Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment.

Systematic review of the literature on dioxins and dioxin-like polychlorinated biphenyls (PCBs)

Introduction

1. In 2018 the European Food Safety Agency (EFSA) published a tolerable weekly intake (TWI) for dioxins and dioxin-like polychlorinated biphenyls (PCBs) of 2 pg TEQ/kg bw/week. This TWI is 7-fold lower than EFSA's previous tolerable intake EFSA (2018).

2. This 7-fold reduction of the tolerable intake would entail that, from a situation in which dietary exposure for most of the UK population is below a level of concern, exposure would instead be at a potentially harmful level. This suggests that current risk management measures for dioxins and dioxin-like PCBs in food, which include regulatory limits and precautionary advice to consumers and are based on the previous tolerable intake, may be inadequate.

Background

1997 IARC evaluation

3. The International Agency for Research on Cancer (IARC) classified TCDD as a Group 1 carcinogen as '"There is sufficient evidence in humans for the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-para-dioxin. The strongest evidence in humans for the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-para-dioxin is for all cancers combined." (Group 1) (IARC, 1997). TCDD is not believed to be mutagenic. In vivo and in vitro genotoxicity

studies of TCDD in human and animal cells have given inconsistent findings, and findings of chromosomal aberrations in humans exposed in vivo to TCDD are equivocal' (IARC, 1997).

2001 COT evaluation

4. In 2001, the COT published a Statement on the tolerable daily intake for dioxins and dioxin-like polychlorinated biphenyls, recommending a tolerable daily intake (TDI) of 2 pg WHO-TEQ/kg bw/day (COT, 2001). The TDI was based upon effects on the developing male reproductive system mediated via the maternal body burden in male offspring rats, specifically reduced sperm production (Faqi et al., 1998 as cited in COT, 2001). The COT considered that this TDI is adequate to protect against other possible effects, such as cancer and cardiovascular effects.

2001 Joint Expert Committee on Food Additives

5. The Joint Expert Committee on Food Additives (JECFA) of the WHO and Food and Agriculture Organisation (FAO) established a provisional tolerable monthly intake (PTMI) of 70 pg/kg bw for dioxins and dioxin-like-PCBs in June 2001. When converted to a tolerable daily basis, this equates to a dose of 2.3 pg/kg bw/day. The PTMI was based on reproductive system toxicity in male offspring rats, as reported by Faqi et al., 1998 and Ohsako et al., 2001 and assumed a threshold mechanism of action for all effects, including cancer (JECFA, 2001).

2012 US Environmental Protection Agency

6. The US Environmental Protection Agency (US EPA) published an assessment of the non-cancer endpoints for dioxins in February 2012, establishing an oral reference dose (RfD) of 0.7 pg/kg bw/day. This was derived from epidemiological studies that evaluated the Seveso cohort who were accidentally directly exposed to TCDD in 1976, with the key studies identified as those from Baccarelli et al., 2008 and Mocarelli et al., 2008 which reported increased thyroid-stimulating hormone (TSH) in neonates and

decreased sperm count and motility in men exposed in childhood (EPA, 2012).

2018 EFSA evaluation

7. In 2018, EFSA published a tolerable weekly intake (TWI) for dioxins and dioxin-like-PCBs of 2 pg TEQ/kg bw/week (EFSA, 2018). This TWI is 7-fold lower than EFSA's previous tolerable weekly intake of 14 pg TEQ/kg bw/week. The EFSA TWI of 2 pg TEQ/kg bw/week is based on data from a Russian Children's study of peripubertal boys and their mothers (Minguez-Alarcon et al., 2017 as cited in EFSA (2018)) with semen quality, following pre- and postnatal exposure, being the critical effect.

2018 IARC evaluation

8. Following an evaluation in 2018 of newly available data for TCDD published since the previous evaluation, IARC supported the previous classification as a Group 1 carcinogen, with a threshold, non-genotoxic mechanism of action (IARC, 2018).

2021 NTP evaluation

9. In the US National Toxicology Programme (NTP) 15th Report on Carcinogens, TCDD was listed as '*known to be a human carcinogen'* based on human and animal data. NTP reiterated the conclusions of the IARC opinion from 1997 (NTP, 2021).

2021 COT interim statement on dioxins

10. In 2021, the COT published an Interim Position Statement presenting a review of the scientific basis and implications for risk management of the EFSA TWI, considering that there were substantial uncertainties over the derivation of the TWI and possible inconsistencies between the animal and human data. The COT noted that the data from a Russian Children's study that identified semen quality following pre- and postnatal exposure, was considered the critical effect but this study appeared inconsistent with the findings in a second study. Therefore, Members considered the Russian study

to provide only a weak data set. Moreover, the COT considered '*there were inconsistencies in the animal data presented in the EFSA opinion and was unclear, in particular, regarding the rationale for the selection of the study to evaluate the critical body burdens'*. Lastly, '*the data presented in EFSA's opinion implied that humans were more sensitive to dioxins than rats. However, this would be inconsistent with the existing body of data on dioxins and knowledge on the relative sensitivity of the human and rat aryl hydrocarbon receptor (AH*R)'.

11. These concerns meant that the COT was unable to endorse the EFSA opinion and considered it necessary to reconsider the evidence base and set its own tolerable intake (COT, 2021a).

12. Therefore, this paper presents data identified from a systematic literature review of dioxins and dioxin-like PCB to allow the Committee to form an opinion on a tolerable intake to ensure the appropriate level of protection for the UK consumer.

2023 COT re-evaluation

13. This re-evaluation of data will focus on male reproductive toxicity and immunotoxicity. The mechanism of action of dioxins via the aryl hydrocarbon receptor (AhR) will also be assessed to investigate species differences related to male reproductive toxicity and immunotoxicity, where possible.

14. The paper will also investigate if carcinogenicity observed with dioxins and dioxin-like PCBs involves a genotoxic mechanism, following previous conclusions by COT in 2001 and EFSA in 2018, that noted that carcinogenicity observed following dioxin exposure is not likely to be associated with direct genotoxicity.

Literature search

15. Searches of the PubMed and Scopus databases for publications relating to male reproductive toxicity, immunotoxicity, carcinogenicity, AhR mechanism of action and other endpoints were performed between

01/02/2017 to 20/10/2022 as described in Annex A. The start date was selected as it is nine months prior to the update (21/11/17) of the literature searches reported in EFSA (2018).

16. Appropriate papers were selected by screening titles and abstracts, based on the inclusion/exclusion criteria described in Annex A.

17. For *in vivo* studies, 13 papers on male reproductive toxicity, 23 papers on immunotoxicity and five papers on carcinogenicity were selected following screening of the title and abstract. For epidemiology, 37 papers on male reproductive toxicity, six papers on immunotoxicity were selected.

18. For other endpoints *in vivo*, eight papers were identified that reported effects on bone, six papers on cardiovascular effects, four on cleft palates, eight on liver effect and two on obesity.

19. The in vivo toxicity data obtained were evaluated using ToxRTool (Klimisch et al., 1997), and epidemiology data were evaluated using the Newcastle-Ottawa (Quality Assessment) Scale (NOS) to ensure data selected were of good quality. It was not possible to evaluate reviews and/or meta-analyses using the NOS, and these were automatically included for further evaluation. Studies that were classified as 1 or 2 using the ToxRTool, based on the Klimisch categories were retained and those scoring 3 or 4 were excluded from further analysis.

20. Overall, nine in vivo papers and 15 epidemiology papers on male reproductive toxicity and four in vivo and three epidemiology papers on immunotoxicity were selected. A summary of these papers is provided in Annex B and C.

21. For AhR, an overview of the generic mode of action of dioxins is provided relating to male reproductive toxicity, immunotoxicity and carcinogenicity as well as a discussion on species differences.

22. Male reproductive toxicity Epidemiology data following accidental human exposures (incident at the herbicide production plant in Seveso, Italy; consumption of contaminated rice oil consumption in Japan (Yusho incident)

and Taiwan (Yucheng incident) and handling and spraying Agent Orange in Vietnam) have shown effects on male reproductive health. Effects reported include reductions in sperm quality, manifested as decreased sperm concentration, total count, decreased motility and total motile count and increased abnormal sperm morphology; reduced oestradiol and increased follicle-stimulating hormone (FSH) levels; and decreased serum testosterone which may interfere with the secretory function of the prostate and seminal vesicles without affecting spermatogenesis. (Brokken & Giwercman, 2014).

Effects on semen parameters

Animal data

Elsayed et al., 2019

23. Elsayed et al. (2019) carried out a study to evaluate the effect of low doses of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on sperm quality in mice. Adult male CD1 mice (6/group) were administered TCDD daily by gavage for 10 days at doses of 0 (negative control (NC) and vehicle control corn oil (VC)), 0.375, 0.75, 1.5 µg/kg bw/day. No study guideline was followed. Mice were sacrificed after 10 days of exposure. Testes and epididymis were weighed. The spermatozoids from cauda epididymis were used for sperm counts, flow cytometry, and the testis were used for morphometric analysis. Various sperm physiological parameters (sperm count, percentage dead sperm, morphology, abnormalities and motility) were assessed as well as cytotoxicity by measuring damage to the integrity of the sperm membrane (7-aminoactinomycin D (7AAD) and apoptosis (Annexin V), complexity (side scatter (SSC) and size (forward scatter (FSC))) of sperm and morphometric analysis.

24. There was no mortality, clinical signs, or significant effect on epididymis or testes weight at any dose. Terminal body weight was transiently reduced by treatment from 0.37 µg/kg bw/day and above.

25. There was a significant decrease in sperm count, an increase in malformations (specifically abnormal tail) and a decrease in motility from

0.375 µg/kg bw/day relative to one or both controls. For the 0.75 and 1.5 µg/kg bw/day groups, there was a significant decrease in normal sperm, malformations (abnormal head) and percentage of dead sperm, relative to one or both controls. There was no dose-response relationship for these endpoints, with the exception of motility.

26. An increase in damage to the integrity of cell membranes leading to cytotoxicity (7AAD) and apoptosis (Annexin V) was seen in the high dose group compared with controls. Significant differences in FSC were seen in all treated groups compared with controls whereas SSC was only increased at 1.5 µg/kg bw/day.

27. The study revealed the toxic effects of low doses of TCDD on sperm quality, membrane integrity and apoptosis in sperm cells in mice at different doses. A consistent dose-response was not seen.

28. Overall, the authors concluded that low doses of TCDD affect the quality of sperm in adult mice and cause toxicity in the male reproductive system.

Erthal et al., 2018

29. Erthal et al. (2018) investigated the effect of TCDD on semen parameters in a developmental study in rats. Pregnant female Sprague-Dawley rats (5/group) were administered TCDD at doses of 0 (Dimethyl sulfoxide (DMSO - vehicle control) or 1 µg/kg bw/day by gavage on gestation day (GD) 15. No study guideline was followed. F1 male offspring (5/group) were sacrificed at either post-natal day (PND) 1 (neonatal) or PND90 (adult) to evaluate early and late testicular damage respectively, through histopathological analysis and determination of sperm count.

30. At PND1 and 90, F1 offspring body weight was significantly increased compared to controls. There was no effect on neonatal or F1 adult sperm count parameters (daily sperm production, number of mature spermatids).

He et al., 2020

31. He et al. (2020) investigated the effects of intrauterine exposure to PCB-118 (2,3′,4,4′,5-pentachlorobiphenyl) on the male reproductive system and sperm epigenetic imprinting in mice.

32. Pregnant female ICR mice (10/group) were administered daily doses of 0 (corn oil), 20 or 100 µg/kg bw/day PCB-118 from GD7.5 to 12.5 (the primordial germ cell migration stage) by gavage (He et al., 2020). No study guideline was followed. F1 offspring were sacrificed at seven weeks and 6-15 male offspring per group were assessed, depending on the endpoint. Reproductive endpoints assessed included histological and morphological effects on the testes as well as distance between seminiferous tubules, DST, height/thickness of seminiferous epithelium, number of spermatogonial cells in seminiferous tubules, sperm morphology, DNA methylation and mRNA expression (DNA methyltransferases (Dnmts) and Ubiquitin-like, with PHD and RING finger domains 1 (Uhrf1)) in the testes.

33. There was no significant difference in body weight of dams, or in body weight or body weight gain of F1 male offspring at any dose, but a significant decrease in dam body weight gain at 100 µg/kg bw/day.

34. Analysis of sperm morphology showed the number of sperm deformities were significantly increased compared to controls at 100 µg/kg bw/day indicating that PCB-118 exposure during pregnancy can affect sperm morphology in male offspring.

35. The authors concluded that exposure to PCB-118 can cause abnormal phenotypes of the sperm of male offspring which can affect the reproductive systems of offspring, which in turn, can reduce fertility.

Meles et al., 2022

36. Meles et al. (2022) investigated sperm apoptosis and necrosis in adult rats. 12-week-old male Wistar rats (6/group) were dosed daily by gavage, for 45 days, with TCDD at 0 (corn oil) or 700 mg/kg bw/day (700,000 µg/kg bw) and four hours later with corn oil (denoted as Group T0). The dosing regimen reflected the wider objective of the study to investigate the ameliorating

effects of α-tocopherol administration four hours after TCDD exposure. On day 46 rats were sacrificed, and blood and testicular samples were collected. No study guideline was followed. Endpoints assessed included live and dead, apoptotic and necrotic spermatozoa counts.

37. A significant decrease in live spermatozoa and a significant increase in dead, apoptotic and necrotic spermatozoa was seen relative to controls.

38. Results indicate that spermatozoa viability was impacted by exposure to TCDD in adult male rats.

Tao et al., 2021

39. Tao et al. (2021) investigated the effect of exposure to PCB-118 during pregnancy on reproductive systems of F1 male mice.

40. Pregnant female ICR mice (7-9/group) were administered PCB-118 by gavage, at daily doses of 0 (vehicle not specified), 20 or 100 µg/kg bw/day from GD8.5 to 13.5. No study guideline was followed. The F1 male offspring (total of 79-81/group; 3-6 offspring/group per endpoint) were used in subsequent experiments. Testes were collected on embryo day (E) 18.5 for protein and gene expression and at 7 weeks for histopathology. Spermatozoa was collected in F1 adults following sexual maturation. Endpoints assessed included testis weight, sperm viability and morphology, histological assessment of the testis (DST, sperm deformity), mRNA expression in testicular tissue, immunohistochemistry (to detect expression of 5 mC in testes) and protein expression.

41. There were no significant differences in maternal body weight relative to controls. Pre-natal exposure to PCB-118 resulted in a significant decrease in the number of spermatogonia and sperm motility at both doses, as well as a significant increase in sperm deformity rate at 100 µg/kg bw/day.

42. Overall, the results showed that PCB-118 increased the sperm deformity rate in the offspring male mice.

Yahia et al., 2018

43. Yahia et al. (2018) investigated the toxicity of TCDF in the testes of adult mice. Sexually mature male albino mice (12/group) were administered TCDF twice weekly by gavage for up to 8 weeks at a dose of 0 (corn oil) or 0.5 µg/kg bw/day. No study guideline was followed. Mice (n=4) were sacrificed at either 2, 4 or 8 weeks after which time sperm DNA integrity was measured and testicular tissue histopathological analysis was carried out.

44. The percentage of DNA damage (fragmented and denatured DNA) in sperm was significantly increased at all time periods.

45. The authors concluded that exposure to TCDF adversely impacts sperm DNA integrity.

Epidemiology data

Amir et al., 2021

46. A comparison of the exposure profiles of infertile (married men actively trying for pregnancy for over 12 months) and fertile males in Pakistan to a number of POPs including dichlorodiphenyltrichloroethane (DDTs) and its metabolites, HCB and PCBs, including the dioxin-like PCB-118, has been reported (Amir et al., 2021). POP levels were measured in hair, blood and urine samples and semen samples were used for semen analysis (sperm count and motility). The WHO guidelines were used to categorise males into normospermic, oligospermic, asthenozoospermic and azoospermic groups. A total of 156 male participants were initially recruited however, following exclusion due to a failure to provide samples, the final cohort (infertile and fertile controls) comprised 111 males. It is unclear from the methodology how the fertile (control) group was recruited.

47. With regards to PCB-118 specifically, this was detected in 69% of all hair samples, 93% of all serum samples and 52.1% of all urine samples at mean ± SE concentrations of 0.554 ± 0.377 pg/mg, 0.039 ± 0.0130 ng/ml and 0.003 ± 0.001 ng/ml respectively. Statistical analysis included the use of ANOVA, bivariate correlation, linear regression, and Pearson’s chi square to

evaluate potential relationships between parameters. No significant differences were found between infertile males and fertile controls with regard to the levels of PCB-118 in hair, serum or urine samples. Although serum levels of PCB-118 was higher in the infertile group when compared to controls, this did not reach statistical significance.

48. A significant negative correlation was reported for PCB-118 in serum and sperm motility (%) (r= -0.212; p=0.041) although no effect was seen on sperm count.

49. Overall, serum levels of PCB-118 were associated with defective semen parameters.

Mínguez-Alarcón et al., 2017

50. Mínguez-Alarcón et al. (2017) assessed the potential impact of environmental exposure to organochlorines (OC) on semen parameters in young Russian men in a longitudinal study. Participants (n=516) were previously enrolled at the age of 8-9 years onto the Russian Children's Study at which time the boys underwent a physical examination, provided a blood sample for OC measurement, and together with his mother or guardian, completed health, lifestyle, and dietary questionnaires. Annual follow-up examinations and completion of questionnaires were made, and at the age of 18-19 years a subset of the cohort (n=133) provided semen samples (1 or 2 samples collected approximately 1 week apart), which were analysed for volume, sperm concentration and motility. The latter was classified according to the WHO classes: rapidly progressive motile (class A), slowly progressive motile (class B), locally motile (class C) or immotile (class D), taking the average value for duplicate measures. Percent motile sperm was defined as the sum of WHO classes A, B, and C. Blood samples taken at recruitment were analysed for levels of 7 polychlorinated dibenzo-p-dioxins (PCDDs, or dioxins), 10 polychlorinated dibenzofurans (PCDFs, or furans), 4 co-planar PCBs (co-PCBs), 6 mono-ortho–substituted PCBs, and 31 other PCBs (non-dioxin-like PCBs).

51. Levels of OCs were expressed as dioxin toxic equivalents (TEQs) on a lipid basis to determine the relative potency of each congener to TCDD before summation and categorised into quartiles due to the potential for nonlinear associations. Statistical analysis used adjusted and non-adjusted linear mixed models to examine the potential association between exposure to individual OCs and semen parameters; models were adjusted for a number of potential confounders: BMI, smoking status, alcohol drinker, season of sample collection, and with regards to percent of motile sperm and total motile sperm count, consideration of abstinence time was also included. Within-person correlations in semen parameters across repeated samples were accounted for using random intercepts. The authors compared semen parameters (total sperm count, sperm concentration, percent motile sperm, total motile sperm count, and semen volume) for men with higher quartiles of serum OC concentrations to those within the lowest quartile.

52. The median (range) for TCDD was 2.9 (0.4–12.1) pg/g lipid and PCDD TEQ was 8.7 (1.0–36.0) pg TEQ/g lipid, with total serum TEQs (21.9 (1.88 – 107)) being almost three times higher than levels reported among European children of similar age.

53. The authors reported a significant association using both adjusted and non-adjusted models between serum TCDD and PCDD TEQs levels at 8 – 9 years of age and decreased semen parameters at 18-19 years of age. Regarding sperm concentrations, the highest quartile of serum TCDD TEQs were 40% lower when compared to the lowest quartile (p-trend = 0.005) when using the adjusted model. Similarly, total sperm count was decreased by 29% (p-trend = 0.05) and the total motile sperm count by 30% (p-trend = 0.05) in the highest quartile of TCDD TEQs, when compared to the lowest quartile. Decreases of 39%, 36% and 40% were also reported for sperm concentration (p-trend = 0.02), total sperm count (p-trend = 0.04), and total motile sperm count (p-trend = 0.05) at the highest quartile when compared with the lowest quartile of PCDD TEQs. No associations were reported for serum PCBs, furans and total TEQs.

54. The authors concluded that ‘higher peripubertal serum TCDD concentrations and PCDD TEQs were associated with poorer semen parameters’.

55. The main strengths of the study identified by the authors include its prospective design and long-term serial follow up of participants which minimises the risk of reverse causation and the collection of two semen samples for the majority of participants. The main limitation of the study was reported as the lack of OC prenatal exposure measurements at the time when sexual differentiation and reproductive tract organisation occurs. In addition, a large number of components were measured and it is not clear whether potential interactions between the different components were evaluated by the authors.

Paul et al., 2017

56. In a case-controlled study, Paul et al. (2017) evaluated the relationship between the levels of twelve individual dioxin-like PCBs in serum and semen parameters in sub-fertile males in Spain. Cases (n = 56; age range 30 – 55) were recruited from couples attending an IVF clinic between May 2012 to June 2014 but excluded due to the presence of known male infertility factors (including: varicocele, post-vasectomy or cryptorchidism, endocrine hypogonadism (abnormal hormonal concentrations), immune infertility, genetic disease, infection, anomalies in the karyotype or Y chromosome microdeletions), leaving a final cohort of n= 50 cases. Participants were divided into two groups based on semen quality criteria, as defined by to the World Health Organization (WHO); low semen quality (n = 24) comprised cases with alteration of at least one parameter in the semen analysis and a control group (n = 26) composed of patients with normal semen quality.

57. Blood serum and semen samples were collected from each subject on the same day. Target analytes included 12 dioxin-like-PCBs from non-ortho PCBs (PCB-77, PCB-81, PCB-126 and PCB-169) and mono-ortho PCBs (PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167 and PCB-189). PCB concentrations were adjusted for total serum lipids and the TEQ calculated. Statistical analysis was carried out using the Mann-Whitney

U-test and multivariate linear regression models with continuous semen parameters as dependent variables and dioxin-like PCBs as predictor or independent variables. Where the concentration of an individual congener was below the limit of detection (LOD), these were reported as equal to their respective LOD however only dioxin-like PCBs with ≥ 60% of values above the LOD were included in the statistical analysis.

58. Total concentrations of dioxin-like PCBs, expressed as pg WHO-TEQ/g lipid, were 22.52 ± 21.2 pg WHO-TEQ/g lipid in the low-quality semen group and 14.00 ± 10.82 pg WHO-TEQ/g lipid in the normal semen quality (control) group. A significant ($p<0.001$) impairment of sperm concentration (24.70 ± 33.97x10^6/mL), total sperm count (38.05 ± 52.84x10^6), motility (20.22 ± 23.55 %), viability (62.28 ± 21.93 %) and morphology (5.36 ± 4.85 %) ($p < 0.001$) was found in the low-quality semen group when compared to the control group. No significant differences were reported for semen volume or levels of cholesterol and triglycerides. Levels of the mono-ortho PCBs were also significantly ($p<0.05$) higher in the low-quality semen group (0.2 ± 0.13 pg WHO-TEQ/g lipid) when compared to the control group (0.15 ± 0.13 pg WHO-TEQ/g lipid). Statistical analysis of serum levels of dioxin-like PCBs expressed as pg/g lipid, reported significantly ($p<0.05$) higher levels of non-ortho PCBs (949.49 ± 624.97 pg/g lipid; $p = 0.020$), total dioxin-like PCBs (7029.96 ± 3023.97 pg/g lipid; $p = 0.028$) and mono-ortho PCBs (6080.46 ± 2754.89 pg/g lipid) in the low-quality semen group when compared to the control group (508.40 ± 324.44, 4805.92 ± 2205.02 and 4297.52 ± 2030.13 pg/g lipid, respectively). Serum levels of PCB-105 were significantly ($p=0.031$) higher in the low-quality semen group when compared to the control group (0.03 ± 0.02 0.01 ± 0.01 pg WHO-TEQ/g lipid respectively), with all other dioxin-like PCBs showing no significant differences between groups.

59. Multivariate regression analysis indicated a number of significant positive and negative correlations, with the negative corelations described as: serum levels of PCB-126 and sperm viability (%) (r=-0.645; 0.013) in the low-quality semen group, semen volume with PCB-118 ($r = -0.539$; $p = 0.031$) and sperm motility (%) with PCB-189 ($r = -0.521$; $p = 0.039$) in the normal-quality semen group. Analysis of the data from the group as a whole showed a

number of statistically significant negative correlations: sperm progress motility (%) with PCB-126 (r =-0.381; p = 0.037) and PCB-189 (r = -0.410; p = 0.024).; sperm viability (%) with PCB-126 (r = - 0.557; p = 0.001), PCB-169 (r = -0.542; p = 0.002) and PCB-189 (r = -0.580; p < 0.001); non-ortho PCBs and sperm viability (%) (r=-0.505; p=0.004); total dioxin-like PCB and sperm viability (%) (r=-0.412; p= 0.023).

60. The authors concluded that serum dioxin-like PCB concentrations may have adverse effects on semen quality, with the negative effects occurring in the late stages of spermatogenesis (during spermiogenesis) and/or epididymal maturation, resulting in the alteration of certain semen parameters such as motility, volume, morphology and viability.

61. The main strength of the study was identified by the authors as the detailed congener-specific PCB analysis which provided an accurate pattern of exposures that may be used to determine exposure pathways. The small number of participants was identified as a significant limitation meaning that the findings should be interpreted with caution.

Petersen et al., 2018

62. Petersen et al. (2018) evaluated the potential effects of exposure to PCBs, including dioxin-like PCB-105, PCB-118 and PCB-156, and perfluorinated alkylated substances (PFASs) via the environment, on semen quality or reproductive hormone levels in Faroese men (n=263; age 24-26 years). At recruitment, participants provided a blood sample for analysis of reproductive hormones and levels of PCBs and PFASs and a semen sample which was assessed for sperm concentration, total sperm count, semen volume, morphology and motility. PCB values were expressed as ∑PCB only and it is not possible to evaluate the effects of the individual dioxin-like PCBs in isolation.

63. There was no association between serum PCB concentration and semen parameters measured.

Effects on testes

Animal data

Elsayed et al., 2019

64. As part of their study on sperm quality in adult mice following exposure to low doses of TCDD (see paragraph 23 for details of this study), Elsayed et al. (2019) also carried out a morphometric analysis of mice testicles.

65. Morphometric analysis revealed a significant decrease in the diameter of seminiferous tubules (DST) from 0.75 µg/kg bw/day and a significant increase in the epithelium of the seminiferous tubules (EST) diameter at 0.375 µg/kg bw/day only.

66. The study revealed the toxic effects of low doses of TCDD on changes to the EST and the DST in mice at different doses. A consistent dose-response was not seen.

Erthal et al., 2018

67. Erthal et al. (2018) investigated the effect of TCDD on testes in a developmental study in neonatal and F1 adult rats (see paragraph 29 for details of this study).

68. TCDD did not cause significant changes in the proportion of the seminiferous cord and interstitial tissue compartments in the neonatal testes, and no abnormal cells were observed in the seminiferous cord and interstitial tissue. TCDD caused a reduced Sertoli cell count in both F1 neonatal and F1 adult tests and there was a significant increase in number of abnormal seminiferous tubules in F1 adults.

69. The authors noted that the reduction in the number of Sertoli cells in both neonatal and adult testes demonstrate the early and persistent effects of TCDD.

He et al., 2020

70. He et al. (2020) investigated the effects of intrauterine exposure to PCB-118 (2,3′,4,4′,5-pentachlorobiphenyl) on the male reproductive system

and sperm epigenetic imprinting in mice (see paragraph 31 for details of this study).

71. There was no significant difference in body weight of dams, or in body weight or body weight gain of F1 male offspring at any dose, but a significant decrease in dam body weight gain at 100 µg/kg bw/day. There was also a significant increase in the testicular coefficient (testes weight / body weight * 100) of F1 male offspring from 20 µg/kg bw/day.

72. Morphological analysis of the testes demonstrated the distance between some seminiferous tubules in the PCB-118 treatment groups was increased, and small vacuoles appeared inside some seminiferous tubules, especially at 100 µg/kg bw/day. Spermatogenic cells at different stages were also disorderly distributed. In addition, the DST and height of the seminiferous epithelium was significantly increased at 100 µg/kg bw/day and the relative number of spermatogonia in the seminiferous tubules was significantly decreased.

73. Relative expression of DNA methyltransferases (Dnmts) in the testes of offspring was decreased in a dose-dependent manner. Compared with the controls, the relative expression of Dnmt, Dnmt3a and Uhrf were significantly reduced at 20 and 100 µg/kg bw/day whereas Dnmt3b and Dnmt3l expression was only significantly decreased at 100 µg/kg/day.

74. DNA methylation was analysed by combined bisulfite restriction analysis (COBRA) to assess the pattern of the differentially methylated region (DMR) region in mouse sperm imprinted genes. Results showed that *H19* was completely digested by both *Taq*$^{\alpha}$ and *Rsa*I in controls, indicating that cytosine was methylated at the recognition site of the two enzymes in the DMR region of *H19*. However, some samples in the treated groups could not be digested, suggesting that inter-uterine exposure to PCB-118 may cause demethylation at some CpG sited in the *H19* MDR of sperm from male offspring. Similarly, all sperm *Gtl2* samples in controls were digested by *Bst*UI whereas some samples of *Gtl2* in the treatment groups, especially at 100 µg/kg/day were not completely digested. For *Snrpn* and *Igf2r* DMRs, all sperm samples in treated

groups were not completely digested, indicating that the cytosine at the DMR region of the imprinted genes was unmethylated.

75. Sodium bisulfite sequencing analysis was also carried out to obtain an accurate methylation pattern of the DMR of the mouse imprinted genes. DNA methylation level in the DMR of *H19* and *Gtl2* were significant reduced in treated groups compared to controls. There was no significant difference between treated groups and controls with respect to methylation of *Snrpn* and *Igf2r* DMRs. The methylation patterns were all consistent with the COBRA analysis.

76. Authors suggested that methylation patterns of *H19* and *Gtl2* in the DMR are probably affected by intrauterine exposure to PCB-118 and concluded that exposure to PCB-118 can cause abnormal phenotypes of the sperm and testicles of male offspring which can affect the reproductive systems of offspring, which in turn, can reduce fertility.

Jin et al., 2018

77. The effect of TCDD on testis inflammation following pre-natal exposure was evaluated by Jin et al. (2018). Pregnant C57BL/6 mice were administered a single dose of TCDD by gavage on E15 at doses of 0 (vehicle not specified) or 10 µg/kg bw.

78. Eight-week old F1 males (n=6/group) were exposed to lipopolysaccharides (LPS) (5 mg/kg) by intraperitoneal (i.p.) injection to induce inflammation, or a saline solution (200 µl/mouse; NC). After 24 h, the mice were sacrificed, and their testes were removed. No study guideline was followed. Endpoints studied included germ cell and testis apoptosis, mRNA and protein levels of pro-inflammatory cytokines, and Klotho expression.

79. To assess the effects of TCDD on germ cell and testes apoptosis, the active caspase3+ cells and TUNEL+ cells in F1 mice testes, respectively were assessed. Both were also significantly increased relative to the LPS-positive controls.

80. To analyse the effects of TCDD on the expression of Klotho in the testes, the paraffin embedded sections of the testis were processed using immunohistochemistry. Klotho was detected in the Sertoli cells, Leydig cells and germ cells and was significantly decreased with LPS and TCDD compared with the LPS-only group.

81. The authors concluded that prenatal exposure to TCDD induces germ cell apoptosis due to the secretion of pro-inflammatory cytokines via autocrine signalling and/or paracrine signalling.

Johnson et al. 2020

82. Johnson et al. (2020) investigated transcriptome-based alterations in fetal pituitary and testis genes/pathways to build an adverse outcome pathway (AOP) linking in utero dioxin exposure in rats to reductions in adult epididymal sperm content. Two study designs (study design 1 GD8-20 repeat dose; study design 2 GD15 single dose) were conducted on time-mated female Cr1:CD(SD) Sprague-Dawley rats (5/group) using TCDD or TCDF. No study guideline was followed.

83. In study design 1, dams were administered either low doses of TCDD or TCDF (0 (corn oil), 0.03, 3 or 1 µg/kg bw) or high doses (0, 3, 6 or 10 µg/kg bw) by gavage on GD8 (loading dose) followed by daily maintenance doses of 0, 0.0003, 0.003 or 0.022 µg/kg bw/day (low doses groups) or 0, 0.066, 0.132 or 0.22 µg/kg bw/day (high dose groups), respectively on GD9-20. The study was terminated at GD20. Due to the small group sizes, reproductive and fetal observations, dam body weight, and dam body weight gain data were not analysed statistically. Endpoints assessed included fetal testes histopathology, and gene expression of follicle stimulating hormone subunit beta (Fshb), luteinizing hormone subunit beta (Lhb), glycoprotein hormones, alpha polypeptide (Cga) in fetal pituatory and inhibin subunit alpha (Inha) in fetal testis.

84. Dams exposed to 6 and 10 µg/kg bw/day TCDD between GD9-20 and those exposed to 10 µg/kg bw/day TCDD and TCDF on GD15 had treatment-

related decreases (16-74%) in body weight gain although body weight was unaffected in all groups.

85. Histopathology analysis of fetal testes revealed no differences in any dose group examined.

86. In study design 2, dams were administered a single dose of 10 µg/kg bw TCDD or TCDF by gavage on GD15. The study was terminated at GD20. Dam body weight was not analysed statistically due to the small group sizes, but the authors noted there was a treatment-related decrease in body weight gain at GD14-17 or 15-18, and GD8-20 or 15-20, for both TCDD or TCDF. There was no treatment-related decrease in body weight in dams exposed to TCDD or TCDF.

87. There was no treatment-related histopathology in fetal testes.

Tao et al., 2021

88. Tao et al. (2021) investigated the effect of exposure to PCB-118 during pregnancy on reproductive systems of F1 male mice (see paragraph 39 for details of this study).

89. There were no significant differences in maternal body weight relative to controls. Pre-natal exposure to PCB-118 resulted in a significant decrease in epithelium thickness and an increased gap in seminiferous tubules at both doses.

90. A significant increase in the height of the spermatogenic epithelium of tubules and DST and a significant decrease in testis weight was also seen at 100 µg/kg bw/day.

91. mRNA expression of Dnmt1, proliferating cell nuclear antigen (Pcna) and stimulated by retinoic acid 8 (Stra8) in the testes of E18.5 offspring was significantly decreased at 20 and 100 µg/kg bw/day, as well as the expression of Stra8 in 7-week old mice.

