7,8-tetraCN	41	149864-82-4						5.5 ± 0.4[e]
1,3,5,7-tetraCN	42	53555-64-9			180		4.0; 4.3	6.1 ± 0.3[e]; 6.2; 6.4
1,3,5,8-tetraCN	43	31604-28-1			131		8.2; 8.3	5.5 ± 0.4[e]; 5.8; 6.0
1,3,6,7-tetraCN	44	55720-42-8			119			5.6 ± 0.4[e]
1,3,6,8-tetraCN	45	150224-15-0						5.1 ± 0.5[e]
1,4,5,8-tetraCN	46	3432-57-3			182–184			5.2 ± 0.5[e]; 8.5[i]
1,4,6,7-tetraCN	47	55720-43-9			139		8.1	5.7 ± 0.4[e]; 5.8; 8.1[i]
2,3,6,7-tetraCN	48	34588-40-4			203–204			5.4 ± 0.3[e]

(Continues)

TABLE A.1 (Continued)

Chlorinated naphthalenes	PCN number	CAS number	Relative molecular Mass (g/mol)	Boiling point (°C)	Melting point (°C)	Vapour pressure (KPa)	Aqueous solubility (μg/L)	Log$K_{o/w}$ octanol/water partition coefficient
Pentachloro naphthalene (pentaCN)		1321-64-8	300					
1,2,3,4,5-pentaCN	49	67922-25-2			169			6.0 ± 0.4[e]
1,2,3,4,6-pentaCN	50	67922-26-3			147			6.1 ± 0.4[e]; 8.9[i]
1,2,3,5,6-pentaCN	51	150224-18-3						6.0 ± 0.4[e]
1,2,3,5,7-pentaCN	52	53555-65-0		313[b]	171	4.2×10^{-6}[b]	7.3	6.3 ± 0.4[e]; 6.9[b]; 8.7[i]
1,2,3,5,8-pentaCN	53	150224-24-1						5.9 ± 0.5[e]; 9.1[i]
1,2,3,6,7-pentaCN	54	150224-16-1						5.9 ± 0.4[e]
1,2,3,6,8-pentaCN	55	150224-23-0						5.55 ± 0.5[e]
1,2,3,7,8-pentaCN	56	150205-21-3						5.8 ± 0.4[e]
1,2,4,5,6-pentaCN	57	150224-20-7						5.7 ± 0.5[e]
1,2,4,5,7-pentaCN	58	150224-19-4						5.8 ± 0.5[e]; 8.9
1,2,4,5,8-pentaCN	59	150224-25-2						5.7 ± 0.5[e]; 9.2[i]
1,2,4,6,7-pentaCN	60	150224-17-2						6.1 ± 0.4[e]; 8.7[i]
1,2,4,6,8-pentaCN	61	150224-22-9						6.0 ± 0.4[e]; 8.8[i]
1,2,4,7,8-pentaCN	62	150224-21-8						6.0 ± 0.4[e]; 9.1[i]
Hexachloro naphthalene (hexaCN)		1335-87-1	335			3×10^{-8}[b]		
1,2,3,4,5,6-hexaCN	63	58877-88-6						6.2 ± 0.5[e]; 10[i]
1,2,3,4,5,7-hexaCN	64	67922-27-4		331[b]	194	9.5×10^{-7}[b]	0.11[b]	6.3 ± 0.5[e]; 7.6[b]; 9.8[i]
1,2,3,4,5,8-hexaCN	65	103426-93-3						6.1 ± 0.6[e]; 10[i]
1,2,3,4,6,7-hexaCN	66	103426-96-6						6.4 ± 0.4[e]; 9.7[i]
1,2,3,5,6,7-hexaCN	67	103426-97-7						6.4 ± 0.4[e]; 9.7[i]
1,2,3,5,6,8-hexaCN	68	103426-95-5						6.1 ± 0.5[e]; 9.8[i]
1,2,3,5,7,8-hexaCN	69	103426-94-4						6.4 ± 0.5[e]; 9.8[i]
1,2,3,6,7,8-hexaCN	70	17062-87-2						6.0 ± 0.5[e]
1,2,4,5,6,8-hexaCN	71	90948-28-0			175–177			6.0 ± 0.6[e]; 7.0[j]; 9.9[i]
1,2,4,5,7,8-hexaCN	72	103426-92-2						6.1 ± 0.6[e]; 9.9[i]

TABLE A.1 (Continued)

Chlorinated naphthalenes	PCN number	CAS number	Relative molecular Mass (g/mol)	Boiling point (°C)	Melting point (°C)	Vapour pressure (KPa)	Aqueous solubility (μg/L)	Log$K_{o/w}$ octanol/water partition coefficient
Heptachloro naphthalene (heptaCN)		32241-08-0	369					
1,2,3,4,5,6,7-heptaCN	73	58863-14-2			160–162			6.4 ± 0.5[e]; 7.7[j]
1,2,3,4,5,6,8-heptaCN	74	58863-15-3		348[b]	194	3.7×10^{-7}[b]	0.04[b]	6.3 ± 0.6[e]; 8.3[b]
1,2,3,4,5,6,7,8-Octachloro naphthalene (octaCN)	75	2234-13-1[k]	404	365[b]	185–197	1.3×10^{-7}[b]	0.08	6.5 ± 0.7[e]; 6.4; 8.4[l]

[a]CAS number for monoCN is identical with CAS number for Halowax 1031.
[b]Indicates estimated value.
[c]Schoene et al. (1984).
[d]Lei et al. (1999).
[e]SciFinder; predicted value for partition co-efficient.
[f]Mackay et al. (1982).
[g]120°C at 11 Torr in Scifinder.
[h]Budavari et al. (1996).
[i]Harner and Bidleman (1998).
[j]Burreau et al. (1997).
[k]CAS number for octaCN is identical with CAS number for Halowax 1051.
[l]Opperhuizen et al. (1985).

TABLE A.2 Physicochemical properties of technical mixtures of PCNs based on the WHO (2001), unless otherwise indicated.

Chlorinated naphthalenes	CAS number	Chlorine content (%)	Chlorinated naphthalene composition (% weight)	Boiling point (as reported)[a] (°C)	Melting point (as reported) (°C)[a]	Vapour pressure (KPa)	Aqueous solubility (μg/L)
Halowaxes							
Halowax 1031	25586-43-0[b]	22	95% mono-, 5% di-	250[c] 259[d]	–25[d]	1.9×10^{-3}[e]	Insoluble[c]
Halowax 1000	58718-66-4	26	60% mono-, 40% di- (SciFinder: chlorinated naphthalene mixture containing 1% naphthalene, 51% monochloro and 48% dichloro derivatives)	250[c]	–33[d]		Insoluble[c]
Halowax 1001	58718-67-5	50	10% di-, 40% tri-, 40% tetra-, 10% penta- (SciFinder: A mixt. of polychlorinated naphthalenes contg. 4% di-Cl, 63% tri-Cl,30% tetra-Cl and 2% penta-Cl derivatives)	308[c]	98 93[d]		Insoluble[c]
Halowax 1099	39450-05-0	52	10% di-, 40% tri-, 40% tetra-, 10% penta- (SciFinder: A chlorinated naphthalene mixture containing 2% dichloro, 48% trichloro, 43% tetrachloro and 7% pentachloro derivatives)	315[c]	102		Insoluble[c]
Halowax 1013	12616-35-2	56	10% tri-, 50% tetra-, 40% penta-	328[c]	120		Insoluble[c]
Halowax 1014	12616-36-3	62	20% tetra-, 40% penta-, 40% hexa- (SciFinder: A chlorinated naphthalene contg. 1% di-Cl, 3% tri-Cl, 10% tetra-Cl, 42% penta-Cl, 38% hexa-Cl & 7% hepta-Cl derivs)	344[c]	137		Insoluble[c]
Halowax 1051	2234-13-1[f]	70	10% hepta-, 90% Octa-	246[d]	185 185–197[d]	6.8×10^{-8}[d] (predicted)	
Nibren waxes	12698-79-2		(SciFinder: A commercial mixt. of chlorinated naphthalenes)				
D88					90		
D116N					113		
D130					135		
Seekay waxes[g]							
68 (R Grade)		46.5					
93 (R Grade)		50					
123 (R Grade)		56.5					
700 (R Grade)		43					
93 (RC Grade)		50					
123 (RC Grade)		56.5					

TABLE A.2 (Continued)

Chlorinated naphthalenes	CAS number	Chlorine content (%)	Chlorinated naphthalene composition (% weight)	Boiling point (as reported)[a] (°C)	Melting point (as reported) (°C)[a]	Vapour pressure (KPa)	Aqueous solubility (μg/L)
Clonacire waxes							
90					90		
115					115		
130					130		
Woskol							

[a]Mean of a range of values reported.
[b]CAS number for Halowax 1031 is identical with CAS number for monoCN.
[c]Brinkman and De Kok (1980).
[d]SciFinder.
[e]Estimated value.
[f]CAS number for Halowax 1051 is identical with CAS number for octaCN.
[g]R Grade = refined or white wax; RC Grade = electrical grade.

APPENDIX B

Literature search to identify studies on PCNs

Literature searches were performed for PCNs, to identify information on physicochemical properties, production and industrial use, environmental fate and levels, analytical methods and occurrence data, toxicokinetics, in vitro toxicological studies, studies in experimental animals, food- and non-food-producing animals, studies on genotoxicity and mode of action, and human data.

Searches were done from 1950 onwards for all areas except from human studies where years from 1900 onwards were considered. The search focus on all types of documents as reflected in Table B.1.

The first literature search was performed in July 2021 and updates of the search were done on 20 March 2022, on 20 May 2023 and on 11 September 2023, using the same search string. Since that date, the literature was monitored to identify studies relevant for the risk assessment until the time of endorsement. The outcome of the searches was saved in separate EndNote files and an automatic duplicate detection run. The references were then screened to remove duplicates. The selection for relevance based on title and abstract and full text was done by EFSA staff and WG members.

Additionally, relevant scientific evaluations by national or international bodies were considered for the current risk assessment, e.g. US-EPA, the UN-EP, ILO and WHO, Dutch Ministry of VROM/DGM, UK-COT, FSAI, RIVM, etc.). When relevant papers were identified during the risk assessment process (e.g. from other studies or reviews), they were also considered. In addition, the draft scientific opinion underwent a public consultation from 24 November 2023 to 14 January 2024. The comments received and how they were taken into account when finalising the scientific opinion were published in Annex E.

TABLE B.1 Details of the literature search.

