National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Method for the derivation of a Human Limit Value for Brominated DiphenylEther-47

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Abstract

Method for the derivation of a Human Limit Value for Brominated DiphenylEther-47

The Human Limit Value (HLV) is defined as the life-long daily human intake of a chemical contaminant which does not result in adverse health effects. The HLV is often based on the extrapolation of animal toxicity to man. With regard to interspecies differences in kinetics a default value of 4 is usually applied in deriving a HLV.
However, as shown for the persistent organic pollutant (POP) 2,3,7,8-TetraChloroDibenzo-*p*-Dioxin (TCDD), the application of this default factor leads to a HLV which does not protect humans against TCDD toxicity. Even more, as TCDD indicates, interspecies differences in POP kinetics may exceed the default factor manifold. POP kinetics therefore should explicitly be incorporated in the extrapolation of animal toxicity of POPs to man. This principle was applied on tetraBromoDiphenylEther-47 (BDE-47). Neurodevelopmental toxicity and thyroid toxicity were identified as the critical endpoints for animal toxicity of BDE-47. Taking the uncertainty in the derivation of the HLV into account, a provisional HLV of 7 ng/kg bw/day is proposed in order to prevent (any) adverse effects of BDE-47 exposure in the human population.

Keywords:
PolyBrominated DiphenylEthers, BDE-47, toxicokinetics, Human Limit Value

Rapport in het kort

Afleiding van de toelaatbare dagelijkse inname van de Broom DiphenylEther-47

De Toelaatbare Dagelijkse Inname (TDI) is de inname van een stof waarbij, bij levenslange blootstelling, geen nadelige effecten op de gezondheid verwacht mag worden. De afleiding van de TDI is vaak gebaseerd op de extrapolatie van bij proefdieren waargenomen toxiciteit naar de mens. Deze extrapolatie bestaat uit correcties voor verschillen in toxicokinetiek (opname, verdeling, uitscheiding) en toxicodynamie (toxisch werkingsmechanisme) tussen proefdieren en de mens Hierbij wordt er standaard vanuit gegaan dat verschillen in toxicokinetiek tussen mens en proefdier niet meer dan een factor 4 bedragen. De afleiding van de TDI voor de dioxine 2,3,7,8-TetraChloroDibenzo-p-Dioxine (TCDD) laat echter zien dat het toepassen van deze factor tot een TDI leidt die de gezondheid onvoldoende beschermt. De belangrijkste reden hiervoor is dat verschillen in de toxicokinetiek van TCDD tussen proefdier en mens veel groter zijn dan de genoemde standaardfactor. De toxicokinetiek van Persistente Organische Milieuverontreinigingen als TCDD, maar ook PolyBroom DiphenylEthers en Perfluorverbindingen, moet daarom expliciet in de afleiding van de TDI opgenomen worden. In navolging van TCDD is dit principe op tetrabroom BDE-47 toegepast. Voor BDE-47 bleken de ontwikkeling van het centraal zenuwstelsel uitmondend in verstoorde gedragsontwikkeling en een verstoorde schildklierfunctie de gevoeligste vormen van diertoxiciteit.
Wanneer met de onzekerheid bij het afleiden van de TDI rekening gehouden wordt kan op basis van deze effecten een voorlopige TDI van 7 ng/kg bw/day berekend worden.

Trefwoorden: PolyBroom DiphenylEthers, BDE-47, toxicokinetiek, toelaatbare dagelijkse inname

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Summary

Summary

Derivation of Human Limit Values: Default approach

In case of chronic exposure, the Human Limit Value (HLV) is defined as the life-long daily human intake which does not result in adverse health effects, even in the sensitive human.

Often the HLV is based on the extrapolation of animal toxicity to man. This extrapolation starts with the selection of a contaminant's critical toxicity endpoint in the animal and its corresponding Point - of - Departure (PoD). Traditionally, the No-Observed-Adverse-Effect-Level (NOAEL) is taken as PoD. Here, the NOAEL is defined as the highest administered dose which did not lead to toxicologically significant toxicity in exposed animals when compared to untreated controls.

In order to obtain the HLV, the NOAEL is divided by Assessment Factors (AFs). In this calculation separate AFs are used for interspecies- (average animal to average human, default value: 10) and intraspecies extrapolation (average human □ sensitive human, default value: 10), leading to an "overall" default AF of 100.
Additional AFs may be applied to account for differences between the exposure conditions in the animal experiment and the human exposure, e.g. when the NOAEL is derived from a subchronic animal, generally a subchronic to chronic AF is applied.

The HLV then is calculated as:

$$HLV = \frac{NOAEL}{AF_{\text{inter}} \times AF_{\text{intra}} \times \cdots \times AF_i}$$

Chemical specific information on toxicokinetics and toxicodynamics can be incorporated into the extrapolation procedure by subdividing the default AFs into subfactors covering toxicokinetics and toxicodynamics. This leads to the following revised calculation of the HLV:

$$HLV = \frac{NOAEL}{AF_{\text{inter},kin} \times AF_{\text{inter},dyn} \times AF_{\text{intra},kin} \times AF_{\text{intra},dyn} \times \cdots \times AF_i}$$

Conveniently staying within a factor of 10, the interspecies AF may be subdivided into a default factor of 4 for kinetics ($AF_{inter,kin}$) and 2.5 for dynamics ($AF_{inter,dyn}$)(Renwick *et al.*, 1993). The intraspecies factor may be subdivided into two equal default factors of $\sqrt{10}$ for kinetics and dynamics (IPCS, 1994).
The NOAEL approach is deterministic, i.e. the NOAEL itself and the AFs have a fixed value.

Human Limit Values: Persistent Organic Pollutants

Persistent Organic Pollutants (POPs) are characterized by their slow removal from the body and, consequently, their bioaccumulating properties after repeated exposure.
As a result, the dose metric associated with the health risk of POPs gradually changes from the daily (external) intake to the accumulated amount in the body. Regarding the latter, the Whole Body Concentration (WBC) provides a generic dose metric for POP which accumulate in body lipid[1]. Adopting the latter dose metric as the starting point for the calculation of the HLV ("WBC approach"), leads the following modification of the interspecies extrapolation of animal toxicity.
First the NOAEL in the (average) animal and its corresponding WBC are determined (by calculus or measurement)(step 1 and 2). The animal WBC then is extrapolated to man, i.e. the chronic (external) exposure leading to this WBC in the average human is calculated (step 3: interspecies extrapolation of toxicokinetics). Finally, the calculated human exposure is corrected for interspecies differences in toxicodynamics and extrapolated from the average human to the sensitive human (intraspecies extrapolation)(step 4).

The explicit quantification of interspecies differences in toxicokinetics (step 3 above) may lead to an AF which grossly exceed the default factor of 4 for interspecies differences in toxicokinetics and, hence, may result in an "overall" AF which significantly exceeds its default value of 100.

The classical case in which an AF for interspecies differences in kinetics was explicitly quantified is the dioxin 2,3,7,8-TetraChloroDibenzo-p-Dioxin (TCDD). The derivation of TCDD's HLV was based on two animal studies showing single dose acute NOAEL's of 15.4 ng/kg bw and 9.3 ng/kg bw. This resulted in HLV values of 2.5 resp. 1.5 pg/kg bw/day (WHO, 1998; SCF, 2000; 2001; JECFA/WHO, 2002, 2005)[2].
The resulting NOAEL/HLV ratios grossly exceeded the default value of 100 more 60-fold. As shown in the Main Document this difference is mainly caused by the different elimination kinetics of TCDD in rodents and humans, TCDD's half-life in rodents being 20 days and more than 7 years in humans.

As TCDD lower brominated PolyBrominated Diphenyl Ethers (PBDEs) show significant interspecies differences in elimination kinetics (von Meyerink *et al.*, 1990; Geyer *et al.,* 2004; Bakker et al., 2008). For this reason the method as applied on the derivation of the HLV of TCDD was applied on the tetrabromo PBDE BDE-47.
Neurodevelopmental toxicity in mice and thyroid toxicity in the rat were identified as critical endpoints for animal toxicity of BDE-47. In compliance with EFSA recommendations animal toxicity was analyzed by means for BenchMarkDose (BMD) modeling. The obtained animal BMDs were extrapolated into a human BMD of 130 ng/kg bw/day (90% CI: 32 – 470) for neurodevelopmental toxicity and 22 ng/kg bw/day (90% CI: 7.2 - 70) thyroid toxicity (see Table 1 for the used PoDs and the applied AFs upon)[3]. By taking the lower bound of the human dose levels associated with either

[1] For details, see Section 2 of the main document
[2] For details, see Section 3 of the main document
[3] For details, see Section 4 of the main document

neurodevelopmental toxicity or thyroid toxicity the uncertainty in the derivation of the HLV is taken into account. Based on these results a provisional HLV of 7 ng/kg bw/day is therefore proposed in order to prevent (any) adverse effects of BDE-47 exposure in the human population.

Conclusions

- The derivation of the HLV for 2,3,7,8-TCDD clearly showed that the risk assessment of POPs of the repeated exposure to these compounds should be based on the accumulated amount of these compounds in the body. Furthermore, the explicit quantification of interspecies differences in kinetics in terms of an interspecies AF for differences in kinetics is a *conditio sine qua non* for the derivation of HLV values for POPs. In this context using the application of the default AF for interspecies differences in toxicokinetics may lead to HLVs which may grossly underestimate the toxicological risk of POP exposure in humans.

- Neurodevelopmental toxicity and thyroid toxicity were found as the critical toxic endpoints of BDE-47 in animals. Taking the Whole Body Concentration as the starting point for interspecies extrapolation (of thyroid toxicity) a provisional HLV of 7 ng/kg bw/day is proposed.

Glossary

Abbreviations

A	Amount
AF	Assessment Factor
C	Concentration
D	Dose (amount/kg bw/day)
F	Fraction
PoD	Pont of Departure
WBC	Whole Body Concentration

Indices

a	animal
a to c	acute to chronic
abs	absorption
back	background
dyn	dynamics
el	elimination
fat	adipose tissue
h	human
inter	interspecies
intra	intraspecies
kin	kinetics
p.o.	per os
r	repeated
s	single

1. Introduction

In the absence of suitable human data, the chronic exposure level at which no adverse effects are thought to occur in humans (Human Limit Value, HLV) is obtained by extrapolating toxicity data from experimental animals to man. Derivation of a HLV starts with a dose-response assessment, i.e. an analysis of the relationship between the dose of a compound and its critical toxicological endpoint. When this relationship is known for the critical endpoint, a so-called Point of Departure (PoD) can be derived. The PoD is defined as the highest *administered* dose which does not result in adverse health effects in the *average/typical* experimental animal. Often a No-Observed-Adverse-Effect-Level (NOAEL) or Bench Mark Dose (BMD) is considered as PoD. To account for differences between the exposure conditions in the animal experiment and the human exposure situation, intrinsic differences between the animal and man, and differences within the population of interest (general population vs. "sensitive" subpopulations), Assessment Factors (AFs) are applied to the PoD. For an extensive description of the hazard characterization process the reader is referred to Bokkers (2009) and Dybing (2002). For the "sensitive" human the derivation of an HLV is summarized by:

$$HLV = \frac{PoD}{AF_{\text{inter}} \times AF_{\text{intra}} \times \cdots \times AF_i} \qquad (1.1)$$

The interspecies assessment factor ($AF_{interspecies}$) is generally assumed to correct for the *combined* differences in toxicokinetics and toxicodynamics between the *average* animal and the *average* human (default: 10). The intraspecies assessment factor ($AF_{intraspecies}$) corrects for corresponding differences between the *average* and the "sensitive" human (default: 10). Additional assessment factors (AF_i) may be applied, e.g. in the case where the exposure duration in the animal experiment differs from that in the human population, when the toxicological information of the compound of interest shows deficiencies, e.g. by the absence of chronic toxicity data, or when a Lowest Observed Adverse Effect Level (LOAEL) instead of a No Observed Adverse Effect Level (NOAEL) is available from an animal study.

Traditionally, the HLV is derived by taking the NOAEL from an animal experiment as PoD. The NOAEL approach is deterministic, i.e. PoD and AFs have one constant value. Alternatively, the HLV may be deduced by means of dose-response modeling of toxicity data. Given the dose-response model, a certain Critical Effect Size (CES, e.g. a 5 or 10% reduction in body weight) is set to calculate the corresponding BMD and its corresponding uncertainty distribution. The BMD distribution is then taken as the PoD and an $AF_{interspecies}$ distribution, instead of a single default value, then leads to the equivalent BMD distribution for the average human being. Then, applying an $AF_{intraspecies}$ distribution to the BMD distribution for the average human results in a BMD distribution for the "sensitive" human. Given the uncertainty in the BMD a lower percentile (e.g. the 5^{th} percentile) of this distribution can then be designated as the HLV. Note that all distributions described above relate to uncertainty only and not to variability.

In an attempt to move away from standard default values in cases where more incisive data are available, it was proposed to subdivide each of the 10-fold default AFs for inter- and intraspecies differences into subfactors covering kinetics and dynamics. Conveniently staying within a factor of 10, the combined interspecies AF was subdivided into a default factor of 4 for differences in kinetics ($AF_{interspecies,kin}$) and 2.5 for differences in dynamics ($AF_{interspecies,dyn}$). The intraspecies factor was subdivided into two equal default factors of $\sqrt{10}$ for differences in kinetics and dynamics (Renwick, 1993; Renwick and Lazarus, 1998, WHO,1994). The explicit expression of these differences then leads to the following revised calculation of the HLV:

$$HLV = \frac{PoD}{AF_{inter,kin} \times AF_{inter,dyn} \times AF_{intra,kin} \times AF_{intra,dyn} \times \cdots \times AF_i} \quad (1.2)$$

In chemical risk assessment, inter- and intraspecies differences in toxicokinetics and/or toxicodynamics are seldom explicitly incorporated in the calculation of HLVs. The reason for this lies in the absence of suitable kinetic and/or toxicity data which can be used to quantify the corresponding AFs (also known as Chemical Specific Adjustment Factors, CSAFs) and the general belief in the toxicological community that the used default factors sufficiently cover inter- and intraspecies differences in toxicokinetics and toxicodynamics. However, in the case that available data indicate otherwise, a modified extrapolation procedure has to be followed. As an example of this procedure the class of Persistent Organic Pollutants (POPs) will be presented here. POPs take their name from their extreme slow removal from the mammalian body once they have entered it. The classical case here is the dioxin 2,3,7,8-TetraChloroDibenzo-p-Dioxin (TCDD). TCDD shows, both in the rat and in man, bioaccumulating properties with significant interspecies differences in toxicokinetics. As a result the Whole Body Concentration (WBC) and, hence, its constituting organ concentrations, will gradually increase after chronic exposure until eventually a stable, "steady state", level is reached. In man, such a situation may be achieved after around 30-35 years, whereas in the rat this only takes 80-100 days. During this time period, which spans around 60% and 90% of the human and rat life time respectively, the dose metric associated with the toxic risk gradually changes from the daily (external) intake to its accumulated match, i.e. the amount of the chemical which has accumulated in the body. Regarding the latter, the WBC is usually taken as a generic dose metric for a chemical's accumulation in the body and its constituting organ concentrations cq. induced organ specific toxicity. For this reason the WBC was taken as the starting point for the derivation of TCDD's HLV value (WHO, 1998; SCF, 2000; 2001; JECFA/WHO, 2002, 2005). Basically, this derivation deviates from the more classical approach in that it explicitly expresses the interspecies AF for differences in toxicokinetics, instead of using the default factor of 4.
Up to now the case of TCDD stands as a separate case. However, other POPs which show significant interspecies differences in kinetics like Poly Brominated Diphenyl Ethers (PBDEs) and perfluorinated compounds (PFOS/PFOA) are likely candidates to have a refined extrapolation procedure too.

The present report reviews the use of the WBC as the starting point for the derivation of the HLV of POPs. The basics of this methodology are presented in Section 2. Its practical application is illustrated on the derivation of the HLVs of 2,3,7,8-TCDD (section 3, deterministic approach) and BDE-47 (section 4, probabilistic approach).

2. Interspecies scaling of POP kinetics

2.1 Whole Body Concentration Approach

As mentioned in the Introduction, an HLV relates to a life-long, daily human exposure which is derived from an (externally) administered dose metric in the animal, i.e. a NOAEL or a BMD. Traditionally an HLV calculation is performed by dividing an animal NOAEL/BMD by (the product of) AFs. However, when the relevant dose metric is the WBC, the extrapolation procedure has to be modified.
Firstly, the NOAEL/ BMD has to be converted to its corresponding animal WBC. Secondly, this animal WBC is extrapolated to its corresponding human WBC. Thirdly the human WBC is related to its corresponding chronic human external daily dose. In this way the chronic daily intake is obtained which, in humans leads to a WBC corresponding with the NOAEL or the BMD in the animals. This intake is corrected for interspecies differences in toxicodynamics and intraspecies extrapolation to result in the HLV.

