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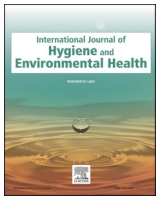

Declining semen quality and polybrominated diphenyl ethers (PBDEs): Review of the literature to support the derivation of a reference dose for a mixture risk assessment

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ABSTRACT

To support a mixture risk assessment for chemicals that interfere with male reproductive health, we reviewed the literature to identify studies of polybrominated diphenyl ethers (PBDEs) and poor semen quality. Several epidemiological studies have shown associations of PBDE exposures with declining semen quality, non-descending testes and penile malformations. In rodent studies, poor semen quality, changes in testosterone levels and reproductive tissues have been observed. *In vitro* studies with reporter gene constructs show PBDE congeners as androgen receptor antagonists, and mixture studies in these systems have demonstrated that PBDE congeners act together with other androgen receptor antagonists. These observations led us to attempt the estimation of reference doses for specific PBDE congeners that can be used in a future mixture risk assessment for deteriorations of semen quality. While epidemiological studies provide support for such associations, they were uninformative for derivations of reference doses, due to the incompatibility of dose metrics used in exposure assessments. We therefore based our estimates on animal studies. Using a rigorous confidence rating approach, we found robust evidence that BDE-47 produced reductions in semen quality. We identified only one high confidence study of BDE-99 and accordingly evaluated the strength of evidence as moderate. One high confidence, and several medium confidence experimental studies observed declines in semen quality after BDE-209 exposure. Using established risk assessment procedures, we estimated that BDE-47 exposures below 0.15 μg/kg/d are unlikely to lead to reductions in semen quality. The corresponding exposures for BDE-99 and BDE-209 are 0.003 μg/kg/d and 1000 μg/kg/d. It is planned to use these estimates as reference doses in a mixture risk assessment of deteriorations in semen quality, involving multiple other chemicals also contributing to poor semen quality.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of organobromine chemicals used as flame-retardants in a wide range of products such as plastics, textiles and electronic equipment. There are 209 congeners which all share a common structural motif of two phenyl rings linked by an oxygen atom. PBDEs have been sold as commercial mixtures, named pentaBDE, octaBDE and decaBDE in reference to their average bromine content.

PBDE congeners differ in their chemical stability but are generally persistent and bioaccumulative. Due to their widespread use in the past, they are ubiquitous environmental contaminants. They accumulate in human and animal tissues. The production and use of hexaBDE, heptaBDE, tetraBDE, pentaBDE and decaBDE has been restricted under the Stockholm Convention on Persistent Organic Pollutants (POPs) (Sharkey et al., 2020).

Human exposure to PBDEs occurs through the diet, through inhalation and ingestion of dust, and by dermal contact. The European Food Safety Authority (EFSA) found the main route of exposure to be food of animal origin with a high lipid content such as meat, fish and dairy products (EFSA 2011).

PBDE congeners and their commercial mixtures have endocrine disrupting properties. *In vitro* assays with reporter gene constructs have revealed androgen receptor (AR) antagonist properties of several congeners (BDEs-19, -28, -38, -39, -47, -49, -79, -99, -100, -127, -153, -155, -181, -190) (Ermler et al., 2010; Harju et al., 2007; Stoker et al., 2005). They also interfere with male reproductive development, as

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Abbreviations

AF	Assessment factor
AR	Androgen receptor
BB	Body burden
BMDL	Benchmark dose level
BPA	Bisphenol A
EFSA	European Food Safety Authority
EHDI	Estimated human daily intake
GD	Gestational day
HBGV	Health-based guidance value
HI	Hazard Index
LOAEL	Lowest observed effect level
NOAEL	No observed effect level
NTP	National Toxicology Program
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PECO	Populations Exposures Comparators Outcomes
PND	Postnatal day
PoD	Point of departure
POP	Persistent organic pollutant
RfD	Reference dose
RoB	Risk of Bias

demonstrated in rodent studies where they produce declines in semen quality, changes in reproductive tissue and hormone levels (Zhang et al., 2020). Several epidemiological studies show associations of PBDE exposures with declining semen quality (Akutsu et al., 2008; Albert et al., 2018; Yu et al., 2019), non-descending testes (Goodyer et al., 2017; Main et al., 2007) and penile malformations (hypospadias) (Poon et al., 2018).

Experimental mixture studies have shown that PBDEs can act in concert with other AR antagonists *in vitro* (Orton et al., 2014). Although supporting *in vivo* studies are missing, it is conceivable that PBDEs will contribute to anti-androgenic mixture effects also *in vivo*. Multiple other chemicals are known to interfere with normal male reproductive development and health. These include phthalates, bisphenol A (BPA), parabens, some azole pesticides, polychlorinated biphenyls and dioxins, as well as analgesics (Kortenkamp 2020). Mixture effects of combinations of some of these anti-androgens have been shown *in vivo*, with effects ranging from retained nipples in male offspring (Axelstad et al., 2014) to declines in semen quality (Axelstad et al., 2018). Furthermore, human exposure to anti-androgens is widespread (Apel et al., 2020; Bauer et al., 2021; EFSA 2018; Koch et al., 2012; Moos et al., 2017). As co-exposures to some or all of these chemicals are a reality (Frederiksen et al., 2020), the impacts of possible mixture effects on male reproductive health warrant systematic examination. PBDE congeners must be included in such an assessment.

The risks from exposures to multiple compounds in chemical risk assessment can be assessed by using the Hazard Index (HI) approach (Teuschler and Hertzberg 1995). The HI is the sum of so-called Hazard Quotients, the ratio of exposure and a reference dose or health-based guidance value (HBGV) for specific toxicities of all chemicals considered together in the assessment. By evaluating this sum against a reference value of 1, the HI expresses fold-exceedances of combined "acceptable" chemical exposures. To achieve consistency in the assessment and to reduce uncertainties, it is important that the reference doses selected for the Hazard Quotient are for similar, ideally identical, toxicity endpoints. A mixing of reference doses related to different toxicities is not advisable as this would introduce bias in the mixture risk assessment.