Databases interrogated:	WoS – Web of Science, PubMED and SciFinder
Year:	1950-date of the search (26.7.2021)
Languages:	English
Type of documents:	Peer-reviewed primary research studies (i.e. studies generating new data), systematic reviews, reviews, case reports and case studies, meta-analyses, extended abstracts, conference proceedings, book chapters and PhD theses, expert opinions, editorials, letters to the editor
Keywords in the search strings:	WoS – Web of Science, PubMED: "Polychlorinated naphthalene*" OR "*chlorinated naphthalene*" OR chloronaphthalene* OR chloro-naphthalene* OR *chloronaphthalene* OR *chloro-naphthalene* OR halowax* OR "nibren wax*" OR "Seekay wax*" OR "See-kay wax*" OR monoCN OR monoCNs OR mono-CN OR mono-CNs OR "mono CN" OR "mono CNs" OR DiCN OR DiCNs OR Di-CN OR Di-CNs OR "di CN" OR "di CNs" OR TriCN OR TriCNs OR Tri-CN OR Tri-CNs OR "Tri CN" OR "Tri CNs" OR tetraCN OR tetraCNs OR tetra-CN OR tetra-CNs OR "tetra CN" OR "tetra CNs" OR pentaCN OR pentaCNs OR penta-CN OR penta-CNs OR "penta CN" OR "penta CNs" OR hexaCN OR hexaCNs OR hexa-CN OR hexa-CNs OR "hexa CN" OR "hexa CNs" OR heptaCN OR heptaCNs OR hepta-CN OR hepta-CNs OR "hepta CN" OR "hepta CNs" OR OctaCNOr OctaCNs OR Octa-CN OR Octa-CNs OR "octa CN" OR "octa CNs" SciFinder: CAS NUMBER: 25586-43-0 OR 90-13-1 OR 91-58-7 OR 2050-69-3 OR 2198-75-6 OR 1825-31-6 OR 1825-30-5 OR 2050-72-8 OR 2050-73-9 OR 2050-74-0 OR 2050-75-1 OR 2065-70-5 OR 2198-77-8 OR 1321-65-9 OR 50402-52-3 OR 50402-51-2 OR 55720-33-7 OR 51570-44-6 OR 55720-34-8 OR 55720-35-9 OR 51570-43-5 OR 55720-36-0 OR 55720-37-1 OR 55720-38-2 OR 2437-55-0 OR 55720-39-3 OR 55720-40-6 OR 1335-88-2 OR 20020-02-4 OR 53555-63-8 OR 55720-41-7 OR 51570-45-7 OR 67922-21-8 OR 67922-22-9 OR 67922-23-0 OR 67922-24-1 OR 53555-64-9 OR 31604-28-1 OR 55720-42-8 OR 3432-57-3 OR 55720-43-9 OR 1321-64-8 OR 67922-25-2 OR 67922-26-3 OR 53555-65-0 OR 1335-87-1 OR 58863-15-3 OR 2234-13-1 OR 25586-43-0 OR 58718-66-4 OR 58718-67-5 OR 39450-05-0 OR 12616-35-2 OR 12616-36-3
N hits	A total of 1038 references (after duplicate removal in EndNote and DistillerSR) underwent title/abstract screening and were divided per field of interest: • Chemical identification, characterisation and formation • Environmental fate • Toxicokinetics • Toxicological studies in vitro • Toxicological studies in experimental animals • Studies on food- and non-food-producing animals • Genotoxicity and mode of action • Human studies • Analytical methods • Occurrence data in feed and food
Grey literature:	Assessments done by national and international bodies (e.g. in Google or from papers)

APPENDIX C

Intakes and composition of diets used to estimate animal exposure to PCNs

The feed intake and the diet composition used to estimate the exposure to PCN of the animal species considered in this report were derived from information extensively described by the CONTAM Panel in previous Scientific Opinions on the risks for animal and public health (EFSA CONTAM Panel, 2011, 2012) and modified by the CONTAM Panel in May 2023 in line with current with common practices and published guidelines. In particular, the amendments aimed at a harmonisation between CONTAM Panel and the FEEDAP Panel when dealing with compounds in feed. The estimated feed intakes are based on published guidelines on nutrition and feeding (EFSA FEEDAP Panel, 2017; Leeson and Summers, 2008; NRC, 2007).

Diets, feed intakes and body weights for the various animal species and categories are summarised in this Appendix.

In addition, the diets for food-producing and non-food-producing animals also include the calculated lower bound (LB) and upper bound (UB) mean and high concentrations for PCN, based on the LB and UB mean and high (P90–P95) concentrations in the feedingstuff reported in Table 8.

C.1 | FEED INTAKE

C.1.1 | Cattle, sheep, goats and horses

TABLE C.1 Default values for live weight and feed intake of ruminants and horses.

	Live weight (kg)	Feed intake (kg/day)		Reference
		Dry matter (DM)	Complete feed[a] (CF)	
Cattle				
Dairy cows	650	20.0	22.7	EFSA (2017)
Cattle for fattening	400	8.0	9.2	EFSA (2017)
Veal calves	100	1.89	2.0[b]	EFSA (2017)
Small ruminants				
Dairy sheep/goat	60.0	1.20	1.36	EFSA (2017)
Lambs for fattening	20.0	1.10	1.25	NRC (2007)
Horses				
All categories	400	8.00	9.1	EFSA (2017)

[a]88% dry matter. The reference to PCN doses in 'feed' in this Opinion needs to be understood as 'complete feed', as laid down in Reg (EC) 767/2009 ('compound feed which, by reason of its composition, is sufficient for a daily ratio').

[b]Milk replacer (94.5% dry matter).

C.1.2 | Pigs, poultry, fish and rabbit

TABLE C.2 Default values for live weight and feed intake of pigs, poultry, fish and rabbits.

	Live weight (kg)	Feed intake (kg/day)		Reference
		Dry matter (DM)	Complete feed (CF)	
Pigs				
Piglets (weaned)	20	0.88	1.0	EFSA (2017)
Pigs for fattening	60	2.20	2.5	EFSA (2017)
Sows, lactating	175	5.28	6.0	EFSA (2017)
Poultry				
Chickens for fattening	2.0	0.158	0.18	EFSA (2017)
Laying hens	2.0	0.106	0.12	EFSA (2017)
Turkeys for fattening	3.0	0.176	0.20	EFSA (2017)
Ducks, fattening	3.0	0.132	0.15	Leeson Summers (2008)
Fish				
Salmonids	0.12	0.0021	0.0024	EFSA (2017)
Rabbits				
Rabbits for fattening	2.0	0.10	0.114	EFSA (2017)

C.1.3 | Dogs and cats

TABLE C.3 Default values for live weight and feed intake of dogs and cats.

	Live weight (kg)	Feed intake (kg/day)		Reference
		Dry matter (DM)	Complete feed (CF)	
Dogs	15	0.25	0.284	EFSA (2017)
Cats	3	0.06	0.068	EFSA (2017)

C.2 | DIET COMPOSITION AND PCN CONCENTRATION ESTIMATES

C.2.1 | Cattle, sheep, goats and horses

TABLE C.4 Composition of complementary[a] feed for bovines.

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	Dairy cow	Cattle for fattening		Dairy cow	Cattle for fattening
Cereal grains and products derived thereof	55	60	Wheat	15	
			Wheat feed	10	10
			Barley	20	40
			Maize gluten feed	10	10
Oil seeds, oil fruits and products derived thereof	26	22	**Linseed**	**25**	**20**
			Rapeseed meal	**0.00**	**0.00**
			Vegetable oils and fats	1	2
Tubers, roots and products derived thereof	11	15	Sugar beet pulp	8	12
			Molasses	3	3
Legume seeds and products derived thereof	5		Beans	5	
Minerals and products derived thereof	2.5	2.5	Mineral salts	2.5	2.5
Feed additives	0.5	0.5	Premix	0.5	0.5
Concentrate: Forages (DM)				**70: 30**	**20: 80**
PCN[b]					
Mean lower bound (ng/kg feed)				0.002	0.000
Mean upper bound (ng/kg feed)				0.003	0.001
High lower bound (ng/kg feed)				0.001	0.001
High upper bound (ng/kg feed)				0.004	0.001

Substitutions of feed materials are highlighted in bold.

[a]For bovines, caprines, ovines and horses the term "complementary" is used to indicate that for these animals the diet should be complemented with forages and roughages.

[b]PCN concentration present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 8).

TABLE C.5 Composition of complementary feed for caprines.

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	Dairy goat	Kids for fattening		Dairy goat	Kids for fattening
Cereal grains and products derived thereof	70	70	Wheat feed	10	10
			Barley	25	20
			Oats	35	40
Oil seeds, oil fruits and products derived thereof	22	22	**Linseed**	**20**	**20**
			Rapeseed meal	**0.00**	**0.00**
			Vegetable oils and fats	2	2
Tubers, roots and products derived thereof	5	5	Sugar beet pulp	2	2
			Molasses	3	3
Minerals and products derived thereof	2.5	2.5	Mineral salts	2.5	2.5
Feed additives	0.5	0.5	Premix	0.5	0.5
Concentrate: Forages (DM)				**75: 25**	**40: 60**
PCN					
Mean lower bound (ng/kg feed)				0.002	0.001
Mean upper bound (ng/kg feed)				0.004	0.002
High lower bound (ng/kg feed)				0.002	0.001
High upper bound (ng/kg feed)				0.005	0.002

Substitutions of feed materials are highlighted in bold.

TABLE C.6 Composition of complementary feed for ovines.

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	Dairy sheep	Lambs for fattening		Dairy sheep	Lambs for fattening
Cereal grains and products derived thereof	47	70	Wheat	14	20
			Wheat feed	15	10
			Barley	18	20
			Oats		20
Oil seeds, oil fruits and products derived thereof	20	20	**Linseed**	**19.00**	**19.00**
			Rapeseed meal	**0.00**	**0.00**
			Sunflower meal	**0.00**	**0.00**
			Vegetable oils and fats	1	1
Tubers, roots, and products derived thereof	20	5	Sugar beet pulp	10	2
			Molasses	5	3
Legume seeds and products derived thereof	10	2	Beans	10	2
Minerals and products derived thereof	2.5	2.5	Mineral salts	2.5	2.5
Feed additives	0.5	0.5	Premix	0.5	0.5
Concentrate: Forages (DM)				**35: 65**	**50: 50**
PCN					
Mean lower bound (ng/kg feed)				0.001	0.001
Mean upper bound (ng/kg feed)				0.001	0.002
High lower bound (ng/kg feed)				0.001	0.001
High upper bound (ng/kg feed)				0.001	0.002

Substitutions of feed materials are highlighted in bold.

TABLE C.7 Composition of complementary feed for horses.

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Cereal grains and products derived thereof	82	Oats	40
		Oat feed	12
		Wheat feed	30
Tubers, roots, and products derived thereof	5	Molasses	5
Legume seeds and products derived thereof	10	Beans	10
Minerals and products derived thereof	2.5	Mineral salts	2.5
Feed additives	0.5	Premix	0.5
Concentrate: Forages (DM)			**25: 75**
PCN			
Mean lower bound (ng/kg feed)			0.000
Mean upper bound (ng/kg feed)			0.000
High lower bound (ng/kg feed)			0.000
High upper bound (ng/kg feed)			0.000

C.2.2 | Pigs, poultry, fish and rabbit

TABLE C.8 Composition of complete feed for piglets, pigs for fattening and lactating sows.