The determination and extrapolation of a WBC from animals to man inevitably needs kinetic modeling. In this context, (apparent) one-compartmental kinetic modeling may suffice for the interspecies scaling of POP kinetics as long as the interest lies in kinetics on a time scale of years rather than days and as long as the distribution kinetics do not differ much between the animal and man (see below, also see Appendix 1 for additional justification of one-compartment modeling in deriving an HLV of POPs and lipid partitioning as the distribution mechanism of POPs in mammals, van der Molen, 1996, 1998, 2000, see also JECFA/WHO 2002 and Zeilmaker and Van Eijkeren, 1997 and references herein). Hence one compartmental modeling has repeatedly been applied for the interspecies extrapolation of low dose 2,3,7,8-TCDD toxicity in the calculation of the HLV (WHO, 1998; SCF, 2000; 2001; JECFA/WHO, 2002, 2005).

2.2 One compartmental modeling

Given one-compartment kinetic modeling and a repeated daily oral intake D_r (amount/kg bw/day) of a chemical, the time course of the WBC, *WBC(t)*, is given by:

$$WBC(t) = \frac{F_{abs} \cdot D_r}{k_{el}} \cdot (1 - e^{-k_{el} \cdot t}) \qquad (2.1)$$

with:

D_r	repeated daily intake (amount/kg bw/day)
k_{el}	elimination rate constant (day^{-1})
F_{abs}	absorption fraction of the daily intake (without dimension)
t	exposure duration (days)

For this model the following relationship holds for k_{el} and the half-life $t_{1/2}$:

$$\ln(2) = k_{el} \cdot t_{1/2} \tag{2.2}$$

The half-life is the time needed for the WBC to half itself after the chemical exposure has stopped. Note that the half-life is not a model parameter per se, whereas k_{el} is, but an entity which can experimentally be observed and which, as such, can be used to identify k_{el}.

Eventually the WBC will reach a constant, "steady state" level. This level, which virtually is reached after 4-5 times a chemical's half-life, then is determined as:

$$WBC_{ss} = \frac{F_{abs} \cdot D_r}{k_{el}} \tag{2.3}$$

3. Interspecies extrapolation of 2,3,7,8-TCDD toxicity

Section 3.1 presents a careful reconstruction of the interspecies extrapolation of 2,3,7,8-TCDD toxicity as performed by JECFA/WHO (2002; 2005) in the derivation of the HLV of this compound.
A justification of the parameter values used can be found in JECFA/WHO (2002; 2005) and Winter-Sorkina *et al.* (2006).

3.1 Extrapolation of acute animal exposure to chronic human exposure

Basically the derivation of the HLV of 2378-TCDD consists of the following four consecutive steps:

- Selection of the most sensitive toxic endpoint and its corresponding dose which does not result in adverse health effects (PoD, e.g. a NOAEL in the (average) animal.
- Determination of the WBC corresponding with the animal PoD.
- Calculation of the chronic, daily, human intake which leads to a WBC which is equivalent to the WBC at the level of the PoD in the (average) animal (see step 2)(interspecies extrapolation of kinetics).
- In this step the dose calculated in step 3 is corrected for intraspecies differences in toxicodynamics and intraspecies extrapolation, to result in a HLV for the sensitive human.

In the following each of the mentioned steps is worked out in detail.

Identification of the critical toxic endpoint (step 1)

Two rodent studies showing an *acute, single dose*, effect at low doses were identified;

- Ohsako *et al.* (2001, single p.o. dose study)

In the Ohsako study pregnant rats were given one single oral dose of TCDD (0-800 ng/kg bw) on day 15 of gestation (GD15) and male offspring were examined on day 49 and 120 after parturition. In this study no reproductive toxicity in offspring was observed at a single (external) dose ($D_{s,a}$) level as low as 12.5 ng/kg bw.

- Faqi *et al.* (1998, multiple s.c. doses)

In the Faqi study dams were treated subcutaneously before mating and throughout mating, pregnancy and lactation. The animals received an initial loading dose of 25, 60 or 300 ng TCDD/kg bw 2 weeks prior to mating, followed by weekly maintenance doses of 5, 12 or 60 ng/kg bw. Effects on male reproduction were studied on Postnatal Days (PND) 70 and 170. Even at the lowest dose combination tested, disturbed sperm production was found (loading

dose: bolus of 25 ng/kg bw; maintenance dose: bolus of 5 ng/kg bw once a week).
The Ohsako study identified GD 15 as *a* critical time window for single dose induced reproductive toxicity and its corresponding WBC. The Faqi study provides additional data for the calculation of the WBC on GD15.
The determinant for the induced toxicity of course is the fetal exposure on GD 15. This exposure can be derived by using linear relationships between the administered maternal dose at GD 15 and the resulting fetal exposure. These relationships, which are available for single as well as repeated exposure of dams to TCDD (JECFA, 2002, as referred to in Winter-Sorkina, 2006), indicate that a single bolus dose and a repeated low dose which lead to the same maternal WBC lead to a different fetal concentration, with fetal exposure after a single bolus dose being (slightly) higher than after repeated exposure[4].
Consequently, in order to lead to the same fetal exposure the maternal WBC after repeated exposure may be higher than after exposure to a single bolus. Given the relationships mentioned above, a factor ($F_{a\square c}$) of 1.7 appeared to correct a maternal WBC after a single bolus dose to a maternal WBC after repeated dose which both lead to the same fetal exposure on GD 15[5].

Calculation of the Whole Body Concentration on GD15 (step 2)

Ohsako study
Given an administered acute p.o. dose of 12.5 ng/kg bw, an experimentally observed absorption fraction of 0.61 for the oral route of administration and an acute to chronic factor of 1.7 results in a GD 15 chronic WBC of 13 ng/kg bw. As animal feed contains trace amounts of TCDD the WBC was augmented with a background (WBC_{back}) of 3 ng/kg bw[6], resulting in a *net chronic* WBC of 16 ng/kg bw.

Faqi study
In the Faqi study, the s.c. dosing schedule was: a loading dose of 25 ng/kg bw at 14 days before mating and maintenance doses of 5 ng/kg bw 7 days before mating, at mating and at GD 7 and GD 14. Given one-compartmental modeling, complete absorption for the s.c. route of administration and a half-life of 21 days for TCDD in the rat a remaining *net* WBC of 20 ng/kg bw at GD 14 can be calculated (for calculation, see Winter-Sorkina *et al.*, 2006). Adding to this the final maintenance dose of 5 ng/kg bw administered at GD 14 an effective maternal WBC of 25 ng/kg bw is obtained for GD 15[7].
Again, as animal fed contains trace amounts of TCDD the WBC was augmented with a background of 3 ng/kg bw, resulting in a *net chronic* WBC of 28 ng/kg bw.

[4] Though JECFA does not examine the matter further the observed effect likely results from Ah-receptor dependent hepatic P450 induction. This effect, which already occurs in the rat liver at dose levels below 0.1 ng/kg bw, increase with the duration of the exposure. The induction leads to increased hepatic sequestration of TCDD which in turn may lead to a reduced exposure of the extrahepatic organs (when compared with the same administered dose level in uninduced rats).
[5] Using non-linearity for the relationship between the administered maternal dose at GD15 and the resulting fetal concentrations resulted in a factor of 2.6. Using this factor led to HLV values of 3.4 pg TEQ/kg bw/day for the Ohsako study and 2.2 for the Faqi study (for details, see de Winter-Sorkina *et al*., 2006).
[6] The animals were assumed to be in "steady state" with respect to their chronic background exposure.
[7] Note that, as the Faqi study rests on repeated exposure, the acute to chronic exposure scaling factor for the embryonic exposure is left out in the calculation of the WBC.

Interspecies extrapolation of kinetics (step 3)

In this step the human daily intake ($D_{r,h}$) is calculated which leads to a WBC during human pregnancy equal to the animal WBC calculated in step 2.
Given the WBC during human pregnancy to have reached a "steady state" one-compartmental modeling $D_{r,h}$ is obtained by rearranging (2.3):

$$D_{r,h} = \frac{WBC_a \cdot \ln 2}{t_{1/2,h} \cdot F_{abs,h}} \qquad (3.1)$$

with:

$D_{r,h}$	daily human intake (amount/kg bw/day) (in the case of	2378-TCDD mainly from food)
WBC_a	Whole Body Concentration as observed in the animal	
$t_{1/2,h}$	half-life of 2378-TCDD in the human body	
$F_{abs,h}$	absorption fraction of the repeated daily human intake	(in the case of 2378-TCDD mainly from food).

Note that, given a half-life of 7 years a "steady state" with respect to TCDD kinetics is virtually expected after about 28 – 35 years of exposure. Taking childbearing age in humans to range from 20 to 45 years implies that the calculated human intake will lead to a WBC which will stay below (age of 20 – 28 years) or just at (age of 28 – 45 years) its animal benchmark. Or, in other words, using equation 3.1 assures that, during reproductive age, the human WBC will not exceed the WBC at the level of the NOAEL of the Ohsako and the Faqi study.

Ohsako study
Substituting 7.6 years for the half-life of TCDD in humans, an absorption fraction of 0.5 for its uptake from food and 16 ng/kg bw as a net chronic "no effect" WBC in the rat, a daily human intake of 8 pg TCDD/kg bw/day was calculated.

Faqi study
Substituting 7.6 years for the half-life of TCDD in humans, an absorption fraction of 0.5 for its uptake from food, 28 ng/kg bw as a net "lowest effect" WBC in the rat and a LOAEL to NOAEL assessment factor of 3 a human daily intake of 4.7 pg TCDD/kg bw/day was calculated.

Interspecies extrapolation of toxicodynamics/intraspecies extrapolation (step 4)

Ohsako study
In concordance with SCF, JECFA concluded that humans may be less sensitive than rats for some effects of TCDD, but the conclusion is less certain for other dioxins, and it cannot be excluded that the most sensitive humans might be as sensitive to the adverse effects of TCDD as rats were in the Ohsako study. JECFA therefore concluded that no *AF* in either direction needs to be applied for inter- and intraspecies differences in toxicodynamics ($AF_{interspecies,dyn} = 1$; $AF_{intraspecies,dyn} = 1$). To compensate for inter-human differences in kinetics an AF of $\sqrt{10}$ was applied ($AF_{intraspecies,kin} = \sqrt{10}$).

As pharmacokinetic modeling was used to scale the WBC in the rat to man the application of an *extra AF* for interspecies differences in kinetics was considered unjustified.

Application of the mentioned AFs then results in a HLV of 8/(1 · 1· $\sqrt{10}$) = 2.5 pg/kg bw/day, equivalent with 75 pg TEQ/kg bw/month.

Faqi study
Applying, as in the Ohsako study, an intraspecies AF an HLV of 4.7/(1 · 1· $\sqrt{10}$) = 1.5 pg/kg bw/day, equivalent with 45 pg TEQ/kg bw/month was calculated.

The HLV thus is the long-term daily intake which leads to a "steady state" Whole Body Concentration (WBC) in the "sensitive" pregnant woman at which no reproductive toxicity (disturbance of sperm production due to intrauterine exposure) is expected in male offspring. In this context "sensitive" corresponds to women who have a relative long half-life for TCDD when compared with the "average woman.

Applied parameter values and Assessment Factors

Ohsako study
In the Ohsako study, a single p.o. dose $D_{a,s}$ was administered on GD15. After absorption, specified by the absorption coefficient $F_{abs,a,p.o}$, the resulting single dose WBC was corrected by means of the scaling factor $F_{a\square c}$ to its chronic equivalent, i.e. the WBC which leads to the same fetal TCDD exposure as the administered single dose or:

$$WBC_{ad\,\min istered,animal,chronic} = D_{a,s} \cdot F_{abs,a,p.o.} \cdot F_{a,a\rightarrow c} \qquad (3.2)$$

The calculated chronic *WBC* was added to the already present background WBC_{back} and the resulting sum was used to calculate the chronic daily human intake $D_{r.h}$ which leads to this *WBC* in humans, or:

$$WBC_{total,animal,chronic} = WBC_{ad\,\min istered,animal,chronic} + WBC_{back} \qquad (3.3a)$$

$$WBC_{total,animal,chronic} = D_{r,h} \cdot \frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{\ln 2} \qquad (3.3b)$$

The human *HLV* value then is calculated as:

$$HLV = \frac{D_{r,h}}{AF_{\text{int}er,dyn} \cdot AF_{\text{int}ra,kin} \cdot AF_{\text{int}ra,dyn}} \qquad (3.4)$$

When expressing WBC_{back} in terms of a single p.o. administered background dose $D_{s,s,back}$, i.e.

$$WBC_{back} = D_{s,a,back} \cdot F_{abs,a,p.o} \cdot F_{a,a\to c} \tag{3.5}$$

one arrives at:

$$HLV = \frac{(D_{s,a} + D_{s,a,back}) \cdot F_{abs,a,p.o.} \cdot F_{a,a\to c} \cdot \ln 2}{t_{1/2,h} \cdot F_{abs,h,p.o} \cdot AF_{\mathrm{inter},dyn} \cdot AF_{\mathrm{intra},kin} \cdot AF_{\mathrm{intra},dyn}} \tag{3.6}$$

Rearranging (3.6) then gives:

$$HLV = \frac{(D_{s,a} + D_{s,a,back})}{\frac{1}{F_{a,a\to c}} \cdot \frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{F_{abs,a,p.o.} \cdot \ln 2} \cdot AF_{\mathrm{inter},dyn} \cdot AF_{\mathrm{intra},kin} \cdot AF_{\mathrm{intra},dyn}} \tag{3.7}$$

thereby defining $AF_{inter,kin}$ as:

$$AF_{\mathrm{inter},kin} = \frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{F_{abs,a,p.o.} \cdot \ln 2} \tag{3.8}$$

Parameter values and assessment factors

$D_{s,a}$	Single p.o. animal dose administered at GD 15 to pregnant dam
WBC_{back}	Background Whole Body Concentration present in pregnant dam at GD15
$D_{s,a,back}$	Acute p.o. animal dose equivalent to the background Whole Body Concentration present in the pregnant dam at GD15 ($D_{s,a,back} = \frac{WBC_{back}}{F_{abs,a,p.o.} \cdot F_{a,a\to c}}$)
$F_{abs,a,p.o.}$	Absorption fraction of orally administered TCDD in the animal
$F_{,a\square c}$	Factor scaling the *WBC* in dams after acute administration to a chronic *WBC* which leads to the same fetal exposure as the acute administration (intraspecies acute to chronic scaling of kinetics in the animal)
$F_{abs,h,p.o.}$	Absorption fraction of TCDD from human food
$t_{1/2,h}$	Terminal half-life of TCDD in humans
$AF_{inter,kin}$	Average rat to average man Assessment Factor for interspecies differences in TCDD toxicokinetics
$AF_{inter,dyn}$	Average rat to average man Assessment Factor for interspecies differences in TCDD toxicodynamics
$AF_{inter,kin}$	Average man to sensitive man Assessment Factor for intraspecies differences in TCDD toxicokinetics
$AF_{intra,dyn}$	Average man to sensitive man Assessment Factor for intraspecies differences in TCDD toxicodynamics

Parameter/Assessment factor	Value	Source
$D_{s,a}$	12.5 ng/kg-bw	Experimental
WBC_{back}	3 ng/kg-bw	Assumption
$D_{s,a,back}$	2.9 ng/kg-bw	Calculated
$F_{abs,a,p.o}$	0.61	Experimental
$F_{,a\ to\ c}$	1.7	Experimental
$F_{abs,h,p.o.}$	0.5	Assumption
$t_{1/2,h}$	7.6 year	Experimental
$AF_{inter,kin}$	3280 day	Calculated
$AF_{inter,dyn}$	1	Default assumption
$AF_{intra,kin}$	$\sqrt{10}$	Default assumption
$AF_{intra,dyn}$	1	Default assumption

Note that in the Ohsako study the *effective acute p.o*. NOAEL at GD15 in the rat consists of the sum $D_{s,a}$ + $D_{s,a,back}$, i.e. 15.4 ng/kg bw. The corresponding *chronic* WBC without effect in the average rat is 16 ng/kg bw(= 15.4 x 1.7 x 0.61). As no interspecies differences in toxicodynamics are assumed between the average rat and the average human and between the average and the sensitive human, i.e.
$AF_{interspecies,dyn}$ = 1 and $AF_{intraspecies,dyn}$ = 1, the *chronic* WBC without effect in the average rat, the average man and the sensitive human in all cases is 16 ng/kg bw. The "true" differences between the average and the sensitive human stems from kinetic differences, with the sensitive human having a half-life of $\sqrt{10}$ times that of the average human. In this context the HLV of 2.5 pg/kg bw/day is expected to lead to a
WBC of just 16 ng/kg bw in the sensitive human (= 2.5 x 0.5 x $\sqrt{10}$ x 7.6 x 365/ln2).
In the case of the average human, this is achieved at an exposure of $\sqrt{10}$ x 2.5 pg/kg bw/day. So, as prescribed by the extrapolation procedure, the average and the sensitive human differ by a factor of $\sqrt{10}$ in the external exposure which leads to the WBC which is expected to be without reproductive risk.