PBDEs not only interfere with the male reproductive system but also produce a wide range of other toxicities. In their assessment of four PBDE congeners (BDE-47, -99, -153, and -209), EFSA identified neurodevelopmental toxicity as the critical toxicity and derived corresponding points of departure (PoDs) (EFSA 2011). However, these values are not suitable as reference doses to build HIs in a mixture risk assessment for disruption of male reproductive health. To derive reference doses for such an assessment, it is necessary to search for appropriate studies of PBDE effects on the male reproductive system.

In this review we examined the literature with the aim of locating studies of the adverse effects of PBDE exposures on male reproductive development. We were interested in deriving corresponding reference doses for specific PBDE-congeners. To be able to utilise existing PBDE exposure data which is available for individual congeners, it was necessary to search for congener-specific toxicity data (EFSA 2011). We were particularly interested in aligning the mixture risk assessment with currently observed deteriorations in semen quality in Western countries (Levine et al., 2017). We therefore selected semen quality as the basis for deriving the PBDE reference doses (exposures no longer associated with declines in semen quality) and reviewed the literature for relevant experimental studies. We were able to build on the systematic review by (Zhang et al., 2020). We also considered the epidemiological literature but found this to be of limited use for deriving a reference dose, for several reasons. First, the dose metric in epidemiological studies is often PBDE tissue levels, especially hair, which complicates conversion to daily intakes, the metric used in most exposure assessments. Second, epidemiological studies do not normally allow attribution of effects to specific PBDE congeners. We therefore focused on experimental studies with animals and assessed the strength of evidence for links between PBDE exposure and declines in semen quality.

2. Materials and methods

2.1. Literature search

Through a scoping search of the literature we identified a recent systematic review and meta-analysis on the toxicity of PBDEs on the rodent male reproductive system (Zhang et al., 2020). Instead of conducting another full systematic review of the literature on the adverse effects of PBDEs on male reproduction, we opted for using this review as the basis for identifying relevant studies and as a starting point for an update. We complemented the records in Zhang et al. (2020) with additional literature searches for the period after 2020, by conducting citation searches of papers describing PBDE effects on semen quality. Briefly, we generally conducted our study according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement (Shamseer et al., 2015). For inclusion of studies we used the PECO principle (Populations: laboratory mammalian species; Exposures: PBDEs by oral gavage, drinking water or diet; Comparators: animals not exposed to PBDEs; Outcomes: semen quality parameters, Supplementary Table 1). Additional literature searches for studies post 2020 were performed in PubMed and Web of Science using the keywords "PBDE", "Polybrominated diphenyl ether", "semen", "sperm", "semin", "reproduction", using MeSH terms and wildcards as appropriate. We also used search alerts in Web of Science, as well as references cited in the EFSA Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food (EFSA 2011). A flow diagram with details of the selection process is shown in Fig. 1.

The focus of our analysis was on mammalian animal studies of the effects of PBDEs on semen parameters. Studies that analysed sperm parameters such as count, concentration, motility, morphology or vitality, but not sperm DNA damage or aneuploidy were included in our analysis. Studies with non-mammalian test species were excluded. Data

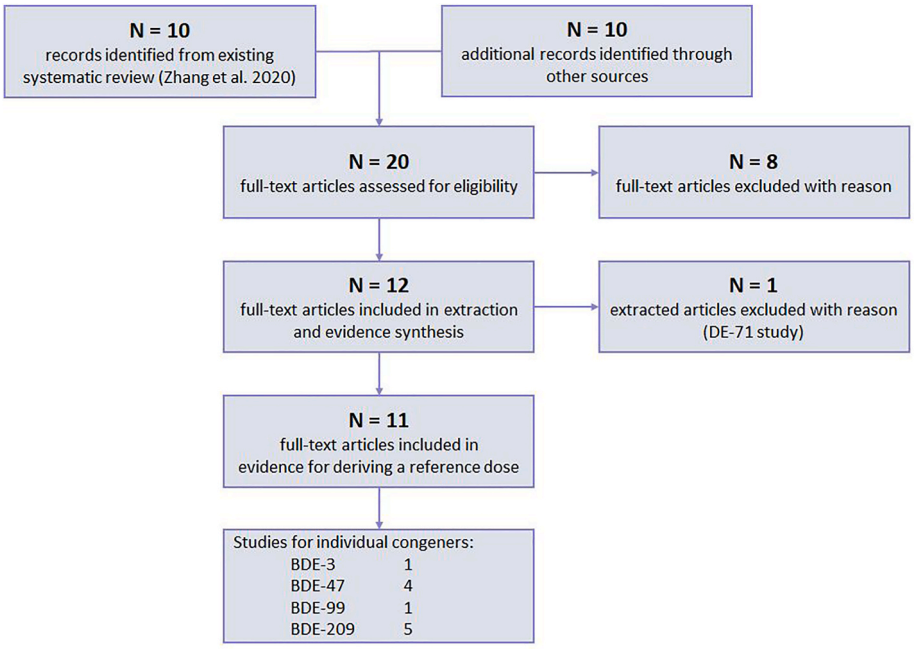

Fig. 1. Literature flow diagram for animal studies of PBDE exposures and semen quality.

from exposures during the sensitive window of exposure for male reproductive toxicity was used, but in the absence of foetal exposure studies, data from postnatal, juvenile, or adult animals were also considered. The eligibility criteria for experimental studies with laboratory animals are listed in Table 1.

2.2. Data extraction

The studies identified in the systematic literature review by Zhang et al. (2020) and additional searches were used to compile self-reported data on the respective PBDE doses related to no observed adverse effect levels (NOAELs) or lowest observed adverse effect levels (LOAELs), or effect doses at predetermined effect magnitudes (benchmark dose levels, BMDL).