92. There was a significant decrease in protein expression of DNMT1 and PCNA in E18.5 mice at 100 µg/kg bw/day. There was also a decrease in

protein expression of DNMT1 and STRA8 in 7-week old mice at 20 and 100 µg/kg bw/day, and in PCNA at 100 µg/kg bw/day.

93. In terms of global methylation levels in embryonic and sexually mature testis of F1 mice, a significant decrease in relative cytosine (5 mC) level was seen at 20 and 100 µg/kg bw/day in E18.5 mice, and at 100 µg/kg bw/day in 7-week old mice.

94. The authors considered that PCB-118 exposure during pregnancy affected the reproductive health of F1 male mice, mainly reflected in the increase in the DST in the testes of offspring, and that the testes were damaged during exposure at the embryonic stage.

Yahia et al., 2018

95. Yahia et al. (2018) investigated the toxicity of TCDF in the testes of adult mice (see paragraph 43 for details of this study).

96. Histopathology analysis showed that treated mice displayed histopathological changes, including degenerative changes of the seminiferous tubules with formation of multinucleated giant cells after 2 weeks and germ cells were exhausted and degenerated. After 4 weeks there was a great exhaustion of germinal epithelium with only a few layers seen in most of the tubules, and detachment of the germ cells from the basal lamina was also prominent.

97. The authors concluded that exposure to TCDF causes histopathological alterations of the testes.

Zhang et al., 2020

98. Zhang et al. (2020) investigated the effects of maternal exposure to PCB-118 during pregnancy on the testes of offspring. Pregnant female ICR mice (33-38/group) were administered PCB-118 by gavage at daily doses of 0 (corn oil), 20 or 100 µg/kg bw/day from GD7.5 to 12.5 (the primordial germ cell migration stage). No study guideline was followed. F1 offspring were sacrificed on PND7, 14, 21, 28 and 35 and testes were isolated. Histopathology was carried out on 6-10 males per group, and global

methylation measured on 3-6 males per group using 5-methylcytosine (5-meC) levels to assess the methylation of genomic DNA.

99. There was no significant difference, relative to controls, in the gestational body weight gain of dams at any dose, total birth weight of litters, the body weight of F1 male mice (n=10-12/group) from birth to PND35, or sex ratio. Testicular organ coefficient (testes weight / body weight * 100%) was significantly decreased on PND28 at 20 µg/kg bw/day and on PND21 and PND35 (not at PND28) at 100 µg/kg bw/day. Changes in testicular morphology were also seen at both doses, including cell detachment, vacuolisation and enlarged gaps in the seminiferous tubules on PND21, PND28 and PND35, as well as a significant decrease in the mean diameter of seminiferous tubules.

100. There were no differences in global methylation as measured by testicular 5-meC levels on PND7 and PND14 at either dose, but levels were significantly decreased from PND21 at 20 µg/kg bw/day, and from PND28 at 100 µg/kg bw/day.

101. At 20 µg/kg bw/day expression of Dnmt 1 and Dmnt3a (from PND21), Dmnt3b (at PND28 only) and Uhrf1 (from PND7) mRNA was significantly decreased compared with controls and at 100 µg/kg bw/day, expression of Dnmt1 (at PND14, 21 and 35), Dmnt3a (from PND28), Dmnt3b (at PND21 and 35) and Uhrf1 (at PND14, 21 and 35) mRNA was significantly decreased. Gene expression at other time points were not significantly different to controls.

102. Protein expression of DNMT3 (on PND21) was significantly decreased at 20 µg/kg bw/day and DNMT1 and DNMT3A was significantly decreased at 100 µg/kg bw/day. On PND28, DNMT1 protein levels were significantly decreased at both doses and DNMT3A was decreased at 100 µg/kg bw/day. On PND35 DNMT3A expression was significantly decreased at both doses.

103. The authors concluded that exposure of pregnant mice to PCB-118 can affect DNA methylation levels of testicular tissue, and lead to testicular morphological damage in exposed F1 offspring.

Epidemiology data

Desalegn et al., (2021)

104. In a case-cohort design study, Desalegn et al. (2021) examined whether perinatal exposure to endocrine-disrupting chemicals (EDCs) during the critical period of testicular descent may increase the risk of cryptorchidism and male fertility. Participants were recruited from 2606 mother-infant pairs enrolled on The Norwegian Human Milk Study (HUMIS, 2002–2009), of which 1326 were mother-son pairs, which aims to measure levels of POPs in breastmilk and to investigate possible health effects associated with high levels. Of the available mother-son pairs, data on levels of POPs in the mothers' breast milk was available for 641, who were included in the present study.

105. Breast milk samples, collected in line with the WHO guidance, were assessed for levels of 27 potential EDCs including the dioxin-like PCBs PCB-105, PCB-114, PCB-118, PCB-156, PCB-157, PCB-167 and PCB-189. Cryptorchidism was evaluated by mothers at 1, 6, 12, 24 months using a self-administered questionnaire and categorised by the authors based on the timing of the presentation as either:

- Congenital cryptorchidism - cryptorchidism based on mother's report at one month after birth.
- Recurrent cryptorchidism- cryptorchidism at birth that spontaneously descends and then reascends.
- Persistent cryptorchidism - cryptorchidism reported both at age 1 and 2 years, including receipt of orchiopexy.
- Ever-reported cryptorchidism - cryptorchidism reported at 1, 6, 12 or 24 months.

106. Statistical analysis included variable selection via elastic net logistic regression to identify the best predictors of cryptorchidism and multivariable logistic regression models to determine effect estimates. The authors used separate ordinary least squares logistic regression models for the single pollutant analysis to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for congenital, recurrent, persistent and ever-reported cryptorchidism,

controlling for the potential confounders identified by a directed acyclic graph. Two versions of the model were used, an unadjusted (crude estimate) model and an adjusted model controlling for appropriate confounders.

107. Congenital cryptorchidism was reported in 6.1% of the total HUMIS cohort, with half spontaneously descending within six months of birth, after which prevalence stabilised to between 2.2 and 2.4%. The prevalence of recurrent and persistent cryptorchidism was 8% and 1.6% respectively and that of ever-reported cryptorchidism was 12.2%. In the ordinary least squares logistic regression, PCB-114, PCB-156, PCB-157 were significantly associated with congenital cryptorchidism (adjusted OR=1.36, 95% CI: 1.05-1.77; 1.32, 1.03-1.70; 1.34, 1.03-1.74 respectively). A significant association was also reported for PCB-114, PCB-118, PCB-167 with recurrent cryptorchidism (adjusted model, 1.88, 1.00-3.50; 1.60, 1.07-2.36; 1.73, 1.06-2.82) and PCB-114 with ever-reported cryptorchidism (1.14, 0.57-2.29). None of the PCBs were associated with persistent cryptorchidism. The multipollutant analysis based on elastic net logistic regression selected PCB-74, PCB-114, PCB-194 and β-HCH in at least 50% of the imputed datasets (n = 100 imputations) as the best predictors among the 27 chemicals for congenital cryptorchidism.

108. The authors concluded that for the group as a whole, infants with the highest exposure to breast milk concentrations of the dioxin-like PCB-114, the non-dioxin-like PCBs-74 and PCB-194 and β-HCH had increased odds of congenital cryptorchidism, and ever-reported cryptorchidism (PCB-194 only (non-dioxin-like PCB)). Although a number of individual PCB congeners were significantly associated with congenital or recurrent cryptorchidism in the unpenalised logistic regression, only PCB-114 and non-dioxin-like PCB-74, and PCB-194 were selected as predictors of congenital cryptorchidism using a variable selection method. The false associations may have been due to confounding by highly correlated chemicals. Several advantages of the study identified by the authors include: as the cohort was recruited from across Norway it is representative of the general population; measurements of EDCs in breast milk correlate with umbilical cord, and maternal serum, and can be a suitable proxy for prenatal and postnatal exposure and a good indicator of

body burden for persistent compounds; reduced chance of confounding from correlated co-exposure due to the use of a variable selection method for the multi-pollutant analysis; and the assessment of cryptorchidism at several time points after birth.

109. The main limitations identified by the authors were that the outcome of cryptorchidism was based on maternal report and that levels of EDCs were determined postpartum and not during the first trimester, when testicles develop.

Desalegn et al., 2022

110. In a follow-up case-control study, Desalegn et al. (2022) evaluated the potential association between AhR activation in breast milk extracts and cryptorchidism in infant boys. Participants were recruited from the HUMIS cohort (as described above) with a total of 91 mother-boy pairs being included who had reported cryptorchidism at either 1-, 6-, 12-, or 24 months after delivery, and had adequate breast milk volume for analysis; 108 controls with adequate breast milk samples for analysis were randomly selected from the mother-son study population who had not reported cryptorchidism. As previously (Desalegn et al., 2021), breast milk samples were assessed for levels of 27 potential EDCs including the dioxin-like PCBs PCB-105, PCB-114, PCB-118, PCB-156, PCB-157, PCB-167 and PCB-189. Cryptorchidism was evaluated by mothers at 1, 6, 12, 24 months using a self-administered questionnaire and categorised as congenital cryptorchidism; recurrent cryptorchidism; persistent cryptorchidism; and ever-reported cryptorchidism. In addition, chemically- and biologically stable AhR activity (pg 2,3,7,8- TCDD equivalent (TEQ)/g lipid) was determined.

111. Statistical analysis was carried out using Pearson’s chi-square test for binary or categorical variables, and the Wilcoxon rank-sum test for continuous variables. Logistic regression models were used to estimate the ORs and 95% CIs for congenital, recurrent, persistent and ever-reported cryptorchidism, minimally controlling for the potential confounders, as identified by a directed acyclic graph (DAG), of maternal education (low/medium, high) as a proxy for socio economic status (SES), maternal age

(continuous), pre-pregnancy BMI (kg/m2), smoking (yes/no), alcohol consumption in pregnancy (yes/no), nulliparity (yes/no), and selected EDCs (β-HCH, PCB-74, PCB-114, PCB-194). Missing data was imputed using multiple imputation by chained equations with predictive mean matching. Additional confounders were also evaluated using a sensitivity analysis. Multivariate linear regression was used to estimate the relationship between levels of EDCs in breast milk and AhR activity, controlling minimally for the confounders of education as a proxy for socioeconomic status, pre-pregnancy BMI, and smoking and alcohol consumption during pregnancy, as identified using a DAG.

112. AhR activity in breast milk samples was not significantly different between cases with cryptorchidism and controls. However, AhR activity was (borderline) significantly associated with all dioxin-like PCBs (PCB-105 adjusted model, β=1.01, 95%CI: -0.09 – 2.11; PCB-114, 0.73, 0.19-1.28; PCB-118, 0.91, 0.12-1.70; PCB-156, 0.77, 0.19-1.35; PCB-157, 0.76, 0.12-1.39; PCB-167, 1.26, 0.39 – 2.13; PCB-189, 0.97, -0.11 – 2.05). The authors concluded that the study showed no association between AhR activity and cryptorchidism in the Norwegian cohort, however as all dioxin-like PCBs were associated with AhR activity this is consistent with a possible role for dioxin-like PCBs in the observed AhR activity. The main strength of the study was identified by the authors as the use of breast milk samples which allowed measurement of all sources of dioxin exposure and is known to be a suitable proxy for prenatal and postnatal exposure. The main limitation of this study was identified as the determination of the outcome, cryptorchidism, which was based on maternal report from self-administered questionnaires.

Effects on hormone levels

Animal data

Erthal et al., 2018

113. Erthal et al. (2018) investigated the effect of TCDD on plasma testosterone concentrations in a developmental study in rats (see paragraph 29 for details of this study).

114. There was no effect on plasma testosterone concentrations in F1 neonates on PND1 or in F1 adults on PND90.

Johnson et al., 2020

115. Johnson et al. (2020) investigated transcriptome-based alterations in fetal pituitary and testis genes/pathways to build an adverse outcome pathway (AOP) linking in utero dioxin exposure in rats to reductions in adult epididymal sperm content (see paragraph 82 for details of this study).

116. In study design 1, at 6 and 10 µg/kg bw TCDD, a significant decrease in gene expression of fetal pituatory Fshb and a corresponding decrease in fetal testis Inha was observed. There was no change in Lhb or Cga expression with TCDD or TCDF. The Lhb mean expression trended lower but was not statistically significant compared to controls.

117. In study design 2, a significant decrease in gene expression of fetal pituitary Fshb and fetal testis Inha was seen with TCDD but no effect was seen with TCDF. There was no change in Lhb or Cga expression with TCDD or TCDF.

118. Overall, the authors concluded that the results from the studies suggest an AOP for dioxin-induced rat male reproductive toxicity involves direct targeting of the perinatal pituitary and testis, which together cause reduced perinatal pituitary follicle stimulating hormone (FSH) and luteinizing hormone (LH) production and testis androgen production, and Sertoli cell proliferation and ultimately, reduced spermatogenic output in the adult rat.

Epidemiology data

Berghuis et al., 2022

119. Berghuis and Roze (2019) carried out a prospective longitudinal cohort study, as part of the Dutch Development at Adolescence and Chemical Exposure (DACE) study, to assess whether prenatal exposure to environmental PCB and OH-PCB are associated with reproductive hormone levels and pubertal characteristics in 13- to 15-year-old children. The DACE study followed up two previous cohorts, Risk of Endocrine Contaminants on

Human Health (RENCO) study (104 mother–infant pairs recruited 1998 to 2000) and the Groningen-Infant-COMPARE (Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogens; GIC) study (90 mother–infant pairs recruited 2001 and 2002). The current study invited children from both cohorts. Participants were excluded based on a lack of data for prenatal POP levels, a diagnosis of a congenital syndrome after recruitment and emigration. Of the 188 children invited, 101 (53.7%) participated, 44 (23.4%) declined the invitation, and 43 (22.9%) did not respond. The final study group consisted of 55 boys and 46 girls.

120. Levels of POPs were measured in maternal blood samples taken during the second and/or third trimester of pregnancy and included the dioxin-like PCBs PCB-105, PCB-118 and PCB-156. Blood samples were taken from children at follow up for the measurement of the reproductive hormones oestradiol (E2), LH, FSH, anti-müllerian hormone (AMH), sex hormone-binding globulin (SHBG), inhibin B, testosterone and free testosterone. Pubertal development was determined as stages according to Marshall and Tanner, including stages for pubic hair, genital development in boys and breast development in girls. Assessment of Tanner stages was made by a trained paediatric endocrinologist in clinic or using a validated method for self-assessment. In addition, testicular volume was assessed using a Prader orchidometer in clinic or by the participant and all participants. completed questionnaires on pubertal onset.

121. Statistical analysis comprised t-tests to compare POP levels between cohorts and in children with and without onset of pubertal characteristics. Where samples were below the LOD, levels were estimated as LOD/2. Correlations were assessed using Spearman’s rank correlation test and Pearson’s correlation test. Multivariable regression analyses was used to assess associations between prenatal POP levels and levels of reproductive hormones, testicular volume, Tanner stages, and self-reported ages at onset of pubertal characteristics. Adjustments were made for a number of confounders as follows: the age at examination (in months) and BMI for reproductive hormone levels; the age at examination (in months), BMI,

maternal age at menarche (only for girls), timing of growth spurt of the father (only for boys; early versus average/late compared to peers), and assessor of Tanner stages (clinic versus participant) were considered for analysis of Tanner stages; BMI, maternal age at menarche (only for girls), and the timing of growth spurt of the father (only for boys; early versus average/late compared to peers) for the analysis of self-reported ages at onset of pubertal characteristics.

122. With regards to the assessment of specific associations between prenatal dioxin-like PCB levels and pubertal development in boys, PCB-105 was significantly ($p<0.05$) positively associated with levels of testosterone ($\beta=0.5$, $p=0.03$), PCB-118 was significantly positively associated with levels of inhibin B ($\beta =0.45$, $p=0.03$) and PCB-156 was significantly positively associated with levels of testosterone ($\beta=0.61$, $p=0.00$) and free testosterone ($\beta =0.53$, $p=0.02$) in 13-15 year old boys.

123. The authors concluded that ‘higher prenatal [total] PCB exposure could be associated with more advanced pubertal development in 13- to 15-year-old children’ and, from the data provided, this may apply to some dioxin-like PCBs.

124. The main strength of the study given by the authors is that the assessment of pubertal development was carried out both biochemically and clinically which allowed evaluation from both types of measurement. Limitations were identified as the possibility of chance findings due to the large number of comparisons carried out and potential bias during recruitment of the pregnant women resulting in lower levels of POP exposure than may be found in the general population as a whole.

Dong et al., 2020

125. Dong et al. (2020) evaluated whether perinatal exposure to dioxins affects steroid hormone levels in preschool-aged children from an e-waste recycling region in China. Mother-infant pairs (n = 50) were recruited from an area with high levels of e-waste recycling between July and September 2015. Mothers were aged between 25 and 30 years of age and were healthy

primiparas with no obvious complications during pregnancy, had a normal single birth delivery, and were breastfeeding. Of these, 8 pairs were excluded due to relocation, leaving 42 pairs in the study at follow up when the children were 4 years old.

126. Breast milk samples were taken from mothers at 4 weeks postpartum for measurement of levels of PCDDs/DFs, which were taken as indicators of perinatal exposure to dioxins in the infants. These were expressed as mass concentrations and TEQs of the WHO 2005 toxic equivalent factors with undetected dioxin levels treated at half of the limit of detection. At 4 years of age, fasting serum samples were collected from each child for measurement of the steroid hormones dehydroepiandrosterone (DHEA), testosterone, androstenedione (A-dione) and progesterone. Statistical analysis was carried out for the PCDD/Fs congeners with detection rates of >80%.

127. Multivariate linear regression (MLR) models were used to evaluate the potential associations between dioxin congener levels and steroid hormone levels. Due to the small number of participants, bootstrapping was used to derive robust coefficient estimates (with 2000 iterations) for dioxin congeners and the bias-corrected and accelerated bootstrap 95% confidence interval (CI); results were considered as statistically significant when the 95% CI values did not contain zero. The MLR models were also adjusted for potential confounders including: residency (years), education (three levels: below junior high school, senior high school, and college or above), body mass index (BMI) of the mother (during 4–6 weeks of pregnancy), gestational age (weeks), full breastfeeding (months), and the child's BMI (kg/m^2). Principal component analysis (PCA) using the varimax procedure was utilised for multiple pollutants modelling.

128. In boys, the geometric mean (GM) steroid hormone levels of testosterone, DHEA, A-dione, and progesterone were 48.5 ± 2.0 pg/mL, 262.6 ± 1.8 pg/mL, 64.0 ± 2.1 pg/mL, and 66.6 ± 1.5 pg/mL, respectively. A significant negative association was found between serum levels of TCDD, 1,2,3,7,8-pentachlorodibenzo-P-dioxin (PeCDD), and 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF) and testosterone levels (β= -0.712 (-1.465,

-0.308); β= -0.813 (-1.658, -0.096); β= -0.636 (-1.059, -0.302) respectively). A significant negative association was also reported between testosterone levels in boys with the total PCDDs and total TEQ of PCDDs/DFs (β= -0.842, 95% CI: -1.629, -0.218; β= -1.425, 95% CI: -2.656, -0.632 respectively).

129. The authors concluded that perinatal exposure to dioxins modifies steroidogenesis in preschool-aged children, although the long-term impact of this could not be determined in the current study. Limitations were reported by the authors as the single sampling point, non-evaluation of the potential interference of dioxins on the steroid hormones that are biosynthesized in the fetal adrenal glands, and the small cohort size.

Shi et al., 2020

130. The potential association between exposure to dioxins resulting from e-waste recycling activities and steroid hormone equilibrium in adult males in China has been investigated (Shi et al., 2020). The study recruited males (n=74) from hospital staff in 2017, with the following inclusion criteria: aged above 60 years; resident in the Luqiao District in Taizhou City for more than 50 years (i.e. before commencement of e-waste recycling activities); no hormone therapy or hormone-disrupting medications within 3 months before enrolment. A blood sample was taken at recruitment and levels of DHEA, testosterone, dihydrotestosterone (DHT) and A-dione were determined. Levels of 17 2,3,7,8-substituted congeners of PCDDs and PCDFs were also determined from the serum sample.

131. Statistical analysis utilised a general linear model to examine any associations of the dioxins and hormone levels, and enzyme activity. Covariates were BMI, alcohol drinking (yes or no), smoking habits (yes or no), age, and residency and dioxin levels were categorical variables based on quartiles of dioxin levels. The results were reported as adjusted means (the means adjusted for all the terms in the models) with 95% CI and the lowest tertile was used as the reference group.

132. Levels of DHEA were significantly higher in the low PCDFs-TEQ group (1933 vs. 1447 pg/ml; $p<0.05$) and in the low PCDD/PCDFs-TEQ group (1996

vs. 1360 pg/ml; $p<0.01$) when compared to the reference group. A significantly higher level of A-dione was found in men with high serum PCDFs-TEQ (2404 vs. 1848; $p<0.05$). No significantly higher (or lower) DHT and testosterone levels were noted in dioxin groups.

133. The authors conclude that the findings 'suggest that specific dioxin exposure may disturb normal DHEA, A-dione levels, and enzyme activity in the general adult male population in an e-waste region of China'. Several limitations are noted by the authors including the age of participants as findings may not be generalised to other age groups. In addition, the study design allowed only one sample point to be included and as there were a small number of participants this may have introduced bias in the coefficient estimates, and findings might be observed due to chance.

Eskenazi et al., 2017

134. In a prospective cohort study, Eskenazi et al. (2017) evaluated the potential relationship between in utero and childhood exposure to DDT, polybrominated diphenyl ether (PBDE) flame retardants, and PCBs including the dioxin-like PCBs PCB-105, PCB-118, PCB-156, PCB-157, PCB-167, and PCB-189, and reproductive hormone levels in adolescent boys. Participants were enrolled as part of the CHAMACOS 1 (recruitment October 1999 and October 2000 and followed to 9 years of age) and CHAMACOS 2 (recruitment 2009 – 2011 from CHAMACOS 1 cohort and followed to 12 years of age; n=163 boys) studies in the US.

135. Measurement of dioxin-like PCBs, and other chemicals of interest, were determined in maternal serum collected during pregnancy or at delivery, and from both mothers and sons at 9 years of age. At 12 years of age, levels of FSH, LH, and total testosterone were also determined in serum samples (taken before 9 AM). Where levels were below the LOD but a signal was detected, the reported value was used and for levels below the LOD where no signal was detected, levels were imputed at random based on a log-normal probability distribution below the LOD via maximum likelihood estimation. Statistical analysis was only carried out for PCBs detected in >75% of maternal and/or child serum samples.

136. Associations were estimated by linear regression models adjusted for the following covariates, chosen based on a directed acyclic graph: maternal education and family poverty at age 9; child age at hormone assessment; cohort (CHAM1 or 2 if applicable). Models with maternal exposures also included maternal pre-pregnancy BMI. Child's age was treated as a continuous variable; family poverty, maternal education and pre-pregnancy BMI, and cohort were parameterised as categorical variables. Averaged maternal BMI values were used where BMI values were missing. Tanner genital development stage was also evaluated and included in sensitivity analysis to determine whether in utero chemical exposure was related to hormone levels within a given pubertal stage.

137. The authors reported that a 10- fold increase in total prenatal ΣPCBs was significantly associated with a 64.5% increase (95% CI: 8.6, 149.0; $p<0.05$) in FSH, however this was primarily driven by non-dioxin-like congeners and was not altered by inclusion of Tanner stage data. Exposures measured in the children's serum at 9 years also showed associations between ΣPCBs and testosterone (108.9% increase; 95% CI: 7.1, 307.2), however, the authors noted that these associations may be mediated by child BMI.

138. The authors concluded the data indicated some association between maternal prenatal blood concentrations of PCBs and some alterations in serum hormone concentrations in adolescent boys. This appeared to be driven by the non-dioxin-like PCBs and was considered to be mediated by child BMI.

139. The strengths of the study are reported by the authors as the longitudinal design, the use of measured exposure data and the availability of covariate information for mothers and children. Limitations were also considered and related to measurements of chemicals other than PCBs.

Miyashita et al., 2018a

140. Miyashita, Araki, et al. (2018) evaluated whether prenatal exposure to dioxin-like compounds irreversibly affect fetal reproductive and steroid

hormone synthesis. Participants were previously enrolled in the Japanese Sapporo Cohort of the Hokkaido Study on Environment and Children's health as 23–35 weeks pregnant native Japanese women attending an obstetrics and gynaecology hospital in Sapporo, Hokkaido, Japan between July 2002 and October 2005. Of the total cohort of 1,347 pregnant women, the current study initially recruited 514 mother-children pairs with the final cohort comprising 183 pairs for which maternal blood levels of dioxin-like compounds and reproductive and steroid hormone levels in cord blood were available. Levels of 29 individual dioxin-like compounds congeners (categorised into 4 groups of 7 PCDDs, 10 PCDFs, 4 non-ortho PCBs, and 8 mono-ortho PCBs) were measured in maternal blood samples and concentrations of progesterone, E2, testosterone, androstenedione, DHEA, cortisol, cortisone, SHBG, LH, FSH, prolactin, inhibin B, and insulin-like factor-3 (INSL3) in cord blood samples.

141. Dioxin-like compounds concentrations were expressed as total lipid content (pg/g lipids) with those below the LOD being taken as half of the individual LOD. Dioxin-like compound levels were divided into four quartiles and were analysed as ordinal variables, and linear contrast coefficients of – 3, − 1, + 1, and + 3 were assigned to the 1st, 2nd, 3rd, and 4th quartiles, respectively. TEQ values were also calculated for each dioxin-like congener by multiplying the concentration of individual dioxin-like compounds congeners by the corresponding toxic equivalency factors. Spearman's correlation coefficient was used to assess relationships between the continuous variables (concentrations of maternal dioxin-like compounds and cord blood hormones) and the continuous variables (maternal age, pre-pregnancy BMI, gestation week at which blood was collected during pregnancy, gestational age, and infant birth weight) and Mann–Whitney U tests were used to evaluate any relationships between maternal dioxin-like compounds or cord blood hormone levels and categorical valuables.

142. Potential associations between maternal dioxin-like compounds concentrations and infant cord blood hormone levels were assessed using multivariate linear regression models, which were adjusted for the following confounders: maternal age at delivery (continuous), parity (≥1 or 0), smoking

behaviour during pregnancy (yes or no), alcohol consumption during pregnancy (yes or no), annual household income (< or ≥ 5 million yen), gestation week at which blood was sampled during pregnancy (continuous) and sex of the cord blood sample. To evaluate the change in concentrations of cord blood hormones for each increase in the quartile dioxin-like compounds level, the 1st quartile was compared to the 2nd, 3rd, and 4th quartiles, and the p-values were adjusted using Bonferroni's correction ($p < 0.0166$).

143. In males, the authors reported a significant negative association of sub-total PCDDs with inhibin B levels (β= -0.34 95% CI:-0.61, -0.07; $p<0.05$) and a significant positive association (0.47 (0.07, 0.86; $p<0.05$) with DHEA levels. Sub-total PCDFs were significantly negatively associated with inhibin B (-0.35 (-0.64, -0.06); $p<0.05$). Sub-total non-ortho-PCBs were also significantly negatively associated with inhibin B (-0.26 (-0.41, -0.10); $p<0.01$), T/E2 (-0.22(-0.42, -0.03); $p<0.05$), cortisol (-0.46 (-0.96, 0.04); $p<0.05$) and SHBG levels (-0.11 (-0.20, -0.02); $p<0.05$) and significantly positively associated with DHEA levels (0.30 (0.07, 0.54); $p<0.05$). Sub-total mono-ortho-PCBs were significantly negatively associated with inhibin B (-0.24 (-0.45, -0.02); $p<0.05$) and significantly positively associated (0.29 (0.01, 0.57); $p<0.05$) with FSH levels. Total dioxin-like compounds were significantly negatively associated with inhibin B (-0.36 (-0.61, -0.11) $p<0.01$) and SHBG (-0.15 (-0.29, -0.01); $p<0.05$) levels and significantly positively associated (0.46 (0.09, 0.83); $p<0.05$) with DHEA levels.

144. The authors concluded that prenatal exposure to dioxin-like compounds alters steroidogenesis and suppresses the secretion of inhibin B in male cord blood.

145. The main strength of the study was identified as the large number of dioxin-like congeners and steroid hormones measured to determine prenatal exposure.

146. Limitations were identified as the change in concentrations of steroid and reproductive hormones that occur immediately before and after birth and due to pregnancy complications, such as placental weight changes and

preeclampsia, which may have affected levels. In addition, maternal dioxin-like compounds were measured in the 2nd and 3rd trimesters, but the critical window for exposure effects on fetal hormones is considered to occur during the first trimester. Assigning measurements below the LOD as one-half of the LODs may have also introduced bias in the association between total dioxin-like compounds and SHBG, as well as PCDFs, mono-ortho PCBs, and FSH.

Oanh et al., 2018

147. The potential association between exposure to polychlorinated dibenzodioxin/dibenzofuran via the mother's breast milk and androgen levels in Vietnamese children aged 5 years has been investigated (Oanh et al., 2018). Participants (n =60) of the study were previously recruited in September 2008 as breastfeeding mothers of 4 – 16-week old infants, with residence in the Phu Cat district (dioxin 'hot-spot') for > 5 years; non exposed breastfeeding mothers (n=63) were recruited from the Kim Bang district (non-sprayed area). At recruitment, breast milk samples were taken and levels of seventeen dioxin congeners (7 dioxin and 10 furan congeners) were determined. Dioxin congener levels were converted to pg/g of lipid and to TEQs using the World Health Organization TEF. Where dioxin levels were below the LOD, a value equal to half the LOD was used.

148. Children were followed up until the age of 5 years (2013) at which time serum samples were collected for measurement of DHEA, DHT, cortisol, cortisone, 17-OH-progesterone (17-OH-P4), P4, A-dione and T. The final cohort at 5 years of age for which maternal dioxin measurements were available, comprised 85 mother–child pairs (35 pairs from Phu Cat, with M:F of 19:16, and 50 pairs from Kim Bang, with M:F of 23:27).

149. Statistical analysis comprised use of the student's t-test or Wilcoxon's signed rank test in case of a normal or non-normal distribution, as determined by the Shapiro–Wilk test, to determine the mean difference for reach indicator. Associations between breast milk dioxin congener and serum hormone and enzyme levels were determined using Pearson correlation coefficients and Spearman correlation coefficients, depending on the distribution. The potential influence of confounding factors was determined using multiple regression

analysis with steroid hormones as the dependent variates and child age, child sex, full breastfeeding period, child BMI, and dioxin congeners in breast milk as independent variates. The Benjamini-Hochburg procedure was applied to adjust p-value for multiple testing.

150. When expressed as pg/g of lipid, the level of all dioxin congeners, with the exception of 2,3,7,8-TeCDF, were significantly higher ($p<0.05$; from 2- to 10-fold) in breast milk samples from mothers located in the hotspot when compared to breast milk samples from mothers in the non-sprayed area. In addition, the TEQs of total PCDDs, PCDFs, and PCDDs/Fs were significantly higher ($p<0.05$; 3-fold) in the hotspot than in the non-sprayed area. Cortisone, DHEA and testosterone concentrations in serum were significantly lower ($p=0.012$, <0.001 and <0.001 respectively) in children from the hotspot than those from the non-sprayed area, with no sex-related differences in the testosterone and DHEA concentrations. Significantly higher ($p=0.006$) levels of A-dione were seen in children from the hotspot than in those from the non-sprayed area, with no significant differences reported for cortisol, 17-OH-P4, and P4 between the two areas and in both sexes.

151. Multiple regression analysis showed a significant ($p = 0.019$ to <0.001) negative correlation between DHEA and all dioxin congeners, with the exception of TCDF and OCDF. Similarly, a significant ($p=0.002$ to <0.001) negative correlation was found between testosterone levels and all dioxin congeners, with the exception of TCDF, 1,2,3,7,8,9-HxCDF and OCDF. A significant ($p=0.039$ to <0.001) positive association was seen between levels of A-dione and all dioxin congeners, with the exception of TCDD and 1,2,3,4,6,7,8-HpCDD.

152. The authors concluded that the findings support disruptions in androgen levels, including decreases in testosterone and DHEA levels and an increase in A-dione level, associated with highly chlorinated dioxin congeners in both male and female Vietnamese children at the age of 5 years.

153. The main limitations identified by the authors is the use of mothers breast milk to estimate dioxin exposure of the children which is less accurate

than direct serum measurement of children's blood levels and the small sample size which may have limited statistical power.

Oyama et al., 2021

154. In a follow up study to that reported by Oanh et al. (2018), as discussed above, Oyama et al. (2021) evaluated the potential for sex-related androgen disruption in 7-year old Vietnamese children. The study included 96 mother-child pairs residing in a dioxin hot spot area (n=45, M:F 25:20) and a non-sprayed area (n=51, M:F 21:30) in Vietnam. As previously, breast milk samples were taken at recruitment and levels of seventeen dioxin congeners (7 dioxin and 10 furan congeners) were determined. Dioxin congener levels were converted to pg/g of lipid and to TEQs using the World Health Organization toxic equivalency factor (TEF). Where dioxin levels were below the LOD, a value equal to half the LOD was used. Serum samples were taken from the 7 year old children for measurement of DHEA, DHT, cortisol, cortisone, 17-OH-progesterone (17-OH-P4), P4, A-dione and testosterone.

155. Statistical analysis determined the difference of the mean in each indicator using the Student's t-test for the normally distributed data and the Mann-Whitney U test for the non-normally distributed data. Pearson correlation coefficients and Spearman correlation coefficients were calculated between the dioxin congener and hormone levels, as well as the enzyme activity and a multiple regression analysis was performed to consider the effects of the multiple factors, with the Benjamine – Hochburg procedure applied to adjust the p-value for the multiple analysis. For the multiple regression analysis, body measurements (height, weight, and head and chest circumferences) and steroid hormones (DHEA, A-dione, testosterone, P4, 17OH progesterone, cortisol, and cortisone) were selected as dependent variables, while mother's age, residence, full breastfeeding period, BMI, and dioxin congeners were selected as independent variables.