Faqi study
In the Faqi study s.c. doses were repeatedly administered before mating as well as up to GD 14 of pregnancy. The net resulting *WBC* in dams at GD15 was calculated and added to the already present background WBC_{back}. The resulting sum, i.e. $WBC_{administered,animal,chronic}$, was considered to have arisen from a chronic dosing schedule, i.e. $F_{a,a\square c}$ is not needed, and therefore was, in concordance with the Ohsako study, used to calculate the chronic daily human intake $D_{r,h}$ which leads to this (summed) *WBC*.

The chronic daily human intake $D_{r,h}$ which leads to $WBC_{administered,animal,chronic}$ in humans, then is:

$$\frac{WBC_{total,animal,chronic}}{AF_{LOAEL\rightarrow NOAEL}} = D_{r,h} \cdot \frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{\ln 2} \qquad (3.9)$$

Modifying equation (3.9) then leads to:

$$HLV = \frac{(D_{s,a} + D_{s,a,back}) \cdot F_{abs,a,s.c.}}{AF_{LOAEL\rightarrow NOAEL} \cdot \frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{\ln 2} \cdot AF_{\mathrm{inter},dyn} \cdot AF_{\mathrm{intra},kin} \cdot AF_{\mathrm{intra},dyn}} \qquad (3.10)$$

Rearranging (3.10) then gives:

$$HLV = \frac{(D_{s,a} + D_{s,a,back})}{AF_{LOAEL\rightarrow NOAEL} \cdot \frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{\ln 2 \cdot F_{abs,a,s.c.}} \cdot AF_{\mathrm{inter},dyn} \cdot AF_{\mathrm{intra},kin} \cdot AF_{\mathrm{intra},dyn}}$$

thereby defining $AF_{inter,kin}$ as:

$$AF_{\mathrm{inter},kin} = \frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{F_{abs,a,s.c.} \cdot \ln 2} \qquad (3.11)$$

Parameter/Assessment factor	Value	Source
$D_{s,a}$	25 ng/kg-bw	Calculated
WBC_{back}	3 ng/kg-bw	Assumption
$D_{s,a,back}$	3 ng/kg-bw	Equivalent to WBC_{back}
$F_{abs,a,s.c.}$	1	Assumption
$F_{a\ to\ c}$	1	Assumption
$F_{abs,h,p.o.}$	0.5	Assumption
$t_{1/2,human}$	7.6 years	Experimental
$AF_{LOAEL\ to\ NOAEL}$	3	Default assumption
$AF_{interspecies,\ kin}$	2001 day	Calculated
$AF_{interspecies,dyn}$	1	Default assumption
$AF_{intraspecies,kin}$	$\sqrt{10}$	Default assumption
$AF_{intrasoecies,dyn}$	1	Default assumption

Note that in the Faqi study the *effective chronic s.c.* LOAEL at GD15 consists of the sum $D_{s,a}$ + $D_{s,a,back}$, i.e. 28.0 ng/kg bw and the extrapolated *effective chronic s.c.* NOAEL in the average rat 9.3 ng/kg bw (= 28/3). As complete absorption is assumed for the subcutaneous route of administration, i.e. $F_{abs,a.s.c.}$= 1, and the Faqi dosing protocol is assumed to mimic chronic fetal exposure, i.e. $F_{a\square c}$ = 1, the chronic WBC without effect in the average rat then also is 9.3 ng/kg-bw. As mentioned above for the Ohsako study, this WBC holds for the average rat, average man as well as sensitive man.

3.2 Extrapolation of repeated animal exposure to chronic human exposure

The extrapolation procedure presented in the foregoing paragraph leads (via the WBC) to a straightforward extrapolation of a *single, acute, dose* (in the Ohsako study: $D_{s,a}$ + $D_{s,a,back}$) into a *repeated, chronic*, human *intake* ($D_{r,h}$).
The selection of an acute single dose animal toxicity study as PoD for the calculation of a human HLV value however is rare and in practice is limited to reproductive toxicity studies like the Ohsako study. Merely repeated dose studies are selected as studies which deliver the PoD (note that the Faqi study in fact is a repeated dose study!). In these cases the WBC corresponding with the PoD may correspond with a "pre-steady state" in the case of sub-acute or semi-chronic exposure (equation 2.1) or a "steady state" in the case of chronic exposure (equation 2.3). Both cases lead to the explicit definition of an acute to chronic AF in the animal and an AF for interspecies differences in kinetics. As an example the extrapolation of the GD15 WBC in the Ohsako and the Faqi study will be re-presented here (the acute WBC's corresponding to GD15 will be reformulated as if they originated from a chronic daily exposure).

In the Ohsako study the time period between birth of a dam and GD15 of a dam's pregnancy is assumed to be long enough to let a repeated daily exposure reach a "steady state" in the dam's body (TCDD: half-life 20 days, a "steady state" being reached after an exposure period of 80 – 100 days, pregnancy assumed to be reached at an age beyond 100 days). Taking the WBC at GD15 (steps 1 and 2 in the foregoing paragraph) this WBC is expressed in terms of its corresponding repeated, daily, exposure in the (average) animal (step 3). This step basically extrapolates an acute exposure in the animal into its corresponding repeated equivalent, i.e. the repeated exposure which leads to the same WBC as after the acute exposure. Next the repeated, daily, exposure in the (average) animal is extrapolated to man leading to the repeated chronic, daily, exposure in the (average) human which does not cause adverse effects (step 4, comparable with step 3 in the foregoing paragraph). Finally the human exposure is corrected for interspecies differences in toxicodynamics and intraspecies extrapolation, resulting in the HLV for the sensitive human (step 5, interspecies extrapolation of toxicodynamics and intraspecies extrapolation, comparable with step 4 in the foregoing paragraph).

In the following each of the mentioned steps is worked out in detail.

<u>Extrapolation of exposure duration (animal, step 3)</u>

Ohsako study
The repeated daily p.o. intake by the animal $D_{r,a}$ which leads to the net chronic WBC at the level of the NOAEL in the Ohsako study, is:

$$D_{r,a} = \frac{WBC_{total,animal,chronic} \cdot \ln(2)}{t_{1/2,a} \cdot F_{abs,a,p.o.}} \qquad (3.12)$$

with:

$D_{r,a}$	Repeated daily p.o. animal intake per kg bw of TCDD
$F_{abs,a,p.o.}$	Absorption fraction of the daily p.o. intake in the animal
$WBC_{total,animal,chronic}$	Total WBC at the NOAEL in the animal after chronic exposure
$t_{1/2,a}$	Terminal half-life of TCDD in the animal

Relating the chronic external daily dose in the animal $D_{r,a.}$ in 3.12 via $WBC_{total,anima,chronic}$ (equation 3.3a) to the administered external p.o. dose, i.e. $D_{a,s} + D_{a,s,back}$ at GD15 (equations 3.2 and 3.5) then gives:

$$D_{r,a} = \frac{(D_{a,s} + D_{a,s,back}) \cdot F_{a\to c} \cdot F_{abs,a,p.o.} \cdot \ln(2)}{t_{1/2,a} \cdot F_{abs,a,p.o}} \qquad (3.13)$$

Basically equation (3.13) relates the single administered (total) p.o. dose at GD15 to its corresponding chronic daily animal dose which leads to exactly the same (total) *WBC* as occurring after a single administration. In classical toxicology the ratio between $D_{a,s} + D_{a,s,back}$ and $D_{r,a}$ stands for the acute to chronic AF ($AF_{a,a\ to\ c}$) in the animal experiment, or:

$$AF_{a,a\to c} = \frac{D_{a,s} + D_{a,s,back}}{D_{r,a}} = \frac{t_{1/2,a}}{F_{a\to c} \cdot \ln 2} \qquad (3.14)$$

Given a value of 1.7 for $F_{a\ to\ c}$ and 20 days for the half-life of 2378-TCDD in the rat then gives a value of 16.9.

Faqi study
The (external) repeated daily p.o. intake by the animal $D_{r,a}$ which leads to the net chronic WBC at the level of the NOAEL in the Faqi study, is:

$$D_{r,a} = \frac{WBC_{total,animal,chronic} \cdot \ln(2)}{t_{1/2,a} \cdot F_{abs,a,p.o.} \cdot AF_{L\to N}} \qquad (3.15)$$

with:

$D_{r,a}$.	Repeated daily p.o. animal intake per kg bw of TCDD
$F_{abs,a,p.o.}$	Absorption fraction of the daily p.o. intake in the animal
$WBC_{total,animal,chronic}$	Total WBC at the LOAEL in the animal after chronic exposure
$t_{1/2,a}$	Terminal half-life of TCDD in the animal
$AF_{L\ to\ N}$	LOAEL to NOAEL Assessment Factor

In concordance with equation 3.11 relating the chronic external daily dose in the animal $D_{r,a.}$ in 3.13 to the administered external p.o. dose, i.e. $D_{a,s} + D_{a,s,back}$ at GD15 then gives:

$$D_{r,a} = \frac{(D_{s,a} + D_{s,a,back}) \cdot F_{abs,a,s.c.} \cdot \ln(2)}{t_{1/2,a} \cdot F_{abs,a,p.o.} \cdot AF_{L\to N}} \qquad (3.16)$$

Basically equation (3.14) relates the single administered (total) external p.o. dose at GD15 to its corresponding chronic daily external dose in the animals which leads to exactly the same (total) *WBC* as occurring after a single administration. In classical toxicology the ratio between $D_{a,s}$ + $D_{a,s,back}$ and $D_{r,a}$ stands for the acute to chronic AF ($AF_{a\ to\ c}$) in the animal experiment, or:

$$AF_{a\to c} = \frac{D_{a,s} + D_{a,s,back}}{D_{r,a}} = \frac{t_{1/2,a} \cdot F_{abs,a,p.o}}{\ln(2) \cdot F_{abs,a,s.c.}} \qquad (3.17)$$

Given a value of 20 day for the half-life of 2378-TCDD in the rat and values of 0.61 and 1 for the p.o. resp. s.c. absorption in the rat then gives a value of 17.6 for $AF_{a\to c}$ (note that $AF_{a\to c}$ is a study specific AF which only applies to the Faqi study! Furthermore note that the $AF_{a\ to\ c}$ has the dimension day).

Interspecies extrapolation of kinetics (step 4)

For extrapolating $D_{r,a}$ to man, i.e. to obtain the life-long daily human p.o. dose which leads to the same total WBC as in the animal ($D_{r,h}$), an interspecies kinetic AF ($AF_{inter,kin}$) is needed. Assuming the WBC in the rat and in man to have reached a "steady state" level during pregnancy $D_{r,a}$ and $D_{r,h}$ scale towards each other as:

$$D_{r,h} = \frac{\left(\frac{D_{r,a} \cdot t_{1/2,a} \cdot F_{abs,a,p.o.}}{\ln(2)} \right) \cdot \ln(2)}{t_{1/2,h} \cdot F_{abs,h,p.o.}} \qquad (3.18)$$

with:

- $D_{r,a}$ repeated daily intake per kg bw without adverse effect in of the *average* animal
- $D_{r.h}$ repeated daily intake per kg bw without adverse effect in the *average*human

Equation 3.18 can be rewritten as:

$$D_{r,h} = \frac{D_{r,a}}{\left(\frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{t_{1/2,a} \cdot F_{abs,a,p.o.}} \right)} \qquad (3.19)$$

Here the denominator (between brackets) equals the interspecies AF for toxicokinetics ($AF_{inter\ ,kin}$):

$$AF_{\mathrm{inter},kin} = \frac{t_{1/2,h} \cdot F_{abs,h,p.o.}}{t_{1/2,a} \cdot F_{abs,a,p.o.}} \qquad (3.20)$$

For the oral route of administration, the substitution of $t_{1/2,animal}$ = 20 days, $F_{abs,a,p.o.}$ = 0.61, $t_{1/2,human}$ = 2774 days and $F_{abs,h,p.o.}$ = 0.5 then gives a value of 114 for $AF_{inter,kin}$. Note that the value of this AF is generic for rat-to-human extrapolation of TCDD toxicity data, i.e. applies to both the Ohsako and the Faqi study and all other rat toxicity studies of 2378-TCDD.

Interspecies extrapolation of toxicodynamics/intraspecies extrapolation (step 5)

Ohsako study
In general a HLV is obtained by applying a product of AFs on a PoD (see Introduction). In the case of the Ohsako study this leads to:

$$HLV = \frac{PoD}{AF_{a\to c} \cdot AF_{\mathrm{inter},kin} \cdot AF_{\mathrm{inter},dyn} \cdot AF_{\mathrm{intra},kin} \cdot AF_{\mathrm{intra},dyn}} \qquad (3.21)$$

Taking the sum of $D_{a,s}$ + $D_{a,s,back}$ as PoD then gives:

$$HLV = \frac{D_{s,a} + D_{s,a,back}}{AF_{a\to c} \cdot AF_{\mathrm{inter},kin} \cdot AF_{\mathrm{inter},dyn} \cdot AF_{\mathrm{intra},kin} \cdot AF_{\mathrm{intra},dyn}} \qquad (3.22)$$

Substituting the effective p.o. NOAEL (= $D_{s,a}$ + $D_{s,a,back}$) of this study of 15400 pg/kg bw, 16.9 as $AF_{a->c}$, 114 for $AF_{interspecies,kin}$, 1 for inter- and intraspecies toxicodynamic AFs ($AF_{interspecies,dyn}$; $AF_{intraspecies,dyn}$),and $\sqrt{10}$ for the inter-human differences in kinetics ($AF_{intraspecies,kin}$) then results in a HLV of 2.5 pg/kg bw/day (Quod Est Demonstrandum).

Parameter/Assessment factor	Value	Source
$D_{s,a}$	12.5 ng/kg-bw	Experimental
WBC_{back}	3 ng/kg-bw	Assumption
$D_{s,a,back}$	2.9 ng/kg-bw	Calculated
$F_{abs,a,p.o}$	0.61	Experimental
$F_{a\ to\ c}$	1.7	Experimental
$F_{abs,h,p.o.}$	0.5	Assumption
$t_{1/2,h}$	7.6 years	Experimental
$t_{1/2,a}$	20 days	Experimental
$AF_{a\ to\ c}$	16.9 day	Calculated
$AF_{interspecies,kin}$	114	Calculated
$AF_{interspecies,dyn}$	1	Default assumption
$AF_{intraspecies,kin}$	$\sqrt{10}$	Default assumption
$AF_{intraspecies,dyn}$	1	Default assumption

Faqi study

In concordance with the Ohsako study the general approach to derive a HLV in the Faqi study is:

$$HLV = \frac{PoD}{AF_{a\to c} \cdot AF_{L\to N} \cdot AF_{\mathrm{inter},kin} \cdot AF_{\mathrm{inter},dyn} \cdot AF_{\mathrm{intra},kin} \cdot AF_{\mathrm{intra},dyn}} \qquad (3.23)$$

Taking the sum of $D_{a,s}$ + $D_{a,s,back}$ as PoD then gives:

$$HLV = \frac{(D_{s,a} + D_{s,a,back})}{AF_{a\to c} \cdot AF_{L\to N} \cdot AF_{\mathrm{inter},kin} \cdot AF_{\mathrm{inter},dyn} \cdot AF_{\mathrm{intra},kin} \cdot AF_{\mathrm{intra},dyn}} \qquad (3.24)$$

Substituting the effective s.c. LOAEL (= $D_{s,a}$ + $D_{s,a,back}$) of this study, i.e. 28000 pg/kg bw, and 17.6 for$AF_{a\ to\ c}$, 3 for $AF_{L->N}$, 114 for $AF_{1,kin}$, 1 for inter- and intraspecies toxicodynamic AFs ($AF_{inter,dyn}$; $AF_{intra,dyn}$), and $\sqrt{10}$ for the inter-human differences in kinetics ($AF_{intra,kin}$) then results in a HLV of 1.5 pg/kg bw/day.

Parameter/Assessment factor	Value	Source
$D_{s,a}$	25 ng/kg-bw	Calculated
WBC_{back}	3 ng/kg-bw	Assumption
$D_{s,a,back}$	3 ng/kg-bw	Equivalent to WBC_{back}
$F_{abs,a,s.c.}$	1	Assumption
$F_{a\ to\ c}$	1	Assumption
$F_{abs,h,p.o.}$	0.5	Assumption
$t_{1/2,h}$	7.6 years	Experimental
$t_{1/2,a}$	20 days	Experimental
$AF_{L\ to\ N}$	3	Default assumption
$AF_{inter,kin}$	114	Calculated
$AF_{a\ to\ c}$	17.6 day	Calculated
$AF_{inter,dyn}$	1	Default assumption
$AF_{intra,kin}$	$\sqrt{10}$	Default assumption
$AF_{intra,dyn}$	1	Default assumption

4. Provisional Human Limit Value of BDE47

As 2,3,7,8-TCDD the flame retardant 2,2',4,4'-bromodiphenyl ether (BDE-47) is a persistent organic pollutant in experimental animals and humans. BDE-47 shows significant interspecies differences in elimination kinetics (Bakker *et al.*, 2008; Geyer *et al.*, 2004; von Meyerink *et al.,* 1990) and accumulates solely in organ lipid (Sanders *et al.*, 2006; Staskal *et al.*, 2006a). These features make BDE-47 a likely candidate too for the application of the WBC as the starting point for the interspecies extrapolation of animal toxicity, i.e. the derivation of a HLV. In this context the extrapolation procedure presented in the foregoing paragraph therefore was directly applied on BDE-47. However, in the case of 2,3,7,8-TCDD the extrapolation procedure is overtly conservative. The reason for this is the application of worst case, deterministic, values for parameters that are used as input, such as the PoD, AFs, and kinetic parameters. This procedure leads to the piling up of worst case assumptions. As an alternative a probabilistic approach may be applied. In such an approach, the input parameters just are considered uncertain, i.e. are characterized as variables with a characteristic uncertainty distribution. The uncertainty in the assessment can be evaluated using Monte Carlo simulation. This yields a HLV in humans with uncertainty being characterized by a Confidence Interval (CI). Taking the uncertainty into account, the lower confidence bound of this dose may be regarded as the HLV (Baird *et al.*, 1996; Bokkers, 2007; 2009; Slob and Pieters, 1998; Vermeire *et al.*, 1999).