The data was extracted into a template based on the one described in the National Toxicology Program (NTP) OHAT 2019 handbook (NTP OHAT 2019) and adapted for animal studies on declining semen quality and exposure to BPA (Kortenkamp et al., 2022; Martin et al., 2021). The data items included elements to summarise the study design, experimental model methodology and results. Some additional minor changes were made regarding the chemical identity and purity and the final data items are listed in the Supplement (Supplementary Table 2).

2.3. Study evaluation

The internal validity of the animal studies was appraised through a risk of bias (RoB) assessment based on a protocol defined for BPA studies by EFSA (EFSA 2017a, 2019) and further developed in the protocol for a systematic review on and declining semen quality (Kortenkamp et al., 2022; Martin et al., 2021). We utilised the NTP OHAT RoB Tool (NTP OHAT 2019). We adapted the assessment further to evaluate the studies we identified for PBDE exposure and male reproductive toxicity endpoints. The key elements of assessment included exposure characterisation (including purity and stability of test compounds, and absence of contaminations), outcome assessment (blinding of the outcome assessors) and power of detecting effects (sufficient number of animals per

Table 1
Eligibility criteria for experimental studies.

		Inclusion criteria	Exclusion criteria
Populations	Laboratory mammalian species including rats, mice, rabbits, guinea pigs, dogs and monkeys	Mammalian species	Non-mammalian test species such as fish or amphibians
Exposures	Polybrominated diphenyl ethers (PBDEs)	Administered by gavage, via drinking water or through the diet	Administered subcutaneously or intraperitoneally
Comparators	Animals not exposed to PBDE	Control group (same species as exposure group (s))	No control group
Outcomes	Semen quality	• Total sperm count • Sperm concentration • Sperm motility • Sperm morphology • Sperm vitality	• Sperm DNA damage • Aneuploidies • Fertility and fertilization outcomes

Criteria for the eligibility of experimental studies on the effects of PBDE exposure of laboratory animals on semen quality.

dose group). Due to the nature of the effects we additionally included a key element for laboratory proficiency (use of a reliable and sensitive animal model and inclusion of a positive control). We considered the characterisation of PBDE exposures as critical, particularly the purity of test compounds and the measurement of contaminants. The presence of dioxins and furans as contaminants is often observed and these compounds can exert similar toxic effects as PBDEs or might even mask effects exhibited by the PBDEs. We considered the use of phytoestrogen-free chow (i.e. soy-free feed) to be relevant for examinations of semen quality. Accordingly, we included this aspect in the RoB assessment, but did not consider it a key element.

A detailed list of all the elements of the RoB assessment can be found in Supplementary Table 3.

Each RoB element was evaluated using the NTP OHAT scores: ++ *definitely low risk of bias*; + *probably low risk of bias*; ~ *probably high risk of bias*; ~~ *definitely high risk of bias*. We used a tiered system to rate the studies, adopted from the system described by EFSA (EFSA 2019). This comprises three tiers, and each study was allocated to one tier as follows: *TIER 1 – high confidence,* where all key elements were scored + or ++ AND no more than one additional question was scored ~ or ~~; *TIER 2 – medium confidence* was assigned to all combinations not covered by *TIER 1* or *3*; the lowest tier, *TIER 3 – low confidence* was used when any one of the key elements was scored ~ or ~~ OR more than 50% of the additional questions were scored ~ or ~~.

2.4. Data synthesis

The findings and characteristics of eligible studies were summarised in a narrative synthesis. The data synthesis included summaries of PBDE exposure ranges (not) associated with declines in semen quality in animal studies as concluded from the derived NOAELs or LOAELs. Only studies we rated as high or medium confidence (*Tier 1* and *Tier 2*) were included in the summary. Studies that were allocated to *Tier 3* were not further analysed in detail.

2.5. Evidence synthesis

We synthesised the evidence from animal studies, using frameworks previously devised for BPA and phthalates (EFSA 2019; Radke et al., 2018). The evidence was categorised as *Robust* if multiple studies with a *Tier 1* confidence rating showed similar adverse effects. Any evidence not explained by study design or difference in animal model was considered of lower confidence, *Tier 2* or *Tier 3*. We rated the evidence as *Moderate* when it was insufficiently strong for *Robust*, but contained at least one *Tier 1* study and additional information supporting the findings. The rating of *Slight* was given in situations where studies suggested a possible decline in semen quality, but with weak or conflicting findings. *Indeterminate* was used for inconsistent, weak or conflicting findings. *Compelling evidence of no effect* was assigned when studies with high confidence ratings consistently demonstrated a lack of biological effects across species, sexes and exposure levels.

2.6. Derivation of a reference dose for individual PBDE congeners for declines in semen quality

In deriving a reference dose for individual PBDE congeners we followed the procedure used by EFSA for other toxicity endpoints (EFSA 2011). Each study where a PoD was discernible, was considered for the derivation of a reference dose. The PoDs under consideration were NOAELs or BMDLs.

Where a NOAEL was reported, but in addition sufficient data was available for BMDL modelling, we employed both, by using the PROAST tool via the EFSA web application (https://efsa.openanalytics.eu/) (EFSA 2017b) and the US-EPA tool BMDS3.x (https://www.epa.gov/bmds) (US EPA 2012).

In cases where no BMDL could be derived, either due to insufficient data or because the models did not deliver a BMDL, we used the reported NOAEL. If only a LOAEL could be estimated from the available data, the NOAEL was extrapolated using a standard assessment factor (AF = 3).

PBDEs are persistent compounds which bioaccumulate in tissues. They can exhibit different kinetic properties in different species which is relevant for extrapolations from rodent studies to humans. To scale the doses across different species we used the body burden approach. The body burden approach has previously been applied to derive health based guidance values for dioxins and dioxin-like polychlorinated biphenyls (PCBs) (EFSA 2015, 2018) and was the basis for margin-of-exposure considerations for PBDEs (EFSA 2011). We employed this approach to estimate rodent body burdens of PBDE congeners associated with PoDs ("critical" body burden). These were then used to derive human intake estimates which would lead to a human body burdens equivalent to the critical body burden in rodents.