156. Breast milk samples from mothers living in the hotspot area had significantly higher (all $p<0.001$) levels (3-10 fold) of all dioxin congeners, with the exception of TCDF and 2,3,4,7,8-PeCDF, when compared with samples from mothers living in the non-sprayed area. The highest differences were

associated with the highly-chlorinated furan-types, 1,2,3,4,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and 1,2,3,4,7,8,9-HpCDF (6.5–9.4 fold higher). The total PCDD, PCDF, and TEQ total PCDD/Fs were also significantly higher ($p<0.001$; 3.0–3.6 fold) in the hotspot area when compared with the non-sprayed area. With regards to steroid hormone levels, in boys testosterone concentrations were significantly ($p<0.001$) supressed to 66.7% of those in the non-sprayed area. In boys only, 17β-HSD and testosterone levels had a strong inverse correlation with the TEQ total PCDD/Fs level in the breast milk ($r = -0.47$, $p = 0.001$, and $r = -0.62$, $p < 0.001$, respectively). A positive correlation ($r = 0.49$, $p < 0.001$, and $r = 0.56$, $p < 0.001$) was observed between the progesterone and TEQ total PCDD/Fs and for DHEA ($r = 0.5$, $p < 0.001$) with the TEQ total PCDD/Fs in the breast milk. No significant correlation was observed for cortisol and cortisone concentrations with the dioxin congeners.

157. DHEA levels in boys were significantly higher ($p<0.01$) from the hotspot area than from the non-sprayed area, a reversal of findings found at aged 5 years, and there was no difference between serum levels of cortisol, cortisone and 17-OH progesterone between the two areas. Serum E2 levels in the 7-year-old boys could not be sufficiently measured. A strong positive correlation was reported between testosterone levels for boys from both the hotspot and un-sprayed areas ($r = 0.758$, $p < 0.001$). Multiple regression analysis of the data for boys showed a strong reverse correlation ($p<0.01$ to <0.001) between the levels of testosterone with all dioxin congeners.

158. The authors concluded that the findings indicated a delay in the expression of the testosterone levels with growth in the 7-year old boys, associated with exposure to dioxins. The main limitation discussed by the authors is the uncertainty of whether changes in testosterone, androstenedione, and DHEA kinetics are due to postnatal exposure to dioxins or the effects of exposure to dioxin during pregnancy.

Petersen et al., 2018

159. Petersen et al. (2018) evaluated the potential effects of exposure to PCBs, including dioxin-like PCB-105, PCB-118 and PCB-156, and

perfluorinated alkylate substances (PFASs) via the environment, on semen quality or reproductive hormone levels in Faroese men (n=263; age 24-26 years) (see paragraph 62 for details of this study).

160. The authors reported a positive association between ∑PCBs (and perfluorooctane sulfonate (PFOS)) and levels of SHBG and LH. In addition, total testosterone was positively associated with ∑PCB.

161. The authors suggested that the association of testosterone with PCB 'may represent a compensatory adaption to elevated SHBG levels to maintain an unchanged free testosterone concentration'. In addition, 'the positive association to LH for both PCBs and PFOS may indicate a direct adverse effect on the testosterone producing Leydig cells'.

Van Luong et al., 2018

162. An assessment of the levels of reproductive hormones in a cohort of males living adjacent to a dioxin-contaminated area in Vietnam has been reported (Van Luong et al., 2018). Males were recruited (n=42) in 2014 with the following inclusion criteria: residence for >15 years in the area; between 20 – 50 years of age; not have received hormone therapy of hormone-disrupting medications within one month prior to enrolment. Blood samples were taken at recruitment for measurement of 17 2,3,7,8-substituted congeners of PCDDs and PCDFs and four non-ortho PCBs; where levels were below the LOD, congeners were assigned a value half of the individual limit. All measurements of individual congeners were expressed as pg/g fat and the cumulative TEQ values were determined for PCDDs, PCDFs, PCDDs/Fs, PCBs, and PCDDs/Fs/PCBs, as per WHO guidance. Serum samples were used for the determination of levels of FSH, LH, progesterone, prolactin, E2 and total testosterone.

163. When compared to analytical reference levels, seven men had low levels of testosterone (<250 ng/dL), and nine men had high levels of prolactin (>9.7 ng/mL). In addition, the levels of 2,3,7,8-TetraCDD levels and TEQs of PCDDs/Fs (7.3 and 34.0 pg/g fat respectively) were higher than reported for men in another dioxin-contaminated area (Phu Cat; 2.6 pg/g fat for 2,3,7,8-

TetraCDD and 26.0 pg/g fat for sum TEQs of PCDDs/Fs) and for men living in Kim Bang, an unsprayed area (1.5 pg/g fat for 2,3,7,8-TetraCDD and 9.2 pg/g fat for sum TEQs of PCDDs/Fs). Statistical analysis was carried out to identify any associations of dioxin concentrations and reproductive hormone levels using a partial correlation model, with adjustments for the potential confounders of age, BMI, and smoking status (yes = 1, no = 2); confounders were identified using Pearson's correlation and from published literature.

164. A positive correlation was reported for all dioxin congeners with levels of prolactin, with significance achieved for the PCDDs 2,3,7,8-TetraCDD (r=0.477; p=0.003), 1,2,3,7,8-PentaCDD (0.378; 0.021), 1,2,3,6,7,8-HexaCDD (0.415; 0.011) and 1,2,3,4,6,7,8-HeptaCDD (0.405; 0.013), for the PCDF congeners 1,2,3,6,7,8-HexaCDF (0.328; 0.047) and 1,2,3,4,6,7,8-HeptaCDF (0.331; 0.045) , for the PCB T4CB #77 (0.368; 0.025) and for the sum TEQ of PCDDs (0.468;0.004), PCDDs/Fs (0.458; 0.004), and PCDDs/Fs/PCBs (0.445; 0.006). A negative correlation was seen between the levels of all congeners and testosterone, with significance achieved for the PCDDs 1,2,3,7,8-PentaCDD (-0.33; 0.046) and 1,2,3,7,8,9-HexaCDD (-0.343; 0.038), the PCDF congeners 2,3,7,8-TetraCDF(-0.451; 0.005), 1,2,3,7,8-PentaCDF (-0.335; 0.043), 2,3,4,6,7,8-HexaCDF (-0.386; 0.018), 1,2,3,4,6,7,8-HeptaCDF (-0.465; 0.004), 1,2,3,4,7,8,9-HeptaCDF (-0.36; 0.028), OctaCDF (-0.343; 0.037), the PCB congeners T4CB #81 (-0.408; 0.012) and P5CB #126 (-0.337; 0.042) and the TEQs of PCDFs (-0.376; 0.022) and PCBs (-0.339; 0.04). Levels of all dioxins were negatively associated with oestradiol, with the exception of P5CB #126, with significance for the PCDD 1,2,3,4,6,7,8-HeptaCDD (-0.346; 0.036) and the PCDF congeners 1,2,3,6,7,8-HexaCDF (-0.369; 0.025) and 1,2,3,4,6,7,8-HeptaCDF (-0.377; 0.021). There were no significant correlations between FSH, LH, or progesterone levels and any dioxin congeners.

165. The authors concluded that the cohort of men had elevated levels of dioxins in their blood when compared to another dioxin hotspot and an unsprayed area. The findings showed that exposure to some dioxins may increase levels of prolactin and decrease levels of testosterone in men.

166. The main limitation identified by the authors was the inability to establish causal relationships, as the study was cross-sectional and without a comparison group and only one blood sample was taken for hormone and dioxin measurements. In addition, the small sample size was noted and the use of multiple statistical tests which may have introduced chance findings.

Summary

167. From the in vivo studies identified, either adult male mice or rats were exposed by gavage, or F1 rats were exposed prenatally to TCDD, TCDF or PCB-118. The male reproductive effects investigated include those on sperm parameters (sperm count and motility, sperm malformations), effects on testes (morphological and histopathological changes in seminiferous tubules and/or epithelium, Sertoli cell count, testicular coefficient, morphological abnormalities in the testes) and effects on hormone levels (serum testosterone, gonadotrophin, INHA).

168. Sperm parameters (sperm count, percentage dead sperm, morphological abnormalities and motility) were affected in all adult mice by TCDD and TCDF exposure, and F1 adult mice with PCB-118. Results in rats were mixed as the number of live spermatozoa was decreased in adult rats exposed to TCDD but no effects were seen on daily sperm production or number of mature spermatids in neonatal or F1 adult rats following a single exposure to TCDD on GD15.

169. Effects on the testes were reported in adult mice following exposure to TCDD and TCDF and F1 mice following exposure to TCDD and PCB-118. However, effects in rats were mixed. In neonatal (PND1) rat testes, no histopathological changes (no abnormal cells in the seminiferous cord and interstitial tissue and no changes in the percentage seminiferous cord and interstitial tissue compartments) were reported following exposure to a single dose of 1 µg/kg bw/day TCDD on GD15. However, TCDD exposure was associated with a reduced Sertoli cell count in both F1 neonatal and F1 adult testes and there was a significant increase in the number of abnormal seminiferous tubules in F1 adults. In contrast, no histological changes (no

further details given) were seen in neonatal and F1 rats when exposed to a loading dose of up to 10 µg/kg bw TCDD or TCDF on GD8 followed by a maintenance dose of 0.22 µg/kg bw/day on GD9-20 or those exposed to 10 µg/kg bw TCDD or TCDF as a single dose on GD15.

170. With regard to an effect of exposure on hormone levels, no effects were seen on testosterone levels in neonatal or F1 rats following in utero exposure to TCDD. In fetal tissues, significant decreases in gene expression of fetal pituatory Fshb and fetal testis Inha were reported. No significant effects were seen with TCDF on testosterone levels or in the gene expression of Lhb or Cga. Authors suggest that the perinatal reductions in pituitary gonadotropin gene expression led to a reduction in circulating LH, testis steroidogenic gene expression, and testis testosterone production.

171. From epidemiology studies, the main reprotoxic effects investigated in males were reduced semen quality, cryptorchidism, changes in hormone levels and pubertal development.

172. Many studies measured levels of the chemical of interest in breast milk which have been reported to correlate with levels in umbilical cord, and maternal serum; breast milk is therefore considered a suitable proxy for prenatal and postnatal exposure and a good indicator of body burden for persistent compounds.

173. Evidence from epidemiological studies in adults on semen quality has provided mixed results, with both declines and improvement in semen parameters having been reported.

174. Negative correlations were reported for TCDD in terms of sperm motility and sperm count. However, response to dioxin-like PCBs was mixed. Negative correlations were reported with PCB-126 and sperm viability, PCB-118 with semen volume and PCB-189 with sperm motility and authors concluded the dioxin-like PCBs caused alteration of semen parameters such as motility, volume, morphology and viability. A significant negative correlation was also reported for PCB-118 sperm motility although no effect was seen on

sperm count. Other studies reported exposure to PCB-105, PCB-118 and PCB-156 had no effect on semen parameters in Faroese men.

175. Effects may be dependent on the individual congener, however not all congeners have been evaluated. The main limitations of the studies include small cohort size, imprecision of sperm measurements and limited evaluation of the clinical relevance of the response.

176. One study was identified that considered the effects of POPs on occurrence of cryptorchidism which included evaluation of seven dioxin-like PCBs. Although significant associations were determined for a number of dioxin-like PCBs, only PCB-114 (also non-dioxin-like PCB-194 and β-HCH) was identified as the best predictor for congenital cryptorchidism. A further study reported that AhR activity in breast milk samples was not significantly different between cases with cryptorchidism and controls. However, all the dioxin-like PCBs measured in the samples showed (borderline) significant associations with AhR activity. Limitations of the study include maternal reporting of the outcome of cryptorchidism and determination of the chemicals of interest during the third trimester or postpartum whilst the most sensitive period for testicular development is the first trimester.

177. A number of studies investigated effects of dioxin and dioxin-like PCBs on hormone levels in male children or male adults with varying results. In most studies a negative association with testosterone levels was reported following exposure to dioxin or dioxin-like PCBs, but in some studies, a positive association was reported or no effects were seen.

178. Results for the effect on inhibin B and DHEA were also inconsistent as some studies showed a positive association whereas some showed a negative correlation. A-dione was increased in two studies.

179. When the epidemiology studies are considered as a whole, a number of limitations can be identified for the data set:

- Small sample size of most studies.

- Multiple statistical tests were carried out on a number of chemicals meaning that some of the significant results could have happened by chance, unless specifically adjusted for (around 2/3 of studies adjusted for this).

- The cross-sectional nature of many of the studies. Serial samples were not collected so it isn't known if levels have been high for long periods or just at the time the sample was taken.

- As studies looked at a single sample/exposure the potential result of subsequent exposures was not considered.

- In regression analysis it appears that very few studies consider the potential effects of exposure to other chemicals. For example, exposure post-Vietnam, and also the impact of maternal prenatal blood concentrations on hormone levels in adolescent boys (no mention of what boys were exposed to during childhood).

- Self-reporting of diagnoses could introduce significant bias.

Immunotoxicity

180. TCDD has been shown to have various immunotoxic effects such as thymic involution, decreased host resistance to pathogens and tumors, suppressed fetal lymphocyte development and maturation. It also suppresses adaptive immune responses, including antibody production, T cell differentiation, cytotoxic T lymphocyte (CTL) activity and delayed hypersensitivity responses (Gutierrez-Vazquez & Quintana, 2018). Whilst AhR is not needed for the development of a functional immune system, its absence prevents the immunosuppressive effects of TCDD (Marshall & Kerkvliet, 2010).

Animal data

Jin et al. (2018)

181. The effect of TCDD on testis inflammation following pre-natal exposure was evaluated by Jin et al. (2018). Pregnant C57BL/6 mice were administered a single dose of TCDD by gavage on E15 at doses of 0 (vehicle not specified) or 10 µg/kg bw.

182. To establish the inflammation model, eight-week old F1 males (n=6/group) were exposed to lipopolysaccharides (LPS) (5 mg/kg) by intraperitoneal (i.p.) injection to induce inflammation, or a saline solution (200 µl/mouse; NC). After 24 h, the mice were sacrificed, and their testes were removed. No study guideline was followed. Endpoints studied included germ cell and testis apoptosis, mRNA and protein levels of pro-inflammatory cytokines (interleukin (IL)-1β, IL-18, IL-12), and Klotho expression.

183. The real time PCR results showed that LPS treatment increased the mRNA levels of IL-1β, IL-18, and IL-12 to 5.1-, 4.7-, and 3.6-fold, respectively, compared with the controls whereas the combination of TCDD and LPS significantly enhanced the expression of IL-1β, IL-18, and IL-12 to 45%, 42%, and 190%, respectively, compared with the LPS group. Similarly, the protein levels of pro-inflammatory cytokines increased with LPS alone and the combined treatment of TCDD and LPS, significantly enhanced the cytokines' production compared with the LPS group (1.6-, 1.8-, and 2.6-fold, respectively). Such an increase in cytokines led to an increase in apoptosis as the number of caspase-3-positive apoptotic cells per seminiferous tubule was higher in LPS-treated mice compared to controls, and also higher following LPS+TCDD treatment compared with LPS alone. The number of TUNEL-positive cells was also higher following LPS+TCDD treatment.

184. Authors noted that pre-treatment with TCDD did not increase AhR suggesting that TCDD may affected inflammation in the testis by an AHR-independent mechanism and speculated that LPS+TCDD initiated oxidative stress to promote inflammation.

185. Overall, author concluded that pre-treatment with TCDD exacerbated testicular inflammation by promoting the production of pro-inflammatory cytokines, which might affect the testicular microenvironment and induces germ cell apoptosis.

Kakutani et al., 2022

186. Kakutani et al. (2022) investigated long-term exposure to TCDD on antigen-specific antibody production in mice. Adult female BALB/c mice (10/group) were administered a mixture of ovalbumin (OVA; 100 µg/mouse) and TCDD at daily doses of 0 (OVA), 0.0005, 0.0055, 0.05, 0.1 or 0.5 µg/kg bw/day (originally reported in ng/kg bw/day) by gavage for 10 weeks. No study guideline was followed. At week 10, serum and mucosal secretions (fecal extracts and vaginal washes) were collected, and titrations of OVA-specific antibody were determined by enzyme-linked immunosorbent assay (ELISA) to assess if TCDD induced antigen-specific humeral responses. Spleen and mesenteric lymph nodes were collected for lymphocyte preparation and mRNA levels of cytokines were measured to assess if TCDD induced Th1- (IFN-γ and IL-2) or Th2- (IL-4, IL-3 and IL-10) type responses.

187. There was no effect on mortality, body weight, or spleen/body weight ratio at any dose.

188. Antibody titres of serum OVA-specific immunoglobulin G (IgG) increased in a dose-dependent manner at week 10 below 0.5 µg/kg bw/day and was significantly increased compared to controls at 0.1 µg/kg bw/day. There was no significant effect on fecal or vaginal immunoglobulin A (IgA) titers at any dose.

189. The OVA-specific IgG1 (a Th2 response) and IgG2a (a Th1 response) responses in the serum were significantly increased compared to controls following TCDD administration as well as titres of IgG1 antibodies. For all cytokines measured except for IL-10, a dose-dependent increase was observed by restimulation of OVA and a decrease in cytokine production was observed at 0.5 µg/kg bw/day. IL-17, which is related to Th17, showed similar results. These results suggested that oral exposure to TCDD enhances the

production of various cytokines, resulting in abnormal antigen-specific antibody production.

190. The authors noted that TCDD had an immunostimulatory effect at low doses and concluded that low doses of TCDD might show adjuvanticity and high doses may cause immunosuppression.

Li et al 2019

191. Li et al. (2019) investigated the potential toxic effect of TCDD on the development and function of Type 3 innate lymphoid cells (ILC3s) in the colon in vivo. Pregnant 8-10 week old C57BL/6 mice (number/group not stated) were administered TCDD by gavage at daily doses of 0 (DMSO), 0.1 or 10 µg/kg bw/day on ED0.5, ED12.5 and PND7. No study guideline was followed. F1 offspring were sacrificed at 10 weeks old and dams at age 23-25 weeks, and the colon was harvested to evaluate alterations in ILC3 differentiation and function. Body weight, organ weight, and organ index were measured in offspring only. Endpoints were measured in 8-14 offspring and 3-6 dams.

192. Significant mortality of pups occurred only at high doses within one or two days of birth (control: 1/16 pups; 0.1 µg/kg bw/day: 1/33 pups; 10 µg/kg bw/day: 25/40 pups).

193. At a maternal dose of 0.1 µg/kg bw/day, offspring had a significantly decreased spleen weight and spleen index (% body weight) relative to controls. There were no significant changes in the offspring spleen weight or index at 10 µg/kg bw/day, and no significant effect on offspring body weight, liver weight or liver index (% body weight) at either dose.

194. Changes in ILC3 differentiation were seen at 10 µg/kg bw/day (no significant effect at 0.1 µg/kg bw/day), with the percentage of ILC3s being significantly decreased in offspring but not in dams. At 0.1 and 10 µg/kg bw/day there was a significant decrease in the expression of retinoic acid receptor-related orphan receptor (RORγt) in ILC3s in offspring (not dose-dependent) and dams. The percentage of natural cytotoxicity receptor NKp46+ ILC3s was significantly increased in offspring at 0.1 and 10 µg/kg bw/day but was only significantly increased in dams at 0.1 µg/kg bw/day.

195. To investigate the effect of TCDD on ILC3 function, the percentages of IL-17a-, interferon (IFN)-γ-, and IL-22-producing ILC3s and the Median fluorescence intensity (MFI) of such cytokines was assessed.

196. The percentage of IL-17a^{+} ILC3s was significantly increased at 10 µg/kg bw/day in offspring but only at 0.1 µg/kg bw/day in dams. The MFI of IL-17a in these IL-17a+ILC3s remained unchanged in both offspring and mothers. In contrast, the percentage of IFN-γ+ ILC3s was significantly increased in mothers at 10 µg/kg bw/day and the MFI of IFN-γ in IFN-γ+ ILC3s was significantly decreased but there was no significant effect in offspring at any dose. Neither the frequency of IL-22+ ILC3s nor the IL-22 expression level was changed after TCDD exposure in offspring or mothers.

197. The authors concluded that long-term maternal exposure to TCDD causes alterations in ILC3 differentiation and production of cytokines in the colon. Moreover, distinct changes in ILC3s and ILC3 subsets are produced in the mother and offspring in response to maternal TCDD exposure.

Lowery et al. (2021)

198. Lowery et al. (2021) investigated how TCDD exposure in adulthood, which negatively impacts immune cells in the periphery, affects microglial characteristics in mouse cortex.

199. To assess baseline microglial characteristics, C57B1/6J mice (6/group and 2 of each sex between ages P60 and P120) were administered TCDD (10 µg/kg) or olive oil via gavage, an exposure paradigm that is immunosuppressive in the peripheral immune system. Twenty-four hours later mice were challenged with LPS (0.75 mg/kg) or saline via i.p. injection to determine whether TCDD dysregulated microglial responses to insult as with peripheral immune responses. Brains were harvested after 24 hours for quantification of microglia density, distribution and morphology. Microglia were evenly distributed across the cortical region in all groups, and no significant effect of acute TCDD exposure or LPS treatment on the density or spacing of microglia was seen. In addition, no sex-specific differences on microglial response was seen, although an effect on microglial clustering was reported.

200. A second cohort of mice was generated for RT-qPCR analysis. Each group consisted of 3 males and 3 females between the ages of P120 and P130. Mice were administered TCDD (10 µg/kg) or olive oil via gavage and 24-hours later were challenged with LPS (4 mg/kg) or saline via i.p. injection. The higher LPS dose was used to ensure the microglia pro-inflammatory response was robust to detect priming or suppression following TCDD exposure. Forty-eight hours after LPS administration, brains were harvested for RT-qPCR analysis for the pro-inflammatory cytokines tumour necrosis factor (TNF)-α, IL-6 and IL-1β.

201. Results showed that LPS induced a significant increase in all three cytokines in both males and females, while there was no effect of TCDD either alone or in combination with LPS, demonstrating that acute, adult TCDD exposure neither induces nor primes or suppresses the microglial inflammatory response.

202. Authors concluded that the results confirm at the molecular level our findings that TCDD exposure in adulthood does not cause overt changes in the microglial inflammatory response.

Epidemiology data

Arisi et al., 2021

203. In a cross-sectional study, Arisi et al. (2021) evaluated the prevalence of skin disorders in a highly PCB polluted area (Brescia) in northern Italy, with locally produced food as the main source of human contamination. PCBs, including dioxin-like-PCBs, can accumulate in all skin cell populations and therefore have the potential to induce inflammatory and/or tumoural skin diseases (Furue et al., 2014 as cited in Arisi et al. (2021)).

204. A random sample (n = 189) of the general population living in Brescia, previously enrolled in a survey aimed to investigate PCB contamination of residents in the area, underwent PCB serum measurement. Three groups of participants (n=63 per group) were identified according to the tertiles of the total PCB serum concentration, forming Groups A, B and C (highest, medium

and lowest total PCB serum values, respectively) and underwent a dermatological evaluation.

205. Enrolled subjects were all Caucasians and residing in the town for more than five years. A total PCB level was calculated by summing the serum values of each of 33 PCB congeners including the dioxin-like PCBs PCB-77, PCB-81, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-156, PCB-157, PCB-167, PCB-169, PCB-189. Following dermoscopic evaluation (no further details given), inflammatory diseases were noted as seborrheic dermatitis, atopic dermatitis, psoriasis, and/or skin mycoses.

206. No significant increase in the frequency of skin inflammatory disease and mycosis was identified between groups A, B and C. The strength of association between skin disorders and serum levels of polychlorinated biphenyls, based on OR and 95% CI, adjusting for age, gender and sun exposure, was also not significant (1.00 (0.99 – 1.02)) for skin inflammatory disease and mycosis.

207. It is not possible to draw any conclusions from this study as only total PCBs were reported. Based on previous literature, the authors stated that 'the sum of dioxin-like PCBs contributed to less than one quarter of the total PCBs, also in subjects with the highest levels of PCB serum levels. Therefore, the relatively low concentration of dioxin-like PCBs in our subjects may have not been sufficient to determine an increased risk of skin lesions'. The low number of participants, and therefore low number of skin lesions, was identified as the main limitation by the authors.

Miyashita et al., 2018b

208. The potential effects of prenatal exposure to dioxin-like compounds on the levels of immunoglobulin E (IgE) in cord blood at birth and the frequency of allergies and infections in offspring at 3.5 (n=327) and 7 (n=264) years in Japan has been investigated (Miyashita, Bamai, et al., 2018). Pregnant Japanese women, who had previously enrolled on the Hokkaido Study on Environment and Children's Health were invited to take part (n = 5140, and 504 were judged eligible for participation in the current study; ten subjects

were excluded due to miscarriage, stillbirth, relocation, or voluntary withdrawal from the study until birth).

209. The concentration of dioxin-like compounds in maternal blood (n=426) was measured in the third trimester of pregnancy, and levels of IgE measured in cord blood at birth, as part of the Hokkaido Study. At 3.5 and 7 years post-delivery, mothers completed a self-administered questionnaire to collate information on 'breast-feeding, environmental exposure to tobacco smoke, keeping pets in the home, living environment, day-care attendance, infant vaccination, and previous or current medical history of infant allergies and infectious symptoms aged up to 3.5 and 7 years'. The response rate at 3.5 years was 77.8% (345 respondents from 443 invitations) and at 7 years was 71% (281 respondents from 396 invitations).

210. The authors used the Spearman correlation test, the Mann–Whitney U test, and the Kruskal–Wallis test to investigate possible associations between concentrations of maternal dioxin-like compounds, cord blood IgE, characteristics of participants, and children's health outcomes. Multivariate analyses were adjusted for maternal age, BMI, parity (primiparous/multiparous), education, smoking during pregnancy, blood lipid, allergic history, infant gender, birth season, distance from home to highway and household income and the blood sampling period. Crude and adjusted logistic regression analyses were performed where adjusted model 1 included confounding variables of maternal factors and adjusted model 2 additionally included confounding environmental factors after birth. The dependent variable for the linear regression analysis was blood levels of dioxin-like compounds and for the logistic regression analysis it was risk of allergies.

211. Median dioxin-like compounds concentrations (comprising PCDDs: TCDD, PeCDD, HxCDD, HpCDD, OCDD; PCDF: TCDF, PeCDF, HxCDF, HpCDF, OCDF; Co-PCBs: 81, 77, 126, 169) among the three participant groups were: 14.0 TEQ pg/g lipid (interquartile range, IQR 10.1–18.0) at birth: 14.2 TEQ pg/g lipid (IQR 10.3–18.9) in children at age 3.5 years; and 15.0 TEQ pg/g lipid (IQR 11.0–20.0) in children at age 7 years. dioxin-like compounds concentration ranges were not significantly different between the

three participant groups. The median IgE concentration of the group at birth was 0.21 IU/mL (IQR 0.08–0.55).

212. The concentrations of dioxin-like compounds in maternal blood were significantly associated with maternal factors, including age ($p<0.01$), parity ($p<0.01$), educational level ($p<0.01$), and gestational period that blood was sampled ($p<0.05$). The concentrations of cord blood IgE were significantly associated with maternal factors, including pelagic fish intake and allergic history; and with infant gender (all $p<0.05$). No associations were observed between cord blood IgE concentration and allergies or infections in children aged up to 3.5 and 7 years.

213. In the logistic regression analysis adjusted for confounding factors (as models 1 and 2 above), the authors reported a positive association between maternal dioxin-like compounds concentration and the incidence of wheezing at 7 years of age among all participants (Model 1 odds ratio (OR)= 8.09 (95% confidence interval (CI), 1.57 to 41.68) $p<0.05$; Model 2 OR= 7.81 (1.42 to 42.94) $p<0.05$)). Among boys, significant associations were observed between maternal dioxin-like compounds concentrations and wheezing in the adjusted models at 3.5 years [adjusted model 2 OR wheezing = 0.03 (0.00 to 0.94) $p<0.05$], which was almost significant at 7 years (12.05 (0.99-146.37).

214. For infections as a whole, children up to age 7 showed higher maternal dioxin-like compounds concentrations compared with non-infected children aged up to 7 years ($p<0.05$). IgE concentration in cord blood decreased significantly ($p<0.05$) with high maternal dioxin concentration among boys, as shown by regression analysis adjusted for confounding factors [adjusted β; −0.87 (95%CI, −1.68 to −0.06) among boys].

215. The authors concluded that ‘prenatal exposure to dioxin-like compounds may modify offspring immune responses and result in increased risk of allergy among children at school age. Moreover, male infants may be more susceptibility to maternal exposure to dioxin-like compounds’.

216. Limitations of the study were recognised as a small sample size and that dioxin-like compounds levels in breast milk were measured, which may

have provided reliable data for direct assessment of prenatal dioxin-like compounds exposure levels.

Margetaki et al., 2022

217. Margetaki et al. (2022) examined a potential association between prenatal exposure to POPs and allergic outcomes in offspring in early and mid-childhood. Mother-child pairs living in Crete were recruited as part of the Rhea birth cohort, with 1110 maternal blood samples being analysed for POP exposure at around 12 weeks of pregnancy (mean ± standard deviation (SD), 11.96 ± 1.49 weeks). Total PCB concentrations were calculated from the sum of the concentrations of six individual PCB congeners (PCB-118, PCB-138, PCB-153, PCB-156, PCB-170 and PCB-180) including the dioxin-like PCBs PCB-118 and PCB-156. Levels of hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE), and polybrominated diphenyl ether (BDE-47) were also determined but are not relevant to this review.

218. Information regarding allergy-related outcomes were collected for offspring at 4 and 6 years of age, with a total of 682 and 454 children being evaluated respectively. The current status of wheeze, asthma, eczema, and rhinitis occurrence was obtained by questionnaires adapted from the International Study on Asthma and Allergy in Childhood (ISAAC; Asher et al., 1995 as cited in Margetaki et al. (2022)).

219. Poisson models with log link and robust standard errors were used to estimate Risk Ratios (RRs) and 95% CIs for the associations between the POP exposures and binary outcome variables. A multiplicative interaction term between each exposure and child sex was applied to the models when evaluating sex-specific associations. All models were adjusted for possible confounders, including, child age (years) and sex (male/female), maternal age (years), history of maternal and paternal atopy (asthma, rhinitis and eczema; yes/no), maternal BMI pre-pregnancy (kg/m^2), maternal level of education (12 years) and parity (nulliparous/multiparous).

220. The authors noted that in girls 'total PCBs were associated with increased risk for current eczema at 4 years (RR (95%CI): 2.1 (1.1, 4.2) and p-interaction = 0.028)'. It is not possible to draw any conclusions from this study as only total PCBs were reported, of which only two were of relevance to this review. No statistical analysis for individual PCBs was carried out.

Summary

221. Four in vivo studies were found relating to immunotoxicity. In all studies, cytokine responses were affected. TCDD exacerbated inflammation by increasing pro-inflammatory cytokines in the spleen, testes and colon but not in the brain and one study suggested that in adult mice, TCDD had an immunostimulatory effect at low doses whereas at high doses it may cause immunosuppression. TCDD also increased antigen-specific humeral responses by increasing antibody production and affected cellular immunity as indicated by the increase in cytokines.

222. Many of the epidemiology studies evaluated total PCB levels only hence it was not possible to determine effects due to individual dioxin-like PCBs.

223. Although some progress has been made in the understanding of antiviral immune responses and how these are evaded by viruses, it remains unclear how environmental exposures to dioxins and/or dioxin-like PCBs may impact on antibody responses in humans.

224. The potential for prenatal exposure to specific dioxins or dioxin-like PCBs to modify offspring immune responses, resulting in an increased risk of allergies in school age children, and in particular males, has been reported. A number of study limitations were apparent however, which may impact on these findings.

Other endpoints seen in vivo

Bone effects

Brankovic et al., 2017, 2019, 2020

225. The effects of lactational exposure to PCB-169 on a number of bone parameters were investigated in a series of papers by Brankovič and colleagues (Brankovič et al., 2017, 2019, 2020). After delivery lactating female Wistar rats (4/group) were administered loading doses of 0 (olive oil) or 2 mg/kg bw/day) (2000 µg/kg bw/day) followed by two maintenance doses of 0.5 mg/kg bw/day (500 µg/kg bw/day) on PND6 and PND14, by i.p. injection. Offspring were euthanised during the suckling period (PND9), at weaning (PND22) or during the pubertal period (PND42). After euthanasia femurs were collected for analysis. Dams in the control group were administered maintenance doses of olive oil on PND6, PND12, PND14 and PND17 by i.p. injection.

Brankovic et al., 2017

226. Brankovič et al. (2017) investigated changes in growth rate, geometry, serum, and bone biochemical parameters and biomechanics of juvenile rat femur induced by lactational exposure to PCB-169. Femurs from 22-day-old offspring were analysed for bone geometry, biomechanics and mineral composition as well as levels of calcium, phosphate and alkaline phosphatase in serum.

227. Lactational exposure to PCB-169 resulted in shorter and thinner femurs, reduced endosteal and periosteal perimeters, smaller total cross-sectional and medullary areas. Levels of bone markers in serum and calcium levels in the bone decreased. Femur mechanical properties were not significantly changed.

228. Authors concluded that the data demonstrate that PCB-169 caused bone alterations in lactationally-exposed offspring as exposure resulted in significantly shorter and thinner femurs, significantly decreased endosteal and periosteal perimeters, smaller total cross-sectional and medullary areas, and

significantly lowered serum bone marker levels and calcium levels in the bone, while femur mechanical properties were not significantly altered.

229. The lowest dose at which effects of PCB-169 were seen was at a loading dose of 2000 µg/kg bw/day followed by two maintenance doses of 500 µg/kg bw/day on PND6 and PND14 (single dose tested).

Brankovic et al., 2019

230. Brankovič et al. (2019) investigated bone geometry, biomechanics and mineral composition in femurs from 42-day-old offspring. Decreased somatic mass and femur size, i.e., mass, periosteal circumference and cross-sectional area were observed in the PCB-169-treated animals. Treatment also resulted in harder and more brittle bones containing higher amounts of minerals.

231. Authors concluded that bone alterations observed on PND42 were induced by PCB-169.

232. The lowest dose at which effects of PCB-169 were seen was at a loading dose of 2000 µg/kg bw/day followed by two maintenance doses of 500 µg/kg bw/day on PND6 and PND14 (single dose tested).

Brankovic et al., 2020

233. Brankpvic et al. (2020) investigated the effects of lactational exposure to PCB-169 on longitudinal femur growth at the distal epiphyseal growth plate (EGP) in young rats on PND9, 22, and 42. The femurs of offspring were used to estimate growth rate. Histomorphometric analysis on the distal femur included the thickness of the EGP and zones of proliferation and hypertrophy with calcification. Stereometry was used to determine trabecular bone volume density.