4.1 Identification of the critical toxic endpoint(s)

The toxicity of BDE-47 has recently been reviewed by US-EPA (2008). This review identified the developing nervous system as the most sensitive target organ, with disturbance of behavioral effects being the critical endpoint in neonatal (male) mice following a single exposure to BDE-47 (Eriksson *et al.*, 2001).
As EPA's review summarized all relevant toxicity studies up to November 2007 this report updates this review to December 2009. To be included in the present analysis, the toxicity studies need to meet four general quality criteria:

(1) The experiment should include at least two dose groups and a control group with at least three subjects per group.

(2) In the absence of suitable human data, to enable extrapolation to humans, a toxicity study should be performed *in vivo* in mammals (in this context mammals are considered surrogates for humans).

(3) The experimental setup should be described in sufficient detail, e.g.: species, strain, sex, body weight and age of the subjects, route of exposure, duration of exposure, should be given to enable the estimation of the whole body BDE-47 concentration.

(4) The experimental results should be presented in sufficient detail to enable dose-response analysis. Preferably, results should be presented in a table as raw data. However, summary data (mean, standard deviation, and sample size) can also serve as input. Data may be retrieved from graphs using scanning software.

Next to the already mentioned study of Eriksson *et al.* (2001) four additional studies were obtained which met the criteria above: Abdelouahab *et al.* (2009), He *et al.* (2009), Richardson *et al.* (2008) and Suvorov *et al.* (2009). A summary of these studies is given below.

Eriksson study
The Eriksson (2001) study identified neurodevelopmental toxicity on the developing brain of neonatal mice as a sensitive toxic effect of BDE-47. In this study neonatal mice were exposed by gavage to a single dose (0, 0.7 and 10.5 mg/kg bw) at PND 10. BDE-47 was administered in a 20 % weight:water peanut oil emulsion. At the age of 2 and 4 months the mice were tested on their spontaneous motor behavior and habituation capability, i.e. the ability to explore a new environment. BDE-47 dose-dependently disturbed the habituation behavior of the mice.

He study
The He (2009) study identified neurodevelopmental toxicity on the developing brain of neonatal rats as a sensitive toxic effect of BDE-47. Rats were exposed to a single oral gavage dose of vehicle (corn oil), 1, 5, or 10 mg BDE-47/kg bw at PND 10. At 2 months of age the total distance swam by rats (6 per sex) to reach an escape platform was increased and the ratio of distance taken in the platform quadrant to total distance was notably decreased in all treated groups in the water maze experiment compared to the control. Furthermore, structural neuron alterations were observed.

Abdelouahab study
Abdelouahab *et al.* (2009) exposed pregnant sheep i.v. to vehicle (emulsifier cremophore EL/ethanol 95 %/sterile water: 1:1:8, v/v) or BDE-47 (0.2, 2, and 20 µg/kg bw/week) from the 5^{th} to 15^{th} week of gestation. At delivery (19^{th} week of gestation) a decrease in total T3 in cord blood was found. BDE-47 concentrations were measured in subcutaneous fat at delivery.

Richardson study
In the Richardson (2008) study mice were exposed by gavage at the age of 9 weeks to 3, 10, or 100 mg BDE-47/kg bw/day dissolved in corn oil for four consecutive days. At 24 hours after the last dose was administered a decrease in the total T4 serum level was found.

Suvorov study
In Suvorov (2009) rat dams were exposed to vehicle (emulsifier cremophore EL/ethanol 95 %/sterile water: 1:1:8, v/v) or BDE-47 (0.002, 0.02 and 0.2 mg/kg bw) each 5 days from GD 15 to PND 20 by i.v. injections. Total (T) and free (F) T3 and T4 blood concentrations and BDE-47 concentrations in adipose tissue were measured in pups and dams on PND 27.

4.2 Dose-response analysis of selected toxicity

The uncertainty in the PoD can be characterized by dose-response analysis with the PROAST software (Slob, 2002). This results in a benchmark dose (BMD), which relates to a particular (predefined) critical effect size (CES). Two families of nested dose response models, the Exponential and Hill models, present in PROAST were fitted to the (continuous) toxicity data. This procedure accounts for the incorporation of model uncertainty in the dose-response analysis. For the evaluated endpoints, both models resulted in acceptable fits (based on the log-likelihood criteria, see Slob, 2002). The bootstrap technique (1000 runs) was used to generate a BMD distribution for each model (Moerbeek *et al.*, 2004). Both BMD distributions were subsequently combined to generate an overall BMD distribution. In the applied probabilistic risk assessment approach, the whole uncertainty distribution around the BMD is used as an input (Bokkers, 2009; Slob and Pieters, 1998).

The quality of the dose-response data was checked by applying the criteria for the application of dose-response modeling in risk characterization as developed by EFSA (2009). Dose-response data are considered poor, and therefore not informative, when one (or more) of the following criteria are met:

1) the confidence interval around the BMD is wide
2) different models result in widely different BMDL values
3) the BMD is estimated by extrapolation outside the range of observation, such that the BMD(L) would then depend heavily on the model used.

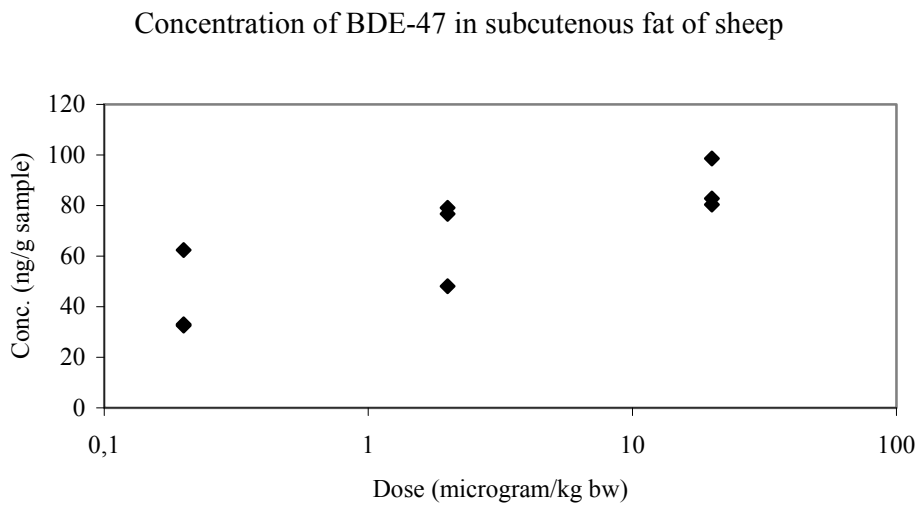
Criteria to judge the adequacy of the dose-response data on the basis of the range of BMDL values obtained (criterion no. 2) have so far not been established. EFSA (EFSA, 2009) proposes that, as a general rule, dose-response data should not result in a range of BMDL values from different accepted models that substantially exceeds one order of magnitude. The other two criteria are not quantified either. For consistency reasons, we propose that criteria no. 1 and 3 should meet this requirement too. Thus, the upper and lower limits of the 90% CI should not exceed one order of magnitude. Furthermore, the BMD should not be 10 times higher than the highest applied dose level, or 10 times lower than the lowest applied dose level.

The outcome of the dose-response analyses is presented in detail in Technical Annex 2. Here we suffice by summarizing the BMD distributions of the various studies by their best estimates and CIs in Table 4.1.

In the Eriksson study, the endpoint resulting in the lowest BMD was rearing. A single dose of 0.184 mg/kg bw (90% CI: 0.072-0.306) resulted in a 5% decrease in rearing. The CES of -5% was applied as a default. It is assumed that changes in activity less than 5% are not adverse. The analysis of the Eriksson study was in concordance with the mentioned EFSA criteria.

Both endpoints in the He study did not meet the quality criteria for a BMD analysis. A closer look at the data (see Appendix) showed that the doses applied in this study are too high. There are no doses which approximately result in the response of interest, i.e. the CES of 5%. Therefore, the He study was not further used in the derivation of a HLV.

In the studies of Abdelouahab (2009) and Suvorov (2009), the BMDs are expressed as adipose tissue concentrations. Strictly speaking the term benchmark concentration would be more appropriate, however, for simplicity sake we will refer to these concentrations as BMD.
The Abdelouahab data display only a marginal 3-fold increase in adipose tissue concentration over a 100-fold increase in the administered dose level (see Figure below). In the case of the Suvorov study the adipose tissue concentration increased from 7 $\pm$ 2 ng/g lipid in untreated controls to 12.6 $\pm$ 3.6, 21.0 $\pm$ 2.6 and 234.3 $\pm$ 176.7 ng/g lipid in the 0.002, 0.02 and 0.2 mg/kg bw dose groups. From this it can be concluded that, in contrast to the Suvorov study, the adipose tissue measurements in the Abdelouahab study barely contain information on the administered dose level. This, of course, poses question marks on the validity of the adipose tissue measurements in the Abdelouahab study as indicator of the administered dose levels. As the adipose tissue data of the Abdelouahab study do contain dose-response BMD information (see below) these data were further analysed, however, for reasons of completeness only.



The concentration of BDE-47 in subcutaneous fat of pregnant sheep at delivery (19th week of gestation). Exposure: weekly intravenous injections to 0, 0.2, 2 and 20 µg/kg bw/week during the 5th to the 15th week of gestation. Study: Abdelouahab (2009).

Based on the Abdelouahab and Suvorov studies, BMDs of 16.8 (90% CI: 10.7-28.5) and 267 (90% CI: 174-558) ng/kg fat were obtained, respectively. The CES of -10% was obtained from Van der Ven *et al.* (2006). The BMD analysis of both the Abdelouahab and the Suvorov study were in concordance with the mentioned EFSA criteria.

In the Richardson study, a BMD of 18.69 (90% CI: 15.11-21.82) mg/kg bw/day was derived for a 10% decrease of thyroid hormone concentration. Note that a comparison of this BMD with those obtained from the Abdelouahab and Suvorov studies is not straight forward because the experimental setup and dose units differ. At a later stage in the hazard characterization the results of these three studies can be compared. Only then it can be determined which one is the most critical thyroid hormone study.
The BMD analysis of the Richardson study was in concordance with the mentioned EFSA criteria.

Table 4.1 Overview of BMDs and 90% CIs (benchmark dose lower limit: BMDL, benchmark dose upper limit: BMDU. Critical effect sizes (CES) are negative or positive depending on a decreasing or increasing response. Bold BMDs are used for further hazard characterization.

Endpoint	Model[a]	CES (%)	BMD	90% CI		Unit	Reference
				BMDL	BMDU		
Neurodevelopmental toxicity (locomotion) (40-60 min)	E	+5	0.173	0.158	0.191	mg/kg bw	Eriksson (2001)
	H	+5	0.530	0.265	0.541		
Neurodevelopmental toxicity (rearing) (0-20 min)	E	-5	0.278	0.251	0.312		
	H	-5	0.087	0.067	0.114		
	E & H	-5	0.184	0.072	0.306		
Neurodevelopmental toxicity (rearing) (40-60 min)	E	+5	4.93	0.512[b]	8.58[b]		
	H	+5	0.576	0.290	0.589		
Neurodevelopmental toxicity (total activity) (0-20 min)	E	-5	0.772	0.659	0.934		
	H	-5	0.531	0.422	0.688		
Neurodevelopmental toxicity (total activity) (40-60 min, age: 2 months)	E	+5	0.732	0.566	1.04		
	H	+5	1.00	0.698	1.306		
Neurodevelopmental toxicity (total activity) (40-60 min, age: 4 months)	E	+5	0.470	0.395	0.579		
	H	+5	0.757	0.410	0.892		
Neurodevelopmental toxicity (location navigation)	E	+5	0.075[c]	0.049	0.109	mg/kg bw	He (2009)
	H	+5	0.051[c]	0.025	0.087		
Neurodevelopmental toxicity (space exploration)	E	+5	0.212	0.017[b]	0.680[b]		
	H	+5	0.166	$5.6*10^{-11}$ [b]	0.741[b]		

[a] E = exponential model, H = Hill model, E & H = results of both models combined
[b] BMDL is more than 10 times smaller than the BMDU, quality criterion 1
[c] BMD is more than 10 times smaller than the lowest dose tested, quality criterion 3.

Table 4.1 (continued)

Endpoint	Model[a]	CES (%)	BMD	90% CI		Unit	Reference
				BMDL	BMDU		
Thyroid toxicity (TT3)	E	-10	18.16	13.15	29.37	ng/g fat	Abdelouahab (2009)
	H	-10	15.12	9.956	26.85		
	E & H	-10	16.8	10.7	28.5		
Thyroid toxicity (TT4)	E	-10	20.45	19.04	22.08	mg/kg bw/day	Richardson (2008)
	H	-10	16.20	14.79	17.83		
	E & H	-10	18.69	15.11	21.82		
Thyroid toxicity (TT4 & FT4)	E	-10	266	177	542	ng/g fat	Suvorov (2009)
	H	-10	267	172	557		
	E & H	-10	267	174	558		

[a] E = exponential model, H = Hill model, E & H = results of both models combined
[b] BMDL is more than 10 times smaller than the BMDU, quality criterion 1
[c] BMD is more than 10 times smaller than the lowest dose tested, quality criterion 3.

4.3 Deriving the benchmark dose in humans

As mentioned in the previous paragraph, two critical toxic effects emerge for BDE-47 toxicity: neurodevelopmental toxicity as induced by exposure during neonatal life (Eriksson and He) or thyroid toxicity as induced during young adulthood (Richardson), by prenatal exposure (Abdelouahab) or by combined pre-/postnatal exposure (Suvorov). With regard to human risk, the effects represent *direct* exposure during neonatal or young adulthood (studies of Eriksson and Richardson) and *indirect* exposure of offspring during pregnancy and directly after birth (Abdelouahab and Suvorov). BMD_hs were derived based on the animal BMDs from the studies of Eriksson, Abdelhouahab, Richardson, and Suvorov (as mentioned above the results of the He study did not meet the quality criteria for dose-response modeling).

Kinetics
The explicit incorporation of interspecies differences in the accumulation kinetics of BDE-47 as outlined in chapters 2 and 3 needs several kinetic parameter values (fraction absorbed in the animal and human food, the elimination rate constant in the animal and man, the body fat fractions of rat and sheep). Table 4.2 presents these parameter values as compiled from the literature. Based on these values beta and lognormal distributions were constructed to allow for the uncertainty of the fractions absorbed and the half-lives respectively. Due to the limited information on absorption and half-life it was assumed that the confidence intervals of the rodents rat and mouse half-lives are the same.
Beta distributions were constructed for absorption fractions, because these distributions are restricted to include values from zero to one. Based on the values in Table 4.2 a minimum (min), maximum (max), and most likely (ml) value was derived, which enables approximation of mean (μ) and variance (σ), and subsequently of α and β:

$$\mu = \frac{min + 4 \cdot ml + max}{6}$$

$$\sigma = \frac{max - min}{6}$$

$$\alpha = \mu\left(\frac{\mu(1-\mu)}{\sigma^2} - 1\right)$$

$$\beta = (1-\mu)\left(\frac{\mu(1-\mu)}{\sigma^2} - 1\right)$$

Half-lives are restricted to positive values with values reaching from zero to (in principle) infinity. Log-normal distributions suit this conditions well. The most likely value is set as mean (on log-scale), and the SD is derived by:

$$SD = \frac{max - ml}{z(p)},$$

where SD, max, and ml are on log-scale and z(p) is a high percentile of the standard normal distribution. Here, we used the 95th percentile, which is 1.645.

The distributions and their summary data[8] are presented in Table 4.3.
Information about the fraction absorbed and half-life in sheep was not available. This hampers the explicit derivation of an interspecies (sheep-to-human) AF. However, the concentration of BDE-47 in adipose tissue of sheep was reported by Abdelouahab, which enables incorporation of kinetics. This will be further discussed below.
Note that half-life ($t_{½}$), as reported in the tables, can be converted to elimination rate (k_{el}), as applied in the various equation, as follows:

$$k_{el} = \frac{\ln 2}{t_{1/2}}$$

The fraction body fat of the average pregnant rat lies between 0.06 and 0.12 (Fisher *et al.*, 1989; 1991). For pregnant sheep the average fraction is 0.19 with a 90% CI of 0.16-0.22 (Foot and Greenhalgh, 1970). Based on these data beta distributions were constructed to describe the body fat fraction (Table 4.4).