We first estimated the body burden at the experimental PoD in the animal study. For studies which used a single oral PBDE dose, the body burden was derived by multiplying the PoD with the fraction of the compound absorbed into the animal body (Equation (1)). The absorbed fraction was derived from the oral absorption of the chemicals. For repeat administration studies, the body burden at the end of treatment was estimated by taking account of the absorption as well as the half-life of the chemical in the animal body. All kinetic parameters were collected from (EFSA 2011).

$$BB_a = F_{abs,a} \cdot PoD \quad (1)$$

with BB_a = body burden in the animal (amount/kg bw); $F_{abs,a}$ = fraction of chemical which is absorbed into the animal body; and PoD = point of departure, such as BMDL or NOAEL.

In a second step, we estimated the equivalent human daily intake (EHDI) by using the assumptions outlined in the EFSA opinion on PBDEs. Accordingly, we used a one compartment model to calculate the EHDI by multiplying the animal body burden derived in step one (Equation (1)) with the rate constant for the elimination from humans, divided by the fraction of compound absorbed into the human body (Equation (2)).

$$EHDI = \frac{BB_a \cdot k_{el,h}}{F_{abs,h}} \quad (2)$$

with $k_{el,h}$ = rate constant for removal from human body (1/day) and $F_{abs,\,h}$ = Fraction of chemical absorbed into the human body. In the one compartment model $k_{el,h}$ can be calculated according to Equation (3).

$$k_{el,h} = \frac{ln2}{t_{½\ ,\ h}} \quad (3)$$

with $t_{1/2,h}$ = halflife of excretion in humans. After substituting $k_{el,h}$ in Equation (2) with Equation (3) the EHDI was calculated according to Equation (4).

$$EHDI = \frac{BB_a \cdot \ln 2}{t_{½\ ,h} \cdot F_{abs,\,h}} \quad (4)$$

An additional assessment factor (AF = 2.5) was then applied to the EHDI to derive the reference dose for the individual PBDE (EFSA 2011). The AF of 2.5 was used to account for inter-species differences (EFSA 2011; WHO 1999). No further AFs were considered to be required because i) the reference dose was derived from developmental toxicity and the body burden applied to the entire human lifespan; and ii) the longest possible half-lives for the congeners were used, resulting in conservative estimates (EFSA 2011).

For PBDE congeners (such as BDE-209) with similar toxico-kinetics in rodents and humans, the body burden approach is not required, and the external PoD derived from the rodent study can be directly used as EHDI. The reference dose can then be calculated directly by application of an additional assessment factor. This is for instance the case for BDE-209 (AF = 100) (EFSA 2011).

3. Results

The selection process for animal studies to be included for the estimation of reference doses for PBDEs relevant for declines in semen quality is shown in Fig. 1.

3.1. Study selection and evaluation

Overall, 12 studies of PBDE congeners and their effect on semen quality *in vivo* were identified. Four of those studies were included in the systematic review by Zhang et al. (2020). Eight additional studies were identified through further searches, by citation searches and search alerts. One of the retrieved studies was conducted with the commercial PBDE mixture DE-71, whilst 11 studies investigated the effects of individual PBDE congeners. One study examined the effects of BDE-3 (Wei et al., 2018), four studies those of BDE-47 (Khalil et al., 2017; Li et al., 2021; Wang et al., 2013; Zhang et al., 2013), one study examined BDE-99 (Kuriyama et al., 2005) and five studies looked at BDE-209 (Miyaso et al., 2012; Sarkar et al., 2016, 2019; Tseng et al., 2006, 2013). All these records were included in the data extraction process.

One eligible study investigated the effects of the commercial PBDE mixture DE-71 on various sperm parameters (Van der Ven et al., 2008). This study identified a BMDL of 9.6 mg/kg/d for DE-71 on sperm morphology. Due to the lack of information on specific PBDE congeners, we could not include this study in our efforts of deriving a reference dose but considered it as supporting evidence for the adverse effects of PBDEs on male reproduction.

To evaluate the internal validity of studies on individual congeners we conducted a RoB analysis (Table 2).

The only eligible study on BDE-3 in mice (Wei et al., 2018) did not provide information about the purity of the test compound and lacked characterisations in terms of contaminations. We therefore rated this study as of low confidence (*Tier 3*).

We identified four studies that investigated semen parameters after exposure to BDE-47 (Khalil et al., 2017; Li et al., 2021; Wang et al., 2013; Zhang et al., 2013). The only mouse study on BDE-47 and semen quality raised concerns as the purity of the compound was not reported. Accordingly, we assigned *Tier 3* (Wang et al., 2013). The remaining three studies were conducted in rats. Two of these ranked "definitely low" or "probably low risk" on all points and where thus evaluated as high confidence studies and assigned to *Tier 1* (Li et al., 2021; Zhang et al., 2013). The study by Khalil et al. (2017) used soy containing diet and lacked a conflict of interest statement. We considered it to be of medium confidence and assigned it to *Tier 2*.

One study investigated the effect of BDE-99 on semen quality in rats (Kuriyama et al., 2005). This study scored "definitely low" or "probably low risk" on all key assessment elements and most of the other assessment aspects, except for the use of soy feed, and was thus rated as high confidence (*Tier 1*). We utilised this study to estimate a reference dose.

Our search returned five studies which investigated BDE-209, all of them examining semen quality in mouse models (Miyaso et al., 2012; Sarkar et al., 2016, 2019; Tseng et al., 2006, 2013). Two of these studies did not provide any information on the purity of their test compound or whether potential contaminants were assessed and thus were rated low confidence studies in *Tier 3* (Kim et al., 2009; Zhai et al., 2019). These studies also used soy containing diet and lacked conflict of interest statements. Two of the studies scored as "probably high" and "definitely high risk" on one or two non-key elements (see Table 2) and were thus of medium confidence and assigned to *Tier 2* (Sarkar et al., 2016; Tseng et al., 2006). Both employed soy containing diet for their experimental procedures and lacked a conflict of interest statement, and one had additional "probable high risk" due to a lack of information on randomisation and blinding (Tseng et al., 2006). The remaining study ranked "definitely low" or "probably low risk" on all assessment points and was therefore considered to be of high confidence (*Tier 1*) (Sarkar et al., 2019).