Groups according to REG (EU) 2022/1104	% of diet				Composition (%)		
	Piglet	Pig	Sow	Feed material	Piglet	Pig	Sow
Cereal grains and products derived thereof	68	77	75	Wheat	48	48	50
				Wheat feed		9	14
				Barley	20	20	11
Oil seeds, oil fruits and products derived thereof	26	16	18	**Linseed**	**25.00**	**15.00**	**16.00**
				Rapeseed meal	**0.00**	**0.00**	
				Vegetable oils and fats	1	1	2
Tubers, roots and products derived thereof	3	4	4	Sugar beet pulp			
				Molasses	3	4	4
Minerals and products derived thereof	2.5	2.5	2.5	Mineral salts	2.5	2.5	2.5
Feed additives	0.5	0.5	0.5	Premix	0.5	0.5	0.5
PCN							
Mean lower bound (ng/kg feed)				0.002	0.001	0.002	
Mean upper bound (ng/kg feed)				0.004	0.003	0.004	
High lower bound (ng/kg feed)				0.001	0.001	0.003	
High upper bound (ng/kg feed)				0.004	0.003	0.005	

Substitutions of feed materials are highlighted in bold.

TABLE C.9 Composition of complete feed for chickens, turkeys and ducks for fattening.

Groups according to REG (EU) 2022/1104	% of diet			Feed material	Composition (%)		
	Chickens	Turkeys	Ducks		Chickens	Turkeys	Ducks
	For fattening				For fattening		
Cereal grains and products derived thereof	75	65	65	Wheat	38	30	35
				Wheat feed	1		5
				Barley		35	25
				Maize	36		
Oil seeds, oil fruits and products derived thereof	20	20	20	**Linseed**	**15**	**16**	**18**
				Vegetable oils and fats	5	4	2
Tubers, roots and products derived thereof	2	2	3	Molasses	2	2	3
Forage dehydrated		10	9	**Green meal**		10	9
Minerals and products derived thereof	2.5	2.5	2.5	Mineral salts	2.5	2.5	2.5
Feed additives	0.5	0.5	0.5	Premix	0.5	0.5	0.5
PCN							
Mean lower bound (ng/kg feed)					0.003	0.019	0.017
Mean upper bound (ng/kg feed)					0.006	0.041	0.036
High lower bound (ng/kg feed)					0.007	0.056	0.048
High upper bound (ng/kg feed)					0.009	0.065	0.056

Substitutions of feed materials are highlighted in bold.

TABLE C.10 Composition of complete feed for laying hens.

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Cereal grains and products derived thereof	65.00	Maize	25.00
		Wheat	30.00
		Wheat middlings	10.00
Oil seeds, oil fruits and products derived thereof	20.00	**Linseed**	**18.00**
		Rapeseed	**0.00**
		Vegetable oils and fats	2.00
Forage dehydrated	3.00	**Green meal**	3.00
Tubers, roots and products derived thereof	2.00	Molasses	2.00
Minerals and products derived thereof	9.50	Mineral salts	9.50
Feed additives	0.50	Premix	0.50
PCN			
Mean lower bound (ng/kg feed)			0.007
Mean upper bound (ng/kg feed)			0.015
High lower bound (ng/kg feed)			0.018
High upper bound (ng/kg feed)			0.022

Substitutions of feed materials are highlighted in bold.

Note: Ash corrected: High dietary calcium necessary for eggshell therefore major changes made.

TABLE C.11 Composition of complete feed for salmons.[a]

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Fish, other aquatic animals and products derived thereof	60.00	Fish meal	33.00
		Fish oil	23.00
		Fish ensiled	4.00
Oil seeds, oil fruits and by-products	27.00	**Linseed**	**15.00**
		Vegetable oil and fat	12.00
Cereal grains products derived thereof	10.00	Wheat gluten	10.00
Minerals and products derived thereof	3.00	Mineral salts	3.00
Feed additives		Premix	
PCN			
Mean lower bound (ng/kg feed)			0.005
Mean upper bound (ng/kg feed)			0.010
High lower bound (ng/kg feed)			0.017
High upper bound (ng/kg feed)			0.019

Substitutions of feed materials are highlighted in bold.

[a]Ellingsen, H., Olaussen, J. O., & Utne, I. B. (2009). Environmental analysis of the Norwegian fishery and aquaculture industry—A preliminary study focusing on farmed salmon. Marine Policy, 33, 479–488.

TABLE C.12 Composition of complete feed for rabbits for fattening.

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Cereal grains and products derived thereof	25.00	Wheat	10.00
		Corn	5.00
		Wheat middlings	10.00
Forage dehydrated	20.00	**Green meal**	**20.00**
Oil seeds, oil fruits and products derived thereof	30.00	**Linseed**	**23.00**
		Soybean meal	**0.00**
		Soya (bean) hulls	7.00
Tubers, roots and products derived thereof	20.00	Sugar beet pulp	18.00
		Molasses	2.00
Land animal products and products derived thereof	2.00	Fat	2.00
Minerals and products derived thereof	2.50	Mineral salts	2.50
Feed additives	0.50	Premix	0.50
PCN			
Mean lower bound (ng/kg feed)			0.114
Mean upper bound (ng/kg feed)			0.133
High lower bound (ng/kg feed)			0.040
High upper bound (ng/kg feed)			0.085

Substitutions of feed materials are highlighted in bold.

C.2.3 | Dogs and cats

TABLE C.13 Composition of complete feed for dogs.

Groups according to REG (EU) 2022/1104	% of diet			Composition (%)	
	With meat	Vegetarian	Feed material	With meat	Vegetarian
Land animal products and products derived thereof	35.00		Animal by-products	2.50	
			Fish meal	5.00	
			Hydrolysed animal products[a]	1.00	
			Fat	5.00	
Cereal grains and products derived thereof	50.00	45.00	Rice	20.00	40.00
			Oats	10.00	
			Barley	10.00	
			Wheat protein	10.00	5.00

TABLE C.13 (Continued)

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	With meat	Vegetarian		With meat	Vegetarian
Oil seeds, oil fruits and products derived thereof	4.00	20.00	**Linseed**	**1.00**	**15.00**
			Soybean meal	**0.00**	**0.00**
			Vegetable oil and fat	3.00	5.00
Tubers, roots, and products derived thereof	5.00	15.00	Sugar beet pulp	5.00	5.00
			Potato protein		10.00
Forages and roughage, and products derived thereof	3.00	2.00	Herbs	3.00	2.00
Legume seeds and products derived thereof		10.00	Peas		5.00
			Carobs		5.00
Milk products and products derived thereof		5.00	Milk protein powder		5.00
Minerals and products derived thereof	2.50	2.50	Mineral salts	2.50	2.50
Feed additives	0.50	0.50	Premix	0.50	0.50
PCN					
Mean lower bound (ng/kg feed)				0.001	0.002
Mean upper bound (ng/kg feed)				0.002	0.004
High lower bound (ng/kg feed)				0.004	0.007
High upper bound (ng/kg feed)				0.005	0.008

Substitutions of feed materials are highlighted in bold.

[a]Includes poultry meal, lambs meal and fish meal.

TABLE C.14 Composition of complete feed for cats.

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Land animal products and products derived thereof	40.00	Animal by-products[a]	35.00
		Hydrolysed animal products[a]	1.00
		Fat	4.00
Cereal grains and products derived thereof	30.00	Rice	10.00
		Wheat middlings	10.00
		Wheat protein	10.00
Oil seeds, oil fruits and products derived thereof	5.00	**Linseed**	**5.00**
Tubers, roots and products derived thereof	12.00	Sugar beet pulp	2.00
		Potato protein	10.00
Legume seeds and products derived thereof	10.00	Peas	5.00
		Carobs	5.00
Minerals and products derived thereof	2.50	Mineral salts	2.50
Feed additives	0.50	Premix	0.50
PCN			
Mean lower bound (ng/kg feed)			0.000
Mean upper bound (ng/kg feed)			0.001
High lower bound (ng/kg feed)			0.000
High upper bound (ng/kg feed)			0.001

Substitutions of feed materials are highlighted in bold

[a]Includes poultry meal, lamb meal and fish meal.

C.3 | GROUPS OF FEED MATERIALS[33]

The list identifies which feed materials could be considered when the groups of feed materials are attributed to a compound feed for target animals.

[33]As per Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials.

Cereal grains and products derived thereof

Cereals:	Barley, maize, oats, broken rice, rye, triticale, wheat.
By-products:	From dry milling: middling's, feed, flakes, bran, hulls. From wet milling: starch, germ meal, gluten feed, gluten. From fermentation: DDG, DDGS, brewer's grains.

Oil seeds, oil fruits, and products derived thereof

Oil seeds:	Cotton seed, linseed, rape seed, soya beans, sunflower seed.
Main products:	Expeller, solvent extracted meal, extruded/toasted beans, flakes.
By-products:	Hulls, protein concentrate

Legume seeds and products derived thereof

Legumes seeds:	Beans, lentils, sweet lupins, peas.
By-products:	Protein/protein concentrate, germ, flakes, hulls.

Tubers, roots and products derived thereof

	Sugar beet, potatoes.
By-products:	Molasses, beet pulp, protein, inulin.

Other seeds and fruits, and products derived thereof

	Acorn, almond, buckwheat, red clover seed, white clover seed.
By-products.	Apple pulp, citrus pulp, grape pulp, middling's, bran/hulls, pectin.

Forages and roughage, and products derived thereof

	Beet leaves, green silage, lucerne (alfalfa) meal.
By-products:	Hay, straw, maize silage.

Other plants, algae, fungi and products derived thereof

	Algae, seaweed, fungi.
By-products.	Sugar cane molasses, cellulose.

Milk products and products derived thereof

Butter, buttermilk, skimmed milk powder, whey/whey powder, delactosed (and demineralised) whey, casein, whey protein, lactose, whey permeate.

Land animal products and products derived thereof

Animal by products, animal fat, blood meal, feather meal, gelatine, egg products, dried, Terrestrial invertebrates.

Fish, other aquatic animals and products derived thereof

Crustacea meal, fish meal, fish solubles, fish protein, fish oil, krill protein concentrate.

Minerals and products derived thereof

Products and co-products obtained by fermentation using microorganisms

Yeast (brewer's yeast), single cell protein (bacterial or fungal origin).

Miscellaneous

Products from the bakery and pasta industry, fruit syrup, dextrose, fructose, xylose, lactulose, Gluco/fructo-oligosaccharides, starch, dextrins, sorbitol, Fatty acids esterified with glycerol, soap stocks, glycerine, propylene glycol, chondroitin sulfate.