[8] Based on 100,000 random samples of the proposed parametric distribution

Table 4.2 Absorption fractions and half-lives for BDE-47 as obtained from literature.

Species	Vehicle	Route	F_{abs}	$t_{½}$ (95% CI) (days)	Reference
Human	na[a]	na		767-803[d]	(Bakker *et al.*, 2008)
Human	na[b]	na	0.96	664 (556-926)	(Geyer *et al.*, 2004)
Human	na[c]	na		1096	(Geyer *et al.*, 2004)
Rat	nr	nr		21.4	(Geyer *et al.*, 2004)
Rat	nr	p.o.,	>0.98		(Meijer *et al.*, 2003)
Rat, female	Peanut oil	p.o.		29.9 (26.8-33.1)	(von Meyerinck *et al.*, 1990)
Rat, male	Peanut oil	p.o.		19.1 (16.5-21.7)	(von Meyerinck *et al.*, 1990)
Mouse	emulsifier cremophore EL/ethanol 95 %/sterile water and corn oil	i.v. and p.o.	0.85		(Sanders *et al.*, 2006)
Rat	As above	as above	0.75		(Sanders *et al.*, 2006)
Mouse	Corn oil	p.o.	0.82	23	(Staskal *et al.*, 2005)

nr: not reported

na: not available, because values are obtained indirectly

[a] derived from Dutch human body burden and daily intake

[b] derived from Swedish human body burden and daily intake

[c] derived from rat data

[d] minimum and maximum values

Table 4.3 Absorption fraction and half-life uncertainty distributions

Species	Parameter	Distribution[a]	Summary data of this distribution	
			Median	90% CI
Mouse	F_{abs}	beta(173, 30.5)	0.85	0.81-0.89
	$t_{½}$	ln(3.1, 0.18)	22.2	16.5-29.8
Rat	F_{abs}	beta(52.4, 14.5)	0.79	0.70-0.86
	$t_{½}$	ln(3.2, 0.18)	24.5	18.2-33.0
Human	F_{abs}	beta(141,6.4)	0.96	0.93-0.98
	$t_{½}$	ln(6.67, 0.014)	788	770-807

[a] beta(α,β), ln = lognormal(mean, sd on log-scale)

Table 4.4 Uncertainty distributions of the fat fraction

Species	Distribution[a]	Summary data of this distribution	
		Median	90% CI
Rat	beta(36, 393)	0.08	0.06-0.11
Sheep	beta(73, 311)	0.19	0.16-0.22

[a] beta(α,β)

4.3.1 Eriksson study

Taking PND 10 as a critical window for the induction of neonatal neurodevelopmental toxicity in mice, the single p.o. dose, i.e. the BMD of 0.184 mg/kg bw, obtained from the Eriksson study can be converted to a WBC by multiplying with the fraction of the oral dose that is absorbed:

$$WBC_a = D_{s,a} \cdot F_{abs,a} \qquad (4.1)$$

with:

WBC_a	Whole Body Concentration in the average animal (at PND 10)
$D_{s,a}$	Single dose in the average animal
$F_{abs,a}$	Absorption fraction of the single p.o. dose by the animal

The resulting WBC_a is 150 µg/kg bw (90% CI: 60-260).

Acute-to-chronic extrapolation

In accordance with equation 2.1, this PND 10 WBC_a can be expressed in terms of an (external) repeated dose which leads to this WBC_a at PND 10:

$$D_{r,a} = \frac{WBC_a \cdot k_{el,a}}{F_{abs,a} \cdot (1 - e^{-k_{el,a} \cdot t})} \qquad (4.2)$$

with:

$D_{r,a}$	Repeated dose in the average animal
$k_{el,a}$	elimination rate in the average animal
t	Repeated dose duration (time), in this situation from birth to PND 10, i.e. 10 days.

Substituting (4.1) into (4.2) then gives:

$$D_{r,a} = \frac{D_{s,a} \cdot k_{el,a}}{(1 - e^{-k_{el,a} \cdot t})} \tag{4.3}$$

The resulting $D_{r,a}$ is 21 µg/kg bw/day (90% CI: 8-36).

Expressing an single dose ($D_{s,a}$) into a repeated dose rate ($D_{r,a)}$ in fact comes, in more traditional terms, down to an acute-to-chronic extrapolation which can be expressed by an acute-to-chronic AF ($AF_{a \text{ to } c}$). The $AF_{a\square c}$ can be obtained by:

$$AF_{a \to c} = \frac{D_{s,a}}{D_{r,a}} = \frac{(1 - e^{-k_{el,a} \cdot t})}{k_{el,a}} \tag{4.4}$$

which gives an $AF_{a\square c}$ of 8.6 days (90% CI: 8.2-8.9).

Interspecies extrapolation of kinetics

Rearranging equation 4.2 gives for the WBC in the animal at PND 10:

$$WBC_a = \frac{D_{r,a} \cdot F_{abs,a} \cdot (1 - e^{-k_{el,a} \cdot t})}{k_{el,a}} \tag{4.5}$$

Whereas the time window in humans corresponding with the time-window of PND 10 for neurodevelopmental toxicity in mice is not exactly known it certainly lies before adulthood is reached. Now, given a half-life of around 2 years in humans a chronic exposure in man is expected to lead to a steady state at the age of 10-12 years. Assuming the human exposure to reach a steady state which equals the WBC at PND 10 in mice thus guarantees that the human WBC before the age of 10-12 years will stay below the PND 10 WBC level in mice and just at this level beyond the age of 12 years. The chronic daily human exposure which meets this criterion is obtained by solving:

$$WBC_a = \frac{D_{r,h} \cdot F_{abs,h}}{k_{el,h}} \tag{4.6}$$

with:

$D_{r,h}$	Repeated dose in the average human
$F_{abs,h}$	oral absorption fraction by human
$k_{el,h}$	elimination rate in the average human

Note that the calculated $D_{r,h}$ garantees that in the time period between birth and adult age the WBC never exceeds the PND 10 WBC in mice. Were PND 10 in mice to correspond with the age period between 0-12 years in man the approach taken is to be considered conservative, i.e. to lead to an overestimation of $D_{r,h}$ and, consequently, the HLV. This, of course, is to be considered as a legitimate application of the precaution principle.

Combining equations 4.5 and 4.6 then gives the interspecies kinetic extrapolation factor ($AF_{interspecies,kin}$):

$$AF_{\text{int}erspecies,kin} = \frac{D_{r,a}}{D_{r,h}} = \frac{k_{el,a} \cdot F_{abs,h}}{k_{el,h} \cdot F_{abs,a}} \cdot \frac{1}{(1-e^{-k_{el,a} \cdot t})} \qquad (4.7)$$

Substitution of $k_{el,a}$, $F_{abs,a}$, $k_{el,h}$, and $F_{abs,h}$ with the values given in table 5.3 results in 149 (90% CI: 139-162) for the (mouse-to-human) $AF_{interspecis,kin}$

Interspecies extrapolation of dynamics

According to the subdivision of the interspecies factor into a kinetic and a dynamic part (IPCS, 1994), the AF for interspecies dynamics should be 2.5. We assume that this factor is regarded as a worst case assumption and factors of less than 2.5 and even less than 1 are plausible as well. Therefore an uncertainty distribution was constructed with median 1 and an upper (95%) confidence limit of 2.5: ln(0,0.56).

Deriving the BMD_h

The general approach to derive an HLV is applied according to equation (1.2). However, here the uncertainties in the assessment are incorporated. This results in a benchmark dose in humans with a confidence interval. Basically the PoD distribution is divided by several AF distributions accounting for acute-to-chronic and interspecies extrapolation:

$$BMD_h = \frac{PoD}{AF_{a \to c} \cdot AF_{\text{int}erspecies,kin} \cdot AF_{\text{int}erspecies,dyn}} \qquad (4.8)$$

Substituting distributions for $D_{s,a}$ as PoD, $AF_{a\ to\ c}$, $AF_{interspecies,kin,}$, and $AF_{interspecies,dyn}$ results in a BMD_h and its 90% CI of 0.13 (0.032-0.47) µg/kg bw/day. In this extrapolation no intraspecies AF is applied. The reason for this is that the toxicity experiments were already performed in sensitive subpopulation, i.e.neonatal animals. These animal toxicity data are extrapolated to an equivalent human population, i.e. young children, which are already considered as sensitive subpopulation.

4.3.2 Abdelouahab and Suvorov studies

Both the Abdelouahab and Suvorov studies provide an internal dose metric which can directly be linked to the toxicity of interest: the concentration of BDE-47 in maternal adipose tissue at delivery (Abdelouahab) or at the end of the relevant exposure period, i.e. PND 27 (Suvorov).

The concentrations in adipose tissue as obtained from the Abdelouahab and Suvorov studies, 16.8 and 267 ng/kg fat respectively (Table 5.1), need to be converted to WBCs. Several distribution studies (Orn and Klasson-Wehler, 1998; Sanders *et al.*, 2006; Staskal *et al.*, 2005; Staskal *et al.*, 2006a, b) indicate that a large fraction (>0.8) of BDE-47 in the body resides in the adipose tissue. As a conservative assumption, we pose that nearly all of the BDE-47 present in a body accumulates in the adipose tissue. Therefore, the adipose tissue concentrations can be multiplied by the body fat fraction to obtain the WBCs:

$$WBC_a = C_{fat,a} \cdot F_{fat,a} \quad (4.9)$$

with:

$C_{fat,a}$	adipose tissue concentration in the average (maternal) animal
$F_{fat,a}$	Fraction adipose tissue in the average (maternal) animal

The resulting WBC_a for rat is 23 µg/kg bw (90% CI: 13-49) (Suvorov). The WBC_a for sheep is 4.5 µg/kg bw (90% CI: 2.5-8.5) (Abdelouahab). Note that this concentration is considered as the maternal WBC at the BMD level.

The WBC_a can be expressed in terms of a chronic (external) intake by:

$$D_{r,a} = \frac{WBC_a \cdot k_{el,a}}{F_{abs,a}} \quad (4.10)$$

For the Suvorov study the resulting $D_{r,a}$ for rat is 0.82 µg/kg bw/day (90% CI: 0.44-1.9) for the rat. This $D_{r,a}$ for rat is considered as PoD.

Unfortunately, in the case of the Abdelouahab study, the $D_{r,a}$ could not be calculated. The reason for this is the absence of basic kinetic index numbers for BDE-47 in sheep, i.e. the half-live and the absorption fraction of BDE-47 in sheep.
However, as shown in sections 2-4 the derivation of $D_{r,a}$ in calculating $D_{r,h}$ may be circumvented by skipping equation (4.10) and directly go from equation (4.9) to equation (4.11) (see below).

Interspecies extrapolation of kinetics

Suvorov study
Extrapolating the $D_{r,a}$ as obtained in the Suvorov study needs interspecies extrapolation of steady state kinetics. As in the animal the critical exposure window consists of pregnancy and the breast feeding period steady state WBC kinetics are a reasonable assumption with regard to women of childbearing age. As mentioned before, the interspecies assessment factor $AF_{interspecies,kin}$ as in equation (3.18) may be used for this purpose. Substitution of $t_{1/2,a}$ = 24.5 days, $F_{abs,a,p.o.}$ = 0.79, $t_{1/2,h}$ = 788 days and $F_{abs,h,p.o.}$ = 0.96 (see Table 4.3) results in a (rat-to-human) $AF_{interspecies,kin}$ of 39 (90% CI: 29-54).

Abdelouahab study
In the case of the Abdelouahab study WBC in the animals has to be linked directly to $D_{r,h}$ by:

$$D_{r,h} = \frac{WBC_a \cdot k_{el,h}}{F_{abs,h}} \quad (4.11)$$

The $D_{r,h}$ based on the sheep data is 0.004 µg/kg bw/day (90% CI: 0.002-0.008). This $D_{r,h}$ is considered as PoD.

Interspecies extrapolation of dynamics

According to the subdivision of the interspecies factor into a kinetic and a dynamic part (IPCS, 1994), the AF for interspecies dynamics should be 2.5. We assume that this factor is regarded as a worst case assumption and factors of less than 2.5 and even less than 1 are plausible as well. Therefore an uncertainty distribution was constructed with median 1 and an upper (95%) confidence limit of 2.5: ln(0,0.56).

Deriving the BMD_h

Suvorov
Basically the PoD is divided by several AFs accounting for interspecies extrapolation:

$$BMD_h = \frac{PoD}{AF_{\text{int}erspecies,kin} \cdot AF_{\text{int}erspecies,dyn}} \quad (4.12)$$

Substituting the $D_{r,a}$ mentioned above as PoD and $AF_{interspecies,kin}$, and $AF_{interspecies,dyn}$ then gives the BMD_h . As in the Eriksson study, an intraspecies AF was not applied. The reason for this is that the toxicity experiments were already performed in a sensitive subpopulation, i.e. the pregnant dam. These animal toxicity data are extrapolated to an equivalent human population, i.e. pregnant women, which are already considered as sensitive subpopulation.

In this way a BMD_h distribution with median 0.022 µg/kg bw/day and 90% CI 0.0072-0.070 µg/kg bw/day was obtained.

Abdelouahab
As $D_{r,h}$ already is a human dose, it only has to be corrected for interspecies differences in dynamics extrapolation. An intraspecies AF is not applied. The reason for this is that the toxicity experiments were already performed in a sensitive subpopulation, i.e. the pregnant dam. These animal toxicity data are extrapolated to an equivalent human population, i.e. pregnant women, which are already considered as sensitive subpopulation. Taking the $D_{r,h}$ as the PoD the BMD_h then is calculated as:

$$BMD_h = \frac{PoD}{AF_{\text{int}erspecies,dyn}} \quad (4.13)$$

In this way a BMD_h distribution with median 0.0030 µg/kg bw/day and 90% CI 0.0010-0.0087 µg/kg bw/day was obtained.

4.3.3 Richardson study

At 9 weeks of age, mice were exposed for 4 days to BDE-47 with thyroid toxicity being determined 24 hours after the last exposure. Given this short exposure period cumulative accumulation of the administered BDE-47 in the body is a reasonable assumption. The BMD obtained from the Richardson study can be converted to a WBC by multiplying with 4 times the fraction of the oral dose that is absorbed because the half life of BDE-47 in mice is longer than 4 days ((Staskal *et al.*, 2005), see Table 4.2).

$$WBC_a = 4 \cdot D_{r,a} \cdot F_{abs,a} \qquad (4.14)$$

The resulting WBC_a is 63 (90% CI: 51-75) mg/kg bw/day. The $D_{r,h}$ relating to this WBC can be derived with equation (5.11) resulting in a value of 0.058 (90% CI:0.047 - 0.069) mg/kg bw. The $AF_{interspecies,kin}$ is obtained by applying equation 5.7). This results in a (mouse-to-human) $AF_{interspecis,kin}$ of 320 (90% CI: 300-340).

Interspecies extrapolation of dynamics
According to the subdivision of the interspecies factor into a kinetic and a dynamic part (IPCS, 1994), the AF for interspecies dynamics should be 2.5. We assume that this factor is regarded as a worst case assumption and factors of less than 2.5 and even less than 1 are plausible as well. Therefore an uncertainty distribution was constructed with median 1 and an upper (95%) confidence limit of 2.5: ln(0,0.56).

Intraspecies extrapolation
In contrast to the other studies, the Richardson study is not performed with a sensitive subpopulation. Therefore, an intraspecies AF is required. A log-normal distribution with a geometrical mean of 3 (+1) and a geometrical standard deviation (GSD) of 1.6 according to Slob and Pieters (1998) is applied. The shift of +1 ensures that the intraspecies AF is always larger than 1, as sensitive humans cannot be less sensitive than the average human. Furthermore the distribution was developed to be consistent with the default AF of 10. Hence, the GSD of the distribution is chosen such that its 99th percentile equals 10.

Deriving the BMD_h
The general approach to derive an HLV is applied according to equation (1.2). However, here the uncertainties in the assessment are incorporated. This results in a benchmark dose in humans with a confidence interval. Basically the PoD distribution is divided by several AF distributions accounting for inter- and intraspecies extrapolation:

$$BMD_h = \frac{PoD}{AF_{\text{int}erspecies,kin} \cdot AF_{\text{int}erspecies,dyn} \cdot AF_{\text{int}raspecies}} \qquad (4.15)$$

Substituting distributions for $D_{r,a}$ as PoD, $AF_{interspecies,kin,}$, $AF_{interspecies,dyn}$, and $AF_{intraspecies}$ results in a BMD_h and its 90% CI of 14 (5-41) µg/kg bw/day.