3.2. Overall study confidence ratings

Overall, four of the 11 studies included in the analysis were assigned to *Tier 1* (high confidence). These included two of the BDE-47 studies, the only BDE-99 study and one BDE-209 study. Three of the 11 studies were assigned to *Tier 2* (medium confidence), including one BDE-47 study and two BDE-209 studies. We allocated a "definitely high risk" in all these studies because they lacked a conflict-of-interest statement. The remaining four studies all obtained a rating of low confidence (*Tier*

Table 2
Outcome of RoB analysis for BDEs -3, -47, -99 and -209.
Shown is the scoring for each Risk of Bias (RoB) element for the selected animal studies. Questions in red represent key element, questions in green are the remaining elements.

The studies were rated as follows: definitely low risk of bias, DLR, in dark green; probably low risk of bias, PLR, in light green; probably high risk of bias, PHR, in yellow; definitely high risk of bias, DHR, in red. The RoB Tier assigned to each study is shown at the bottom. More information on the elements of the RoB assessment is shown in the detailed list in Supplementary Table 3.

RoB analysis for PBDEs		PBDE:	BDE 3	BDE 47				BDE 99	BDE 209				
		Author:	Wei et al.	Zhang et al.	Khalil et al.	Wang et al.	Li et al.	Kuriyama et al.	Miyaso et al.	Tseng et al.	Sarkar et al.	Tseng et al.	Sarkar et al.
		Year:	2018	2013	2017	2013	2021	2005	2009	2011	2016	2006	2019
Detection bias	1. Was exposure sufficiently characterised, including purity and stability of test substance?		PHR	PLR	PLR	PHR	PLR	PLR	PHR	PHR	DLR	DLR	DLR
Detection / Performance bias	2. Where the outcome assessors blinded to study groups?		PLR	PLR	DLR	PLR	PLR	PLR	PLR	PLR	PLR	DLR	PLR
Detection bias	3. Was the number of animals per dose group sufficient?		DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR
Detection bias	4. Was a reliable and sensitive animal model used and a positive control included the showed an effect?		PLR	PLR	PLR	PLR	PLR	DLR	PLR	PLR	PLR	PLR	PLR
Selection bias	5. Was administered dose adequately randomised?		PLR	DLR	PLR	DLR	PLR	PLR	PLR	PLR	PLR	PHR	PLR
Selection bias	6. Was allocation to study groups adequately concealed?		PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR
Performance bias	7. Were experimental conditions identical across study groups?		DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR
Performance bias	8. Was exposure consistently administered across treatment groups (method, time frame etc)?		DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR	DLR
Performance bias	9. Was the diet soy-free or soy-poor?		PLR	PLR	PHR	PLR	PLR	PHR	PHR	PHR	PHR	PHR	PLR
Attrition bias	10. Were outcome data complete without attrition or exclusion?		PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	DLR
Detection bias	11. Were reliable and sensitive methods used for investigating the selected endpoint?		DLR	PLR	DLR	DLR	DLR	PLR	PLR	DLR	DLR	DLR	DLR
Detection bias	12. Were measurements collected at suitable timepoints?		PLR	PLR	DLR	PLR	PLR	DLR	PLR	DLR	PLR	DLR	DLR
Detection bias	13. Were statistical methods appropriate & can we be confident about the estimation of doses associated with low effects (NOAEL, LOAEL etc)?		DLR	DLR	PLR	PLR	DLR	DLR	PLR	DLR	PLR	PLR	PLR
Selective reporting bias	14. Have all study outcomes been reported?		PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR	PLR
Selective reporting bias	15. Have funding sources and conflicts of interest been reported?		DLR	DLR	DHR	DHR	DLR	DLR	DHR	DHR	DHR	DHR	DLR
		RoB TIER:	3	1	2	3	1	1	3	3	2	2	1

Table 3
Evaluation of experimental animal studies on semen quality and additional male reproductive endpoints after treatment with PBDE.
Colours: Key appraisal elements – Dark green: definitely low risk; light green: probably low risk; light red: probably high; (dark red: definitely low risk – not applicable). Study outcomes – Yellow: highlight of problematic finding despite medium rating.

Abbreviations: Key appraisal elements – PTU: Propylthiouracil.

	Study description			Key appraisal elements				Study outcomes		Study evaluation	
Reference	Species, strain	Outcome measures	PBDE congener	Purity of chemical, check for contamination	Randomisation, concealment, blinding	Number of animals per group	Model sensitivity, positive control	Outcomes	Comments	Tier	Overall confidence
Wei at al. 2018	Mouse, C57BL/6J gpt delta mice	Sperm count, vitality, morphology	**BDE-3**	not reported	yes	6	no positive control	Decrease in sperm count		**3**	Low
Zhang et al. 2013	Rat, Sprague Dawley	Daily sperm production	**BDE-47**	>98.7%	yes	10	no positive control	Decrease in daily sperm production	Serum testosterone also decreased	**1**	High
Khalil et al. 2017	Rat, Wistar	Sperm motility and morphology, daily sperm production	**BDE-47**	100%	yes	21 (3 per litter, 7 litters per group)	no positive control	Disruption of sperm morphology	Only one dose tested!	**2**	Medium
Wang et al. 2013	Mouse, B6 mice	Sperm morphology, motility, capacitation	**BDE-47**	not reported	yes	10	no positive control	Disruption of sperm capacitation and some motility parameters		**3**	Low
Li et al. 2021	Rat, Sprague Dawley	Sperm quantity and motility parameters	**BDE-47**	99.90%	yes	9	no positive control	Disruption of sperm motility		**1**	High
Kuriyama et al.2005	Rat, Wistar, HsdCpb:WU strain	Spermatid number, sperm count and morphology	**BDE-99**	98%	yes	16-20	PTU (for thyroid endpoint)	Decrease in sperm number and production	Sperm quality was not affected	**1**	High
Tseng et al. 2011	Mouse, CD-1	Sperm count, motility, motion analysis, morphology	**BDE-209**	not reported	yes	15	no positive control	Disruption of sperm morphology	No changes in count or motility	**3**	Low
Miyaso et al.2012	Mouse, ICR	Sperm count	**BDE-209**	not reported	yes	5	no positive control	Decreased sperm count	Sperm count only decreased at lowest concentration; Testes weight also decreased	**3**	Low
Sarkar et al. 2016	Mouse, Parkes (P) strain	Sperm number and viability	**BDE-209**	>98% analytical grade	yes	9	no positive control	Decreased sperm count and viability	Testis weight also decreased	**2**	Medium
Tseng et al. 2006	Mouse, CD-1	Sperm motility, motion, morphology	**BDE-209**	98%	not reported	10	no positive control	Disruption of sperm morphology	No changes in count or motility	**2**	Medium
Sarkar et al. 2019	Mouse, Parkes	Sperm count, motility, viability, morphology	**BDE-209**	> 98%	yes	7	no positive control	Decrease in sperm number, motility, and morphology	All parameters at all concentrations affected	**1**	High