234. PCB-169 affected longitudinal bone growth in the early postnatal period by interfering with chondrocytes in the EGP zone of proliferation and, to a lesser extent, the zone of hypertrophy. Morphometric alterations in EGP structure diminished until puberty. The slow growth rate continued until PND42.

235. It was concluded that PCB-169 caused age-dependent effects on femur growth rate and histomorphometric characteristics.

236. The lowest dose at which an adverse effect was seen at a loading dose of 2000 µg/kg bw/day followed by two maintenance doses of 500 µg/kg bw/day on PND6 and PND14 (single dose tested).

Fader et al., 2018

237. Fader et al. (2018) investigated the effect of 0.01-30 TCDD µg/kg bw/day on the femoral morphology of male and female juvenile C57BL/6 mice (5-8/group), gavaged every 4 days for 28 days. RNA-Sequencing (RNA-Seq) was used to investigate gene expression changes associated with the resultant phenotype.

238. TCDD dose-dependently increased trabecular bone volume fraction (BVF) 2.9- and 3.3-fold in male and female femurs, respectively. Decreased serum tartrate-resistant acid phosphatase (TRAP) levels, as well as a reduced osteoclast surface to bone surface ratio and repression of femoral proteases (cathepsin K, matrix metallopeptidase 13) indicates that TCDD impaired bone resorption.

239. Increased osteoblasts at the trabecular bone surface were correlated with a reduction in the number of bone marrow adipocytes. Authors suggested that AhR activation may direct mesenchymal stem cell differentiation towards osteoblasts rather than adipocytes. Femoral expression of transmembrane glycoprotein NMB (Gpnmb; osteoactivin) was dose-dependently induced by TCDD and increased serum levels of 1,25-dihydroxyvitamin D3 correlated with the renal induction of 1α-hydroxylase Cyp27b1, suggesting a possible contribution to impaired bone resorption.

240. Overall, the authors concluded that TCDD alters the balance between bone resorption and formation in juvenile mouse femurs, which results in increased bone mass with reduced marrow adiposity.

241. The lowest dose at which an adverse effect was seen was 1 µg/kg bw/day, which caused a significant decrease in trabecular spacing in femurs of male mice.

Ronis et al., 2020

242. Ronis et al. (2020) investigated the effect of PCB-126 on skeletal toxicity. Groups of 5-6 week old male Sprague-Dawley rats (12/group) were administered PCB-126 at a single dose of 0 (soy oil) or 5 µmol/kg bw (1630 µg/kg bw/day) by i.p. injection and sacrificed after 4 weeks.

243. PCB-126 exposure reduced long bone length, diameter and surface area but increased trabecular thickness and volume. Serum osteocalcin, a marker and a regulator of bone formation, was reduced but PCB exposure had no effect on the bone resorption marker RatLaps.

244. PCB-126 exposure also caused hypocalcemia and significant increases in serum parathyroid hormone (PTH) whereas serum phosphorous remained unchanged. Hyperparathyroidism was accompanied by an increase in expression of mRNAs of vitamin D3 metabolizing cytochrome P450 enzymes CYP27B1 and CYP24 in the kidney. Reduced body weight, serum IGF-1 and hepatic expression of mRNAs encoding of the male-specific GH-pattern regulated CYP2C11 and CYP3A2 were also reported following PCB exposure compared to controls.

245. Authors suggested that skeletal toxicity after exposure to PCB-126 is a result of disruption of calcium homeostasis and the growth hormone (GH)–insulin-like growth factor (IGF) (GH-IGF-1) axis and involves direct AhR-mediated effects on bone formation. Overall, it was concluded that the data demonstrate that skeletal toxicity occurs in male rats following exposure to PCB-126, which is consistent with epidemiological evidence. These effects appear to be mediated by endocrine disruption as well as PCB-126 exerting direct effects on bone turnover and bone cell differentiation.

246. The dose at which an adverse effect seen was 5 µmol/kg bw (1630 µg/kg bw/day).

Williams et al., 2020

247. Williams et al. (2020) investigated the effect of PCB-126 on skeletal toxicity. PCB-126 at a single dose of 0 (corn oil) or 5 µmol/kg bw (1630 µg/kg bw/day) was administered to 4-week old male and female wild type (WT) and AhR knockout rats (AhR -/-) Sprague Dawley rats (25-28/group) by i.p. injection. Animals were sacrificed after 4 weeks after which bone length was measured and bone morphology was assessed by microcomputed tomography and dynamic histomorphometry.

248. Reduced bone length was the only genotype-specific effect and was only observed in males. WT rats exposed to PCB-126 had reduced serum calcium, reduced tibial length, cortical area, and medullary area compared to controls. Reduced bone formation rate was also seen in females which was consistent with inhibition of endosteal and periosteal bone growth. Such effects were not seen in AhR -/- rats.

249. Gene expression in bone marrow and the bone shaft showed that approximately 75% of the PCB-regulated genes appeared AhR dependent. Indian hedgehog (Ihh) and connective tissue growth factor (Ctgf/Ccn2), which regulate chondrocyte proliferation and differentiation in the bone growth plate and cell-matrix interactions were significantly induced by PCB-126.

250. Overall, the authors concluded that skeletal toxicity occurs in rats following exposure to PCB-126 and such effects appear to be mediated indirectly via AhR-mediated endocrine disruption, and also directly via AhR-mediated effects on growth plate function, bone cell matrix interactions and bone formation.

251. The dose at which an adverse effect seen was 5 µmol/kg bw (1630 µg/kg bw/day).

Summary

252. In the in vivo studies identified, adult male and female mice or rats, male and female juvenile mice, or lactating rats were administered TCDD, PCB-126 or PCB-169 by gavage or i.p. injection.

253. Only one study was found relating to TCDD exposure, which showed an altered balance between bone resorption and formation in juvenile mouse femurs, resulting in increased bone mass with reduced marrow adiposity.

254. Several studies investigated the effects of PCB-169. On PND22, lactationally-exposed offspring exhibited shorter and thinner femurs, decreased somatic mass and femur size, i.e., mass, periosteal circumference and cross-sectional area, as well as lower serum bone marker levels and calcium levels. At PND42, weaned offspring also showed decreased somatic mass and femur size following PCB-169 treatment, as well as harder and more brittle bones containing higher amounts of minerals. PCB-169 also affected longitudinal bone growth in the early postnatal period and such slow growth rate continued until PND42.

255. Several studies also investigated the effect of PCB-126. Both studies showed exposure to 1630 µg/kg bw/day PCB-126 was associated with hypocalcemia in rats, which resulted in skeletal toxicity (reduced long bone length, diameter and surface area).

256. The lowest dose at which effects on bone parameters were seen for TCDD was 1 µg/kg bw/day when male and female juvenile mice were dosed every 4 days for 28 days (see Fader et al. (2018)).

257. For PCB-126, skeletal toxicity was seen at 5 µmol/kg bw (1630 µg/kg bw/day) when male rats were administered a single dose (see Ronis et al. (2020) and Williams et al. (2020)).

258. The lowest dose at which effects were seen for PCB-169 was for lactating rats administered a loading dose of 2000 µg/kg bw/day followed by two maintenance doses of 500 µg/kg bw/day on PND6 and PND17 (see Brankovič et al. (2019); Brankovič et al. (2020); Brankovič et al. (2017).

Cardiovascular effects

Bey et al., 2022

259. Bey et al. (2022) investigated the effect of TCDD on the formation of atherosclerotic lesions, histological parameters and critical atherosclerotic markers in aorta. Twenty-week and 27-week old mixed sex groups of male and female ApoE KO mice (10/group) were administered TCDD by i.p. injection at doses of 0 (corn oil) or 1 µg/kg bw/week (converted to 0.14 µg/kg bw/day) for eight weeks.

260. Atherosclerotic lesions, histological parameters and critical atherosclerotic markers in the aorta were assessed.

261. TCDD increased atherogenic lesions leading to a switch of vascular smooth muscle cells (VSMCs) from a contractile to a pro-atherogenic phenotype, which promotes atherosclerotic plaque development, and increased expression of the proatherosclerotic marker vascular cell adhesion molecule VCAM1. AhR activation accelerated the formation of atherosclerotic plaques with sex and age differences due to the phenotypical switch of VSMCs.

262. Authors suggested there to be a gender difference in the effects of TCDD on the progression of atherosclerotic plaques, and that the stage of atherosclerosis development also seems be a differentiating factor.

263. The lowest dose at which effects of TCDD were seen was 0.14 µg/kg bw/day (the only dose tested).

Ciftci et al., 2018

264. Ciftci et al. (2018) investigated the protective effects of beta-glucan against TCDD-induced cardiotoxicity in rats by measuring biochemical parameters (oxidant/antioxidant parameters) and histopathological changes.

265. Adult female Sprague-Dawley rats (8/group) were administered TCDD for 21 days at doses of 0 (distilled water) or 2 µg/kg bw/week (converted to 0.29 µg/kg bw/day) by gavage. Rats were sacrificed after 21 days and heart tissues were collected.

266. In rats exposed to TCDD, histopathological damage of heart tissue was visually evident as eosinophilic stained and pyknotic nuclei cells were seen as well as necrosis, hemorrhage mononuclear cell infiltration, oedema, vascular congestion and vacuolisation. A decrease in levels of glutathione (GSH), catalase (CAT), glutathione peroxidase (GSH-Px), and SOD were also reported, which were increased with beta glucan administration.

267. The authors concluded that TCDD leads to oxidative and histopathological damages in heart tissue of rats. Moreover, beta-glucan had strong antioxidative potentials and partially prevented cardiotoxic effects of TCDD on histopathological changes.

268. The lowest dose at which effects of TCDD were seen was 0.29 µg/kg bw/day (single dose tested).

Fujisawa et al., 2019

269. Fujisawa et al. (2019) investigated TCDD-induced cardiotoxicity in the development period in mice. One day after delivery, nursing C57BL/6J mice (3-5/group) were administered TCDD by gavage, at doses of 0, 20 or 80 µg/kg bw/week (converted to 0, 2.89 or 11.43 µg/kg bw/day). On PND7 and PND21 male offspring were sacrificed, and hearts were examined by histological and gene expression analysis.

270. At 11.43 µg/kg bw/day, pup body weight was significantly decreased on PND7 and PND21, and heart weight on PND21 and heart-to-body weight ratio was significantly increased. Macroscopic and histological examination of the heart revealed morphological changes at 2.89 µg/kg bw/day relative to controls, although these were more evident at 11.43 µg/kg bw/day. At 11.43 µg/kg bw/day, animals showed left ventricular remodelling on PND7 and heart hypertrophy on PND21. This was accompanied by fibrosis and increased expression of associated genes (natriuretic peptide), β-myosin heavy chain, and endothelin-1.

271. The authors concluded that the results suggested that TCDD directly induces cardiotoxicity in the postnatal period, represented by progressive ventricular hypertrophy.

272. The lowest dose at which effects of TCDD were seen was 2.89 µg/kg bw/day (the lowest dose tested).

Nanakali et al., 2020

273. Nanakali (2020) investigated TCDD-induced oxidative damages and histological changes in the rat heart, with the primary objective of the study to investigate the preventative effects of quercetin. Adult male Wistar rats were administered daily doses of 0 (corn oil) or 20 µg/kg bw/day TCDD for 60 days by gavage. Blood and heart samples were collected when animals were sacrificed at 60 days.

274. The serum cardiac troponin I (cTnI) level (to represent myocardial damage) and the heart lipid peroxidation (measured by MDA) significantly increased, and the heart antioxidant profile (SOD, CAT, GSH) significantly decreased compared to controls. Histopathological changes were observed in TCDD-treated rats, including in the interventricular septum and the left ventricle. The main changes included myocardial congestion, hemorrhage and edema.

275. Authors concluded the key findings of the study were that TCDD induces cardiotoxicity by inducing oxidative damage and histological changes and that quercetin could alleviate heart oxidative damage and histological changes induced by TCDD.

276. The lowest dose at which effects of TCDD were seen was 20 µg/kg bw/day (single dose tested).

Petriello et al., 2018

277. Petriello et al. (2018) investigated the effect of PCB-126 on accelerated atherosclerosis and inflammation in mice fed a low-fat diet. Adult male Ldlr–/– mice (30/group) were fed a low-fat atherogenic diet from week 0 to study termination and were administered PCB-126 by gavage at weeks 2 and 4 at doses of 0 (safflower oil) or 1 µmol/kg bw (converted to 326.4 µg/kg bw/day). Mice were sacrificed (n=10) at weeks 8, 10, or 12 post first gavage, depending on the endpoint studied.

278. Mice exposed to PCB-126 exhibited significantly increased plasma inflammatory cytokine levels, circulating biomarkers of cardiovascular disease, the number of circulating platelets and red blood cell counts, and had significantly increased accumulation of hepatic fatty acids, and accelerated atherosclerotic lesion formation in the aortic root. PCB-126 also significantly increased circulating neutrophils, monocytes, and macrophages.

279. Overall, the authors concluded that PCB-126 increased inflammation and accelerated atherosclerosis in mice.

280. The lowest dose at which effects of PCB-126 were seen was 1 µmol/kg bw/week (single dose tested).

Wang et al., 2021

281. Wang et al. (2021) investigated the effect of PCB-126 on cardiac injury, characterised by metabolomics and general pathophysiological endpoints. Adult male C57BL/6 mice were administered doses by gavage of 0 (corn oil), 0.5 or 50 µg/kg bw/week (converted to 0.07 or 7.14 µg/kg bw/day) for eight weeks.

282. Metabolomic analysis (only conducted in mice exposed to 0.07 µg/kg bw/day) of the heart tissue identified 59 differential metabolites that were involved in lipid metabolism, amino acid metabolism, and the tricarboxylic acid (TCA) cycle. Typical metabolomic characteristic of cardiac hypertrophy was reflected by a significant accumulation of fatty acids (e.g. palmitic, palmitoleic, and linoleic acid), and disturbance of carbohydrates including D-glucose and intermediates in TCA cycle (fumaric, succinic, and citric acid). Exposure also significantly increased glycine and threonine, the amino acids necessary for the productions of collagen and elastin. PCB-126-exposed mice exhibited significant upregulation of collagen synthesis enzymes and extracellular matrix proteins, indicative of cardiac fibrosis, and the expression of genes related to TGFβ/PPARγ/MMP-2 signaling pathway was perturbed in the PCB126-treated hearts.

283. Myocardial fibers arrangement was shown to be disordered in the hearts of mice at 0.07 or 7.14 µg/kg bw/day, and at 7.14 µg/kg bw/day heart

weight/body weight ratio, and the heart weight/length of tibia ratio, was significantly increased.

284. Authors concluded that PCB-126 exposure causes myocardial injury and disrupts cardiac metabolism correlated with hypertrophy and fibrosis.

285. The lowest dose at which effects of PCB-126 were seen was 0.07 µg/kg bw/day (lowest dose tested).

Summary

286. In the in vivo studies identified, either adult male and female mice or rats, or lactating mice were dosed by gavage or i.p. injection with TCDD or PCB-126.

287. TCDD exposure was associated with a number of cardiovascular related effects, including: increased inflammation and atherogenic lesions in mice; oxidative and histopathological damage in heart tissue of rats; cardiotoxicity in the postnatal period, represented by left ventricular remodelling in F1 rat offspring on PND7 and heart hypertrophy on PND21; myocardial congestion, haemorrhage and oedema in adult rats; myocardial injury in rats; and disruption of cardiac metabolism, correlated with hypertrophy and fibrosis in rats.

288. The lowest dose at which effects were seen for TCDD was 0.14 µg/kg bw/day when adult male and female mice were dosed weekly for eight weeks (see Bey et al. (2022)) or 326.4 µg/kg bw/day when adult male mice were dosed at weeks 2 and 4 (see Petriello et al. (2018)). The lowest dose of PCB-126 at which cardiotoxic effects were seen was 0.07 µg/kg bw/day when adult male mice were dosed weekly for eight weeks (see Wang et al. (2021)).

Cleft palate

Gao et al., 2020

289. Gao et al. (2020) carried out a study investigating the role and molecular mechanism of TCDD-induced cleft palate. Pregnant C57BL/6 female mice (15/group) were administered TCDD by gavage at doses of 0

(corn oil) or 64 µg/kg bw on day E10. Mice were sacrificed on day E16 and fetuses collected for morphological and histological analysis.

290. Incidence of cleft palate in the TCDD-treated group was 100% compared to 0% in the control group. Morphological observation revealed that the palatal shelves of fetal mice had palatal wrinkles and fused together in the control group but not in the TCDD-treated group. Histology analysis revealed that the palate shelves contacted each other in the control group but had failed to contact each other and led to cleft palate in the TCDD-treated group.

291. The lowest dose at which effects of TCDD were seen was 64 µg/kg bw (single dose tested).

He et al., 2021

292. He et al. (2021) investigated the relationship between MEG3 and the proliferation of palatal mesenchymal cells and the underlying molecular mechanism by establishing fetal cleft palate with TCDD (64 µg/kg) in mice. Adult C57BL/6 N mice (8/group) were administered TCDD at dose of 0 (vehicle and corn oil) or 64 µg/kg bw on GD10 by gavage. Mice were sacrificed on GD13, 14, and 15 and the fetal palatal shelves were collected for histochemical analysis.

293. The results revealed that maternally expressed gene 3 (MEG3) was highly expressed during the critical period of CP formation and that the fetal mesenchymal proliferation was significantly inhibited at certain critical periods in TCDD-exposed mice.

294. Crosstalk between MEG3 and the TGF-β/Smad pathway occurred, such that the inhibition of the transforming growth factor-β (TGF-β)/Smad pathway was induced by TCDD.

295. Overall, authors suggested that TCDD-induced cleft palate may be caused by MEG3 inhibition of the proliferation of palatal mesenchymal cells involving the TGFβ/Smad pathway.

296. The lowest dose at which effects of TCDD were seen was 64 µg/kg bw (single dose tested).

Satake et al., 2022

297. Satake et al. (2022) investigated the preventive effect of quercetin intake on the TCDD-induced cleft palate and its mechanism of action.

298. In the dose-range finding study and confirmatory experiment, pregnant ICR mice (3/group) were administered TCDD at dose of 0 (olive oil), 10, 15, 20, 25, 30, 35 or 40 µg/kg bw on GD12 by gavage. Mice were sacrificed on GD16 and fetuses were collected (n=36-41/group) to evaluate gross findings and histological analysis.

299. Embryonic lethality was observed in the groups exposed to TCDD concentrations above 30 µg/kg bw. Incidence of cleft palate was 10.8% at 10 µg/kg bw and the authors concluded that the incidence increased in a concentration-dependent manner. Based on these results, the TCDD concentration of 25 µg/kg that induced the development of cleft palate, but did not demonstrate embryonic lethality, was used in subsequent experiments.

300. In the main study, pregnant ICR mice (3/group) were administered TCDD at dose of 0 (olive oil) or 25 µg/kg bw on GD12 by gavage. Mice were sacrificed on GD16 and fetuses were collected to evaluate the incidence of cleft palate.

301. Incidence of cleft palate was significantly increased (92.1%) relative to the controls (0%) at 25 µg/kg bw. Quercetin reduced the development of TCDD-induced cleft palate in fetal mice.

302. In conclusion, the results suggest that quercetin intake by pregnant mice can prevent cleft palate in fetal mice.

303. The lowest dose at which effects of TCDD were seen was 10 µg/kg bw (lowest dose tested in the dose-range finding study).

Summary

304. In the in vivo studies identified, pregnant mice were dosed by gavage with TCDD. All studies showed an increased incidence of cleft palates in offspring following in utero exposure.

305. The lowest dose at which effects were seen for TCDD was 10 µg/kg bw when pregnant mice were dosed on GD12 only (see Satake et al. (2022)).

Liver effects

Abdulkareem & Nanakali., 2020

306. The effect of TCDD on indices of oxidative stress, liver enzymes activity and the expression of CYP1A1 and the effects of mutagenesis of TCDD-induced liver toxicity were investigated by Abdulkareem and Nanakali (2020). The primary aim of this study was to investigate the protective effects of antioxidant quercetin against TCDD-induced hepatotoxicity.

307. Adult male Wistar rats (6/group) were administered TCDD daily by gavage for 90 days at doses of 0 (corn oil) or 10 µg/kg bw/day. Animals were sacrificed after 90 days and livers were collected. Liver enzymes (alkaline phosphatase (ALP), alanine and aspartate aminotransferase (ALT and AST)) were measured in serum to evaluate liver function. Lipid peroxidation was measured by MDA production and antioxidant profiles including SOD, CAT and glutathione reductase (GHS-R) were measured in liver. Gene expression of CYP1A1 was also evaluated.

308. There was a significant decrease in body weight, and a significant increase in liver weight following TCDD administration. Levels of AST, ALT and ALP were also all significantly increased in the TCDD-treated group compared with controls.

309. The level of oxidative stress biomarker MDA in the liver was significantly increased, while levels of GSH-R, SOD and CAT were significantly decreased. mRNA expression level of CYP1A1 in liver cells increased, but was not significantly different to controls.

310. The authors concluded that TCDD caused oxidative effects in rat liver.

311. The lowest dose at which effects of TCDD were seen was 10 µg/kg bw/day (single dose tested).

Basak et al., 2022

312. Basak Turkmen et al. (2022) investigated the ameliorating effects of beta-glucan on liver tissues of rats with TCDD-induced toxicity. Adult female Sprague-Dawley rats (6/group) were administered TCDD by gavage at doses of 0 (carboxymethylcellulose) or 2 µg/kg bw/week (converted to 0.29 µg/kg bw/day) for three weeks.

313. Thiobarbituric acid reactive substance (TBARS) and GSH levels were significantly increased, and SOD, CAT and glutathione peroxidase (GPx) activities were significantly reduced in liver tissues. In the liver tissue, hemorrhage under Glisson's capsule, eosinophilic and pyknotic nuclei, mononuclear cell infiltration, vascular congestion, sinusoid dilation and hemorrhage were seen.

314. The dose at which effects of TCDD were seen was 0.29 µg/kg bw/day (single dose tested).

Duval et al., 2017

315. The effects of TCDD on non-alcoholic fatty liver disease (NAFLD) in diet-induced obese mice were investigated by Duval et al. (2017). Eight-week old male C57BL/6J mice (30/group) were fed either a low-fat diet (LFD) or a high-fat diet (HFD) for 14 weeks. Mice were administered TCDD by i.p. injection at doses of 0 (corn oil) or 5 µg/kg bw/week (converted to 0.71 µg/kg bw/day) for the final six weeks of the two diets.

316. Significantly increased triglyceride levels were seen and, along with liver histology indicated that exposure of HFD-mice to TCDD worsened hepatic steatosis, as compared to either HFD controls or a TCDD-treated mice on a LFD. The mRNA levels of key genes of hepatic lipid metabolism were also significantly altered. Increased liver collagen staining and serum transaminase levels also showed that TCDD induced liver fibrosis in mice fed a HFD. TCDD in LFD- mice significantly increased the expression of several inflammation and fibrosis marker genes but was not significantly different to HFD-mice.

317. The authors concluded that TCDD amplifies the impairment of liver functions observed in mice fed a HFD as compared to a LFD and the results provide new evidence that environmental pollutants promote the development of liver fibrosis in obesity-related NAFLD in C57BL/6J mice.

318. The dose at which effects of TCDD were seen was 0.71 µg/kg bw/day (single dose tested).

Erdemli et al., 2018

319. Erdemli et al. (2018) investigated the ameliorating effects of thymoquinone (TQ) on TCDD-induced hepatotoxicity. Wistar rats (age not specified; 10/group) were administered TCDD by gavage at daily doses of 0 (corn oil or physiological saline) or 1 µg/kg bw/day for 30 days. Additional study groups were co-administered TCDD with TQ at doses of 1 µg/kg bw/day TCDD with 50 mg/kg TQ.

320. Histopathological changes seen in the liver including thickening of Glisson's capsule, intracytoplasmic vacuolization in hepatocytes, sinusoidal dilation, vascular and sinusoidal congestion and inflammatory cell infiltration. TCDD administration significantly increased MDA, total oxidant status (TOS), ALT, AST and ALP levels in rat liver tissue and significantly reduced GSH, SOD, CAT and total antioxidant status (TAS) levels, all of which were eliminated by TQ.

321. The authors concluded that TCDD administration caused oxidative stress in rat liver and TQ administered with TCDD prevented TCDD-induced hepatotoxicity.

322. The dose at which effects of TCDD were seen was 1 µg/kg bw/day (single dose tested).

Han et al., 2017

323. Han et al. (2017) investigated the effect of TCDD on hepatic inflammation, hepatic stellate cell (HSC) activation and liver fibrosis. Six-week-old male C57BL/6 mice (9/group) were administered TCDD by a single

intraperitoneal (i.p.) injection per week for 6 weeks at doses of 0 (corn oil), 10 or 25 µg/kg bw/week (converted to 1.43 or 3.60 µg/kg bw/day).

324. TCDD-exposed mice showed extensive disruption of liver architecture, including hepatocellular necrosis, inflammatory cell infiltration, and fibrosis.

325. There was a statistically significant increase in liver fibrosis and necroinflammatory scores following TCDD exposure, indicated by an increased expression of α-smooth muscle actin (α-SMA; a marker of activated hepatic HSCs) from 1.43 µg/kg bw/day, and a significant increase in the levels of the fibrosis marker Coll1A1 and HSC marker synaptophysin (SYP), all indicating that TCDD can induce fibrotic effects in mice liver. In mouse liver mRNA levels of α-SMA were significantly increased from 3.60 µg/kg bw/day, and RNA levels of Coll1A1 were significantly increased significantly increased following in vivo exposure at 25 µg/kg bw/week, indicating that TCDD up-regulates the expression of liver fibrosis markers in vivo. The expression of phosphorylated Akt (p-Akt) was enhanced in a dose-dependent manner in mouse liver although the protein level remained unchanged.

326. The authors concluded that TCDD was shown to stimulate liver inflammation through the Akt/nuclear factor kappa B (NF-kB) p65 signalling pathway.

327. The lowest dose at which effects of TCDD were seen was 1.43 µg/kg bw/day (lowest dose tested).

Li et al., 2022

328. Li et al. (2022) investigated the ameliorating effects of ginsenoside Rg1 on the effects of TCDD on liver injury. Male (age not specified; 10/group) ICR mice were administered TCDD by i.p. injection at doses of 0 (DMSO) or 30 µg/kg bw/week (converted to 4.30 µg/kg bw/day) for 42 days.

329. There was a significant increase in serum AST, ALT and ALP levels and significantly increased relative liver weight. In addition, there was evidence of histopathological changes including mild interstitial hyperplasia and vascular proliferation in foci, evidence of lymphocytes adhering to the

walls of dilated vasculature, and individual hepatocyte degeneration and necrosis were seen. Ginsenoside Rg1 significantly decreased the levels of AST and ALT and ameliorated liver histological changes.

330. The authors concluded that ginsenoside Rg1 partially alleviates TCDD-induced liver injury, possibly by competing with TCDD for the AhR binding site as a partial agonist.

331. The lowest dose at which effects of TCDD were seen was 4.30 µg/kg bw/day (single dose tested).

Shan et al., 2020

332. The effects of PCB-156 on non-alcoholic fatty liver disease (NAFLD) in diet-induced obese mice were investigated by Shan et al. (2020). Eight-week old male C57BL/6 mice were administered PCB-156 by i.p. injection on weeks 4, 6, 8 and 10 at doses of 0 (corn oil) or 55 mg/kg bw/week (55000 µg/kg bw/week, equivalent to 7857.1 µg/kg bw/day), before sacrifice at 12 weeks. At each dose, mice (5/group) were either fed a control diet (CD) or a HFD.

333. In the CD-fed group there was increased intra-abdominal fat mass, hepatic lipid levels and dyslipidemia, and in the HFD-fed group, aggravated NAFLD. Genes expression involved in lipid metabolism pathways, such as lipogenesis, lipid accumulation and lipid ß-oxidation, were significantly increased in liver tissues exposed to PCB-156 in both the CD-fed group and the HFD-fed group. In addition, the cytochrome P450 pathway, PPARs and the glutathione metabolism pathway were activated following exposure to PCB-156.

334. The authors stated the data suggested PCB-156 could promote NAFLD development by altering the expression of genes related to lipid metabolism and inducing oxidative stress.

335. The lowest dose at which effects of TCDD were seen was 7857 µg/kg bw/day (single dose tested).

Vieira Silva et al., 2022

336. Vieira Silva et al. (2022) investigated the effects of PCB-156 during a 90-day study in accordance with OCED 408 (OECD, 2018). Male and female Sprague-Dawley rats (age not specified) were administered PCB-156 at doses of 0 (Lab Chow 5002 diet), 0.01, 0.1, 1 or 10 mg/kg bw/day (0, 10, 100, 1000 or 10,000 µg/kg bw/day) for 90 days via the diet.

337. Dose-dependent increases in liver weight were seen in both sexes as well as liver ethoxyresorufin-O-deethylase (EROD), pentoxyresorufin-O-dealkylase (PROD) and uridine 5′ -diphospho-glucuronosyltransferase; (UDPGT) enzyme activities. Apolar retinoid concentrations were significantly decreased in the liver and histopathological examination of the liver showed mild to moderate dose-related changes compared to controls.

338. The authors concluded that the retinoid endpoints should be further evaluated for a causal relationship to PCB-induced liver toxicity and derived a lowest observed adverse effect level (LOAEL) of 10 µg/kg bw/day based on reduced apolar liver retinoid concentrations. The lower confidence limit of the benchmark dose for a 5% decrease (BMDL5) was 0.0009 and 0.0007 ppm in males and females respectively, corresponding to a daily dose of 0.06 µg/kg bw/day.

Summary

339. In the animal studies identified, adult male or female rats, or male mice were dosed with TCDD or PCB-156.

340. TCDD was associated with oxidative effects and histopathological changes in rat liver, and liver damage, inflammation and fibrosis in mouse liver. TCDD was also associated with an amplified impairment of liver function and could promote the development of NAFLD in animals fed HFD. PCB-156 reduced apolar liver retinoid concentrations in rats compared to controls as well as causing histological changes and liver damage.

341. The lowest dose at which hepatotoxic effects were seen for TCDD was 0.29 µg/kg bw/day when adult male rats were dosed weekly for three weeks (see Basak Turkmen et al. (2022)), and for PCB-156 at 10 µg/kg bw/day when

male and female rats were dosed daily for 90-days (see Vieira Silva et al. (2022)).

Obesity

Brulport et al., 2017

342. Brulport et al. (2017) carried out a study to investigate the obesogenic effect of chronic exposure to TCDD. Adult male and female C57BL/6J mice (10/group) were fed with a HFD and administered TCDD by gavage at dose of 0 (corn oil) or 1 µg/kg bw/week (converted to 0.14 µg/kg bw/day) from weeks 10 to 42. No study guideline was followed.

343. TCDD induced a significant body weight gain in adult mice fed a HFD (7% in males and 8% in females) after 23 weeks in males and 35 weeks in females. Fat mass significantly increased, regardless of sex (13% in male and 11% in female) and showed strong correlation with body weight in both sexes. A sex effect was observed in the fat mass distribution in adipose tissue visceral adipose tissue (VAT) fat pad weight was significantly decreased in males (11%) but significantly increased in females (14%). TCDD did not affect glucose, insulin, no esterified fatty acids (NEFA), cholesterol, triglycerides in plasma in either sex. In contrast, leptin was significantly decreased in females following TCDD exposure.

344. At sacrifice, relative liver weight in males was significantly increased compared with controls, but hepatic triglyceride content was unchanged. Conversely, in females, liver weight was slightly, albeit not significantly, increased but hepatic triglycerides content increased significantly.

345. The authors concluded that TCDD is obesogenic after chronic exposure in adult mice fed a HFD.

346. The lowest dose at which effects of TCDD were seen was 0.14 µg/kg bw/day (single dose tested).

Hoyeck et al., 2020

347. Hoyeck et al. (2020) investigated short- and long-term impact of transient low-dose TCDD exposure during pregnancy and lactation on glucose homeostasis and beta cell function in female mice.

348. In Cohort 1, 8-week old female C57BL/6 mice (3-14/group) were administered TCDD by subcutaneous (s.c.) injection at doses of 0 (corn oil) or 0.02 µg/kg bw twice per week (original dose reported as 20 ng/kg bw, equivalent to 0.006 µg/kg bw/day) starting one week prior to pairing and throughout mating and pregnancy. Mice were sacrificed on PND1 and the whole pancreas was harvested for histological analysis.

349. In Cohort 2, 8-week old female C57BL/6 mice were administered corn oil or TCDD for one week prior to pairing with male mice and lasting throughout mating, pregnancy and lactation, by subcutaneous injection. Litters were culled to 6 pups/litter at PND1. Metabolic assessments were conducted on chow-fed dams (“TCDDChow” dams) for 6-10 weeks following the last TCDD exposure at weaning (“Post-TCDD” window). A subset of dams was then transferred to a 45% HFD or remained on a standard chow diet for an additional 11 weeks (“Metabolic challenge” window). Whole pancreas, liver, perirenal fat, and placenta were harvested from a subset of mice at approximately day 15.5 of pregnancy (GD15.5). Pancreas and hypothalamic brain tissue were collected at week 11 of the metabolic challenge.

350. TCDD-exposed dams were transiently hypoglycemic on PND1 compared to control dams, but otherwise had normal fasting glycemia during TCDD exposure and for 10 weeks after exposure had ended (“Post-TCDD” window). There was no effect of TCDD on body weight or food intake during pregnancy, but TCDD-exposed dams were transiently significantly heavier than controls at mid-lactation with no change in food intake. There was a significant increase in body weight in TCDD-exposed dams starting five weeks after their last exposure. The authors speculated that female mice exposed to TCDD during pregnancy and lactation might be more susceptible to weight gain when challenged with an obesogenic diet (45% HFD).

351. In Cohort 2 during the “Metabolic challenge” window, the increased body weight in TCDDChow dams returned to control levels, but dams on the TCDD-HFD showed a rapid and sustained significant increase in body weight starting 1 week after being transferred to HFD, whereas control HFD dams only had a modest significantly increased body weight after ~10 weeks of HFD feeding. Both HFD-fed groups had significantly increased percentage fat mass and decreased percentage lean mass compared to chow-fed dams after 10 weeks of HFD feeding, but these changes in body composition were exacerbated in TCDD-HFD dams compared to control HFD dams. Leptin levels were significantly increased in control dams fed a HFD compared to those fed a normal diet. In contrast, TCDD-exposed animals fed a HFD only a showed a non-significant increased trend compared to those fed chow and was significantly lower than HFD-fed controls.