Table 1 Derivation of the HLV value of PBDE-47: Applied Assessment Factors on animal neurodevelopmental toxicity and thyroid toxicity (median with 90% Confidence Interval between brackets)

Study	Eriksson	Suvorov	Abdelouahab
Critical toxicity	Neurodevelopmental toxicity	Thyroid toxicity	Thyroid toxicity
Species	mouse	rat	sheep
PoD			
BMD (animal, µg/kg bw)	184 (72 – 306)	23 (13 - 9)	4.5 (2.5 - 8.5)
Assessment Factors			
Acute to Chronic (animal) Inter, kinetics Inter, dynamics Intraspecies	8.6 (8.2 – 8.9) 149 (139 – 162) 1 (P95: 2.5) 1	28.0 39 (29 - 54) 1 (P95: 2.5) 1	not determined not determined 1(P95: 2.5) 1
Human BMD (ng/kg bw/day)	130 (32 - 470)	22 (7.2 - 70)	3 (1 – 8.7)

5. Discussion

This report describes the derivation of an HLV for BDE-47 based on the extrapolation of animal toxicity data, i.e. (developmental) neurodevelopmental toxicity and thyroid toxicity. The derivation was in concordance with earlier extrapolations of POP toxicity, i.e. 2,3,7,8-TCDD (WHO, 1998; SCF, 2000; 2001; JECFA/WHO, 2002, 2005) and BDE-99 (Bakker *et al.,* 2008). It explicitly incorporates interspecies differences in kinetics in the extrapolation procedure, and was further extended by including uncertainties in a more realistic way. In the present study, input parameters (e.g. PoD, AFs and kinetics) were considered uncertain and were characterized by means of an uncertainty distribution. For example an NOAEL was replaced by a BMD and its corresponding CI and kinetic parameters and AFs replaced by uncertainty distributions.

Human BMD values and HLV
The analysis of BDE-47 animal toxicity revealed neurodevelopmental toxicity and disturbance of thyroid functioning as the most sensitive toxicity endpoint.
In the case of neurodevelopmental toxicity (CES: 5%) in mice a human BMD of 130 ng/kg bw/day (90% CI: 32-470) was obtained.

Based on effects on thyroid effects (CES: 10%) in sheep a human BMD was obtained of 3 ng/kg bw/day (90% CI: 1-8.7). A human BMD of 22 ng/kg bw/day (90% CI: 7.2-70) was obtained for thyroid toxicity in the rat.
The extrapolation of the thyroid toxicity in sheep clearly led to the lowest human BMD. However, the underlying sheep study suffers from serious methodological flaws with respect to the dose metric used as the staring point for the derivation of the HLV, i.e. the maternal adipose tissue concentration not reflecting the administered dose regime. For this reason it is felt that the (aberrant?) kinetics of BDE-47 in sheep should first be clarified before the calculated human BMD value can serve as the HLV value.
Therefore, a value of 7.2 ng/kg bw/day based on thyroid toxicity in the rat is proposed as the human BMD for thyroid toxicity.

The human BMDs of 7.2 ng/kg bw/day as derived for thyroid toxicity is lower than the corresponding value of 32 ng/kg bw/day for neurodevelopmental toxicity, indicating thyroid toxicity as a more sensitive toxic effect than neurodevelopmental toxicity. So, in this context an HLV based on neurodevelopmental toxicity is not expected to be protective for thyroid toxicity as well. Proceeding from a health protective point of view, a provisional HLV of 7 ng/kg bw/day therefore seems warranted.

Endpoint Neurodevelopmental toxicity in mice
Various studies and reviews support the identification of neurodevelopmental toxicity and thyroid toxicity as the most sensitive endpoints for BDE-47 toxicity (Costa and Giordano, 2007; Costa *et al.*, 2008; Dingemans *et al.*, 2007; Dingemans *et al.*, 2008; Dunnick and Nyska, 2009; Fonnum and Mariussen, 2009; Tagliaferri *et al.*, 2009). *In vitro* studies or studies performed with non-mammalian species can be used to gain insight in the mode of action and the kinetics of BDE-47, however, they are not yet sufficient to provide a PoD for quantitative hazard characterization.
Furthermore, in accordance with the Eriksson (2001) study, Gee and Moser (Gee and Moser, 2008; 0, 1, 10, or 30 mg/kg bw)) and He *et al*. (He *et al.*, 2009, 0, 1, 5 and 10 mg/kg bw, 2009) exposed mice to a single oral dose of BDE-47 on

PND 10. In these studies behavioral endpoints assessing sensory and motor maturation were examined. The results support the lasting changes in motor activity in mice as reported by Eriksson. Unfortunately, the results as reported by Gee and Moser are of insufficient quality to perform a dose-response analysis. In the study of He the applied doses are too high. This resulted in a lack of information in the non-adverse dose (range) and consequently this study is not suitable to perform quantitative hazard characterization.

Need for additional thyroid toxicity studies in sheep
Sheep have several characteristics which make a suitable animal model for humans. The authors of the sheep study (Abdelouahab *et al.*, 2009) already indicate that there are many similarities in physiology of gestation between sheep and human, for example the thyroid hormone regulated growth and differentiation of the brain (Fisher, 1991). The thyroid system ontogenesis is much more similar between sheep and humans compared to rats and humans (Cudd *et al.*, 2002), with rat and man differing significantly (Van Raaij *et al.*, 2002).Furthermore, the smaller number of offspring in sheep compared to rodents or pigs is thought to mimic the human situation better.

Comparison with US EPA evaluation
In 2008, US-EPA conducted a hazard characterization for BDE-47 based on the study of Eriksson *et al.* (2001). In this characterization, an animal BMD of 0.35 mg/kg bw was calculated, which was divided by a total uncertainty factor of 3000 to arrive at a human BMD of 100 ng/kg bw/day. The total uncertainty factor consisted of a subfactor for acute-to-chronic extrapolation of 3 and three factors of 10 for interspecies, intraspecies and (otherwise unspecified) database uncertainty.

Starting with an twofold lower animal BMD of 0.18 mg/kg bw (90% CI: 0.072 – 0.308) an acute to chronic assessment factor of 8.6 , an interspecies assessment factor of 149 for kinetics and neglecting intraspecies extrapolation, the present study resulted in a human BMD of 130 ng/kg bw/day (90% CI: 32 – 470 ng/kg bw/day).
So, though the human BMD for neurodevelopmental toxicity calculated in this study did not differ much from the one calculated by US-EPA, it stems from quite different extrapolation approach, i.e. a semi-quantitative (US-EPA) vs. a full quantitative approach (this study). For example, in the US-EPA extrapolation mouse/human differences in kinetics were (tacitly) be assumed to be in concordance with the proposed default value of 4 (IPCS, 1994). However, the current study clearly identified interspecies differences in kinetics to amount a factor of 149 (90% CI: 139-162).

6. Conclusion

This study confirms earlier findings with 2,3,7,8-TCDD that the explicit quantification of interspecies differences in kinetics is a *conditio sine qua non* for the derivation of HLV values for POPs. In this context using a default AF of 4 for interspecies differences in toxicokinetics may lead to HLVs which are not sufficiently low, i.e. their use in risk assessment may result in a gross underestimation of the toxicological risk of POPs in humans.

This study extends earlier derivations of HLVs for POPs (2,3,7,8-TCDD; BDE-99) in that it avoids an overly conservative extrapolation of animal toxicity from animals to man (replacement of an extrapolation on the basis of a NOAEL and deterministic AFs though an extrapolation on the basis of a BMD and probabilistic AFs).
Neurodevelopmental toxicity and thyroid toxicity were found as the most sensitive animal toxicity. Based on thyroid toxicity a provisional HLV of 7 ng/kg bw/day seems warranted for BDE-47.

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Technical Annex 1

Whole Body Concentration as dose metric for POP toxicity

The WBC reflects the accumulation of a chemical at the level of the total body, whereas toxicity is induced at the level of individual organs. This raises the question to what extent the WBC really reflects organ specific exposure and its associated toxicity. Answering this question is as follows. Given the abundant information on the distribution kinetics of the dioxin 2,3,7,8-TCDD this compound is taken as a default here. As it is likely that the distribution of a single dose of TCDD is completed within a few hour or days in humans and the focus on the calculation of a HLV is on a time scale of years rather than days a pseudo equilibrium, i.e. instantaneous equilibrium between the blood and the organs, at any time of human life may be assumed (van der Molen *et al.*, 1996; van der Molen, 1998).

Assuming toxicity in the i^{th} organ to be direct related to a chemical's organ concentration answering the question raised above basically come down to relating the time-courses of the organ concentration $C_i(t)$ to the $WBC(t)$. Here, the most straightforward (modeling) way is to relate $C_i(t)$ directly to the concentration in blood $C_{bl}(t)$ by means of a partition coefficient p_i, or:

$$C_i(t) = p_i \cdot C_{bl}(t) \qquad \text{(A.1)}$$

with:

p_i	the organ-blood partition coefficient
$C_{bl}(t)$	the concentration in the blood (amount/kg)

$C_i(t)$ however also equals:

$$C_i(t) = \frac{A_i(t)}{V_i} \qquad \text{(A.2)}$$

Furthermore for $WBC(t)$ it holds:

$$WBC(t) = \frac{\sum_i A_i(t)}{\sum_i V_i} \qquad \text{(A.3)}$$

Substituting A.1 and A.2 into A.3 then gives:

$$WBC(t) = C_{bl}(t) \cdot \frac{\sum_i p_i V_i}{\sum_i V_i} \qquad \text{(A.4)}$$

Given the ratio $\frac{V_i}{\sum_i V_i}$ as f_i , i.e. the fraction of the i^{th} organ compartment of the total body weight, rewriting equation A.4 then relates the *WBC(t)*, via $C_{bl}(t)$ and f_i , to the organ specific exposure $p_i \cdot Cbl(t)$:

$$WBC(t) = C_{bl}(t) \cdot \sum_i p_i f_i \qquad \text{(A.5)}$$

Basically equation A.5 states that, given the *WBC(t),* organ specific exposure is determined by the combination of an organ's affinity for a chemical relative to the blood (as reflected by the organ's partition coefficient p_i) and the organ's fraction of the total body weight (as reflected by f_i). Consequently the WBC will lead to the same organ exposure in different species when organs partition coefficients and relative organ fractions are the same. In this context interspecies differences in p_i en f_i there fore are considered as "residual uncertainty" with respect to the use of the *WBC(t)* as dose metric for organ exposure.
Of course, the concentration in the blood is determined by the combined effect of these two parameters over all *i* organ compartments.

Regarding the interspecies extrapolation of the *WBC(t)* as a generic dose metric for organ specific toxicity equation A.5 also states that, in order to lead to the same organ exposure, the parameters p_i and f_i should have similar values in various species.
Regarding POPs the partition coefficients p_i in general are determined by the organ lipid content. Assuming this content constant within a species the partition coefficients likely have a constant value too, with interspecies differences being caused by differences in organ lipid content and relative organ fraction. However, here the partition coefficient of the rodent liver, i.e. p_l, may be the exception to the rule here. The reason for this is that, next to lipid partitioning, the rodent liver possesses a specific protein binding mechanism which by far may overshadow lipid partitioning. This mechanism, known as hepatic sequestration, consists of the (dose- and time dependent) binding of ligands to the hepatic Ah-receptor, followed by the induction of Ah-receptor induced P450 proteins and binding of the Ah-receptor ligand to the induced P450 proteins, thereby increasing the liver's affinity for the Ah-receptor ligand. In the case of the Ah-receptor ligand 2,3,7,8-TCDD this mechanism has been found to be the dominant determinant of the hepatic partition coefficient. As the binding of the dioxin 2,3,7,8-TCDD to the Ah-receptor is dose- and time-dependent it follows that this holds for the hepatic partition coefficient too. So, regarding Ah-receptor ligands such as 2,3,7,8-TCDD which show a high affinity for Ah-receptor binding and binding to Ah-receptor dependent induced P450 proteins equation A.5 needs reformulation in terms of a constant partition coefficients for the extrahepatic organs and a dose-/time-dependent hepatic partition coefficient. This however,

needs the extension of partitioning modeling beyond simple lipid partitioning, i.e. needs the mechanistic modeling of ligand-Ah-receptor interactions, ligand P450 interactions and, at short time scale, organ blood flows, or in other words, full PBPK modeling.

In extremis (high exposure/short exposure or low exposure/long exposure), hepatic sequestration may lead to the liver as the primary site of TCDD disposition. In this case the "wet weight" liver tissue concentration may outweigh the "wet weight" adipose tissue concentration 10-fold (for comparison, in the absence of hepatic sequestration, the adipose tissue outweighs the liver by a factor of 10!).
Hepatic sequestration of course causes a shift in the contribution of the organ compartments to the WBC, with the contribution of the extra-hepatic organs decreasing at increasing hepatic sequestration. A typical (empirical) example of this mechanism is found in the fetal exposure in the rat after acute or chronic exposure of dams: a single bolus dose and a repeated low maternal (equivalent) dose leading to the same maternal WBC will lead to a higher fetal exposure after a single bolus dose than after a repeated dose. Mechanistically this observation can be explained by hepatic sequestration after chronic dosing leading to a relative low fraction of the WBC to reside in the blood and the extrahepatic organs. Consequently, in order to lead to the same fetal exposure, the maternal WBC after repeated exposure may be higher than after exposure to a single bolus.

In the case of PBDEs the affinity for the Ah-receptor is low and kinetic experiments have not given any indication for the hepatic sequestration of these compounds (so f.e. Staskal 2005, 2006a,b). In the case of PBDEs equation A.5 is therefore considered valid in relating the *WBC(t)* to organ exposure after single and repeated exposure in rodents as well as in humans

Adipose tissue as dose surrogate for the WBC

In practice organ specific concentrations as calculated from the WBC may be available for a limited number of organs, whereas toxicity data just may be available for other organs. In these cases the adipose tissue concentration, rather than the WBC, may be used as a dose surrogate for the exposure of extrahepatic organs (in cases where the WBC does not reflect hepatic sequestration). For example,
in the case of thyroid toxicity the adipose tissue concentration may be used as a dose surrogate for the thyroid concentration. The justification is as follows.

Assuming (for the sake of simplicity) "steady state" conditions with respect to POP exposure and thyroid toxicity (*E*) to be induced this toxicity depends on the total "steady state" thyroid concentration ($C_{thy,ss}$), or:

$$E_{thy} = f(C_{thy,ss}) \quad \text{(A.6)}$$

Furthermore, assume $C_{thy,ss}$ to be a function of the total "steady state" adipose tissue concentration ($C_{f,ss}$):

$$C_{thy,ss} = f'(C_{f,ss}) \quad \text{(A.7)}$$

and:

$$E_{thy} = f(f'(C_{f,ss}) \tag{A.8}$$

Now, assuming the lipid content of the thyroid and the adipose tissue to be constant the ratio between the ratio of the "steady state" concentrations of these organs is characterized by the ratio of their lipid partition coefficients:

$$\frac{C_{thy,ss}}{C_{f,ss}} = \frac{P_{thy}}{P_f} \tag{A.9}$$

So, in the case of a linear (or at low doses approximately linear) relationship between thyroid toxicity and the thyroid concentration the induction of thyroid toxicity may be expressed as a linear function of the concentration in the adipose tissue:

$$E = \alpha \cdot \frac{P_{thy}}{P_f} \cdot C_{f,ss} \tag{A.10}$$

A more complicated situation occurs when the relationship between thyroid toxicity and the thyroid concentration is non-linear. For example, assuming the following dose-response relationship to hold for thyroid toxicity and the thyroid concentration:

$$E = a \cdot [c - (c-1) \cdot \exp(-b \cdot C_{thy,ss})] \tag{A.11}$$

then leads to the following equivalent dose-response relationship between induced thyroid toxicity and the concentration in the adipose tissue:

$$E = a \cdot [c - (c-1) \cdot \exp(-b \cdot \frac{P_{thy}}{P_f} C_{f,ss})] \tag{A.12}$$

Technical Annex 2

Dose-response modeling of BDE-47 toxicity

Eriksson study

Data collection and modeling strategy

In contrast to US-EPA the raw data were retrieved from figures 1 and 2 instead of analyzing the processed data from table 1. US-EPA considered the change in the mean equal to 0.5, 1, and 1.5 times the standard deviation of the control group the relevant (adverse) response, i.e. benchmark response (BMR). In our analysis we did not let the BMR depend on the variance and error in the control group, but considered a particular change in (continuous) response as being adverse. This change in response is termed critical effect size (CES) (Slob, 2002). The software package PROAST[9] version 20.2 running in R version 2.8.1 was used to derive the BMDs according to the methods described by Slob (2002). Two families of nested models, the so called Exponential model family and the Hill model family, are currently available to describe continuous data. Results of both models are used to account for model uncertainty, i.e. the ability of different models to describe the same data set.

Experimental protocol

The principle of the test is that mice, being placed in a new surrounding, spontaneously start with the exploration of their new surroundings. In this they make all kinds of (horizontal and vertical) movements. However, after a certain time, this behavior stops: the exploration has been completed.
In testing the ability of BDE-47 to disturb this behavior a single oral BDE-47 dose (0, 0.7 and 10.5 mg/kg bw) was administered to male NMRI mice at PND 10. At the age of 2 and 4 months the mice were tested on their spontaneous motor behavior and habituation capability, i.e. the ability to explore a new environment. The test consisted of placing the mice for 60 minutes in a new cage followed by automatically measuring their locomotion, i.e. locomotor activity as measured by the number of horizontal movements, rearing activity, i.e. motor activity as measured by the number of vertical movement, and total motor activity, i.e. cage vibrations as measured by motor activity in the cage, for the following three time periods: 0-20, 40-60 and 40-60 minutes. All motor activity was expressed as the number of times ("counts") mice crossed a grid horizontal or vertical infrared beams in the cage.