3). In all cases this was due to a lack of information on the purity of the tested PBDE congener and the potential for contaminants. The *Tier 3* studies comprised the BDE-3 study, one BDE-47 and two BDE-209 studies.

3.3. Evidence synthesis

The evaluation of the studies is summarised in Table 3. The table shows that all of the studies observed some adverse effects on semen quality after administration of individual PBDE congeners. All studies showed declines in various semen parameters, irrespective of their confidence rating.

In the BDE-3 study, a decline in semen quality was observed, however, the study was ranked as low confidence and was therefore not included in the derivation of a reference dose. Due to the low confidence of the only available study, we rated the evidence for an effect of BDE-3 on semen quality as *Slight*.

All four studies that tested BDE-47 consistently reported disrupted sperm parameters and only one of these studies was rated as low confidence. Two studies were of high and one of medium confidence. Thus, the evidence that BDE-47 exposures lead to declines in semen quality is considered to be *Robust*.

We identified only one study investigating the effect of BDE-99 on sperm parameters. We rated this study as high confidence. Due to the lack of additional studies, we evaluated the evidence for semen quality declines from BDE-99 as *Moderate*.

The effects of BDE-209 on semen quality were studied the most and were consistently found to be adverse. The evaluation of the studies only found one to be of high confidence, with an additional two being of medium confidence. Two studies on sperm parameters scored low confidence. Due to the consistency of adverse findings for BDE-209 but the scarcity of high-quality studies, we considered the evidence for association between BDE-209 and semen quality declines to be *Moderate*.

3.4. Derivation of reference doses for declines in semen quality for BDE-47, -99 and -209

We derived reference dose values for three PBDE congeners, BDE-47, -99 and -209. Where data such as responses from three or more different dose groups were available, we attempted BMD modelling to estimate a $BMDL_5$. However, even for studies with sufficient numbers of dose groups, no adequate model could be fitted and the resulting $BMDL_5$

Table 4
Derivation of reference doses from rodent studies that full-filled all inclusion criteria and passed RoB assessment.

Congener/Study	Tier	Species	LOAEL (μg/kg/d)	NOAEL (μg/kg/d)	BB at NOAEL (μg/kg/d)	EHDI (μg/kg/d)	RfD (μg/kg/d)
BDE-47 Zhang et al. (2013)	1	Rat	1.00E+03	30[a)]	500	0.374	**0.15**
BDE-47 Li et al. (2021)	1	Rat	1.00E+03	100[a)]	2.00E+03	1.497	0.6
BDE-99 Kuriyama et al. (2005)	1	Rat	60	*20*[b)]	15	0.00721	**0.003**
BDE-209 Sarkar et al. (2016)	2	Mouse	9.50E+05	7.50E+05	n.a.	7.50E+05	7500
BDE-209 Tseng et al. (2006)	2	Mouse	5.00E+05	1.00E+05	n.a.	1.00E+05	**1000**

The reference doses chosen for mixture risk assessment are shown in bold.
LOAEL: Lowest observed adverse effect level; NOAEL: No observed adverse effect level; BB: Critical body burden; EHDI: Estimated human daily intake associated with rodent BB at NOAEL; RfD: Reference dose derived by dividing the EHDI by 2.5 for (BDE-47 and BDE-99) or 100 (BDE-209).
The NOAEL values shown in italics are extrapolations from studies where only a LOAEL, but no NOAEL was observed. A NOAEL was extrapolated by dividing the LOAEL by a factor of 3.
[a)] Repeat administration, BB estimated taking absorption and excretion into account.
[b)] Single administration.

values had too wide confidence intervals to be reliable. We therefore decided to use NOAEL values as PoDs for all congeners. If only a LOAEL was available, the NOAEL was extrapolated by using an AF of 3.

Table 4 shows the PoDs derived from the studies which we included in the calculation of reference dose values.

