Bundesinstitut für Risikobewertung

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"General observations" on the opinion „Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food", as drafted by the EFSA Panel on Contaminants in the Food Chain (CONTAM)

Preface

This document provides the view of the BfR on the relevant human studies on lower sperm concentrations in association with TCDD/dioxin exposure during childhood. As this was considered as critical effect for the derivation of a HBGV by the CONTAM Panel, other effects, e.g. on sperm motility or hormone levels, are not discussed here.

1. Human studies examining associations between dioxin levels in childhood and adult sperm concentrations

In the following chapter, the results of three human studies examining associations between dioxin levels in childhood and adult sperm concentrations are summarized, and remarks are made regarding critical aspects.

Study of Mocarelli et al. (2008)

Males exposed to TCDD during the Seveso accident in 1976 were studied at three age groups, infancy/prepuberty (1–9 years, mean age at exposure 6.2 years), puberty (10–17 years, mean age at exposure 13.2 years), and adulthood (18–26 years, mean age at exposure 21.5 years), using 1976 serum TCDD levels and semen samples collected 22 years later. Results were compared to healthy age-matched control men (n=184).
The 71 males exposed at the age 1-9 years (median TCDD 210 pg/g fat, age 22–31 years) had lower sperm concentrations compared to control men (53.6 vs. 72.5 million/mL, i.e. -25.7 %; p = 0.025). The data were split into quartiles based on serum TCDD levels, showing median levels of 68, 142, 345, and 733 pg/g fat, and sperm concentrations of 55, 55, 57 and 48 million/mL, respectively, as compared to the 73 million/mL in the control group (sperm concentrations estimated by the CONTAM Panel from Figure 3a of the paper). Accordingly, there was no dose-response association among the exposed. In contrast to the group 1-9 years of age, the 44 males exposed at the age of 10-17 years (median TCDD 164 pg/g fat, age 32–39 years) showed higher sperm concentrations compared to control men (81.9

vs. 60.8 million/mL), but the difference was not significant (p = 0.231). No associations were observed in 20 men exposed as adults (median TCDD 123 pg/g fat, age 40–47 years).

Remarks:

- A relatively broad age span of 1-9 years was chosen without reasonable explanation. In this context, the question of the relevant sensitive window during development arises. Many authors refer to the “neonatal minipuberty”, including Mocarelli et al. in their second paper (Mocarelli et al. 2011). In their first paper (Mocarelli et al. 2008), the authors refer to Sharpe et al. (2003), describing developmental stages of the reproductive system and sensitive periods for the maturation of Sertoli cells (birth, the “neonatal-infantile” period ending with 12-18 months, and puberty with an age of 10-13 years). Considering the mean age of 6.2 years in the group 1-9 years old, only relatively few of the males are expected to been exposed during the first 1.5 years of life.
- Regarding the dose-response relationship, no dose-dependency was observed for the TCDD quartiles of the exposed males 1-9 years old at exposure. The difference in sperm concentrations was observed between exposed and control men only. According to the calculations of the CONTAM Panel (Table 12), a non-TCDD background of 51 pg total TEQ/g fat has to be added to the TCDD levels, resulting in mean total TEQ levels of 119 pg/g fat in the exposed males of the first quartile, and of 51-66 pg total TEQ/g fat in the controls (TEQ levels were calculated using WHO2005-TEFs throughout this document).
- Sixteen exposed males with TCDD levels above 2000 pg/g fat (10 males in the group 1–9 years and 6 males in the group 10–17 years of age in 1976) were excluded from the evaluation without explanation.

<u>Study of Mocarelli et al. (2011)</u>

Sperm quality was investigated in 39 sons (mean age, 22.5 years, 21 were breast-fed and 18 formula-fed, maternal TCDD mean 19 and 27 pg/g fat, respectively) born between 1977 and 1984 to mothers exposed to dioxin during the Seveso accident in 1976, and 58 comparisons (mean age, 24.6 years, 36 were breast-fed and 22 formula-fed) born to mothers exposed to background dioxin only (TCDD about 10 pg/g fat). Maternal dioxin levels at conception were extrapolated from the concentrations measured in serum in 1976 (exposed only). The 39 exposed men had significantly lower sperm concentrations relative to the 58 men in the comparison group (61.5 vs. 88.7 million/mL, -31 %, p = 0.01). A stronger significant association was observed when comparing the 21 breast-fed exposed sons and the 36 breast-fed comparisons (54.4 vs. 99.6 million/mL, -45 %, p = 0.002). In contrast, no statistically significant differences were observed between the 18 exposed formula-fed men and the 22 formula-fed comparisons (p = 0.53) on the one hand as well as the 21 exposed breast-fed