C.4 | ESTIMATED INTAKE OF PCN[34] USING MEAN AND HIGH, LB AND UB PCN CONCENTRATIONS IN FEEDINGSTUFFS

TABLE C.15 Estimated exposure to PCN using Mean LB/UB and High LB/UB by a 650-kg body weight lactating dairy cow, a 400-kg body weight Cattle for fattening and a 100-kg body weight Veal calf, using model diets.

	Exposure											
	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day
	High		Mean		High		Mean		High		Mean	
	Dairy cow				Cattle for fattening				Veal calf[a]			
Model diet plus forages												
LB	0.022	< 0.001	0.030	< 0.001	0.005	< 0.001	0.004	< 0.001	n/a	n/a	n/a	n/a
UB	0.073	< 0.001	0.067	< 0.001	0.010	< 0.001	0.008	< 0.001	n/a	n/a	n/a	n/a

[a]For veal calf, it was not possible to estimated exposure to PCN no samples of milk replacer were reported for this animal category.

TABLE C.16 Estimated exposure to PCN using mean LB/UB and high LB/UB by a 60-kg body weight Dairy sheep/goat, by a 20-kg body weight lamb for fattening and 400-kg body weight horse, using model diets.

	Exposure											
	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day
	High		Mean		High		Mean		High		Mean	
	Dairy sheep/goats				Lambs for fattening				Horses[a]			
Model diet plus forages												
LB	0.001/0.003	< 0.001/< 0.001	0.001/0.002	< 0.001/< 0.001	0.001	< 0.001	0.001	< 0.001	n/a	n/a	n/a	n/a
UB	0.002/0.005	< 0.001/< 0.001	0.002/0.004	< 0.001/< 0.001	0.002	< 0.001	0.002	< 0.001	n/a	n/a	n/a	n/a

[a]No feed materials among those included in the horse model diet were available to derive an exposure to PCN on horses.

[34]Sum of hexaCNs.

TABLE C.17 Estimated exposure to PCN using mean LB/UB and high LB/UB by a 20-kg body weight weaned piglet, a 60-kg body weight pig for fattening and a 175-kg body weight lactating sow using model diets.

	Exposure											
	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day
	High		Mean		High		Mean		High		Mean	
	Weaned piglet				Pigs for fattening				Lactating sow			
Model diet												
LB	1.408	0.070	1.892	0.095	3.520	0.059	3.190	0.053	16.896	0.097	10.138	0.058
UB	4.488	0.224	4.206	0.210	8.140	0.136	6.996	0.117	28.723	0.164	21.754	0.124

TABLE C.18 Estimated exposure to PCN using mean LB/UB and high LB/UB by a 2-kg body chicken for fattening and a 2-kg body weight Laying hen, using model diets and complete feeds.

	Exposure							
	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day
	High		Mean		High		Mean	
	Chickens for fattening				Laying hen			
Model diet								
LB	1.264	0.632	0.481	0.240	2.146	1.073	0.834	0.417
UB	1.607	0.804	0.999	0.499	2.675	1.338	1.770	0.885

TABLE C.19 Estimated exposure to PCN using mean LB/UB and high LB/UB by a 3-kg body weight turkey and a 3-kg body weight duck for fattening, using model diets and complete feeds.

	Exposure							
	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day
	High		Mean		High		Mean	
	Turkeys for fattening				Ducks for fattening			
Model diet								
LB	11.126	3.709	3.886	1.295	7.172	2.391	**2.572**	0.857
UB	12.934	4.311	8.196	2.732	8.461	2.820	5.444	1.815

TABLE C.20 Estimated exposure to PCN using mean LB/UB and high LB/UB by a 2-kg body weight rabbit for fattening and a 0.12-kg body weight salmon, using model diets and complete feeds.

	Exposure							
	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day
	High		Mean		High		Mean	
	Rabbits for fattening				Salmon			
Model diet								
LB	11.364	5.682	4.034	2.017	0.040	0.336	0.012	0.102
UB	13.277	6.638	8.541	4.271	0.045	0.373	0.025	0.206

TABLE C.21 Estimated exposure to PCN using Mean LB/UB and High LB/UB by a 15-kg body weight dog and a 3-kg body weight cat, using model diets and complete feeds.

	Exposure											
	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day	pg/day	pg/kg/bw per day
	High		Mean		High		Mean		High		Mean	
	Dog (meat diet)				Dog (vegetarian diet)				Cats			
Model diet												
LB	1.200	0.080	0.353	0.024	2.000	0.133	0.588	0.039	0.000	0.000	0.021	0.007
UB	1.305	0.087	0.705	0.047	2.175	0.145	1.175	0.078	0.042	0.014	0.048	0.016

APPENDIX D

Previously reported occurrence data in the open literature

TABLE D.1 Studies reporting on occurrence of PCNs in food in European countries in the open literature.

Reference	Country sampling place	Sampling year	Total number of samples, LOD (wet weight) and other info	FOOD category or specific animal parts (number of samples)	Sum of PCNs analysed: Concentration: mean or range (ng/kg wet weight)	HexaCNs or PCN-66/67: Concentration: mean or range (ng/kg wet weight)	Included	Comments
Llobet et al. (2007) and Martí-Cid et al. (2007)	**Spain**	2005	*N* = 42 fish samples (14 species, 3 for each species) LOD = 0.8–2.1 ng/kg	Edible parts	3–227	HexaCNs 0.3–12.2	Tetra – Octa CNs	TetraCNs and PentaCNs major contributors for all species
Martí-Cid et al. (2008)	**Spain**	2006	*N* = 104 food samples LOD = 0.8–2.1 ng/kg	 Fish and seafood Oils and fats Bakery products Dairy products Pulses Cereals Eggs Vegetables Tubers Fruits Meat and meat products	 47.1 21.5 15.3 11.7 10.2 8.0 4.3 2.3 2.2 1.2 2.8	HexaCNs 3.0 1.6 1.0 1.3 0.4 0.2 0.3 0.2 0.2 0.1 0.2	Tetra – Octa CNs	
Domingo et al. (2003)	**Spain**	2000	*N* = 108 food samples LOD = 1–2 ng/kg dry weight	 Meat and meat products (30) Fish and shellfish (16) Vegetables (16) Tuber (4) Fruits (12) Eggs (4) Milk (4) Dairy products (4) Cereals (8) Pulses (4) Oil and fat (6)	 18 39 4 3 0.7 23 0.4 36 71 3 447	HexaCNs 2 7 0.6 0.6 0.2 3 0.05 1 21 1 7	Tetra – Octa CNs	TetraCNs are the predominant homologue for all food groups
Nfon et al. (2008)	**Baltic sea**	1991–1993	*N* = 12 fish samples (Herrings)	Whole fish	NR	NR	NR	Only trophic levels reported

TABLE D.1 (Continued)

Reference	Country sampling place	Sampling year	Total number of samples, LOD (wet weight) and other info	FOOD category or specific animal parts (number of samples)	Sum of PCNs analysed Concentration: mean or range (ng/kg wet weight)	HexaCNs or PCN-66/67 Concentration: mean or range (ng/kg wet weight)	Included	Comments
Koistinen et al. (1989)	**Finland** **Norway** Baltic Sea	1986–1988	*N* = 13 fish samples LOD = < 5 ng/kg	 Salmon Fillets (7) Pike fillets (2) Walleye fillets (1) Cod liver fat (3)	 < 20–10,000 30–700 20–200 < 20–600	HexaCNs < 20–100 30–40 20 < 20–200	Tetra – Hexa CNs	
Isosaari et al. (2006)	**Finland** Baltic Sea Internal lakes	2001–2003	*N* = 156 fish samples (several species)	Edible parts of Sea Fish (42) Lake fish (43)	40 17	NR	PCN-27, 36, 42, 48, 52, 53, 54, 66/67, 68, 70, 71/72, 73, 74, 75	
Falandysz and Fernandes (2020)	**Poland**	1972–2017	*N* = 7 samples of cod liver oil and canned liver Lipid % = oil (62.8) Liver (32.3)	Canned cod liver (2) Cod liver oil (5)	1160–2240 (lipid weight) 2.6–12,600 (lipid weight)	PCN-66/67 196–407 (lipid weight) 0.29–2500 (lipid weight)	PCN-13, 27, 42, 52/60, 53, 63, 65, 66/67, 64/80, 69, 70, 71/72, 73, 74, 75	PCN-42, –52/60, –66/67 were the dominant contributors
Falandysz, Strandberg, Strandberg, Bergqvist, and Rappe (1997)	**Poland** Baltic Sea	1992	*N* = 737 fish samples (different species) Lipid % = 1.3–22.6	Whole fish Lamprey (6) Perch (16) Flounder (15) Mussel (700)	(all lipid weight) 6300–8900 19,000–69,000 36,000–83,000 80,000–110,000	PCN-66/67 (all lipid weight) 400–520 520–1100 540–1200 270–300	Tetra–Octa CNs	
Falandysz et al. (1998)	**Poland** Baltic Sea	1992	*N* = 60 fish samples (three-spined sticklebacks) Lipid % = 2.4–2.7	Whole fish	35,000–130,000 (lipid weight)	PCN-66/67 370–590 (lipid weight)	Tetra–Octa CNs	
Fernandes et al. (2018)	**UK**	NR	*N* = 75 fish samples (7 marine species)	Edible parts	0.7–265	NR	PCN-52/60, 53, 66/67, 68, 69, 71/72, 73, 74, 75	
Fernandes et al. (2010)	**UK**	2007	*N* = 44 food samples LOD = 0.02–0.1 ng/kg	 Meat and meat products Fish Poultry Dairy products Other foods	 1.6 19.9 2.4 1.5 0.2–2.8	PCN-66/67 0.11 2.97 0.13 0.13 0.01	PCN-52, 53, 66/67, 68, 69, 71/72, 73, 74, 75	

(Continues)

TABLE D.1 (Continued)

Reference	Country sampling place	Sampling year	Total number of samples, LOD (wet weight) and other info	FOOD category or specific animal parts (number of samples)	Sum of PCNs analysed Concentration: mean or range (ng/kg wet weight)	HexaCNs or PCN-66/67 Concentration: mean or range (ng/kg wet weight)	Included	Comments
Rose et al. (2015)	**UK**	2008–2009	*N* = 46 fish samples LOD = 0.1 ng/kg	Edible parts of several species	1.1–1198	PCN-66/67 0.4–112	PCN-52/60, 53, 66/67, 68, 69, 71/72, 73, 74, 75	
Fernandes et al. (2011)	**Ireland**	2007–2008	*N* = 100 food samples LOD = 0.01–0.1 ng/kg	Fish Shellfish Milk Eggs Liver Dairy products Animal fat Other foods	21.8 1.1 0.2 0.6 0.9 1.8 3.7 0.6	PCN-66/67 0.13–4.84 < 0.01 0.01–0.04 0.03–0.09 0.01–1.96 0.16–1.42 0.17–2.26 < 0.01–0.14	PCN-52, 53, 66/67, 68, 69, 71/72, 73, 74, 75	
Kannan et al. (2002)	**Italy**	1999	*N* = fish samples (2 species) LOD = 1–75 ng/kg	Tuna fish parts Muscle (7) Liver (5) Fat (3) Swordfish parts Muscle (10) Liver (4)	 7.0–23.6 53.8 552 14.7 62.9	NR	Tri–Octa CNs	
Godéré et al. (2022)	**France**	2005	*N* = 37 fish samples (different species including crustaceans and molluscs) LOD = 0.16 ng/kg	Edible parts	1.9–440	PCN-66/67 0.03–30	Mono–Octa CNs	In total 69 PCNs
Zacs et al. (2021)	**Latvia**	2019–2020	*N* = 64 food samples	Fish and fish products (16) Fish oil (4) Bread (6) Dairy products (10) Meat (11) Vegetable oil (3) Baby food (4) Eggs (9)	 45.7 10.5 9.5 5.3 3.1 3.5 1.4 1.1	HexaCNs 8.85 0.37 0.12 0.12 0.17 0.24 0.03 0.21	Tetra–Octa CNs	26 congeners in total Tetra and PentaCNs were the major contributors

TABLE D.2 Studies reporting on occurrence of PCNs in food in Non-European countries in the open literature.