352. The authors suggested that transient low-dose TCDD exposure during pregnancy impairs metabolic adaptability to HFD feeding, demonstrating that dioxin exposure may be a contributing factor to obesity and diabetes pathogenesis in females.

353. The lowest dose at which effects of TCDD were seen was 0.006 µg/kg bw/day (single dose tested).

Summary

354. Adult male or female mice, or pregnant mice were dosed with TCDD by gavage or s.c. injection. TCDD was associated with significant weight gain in adult male and female mice and pregnant mice fed a HFD.

355. The lowest dose at which effects were seen for TCDD was 0.006 µg/kg bw/day, when mice were dosed twice per week commencing one week prior to pairing and throughout mating and pregnancy (see Hoyeck et al. (2020)).

Mechanism of carcinogenicity

356. In 2001, the COT concluded “…although a precise mechanism for carcinogenesis in laboratory animals or humans could not be elucidated from the available information, the data (i.e. negative genotoxicity in standard

assays, and evidence from studies of mechanisms) suggested that a threshold approach to risk assessment was likely to be appropriate" (COT, 2001). This conclusion is supported by the more recent literature review of in vivo and in vitro data undertaken by EFSA (2018) that "There is no robust evidence that the development of cancer caused by TCDD and other PCDD/Fs in experimental animals is associated with direct genotoxicity". One of the proposed mechanisms of genotoxicity is oxidative stress and DNA damage. It is well established that high dose exposure to TCDD induces oxidative stress, therefore it is hypothesised that such oxidative stress is associated with the mutagenicity observed (Abdulkareem & Nanakali, 2020).

Abdulkareen & Nanakali, 2020

357. Abdulkareem and Nanakali (2020) investigated the effect of antioxidant quercetin on oxidative stress biomarkers and the effect of mutagenesis of TCDD-induced liver toxicity in male rats. Rats (n=6) were treated with corn oil (Group 1), TCDD (10 µg/kg bw/day; Group 2), quercetin (20 mg/kg bw/day; Group 3) or TCDD (10 µg/kg bw/day) and quercetin (20 mg/kg bw/day)(Group 4 and Group 5) , via oral gavage for 90 days. Group 4 was administered TCDD 30 min after quercetin treatment and Group 5 was administered TCDD 30 min before quercetin treatment). Animals were sacrificed at the end of the treatment period and blood and liver samples collected.

358. The activities of antioxidant enzymes (SOD, CAT, and GSH-R) and MDA level (as the biomarker for lipid peroxidation) were measured in the liver.

359. A significant elevation in MDA level was observed in the rats exposed to TCDD, whereas GSH-R, SOD and CAT activities significantly declined in liver tissue compared to controls. Administration of quercetin thirty minutes before TCDD (Group 5) significantly improved SOD, CAT, and MDA levels compared to the TCDD group.

360. Prolonged exposure to TCDD also causes mutatations in the CYP1A1 gene and increased gene expression in liver cells.

361. The authors concluded that TCDD can cause oxidative stress, and mutagenic and oxidative effects in rat liver.

Chen et al., 2020

362. The genotoxicity of PCB-77, PCB-126 or PCB-81 were investigated in Chinese hamster V79-derived cell lines and in the human hepatoma C3A cell line by Chen et al. (2020). V79-derived cell lines, genetically modified to express human cytochrome P450 (CYP) 1A1, CYP1A2 and CYP1B1, and the C3A cell line (subclone of the HepG2 line), which endogenously expresses various cytochomes, were exposed to PCB-77, PCB-126 or PCB-81 at doses of 0, 5, 10, 20, 40 and 80 µM. V79-Mz cells were also used as controls as they do not expresss CYPs, sulfotransferases (SULTs) or UDP-glucuronosyltransferases (UGTs). Cytotoxicity was assessed using the cell counting kit-8 (CCK-8) assay as a measure of cell growth and viability. Micronucleus assays were carried out in accordance with OECD 487 (OECD, 2016) as a measure of clastogenicity.

363. V79-derived cell lines followed treatment regimes of 6 h/18 h, and 18 h/6 h (potentially favourable for reactive metabolites to build up). C3A cell lines followed regimes of 18 h/54 h (short exposure / long recovery) and 54 h/18 h (long exposure / short recovery). To determine the contribution of CYP1B1 on micronucleus formation, C3A cells were co-exposed with the CYP1B1 inhibitor I-2,3',4,5'-tetramethoxystilbene (TMS; 30 nM), 2 h prior to the addition of the test compound to the end of cell recovery period.

364. Differentiation between centromere protein B (CENP-B^+)-positive and centromere protein B (CENP-B^-)-negative micronuclei was also assessed in both cell lines and protein expression of CYP1A1, CYP1A2, CYP1B1, γ-H2AX (as a measure of DNA damage) was meausured by Western blotting in C3A cells.

365. PCB-77 and PCB-126 were virtually non-cytotoxic in all V79 cells whereas PCB-81 showed a concentration-dependent decrease in cell viability, with V79-CYP1B1 showing a greater loss of viability than other cell lines. In C3A cells, under the 54 h/18 h regime, PCB-77 and PCB-126 were non-cytotoxic whereas PCB-81 was mildly cytotoxicity, which was abolished by TMS.

366. When using the 6 h/18 h regime, 80 µM PCB-77 increased micronuclei in V79-CYP1B1 cells but had no effect in the other V79 cell lines. Similarly, PCB-81 induced micronuclei in V79-CYP1B1 line, with a slightly higher potency than PCB-77, but was negative in the other cells. PCB-126 did not induce micronuclei in any of the V79 cell lines.

367. Using the 18 h/6 h regime, PCB-77 and PCB-81 were more potent in inducing micronuclei in V79-CYP1B1 cells when compared the 6 h/18 h regime. PCB-126 was again negative in all cell lines. PCB-81 also induced micronuclei in V79-CYP1A2, but only at the highest test concentration (40 µM).

368. In C3A cells, under the 54 h/18 h regime, PCB-77 and PCB-81 signficantly increased micronuclei at 40 and 80 µM. There was no signficant increase in micronuclei in cells co-exposed to TMS. PCB-126 did not induce micronuclei under either regime, and none of the PCBs tested induced micronuclei under the 18 h/54 h regime.

369. In V79 cells, using the 18 h/6 h regime, 40 µM PCB-77 and PCB-81 (the only dose tested) significantly increased the frequency of CENP-B^-, but not CENP-B^+, micronuclei. Similarly, in C3A cells, using the 54 h/18 h regime, 40 µM PCB-77 and PCB-81 significantly increased the frequency of CENP-B^- micronuclei but not CENP-B^+ micronuclei.

370. Exposure of C3A cells to PCB-77, PCB-81 and PCB-126 also significnatly increased the protein levels of CYP1A1 and CYP1A2 whilst CYP1B1 remained unchanged. PCB-77, PCB-81 and PCB-126 also signficantly increased the level of γ-H2AX protein and co-exposure to TMS reduced levels to that of controls.

371. The authors concluded human CYP1B1 is able to activate PCB-77 and PCB-81 to clastogenic metabolites in V79 and C3A cells, as evidenced by the formation of centromere-free micronuclei and double strand DNA breaks in mammalian cells.

Luukkonen et al., 2017

372. Luukkonen et al. (2017) investigated the effect of TCDD on mitochondrial integrity and whether TCDD can consequently cause induced genomic instability, which may play a role in the carcinogenic process.

373. Human SH-SY5Y neuroblastoma cells were treated with TCDD for 48-h at doses of 0, 0.0001, 0.001, 0.005, 0.01, 0.05, 0.1 and 0.25 µM to investigate immediate effects, or at doses of of 0, 0.0001, 0.001, 0.01, 0.05 and 0.1 µM to investigate delayed effects (8 or 15-days post-exposure). No study guideline was followed. Mitochondrial integrity was evaluated by measuring mitochondrial superoxide production, mitochondrial membrane potential (MMP), and mitochondrial activity. Micronucleus formation was used to assess immediate genetic damage and induced genomic instability (IGI).

374. Immediately after exposure, mitochondiral superoxide potential was signficantly increased at 0.001, 0.05 and 0.25 µM TCDD, although showed no dose-response. All TCDD concentrations caused a decrease in MMP levels, but the dose-response was not significant. The mitochondrial activity of the cells was also increased with all doses, although again did not reach statistical significance. No effects were seen on micronucleus frequency. Eight days post-exposure, at 0.01 and 0.100 µM, MMP level was signficantly decreased. There was no significant effect on mitochondrial superoxide level, mitochondrial activity or micronucleus frequency at any dose.

375. Fifteen days post-exposure, at 0.0001 µM mitochondrial superoxide potential was signficantly increased. There was no significant effect on MMP level, mitochondrial activity or micronucleus frequency at any dose.

376. The authors concluded that although an increased level of mitochondrial superoxide was seen immediately after exposure to TCDD, the results do not support TCDD-induced genomic instability and genetic damage in human SH-SY5Y neuroblastoma cells.

Summary

377. Results supporting a genotoxic mechanism of action for carcinogenicity are equivocal. In vivo, TCDD was associated with increased oxidative stress and mutatations in CYP1A1 in rat liver.

378. In vitro, in genetically modified Chinese hamster V79 cells and human hepatoma C3A cells, human CYP1B1 activated PCB-77 and PCB-81 to clastogenic metabolites, as evidenced by the formation of centromere-free micronuclei and double strand DNA breaks in mammalian cells. Whereas in a study using human SH-SY5Y neuroblastoma cells, authors concluded that the results do not support TCDD-induced genomic instability or genetic damage.

AhR mechanism of action

379. TCDD is one of the most studied AhR ligands. In response to dioxins, AhR activation induces an adaptive metabolic response, mediating a variety of toxic effects including carcinogenicity, hepatotoxicity, thymic involution, birth defects, impaired reproductive capacity, bone effects and immune suppression (Andersson et al., 2002).

380. Evidence that AhR plays a role in dioxin toxicity is twofold. Firstly, the toxic potency of dioxin congeners in vivo correlates with the binding affinity for the AhR. e.g., the TCDD displays the greatest affinity for the AhR and is the most toxic, while the weaker 2,8-dichloro congener has a 300-fold lower affinity and is essentially non-toxic. Secondly, mice harbouring the Ahrb allele, coding for a receptor with a high binding affinity, are more sensitive to dioxin toxicity and the induction of AhR battery genes compared with those harbouring the Ahrd allele, which encodes a receptor with a 10-fold lower affinity. In addition to binding affinities with dissociation constant (KD) values in the picomolar range, TCDD is not appreciably metabolised, thus causing prolonged AhR activation (Stevens et al., 2009).

381. The Aryl hydrocarbon/Dioxin receptor (AhR) has been defined as an environmental-sensor PAS (Period [Per]-Aryl hydrocarbon receptor nuclear translocator [ARNT]-Single minded [Sim]) protein that is structurally included within the class I of basic helix-loop-helix transcriptional regulators. They are polymorphic and known alleles include AhRb-1–3 and AhRd. The receptors have different affinities but all contain a basic helix-loop-helix family as well as PAS and transactivation domains (Cespedes et al., 2010; Mulero-Navarro & Fernandez-Salguero, 2016).

382. The AhR has major roles in xenobiotic-induced toxicity and carcinogenicity (Mulero-Navarro & Fernandez-Salguero, 2016).

383. AhR causes an ‘adaptive metabolic response’ in that cytosolic AHR binds xenobiotic ligands and activates transcription of enzymes that mediate

their biotransformation and excretion. Early experiments revealed that exposure to pollutants led to increases in cytochrome P450 that hydroxylated the pollutant, thereby increasing its water solubility and decreasing its biological residency (Stevens et al., 2009).

Canonical pathway

384. In its inactive state, the AhR receptor resides in the cytosolic compartment of the cell bound to a molecular chaperone complex consisting of different partners, namely two heat shock protein 90 (Hsp90) chaperons (X-associated protein 2 (XAP2) and cellular-sarcoma (c-Src) kinase) and cochaperon p23. These chaperone proteins stabilize the AHR maintaining it in a conformation which is unable to enter the nucleus but optimal for ligand binding (Larigot et al., 2018).

385. Upon ligand binding, the complex undergoes a transformation which exposes the nuclear localization signal (NLS) (in the N-terminal region) and releases c-Src. The AhR then translocate to the nucleus where it dissociates from the chaperons and cochaperons (XAP2, the other Hsp90, and p23) and dimerises with ARNT. The AhR/ARNT heterodimer can bind to specific xenobiotic response elements (XREs) located in the target gene regulatory sequences that are located in the upstream regulatory region of target genes (Larigot et al., 2018; Mulero-Navarro & Fernandez-Salguero, 2016).

386. AhR target genes may be divided into two groups: genes involved in homeostatic control of xenobiotic detoxification and those involved in the control of proliferation, differentiation and inflammation (Wajda, Lapczuk, et al., 2017).

Non-canonical pathway

387. The AhR complex can also bind to non-canonical alternative DNA binding sites and also signals through its binding to other receptors or transcription factors including the estrogen receptor, E2F Transcription Factor 1 (E2F1), retinoblastoma protein (RB), and proto-oncogene c-Maf (c-Maf).

Furthermore, the AHR contributes to intracellular signaling through non-transcriptional pathways (Wang et al., 2020).

Species specificity

388. The sensitivity of AhR to TCDD is species-specific. However, all ligands interact with AhR in the ligand binding domain (LBD) regardless of the species and the mechanism of AhR action is believed to be the same. Moreover, the associated proteins, such as HSP90 and ARNT are similar. Despite such similarities, different species have very different AhR responses (Xu et al., 2021).

389. The ligands that enter the cytoplasm and bind to AhR shuttle into the nucleus, identify the dioxin response elements (DRE) sequence, recruit coactivators, and start downstream gene transcription. However, the binding affinity of the ligands to AhR, the recruited coactivator, and the DRE in the target gene all affect the final AhR response. Such elements all show species diversity, leading to the differences in response (Xu et al., 2021).

390. Murine AhR has a 10-fold higher affinity for TCDD compared with the human AhR suggesting that mice may be more sensitive to the effects of dioxins than humans (Wang et al., 2020). In contrast, Han/Wistar rats have a modified transactivation domain making them less sensitive and able to tolerate 1000-fold higher doses (Brokken & Giwercman, 2014). Available epidemiological evidence and results from in vitro analyses also suggest that humans are significantly less sensitive than most mammalian species with regards to TCDD-dependent toxicity, and although this reduction in sensitivity is due in part to the lower binding affinity reported for the human AhR, other modulatory factors appear to play a key role (Denison et al., 2011).

391. The relative lack of similarity of the carboxy (DNA-binding) terminus between the mouse and human AHRs may result in different co-factor recruitment, specifically LXXLL binding motifs, leading to distinct transcriptional activity. Moreover, murine and human AHRs induce different transcriptional responses to TCDD. Such species-specific ligand selectivity

may explain the differences observed between human and mouse models (Wang et al., 2020).

392. Genetic variations in the rodent AHR have been shown to dramatically alter ligand binding and transactivation by the receptor. For example, a single nucleotide change at codon 375 in the ligand binding domain of the murine AHR reduces the binding affinity for TCDD approximately 10-fold in the resistant DBA/2 strain compared to the more sensitive C57BL/6J strain, which shows a higher CYP1A1 induction and a greater sensitivity to TCDD. Similarly, due to a deletion in the transactivation domain of the rat AHR, the Han/Wistar rat strain is a 1000-fold more resistant to TCDD than the more sensitive Long-Evans rat strain (Brokken & Giwercman, 2014).

AhR and male reproductive toxicity

393. Normal cellular processes, such as the cell cycle, stem cell proliferation, and tissue differentiation are regulated by AHR (Hansen et al., 2014).

394. AHR signaling may interfere with the male reproductive system through several mechanisms, such as by directly affecting steroid hormone levels via induction of CYP1A1 and CYP1B1 that catalyse the conversion of steroid hormones (Brokken & Giwercman, 2014). For example, AHR-ARNT heterodimers are able to inhibit the androgen signaling by binding to androgen response elements adjacent to or overlapping with XRE sequences or by increasing androgen receptor degradation. In addition, the activated AHR pathway can induce apoptosis of testicular germ cells. As AHR pathway proteins are widely distributed in human testicular tissue, this may explain the mechanism by which dioxins and dioxin-like PCBs interfere with spermatogenesis and male fertility, resulting in elevated cell death and reduced sperm count (Merisalu et al., 2007).

395. The presence of AHR in sperm also could provide a mechanism by which dioxins and dioxin-like PCBs could influence sperm function as AHR can reduce ejaculated sperm number and impair semen quality contributing to male infertility (Wei et al., 2018).

396. Hansen et al. (2014) also reported AHR plays a role in the formation of fully functional mature sperm as demonstrated AHR is localized in the acrosome and the principal piece of the sperm flagellum in mature sperm and could give rise to an increase intracellular calcium concentration, which could affect sperm capacitation.

397. Schultz et al. (2003) demonstrated a difference in expression of AHR or ARNT in rat and human testes and concluded '*The wide and most abundant distribution of AHR and ARNT proteins in human testis explains how dioxins can interfere directly with human spermatogenesis and fertility, resulting in cell death and a reduction of testicular weight and sperm count. As, in contrast to the distribution in the rat testis, AHR and ARNT were found in pre- and postmeiotic cells of the seminiferous epithelium, the activation of AHR/ARNT in the human testis may lead to an even wider extent of biological consequences than in the rat testis'*.

398. AHR is expressed in tissue-specific and developmental stage-specific patterns (Hansen et al., 2014).

399. The testes and prostate are some of the most sensitive organs to TCDD toxicity (Schultz et al., 2003; Wajda, Łapczuk, et al., 2017) and intercellular signalling between Sertoli and neighbouring germ cells can be disturbed even after a short exposure period, which leads to a disruption of spermatogenic cells. Single exposures can also reduce the volume of Leydig cells. In addition to direct toxic responses, the AHR/ARNT receptor complex is able to modify hormonal signals by interacting with their response elements on target DNA.

400. Epididymis and vas deferens are less sensitive to TCDD compared to testes and prostate and require 10-fold higher doses to cause the same effect, compared to the prostate (Wajda, Lapczuk, et al., 2017). In rats, mRNA levels of AhR was similar in all parts of the epididymis in rats, contrasting results seen in humans where AhR expression was different. Analysis also showed very strong and strong immunostaining in rat and human epididymis, respectively, which also correlated with protein levels.

401. Differences also occurred in immunohistochemical analysis of AhR monoclonal antibodies between spermatogenic cells at different stages and other cells of testis and epididymis, when human and rat tissues were compared. Half of Sertoli cells in rat testis were AhR-negative, whereas in most human Sertoli cells the reaction was strong, and 60% of rat Leydig cells were negative or with weak staining, contrary to most human Leydig cells that showed a strong AHR-positive reaction. These data correlate with those of Schultz et al. (2003), who also reported the number of immunoreactive cells of the human testis exceeded the number of those in the rat testis, which may be explained by differences between species.

402. Wajda, Łapczuk, et al. (2017) also reported a different ratio of CYP1B1/Cyp1b1 within testis and epididymis both in human and rat. The animal model showed higher level of Cyp1b1 mRNA in testis than in epididymis, whereas in humans higher mRNA level was reported in epididymis than in testis. Authors suggested that the data demonstrates that the reaction to TCDD treatment depends on tissue type, suggesting different induction characteristics of CYP1B1.

403. In contrast to AhR results, Arnt expression in rat tissue was higher in testis than in epididymis, what is also consistent with the above-mentioned report of Roman et al. [21]. The present study revealed that in human tissue ARNT mRNA level was the highest in caput of epididymis (in comparison with testis and cauda of epididymis statistically significant).

404. The time of exposure has an impact on the effects observed. A study on men from Seveso, who were exposed to TCDD during infancy, showed reduced sperm concentration and motility, whereas men exposed during puberty showed opposite effects. No effects were seen in older men (mean age of 21.5 years old when exposed) (Brokken et al., 2014).

405. Indeed, AhR is only expressed in fetal testes at certain development stages such as only germ cells in the first and second trimester (Wajda, Lapczuk, et al., 2017). This indicates that exposure during the fetal or early life period is the most sensitive window of exposure (Brokken & Giwercman, 2014).

406. Reduced androgen levels during the period when Sertoli cells are most dependent on androgens could also explain the permanently decreased sperm counts in adults who were exposed to TCDD before puberty. So although exposure to dioxins or dioxin-like compounds may have a suppressive effect on testosterone levels throughout life, such effects only occur when exposure occurs during reproductive development either in utero or up to puberty (Brokken & Giwercman, 2014).

AhR and immunotoxicity

407. AhR is expressed by all major immune cell types, including B cells, T cells, dendritic cells (DCs), macrophages, granulocytes, and natural killer cells. In particular, the AhR was shown to play a role in the differentiation of a subset of T cells, Th17, which are involved in autoimmune diseases. Recent studies have identified other AhR actions in immune system development. The AhR was found to be expressed in the innate lymphoid cells. These lead to the formation of intestinal lymphoid follicles that are critical for defence against infection and for the development of intraepithelial lymphocytes that are the first defence barrier in skin and intestine. Therefore, AhR is involved in immunotoxicity, regulation of thymocytes and dendritic cell functions, autoimmunity, inflammation and the defence against infections (Barouki et al., 2012; Barouki et al., 2007).

408. In addition, many genes involved in immune regulation contain multiple DREs in their promoter region. Ultimately, the specific effects of AHR activation by TCDD on an immune response are determined by what cell types are involved, the activation status of the cells, and the type of antigenic stimulation (Marshall & Kerkvliet, 2010).

409. In a review, Sabuz Vidal et al. (2021) evaluated mechanisms of chemical-induced immunotoxicity for TCDD through interaction with the nuclear receptors AhR, estrogen receptor (ER) and the peroxisome proliferator-activated receptor (PPAR) in T, B, and dendritic cells.

410. The authors state that the primary outcome of AhR activation by TCDD, and other EDCs, is the impairment of the regulatory T cells

(Treg)/Th17 balance. The complex process of T cell differentiation depends on multiple factors including cytokines, cell-to-cell interactions, and epigenetic modifications (Goswami and Awasthi 2020; Singh et al. 2020 as cited in Sabuz Vidal et al. (2021)). As AHR-dependent effects are ligand-specific, disturbance of the cytokine profile can lead to both anti- or pro-inflammatory outcomes, depending on the ligand (Marshall et al. 2008; Chmill et al. 2010; Benson and Shepherd 2011a; Yang et al. 2016; Al-Ghezi et al. 2019 as cited in Sabuz Vidal et al. (2021)). The interaction of AHR with nuclear factor kappa B (NFkB) has also been suggested as a mechanism by which TCDD controls Treg/Th17 balance (Marshall et al. 2008; Chmill et al. 2010; Gerondakis et al. 2014; Ehrlich et al. 2018; Gao et al. 2020 as cited in Sabuz Vidal et al. (2021)).

411. The molecular mechanisms of immunotoxicity identified in the review for the different NRs and cell types were proposed as potential molecular initiating events (MIEs) and molecular and cellular key events (KEs) for the development of an AOP network for immunotoxicity are presented in Table 1.

Table 1 Molecular mechanisms of immunotoxicity identified in the review

Proposed MIE	**Proposed molecular KE**	**Proposed cellular KE**
Activation AHR in T cells	Inhibition/Activation NFκB Upregulation/downregulation IL-10 Upregulation/downregulation RORγδ Induction/Inhibition STAT3	Induction/Reduction Treg Induction/Reduction Treg Induction/Reduction Th17 Induction/Reduction Th17
Activation AHR in DC	Inhibition/Activation NFκB Upregulation IDO	Increase DC maturation Increase tolerogenic DCs
Activation AHR in B cells mouse	Inhibition Blimp-1 Upregulation SHP-1	Decrease B cell proliferation Reduction Ig production
human	Downregulation LCK	Impair vesicular trafficking Reduction Ig secretion

Data synthesis

412. The lines of evidence for the data integration were drawn from the literature search undertaken from 2017 to 2022. The data synthesis of epidemiological and toxicological evidence follows the approach presented by COT (2021).

Male reproductive toxicity

Effects on semen parameters

413. Effects on semen parameters were investigated in a number of in vivo studies in adult mice and rats or F1 rats exposed prenatally to TCDD, TCDF or PCB-118.

414. The range of effects observed included a decrease in sperm count, motility, and percentage of normal sperm and an increase in sperm malformations, percentage of dead sperm, number of apoptotic and necrotic spermatozoa and percentage of DNA damage (fragmented and denatured DNA) in sperm.

415. Sperm parameters were affected in all adult mice following TCDD and TCDF exposure, and F1 adult mice with PCB-118. Results in rats were mixed as the number of live spermatozoa was decreased in adult rats exposed to TCDD but no effects were seen on daily sperm production or number of mature spermatids in neonatal or F1 adult rats following TCDD exposure on GD15.

416. In human studies, TCDD was reported to have a negative correlation with sperm motility and sperm count, but mixed results were obtained with dioxin-like PCBs. Whilst some studies reported a negative correlation between PCB-126 and sperm viability, PCB-118 with semen volume and sperm motility and PCB-189 with sperm motility, others failed to show a correlation between PCB-118 and sperm count, and PCB-105, PCB-118 and PCB-156 on any seme parameters.

417. The results in adult mice and rats exposed to TCDD showed consistent evidence of adverse effects on semen parameters (sperm count, sperm motility, percentage dead sperm, morphological abnormalities). Similarly, negative correlations between TCDD and sperm count and sperm motility were also observed in epidemiology studies.

418. PCB-118 caused a significant increase in sperm deformities in F1 mice in both studies. In humans, available epidemiological evidence provides inconsistent evidence of the adverse effects of dioxins and dioxin-like PCBs on semen parameters. Negative correlations were reported with PCB-126 and sperm viability, PCB-118 with semen volume and PCB-189 with sperm motility. A significant negative correlation was also reported for PCB-118 and sperm motility although no effect was seen on sperm count. Other studies reported exposure to PCB-105, PCB-118 and PCB-156 had no effect on semen parameters.

419. Overall, a causal relationship in humans between dioxins and dioxin-like PCBs and some semen parameters is possible, which is supported by experimental data, other parameters fail to be consistent across animals and humans.

420. Overall, the available in vivo and epidemiological data indicate a moderate causal association between dioxin and dioxin-like PCB exposure and adverse effects on semen parameters. AhR signaling is known to play a role in interfering with the male reproductive system hence provides a plausible mechanism of action.

421. Table 2 presents a summary of the strengths and weaknesses of the recent data on male reproductive toxicity (semen parameters) and dioxins and dioxin-like PCBs, and the influence of the lines of evidence on the overall conclusion.

Table 2 Summary of the strengths and weaknesses of the data on dioxins and dioxin-like PCBs and male reproductive toxicity (semen parameters) and the influence of the lines of evidence on the overall conclusion

Lines of evidence and their main strengths(S) and weaknesses (W)	**Influence on Conclusion**

Animal data	
S – All studies were considered to be good quality achieving a Klimisch score of 1 or 2 i.e. adequate protocols, number of animals, doses etc were used.	Toxicity on semen parameters is broadly the same in both mice and rats. Animal data are in support of human findings.
S – Adult mice and rats both show adverse effects on sperm parameters (decreased sperm count) following dioxin exposure.	
W – Neither study was carried out according to good laboratory practice or accepted guidelines.	
W – Limited data were found.	
W – The studies had diverse dose ranges and different endpoints were measured.	
W – Effects seen were not consistent across life stages as no effects on sperm parameters (i.e. daily sperm production, number of mature spermatids) were seen in neonatal or F1 adults following prenatal exposure, whereas effects were seen in adult animals in other studies following direct exposure.	
W – The difference in toxicokinetics between animals and humans was not investigated.	
Human data	
S – Reliable and good study design (prospective cohort, longitudinal, case-control studies), long-term follow-up with adequate control for cofounding variables.	Evidence that dioxins and dioxin-like PCBs cause adverse effects on semen parameters in epidemiological studies following environmental exposure. Animal data are in support of human findings.
S - Large cohorts in some studies.	
S – Detailed congener-specific PCB analysis in biological samples in some studies.	
S – Semen effects correlated with levels of dioxins and dioxin-like PCBs in biological samples.	
S – Risk metrics, confidence intervals and/or statistical analysis provided for all studies	
W – Limited prenatal exposure measurements during the period of sexual differentiation.	
W – Limited exposure measurements as single serum measurements usually carried out.	
W – Adverse effects seen are inconsistent across different PCBs.	
W – Dioxins and dioxin-like PCBs are present as mixtures as well as other chemicals that may affect semen parameters.	
W – Limited number of participants in some studies	
W – Prospective cohort studies cannot provide evidence for causal association between dioxins and dioxin-like PCBs and adverse semen parameters.	
Mechanistic data	
S – Established MoA via AhR mechanism.	

Conclusion on causality	Both epidemiological and experimental animal data provide moderate evidence for a causal relationship between dioxins and dioxin-like PCBs and adverse effects on semen parameters. This is further supported by the established MoA via the AhR.

422. The integrated conclusion is in the zone “very likely/almost certain” in **Error! Reference source not found.**.

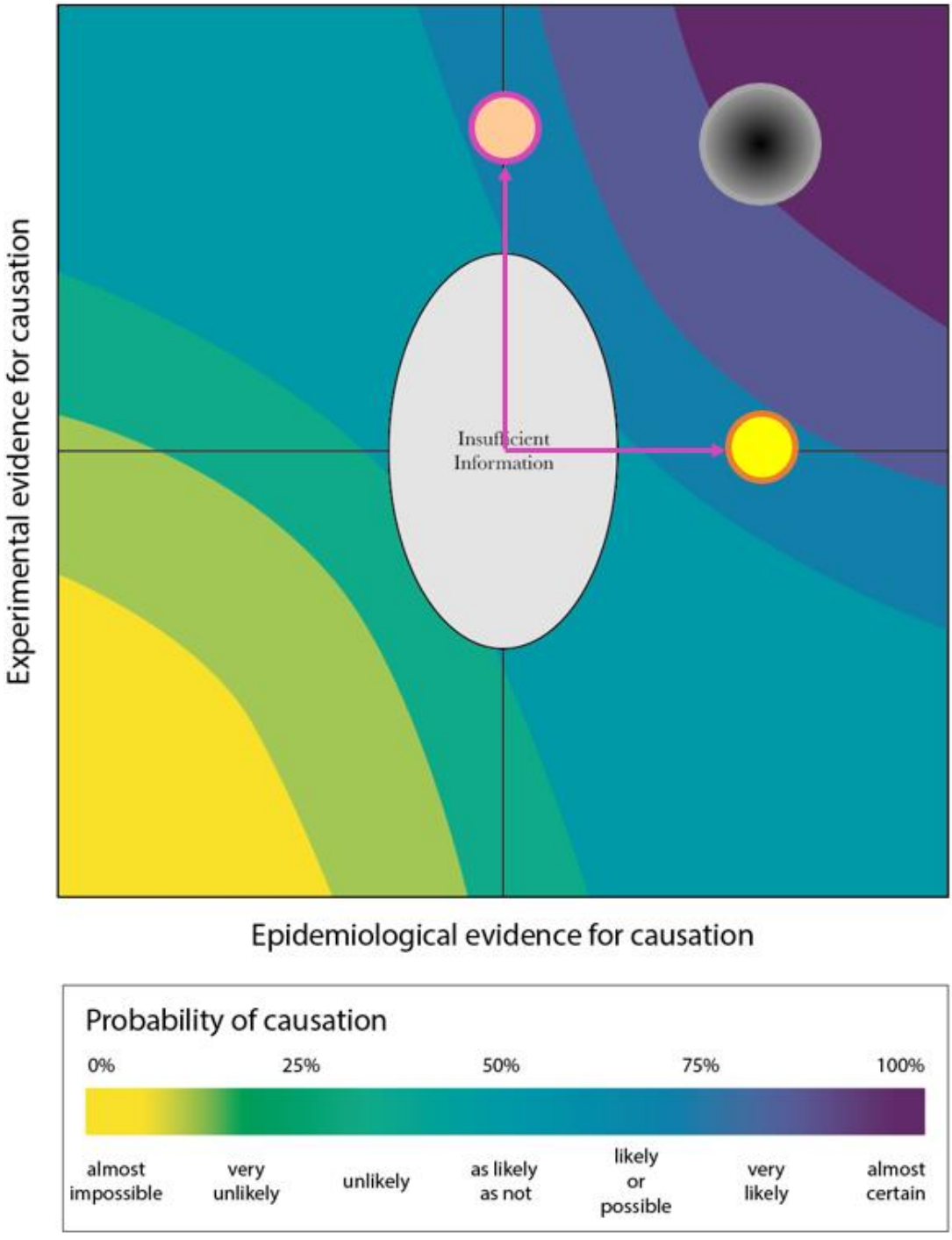

Figure 1. Visualisation of the causality of dioxins and dioxin-like PCBs exposure and male reproductive toxicity (semen parameters). The yellow circle is representative of all epidemiological evidence assessed (n=4); the orange circle of all toxicological evidence assessed (n=6). The grey circle represents the conclusion of causality of the integrated evidence.

Effects on testes

423. Effects on the testes have been reported in a number of in vivo studies in mice and in rats, exposed to either TCDD, TCDF or PCB-118. Doses varied between studies, in pre-natal studies pregnant animals were exposed on different GDs, and different endpoints were measured in mice and rats.

424. The range of effects observed included morphological/histopathological changes to the seminiferous tubules, reduced Sertoli cell counts, increased testicular coefficients and testicular inflammation and decreased testes weights.

425. Effects on testes were not consistent across all studies and varied with species and individual dioxin. In mice, findings were consistent as TCDD and TDCF were associated with effects on testes in adult mice and TCDD and PCB-118 in F1 mice. Findings in rats were mixed as no histopathological changes were seen in neonatal rat testes following TCDD exposure but reduced Sertoli cell counts were seen in neonatal and F1 adult testes as well as abnormal seminiferous tubules. In contrast, no histological changes were seen in neonatal and F1 rats following TCDD or TCDF exposure on GD9-10 (following a loading dose on GD8) or GD15.