BDE-47: We based the derivation of a BDE-47 reference dose on two *Tier 1* studies which all used repeated dose administration in the rat (Li et al., 2021; Zhang et al., 2013). Li et al. (2021) exposed dams in 3 dose groups from 10 days pre-gestation to PND21, covering the critical period of male reproductive development (Gestational Day (GD) 9 to Postnatal Day (PND) 10). Zhang et al. (2013) exposed adult males in 3 dose groups for eight weeks, 6 days per week. The PoDs in these studies were 30 μg/kg/d (NOAEL) (Zhang et al., 2013) and 100 μg/kg/d (NOAEL) (Li et al., 2021). By using the toxicokinetic parameters for BDE-47 ($t_{1/2,a}$ = 23 days, $F_{abs,a}$ = 0.75 for the rat and $t_{1/2,h}$ = 926 days, $F_{abs,h}$ = 1 for the human, see (EFSA 2011)) we first calculated the cumulative critical body burdens at the NOAEL in the rat before estimating the EHDIs for BDE-47. The critical body burdens were 500 μg/kg/d (Zhang et al., 2013) and 2000 μg/kg/d (Li et al., 2021) and the estimated EHDIs were 0.374 μg/kg/d (Zhang et al., 2013) and 1.497 μg/kg/d (Li et al., 2021) respectively. Finally, the reference dose was derived by applying an AF of 2.5 to the EHDIs to account for variability between rodents and humans. Accordingly, the reference doses for BDE-47 (Table 4) were 0.15 μg/kg/d (Zhang et al., 2013) and 0.6 μg/kg/d (Li et al., 2021). Although the study by Zhang et al. (2013) was conducted in adult rats, it produced the lower PoD which we chose as our final estimate.

BDE-99: One (*Tier 1*) study qualified for derivation of a reference dose for BDE-99 (Kuriyama et al., 2005). It covered the critical period of male reproductive development. The study observed a LOAEL of 60 μg/kg/d based on administration of single oral doses in 2 dose groups at GD 6. Therefore, the NOAEL was estimated as 20 μg/kg/d, by application of a factor of 3. Considering an oral absorption in rodents of 75%, we calculated the critical body burden of BDE-99 at PoD by multiplication of the PoD of 20 μg/kg/d with the absorbed fraction as 15 μg/kg/d. With the toxicokinetic parameters for BDE-99 ($t_{1/2,a}$ = 20 days, $F_{abs,a}$ = 0.75 for the rat and $t_{1/2,h}$ = 1442 days, $F_{abs,h}$ = 1) we estimated 0.00721 μg/kg/d as EHDI in accordance with EFSA (2011). By application of an additional factor of 2.5 to account for inter-species variability in rodents and humans this gave a reference dose of 0.003 μg/kg/d (Table 4).

BDE-209: The two studies which we used to derive a reference dose for BDE-209 were conducted in juvenile or adult mice and were rated as medium confidence (*Tier 2*) (Sarkar et al., 2016; Tseng et al., 2006). The study in juvenile mice included 4 dose groups with a treatment duration of 50 days from PND21 (Tseng et al., 2006). In Sarkar et al. (2016), adult mice (12–14 weeks old) received BDE-209 in 2 dose groups for 35 days. The reported NOAELs in the studies were 7.5×10^5 μg/kg/d and 1×10^5 μg/kg/d. For BDE-209 the elimination half-life in animals and humans does not differ markedly and thus the corresponding external PoDs were used as EHDI to estimate the reference doses by application of an uncertainty factor of 100 following EFSA guidance (EFSA 2011). This produced possible reference doses of 7500 μg/kg/d (Sarkar et al., 2016) and 1000 μg/kg/d (Tseng et al., 2006) (Table 4). We had a higher confidence in the value produced by the (Tseng et al., 2006) study as juvenile mice with 4 dose groups were used. Accordingly, we chose the reference dose of 1000 μg/kg/d for BDE-209.

An additional study on BDE-209 which was rated as high confidence (*Tier 1*) (Sarkar et al., 2019) could not be included as BDE-209 administration was postnatally via gavage of the lactating dams which made it difficult to estimate the dosages received by the pups. However, at the maternal dose of 500 mg/kg/d (a LOAEL) reductions in sperm number and motility as well as changes in sperm morphology were seen in the offspring. This LOAEL is similar to those in the studies we used to derive the BDE-209 reference dose.

3.5. Extrapolation to untested PBDE congeners

Of the 209 possible PBDE congeners, relatively few are of environmental relevance, and even fewer have been tested toxicologically to a level required for risk assessments. However, limiting a mixture risk assessment only to toxicologically evaluated congeners while ignoring others that also contribute to human exposures will bias the assessment in the direction of underestimations of risk. To deal with this challenge, we adopted the read-across approach elaborated by us in an earlier PBDE mixture risk assessment for neurodevelopmental toxicity (Martin et al., 2017). Focusing on the congeners for which exposure data are available (EFSA 2011) – BDE-28, -47, -99, -100, -153, -154, -183 and -209 – we assumed that congeners with similar bromine content have similar toxicities. Congeners with similar bromine content also have similar half-lives in rodents and humans. Accordingly, we propose to assign the reference dose for BDE-47 (0.15 μg/kg/d) also to the untested BDE-28, the reference dose of BDE-99 (0.003 μg/kg/d) to BDE-153 and -154, and the reference dose for BDE-209 (1000 μg/kg/d) to BDE-183 and nonaBDE. Extrapolated reference doses that are close to exposure levels indicate a need to prioritise a congener for testing to refine the assessment.

3.6. Comparison with PBDE exposures

The average exposures to BDE-47 via food experienced by adults in Europe are around 0.7 ng/kg/d but can rise to 7.3 ng/kg/d through high consumption of PBDE-contaminated food items and additional high fish

Table 5
Calculation of Hazard Quotients for individual PBDE congeners.

BDE congener	RfD (μg/kg/d)	Average consumption		High consumption	
		Exposure (μg/kg/d)	HQ average	Exposure (μg/kg/d)	HQ high
BDE 28	0.15	0.00017	0.0011	0.00052	0.0035
BDE 47	**0.15**	**0.00072**	**0.0048**	**0.00733**	**0.049**
BDE 99	**0.003**	**0.00035**	**0.12**	**0.00142**	**0.47**
BDE 100	0.003	0.0003	0.1	0.00271	0.90
BDE 153	0.003	0.00026	0.087	0.00095	0.32
BDE 154	0.003	0.00028	0.093	0.00112	0.37
BDE 183	1000	0.00023	2.3E-07	0.001	0.000001
BDE 209	**1000**	**0.00169**	**1.69E-06**	**0.00479**	**4.79E-06**

Hazard Quotients have been calculated for average and high exposure to PBDEs via food, based on published consumption data (EFSA 2011; Martin et al., 2017). RfD: Reference dose; HQ: Hazard Quotient.
Data in bold shows congeners for which the RfD was derived, RfD values for the additional congeners was extrapolated.

consumption (Martin et al., 2017) based on data from EFSA (2011), well below the reference dose of 150 ng/kg/d. Similarly, average exposures to BDE-209 of adults via food are 1.7 ng/kg/d, with extremes of up to 4.8 ng/kg/d (Martin et al., 2017), far removed from the reference dose of 1000 μg/kg/d. The situation is different for BDE-99. Average intakes via food are around 0.35 ng/kg/d, which can reach 1.4 ng/kg/d for consumers of highly PBDE-contaminated food items and additional high fish consumption (Martin et al., 2017). This is relatively close to the reference dose of 2.9 ng/kg/d, yielding Hazard Quotients of 0.12 and 0.47, respectively.