So, where BDE-47 to disturb the mice's spontaneous learning behavior to explore their new surrounding it would shift the time-interval needed for the exploration (with locomotion, rearing activity and total activity as quantitative indicators for the exploration).

[9] Available at www.proast.nl

Locomotion
As shown in Figure A-1 taking the 0-20 minute observation period a clear dose-response on locomotion appeared, with controls and lowest exposed mice showing the highest locomotion activity, i.e. the highest eagerness to explore their new surroundings and highest exposed mice clearly being less eager to explore their new surroundings. In contrast, as might be expected, taking the 40-60 minute observation period the mirror image of this effect is seen: control and lowest exposed mice showing no locomotor activity any more, with highest exposed mice still being active (however, at a relative low level). Clearly, the control and lowest exposed mice had completed the exploration of their new surroundings within the set 60 minute time period, whereas the highest exposed mice had not.

As shown the exposure to BDE-47 clearly led to a decrease in locomotion activity, with this effect being the most prominent in 4 month old mice. Data analysis therefore showed locomotion during the 40-60 min observation period by far to result in the lowest BMDs. This time period was therefore chosen as the time period in which the effect of BDE-47 on locomotion was most pronounced. For this reason this time-period was chosen to quantify BDE-47's effect on locomotion (see Figure A-2).

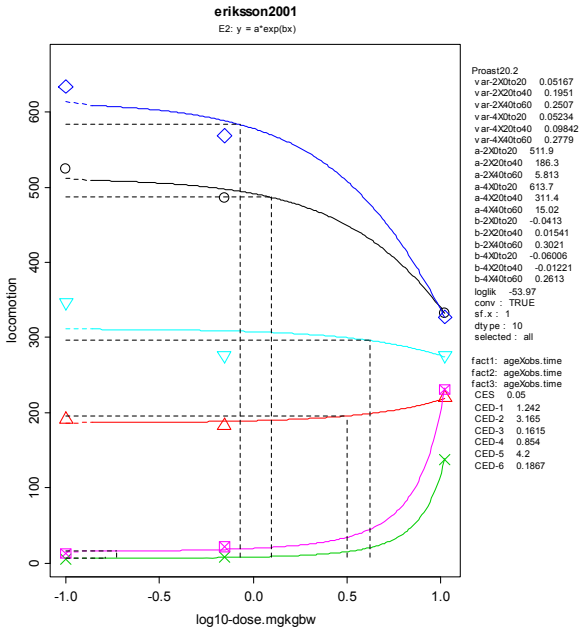

Figure A-1 Dose-response of locomotion (mean counts) against BDE-47 dose. Points plotted at dose = -1 are controls. Upper two curves: interval 0-20 at age 2 and 4 months; middle two curves: interval 20-40 at age 2 and 4 months; lower two curves: interval 40-60 at age 2 and 4 months. Model fit: Exponential model, CES: 5%. Data, mean, SDs, n/dose group =8, are from Eriksson (2001, fig 1 and 2).

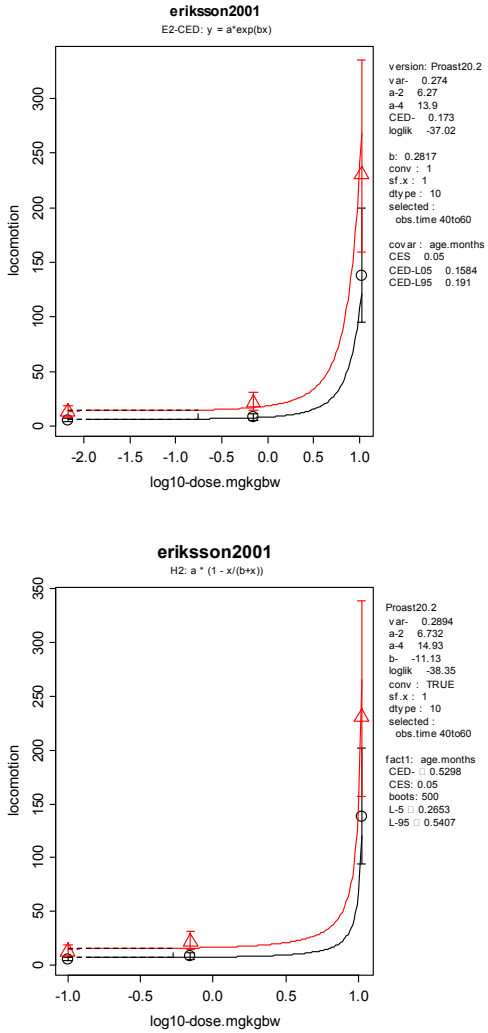

Figure A-2 Dose-response analysis of locomotion (counts) against BDE-47 dose. Points plotted at dose = -1 are controls. For the 40-60 minute observation interval in 2 (circles) and 4 (triangles) month old mice Model fits: Exponential (left) and Hill (right) model, CES: 5%. Data, mean, SDs, n/dose group = 8, are from Eriksson (2001). Obtained BMDs are reported in Table 4.1 of the main text.

Rearing

As shown in Figure A-3 taking the 0-20 minute observation period a clear dose-response on rearing appeared, with controls and lowest exposed mice showing the highest locomotion activity. In concordance with the effect on locomotion the 40-60 minute observation period showed the mirror image of this effect. The lowest BMDs were observed in the intervals 0-20 and 40-60 minutes. These time-windows were therefore analyzed separately (see figures A-4 and A-5).

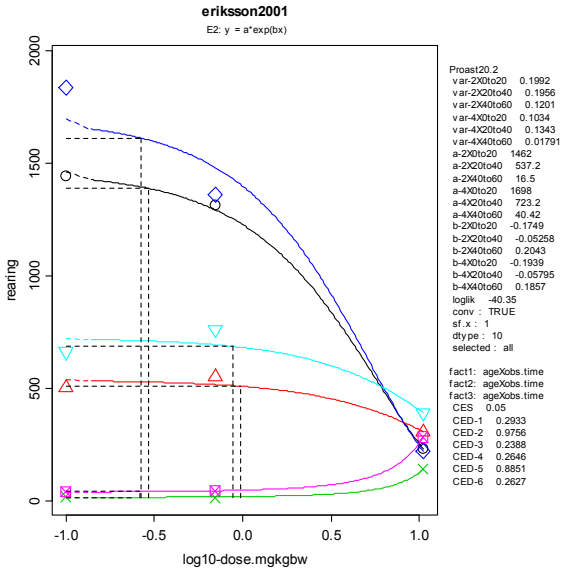

Figure A-3 Dose-response of rearing (mean counts) against BDE-47 dose. Points plotted at dose = -1 are controls. Upper two curves are from interval 0-20 at age 2 and 4 months. Middle two curves are from interval 20-40 at age 2 and 4 months. Lower two curves are from interval 40-60 at age 2 and 4 months. Model fit: Exponential model, CES: 5%. Data, mean, SDs, n/dose group = 8, are from Eriksson (2001).

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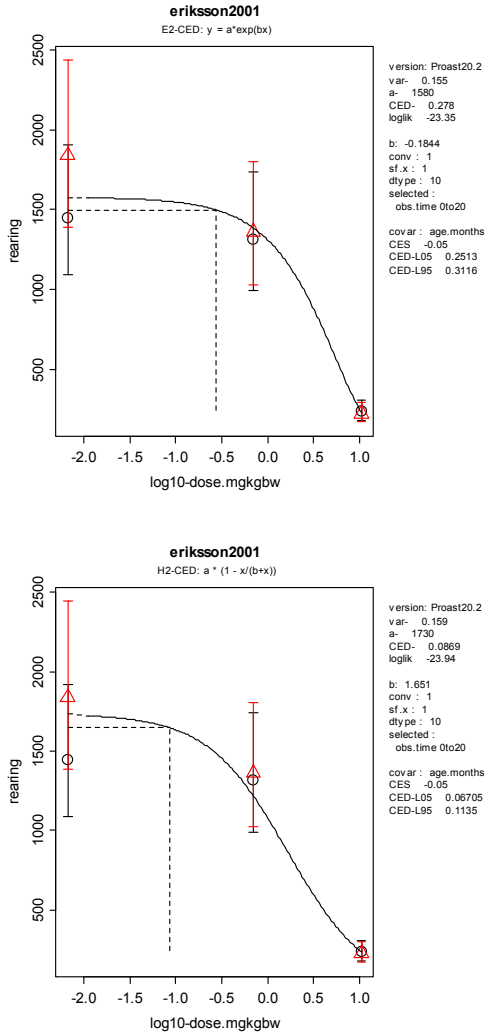


Figure A-4 Dose-response analysis of rearing (counts) against BDE-47 dose for the 0-20 minute observation interval in 2 (circles) and 4 (triangles) month old mice Model fits: Exponential (left) and Hill (right) model, CES: 5%. Data: means and SDs, n/dose group =8. Points plotted at dose = -2 are controls. Obtained BMDs are reported in Table 4.1 of the main text.

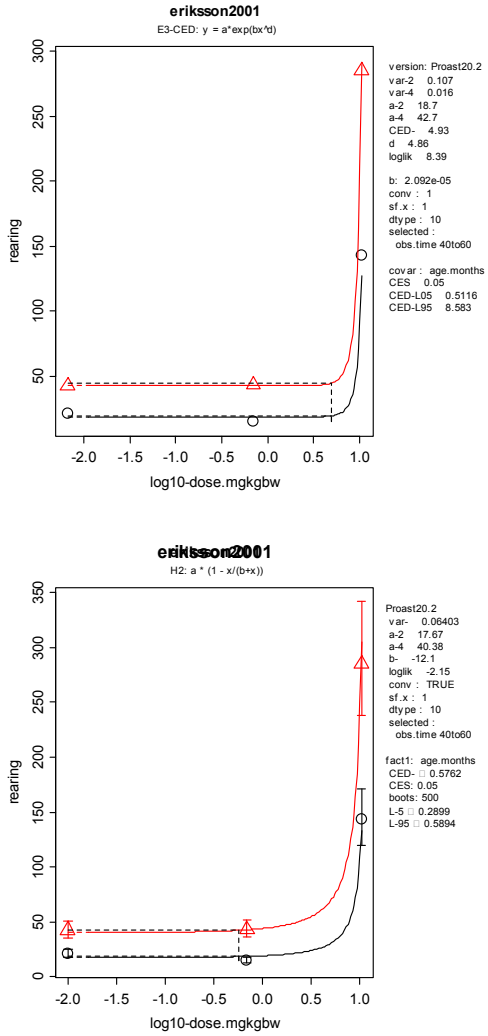


Figure A-5 Dose-response analysis of rearing (counts) against BDE-47 dose for the 40-60 minute observation interval in 2 (circles) and 4 (triangles) month old mice Model fits: Exponential (left) and Hill (right) model, CES: 5%. Data: means and SDs, n/dose group = 8. Points plotted at dose = -2 are controls. Obtained BMDs are reported in Table.4.1 of the main text.

Total activity

As shown in Figure A-6 taking the 0-20 minute observation period a clear dose-response on total activity, with controls and lowest exposed mice showing the highest total activity. In concordance with the effect on locomotion and rearing the 40-60 minute observation period showed the mirror image of this effect.

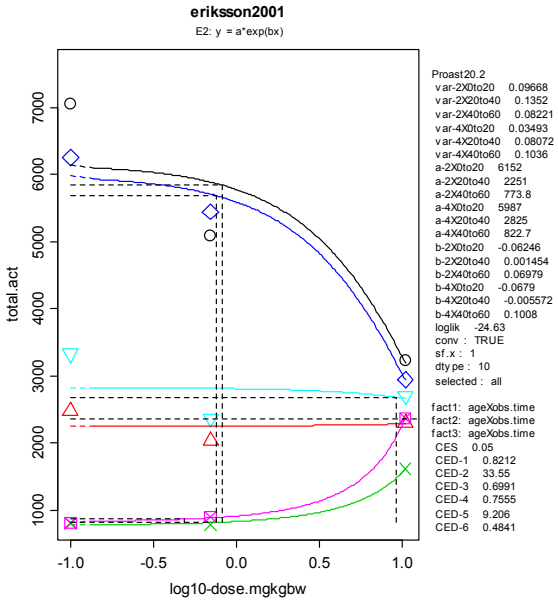


Figure A-6 Dose-response analysis of total activity (counts) against BDE-47 dose. Upper two curves are from interval 0-20 at age 2 and 4 months. Middle two curves are from interval 20-40 at age 2 and 4 months. Lower two curves are from interval 40-60 at age 2 and 4 months. Model fit: Exponential model, CES: 5%. Data: means and SDs, n/dose group =8. Points plotted at dose = -1 are controls.

The lowest BMDs were observed in the intervals 0-20 and 40-60 minutes. These time-windows were therefore analyzed separately (see figures A-7 and A-8).

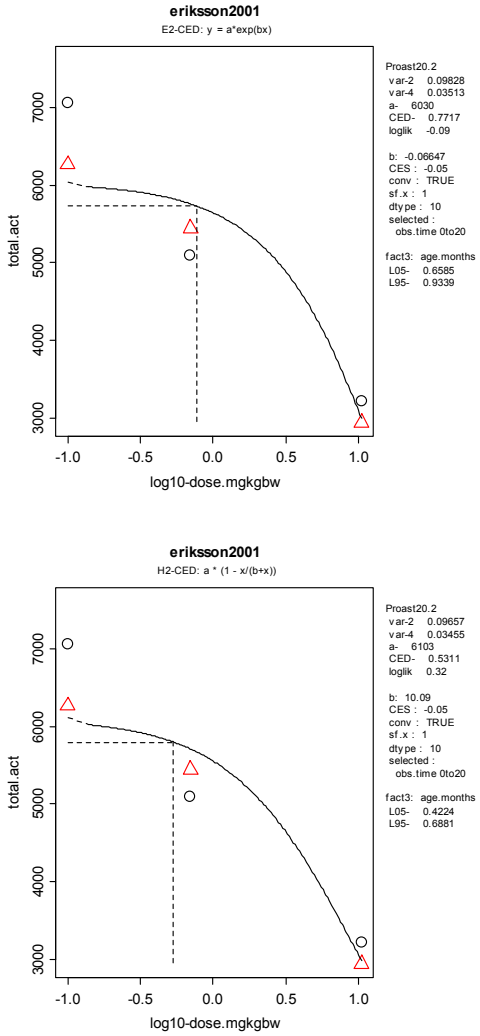


Figure A-7 Dose-response analysis of total activity (counts) against BDE-47 dose for the 0-20 minute observation interval in 2 (circles) and 4 (triangles) month old mice Model fits: Exponential (left) and Hill (right) model, CES: 5%. Data: means and SDs, n/dose group = 8. Points plotted at dose = -1 are controls. Obtained BMDs are reported in Table 4.1 of the main text.

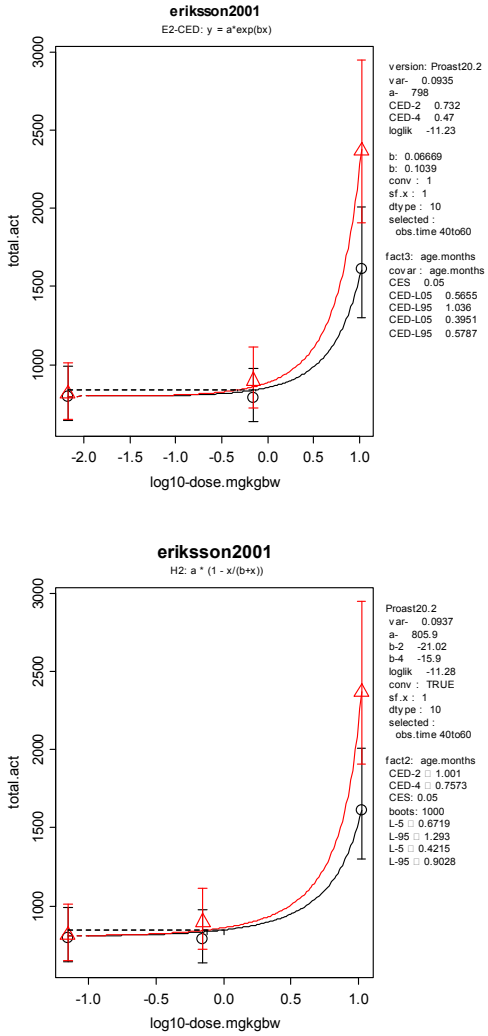


Figure A-8 Dose-response analysis of total activity (counts) against the administered (external) BDE-47 dose for the 40-60 minute observation interval in 2 (circles) and 4 (triangles) month old mice Model fits: Exponential (left) and Hill (right) model, CES: 5%. Data: means and SDs, n/dose group = 8. Points plotted at lowest dose (approx. -1) are controls. Obtained BMDs are reported in Table 4.1 of the main text.