426. A single epidemiological (case-cohort) study has reported the effects of perinatal exposure via breastmilk to mixtures of 27 potential EDC, which included 7 dioxin-like PCBs, and effects on the risk of cryptorchidism and male fertility. The authors concluded that for the group as a whole, infants with the highest exposure to breast milk concentrations of the dioxin-like PCB-114 (and the non-dioxin-like PCBs-74 and PCB-194 and β-HCH) had increased odds of congenital cryptorchidism and only PCB-114 (and the non-dioxin-like PCB-74 and PCB-194) were selected as predictors of congenital

cryptorchidism. However, the incidence of cryptorchidism was based on maternal reports using a self-administered questionnaire.

427. The available in vivo evidence highlights the potential differences between animal species and the effect on testes, although the studies are not directly comparable. Epidemiological evidence provides some evidence of the adverse effects of dioxin-like PCBs on testes although data are limited to one study.

428. Overall, the available in vivo and epidemiological data indicate a weak to moderate causal association between dioxin and dioxin-like PCB exposure and adverse effects on testes. AhR signaling is known to play a role in interfering with the male reproductive system hence provides a plausible mechanism of action.

429. Table 3 presents a summary of the strengths and weaknesses of the recent data on male reproductive toxicity (testes parameters) and dioxins and dioxin-like PCBs, and the influence of the lines of evidence on the overall conclusion.

Table 3 Summary of the strengths and weaknesses of the data on dioxins and dioxin-like PCBs and male reproductive toxicity (testes parameters) and the influence of the lines of evidence on the overall conclusion

Lines of evidence and their main strengths(S) and weaknesses (W)	**Influence on Conclusion**
Animal data	
S – All studies were considered to be good quality achieving a Klimisch score of 1 or 2 i.e. adequate protocols, number of animals, doses etc were used.	Adverse effects on the testes occurs in both mice and rats following pre-natal exposure. However, the endpoints measured and the adverse effects observed vary between limited number of studies. Animal data do not reflect human observations as different effects on the testes were reported.
S – Neonatal and F1 rats and mice show adverse effects on the testes following pre-natal exposure to either TCDD or PCB-118 (including reduced Sertoli cell count, various morphological changes, testicular inflammation and a significant decrease in testes weight). Exposure of adult mice to TCDD and TCDF also show histopathological changes.	
S – Data are available for adult rats and mice and both species show adverse effects on testes.	
S – Data are available for adult and F1 mice and both show adverse effects on testes.	

W – No study was carried out according to good laboratory practice or accepted guidelines.	
W – The studies had diverse dose ranges, pregnant animals were exposed on different GDs, and different endpoints were measured in mice and rats.	
W - Limited data were found for the effects of dioxins and dioxin-like PCBs in rats.	
W – Data are available for F1 neonates in rats although histopathological effects were inconsistent between the two studies with TCDD and no dose response was reported as effects were seen at lower doses but not at higher doses.	
W – The difference in toxicokinetics between animals and humans was not investigated.	
Human data	
S – Reliable and good study design (single case-control study with follow-up), with adequate control for cofounding variables.	Some evidence that dioxins and dioxin-like PCBs cause adverse effects (cryptorchidism) on the testes in epidemiological studies following environmental exposure. Animal data do not reflect human observations as different effects on the testes were reportd.
S - Adequate cohort.	
S – Detailed congener-specific PCB analysis in biological samples (breast milk).	
S – Concentrations of dioxins and dioxin-like PCBs in breast milk is a suitable proxy for prenatal and postnatal exposure.	
S – Risk metrics, confidence intervals and/or statistical analysis provided for the study	
W – Low number of studies were found.	
W – Dioxins and dioxin-like PCBs are present as mixtures as well as other chemicals that may affect semen parameters.	
W – Incidence of cryptorchidism was based on maternal reports using a self-administered questionnaire.	
W – Prospective cohort studies cannot provide evidence for causal association between dioxins and dioxin-like PCBs and adverse effects on testes.	
Mechanistic data	
S – Established MoA via AhR mechanism.	
Conclusion on causality	Both epidemiological and experimental animal data provide moderate evidence for a causal relationship between dioxins and dioxin-like PCBs and adverse effects on testes, albeit different effects were seen in animals and humans. This is further supported by the established MoA via the AhR.

430. The integrated conclusion is in the zone “very likely” in **Error! Reference source not found.**.

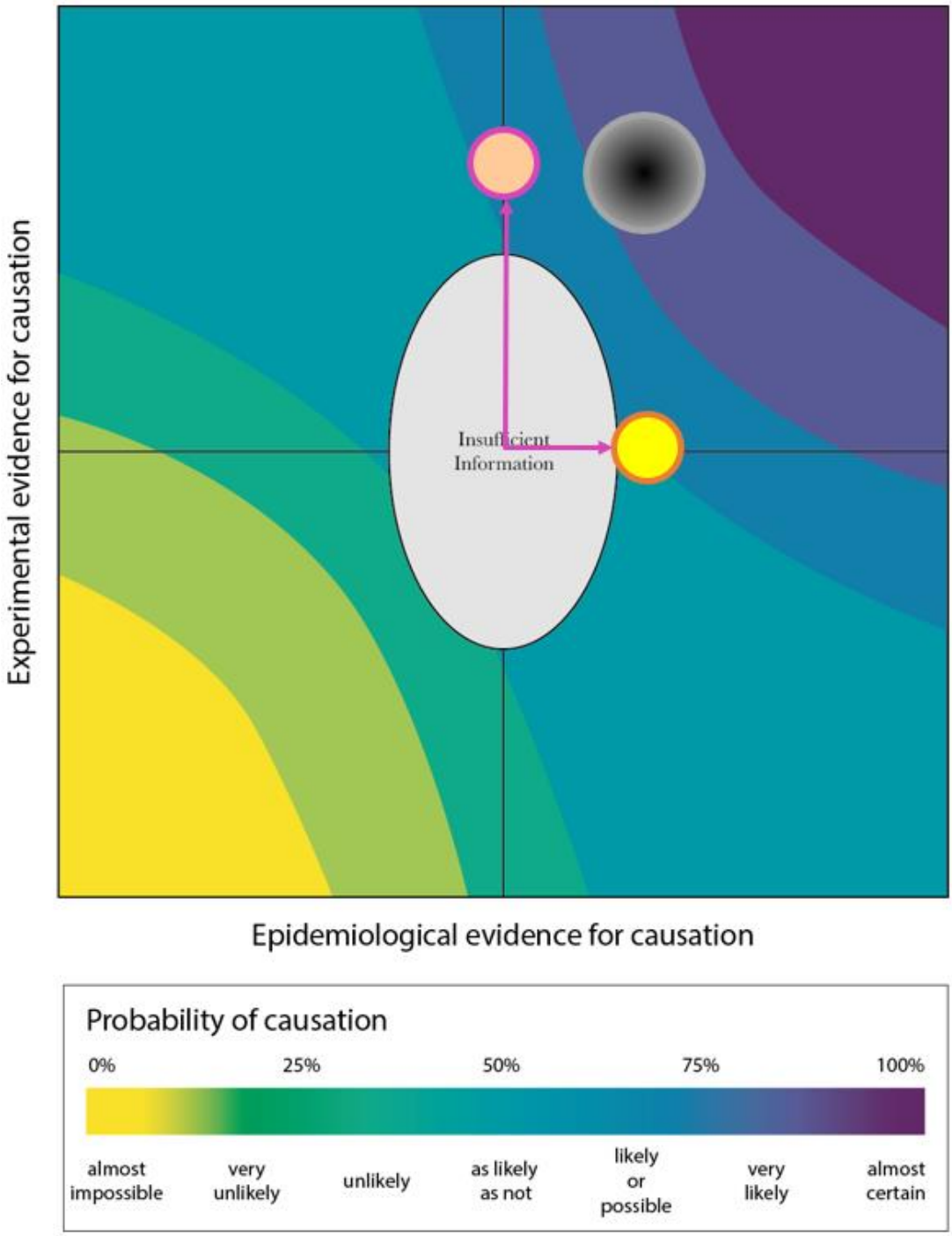

Figure 2. Visualisation of the causality of dioxins and dioxin-like PCBs exposure and male reproductive toxicity (testes parameters). The yellow circle is representative of all epidemiological evidence assessed (n=1); the orange circle of all toxicological evidence assessed (n=8). The grey circle represents the conclusion of causality of the integrated evidence.

Effects on hormones

431. A limited number of studies were identified that evaluated the effects of dioxins and dioxin-like PCBs on hormones. Testosterone levels were investigated in F1 neonatal and adult rats following TCDD exposure and gene expression of Fshb, Lhb, Cga and Inha in fetal pituitary and testes, respectively.

432. TCDD has no effect on plasma testosterone concentrations in F1 neonates on PND1 or in F1 adults on PND90 but significantly decreased gene expression of fetal pituitary Fshb and fetal testis Inha.

433. In human studies, evidence of a causal relationship between dioxins and dioxin-like PCBs and hormone levels was inconsistent as some studies showed a positive association with testosterone, inhibin B and DHEA whereas others showed a negative correlation.

434. Overall, a causal relationship in humans between dioxins and dioxin-like PCBs and hormones is possible, although such data are not strongly supported by experimental data.

435. The available in vivo and epidemiological data indicate a limited causal association between dioxin and dioxin-like PCB exposure and adverse effects on hormone levels. AhR signaling is known to play a role in interfering with the male reproductive system hence provides a plausible mechanism of action.

436. Table **4** presents a summary of the strengths and weaknesses of the recent data on male reproductive toxicity (hormone levels) and dioxins and dioxin-like PCBs, and the influence of the lines of evidence on the overall conclusion.

Table 4 Summary of the strengths and weaknesses of the data on male reproductive toxicity (hormone levels) and dioxins and dioxin-like PCBs and the influence of the lines of evidence on the overall conclusion

Lines of evidence and their main strengths(S) and weaknesses (W)	**Influence on Conclusion**
Animal data	
S – Both studies were considered to be good quality achieving a Klimisch score of 1 or 2 i.e. adequate protocols, number of animals, doses etc were used.	Limited number of in vivo studies were found that reported effects on hormones. Results were inconsistent as the hormones measured varied between the studies and the effect on such hormones differed. There is limited evidence that dioxins and dioxin-like PCBs affect reproductive hormone levels.
W – No study was carried out according to good laboratory practice or accepted guidelines.	
W - Limited data were found for the effects of TCDD in rats as only two studies were found which investigated different hormones. No studies in mice were found.	
W – There is a lack of consistency between the effects caused by TCDD and TCDF.	
W – The difference in toxicokinetics between animals and humans was not investigated.	
Human data	
S – Reliable and good study designs with adequate control for cofounding variables.	Data in humans were inconsistent between studies as some studies showed a positive association with hormone levels whereas others showed a negative correlation. Therefore, there is limited evidence that dioxins and dioxin-like PCBs affect reproductive hormone levels.
S - Adequate cohorts.	
S – Detailed congener-specific dioxins and dioxin-like PCB analysis in biological samples.	
S – Risk metrics, confidence intervals and/or statistical analysis provided for the studies	
W – Dioxins and dioxin-like PCBs are present as mixtures as well as other chemicals that may affect hormone levels.	
W – The effect on testosterone levels is inconsistent across different studies.	
Mechanistic data	
S – Established MoA via AhR mechanism.	
Conclusion on causality	Both epidemiological and experimental animal data failed to show a consistent effect on reproductive hormones and therefore evidence for a causal relationship between dioxins and dioxin-like PCBs.

437. The integrated conclusion is in the zone “unlikely” in Figure 3.

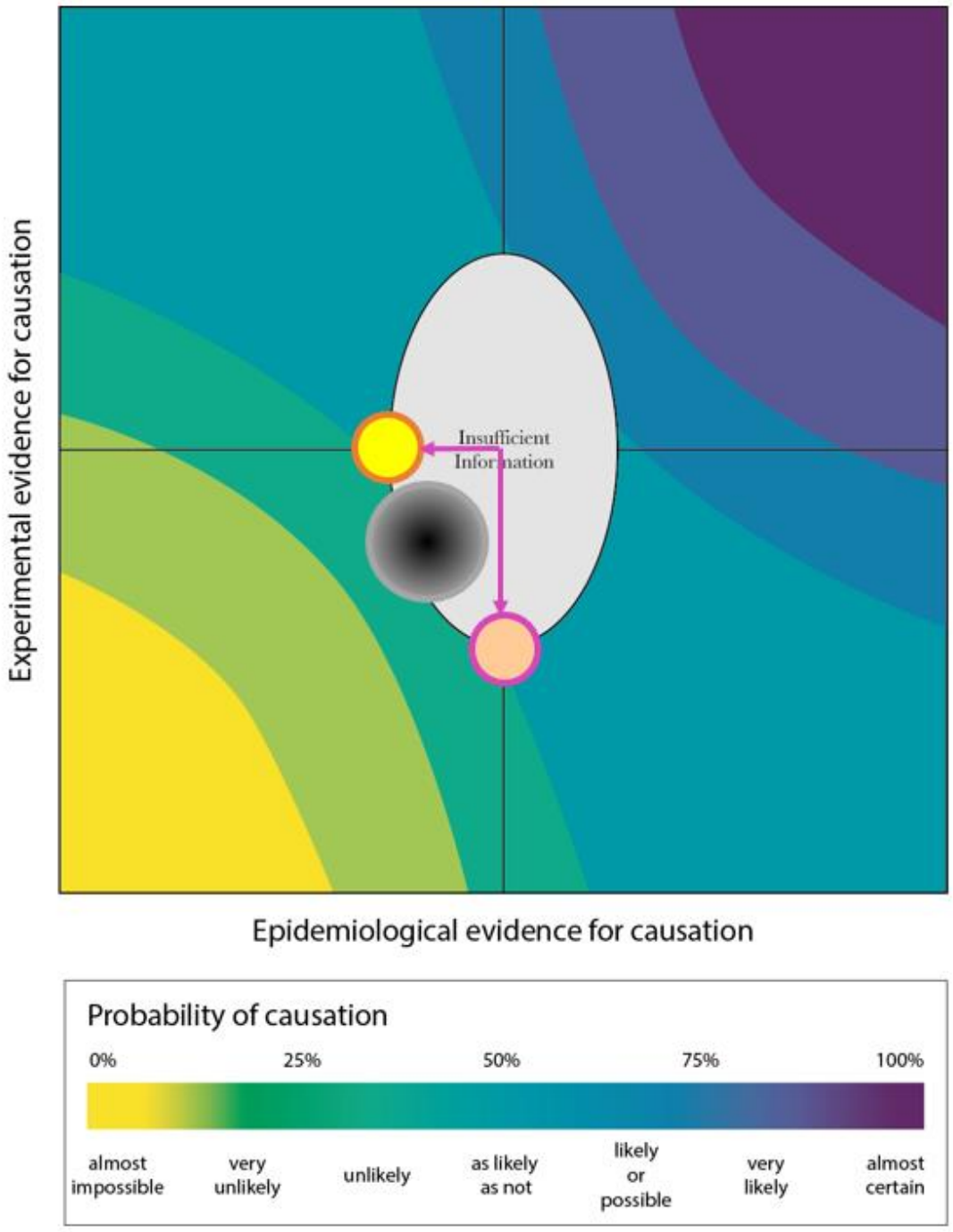

Figure 3. Visualisation of the causality of dioxins and dioxin-like PCBs exposure and male reproductive toxicity (hormone levels). The yellow circle is representative of all epidemiological evidence assessed (n=9); the orange circle of all toxicological evidence assessed (n=2). The grey circle represents the conclusion of causality of the integrated evidence.

Immunotoxicity

438. A limited number of studies were identified that evaluated the immunotoxic effects of dioxins and dioxin-like PCBs and most studies reported an effect on pro-inflammatory cytokines for example, in spleen, testes and colon. In contrast, TCDD failed to cause an effect on cytokine production in microglial cells in the brain.

439. Limited epidemiology data were available hence it remains unclear how environmental exposures to dioxins and/or dioxin-like PCBs may impact on antibody responses in humans. An increased risk of allergies in school age children, and in particular males, has been reported following prenatal exposure to specific dioxins or dioxin-like PCBs, however, a number of study limitations were apparent that may impact on these findings.

440. Overall, epidemiological data indicate that a causal relationship in humans between dioxins and dioxin-like PCBs and immunotoxicity, as a whole, is possible, and such data are strongly supported by experimental data, albeit limited. Due to the limited amount of data, specific immunotoxicological endpoints cannot be compared. AhR signaling is known to play a role in immunotoxicity and hence provides a plausible mechanism of action.

441. Table 5 presents a summary of the strengths and weaknesses of the recent data on immunotoxicity and dioxins and dioxin-like PCBs, and the influence of the lines of evidence on the overall conclusion.

Table 5 Summary of the strengths and weaknesses of the data on immunotoxicity and dioxins and dioxin-like PCBs and the influence of the lines of evidence on the overall conclusion

Lines of evidence and their main strengths(S) and weaknesses (W)	**Influence on Conclusion**
Animal data	
S – All studies were considered to be good quality achieving a Klimisch score of 1 or 2 i.e. adequate protocols, number of animals, doses etc were used.	The effect on pro-inflammatory cytokine production is reported in a number of different studies looking at different organs in mice and rats. Animal data do not reflect human observations as different effects were investigated.
S – Most studies show that adult and F1 mice both show adverse effects on the immune system following exposure to TCDD.	
S – Most studies reported the effect of TCDD on cytokine production so comparisons could be made.	
W – No study was carried out according to good laboratory practice or accepted guidelines.	
W – Limited data were found.	
W – The difference in toxicokinetics between animals and humans was not investigated.	
Human data	
S – Reliable and good study designs with adequate control for cofounding variables.	Evidence that dioxins and dioxin-like PCBs modify immune response in offspring following environmental exposure. Similar effects were not investigated in animals.
S - Large cohorts in some studies.	
S – Detailed congener-specific PCB analysis in biological samples in some studies.	
S – Risk metrics, confidence intervals and/or statistical analysis provided for all studies	
W – Limited exposure measurements.	
W – Adverse effects seen are inconsistent across studies.	
W – Dioxins and dioxin-like PCBs are present as mixtures as well as other chemicals that may affect the immune response.	
W – Limited number of participants in some studies	
Mechanistic data	
S – Established MoA via AhR mechanism.	
Conclusion on causality	Both epidemiological and experimental animal data provide some evidence for a causal relationship between dioxins and dioxin-like PCBs and immunotoxicity albeit different endpoints were investigated in animals and humans.

442. The integrated conclusion is in the zone “as likely as not” in Figure 4.

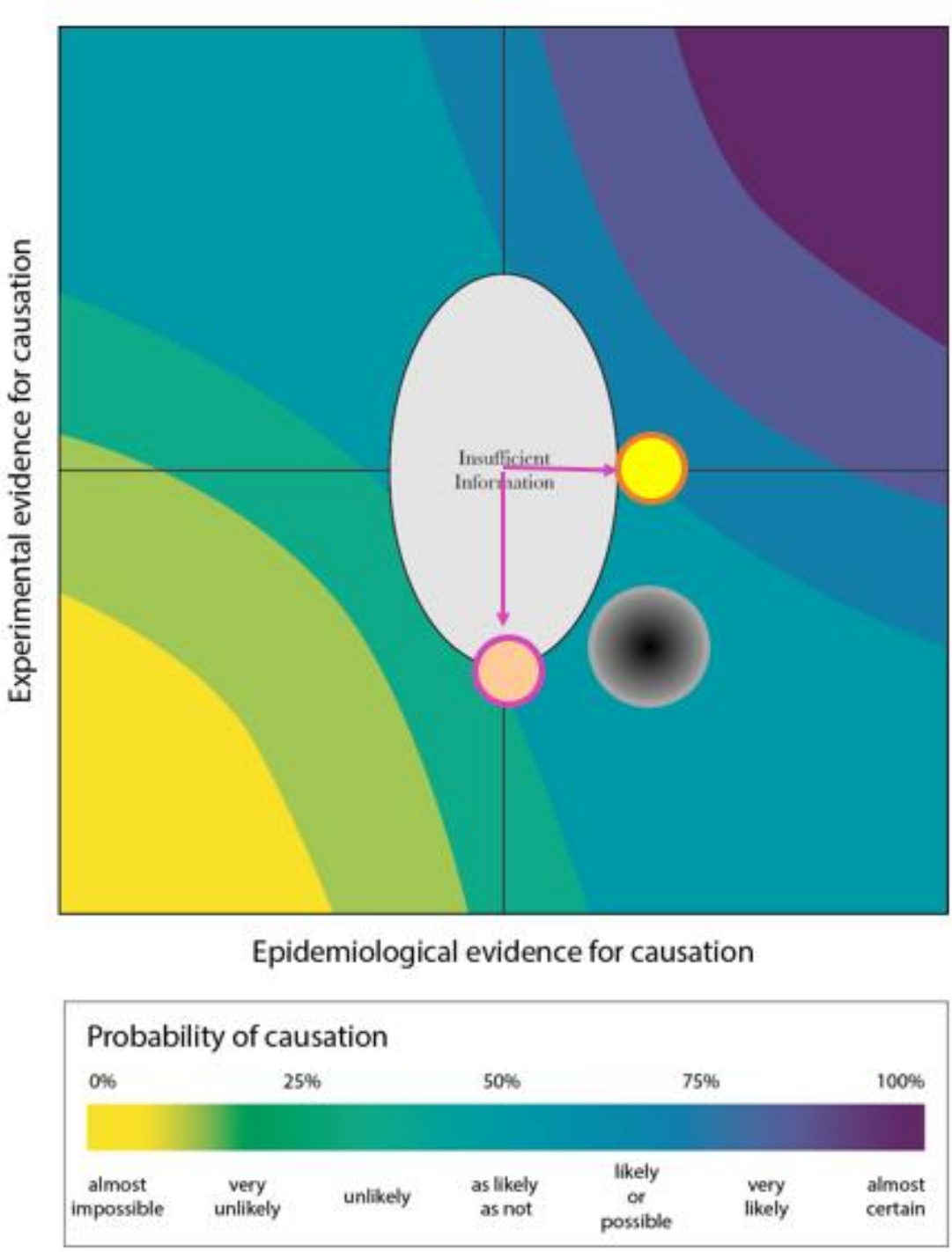

Figure 4. Visualisation of the causality of dioxins and dioxin-like PCBs exposure and immunotoxicity. The yellow circle is representative of all epidemiological evidence assessed (n=4); the orange circle of all toxicological evidence assessed (n=3). The grey circle represents the conclusion of causality of the integrated evidence.

Overall summary

443. With regards to male reproductive effects, following exposure to TCDD, TCDF or PCB-118, a number of effects were noted in both animals and humans including effects on sperm parameters (sperm count and motility, sperm malformations), effects on testes (morphological and histopathological changes in seminiferous tubules and/or epithelium, Sertoli cell count, testicular coefficient, morphological abnormalities in the testes) and effects on hormone levels (serum testosterone, gonadotrophin, INHA). EFSA (2018) also noted semen quality and cryptorchidism to be the main adverse effects as well as pubertal development.

444. Overall, male reproductive toxicity was seen in vivo from a dose of 0.375 µg/kg bw/day for TCDD (decreased sperm count and motility, increased sperm malformations, morphological changes in the seminiferous tubules and the epithelium of the seminiferous tubules), from 0.5 µg/kg bw/day for TCDF (decreased serum testosterone level, increased DNA damage in sperm and degenerative histopathological changes of the seminiferous tubules) and from 20 µg/kg bw/day for PCB-118 (increase in testicular coefficient, morphological changes in the seminiferous epithelium and/or seminiferous tubules, decrease in number of spermatogonia, increase in sperm deformities, and a decrease in sperm motility).

445. From epidemiology studies, the main reprotoxic effects investigated in males were reduced semen quality, cryptorchidism, changes in hormone levels and pubertal development. This is in line with the main effects reported by EFSA (2018), with no additional reprotoxic effects being identified here.

446. Less data were available regarding immunotoxicity, and endpoints investigated in vivo and in epidemiology studies were not aligned. In vivo, TCDD increased inflammation by increasing cytokine responses in spleen, testes, colon but not brain.

447. TCDD also increased humeral and cellular immunity as indicated by the increase in antibody and cytokine production, respectively. EFSA (2018)

concluded that TCDD and dioxin-like PCBs may lead to the dysregulation of components of the immune system and eventually lead to adverse effects such as repressed resistance to infections or effects on autoimmune phenomena.

448. Epidemiology studies reported an increased risk of allergy in school children, although studies had several limitations. This is in line with the main effects reported by EFSA (2018), with no additional immunotoxic effects being identified here.

449. A number of other effects were seen in vivo including effects on bone, cardiovascular system and liver, as well as associations with obesity and cleft palate.

450. Results supporting a genotoxic mechanism of action for carcinogenicity are equivocal. In vivo, TCDD was associated with increased oxidative stress and mutatations in CYP1A1 in rat liver. In vitro, PCB-77 and PCB-81 were activated to clastogenic metabolites, as evidenced by the formation of centromere-free micronuclei and double strand DNA breaks in mammalian cells. However, in an in vitro study using human SH-SY5Y neuroblastoma cells, data did not support TCDD-induced genomic instability or genetic damage.

Conclusion

451. Exposure to dioxins and dioxin-like PCBs appear to be associated with an adverse effect on male fertility as effects on sperm parameters, testes and sex hormones were reported in vivo and in humans, although the biological impact of any effect seen is not clear.

452. Previously, the COT noted data from the Russian Children's study, identifying semen quality following pre- and postnatal exposure, appeared inconsistent with the findings in a second study and considered it only to provide only a weak data set. Data identified in this review also reported an association between dioxins and dioxin-like PCBs and sperm parameters

such as motility, volume, morphology and viability (Amir et al., 2021; Paul et al., 2017) although Petersen et al. (2018) failed to show an association.

453. A number of in vivo studies also reported effects of sperm parameters following exposure to dioxins and dioxin-like PCBs (Elsayed et al., 2019; He et al., 2020; Meles et al., 2022; Tao et al., 2021; Yahia et al., 2018) although Erthal et al. (2018) reported there was no effect on neonatal or F1 adult sperm count parameters (daily sperm production, number of mature spermatids).

454. Immunotoxicity was also observed, although different endpoints were investigated in animals and humans. Exposure was also associated with adverse effects on bone, cardiovascular system and liver and caused obesity and cleft palate.

455. The COT also commented on the data presented in EFSA's opinion that implied that humans were more sensitive to dioxins than rats and noted that this is inconsistent with the existing body of data on dioxins and knowledge on the relative sensitivity of the human and rat AHR.

456. Various studies have demonstrated revealed species-, cell- and region-specific pattern of the AhR system expression in the rat and human testis and epididymis. Overall, greater AHR was reported in human testes compared with rat testes and suggest that activation of AHR/ARNT in humans may lead to greater biological consequences than in rats.

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List of Abbreviations

7AAD	7-Aminoactinomycin D
17-OH-P4	17-OH-progesterone
17β-HSD	17β-hydroxysteroid dehydrogenase
3β-HSD	3β–hydroxysteroid dehydrogenase
5-meC	5-methylcytosine

α-SMA	α-smooth muscle actin
A-dione	Androstenedione
AGDAP	Anal-penile distance
AGI	Anogenital index
AHR / Ahr	Aryl hydrocarbon receptor
AIP	Aryl hydrocarbon interacting protein
ALP	Alkaline phosphatase activity
ALT	Alanine aminotransferase
AMH	Anti-müllerian hormone
AO	Agent Orange
AOP	Adverse outcome pathway
AST	Aspartate aminotransferase
BDE-47	Polybrominated diphenyl ether
BMDL	Lower confidence limit of the benchmark dose
BMI	Body mass index
CAT	Catalase
CCK-8	Cell counting kit-8
CD	Control diet
CENP-B^{-}	Negative centromere protein B
CENP-B^{+}	Positive centromere protein B
CI	Confidence interval
CMM	Cutaneous malignant melanoma
c-MAF	Proto-oncogene c-Maf
COBRA	Combined bisulfite restriction analysis
COT	Committee on Toxicity
CRC	Colorectal cancer
c-Scr	Cellular-sarcoma
cTnI	Serum cardiac troponin I
CYP17 lyase	Cytochrome P450 17,20 lyase
CYP1A1	Cytochrome P450 Family 1 Subfamily A Member 1
CYP1A2	Cytochrome P450 Family 1 Subfamily A Member 2
CYP1B1	Cytochrome P450 Family 1 Subfamily B Member 1

DACE	Dutch Development at Adolescence and Chemical Exposure
DAG	Directed acyclic graph
DC	Dendric cells
DDD	4,4′-dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEPs	Diesel exhaust particles
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DMR	Differentially methylated region
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
Dnmt / DNMT	DNA methyltransferase
DRE	Dioxin response elements
DST	Diameter of seminiferous tubule
E (or ED)	Embryonic day
E2	Oestradiol
E2F1	E2F Transcription Factor 1
EBV	Epstein-Barr virus
EDC	Endocrine disrupting chemical
EFSA	European Food Standards Agency
EGP	Epiphyseal growth plate
ELISA	Enzyme-linked immunosorbent assay
ER	Estrogen receptor
EROD	Ethoxyresorufin-O-deethylase
EST	Epithelium of the seminiferous tubules
FSC	Forward scatter
FSH	Follicle stimulating hormone
Fshb	Follicle stimulating hormone subunit beta
GD	Gestation day
GH	Growth hormone

GM	Geometric mean
GPx	Glutathione peroxidase
GSH-Px	Glutathione peroxidase
GSH-R	Glutathione reductase
HCB	Hexachlorobenzene
HCC	Hepatocellular carcinoma
HCV	Viral hepatitis C
HFD	High fat diet
HR	Hazard ratio
HSC	Hepatic stellate cell
Hsp90	Heat shock protein 90
HxCDF	1,2,3,4,7,8-hexachlorodibenzofuran
HUMIS	Norwegian Human Milk Study
HZ	Interferon
i.p.	Intraperitoneal
IARC	International Agency for Research on Cancer
IFN-1	Interferon Type-I
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IGF	Insulin-like growth factor
IgG	Immunoglobulin G
IGI	Induced genomic instability
Ihh	Indian hedgehog
IL	Interleukin
ILC3s	Type 3 innate lymphoid cells
Inha	Inhibin subunit alpha
INSL3	Insulin-like factor-3
IQR	Interquartile range
IRF7	Interferon regulatory factor 7
IRR	Incidence rate ratios
ISAAC	International Study on Asthma and Allergy in Childhood

IVF	In vitro fertilisation
JECFA	Joint Expert Committee on Food Additives of the WHO and Food and Agriculture Organisation (FAO)
KD	Dissociation constant
KE	Key event
LBD	Ligand binding domain
LDH	Lactate dehydrogenase
LFD	Low fat diet
LH	Luteinizing hormone
Lhb	Luteinizing hormone subunit beta
Lhb	Hormone subunit beta
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LPS	Lipopolysaccharide
MDA	Malondialdehyde
MEG3	Maternally expressed gene 3
MFI	Median fluorescence intensity
MIE	Molecular initiating event
MLR	Multivariate linear regression
MMP	Mitochondrial membrane potential
mRNA	Messenger ribonucleic acid
MSWI	Municipal solid waste incinerator
NAFLD	Non-alcoholic fatty liver disease
NC	Negative control
NDL	Non-dioxin-like
NEFA	No esterified fatty acids
NF-kB / NrkB	Nuclear factor kappa B
NHL	Non-Hodgkin lymphoma
NLS	Nuclear localization signal
NKp46+	Natural cytotoxicity receptor NKp46
NOS	Newcastle-Ottawa (Quality Assessment) Scale
NR	Nuclear receptor

NTP	National Toxicology Programme
OC	Organochlorine
OCDF	Octachlorodibenzofuran
OCP	Organochlorine pesticide
OECD	Organisation for Economic Co-operation and Development
OR	Odds ratio
OVA	Ovalbumin
1,2,3,7,8-pentachlorodibenzo-P-dioxin	PeCDD
P4	Progesterone
p-Akt	Phosphorylated Akt
PAS	Per-Arnt-sim
PBDE	Polybrominated diphenyl ether
PCBs	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-p-dioxins
PCDF	Polychlorinated dibenzofurans
Pcna / PCNA	Proliferating cell nuclear antigen
PFAS	Perfluorinated alkylate substances
PFCs	Perfluorinated chemicals
PFOS	Perfluorooctane sulfonate
PMTI	Provisional tolerable daily intake
PND	Postnatal day
POP	Persistent organic pollutant
PPAR	Peroxisome proliferator-activated receptor
PR	Progesterone receptor
PROD	Pentoxyresorufin-O-dealkylase
PTH	Serum parathyroid hormone
RB	Retinoblastoma protein
RENCO	Risk of Endocrine Contaminants on Human Health
RNA-Seq	RNA sequencing

RORγt	Retinoic acid receptor-related orphan receptor
RR	Relative risk
RRs	Risk Ratios
s.c.	Subcutaneous
SD	Standard Deviation
SES	Socio economic status
SHBG	Sex hormone-binding globulin
SMR	Standardised mortality ratio
SOD	Superoxide dismutase
SSC	Side scatter
SSR	Secondary sex ratio
Stra8 / STRA8	Stimulated by retinoic acid 8
STS	Soft tissue sarcoma
SULTs	Sulfotransferases
SYP	Synaptophysin
T	Testosterone
TAS	Total antioxidant status
TBARS	Thiobarbituric acid reactive substance
TCA	Tricarboxylic acid
TCDD	2,3,7,8-Tetrachlorodibenzodioxin
TCDF	2,3,7,8-Tetrachlorodibenzofuran
TDI	Tolerable daily intake
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
TGF-β	Transforming growth factor-β
TLR	Toll like receptor
TMS	Tetramethoxystilbene
TNF	Tumour necrosis factor
TOS	Total oxidant status
TQ	Thymoquinone
TRAP	Tartrate-resistant acid phosphatase
Treg	Regulatory T cells

TSH	Thyroid stimulating hormone
TWI	Tolerable weekly intake
UDPGT	Uridine 5′ -diphospho-glucuronosyltransferase
UGTs	UDP-glucuronosyltransferases
Uhrf1	Ubiquitin-like, with PHD and RING finger domains 1
US EPA	US Environmental Protection Agency
VAT	Visceral adipose tissue
VC	Vehicle control
VCAM1	Vascular Cell Adhesion Molecule 1
VSMCs	Vascular smooth muscle cells
WHO	World Health Organisation
WQS	Weighted quantile sum
WT	Wild type
XAP2	X-associated protein 2
XRE	Xenobiotic response elements

Annex A

Systematic review of the literature on dioxins and dioxin-like polychlorinated biphenyls

Details of literature search carried out.