We also used the extrapolated values for untested PBDE congeners to derive Hazard Quotients using the available exposure data for average and high consumption (EFSA 2011; Martin et al., 2017). The Hazard Quotients for the additional congers in the average exposure scenario ranged from 2.3×10^{-7} for BDE-183 to 0.12 for BDE-99 (Table 5). For high exposures, the range of Hazard Quotients was 1×10^{-6} for BDE-183 to 0.9 for BDE-100 (Table 5).

The sum of all Hazard Quotients, i.e. the HI for all congeners at average exposures had a value of 0.4, coming relatively close to a value of 1. The HI for the high consumption scenario was 2.1, exceeding the index value of 1.

4. Discussion

Mixture risk assessments for human health endpoints such as male reproductive health require the availability of relevant toxicity data. Here, we derived reference doses for three PBDE-congeners, BDE-47, -99 and -209, from animal studies on declines in semen quality. Although toxicity data for commercial PBDE mixtures and sums of congeners are also available, it was necessary to derive references doses for specific congeners in order to achieve a match with existing exposure data available e.g. from EFSA (EFSA 2011). Accordingly, we propose to utilise the reference doses estimated for BDE-47, -99 and -209 also for the evaluation of other congeners for which toxicity data are missing altogether.

We based our work on a recently published systematic review of the toxicity of PBDEs on the rodent male reproductive system (Zhang et al., 2020). Although the effects of PBDEs on male reproductive development have been studied in several animal studies, with respect to reproductive organ weights, anogenital distance or reproductive hormones, declines in semen quality were not always assessed. This meant that we could rely only on a limited number of studies.

We found that the quality of many eligible studies was compromised by a lack of information on the purity of the PBDE congeners. This is a significant shortcoming as other contaminants frequently found as impurities of PBDEs, such as dioxins, exert similar effects (EFSA 2018). Thus, we could not take account of studies which did not ascertain the absence of such contaminants.

We also did not consider studies that used the commercial mixture DE-71, due to their poorly defined composition. However, the majority of studies with DE-71 support the observations from studies with specific congeners, that PBDEs negatively affect semen quality and other markers of male reproductive development.

Ideally, exposure regimens would have covered the critical period when germline stem cell populations are established (mouse: gestational day 7 to postnatal day 8, rat: gestational day 9 to postnatal day 10). However, studies covering this period were not always available. In such cases (BDE-47: Zhang et al. (2013); BDE-209: Sarkar et al. (2016); Tseng et al. (2006)), we had to make recourse to exposure studies in adult male rodents. Possible concerns that this might have led to accordingly higher estimates of reference doses were not borne out in the case of BDE-47, where the study that covered the period of establishment of germ cell stem populations (Li et al., 2021) produced the higher reference dose.

In support of the observations from rodent studies, there are several reports of adverse effects of PBDEs on male reproductive health from human epidemiological studies. Declines in semen quality associated with elevated levels of PBDEs were observed in men attending fertility clinics (Abdelouahab et al., 2011; Den Hond et al., 2015) as well as in healthy men (Akutsu et al., 2008; Albert et al., 2018; Yu et al., 2019). However, others have found no association of selected congeners with semen parameters (Toft et al., 2014). The choice of congeners is often guided by their use as marker congeners and detection limits, and less by their toxicity and findings are reported linked to the sum of measured PBDEs. Overall, semen quality in men was found to be negatively associated with the individual congeners BDE-47, -100 and -153 and the sum of BDE-47, -99, -100 and -153. Furthermore, prenatal exposure to PBDEs has been linked with disrupted male reproductive development, namely an increase in cryptorchidism (Goodyer et al., 2017; Main et al., 2007) and hypospadias (Poon et al., 2018). However, many of these studies (Goodyer et al., 2017; Main et al., 2007; Poon et al., 2018) measured PBDEs in hair which makes it difficult to estimate daily exposures and to relate these observations to our reference doses.

The reference doses we estimated for declines in semen quality are higher than those which we used in a mixture risk assessment for developmental neurotoxicity of PBDEs (Martin et al., 2017) based on data from EFSA (2011) (BDE-47: 68.8 ng/kg/d versus 150 ng/kg/d for semen quality declines; BDE-99: 1.68 ng/kg/d versus 2.9 ng/kg/d; BDE-209: 17 μg/kg/d versus 1000 μg/kg/d). Thus, evaluated in a chemical-by-chemical approach, current exposures to single PBDE congeners are unlikely to be of concern in terms of declining semen quality. However, we find that the untested congeners BDEs-100, -153 and -154 were present at exposure levels close to the extrapolated reference dose of 2.9 ng/kg/d, indicating that toxicity data for those congeners is required to refine the assessment. Inclusion of all congeners with exposure data resulted in HIs of 0.4 for average and 2.1 for high consumption, indicating that combined exposures to these PBDEs warrants further investigation. It remains to be seen, how the contribution of PBDEs will play out in a risk assessment scenario that takes account of exposures to multiple chemicals implicated in disruptions of male reproductive development (Kortenkamp 2020).

Declaration of competing interest

The authors declare there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113953.

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