Richardson study

Richardson et al (2008) orally exposed mice aged 9 weeks to 3, 10, or 100 mg BDE-47/kg bw/day for four days. BDE caused a 43% decrease in serum TT4 concentrations at the highest dose 24 hours after the last dose. The dose-response of TT4 is presented in Figure A-9. The BMDs and CIs derived from this analysis are presented in Table 5.1 of the main text.

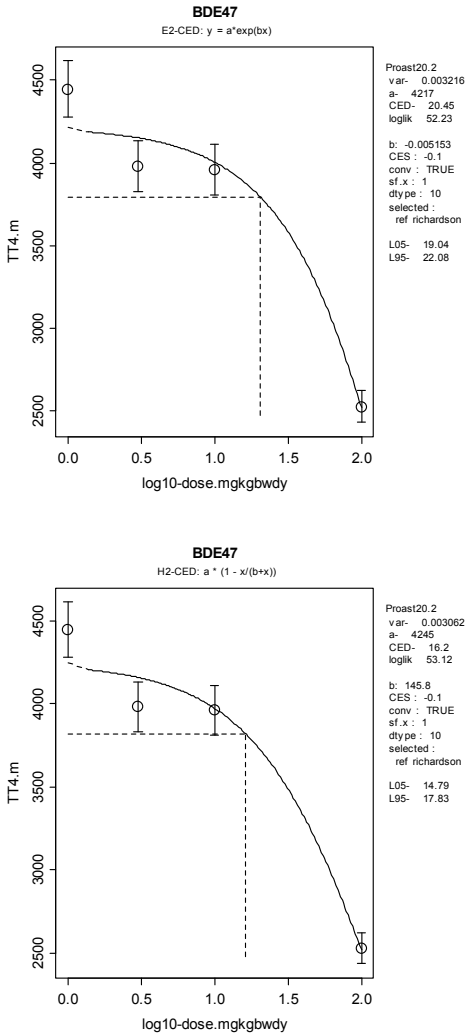


Figure A-9 Dose-response of the TT4 concentrations (ng/dL serum, mean and CI) against the administered (external) BDE-47 dose (mg/kg bw/day). Points plotted at dose = 0 are controls. Model fits: Exponential (left) and Hill (right) model. CES: 10%. Data are from Richardson et al (2008), figure 1, reported as means and SEMs, n/dose group = 9.
Obtained BMDs are reported in Table 4.1 of the main text.

Abdelouahab study

Abdelouahab et al (2009) exposed pregnant sheep i.v. to vehicle or BDE-47 (0.2, 2, and 20 µg/kg bw/week) from the 5th to 15th week of gestation. At delivery the following BDE-47 concentrations were measured (caesarian 1 week after last dose) in adipose tissue of dams and lamb:

Table 1. BDE-47 concentrations (in ng/g sample) in subcutaneous fat in sheep and in neck fat in lambs at delivery

Sheep No.	Group of exposure µg/kg b.w.	Sheep fat tissue concentrations	Lamb fat concentrations		
			lamb 1	lamb 2	lamb 3
1	0.2	62.45	19.22	10.80	16.21
2	0.2	32.99	26.25	21.82	–
3	0.2	32.47	16.35	22.75	–
4	2.0	79.08	44.84	–	–
5	2.0	48.80	35.04	29.70	–
6	2.0	76.71	48.17	18.01	–
7	20.0	82.79	128.65	–	–
8	20.0	80.34	53.98	79.42	–
9	20.0	98.58	not pregnant		

No BDE-47 could be detected in the adipose tissue of unexposed animals.
The hundredfold increase in the external exposure only led to a two-fold increase in the maternal adipose tissue concentration. This anyway indicates the absence of linear accumulation kinetics. The latter is expected under the assumption that BDE-47 has persistent characteristics in sheep, as displayed in rodents with adipose tissue as the main storage of BDE-47 in the body (see f.e. Staskal *et al.*, 2005).
So, though the kinetics of BDE-47 in the Abdelouahab study is not in concordance with classical POP kinetics, a BMD analysis revealed that only a two-fold increase in the maternal BDE-47 adipose tissue concentration could lead to a significant lowering of TT3 in cord blood, suggesting the sheep's thyroid gland to be very sensitive for BDE-47 in the maternal body (see below).
Total and free T3 and T4 levels in cord blood (at delivery) were reported. TT4 concentrations did not show a dose-response effect. However, as shown in Figure A-10, TT3 dose-dependently decreased in cord blood. The BMDs and CIs derived from this study derived from these data are presented in Table 5.1 in the main text.

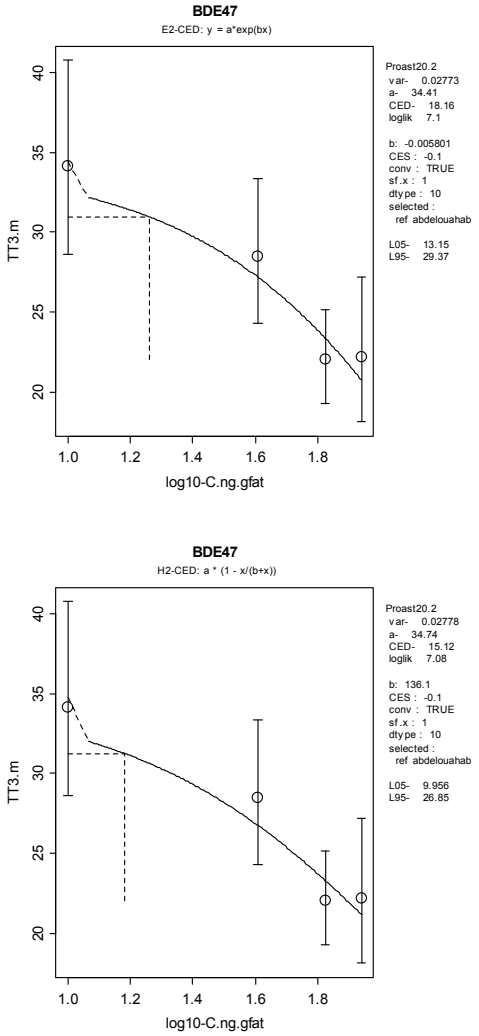


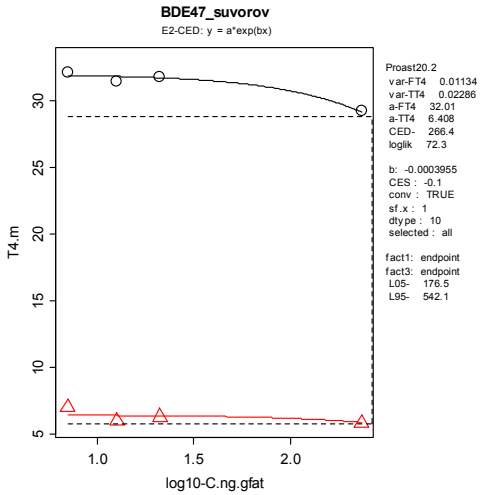
Figure A-10 Dose-response analysis of TT3 concentrations (ng/dL) in cord blood of lambs against the BDE-47 concentration in maternal fat (ng/g fat) at delivery. Model fits: Exponential (left), Hill (right) model. CES: 10%. Data are from Abdelouahab et al (2009), figure 1 and table 1. Obtained BMDs are reported in Table 4.1 of the main text.

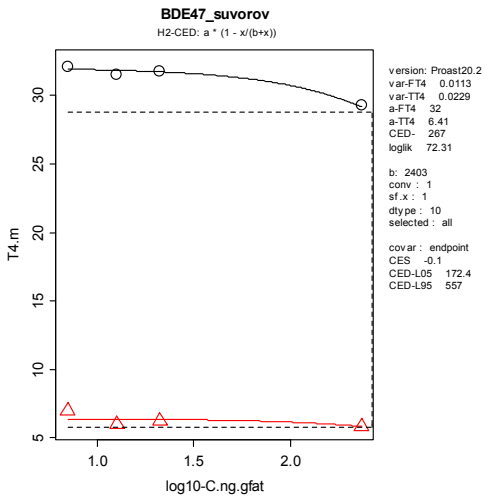
Suvorov study

In Suvorov (2009) rat dams were exposed to vehicle or low-dose BDE-47 (0.002, 0.02 and 0.2 mg/kg bw) each 5 days from GD 15 to PND 20 by iv injections. Spontaneous locomotor activity of pups was assessed using the open field test on PND 15, 20, and 25. Sensorimotor coordination was assessed on PND 30. In addition, Total (T) and Free (F) T3 and T4 blood concentrations and BDE-47 concentrations in maternal adipose tissue were measured in pups and dams on PND 27. Maternal adipose concentrations amounted: 7.0 $\pm$ 0.2 ng/g lipid for controls, 12.6 $\pm$ 3.6 ng/g lipid for the group exposed to 0.002 mg/kg

bw, 21.0 + 2.6 ng/g lipid for the group exposed to 0.02 mg/kg bw and 234.3 ± 167.7 ng/g lipid for the group exposed to 0.2 mg/kg bw.
As in the Abdelouahab study these kinetics do not match classical POP accumulation
kinetics as expected for repeated exposure to BDE-47 in rats. Furthermore, the maternal adipose tissue concentration is prone to considerable uncertainty. Notwithstanding these methodological flaws the data of this study sufficed for BMD analysis of the maternal adipose tissue concentration against TT4 and FT4 in neonatal rats (see Figure A-11). The BMDs and CIs derived from this study derived from these data are presented in Table 5.1 of the main text.

The authors indicate that exposure to BDE-47 results in statistically significant ($p<0.01$, 0.05, or 0.10) changes in several locomotor activity parameters. However, re-analysis of the data did not show (monotonic) dose-responses. The data clearly are too scarce to provide evidence for non-monotonic dose-responses. Thyroid hormone levels were not altered in the exposed dams.





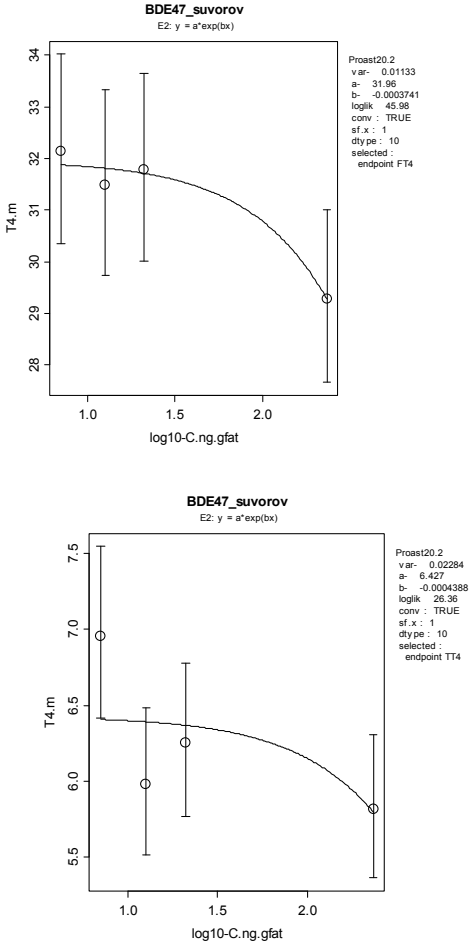


Figure A-11 Dose-response analysis of FT4 (circles, pmol/L) and TT4 (triangles, µg/dL) concentrations in blood of neonatal rats against the BDE-47 concentration in adipose tissue of dams, both on PND 27. Model fits: Exponential (top left), Hill (top right) model. Bottom left and right: details of FT4 and TT4, respectively, fitted with the exponential model. CES: 10%. Data are from Suvorov (2009), table 2. Control is not zero but background fat concentration. BMDs are reported in Table 4.1 of the main text.

He study

The He study (2009) identified neurodevelopmental toxicity on the developing brain of neonatal rats as a sensitive toxic effect of BDE-47. As in the Eriksson study neonatal rats were exposed to a single oral gavage dose of vehicle (corn oil), 1, 5, or 10 mg BDE-47/kg bw at PND 10. At 2 months of age the total distance swam by rats (6 per sex) to reach an escape platform was increased and the ratio of distance taken in the platform quadrant to total distance was notably decreased in all treated groups in the water maze experiment compared to the control. Furthermore, structural neuron alterations were observed.

Both endpoints in the He study did not meet the quality criteria for BMD analysis: the calculated BMD should not be too far outside the experimental range of observation (Figures A12 and A13) and that the width of its CI should not be too wide. of the. A closer look at the data clearly showed that the doses applied in this study are too high to allow for a meaningful BMD calculation at the relevant 5% CES.

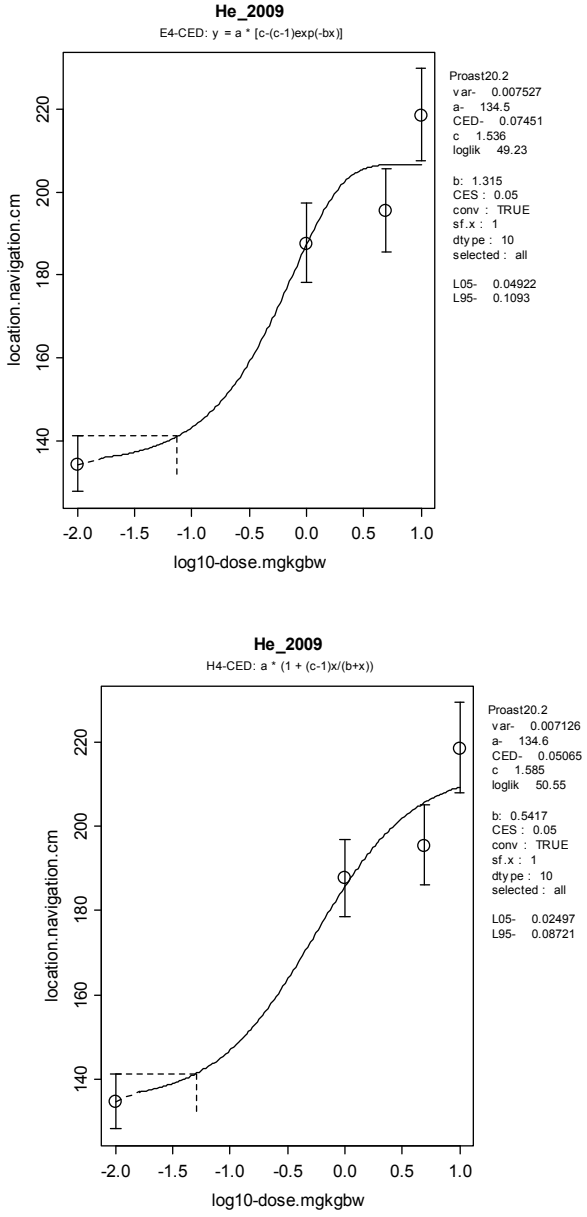


Figure A-12 Dose-response analysis of location navigation distance (cm) of neonatal rats against the dose. Model fits: Exponential (left), Hill (right) model. CES: 5%. Data are scanned from He (2009), figure 1. Points plotted at dose = -2 are controls. BMDs are reported in Table 4.1 of the main text.

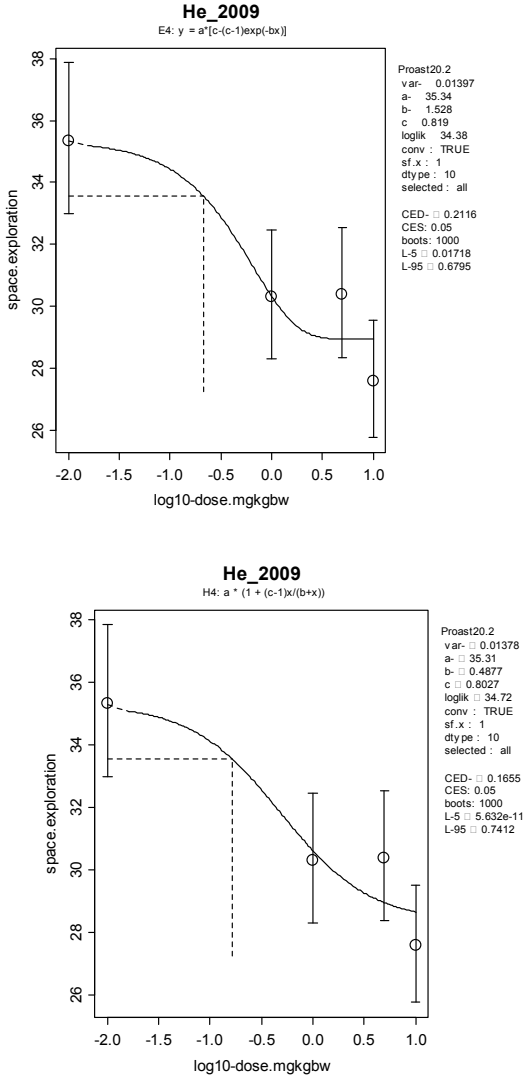


Figure A-13 Dose-response analysis of space exploration (ratio of the distance in the platform quadrant and the total distance (%)) of 2 month old rats against the dose. Model fits: Exponential (left), Hill (right) model. CES: 5%. Data are scanned from He (2009), figure 1. Points plotted at dose = -2 are controls. BMDs are reported in Table 4.1 of the main text.

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