Relevant literature was obtained from Scopus and PubMed for dioxins and dioxin-like PCBs, relating to male reproductive toxicity, immunotoxicity, and other toxicological endpoints (animals only). Both in vivo and epidemiology data were collated. Additionally relevant literature was obtained for the aryl hydrocarbon receptor (AhR) mechanism of action of dioxins and dioxin-like PCBs and mechanism of carcinogenicity. Searches were performed from 2017 to 20/10/2022 in October 2022.

The search terms for Scopus and PubMed are presented below and the inclusion/exclusion criteria (Table **6**, Table **7** and Table 8).

Search 1: male reproductive toxicity

Scopus – in vivo

(TITLE-ABS-KEY (*chlorodibenzodioxin OR *chlorodibenzofuran OR *chlorobiphenyl OR dioxin* OR dioxin AND like OR pcb OR tcdd OR pcdd OR pcdf OR tcdf OR pecdd OR ocdd OR hxcdd OR hxcdf OR hpcdd OR hpcdf OR teq OR "toxic* equivalen*" OR coplanar) AND TITLE-ABS-KEY (rat OR mice OR mouse OR monkey OR pig OR rabbit OR hamster OR dog OR cat OR mink OR hare OR chinchilla OR vivo OR primate OR swine) AND TITLE-ABS-KEY (sperm OR semen OR hormone OR reproduct* OR cryptorchidism OR puberty* OR "sex ratio" OR testosterone OR testis OR anogenital OR preputial OR epididym* OR prostate OR "sex organ")) AND PUBYEAR > 2016 AND PUBYEAR > 2016 AND (EXCLUDE (LANGUAGE , "Japanese") OR EXCLUDE (LANGUAGE , "Korean")): 121

Scopus – epidemiology

(TITLE-ABS-KEY (*chlorodibenzodioxin OR *chlorodibenzofuran OR *chlorobiphenyl OR dioxin* OR dioxin AND like OR pcb OR tcdd OR pcdd OR pcdf OR tcdf OR pecdd OR ocdd OR hxcdd OR hxcdf OR hpcdd OR hpcdf OR teq OR "toxic* equivalen*" OR coplanar) AND TITLE-ABS-KEY (epidemiolog* OR "cohort stud*" OR "case control stud*" OR "adverse effect*" OR "observational stud*" OR "case serie*" OR "case report*" OR "cross sectional stud*") AND TITLE-ABS-KEY ((human) AND (man OR men OR male OR boy)) AND TITLE-ABS-KEY (sperm OR semen OR hormone OR reproduct* OR cryptorchidism OR puberty* OR "sex ratio" OR testosterone OR testis OR anogenital OR preputial OR epididym* OR prostate OR "sex organ")) AND PUBYEAR > 2016 AND PUBYEAR > 2016 AND (EXCLUDE (LANGUAGE , "Japanese")):54

PubMed - in vivo

(((2,3,7,8-Tetrachlorodibenzo-p-dioxin[MeSH Terms] OR tetrachlorodibenzodioxin[MeSH Terms] OR polychlorinated biphenyls[MeSH Terms] OR biphenyls, polychlorinated[MeSH Terms] OR tetrachlorodibenzodioxin[MeSH Terms] OR pcbs[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (chlorodibenzodioxin[Title/Abstract] OR chlorodibenzofuran[Title/Abstract] OR chlorobiphenyl[Title/Abstract] OR dioxin*[Title/Abstract] OR dioxin like[Title/Abstract] OR pcb[Title/Abstract] OR TCDD[Title/Abstract] OR PCDD[Title/Abstract] OR PCDF[Title/Abstract] OR TCDF[Title/Abstract] OR PeCDD[Title/Abstract] OR OCDD[Title/Abstract] OR HxCDD[Title/Abstract] OR HxCDF[Title/Abstract] OR HpCDD[Title/Abstract] OR HpCDF[Title/Abstract] OR TEQ[Title/Abstract] OR "toxic* equivalen*"[Title/Abstract] OR coplanar[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((rat[Title/Abstract] OR mice[Title/Abstract] OR mouse[Title/Abstract] OR monkey[Title/Abstract] OR pig[Title/Abstract] OR rabbit[Title/Abstract] OR hamster[Title/Abstract] OR

dog[Title/Abstract] OR cat[Title/Abstract] OR mink[Title/Abstract] OR hare[Title/Abstract] OR chinchilla[Title/Abstract] OR vivo[Title/Abstract] OR primate[Title/Abstract] OR swine[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR ((Rats[MeSH Terms] OR mice[MeSH Terms] OR guinea pigs[MeSH Terms] OR rabbits[MeSH Terms] OR dogs[MeSH Terms] OR cats[MeSH Terms] OR mink[MeSH Terms] OR hares[MeSH Terms] OR chinchilla[MeSH Terms] OR primates[MeSH Terms] OR swine[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter])))) AND ((Semen[MeSH Terms] OR hormones[MeSH Terms] OR reproduction[MeSH Terms] OR cryptorchidism[MeSH Terms] OR puberty[MeSH Terms] OR sex ratio[MeSH Terms] OR testosterone[MeSH Terms] OR testis[MeSH Terms] OR epididymis[MeSH Terms] OR prostate[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (Sperm[Title/Abstract] OR Semen[Title/Abstract] OR hormone[Title/Abstract] OR reproduct*[Title/Abstract] OR cryptorchidism[Title/Abstract] OR puberty*[Title/Abstract] OR "sex ratio" [Title/Abstract] OR testosterone[Title/Abstract] OR testis[Title/Abstract] OR anogenital[Title/Abstract] OR preputial[Title/Abstract] OR epididym*[Title/Abstract] OR prostate[Title/Abstract] OR "sex organ" [Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))): 699

PubMed - epidemiology

((((("polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms] OR "polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms]) AND "spermatozoa"[MeSH Terms]) OR ("dioxin*"[Title/Abstract] OR "dioxin like"[Title/Abstract] OR "pcb"[Title/Abstract] OR "TCDD"[Title/Abstract] OR "PCDD"[Title/Abstract] OR "PCDF"[Title/Abstract] OR "TCDF"[Title/Abstract]

OR "PeCDD"[Title/Abstract] OR "OCDD"[Title/Abstract] OR "HxCDD"[Title/Abstract] OR "HxCDF"[Title/Abstract] OR "HpCDD"[Title/Abstract] OR "HpCDF"[Title/Abstract] OR "TEQ"[Title/Abstract] OR "toxic equivalen*"[Title/Abstract] OR "coplanar"[Title/Abstract])) AND ("cohort studies"[MeSH Terms] OR "case control studies"[MeSH Terms] OR "case control studies"[MeSH Terms] OR (("ieee int conf automation sci eng case"[Journal] OR "case phila"[Journal] OR "case"[All Fields]) AND "research report"[MeSH Terms]) OR "cross sectional studies"[MeSH Terms] OR "cross sectional studies"[MeSH Terms] OR ("epidemiolog*"[Title/Abstract] OR "cohort stud*"[Title/Abstract] OR "case control stud*"[Title/Abstract] OR "adverse effect*"[Title/Abstract] OR "observational stud*"[Title/Abstract] OR "case serie*"[Title/Abstract] OR "case report*"[Title/Abstract] OR "cross sectional stud*"[Title/Abstract])) AND ("men"[MeSH Terms] OR "male"[MeSH Terms] OR ("man"[Title/Abstract] OR "men"[Title/Abstract] OR "male"[Title/Abstract] OR "boy"[Title/Abstract])) AND ("Semen"[MeSH Terms] OR "hormones"[MeSH Terms] OR "reproduction"[MeSH Terms] OR "cryptorchidism"[MeSH Terms] OR "puberty"[MeSH Terms] OR "sex ratio"[MeSH Terms] OR "testosterone"[MeSH Terms] OR "testis"[MeSH Terms] OR "epididymis"[MeSH Terms] OR "prostate"[MeSH Terms] OR ("Sperm"[Title/Abstract] OR "Semen"[Title/Abstract] OR "hormone"[Title/Abstract] OR "reproduct*"[Title/Abstract] OR "cryptorchidism"[Title/Abstract] OR "puberty*"[Title/Abstract] OR "sex ratio"[Title/Abstract] OR "testosterone"[Title/Abstract] OR "testis"[Title/Abstract] OR "anogenital"[Title/Abstract] OR "preputial"[Title/Abstract] OR "epididym*"[Title/Abstract] OR "prostate"[Title/Abstract] OR "sex organ"[Title/Abstract]))) AND ((humans[Filter]) AND (2017/2/1:2022/10/20[pdat]) AND (english[Filter])):90

Search 2: immunotoxicity

Scopus – in vivo

((TITLE-ABS-KEY (*chlorodibenzodioxin OR *chlorodibenzofuran OR *chlorobiphenyl OR dioxin* OR dioxin AND like OR pcb OR tcdd OR pcdd OR pcdf OR tcdf OR pecdd OR ocdd OR hxcdd OR hxcdf OR

hpcdd OR hpcdf OR teq OR "toxic* equivalen*" OR coplanar) AND TITLE-ABS-KEY (rat OR mice OR mouse OR monkey OR pig OR rabbit OR hamster OR dog OR cat OR mink OR hare OR chinchilla OR vivo OR primate OR swine) AND TITLE-ABS-KEY (immun* OR antibod* OR lymphocyte OR "t cell" OR "b cell" OR "dendric cell" OR igm OR antigen OR mitogen OR "natural killer cell" OR igg OR iga OR ige OR "polymorphonuclear leucocyte")) AND PUBYEAR > 2016 AND PUBYEAR > 2016) AND (EXCLUDE (LANGUAGE , "Chinese")): 199

Scopus – epidemiology

(TITLE-ABS-KEY (*chlorodibenzodioxin OR *chlorodibenzofuran OR *chlorobiphenyl OR dioxin* OR dioxin AND like OR pcb OR tcdd OR pcdd OR pcdf OR tcdf OR pecdd OR ocdd OR hxcdd OR hxcdf OR hpcdd OR hpcdf OR teq OR "toxic* equivalen*" OR coplanar) AND TITLE-ABS-KEY (epidemiolog* OR "cohort stud*" OR "case control stud*" OR "adverse effect*" OR "observational stud*" OR "case serie*" OR "case report*" OR "cross sectional stud*") AND TITLE-ABS-KEY (human OR men OR man OR child* OR wom*n OR male OR female OR adolescent) AND TITLE-ABS-KEY (urine OR serum OR plasma OR haema* OR hema* OR blood OR immun* OR antibod* OR lymphocyte OR "T cells" OR "B cells" OR "Dendric cells" OR igm OR antigen OR mitogen OR "natural killer cells" OR igg OR iga OR ige OR "polymorphonuclear leucocytes")) AND PUBYEAR > 2016 AND (EXCLUDE (LANGUAGE , "Russian")): 206

PubMed – in vivo

(((2,3,7,8-Tetrachlorodibenzo-p-dioxin[MeSH Terms] OR tetrachlorodibenzodioxin[MeSH Terms] OR polychlorinated biphenyls[MeSH Terms] OR biphenyls, polychlorinated[MeSH Terms] OR tetrachlorodibenzodioxin[MeSH Terms] OR pcbs[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (chlorodibenzodioxin[Title/Abstract] OR chlorodibenzofuran[Title/Abstract] OR chlorobiphenyl[Title/Abstract] OR dioxin*[Title/Abstract] OR dioxin

like[Title/Abstract] OR pcb[Title/Abstract] OR TCDD[Title/Abstract] OR PCDD[Title/Abstract] OR PCDF[Title/Abstract] OR TCDF[Title/Abstract] OR PeCDD[Title/Abstract] OR OCDD[Title/Abstract] OR HxCDD[Title/Abstract] OR HxCDF[Title/Abstract] OR HpCDD[Title/Abstract] OR HpCDF[Title/Abstract] OR TEQ[Title/Abstract] OR "toxic* equivalen*"[Title/Abstract] OR coplanar[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((Rats[MeSH Terms] OR mice[MeSH Terms] OR guinea pigs[MeSH Terms] OR rabbits[MeSH Terms] OR dogs[MeSH Terms] OR cats[MeSH Terms] OR mink[MeSH Terms] OR hares[MeSH Terms] OR chinchilla[MeSH Terms] OR primates[MeSH Terms] OR swine[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (rat[Title/Abstract] OR mice[Title/Abstract] OR mouse[Title/Abstract] OR monkey[Title/Abstract] OR pig[Title/Abstract] OR rabbit[Title/Abstract] OR hamster[Title/Abstract] OR dog[Title/Abstract] OR cat[Title/Abstract] OR mink[Title/Abstract] OR hare[Title/Abstract] OR chinchilla[Title/Abstract] OR vivo[Title/Abstract] OR primate[Title/Abstract] OR swine[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter])))) AND ((Immunoglobulin[MeSH Terms] OR antibodies[MeSH Terms] OR lymphocytes[MeSH Terms] OR antigens[MeSH Terms] OR mitogens[MeSH Terms] OR killer cells, natural[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (Immun*[Title/Abstract] OR antibod*[Title/Abstract] OR lymphocyte[Title/Abstract] OR T cells[Title/Abstract] OR B cells[Title/Abstract] OR Dendric cells[Title/Abstract] OR IgM[Title/Abstract] OR Antigen[Title/Abstract] OR Mitogen[Title/Abstract] OR natural killer cells[Title/Abstract] OR IgG[Title/Abstract] OR IgA[Title/Abstract] OR IgE[Title/Abstract] OR polymorphonuclear leucocytes[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))): 365

PubMed – epidemiology

((((("polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms] OR "polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language])) OR (("chlorodibenzodioxin"[Title/Abstract] OR "chlorodibenzofuran"[Title/Abstract] OR "chlorobiphenyl"[Title/Abstract] OR "dioxin*"[Title/Abstract] OR "dioxin like"[Title/Abstract] OR "pcb"[Title/Abstract] OR "TCDD"[Title/Abstract] OR "PCDD"[Title/Abstract] OR "PCDF"[Title/Abstract] OR "TCDF"[Title/Abstract] OR "PeCDD"[Title/Abstract] OR "OCDD"[Title/Abstract] OR "HxCDD"[Title/Abstract] OR "HxCDF"[Title/Abstract] OR "HpCDD"[Title/Abstract] OR "HpCDF"[Title/Abstract] OR "TEQ"[Title/Abstract] OR "toxic equivalen*"[Title/Abstract] OR "coplanar"[Title/Abstract]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]) AND ((((("epidemiolog*"[Title/Abstract] OR "cohort stud*"[Title/Abstract] OR "case control stud*"[Title/Abstract] OR "adverse effect*"[Title/Abstract] OR "observational stud*"[Title/Abstract] OR "case serie*"[Title/Abstract] OR "case report*"[Title/Abstract] OR "cross sectional stud*"[Title/Abstract]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language])) OR (("cohort studies"[MeSH Terms] OR "case control studies"[MeSH Terms] OR "case control studies"[MeSH Terms] OR (("ieee int conf automation sci eng case"[Journal] OR "case phila"[Journal] OR "case"[All Fields]) AND "research report"[MeSH Terms]) OR "cross sectional studies"[MeSH Terms] OR "cross sectional studies"[MeSH Terms]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language])) AND ((((("men"[Title/Abstract] OR "man"[Title/Abstract] OR "child*"[Title/Abstract] OR "woman"[Title/Abstract] OR "women"[Title/Abstract] OR "male"[Title/Abstract] OR "female"[Title/Abstract] OR "adolescent"[Title/Abstract]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language])) OR

(("men"[MeSH Terms] OR "child"[MeSH Terms] OR "women"[MeSH Terms] OR "male"[MeSH Terms] OR "female"[MeSH Terms] OR "adolescent"[MeSH Terms]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language])) AND ((("Urine"[Title/Abstract] OR "serum"[Title/Abstract] OR "plasma"[Title/Abstract] OR "haema*"[Title/Abstract] OR "hema*"[Title/Abstract] OR "blood"[Title/Abstract] OR "immun*"[Title/Abstract] OR "antibod*"[Title/Abstract] OR "lymphocyte"[Title/Abstract] OR "t cells"[Title/Abstract] OR "b cells"[Title/Abstract] OR "dendric cells"[Title/Abstract] OR "IgM"[Title/Abstract] OR "Antigen"[Title/Abstract] OR "Mitogen"[Title/Abstract] OR "natural killer cells"[Title/Abstract] OR "IgG"[Title/Abstract] OR "IgA"[Title/Abstract] OR "IgE"[Title/Abstract] OR "polymorphonuclear leucocytes"[Title/Abstract]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language])) OR (("Urine"[MeSH Terms] OR "serum"[MeSH Terms] OR "plasma"[MeSH Terms] OR "blood"[MeSH Terms] OR "immunoglobulins"[MeSH Terms] OR "antibodies"[MeSH Terms] OR ("lymphocytes"[MeSH Terms] OR "lymphocyte count"[MeSH Terms]) OR "antigens"[MeSH Terms] OR "mitogens"[MeSH Terms] OR "killer cells, natural"[MeSH Terms]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND ((humans[Filter]) AND (2017/2/1:2022/10/20[pdat]) AND (english[Filter])): 239

Search 3: carcinogenicity

Scopus – in vivo

(TITLE-ABS-KEY (*chlorodibenzodioxin OR *chlorodibenzofuran OR *chlorobiphenyl OR dioxin* OR dioxin AND like OR pcb OR tcdd OR pcdd OR pcdf OR tcdf OR pecdd OR ocdd OR hxcdd OR hxcdf OR hpcdd OR hpcdf OR teq OR "toxic* equivalen*" OR coplanar) AND TITLE-ABS-KEY (rat OR mice OR mouse OR monkey OR pig OR rabbit OR hamster OR dog OR cat OR mink OR hare OR chinchilla OR vivo OR primate OR swine) AND TITLE-ABS-KEY (cancer OR

carcin* OR tumor OR tumour OR neoplasm* OR mutagen* OR genotox*)) AND PUBYEAR > 2016 AND PUBYEAR > 2016: 187

Scopus – epidemiology

(TITLE-ABS-KEY (*chlorodibenzodioxin OR *chlorodibenzofuran OR *chlorobiphenyl OR dioxin* OR dioxin AND like OR pcb OR tcdd OR pcdd OR pcdf OR tcdf OR pecdd OR ocdd OR hxcdd OR hxcdf OR hpcdd OR hpcdf OR teq OR "toxic* equivalen*" OR coplanar) AND TITLE-ABS-KEY (epidemiolog* OR "cohort stud*" OR "case control stud*" OR "adverse effect*" OR "observational stud*" OR "case serie*" OR "case report*" OR "cross sectional stud*") AND TITLE-ABS-KEY (human OR men OR man OR child* OR wom*n OR male OR female OR adolescent) AND TITLE-ABS-KEY (cancer OR carcin* OR tumor OR tumour OR neoplasm* OR mutagen* OR genotox*)) AND PUBYEAR > 2016 AND (EXCLUDE (LANGUAGE , "Italian") OR EXCLUDE (LANGUAGE , "Russian")): 78

PubMed – in vivo

(((2,3,7,8-Tetrachlorodibenzo-p-dioxin[MeSH Terms] OR tetrachlorodibenzodioxin[MeSH Terms] OR polychlorinated biphenyls[MeSH Terms] OR biphenyls, polychlorinated[MeSH Terms] OR tetrachlorodibenzodioxin[MeSH Terms] OR pcbs[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (chlorodibenzodioxin[Title/Abstract] OR chlorodibenzofuran[Title/Abstract] OR chlorobiphenyl[Title/Abstract] OR dioxin*[Title/Abstract] OR dioxin like[Title/Abstract] OR pcb[Title/Abstract] OR TCDD[Title/Abstract] OR PCDD[Title/Abstract] OR PCDF[Title/Abstract] OR TCDF[Title/Abstract] OR PeCDD[Title/Abstract] OR OCDD[Title/Abstract] OR HxCDD[Title/Abstract] OR HxCDF[Title/Abstract] OR HpCDD[Title/Abstract] OR HpCDF[Title/Abstract] OR TEQ[Title/Abstract] OR "toxic* equivalen*"[Title/Abstract] OR coplanar[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((Rats[MeSH Terms]

OR mice[MeSH Terms] OR guinea pigs[MeSH Terms] OR rabbits[MeSH Terms] OR dogs[MeSH Terms] OR cats[MeSH Terms] OR mink[MeSH Terms] OR hares[MeSH Terms] OR chinchilla[MeSH Terms] OR primates[MeSH Terms] OR swine[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (rat[Title/Abstract] OR mice[Title/Abstract] OR mouse[Title/Abstract] OR monkey[Title/Abstract] OR pig[Title/Abstract] OR rabbit[Title/Abstract] OR hamster[Title/Abstract] OR dog[Title/Abstract] OR cat[Title/Abstract] OR mink[Title/Abstract] OR hare[Title/Abstract] OR chinchilla[Title/Abstract] OR vivo[Title/Abstract] OR primate[Title/Abstract] OR swine[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter])))) AND ((Cancer[Title/Abstract] OR Carcin*[Title/Abstract] OR Tumor[Title/Abstract] OR tumour[Title/Abstract] OR Neoplas*[Title/Abstract] OR Mutagen*[Title/Abstract] OR Genotox*[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR ("neoplasms"[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))): 859

PubMed – epidemiology

((((2,3,7,8-Tetrachlorodibenzo-p-dioxin[MeSH Terms] OR tetrachlorodibenzodioxin[MeSH Terms] OR polychlorinated biphenyls[MeSH Terms] OR biphenyls, polychlorinated[MeSH Terms] OR tetrachlorodibenzodioxin[MeSH Terms] OR pcbs[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (chlorodibenzodioxin[Title/Abstract] OR chlorodibenzofuran[Title/Abstract] OR chlorobiphenyl[Title/Abstract] OR dioxin*[Title/Abstract] OR dioxin like[Title/Abstract] OR pcb[Title/Abstract] OR TCDD[Title/Abstract] OR PCDD[Title/Abstract] OR PCDF[Title/Abstract] OR TCDF[Title/Abstract] OR PeCDD[Title/Abstract] OR OCDD[Title/Abstract] OR HxCDD[Title/Abstract] OR HxCDF[Title/Abstract] OR HpCDD[Title/Abstract] OR HpCDF[Title/Abstract] OR TEQ[Title/Abstract] OR "toxic* equivalen*"[Title/Abstract] OR coplanar[Title/Abstract] AND

((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((epidemiolog*[Title/Abstract] OR "cohort stud*"[Title/Abstract] OR "case control stud*"[Title/Abstract] OR "adverse effect*"[Title/Abstract] OR "observational stud*"[Title/Abstract] OR "case serie*"[Title/Abstract] OR "case report*"[Title/Abstract] OR "cross sectional stud*"[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (Cohort studies[MeSH Terms] OR case control study[MeSH Terms] OR case control studies[MeSH Terms] OR adverse effects[MeSH Terms] OR case reports[MeSH Terms] OR cross sectional study[MeSH Terms] OR cross sectional studies[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter])))) AND ((men[Title/Abstract] OR man[Title/Abstract] OR child*[Title/Abstract] OR woman[Title/Abstract] OR women[Title/Abstract] OR male[Title/Abstract] OR female[Title/Abstract] OR adolescent[Title/Abstract] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) OR (men[MeSH Terms] OR child[MeSH Terms] OR women[MeSH Terms] OR male[MeSH Terms] OR female[MeSH Terms] OR adolescent[MeSH Terms] AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter]))) AND ((2017/2/1:2022/10/20[pdat]) AND (english[Filter])))) AND (Cancer[Title/Abstract] OR Carcin*[Title/Abstract] OR Tumor[Title/Abstract] OR tumour[Title/Abstract] OR Neoplas*[Title/Abstract] OR Mutagen*[Title/Abstract] OR Genotox*[Title/Abstract] AND ((humans[Filter]) AND (2017/2/1:2022/10/20[pdat]) AND (english[Filter]))): 115

Search 4: other toxicological endpoints

Scopus – in vivo

((TITLE-ABS-KEY (*chlorodibenzodioxin OR *chlorodibenzofuran OR *chlorobiphenyl OR dioxin* OR dioxin AND like OR pcb OR tcdd OR pcdd OR pcdf OR tcdf OR pecdd OR ocdd OR hxcdd OR hxcdf OR hpcdd OR hpcdf OR teq OR "toxic* equivalen*" OR coplanar) AND TITLE-ABS-KEY (rat OR mice OR mouse OR monkey OR pig OR rabbit OR hamster OR dog OR cat OR mink OR hare OR chinchilla OR vivo OR primate OR swine) AND TITLE-

ABS-KEY (toxic* OR "combined effect*" OR "dose depend*" OR repro* OR disrupt* OR "oxidative stress" OR antioxidant OR susceptibil* OR neuro* OR dimorphi*)) AND PUBYEAR > 2016) AND NOT ((TITLE-ABS-KEY (sperm OR semen OR cryptorchidism OR testosterone OR testis OR epididym* OR prostate OR immun* OR antibod* OR lymphocyte OR "t cell" OR "b cell" OR "dendric cell" OR igm OR antigen OR mitogen OR "natural killer cell" OR igg OR iga OR ige OR "polymorphonuclear leucocyte" OR cancer OR carcin* OR tumor OR tumour OR neoplasm* OR mutagen* OR genotox*)) AND PUBYEAR > 2016) AND (LIMIT-TO (LANGUAGE , "english")): 260

PubMed – in vivo

(((((("chlorodibenzodioxin"[Title/Abstract] OR "chlorodibenzofuran"[Title/Abstract] OR "chlorobiphenyl"[Title/Abstract] OR "dioxin*"[Title/Abstract] OR "dioxin like"[Title/Abstract] OR "pcb"[Title/Abstract] OR "TCDD"[Title/Abstract] OR "PCDD"[Title/Abstract] OR "PCDF"[Title/Abstract] OR "TCDF"[Title/Abstract] OR "PeCDD"[Title/Abstract] OR "OCDD"[Title/Abstract] OR "HxCDD"[Title/Abstract] OR "HxCDF"[Title/Abstract] OR "HpCDD"[Title/Abstract] OR "HpCDF"[Title/Abstract] OR "TEQ"[Title/Abstract] OR "toxic equivalen*"[Title/Abstract] OR "coplanar"[Title/Abstract]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language])) OR (("polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms] OR "polychlorinated dibenzodioxins"[MeSH Terms] OR "polychlorinated biphenyls"[MeSH Terms]) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]) AND ((((("rats"[MeSH Terms] OR "mice"[MeSH Terms] OR "guinea pigs"[MeSH Terms] OR "rabbits"[MeSH Terms] OR "dogs"[MeSH Terms] OR "cats"[MeSH Terms] OR "mink"[MeSH Terms] OR "hares"[MeSH Terms] OR "chinchilla"[MeSH Terms] OR "primates"[MeSH Terms] OR "swine"[MeSH Terms]) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language])) OR (("rat"[Title/Abstract] OR "mice"[Title/Abstract] OR

"monkey"[Title/Abstract] OR "pig"[Title/Abstract] OR "rabbit"[Title/Abstract] OR "hamster"[Title/Abstract] OR "dog"[Title/Abstract] OR "cat"[Title/Abstract] OR "mink"[Title/Abstract] OR "hare"[Title/Abstract] OR "chinchilla"[Title/Abstract] OR "vivo"[Title/Abstract] OR "primate"[Title/Abstract] OR "swine"[Title/Abstract]) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND (2017/02/01:2022/10/20[Date - Publication] AND "english"[Language]) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language])) AND ((((("toxic*"[Title/Abstract] OR "combined effect*"[Title/Abstract] OR "dose depend*"[Title/Abstract] OR "repro*"[Title/Abstract] OR "disrupt*"[Title/Abstract] OR "oxidative stress"[Title/Abstract] OR "antioxidant*"[Title/Abstract] OR "susceptibil*"[Title/Abstract] OR "neuro*"[Title/Abstract] OR "dimorphi*"[Title/Abstract]) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language])) OR (("oxidative stress"[MeSH Terms] OR "antioxidants"[MeSH Terms]) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language])) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language])) NOT (("Sperm"[Title/Abstract] OR "semen"[Title/Abstract] OR "cryptorchidism"[Title/Abstract] OR "testosterone"[Title/Abstract] OR "testis"[Title/Abstract] OR "epididym*"[Title/Abstract] OR "prostate"[Title/Abstract] OR "immun*"[Title/Abstract] OR "antibod*"[Title/Abstract] OR "lymphocyte"[Title/Abstract] OR "t cell"[Title/Abstract] OR "b cell"[Title/Abstract] OR "dendric cell"[Title/Abstract] OR "igm"[Title/Abstract] OR "antigen"[Title/Abstract] OR "mitogen"[Title/Abstract] OR "natural killer cell"[Title/Abstract] OR "igg"[Title/Abstract] OR "iga"[Title/Abstract] OR "ige"[Title/Abstract] OR "polymorphonuclear leucocyte"[Title/Abstract] OR "cancer"[Title/Abstract] OR "carcin*"[Title/Abstract] OR "tumor"[Title/Abstract] OR "tumour"[Title/Abstract] OR "neoplasm*"[Title/Abstract] OR "mutagen*"[Title/Abstract] OR "genotox*"[Title/Abstract]) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language]))) AND (2017/01/01:2022/10/20[Date - Publication] AND "english"[Language]): 1018

Inclusion and exclusion criteria

Table 6 Inclusion and exclusion criteria used during primary screening of titles of in vivo studies

	Inclusion criteria	**Exclusion criteria**
Experimental animals	Rats, mice, monkeys, guinea pigs, rabbit, hamster, dog Immunised animals Pathogen infected animals	Other species, transgenic animals In vitro studies Human (epidemiology) studies
Study population	Any experimental animal study, all ages, male and females	None
Route of administration	Oral (feeding, gavage and drinking water studies) Inhalation studies Dermal studies Subcutaneous injection (s.c.) Intraperitoneal injection (i.p.) Intramuscular injection (i.m.)	None
Study duration	Any	None
Chemicals	Dioxins Dioxin-like PCBs	Non-dioxin-like (indicator) PCBs Mixtures with compounds other than the target PCDD/Fs and dioxin-like PCBs (e.g. organochlorinated compounds, brominated flame retardants, etc).
Endpoint	Male reproductive toxicity and immunotoxicity Other toxicity endpoints	Female reproductive toxicity Other toxicity endpoints in epidemiology studies Enzyme induction (e.g. CYP modulation), gene expression or-omics profiles only Co-administration of pro-carcinogens (CON A, DMBA, NKK) Protective effects of certain substances against PCDD/Fs and/or dioxin-like PCB toxicity Exposure data only

Table 7 Data-specific inclusion/exclusion criteria used during primary screening of titles of epidemiology studies

	Inclusion criteria	**Exclusion criteria**
Study design	Cross-sectional studies Cohort studies Case-control studies (retrospective and nested) Case series/Case reports	Animal studies *In vitro* studies
Study population	All populations groups, all ages, males and females Study location: all countries	None
Route of administration	Dietary, dermal, inhalation, transplacental exposure	None
Chemicals	tbc	tbc
Endpoint	Male reproductive toxicity and immunotoxicity	Female reproductive toxicity Gene expression only

		Drug metabolising enzyme Activity/levels only Exposure data only

Table 8 Generic Inclusion and exclusion criteria used during primary screening of titles

Inclusion criteria	**Exclusion criteria**
Articles in English language	Articles in other languages
Articles published from Aug 2015 to present • Scopus from 2017 to 20/10/20 • PubMed from 01/02/2017 to 20/10/2022	Articles published prior to Aug 2015
Meta-analyses, scientific articles, reports and letters that report re-working of data	Systematic reviews of papers prior to 2015 Expert opinions, commentaries, editorials and letters to the editor PhD Theses Extended abstracts, conference proceedings

Annex B

Table 9 In vivo studies for semen parameters

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
TCDD	CD1 mice / male / n=6	0.375, 0.75, 1.5 / gavage / 10 days	↓ bw ↓ sperm count ↑ sperm malformations ↓ sperm motility	-	0.375	Elsayed et al. (2019)
TCDD	Sprague-Dawley rats / female / n=5	0, 1 / gavage / GD15	↑ body weight (F1 offspring)	-	1	Erthal et al. (2018)
PCB-118	ICR mice / female / n=10	0, 20, 100 / gavage / GD7.5 to 12.5	↑ sperm deformities (F1 offspring)	20	100	He et al. (2020)
TCDD	Wistar rats / male / n=6	0, 700,000 / gavage / 42 days	↓ live sperm ↑ dead, apoptotic and necrotic sperm	-	700,000	Meles et al. (2022)
PCB-118	ICR mice / female / n= 7-9	0, 20, 100 / gavage / GD8.5 to 13.5	↓ number of spermatogonia (F1 adults) ↓ sperm motility (F1 adults)	-	20	Tao et al. (2021)
TCDF	Albino mice (strain not specified) / male / n=12	0, 0.5 / gavage / 8 weeks	↑ DNA damage in sperm	-	0.5	Yahia et al. (2018)

Table 10 In vivo studies for effects on testes

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
TCDD	CD1 mice / male / n=6	0.375, 0.75, 1.5 / gavage / 10 days	↑ epithelium of the EST	-	0.375	Elsayed et al. (2019)
TCDD	Sprague-Dawley rats / female / n=5	0, 1 / gavage / GD15	↓ Sertoli cell count (F1 neonatal and F1 adult) ↑ abnormal seminiferous tubules (F1 adult)	-	1	Erthal et al. (2018)
PCB-118	ICR mice / female / n=10	0, 20, 100 / gavage / GD7.5 to 12.5	↑ testicular coefficient (F1 offspring) ↓ Dnmt, Dnmt3a and Uhrf expression	-	20	He et al. (2020)
TCDD	C57BL/6 mice / adult female & F1 males / n=6 F1 males (no. of adult females unknown)	0, 10 / gavage / E15	↑ active caspase3+ cells and TUNEL+ cells (F1 offspring) ↓ Klotho expression	-	10	Jin et al. (2018)
TCDD or TCDF	Sprague-Dawley rats / female / n=5	0, 0.03, 3, 1 / gavage / GD8 (loading dose) 0, 0.0003, 0.003, 0.022 / gavage / GD9-20	No effects seen in F1 fetuses	1	-	Johnson et al. (2020) Study design 1 (low dose)
TCDD	Sprague-Dawley rats / female / n=5	0, 3, 6, 10 / gavage / GD8 (loading dose) 0, 0.066, 0.132, 0.22 / gavage / GD9-20	No effects seen in F1 fetuses	10	-	Johnson et al. (2020) Study design 1 (high dose)
TCDD or TCDF	Sprague-Dawley rats / female / n=5	0, 10 / gavage / GD15	No effects seen in F1 fetuses	10	-	Johnson et al. (2020) Study design 2
PCB-118	ICR mice / female / n= 7-9	0, 20, 100 / gavage / GD8.5 to 13.5	↓ epithelium thickness (F1 offspring)	-	20	Tao et al. (2021)