Reference	Country sampling place	Sampling year	Total number of samples, LOD (wet weight) and other info	FOOD category or specific animal parts (number of samples)	Sum of PCNs analysed	HexaCNs or PCN-66/67	Included	Comments
					Concentration: Mean or range (ng/kg wet weight)	Concentration: Mean or range (ng/kg wet weight)		
Pagano and Garner (2021)	USA Great lakes	2004–2018	*N* = 123 fish samples (trouts and walleye)	Lake trouts Whole fish (111) Walleye Whole fish (12)	139–1641 3947	NR	Tetra–Hexa CNs	In total 41 PCNs
Pagano and Garner (2020)	USA Lake Champlain	2012–2018 Excluding 2014	*N* = 60 fish samples (lake trouts)	Whole fish Fillet Eggs	635 487 154	NR	All PCNs	Tetra, Penta, HexaCNs (93% of average total PCNs)
Pagano and Garner (2019)	USA Cayuga Lake	2011–2017	N = 56 fish samples (lake trouts)	Whole fish (7)	516	NR	All PCNs	
Zhang et al. (2016)	USA Ontario lake	2007	*N* = 11 fish samples (salmon and trouts) LOD = 0.4–1.8 ng/Kg wet weight	Fillets (11) Eggs (11)	285–1666 48–663	PCN-66/67 34–450 5.8–200	PCN-42, 50, 52/60, 53, 54, 64/68, 66/67, 69, 70, 71/72, 73	
Kannan et al. (2000)	USA Great lakes	1996–1997	*N* = 26 fish samples (several species) LOD = 0.2 pg/g	Whole fish Fillet	250–31,400 19–1200	PCN-66/67 87–890 6–180	Tri–Octa CNs	
Gewurtz et al. (2009)	USA Lake Ontario	1979–2005	*N* = 29 fish samples (lake trout) Lipid % = 8.1–18.2	Whole fish	1000–120,000 (lipid weight)	PCN-66/67 1000–59,100 (lipid weight)	PCN-13, 27, 28, 36, 42, 46, 48, 49, 50, 52/60, 53, 54, 63, 64/68, 66/67, 69, 70, 71/72, 73, 74, 75	
Gewurtz et al. (2018)	USA/Canada Great lakes	2006–2013	*N* = 470 fish samples (18 fish species)	Fillet	6–6700	PCN-66/67 < 1–500	PCN-13, 27, 28, 36, 42, 46, 48, 49, 50, 52/60, 53, 54, 63, 64/68, 66/67, 69, 70, 71/72, 73, 74, 75	
McGoldrick et al. (2018)	Canada Great lakes	2010–2015	*N* = 80 fish samples (lake trout and walleye)	Whole fish	171–7660	HexaCNs 18–1230	All PCNs	
Kim et al. (2018)	South Korea	2012–2013	*N* = 165 fish samples (33 fish species including molluscs and crustaceans)	Edible parts of several species	ND-110	HexaCNs ND-14	Tetra–Octa CNs	OctaCN not detected

(Continues)

TABLE D.2 (Continued)

Reference	Country sampling place	Sampling year	Total number of samples, LOD (wet weight) and other info	FOOD category or specific animal parts (number of samples)	Sum of PCNs analysed Concentration: Mean or range (ng/kg wet weight)	HexaCNs or PCN-66/67 Concentration: Mean or range (ng/kg wet weight)	Included	Comments
Kim et al. (2019)	South Korea	NR	*N* = 60 fish samples (crucian carp) LOD = 0.02–2.43 pg/g ww in muscle, 0.03–0.52 pg/g in gonads, 0.01–0.29 pg/g in the liver, and 0.07–0.90 pg/mL in blood	Muscle (60) Gonad (18) Liver (18) Blood (18)	2.2 29.6 25.7 12.0	PCN-66/67* 0.1 1.7 1.6 0.4 (pg/mL)	Tri–Octa CNs	In total 41 PCNs *The values for PCN-66/67 are based on eye visualisation of figure 2 in the study
Jiang et al. (2007)	China	2003–2004	*N* = 23 fish samples (including other marine species) Lipid % = 0.59–2.80	Several species muscle and skin or whole body	93.8–1300 (lipid weight)	NR	Tri–Octa CNs	
Cui et al. (2018)	China	2014	*N* = 122 fish samples (17 species) LOD = 0.010 to 0.45 ng/kg	Only fillet (dorsal muscle tissue)	7.3–214	HexaCNs ND-2.7	All PCNs	20 congeners in total Di, Penta, and TetraCNs (58% of the average total PCNs)
Dong, Li, et al. (2022)	China	2021	*N* = 65 cow milk samples LOD for milk = 0.008–0.38 pg/g lipid weight Lipid % = 3.6	Milk	552 (lipid weight)	NR	All PCNs	
Wang, Zhang, Wang, et al. (2022)	China	2020	*N* = 102 tea samples LOD = 0.001–0.028 ng/Kg dry weight	Tea	36.1 (dry weight)	NR	All PCNs	DiCNs and TetraCNs were the dominant contributors
Wang, Han, Cao, and Yan (2022)	China	NR	*N* = 5 shrimps LOD = 0.00983–0.0661 ng/kg	Edible parts	ND-38.1	NR	PCN-1, –9, –27	
Wang, Zhang, Fan, et al. (2022)	China	2019	*N* = 36 mitten crabs LOD = 0.002–0.032 ng/kg	Edible parts	21.3	NR	All PCNs	DiCNs, TetraCNs and PentaCNs were the dominant contributors
Li et al. (2022)	China	2016–2020	*N* = 192 composite samples (17,280 total samples) LOD = 0.17–0.74 ng/kg	Fish Meat Eggs Milk Legumes Vegetables Cereals Potatoes	27.2 92.0 31.4 22.2 16.8 12.1 7.6 9.9	NR	All PCNs	DiCNs, triCNs, tetraCNs and pentaCNs were the dominant contributors

TABLE D.3 Studies reporting on occurrence of PCNs in feed in Non-European countries in the open literature.

Reference	Country sampling place	Sampling year	Total number of samples, LOD and other info	FEED category (number of samples)	Sum of PCNs analysed: Concentration: mean or range (ng/kg feed)	HexaCNs or PCN-66/67: Concentration: mean or range (ng/kg feed)	Included	Comments
Dong et al. (2018)	China	2016	*N* = 22 samples LOD = 0.13–2.30 ng/kg	Feed meal (12) Meat and bone meal (2) Chicken meal (1) Protein powder (2) Blood meal (1) Whey powder (1) Rapeseed meal (1) Peanut meal (1) Soybean meal (1)	1009 742 482 583 342 147 372 230 28	PCN-66/67 1.91 1.93 1.01 2.40 0.94 0.40 1.07 1.35 0.92	All PCNs	DiCNs and TriCNs were the dominant contributors in all samples
Dong, Zhang, et al. (2022)	China	2021	*N* = 33 samples LOD = 0.004–0.09 ng/kg	Feed ingredients of plant origin for cow	79.6	NR	All PCNs	DiCNs and TriCNs were the dominant contributors in all samples
Wang, Zhang, Fan, et al. (2022)	China	2019	*N* = 22 samples LOD = 0.007–0.051 ng/kg	Feed ingredients for crab	89.5	NR	All PCNs	DiCNs, TriCNs and TetraCNs were the dominant contributors
Guruge et al. (2004)	Japan	2001	*N* = 30 samples LOD = 0.01–0.05 ng/kg (lipid weight) Lipid % = 3.6–100	Feed ingredients (fish oil, fish meal, meat and bone meal)	98–1500 (lipid weight)	PCN-66/67 0.3–21 (lipid weight)	61 tri- to octaCNs	TetraCNs were the predominant homologues in all samples

APPENDIX E

Benchmark Dose (BMD) modelling

This appendix contains the details of the BMD modelling performed on experimental animal data. It consists of an introductory sub-section describing the approach followed in the modelling (**Section 0.1**) and the individual BMD reports per study (**Section 0.2**).

Introduction

In this introduction, a general description of the approach followed in the modelling is given.

Selection of the BMR

The benchmark dose (BMD) is defined as the estimated dose that corresponds with a predefined change in response compared with the background response. The benchmark response (BMR) is the response corresponding with the estimated BMD of interest.

The EFSA guidance on Benchmark Dose Modelling (BMD) in 2022 recommends the estimation of the 90% credible interval while taking into account biological relevance. For the decreased Platelet (PLT) count, the CONTAM Panel agreed to use a benchmark response (BMR) of 20%, aligning with the recommendation in the JMPR guidance document, which designates a change exceeding 20% in PLT count as adverse (JMPR, 2015). Regarding the increased relative liver weight, the CONTAM Panel agreed to use a BMR of 15%, in accordance with the JMPR guidance document, which suggests that a change exceeding 15% in relative liver weight should be considered as adverse (JMPR, 2015).

A 90% confidence interval around the BMD was estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

Software used

Results are obtained using the EFSA web-tool for Bayesian BMD analysis, which uses the R-package [BMABMDR] version 0.0.0.9060 for the underlying calculations.

Specification of deviations from default assumptions

All the results were calculated using the bridge sampling as numerical method, which is more accurate and computational demanding than the Laplace approximation set as a default.