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
			↑ gap in seminiferous tubules (F1 offspring E18.5) ↑ gap in seminiferous tubules (F1 offspring E18.5) ↓ mRNA expression of Dnmt1, Pcna and Stra8 (F1 offspring E18.5) ↓ Stra8 expression (F1 offspring 7 weeks) ↓ protein expression of DNMT1 and STRA8 (F1 offspring 7 weeks)			
TCDF	Albino mice (strain not specified) / male / n=12	0, 0.5 / gavage / 8 weeks	Degenerative changes of the seminiferous tubules with formation of multinucleated giant cells Exhausted and degenerated germ cells Exhaustion of germinal epithelium Detachment of the germ cells from the basal lamina	-	0.5	Yahia et al. (2018)
PCB-118	ICR mice / female / n=33-38	0, 20, 100 / gavage / GD7.5 to 12.5	↓ testicular organ coefficient (F1 offspring) ↑ cell detachment, vacuolisation and enlarged gaps in the seminiferous tubules (F1 offspring) ↓ diameter of seminiferous tubules (F1 offspring) ↓ testicular 5-meC levels (F1 offspring)	-	20	Zhang et al. (2020)

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
			↓ Dnmt 1, Dmnt3a, Dmnt3b, Uhrf1 gene expression (F1 offspring) ↓ DNMT3 protein expression (F1 offspring)			

Table 11 In vivo studies for effects on hormone levels

Substance	**Species / sex / number**	**Dose µg/kg bw/day / route of administration / duration**	**Observed effects at LOAEL**	**NOAEL* (µg/kg bw/day)**	**LOAEL* (µg/kg bw/day)**	**Reference**
TCDD	Sprague-Dawley rats / female / n=5	0, 1 / gavage / GD15	No effects seen	1	-	Erthal et al. (2018)
TCDD or TCDF	Sprague Dawley rats / female / n=5	0, 3, 6, 10 / gavage / GD8 (loading dose) 0, 0.066, 0.132, 0.22 / gavage / GD9-20	TCDD ↓ Fshb pituitary gene expression (F1 fetus) ↓ Inha testis gene expression (F1 fetus) TCDF No effects seen	TCDD 3 TCDF 10	TCDD 6 TCDF -	Johnson et al. (2020) Study design 1 (high dose)
TCDD or TCDF	Sprague-Dawley rats / female / n=5	0, 10 / gavage / GD15	TCDD ↓ Fshb pituitary gene expression (F1 fetus) ↓ Inha testis gene expression (F1 fetus) TCDF No effects seen	TCDD - TCDF 10	TCDD 10 TCDF -	Johnson et al. (2020) Study design 2

Table 12 In vivo studies for effects on immunotoxicity

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
TCDD	C57BL/6 mice / female / n=6 (males)(number of females unknown)	0, 10 / gavage / E15	↑ IL-1β, IL-18, and IL-12 expression (F1 male offspring) ↑ pro-inflammatory cytokines (F1 male offspring) ↑ apoptosis (F1 male offspring) ↑ TUNEL-positive cells (F1 male offspring)	-	10	Jin et al. (2018)
TCDD	BALB/c mice / female / n=10	0, 0.0005, 0.0055, 0.05, 0.1, 0.5 / gavage / 10 weeks	↑ antibody titres of IgG	0.05	0.1	Kakutani et al. (2022)
TCDD	C57BL/6 mice / female / n=not stated	0, 0.1, 10 / gavage / ED0.5, ED12.5 & PND7	↓ spleen weight & index (F1 offspring) ↓ RORγt expression in ILC3s (dams & F1 offspring) ↑ NKp46+ ILC3s (dams & F1 offspring) ↑ IL-17a+ ILC3s (dams)	-	0.1	Li et al. (2019)
TCDD	C57B1/6J mice / male & female / n=6	0, 10 / gavage / single exposure	No effects seen	10	-	Lowery et al. (2021)

Table 13 In vivo studies for other endpoints – bone effects

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
PCB-169	Wistar rats / female / n=4	0, 2000 / i.p / PND0 (loading dose) 0, 500 / i.p / PND6 & PND14	Shorter and thinner femurs, reduced endosteal and periosteal perimeters, smaller total cross-sectional and medullary areas ↓ levels of bone markers in serum ↓ calcium levels in the bone	-	2000	Brankovič et al. (2017)
PCB-169	Wistar rats / female / n=4	0, 2000 / i.p / PND0 (loading dose) 0, 500 / i.p / PND6 & PND14	↓ somatic mass and femur size Harder and more brittle bones containing higher amounts of minerals	-	2000	Brankovič et al. (2019)
PCB-169	Wistar rats / female / n=4	0, 2000 / i.p / PND0 (loading dose) 0, 500 / i.p / PND6 & PND14	↓ longitudinal bone growth Morphometric alterations in EGP structure	-	2000	Brankovič et al. (2020)
TCDD	C57BL/6 mice / male & female / n=5-8	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 / gavage / every 4 days for 28 days	↓ trabecular spacing in femurs of male mice	0.3	1	Fader et al. (2018)
PCB-126	Sprague-Dawley rats / male / n=12	0, 1630 / i.p / single exposure	↓ long bone length, diameter and surface area ↑ trabecular thickness and volume ↓ serum osteocalcin ↑ hypocalcemia	-	1630	Ronis et al. (2020)
PCB-126	Sprague Dawley rats (WT & AhR -/) / male & female / n=25-28	0, 1630 / i.p / single exposure	↓ bone length (males) ↓ serum calcium ↓ tibial length ↓ cortical area ↓ medullary area ↓ bone formation (females) ↑ Ihh & Ctgf/Ccn2	-	1630	Williams et al. (2020)

Table 14 In vivo studies for other endpoints – cardiovascular effects

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
TCDD	ApoE KO mice / male & female / n=10	0, 0.14 (1 µg/kg bw/week) / i.p / weekly for 8 weeks	↑ atherogenic lesions ↑ VCAM1 expression	-	0.14	Bey et al. (2022)
TCDD	Sprague-Dawley rats / female / n=8	0, 0.29 (2 µg/kg bw/week) / gavage / weekly for 21 days	Histopathological damage of heart tissue ↓ GSH, CAT, GSP-Px & SOD levels	-	0.29	Ciftci et al. (2018)
TCDD	C57BL/6J mice / female / n=3-5	0, 2.89, 11.43 (0, 20, 80 µg/kg bw/week) / gavage / single exposure PND1	Morphological changes (macroscopic and histopathological) changes of the heart	-	2.89	Fujisawa et al. (2019)
TCDD	Wistar rats / male / n=4	0, 20 / gavage / 60 days	↓ cTnI levels ↑ MDA levels ↓ SOD, CAT, GSH Histopathological changes of the heart	-	20	Nanakali (2020)
PCB-126	Ldlr–/– mice / male / n=30	0, 326.4 / gavage / weekly at weeks 2 & 4 Low fat diet (LFD)	↑ plasma inflammatory cytokine levels ↑ number of circulating platelets ↑ red blood cell counts ↑ accumulation of hepatic fatty acids Accelerated atherosclerotic lesion formation in the aortic root ↑ circulating neutrophils, monocytes, and macrophages	-	326.4	Petriello et al. (2018)
PCB-126	C57BL/6 mice / male / n=6-12	0, 0.07, 7.14 (0, 0.5, 50 µg/kg bw/week) / gavage / weekly for 8 weeks	↑ accumulation of fatty acids Disturbance of carbohydrates including D-glucose and intermediates in TCA cycle ↑ glycine and threonine	-	0.07	Wang et al. (2021)

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
			Upregulation of collagen synthesis enzymes and extracellular matrix proteins Perturbation of gene expression related to TGFβ/PPARγ/MMP-2 signalling pathway			

Table 15 In vivo studies for other endpoints – cleft palate

Substance	**Species / sex / number**	**Dose µg/kg bw/day / route of administration / duration**	**Observed effects at LOAEL**	**NOAEL* (µg/kg bw/day)**	**LOAEL* (µg/kg bw/day)**	**Reference**
TCDD	C57BL/6 mice / female mice / n=15	0, 64 / gavage / E10	↑ incidence (100%) of cleft palate	-	64	Gao et al. (2020)
TCDD	C57BL/6 N mice / female / n=8	0, 64 / gavage / GD10	↑ MEG3 gene expression Inhibited fetal mesenchymal proliferation Inhibited TGF-β/Smad pathway	-	64	He et al. (2021)
TCDD	ICR mice / female / n=3	0, 10, 15, 20, 25, 30, 35, 40 / gavage / GD12	↑ incidence (10.8%) of cleft palate	-	10	Satake et al. (2022) Dose range-finding
TCDD	ICR mice / female / n=3	0, 25 / gavage / GD12	↑ incidence (92.1%) of cleft palate	-	25	Satake et al. (2022) Main study

Table 16 In vivo studies for other endpoints – liver effects

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
TCDD	Wistar rats / male / n=6	0, 10 / gavage / 90 days	↓ liver weight ↑ AST, ALT & ALP levels ↑ MDA levels ↓ GSH-R, SOD & CAT levels ↑ mRNA expression level of CYP1A1 (not statistically significant)	-	10	Abdulkareem and Nanakali (2020)
TCDD	Sprague-Dawley rats / female / n=6	0, 0.29 (0, 2 µg/kg bw/week) / gavage / weekly for 3 weeks	↑ TBARS & GSH levels ↓ SOD, CAT & GPx activity Haemorrhage under Glisson's capsule, eosinophilic and pyknotic nuclei, mononuclear cell infiltration, vascular congestion, sinusoid dilation and haemorrhage in liver tissue	-	0.29	Basak Turkmen et al. (2022)
TCDD	C57BL/6J mice / male / n=30	0, 0.71 (0, 5 µg/kg bw/week) / gavage / weekly for 6 weeks LFD or high fat diet (HFD)	↑ triglyceride levels (HFD) ↑ hepatic steatosis (HFD) Changes in mRNA levels of key genes of hepatic lipid metabolism (HFD) ↑ liver fibrosis (HFD) ↑ gene expression of inflammation and fibrosis marker genes (LFD)	-	0.71	Duval et al. (2017)
TCDD	Wistar rats / male / n=10	0, 1 / gavage / 30 days	Histopathological changes (thickening of Glisson's capsule, intracytoplasmic vacuolization in hepatocytes, sinusoidal dilation, vascular and sinusoidal	-	1	Erdemli et al. (2018)

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
			congestion and inflammatory cell infiltration) ↑ MDA levels ↑ TOS, ALT, AST & ALP levels ↓ GSH, SOD, CAT & TAS levels			
TCDD	C57BL/6 mice / male / n=9	0, 1.43, 3.60 (0, 10, 25 µg/kg bw/week) / i.p / weekly for 6 weeks	↑ liver fibrosis ↑ hepnecroinflammatory scores ↑ α-SMA expression	-	1.43	Han et al. (2017)
TCDD	ICR mice / male / n=10	0, 4.30 (0, 30 µg/kg bw/week) / i.p. / weekly for 42 days	↑ serum AST, ALT & ALP levels ↑ relative liver weight Histopathological changes (mild interstitial hyperplasia and vascular proliferation in foci, evidence of lymphocytes adhering to the walls of dilated vasculature, and individual hepatocyte degeneration and necrosis)	-	4.30	Li et al. (2022)
PCB-156	C57BL/6 mice / male / n=5	0, 7857 (0, 55,000 µg/kg bw/week) / i.p / on weeks 4, 6, 8 and 10 Control diet (CD) or HFD	↑ intra-abdominal fat mass hepatic lipid levels and dyslipidemia (CD) Aggravated NAFLD (HFD) ↑ expression of genes involved in lipid metabolism pathways (CD & HFD) Activation of cytochrome P450 pathway, PPARs and glutathione metabolism pathway (CD & HFD)	-	7857	Shan et al. (2020)
PCB-156	Sprague-Dawley rats / male & female / n=10	0, 10, 100, 1000, 10,000 / diet / 90 days	↑ liver weight	-	10[1]	Vieira Silva et al. (2022)

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
			↑ EROD, PROD & UDPGT enzyme activities ↓ apolar retinoid concentrations in liver Histopathological changes			

Table 17 In vivo studies for other endpoints – obesity

Substance	Species / sex / number	Dose µg/kg bw/day / route of administration / duration	Observed effects at LOAEL	NOAEL* (µg/kg bw/day)	LOAEL* (µg/kg bw/day)	Reference
TCDD	C57BL/6J mice / male & female / 10/group	0, 0.14 (0, 1 µg/kg bw/week) / gavage / weekly from weeks 10 to 42 HFD	↑ body weight gain ↑ fat mass (HFD) ↓ VAT fat pad weight (males) ↑ VAT fat pad weight (females) ↓ plasma leptin levels (females) ↑ relative liver weight (males) ↑ relative liver weight (females, not statistically significant) ↑ hepatic triglycerides (females)	-	0.14	Brulport et al. (2017)
TCDD	C57BL/6 mice / female / 3-14/group	0, 0.006 (0, 0.2 µg/kg bw/ twice per week) / s.c injection / one week prior to pairing & throughout pregnancy and lactation (weeks 0-7) Control or HFD (dams, from weeks 17-28 during "Metabolic challenge" window)	Transient hypoglycemia (dams PND1) ↑ bw (mid-lactation, transient) ↑ bw (from 5 weeks post exposure "Post-TCDD window") ↑ bw ↑ % fat mass ↓ % lean mass ↓ leptin levels	-	0.006	Hoyeck et al. (2020)

* Derived by Secretariat (unless otherwise stated)

[1] NOAEL / LOAEL derived by author

Annex C

Table 18 Epidemiology studies for effects on semen parameters

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
Amir et al. (2020)	Pakistan, 111, hair, serum, urine, semen	Case controlled	PCB-118	PCB-118 detected in: 69% of hair samples (mean ± SE of 0.554 ± 0.377 pg/mg); 93% of serum samples (0.039 ± 0.0130 ng/ml); 52.1% of urine samples (0.003 ± 0.001 ng/ml). No significant differences in PCB-118 levels in hair, serum or urine between infertile males and fertile controls. Significant negative correlation between PCB-118 in serum and sperm motility (%) (r= -0.212; p=0.041).	Statistical analysis comprised ANOVA, bivariate correlation, linear regression, and Pearson's chi square. No discussion of confounders.	PCBs 28, 52, 101, 138, 153 and 180. op'- DDE, pp'-DDE, op'-DDD, pp'-DDD, op'-DDT and pp'-DDT HCB
Mínguez-Alarcón et al. (2017)	Russian Children's study, 133, semen (at aged 18-19 years), blood (at aged 8 – 9 years)	Longitudinal	TCDD, PCDDs, PCDFs, co-PCBs, mono-ortho–substituted PCBs	Adjusted model findings: significant trend of serum TCDD TEQs and decreased sperm concentration (p-trend = 0.005), sperm count (p-trend = 0.05) and motile sperm count (p-trend = 0.05). significant trend of serum PCDD TEQs and decreased sperm concentration (p-trend = 0.02), total sperm count (p-trend = 0.04), total motile sperm count (p-trend = 0.05).	TEQs calculated (lipid basis according to WHO). Statistical analysis used adjusted and non-adjusted linear mixed models. Confounders identified and accounted for in adjusted model.	non-dioxin-like PCBs (n=31)

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
Paul et al. (2017)	Spain, 50, serum, semen	Case controlled	PCBs -77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189	Serum DL-PCB concentrations higher in the low quality semen group compared with the normal quality semen group (did not reach significance). Significant ($p<0.001$) impairment of sperm concentration, total sperm count, motility, viability and morphology in the low-quality semen group when compared to the normal quality semen group. Sperm concentration and total sperm count were not impaired. Multivariate regression analysis showed negative corelations of serum levels of PCB-126 and sperm viability in the low-quality semen group, semen volume with PCB-118 and sperm motility (%) with PCB-189 in the normal-quality semen group. Analysis of the group data showed statistically significant negative correlations for sperm progress motility (%) with: PCB-126 and PCB-189, sperm viability (%) with PCB-126, PCB-169 and PCB-189; non-ortho PCBs and sperm	TEQs calculated (lipid basis according to WHO). Statistical analysis used Mann-Whitney U-test and multivariate linear regression models (semen parameters as dependent variables and dioxin-like PCBs as independent variables). No discussion of confounders.	None

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
				viability (%); total dioxin-like PCB and sperm viability (%).		
Petersen et al. (2018)	Faroe Islands, 263, serum, semen	Cross-sectional	PCBs - 105, 118, 156	no association between serum ∑PCB concentration and semen parameters measured.	Statistical analysis used ANOVA, chi square, t-test and Spearman correlations. Confounders identified and accounted for in modelling.	PCB 28, PCB 52, PCB 101, PCB 153, PCB 138 and PCB 180. PFOA, PFOS, PFHxS, PFNA, PFDA

Table 19 Epidemiology studies for effects on testes

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
Desalegn et al., (2021)	The Norwegian Human Milk Study, 641 (mother-son pairs), breast milk	Case-cohort	PCB-105 PCB-114 PCB-118 PCB-156 PCB-157 PCB-167 PCB-189	Cryptorchidism was evaluated by mothers at 1, 6, 12, 24 months using a self-administered questionnaire and categorised by the authors. Significant association for PCB-114 (also PCB-74, PCB-194 and β-HCH) had increased odds of congenital cryptorchidism (adjusted model OR=1.36, 95% CI: 1.05-1.77). Significant association for PCB-114, PCB-118, PCB-167 with recurrent cryptorchidism (adjusted model, OR=1.88, 95% CI: 1.00-3.50; 1.60, 1.07-2.36; 1.73, 1.06-2.82 respectively). Significant association for PCB-114 with ever-reported cryptorchidism (1.14, 0.57-2.29). PCB-114 (also PCB-74, and PCB-194) reported as predictors of congenital cryptorchidism.	Statistical analysis used ordinary least squares logistic regression and elastic net logistic regression models. Confounders identified and accounted for in modelling.	5 organochlorine pesticides (OCPs; β-HCH, HCB, p,p′ -DDE, p,p′ -DDT); non-DL PCBs PCB-74, PCB-99, PCB-153, PCB-170, PCB-180, PCB-194, and PCB-138; 6 polybrominated diphenyl ethers ((P)BDEs; BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154); 2 poly- and perfluoroalkyl substances (PFASs; PFOA, PFOS)
Desalegn et al., (2022)	The Norwegian Human Milk Study,	Case controlled	PCB-105 PCB-114	No significant difference in AhR activity in breast milk	Statistical analysis used Pearson's chi-	5 organochlorine pesticides (OCPs; β-HCH,

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
	91 (mother-son pairs), breast milk		PCB-118 PCB-156 PCB-157 PCB-167 PCB-189	samples between cases with cryptorchidism and controls. AhR activity was (borderline) significantly associated with all dioxin-like PCBs, consistent with a possible role for dioxin-like PCBs in the observed AhR activity.	square and the Wilcoxon rank-sum tests and logistic regression modelling. Confounders identified and accounted for in modelling.	HCB, p,p′ -DDE, p,p′ -DDT); non-DL PCBs PCB-74, PCB-99, PCB-153, PCB-170, PCB-180, PCB-194, and PCB-138; 6 polybrominated diphenyl ethers ((P)BDEs; BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154); 2 poly- and perfluoroalkyl substances (PFASs; PFOA, PFOS); AhR activity

Table 20 Epidemiology studies for effects on hormone levels

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
Berghuis et al. (2022)	The Netherlands (Dutch Development at Adolescence and Chemical Exposure (DACE) study), 101, blood (maternal and child aged 13 – 15 years)	Longitudinal cohort	PCB-105 PCB-118 PCB-156	Reproductive hormones measured as oestradiol (E2), LH, FSH, AMH, SHBG, inhibin B, testosterone, free testosterone, albumin. Significant association for prenatal exposure to PCB-105 and levels of testosterone (β=0.5, p=0.03), PCB-118 and levels of inhibin B (β =0.45, p=0.03) and PCB-156 and levels of testosterone (β=0.61, p=0.00) and free testosterone (β =0.53, p=0.02).	Statistical analysis used t-tests, Spearman's rank correlation test, Pearson's correlation test, multivariable regression modelling. Confounders identified and accounted for in modelling.	PCB-153, PCB-138, PCB-146, PCB-170, PCB-180, PCB-183, PCB-187; 3-OH-PCB-153, 3 -OH-PCB-138, 4-OH-PCB-172 4-OH-PCB-107, 4-OH-PCB-146, 4-OH-PCB-187; DDE, PCP, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, HBCDD Reproductive hormones; pubertal development
Dong et al. (2020)	China, 42 (mother-child pairs), breast milk, blood (child at 4 years of age.	Cross-sectional	2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TeCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF,	Significant negative association for serum TCDD, PeCDD, HxCDF and testosterone levels (β= -0.712 (-1.465, -0.308); β= -0.813 (-1.658, -0.096); β= -0.636 (-1.059, -0.302) respectively). Significant negative association for testosterone levels and total PCDDs and total TEQ of PCDDs/DFs (β= -0.842, 95% CI: -1.629, -0.218; β= -1.425, 95% CI: -2.656, -0.632 respectively).	Statistical analysis used multivariate linear regression modelling, principal component analysis. Confounders identified and accounted for in modelling.	Testosterone, DHEA, A-dione, progesterone.

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
			1,2,3,6,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF			
Shi et al., 2020	China, 74, blood	Cross-sectional	2378-TeCDD, 12378-PeCDD, 123478-HxCDD, 123678-HxCDD, 123789-HxCDD, 1234678-HpCDD, OCDD, 2378-TeCDF, 12378-PeCDF, 23478-PeCDF, 123478-HxCDF, 123678-HxCDF, 234678-HxCDF, 123789-HxCDF, 1234678-HpCDF, 1234789-HpCDF, OCDF	Significantly higher DHEA levels in low PCDFs-TEQ group (1933 vs. 1447 pg/ml; $p<0.05$) and in the low PCDD/PCDFs-TEQ group (1996 vs. 1360 pg/ml; $p<0.01$) compared to reference group. Significantly higher A-dione levels in men with high serum PCDFs-TEQ (2404 vs. 1848; $p<0.05$).	Statistical analysis used general linear models. Confounders identified and accounted for in modelling.	DHEA, testosterone, DHT. A-dione
Eskenazi et al., 2017	US (CHAMACOS study), blood (maternal and child at 9 and 12 years of age)	Prospective cohort	PCB-105, PCB-118, PCB-156, PCB-157, PCB-167, PCB-189	At 12 years, 10- fold increase in total prenatal ΣPCBs significantly associated with increased FSH (95% CI: 8.6, 149.0; $p<0.05$) – considered to be driven by non-dioxin-like congeners. At 9 years associations between ΣPCBs and	Statistical analysis used linear regression modelling. Confounders identified and accounted for in modelling.	p,p'-DDT, p,p'-DDE, o,p'-DDT; PBDE congeners (BDEs 17, 28, 47, 66, 85, 99, 100, 153, 154, and 183); PCB congeners 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 110, 128, 138, 146, 149, 151, 153, 170, 172, 177, 178, 180, 183,

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
				testosterone (108.9% increase; 95% CI: 7.1, 307.2) – possibly mediated by child BMI.		187, 194, 195, 196,199, 206, 209 LH, FSH, Testosterone Tanner scale assessments.
Miyashita, et al., 2018a	Japan (Japanese Sapporo Cohort of the Hokkaido Study), 183 (mother-child pairs), blood (maternal) and cord blood	Prospective study	PCDDs: TCDD, PeCDD, HxCDD, HpCDD, OCDD; PCDF: TCDF, PeCDF, HxCDF, HpCDF, OCDF; Co-PCBs: 81, 77, 126, 169	Significant negative association of sub-total PCDDs with inhibin B levels (β= -0.34 95% CI:-0.61, -0.07; p<0.05) and significant positive association (0.47 (0.07, 0.86; p<0.05) with DHEA levels. Significant negative association of sub-total PCDFs with inhibin B (-0.35 (-0.64, -0.06); p<0.05). Significant negative association of sub-total non-ortho-PCBs with inhibin B (-0.26 (-0.41, -0.10); p<0.01), T/E2 (-0.22(-0.42, -0.03); p<0.05), cortisol (-0.46 (-0.96, 0.04); p<0.05) and SHBG levels (-0.11 (-0.20, -0.02); p<0.05) and significant positive associated with DHEA levels (0.30 (0.07, 0.54); p<0.05). Significant negative association of sub-total mono-ortho-PCBs with inhibin B (-	Statistical analysis used Spearman's correlation test, Mann–Whitney U test, multivariate linear regression modelling. Confounders identified and accounted for in modelling.	Progesterone, E2, testosterone, androstenedione, DHEA, cortisol, cortisone, SHBG, LH, FSH, prolactin, inhibin B, and insulin-like factor-3 (INSL3)

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
				0.24 (-0.45, -0.02); p<0.05) and significant positive associated (0.29 (0.01, 0.57); p<0.05) with FSH levels. Significant negative association of total dioxin-like compounds with inhibin B (-0.36 (-0.61, -0.11) p<0.01) and SHBG (-0.15 (-0.29, -0.01); p<0.05) and significant positive association (0.46 (0.09, 0.83); p<0.05) with DHEA.		
Oanh et al., 2018	Vietnam, 123 (60 test, 63 control mother-child pairs), breast milk, blood (child at 5 years of age)	Cohort study	2,3,7,8-TeCDD 1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-HpCDD OCDD 2,3,7,8-TeCDF 1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF 1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-	Significantly higher (p<0.05) levels of all congeners (exception of 2,3,7,8-TeCDF) in breast milk of mothers located in the dioxin contaminated area, compared to controls. TEQs of total PCDDs, PCDFs, and PCDDs/Fs significantly higher (p<0.05) in the dioxin contaminated area, compared to controls. Significantly lower cortisone, DHEA and testosterone levels (p=0.012, <0.001 and <0.001 respectively) in children from the dioxin contaminated area, compared to controls. Significantly higher (p=0.006)	Statistical analysis used student's t-test, Wilcoxon's signed rank test, Pearson correlation and Spearman correlation. Confounders identified and accounted for in modelling.	DHEA, DHT, cortisol, cortisone, 17-OH-progesterone (17-OH-P4), P4, A-dione and Testosterone.

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
			HxCDF 2,3,4,6,7,8-HxCDF 1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF OCDF	levels of A-dione in children from the dioxin contaminated area, compared to controls. Significant (p = 0.019 to <0.001) negative correlation between DHEA and all dioxin congeners, with the (exception of TCDF and OCDF). Significant (p=0.002 to <0.001) negative correlation between testosterone levels and all dioxin congeners (exception of TCDF, 1,2,3,7,8,9-HxCDF and OCDF). Significant (p=0.039 to <0.001) positive association between levels of A-dione and all dioxin congeners- (exception of TCDD and 1,2,3,4,6,7,8-HpCDD).		
Oyama et al., 2021	Vietnam, 96 (mother child pairs, 45 test, 51 control), breast milk, blood (child at 7 years of age)	Cohort study	2,3,7,8-TeCDD 1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-	Breast milk analysis as reported by Oanh et al., 2018. Significantly supressed testosterone concentrations (p<0.001) in children from the dioxin contaminated area, compared to controls. In boys only, 17β-HSD and	Statistical analysis used student's t-test, Wilcoxon's signed rank test, Pearson correlation and Spearman correlation. Confounders identified and	DHEA, DHT, cortisol, cortisone, 17-OH-progesterone (17-OH-P4), P4, A-dione and testosterone.

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
			HpCDD OCDD 2,3,7,8-TeCDF 1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF 1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF 2,3,4,6,7,8-HxCDF 1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF OCDF	testosterone levels inversely correlated with the TEQ total PCDD/Fs ($r = -0.47$, $p = 0.001$, and $r = -0.62$, $p < 0.001$, respectively). Positive correlation ($r = 0.49$, $p < 0.001$) between progesterone and TEQ total PCDD/Fs and for DHEA ($r = 0.5$, $p < 0.001$) with the TEQ total PCDD/Fs. DHEA levels in boys were significantly higher ($p<0.01$) from the dioxin contaminated area, compared to controls - a reversal of findings found at aged 5 years. Significant reverse correlation ($p<0.01$ to <0.001) between testosterone and all dioxin congeners.	accounted for in modelling.	
Petersen et al., 2018	Faroe Islands, 263, blood, semen	Cross-sectional	PCB-105, PCB-118, PCB-156	∑PCBs only reported. Significant positive association between ∑PCB and SHBG, LH, testosterone and the testosterone/estradiol ratio (adjusted regression coefficients of 0.05 (0.004–0.09), 0.06 (0.01–0.11), 0.04 (0.004–0.08) and 0.04 (0.01–0.08) respectively, all $p<0.05$).	Statistical analysis used ANOVA, chi square, t-test, Spearman correlations. Confounders identified and accounted for in modelling.	PCB 28, PCB 52, PCB 101, PCB 153, PCB 138, PCB 180; PFOA, PFOS, PFHxS, PFNA, PFDA

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
Van Luong et al., 2018	Vietnam, 42, blood	Cross-sectional	2,3,7,8-TeCDD 1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-HpCDD OCDD 2,3,7,8-TeCDF 1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF 1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF 2,3,4,6,7,8-HxCDF 1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF OCDF PCB-77, PCB-81, PCB-126, PCB-169	Significant correlation between prolactin and 2,3,7,8-TetraCDD (r=0.477; p=0.003), 1,2,3,7,8-PentaCDD (0.378; 0.021), 1,2,3,6,7,8-HexaCDD (0.415; 0.011) and 1,2,3,4,6,7,8-HeptaCDD (0.405; 0.013). Significant correlation between prolactin and 1,2,3,6,7,8-HexaCDF (0.328; 0.047), 1,2,3,4,6,7,8-HeptaCDF (0.331; 0.045), PCB- 77 (0.368; 0.025), sum TEQ of PCDDs (0.468;0.004), PCDDs/Fs (0.458; 0.004), and PCDDs/Fs/PCBs (0.445; 0.006). Significant negative correlation between testosterone and 1,2,3,7,8-PentaCDD (-0.33; 0.046), 1,2,3,7,8,9-HexaCDD (-0.343; 0.038). Significant negative correlation between testosterone and 2,3,7,8-TetraCDF(-0.451; 0.005), 1,2,3,7,8-PentaCDF (-0.335; 0.043), 2,3,4,6,7,8-HexaCDF	Statistical analysis used partial correlation modelling, Pearson's correlation. Confounders identified and accounted for in modelling.	FSH, LH, progesterone, prolactin, E2 and total testosterone.

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
				(-0.386; 0.018), 1,2,3,4,6,7,8-HeptaCDF (-0.465; 0.004), 1,2,3,4,7,8,9-HeptaCDF (-0.36; 0.028), OctaCDF (-0.343; 0.037) Significant negative correlation between testosterone and PCB-81 (-0.408; 0.012), PCB-126 (-0.337; 0.042), TEQs of PCDFs (-0.376; 0.022) and PCBs (-0.339; 0.04). Significant negative association between oestradiol and 1,2,3,4,6,7,8-HeptaCDD (-0.346; 0.036), 1,2,3,6,7,8-HexaCDF (-0.369; 0.025) and 1,2,3,4,6,7,8-HeptaCDF (-0.377; 0.021).		

Table 21 Epidemiology studies for immunotoxicity

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
Arisi et al., 2021	Itay (Brescia), blood	Cross-sectional	PCB-77, PCB-81, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-156, PCB-157, PCB-167, PCB-169, PCB-189.	Inflammatory diseases noted as: seborrheic dermatitis; atopic dermatitis; psoriasis; skin mycoses. No significant increase in the frequency of skin inflammatory disease and mycosis between groups with high, medium and low ∑PCBs serum levels (adjusted OR=1.00, 95% CI: 0.99 – 1.02).	Statistical analysis used the Kolmogorov-Smirnov test and multivariate logistic regression modelling. Confounders identified and accounted for in modelling.	PCB 28, 31, 52, 101, 128, 138, 153, 170, 180, 194, 206, 209. Dermatological evaluation.
Miyashita et al., 2018b	Japan (Hokkaido Study on Environment and Children's Health), 504, blood, cord blood	Cross-sectional	PCDDs: TCDD, PeCDD, HxCDD, HpCDD, OCDD; PCDF: TCDF, PeCDF, HxCDF, HpCDF, OCDF; Co-PCBs: 81, 77, 126, 169	Allergy related outcomes recorded as food allergy, eczema and wheezing and infections as otitis media and respiratory infection. Significant positive association for maternal DLC and wheezing in children aged up to 7 years [odds ratio (OR); 7.81 (95% confidence interval (CI), 1.42 to 42.9) $p<0.05$]. Significant inverse association for maternal DLC and wheezing in boys at 3.5 years (0.03 (0.00 to 0.94) $p<0.05$). Almost significant in boys at 7 years (12.05 (0.99-146.37).	Statistical analysis used the Spearman correlation test, Mann–Whitney U test, Kruskal–Wallis test and multivariate linear regression modelling. Confounders identified and accounted for in modelling.	IgE Allergies and infections survey

Author	Country, number of subjects, sample type(s)	Study type	Dioxin/DL-PCB measured	Findings	Comments	Other compounds / measurements
				Significant inverse association for maternal DLC and cord blood IgE in boys [partial regression coefficient; −0.87 (95% CI), −1.68 to −0.06 p<0.05].		
Margetaki et al., 2022	Crete (Rhea birth cohort), 682 (mother-child pairs at 4 years), 454 (mother-child pairs at 6 years), blood (maternal)	Cross-sectional	PCB-118 PCB-156	Allergy related outcomes recorded as wheeze, asthma, eczema, and rhinitis occurrence. ∑PCBs associated with increased risk for current eczema at 4 years ((RR (95%CI): 2.1 (1.1, 4.2) p-interaction = 0.028)).	Statistical analysis used Poisson models with log link and robust standard errors. Confounders identified and accounted for in modelling.	PCB-138, PCB-153, PCB-170, PCB-180; HCB; DDT; DDE; BDE-47 Allergies survey.