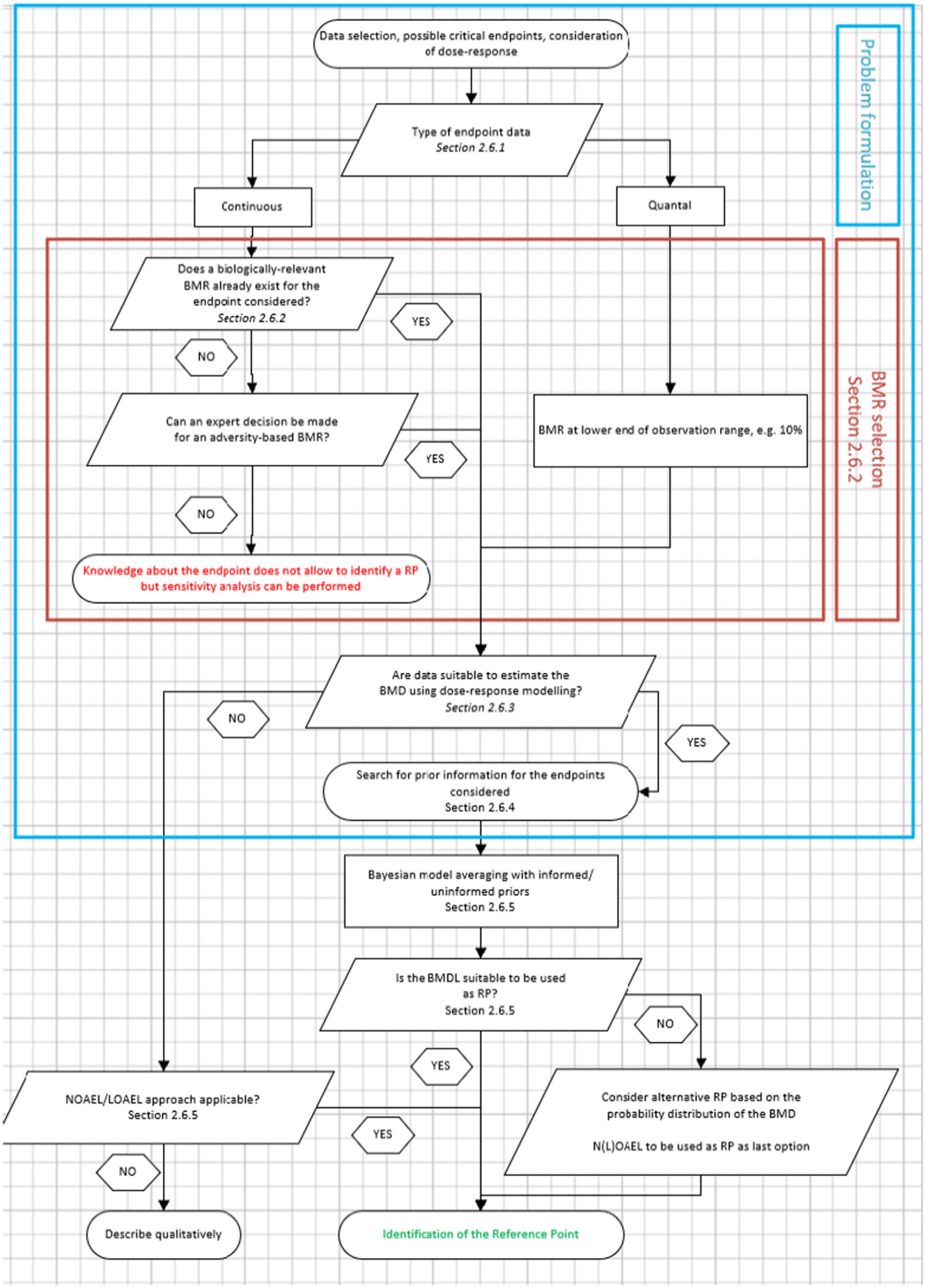
The CONTAM Panel selected the following default models:

Default set of fitted models

Family	Model	$y\|x \sim N(\mu(x), \sigma^2)$	$y\|x \sim LOGN(\mu(x), \sigma^2)$
		Dose–response function $(\mu(x))$	**Dose–response function** $(e^{\mu(x)})$
1a	Exponential[(i)]	$a \cdot \left(1 + (c-1) \cdot \left(1 - e^{-b \cdot x^d}\right)\right)$	$e^{a \cdot \left(1 + (c-1) \cdot \left(1 - e^{-b \cdot x^d}\right)\right)}$
	Inverse Exponential	$a \cdot \left(1 + (c-1) \cdot e^{-b \cdot x^{-d}}\right)$	$e^{a \cdot \left(1 + (c-1) \cdot e^{-b \cdot x^{-d}}\right)}$
	Hill[(ii)]	$a \cdot \left(1 + (c-1) \cdot \left(1 - \frac{b}{b + x^d}\right)\right)$	$e^{a \cdot \left(1 + (c-1) \cdot \left(1 - \frac{b}{b + x^d}\right)\right)}$
	Log-Normal	$a \cdot (1 + (c-1) \cdot \Phi(\log(b) + d \cdot \log(x)))$	$e^{a \cdot (1 + (c-1) \cdot \Phi(\log(b) + d \cdot \log(x)))}$
1b	Gamma[(iii)]	$a \cdot \left(1 + (c-1) \cdot \frac{\gamma(d, b \cdot x)}{\Gamma(d)}\right)$	$e^{a \cdot \left(1 + (c-1) \cdot \frac{\gamma(d, b \cdot x)}{\Gamma(d)}\right)}$
	LMS-two stage	$a \cdot \left(1 + (c-1) \cdot \left(1 - e^{-b \cdot x - d \cdot x^2}\right)\right)$	$e^{a \cdot \left(1 + (c-1) \cdot \left(1 - e^{-b \cdot x - d \cdot x^2}\right)\right)}$
2	Probit increasing	$a \cdot \Phi(c + b \cdot x^d)$	$e^{a \cdot \Phi(c + b \cdot x^d)}$
	Probit decreasing	$a \cdot (1 + \Phi(c)) - a \cdot \Phi(c + b \cdot x^d)$	$e^{a \cdot (1 + \Phi(c)) - a \cdot \Phi(c + b \cdot x^d)}$
	Logistic increasing	$a \cdot \frac{e^{c + b \cdot x^d}}{1 + e^{c + b \cdot x^d}}$	$e^{a \cdot \frac{e^{c + b \cdot x^d}}{1 + e^{c + b \cdot x^d}}}$
	Logistic decreasing	$a \cdot \left(1 + \frac{e^c}{1 + e^c}\right) - a \cdot \frac{e^{c + b \cdot x^d}}{1 + e^{c + b \cdot x^d}}$	$e^{a \cdot \left(1 + \frac{e^c}{1 + e^c}\right) - a \cdot \frac{e^{c + b \cdot x^d}}{1 + e^{c + b \cdot x^d}}}$

Procedure for selection of BMDL

Flowchart to derive a Reference Point from a dose–response dataset of a specified endpoint, using BMD analysis



Selected study

Klimczak et al. (2018) study on female Sprague–Dawley rats exposed orally to a mixture of HexaCN mixture for 90 days (see composition in Table 5).

1. Decrease in platelets (PLT) count in the blood of female Sprague–Dawley rats exposed orally to a mixture of HexaCN mixture for 90 days (see composition in Table 5) – Model averaging (Klimczak et al., 2018).

Data description

The endpoint to be analysed is: PLT count.

Data used for analysis

HexaCNs (mg/kg bw per day)	PLT (10^3 μL)	SD	Animals (*n*)
0.00	809.75	124.65	10
0.03	745.40	60.28	10
0.10	581.60	70.20	10
0.30	422.50	49.87	10

Selection of the BMR

The BMR (benchmark response) used is a 20% change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

Results

Response variable: PLT count.

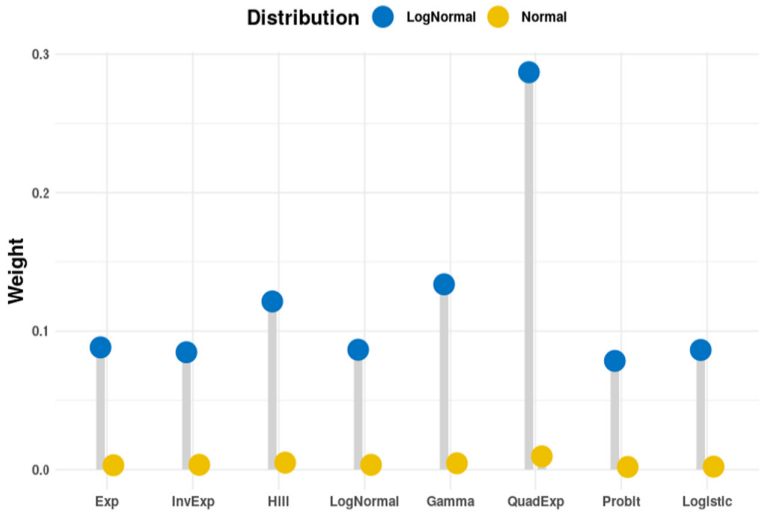
Estimated BMDs per model

Model	BMDL	BMD	BMDU	Model weights	Converged
E4_N	0.049	0.074	0.103	0.003	1
IE4_N	0.048	0.072	0.099	0.003	1
H4_N	0.049	0.072	0.101	0.005	1
LN4_N	0.051	0.073	0.102	0.003	1
G4_N	0.051	0.075	0.105	0.005	1
QE4_N	0.053	0.076	0.110	0.010	1
P4_N	0.052	0.077	0.106	0.002	1
L4_N	0.051	0.077	0.105	0.002	1
E4_LN	0.048	0.075	0.104	0.088	1
IE4_LN	0.051	0.077	0.101	0.085	1
H4_LN	0.049	0.074	0.102	0.121	1
LN4_LN	0.051	0.076	0.102	0.087	1
G4_LN	0.049	0.075	0.104	0.134	1
QE4_LN	0.053	0.075	0.109	0.287	1
P4_LN	0.048	0.075	0.104	0.079	1
L4_LN	0.048	0.075	0.103	0.086	1

Goodness of Fit

Best-fitting model fits sufficiently well (Bayes factor is 1.86e+00).

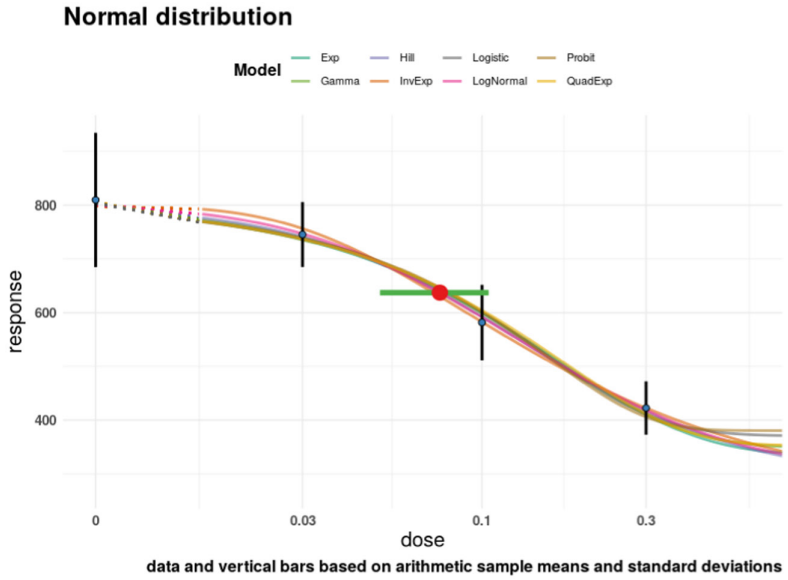
Weights for Model Averaging

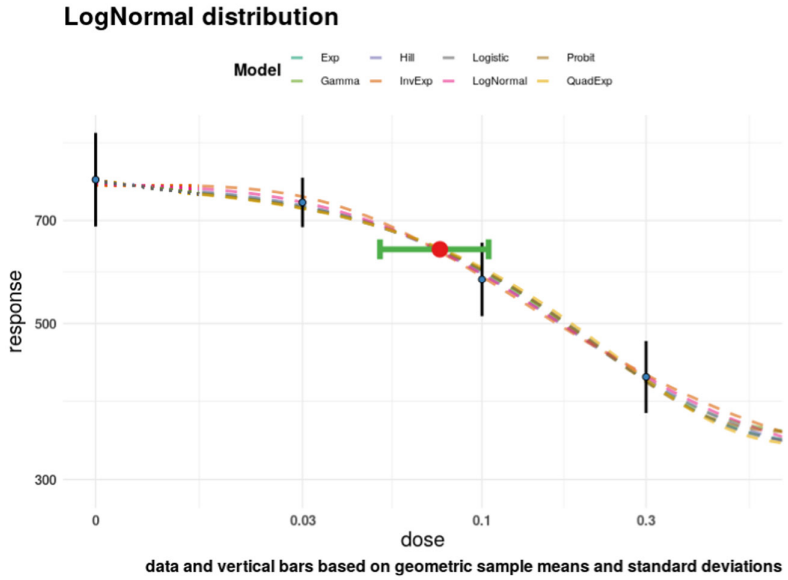


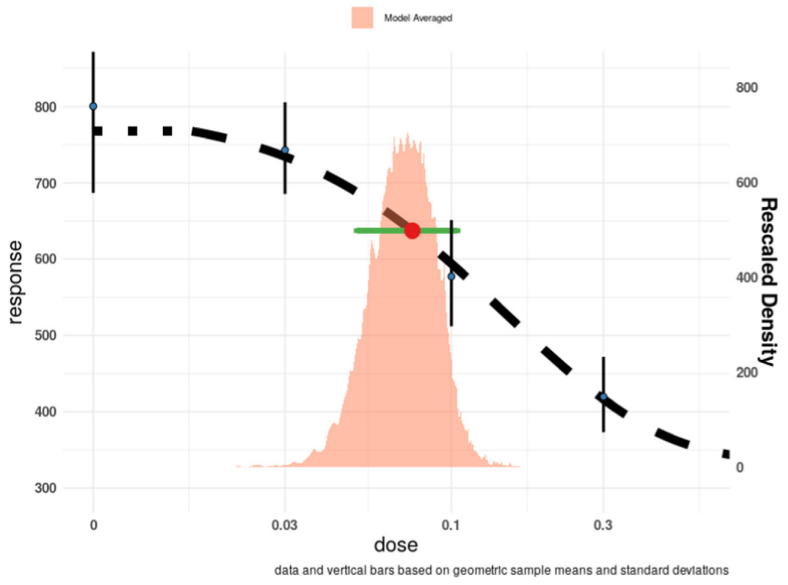
Final BMD Values

Model	Type	$BMDL_{20}$	BMD	$BMDU_{20}$
Model Averaged	BS	0.050	0.075	0.105

Visualisation







2. Increase in liver relative weight of female Sprague–Dawley rats exposed to a mixture of HexaCNs for 90 days (see composition of the mixture in Table 5) – Model averaging (Klimczak et al., 2018).

Data description

The endpoint to be analysed is liver relative weight.

Data used for analysis

HexaCNs (mg/kg bw per day)	Liver weight relative (g)	SD	Animals (*n*)
0.00	3.22	0.17	10
0.03	3.14	0.15	10
0.10	3.45	0.18	10
0.30	5.09	0.55	10

Selection of the BMR

The BMR (benchmark response) used is a 15% change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

Results

Response variable: liver relative weight

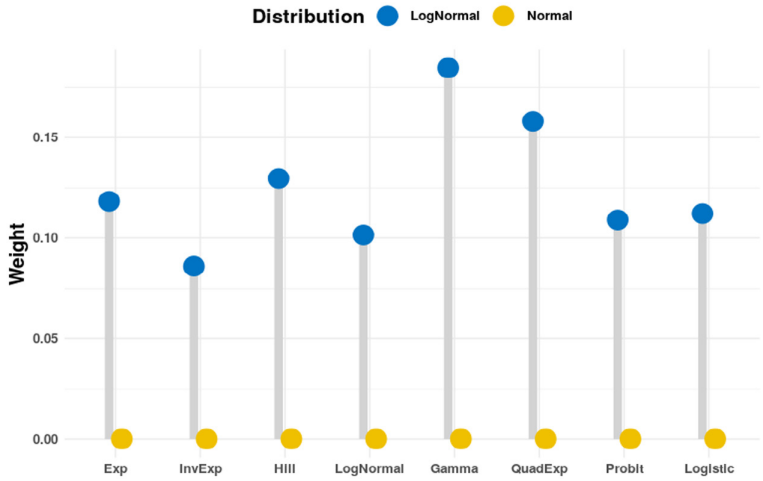
Estimated BMDs per model

Model	BMDL	BMD	BMDU	Model weights	Converged
E4_N	0.104	0.149	0.227	0.000	1
IE4_N	0.106	0.145	0.245	0.000	1
H4_N	0.105	0.147	0.216	0.000	1
LN4_N	0.108	0.148	0.239	0.000	1
G4_N	0.106	0.145	0.205	0.000	1
QE4_N	0.085	0.117	0.141	0.000	1
P4_N	0.103	0.151	0.220	0.000	1
L4_N	0.104	0.153	0.240	0.000	0
E4_LN	0.111	0.147	0.202	0.118	1
IE4_LN	0.108	0.133	0.205	0.086	1
H4_LN	0.110	0.143	0.193	0.130	1
LN4_LN	0.109	0.139	0.201	0.102	1
G4_LN	0.112	0.143	0.184	0.185	1
QE4_LN	0.100	0.129	0.150	0.158	1
P4_LN	0.112	0.148	0.200	0.109	1
L4_LN	0.113	0.150	0.204	0.112	1

Goodness of Fit

Best-fitting model fits sufficiently well (Bayes factor is 2.18e+00).

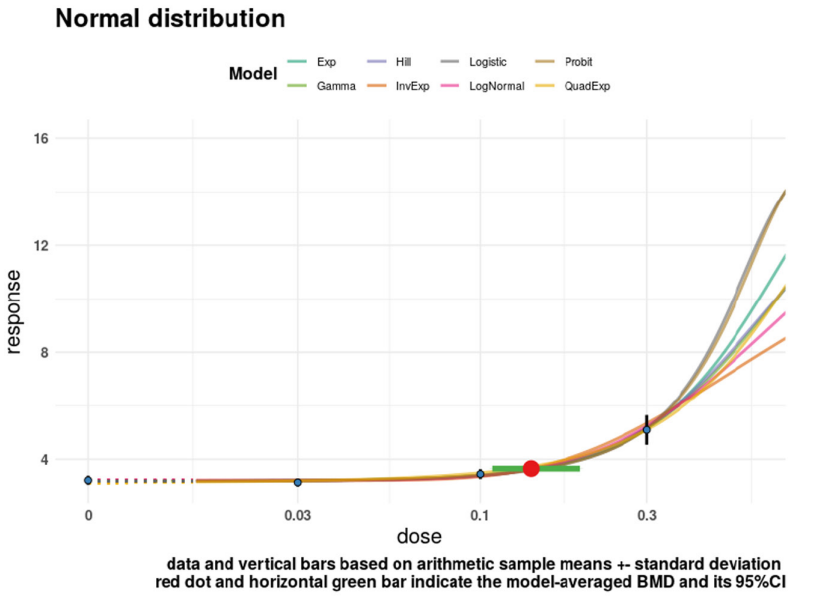
Weights for Model Averaging

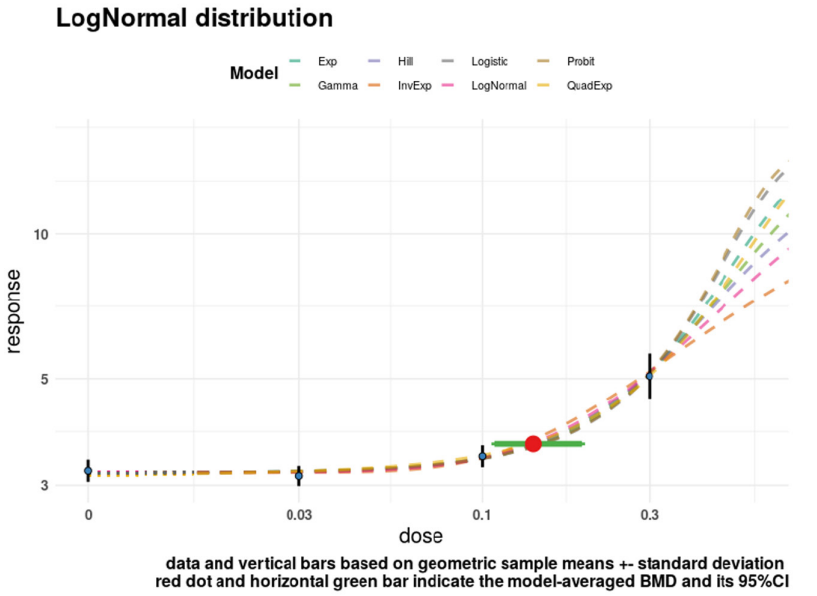


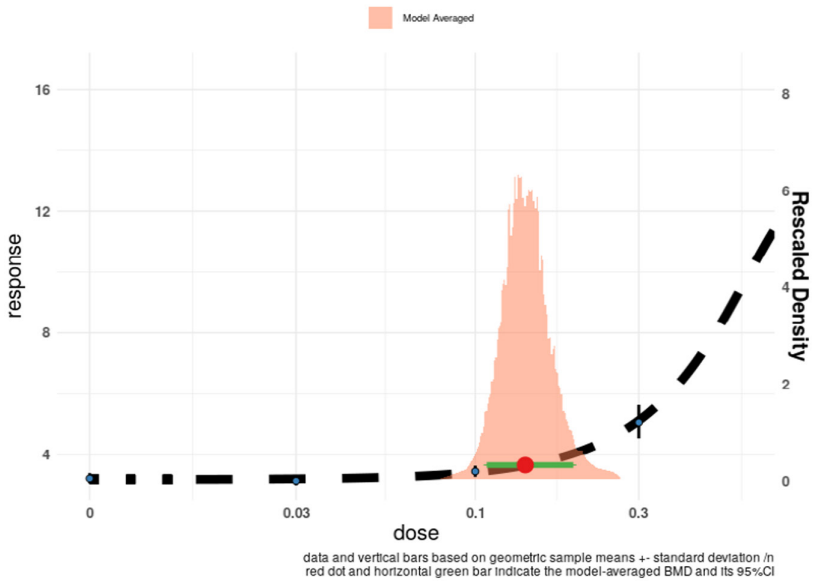
Final BMD values

Model	Type	$BMDL_{15}$	BMD	$BMDU_{15}$
Model Averaged	BS	0.109	0.14	0.191

Visualisation







APPENDIX F

Lists of uncertainties

TABLE F.1 Hazard identification and characterisation for human risk assessment.

Main group	Sub-group	Overarching questions	Sources of uncertainty in the opinion of PCNs in food	Priority ranking of the uncertainty[a]
Hazard identification and characterisation	**ADME**	**Is there uncertainty in any aspect of ADME?**	Data lacking for most congeners. However, absorption was indicated indirectly from the animal studies	1
			Data lacking for most congeners. For PCNs, a body burden approach would probably be appropriate for risk characterisation, but data for this is lacking	2–3 (–)
			The effect of confounders is unknown. It is conceivable that the presence of chemicals which could induce biotransformation enzymes could accelerate metabolism of PCNs, but data on this are lacking	1
			Data lacking for most congeners, but would not be expected to significantly impact the risk assessment	1
			Data lacking for most congeners, but would not be expected to significantly impact the risk assessment as it is not known whether the toxic moiety is the parent compound or metabolite	1–2 (–/+)
			There will be genetic variability, but this is considered to be accommodated by the default uncertainty factor taking into account human variability	1
	Toxicity studies in experimental animals: critical endpoints and critical study design	**Are there limitations in the study design of the studies that can result in uncertainties?**	A two-generation study was not available	3 (–)
			In the critical study only female rats were used, the duration is only for 90 days and a number of standard parameters were not assessed (e.g. histopathology performed only in a few of the organs and tissues required by the OECD TG 408 for a 90-day study)	3 (–)
		Are there uncertainties in the use of the animal model?	There are a number of missing studies and parameters (e.g. chronic study, two-generation study, carcinogenicity study, developmental neurotoxicity study, immunotoxicity study)	3 (–)
	Genotoxicity	**Is there uncertainty on the genotoxicity of the substance?**	The extremely limited information on genotoxicity of PCNs does not allow to conclude on their genotoxic potential	2–3 (only for the genotoxicity assessment)
	Mixture group membership and interactions	**Is there uncertainty on the extent and profile of effects due to co-exposure (e.g. metabolites, interaction of chemicals, combined effects)?**	There is no specific indication that interactions between PCNs will occur. Therefore, according to the EFSA guidance on chemical mixtures (EFSA, 2019), no specific consideration for interactions need to be taken into account	1
			The critical study takes account of the combined effects of the congeners present in the hexaCNs mixture that was tested	
			Possible combined effects from other congeners cannot be excluded due to the lack of data. However, hexaCNs are considered as the most potent ones	
	Dose response analysis of critical endpoints	**Is there uncertainty regarding the dose response analysis e.g. trend occurrence, large data variation, possible covariants?**	The BMD analysis was performed on group means, due to the lack of raw data. The SD for the control group was higher than that of the dose groups	1

[a](+), overestimation of risk; (–), underestimation of risk.

0–U with negligible priority;

1–U with low priority;

2–U with medium priority;

3–U with high priority.

TABLE F.2 Elements of the CONTAM road map and relevance for the uncertainty analysis of the PCNs in food draft Opinion – **OCCURRENCE AND EXPOSURE FOR HUMAN RISK ASSESSMENT.**

Main group	Subgroup	Overarching questions	Sources of uncertainty in the opinion of PCNs in food	Priority ranking of the uncertainty[a]
Occurrence data	**Analytical measurements**	Is there uncertainty due to the performance of the analytical method? This may include identification, sensitivity and recovery	1. For the 3 pairs of hexaCN congeners (including PCN-66/67) this adds considerable uncertainty but most congeners are adequately resolved	1
			2. Assumed that co-eluting congeners are always co-eluting, thus also the congeners reported as singles were reclassified to co-eluting	
			Method not sufficiently sensitive for some congeners and some matrices	0 or 1 (to be confirmed based on the hazard assessment result)
			Methods use internal standards, therefore, recovery is capture well; for those congeners with a corresponding standard. For other congeners it is reasonable to assume that the correction will be similar	1
			Few CRMs and few PTs available on PCNs; activities conducted by EURL give a reasonable confidence in analytical competence	1
	Data reporting	Is there uncertainty on whether there are errors in the reported occurrence data or linked to missing information?	Some unidentified errors in reporting, in particular with regard to food classification and/or expression of the result are likely introduced	1 +/–
			Low number or	2 +/–
			Complete lack of occurrence data for many food categories considered important contributors to the exposure assessment (e.g. various fish species, types of meat, etc.)	3–
			Occurrence data available only from three EU countries and therefore the data do not geographically represent the EU	2 +/–
			Occurrence data were mostly obtained from last 3 years that properly reflects the recent contamination, but overall, the distribution of year of sampling was not optimal due to lack of data from previous years that did not allow to analyse eventual trends in occurrence levels over the years. The occurrence data for some food categories (e.g. seafood) were mostly available from year 2007 that is considered as outdated information	1 +/–
			Extrapolation of the occurrence levels from one specific food category to other, or to entire food category at higher level (e.g. data reported on salmon considered for all other diadromous fish, data reported on cattle milk considered for all types of milk)	2 +/–
		Is there uncertainty in the occurrence data due to lack of data for potentially relevant major food categories?	Depending on PCN congener; there was a lack of occurrence data for different food categories considered major contributors to the overall exposure	1–3 depending on PCN congener when considering individual PCNs exposure scenarios 2 when considering mixture scenario
	Multiple chemicals and metabolites	Is there uncertainty in the occurrence data due to that not **all relevant substances** are reported	Not all congeners are measured and some co-elute	2
			Many of the congeners are reported separately or as congener pairs	1
			Not much info but PCNs are persistent so breakdown products not likely to be major concern	1
	Left censorship	Is there uncertainty in the occurrence data due to extrapolation or use of models?	The occurrence data, in particular with regard to major contributors to the overall exposure, the LODs/LOQs were considered sufficiently low	1–for Lower-bound and + for Upper-bound concentrations
		Is there uncertainty in the occurrence data due to left censorship and the substitution method	High percentage of left censored data	1–2 depending on food category and PCN congener 1 overall when considering the impact on mixture scenario – for Lower-bound and + for Upper-bound concentrations

(Continues)

TABLE F.2 (Continued)

Main group	Subgroup	Overarching questions	Sources of uncertainty in the opinion of PCNs in food	Priority ranking of the uncertainty[a]
Consumption data	**Data reporting**	Is there uncertainty in consumption data, e.g. due to methodology of the dietary survey, weekdays, national recipes?	Little information on the impact of processing available in the literature, however, it is reasonable to assume that PCNs will behave in a similar way such as other POPs (e.g. dioxins)	0 or 1
		Is there uncertainty in the form of the food reported (powder/liquid/reconstituted etc)	Little information on the impact of processing available in the literature, however, it is reasonable to assume that PCNs will behave in a similar way such as other POPs (e.g. dioxins). For a small part of the data reported to EFSA, it is not always clear if these related to the raw or processed food	1
	Representativeness of the data	Is there uncertainty in the representativeness of the consumption data (e.g. of the countries, special population groups, sample size and response rates	Use of default value for consumption of human milk (not consumption data in the EFSA database)	2 (breast-fed infants) + for the high consumption amounts
Dietary Exposure estimates methodology		Is there uncertainty linked to the methodology used for calculating the exposure?	1. Impact of the contribution of the one egg sample with high PCN-69 occurrence to the exposure result 2. Impact of performing exposure based on a mixture of hexaCNs (+ PCN-73) (81% of PCN-66/67-the most potent PCNs) assuming equal potency 3. The impact of coeluting congeners means that we don't have exposure estimates for these individual compounds 4. Occurrence data for some food types, e.g. salmon, extrapolated to all foods of the same family 5. applying of imputation for missing data for the mixture scenario	1. 3 + 2. To be considered in the RC uncertainty analysis 3. 2 4. 1–2 +/– 5. 1 +/–
Non-dietary exposure		Is there uncertainty in the exposure due to other sources than dietary are not included?	There was not enough information available to estimate non-dietary sources of exposure to PCNs from sources such as dust, which has been shown to be important for some population groups for other POPs	1–2 Other sources (dust) are likely to be lower in magnitude compared to dietary

[a](+), overestimation of risk; (–), underestimation of risk.

0–U with negligible priority;

1–U with low priority;

2–U with medium priority;

3–U with high priority.

TABLE F.3 Risk characterisation for human risk assessment.

Main group	Sub-group	Overarching questions	Sources of uncertainty in the opinion of PCNs in food	Priority ranking of the Uncertainty[1]
Risk characterisation	**Uncertainty factors**	Mixtures used in toxicological studies do not resemble the profiles found in food	Reference Point is based on hexaCN mixtures in which a single congener appears to have dominating potency Impact of performing exposure based on a mixture of hexaCNs (+ PCN-73) (81% of PCN-66/67-the most potent PCNs) assuming equal potency	2 (+)

ANNEXES

Annex A: Protocol for PCNs in feed and food

The annex is provided as a separate pdf file containing the protocol for the animal and human risk assessment related to the presence of polychlorinated naphthalenes in feed and food, and is available under the Supporting Information section on the online version of the Scientific output.

Annex B: Dietary surveys and occurrence data for PCNs in feed and food submitted to EFSA

The annex is provided as a separate Excel file containing the occurrence data on PCNs in food and feed and dietary surveys per country and age group available in the EFSA Comprehensive Database, considered in the exposure assessment, and is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.10641455.

Annex C: Human dietary exposure to PCNs

The Annex is provided as a separate Excel file containing the occurrence data on PCNs in food as considered for exposure assessment, chronic dietary exposure to PCNs and the contribution of different food groups to the dietary exposure, and is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.10641509.

Annex D: Protocol and results of Expert Knowledge Elicitation for PCNs

The annex is provided as a separate pdf file containing the Expert Knowledge Elicitation (EKE) protocol and results of the uncertainty analysis for the Opinion on PCNs in feed and food, and is available under the Supporting Information section on the online version of the Scientific output.

Annex E: Outcome of the public consultation

The annex is provided as a separate pdf file containing the List of comments received during the public consultation of the PCN opinion and responses provided by the CONTAM Panel, and is available under the Supporting Information section on the online version of the Scientific output.