men (p = 0.07) on the other hand. The nine breast-fed men belonging to the two highest quartiles of TCDD (> 26 pg/g fat, median 58.9 pg/g fat) did show lower sperm concentrations (*p* = 0.0003) than the 36 breast-fed comparisons, whereas in the 12 breast-fed men in the two lowest quartiles (TCDD < 26 pg/g fat, median 13.1 pg/g fat), the lower sperm concentrations showed borderline significance (p = 0.05) in comparison to the breast-fed controls. Despite the differences described, a dose-response model did not reach statistical significance.
As a remarkable minor (statistically not significant) result, the breast-fed control men had higher sperm concentrations compared to the formula-fed control men (99.6 vs. 81.8 million/mL, respectively, p = 0.51), despite their expected higher exposure in early childhood.

Remarks

- According to the calculations of the CONTAM Panel (Table 12), a non-TCDD background of 64 pg total TEQ/g fat has to be added to the maternal levels in exposed and control men, resulting in total TEQ levels of 83 pg/g fat in the exposed breast-fed men and 74 pg/g fat in the (breastfed) controls; despite this relatively low difference in exposure, the difference in sperm concentrations was highly significant (p = 0.002). Splitting the exposed breast-fed group into “low” and “high” (77 vs. 123 pg/g fat, respectively; Figure 1B) seems to present a more convincing dose-dependency. However, despite the low difference in exposure (77 vs. 74 pg/g fat), the exposed “low” group had significantly lower sperm concentrations compared to the controls (p = 0.05). A dose-response model did not reach statistical significance, maybe due to the low number (n=39) of exposed men.

Study of Minguez-Alarcon et al. (2017)

As part of the “Russian Children’s Study”, 516 boys were enrolled at the age of 8–9 years and followed for up to 10 years. Persistent organochlorine compounds including PCDDs, PCDFs and PCBs were measured in serum. At 18-19 years, 133 young men provided semen samples. Of these men, the median for TCDD, PCDD TEQ, PDDD/F TEQ and total TEQ including DL-PCBs was 2.9, 8.7, 13.5 and 21.9 pg/g fat, respectively. The data on sperm concentrations were split into quartiles based on TCDD or TEQ. Higher quartiles were associated with lower sperm concentrations (*p*-trend ≤ 0.05) in case of TCDD and PCDD TEQ, but not for PCDF TEQ, PCB TEQ and total TEQ. For the evaluation of the CONTAM Panel, additional data on the sperm concentration in association with PCDD/F TEQ were provided by the authors. For the quartiles with median levels of 7.0, 10.9, 15.9 and 32.8 pg/g fat, the corresponding values of sperm concentrations were mean 63.6, 37.4, 40.9 and 41.0 million/mL, respectively (trend: p = 0.04, -41.2 % between first and second quartile, no further decrease at higher exposure). The CONTAM Panel used the median PCDD/F TEQ level of the first quartile (7.0 pg/g fat) as NOAEL and “Point of Departure” for the derivation of the HBGV using kinetic modelling.

Remarks

- The study is part of a large cohort study in the vicinity of a chemical manufacturing plant in the city of Chapaevsk. Until the close-down in 2003, the factory has led to an extensive contamination of the environment with dioxins, but also with organochlorine pesticides, lead and probably other compounds with shorter half-lives not detected in the study. A couple of other effects related to different compounds has been published over the last years (Sergeyev et al. 2017).
  In addition, it should be noted that the dioxin pattern is not comparable to that in central Europe: The PCDD/F TEQ is determined by TCDD to about 20 % (central Europe: about 10 %). For the median of the first quartile, the CONTAM Panel even reported a median TCDD level of 2.5 pg/g fat contributing to 36 % of the PCDD/F TEQ level of 7.0 pg/g fat.
- Regarding the different quartiles based on PCDD/F TEQ, no description is available on the differences in other relevant factors possibly influencing the outcome (e.g. lifestyle characteristics or other chemicals). Therefore, the value of statistical adjustment and the possible residual confounding can't be adequately evaluated.
- In contrast to the expectations on the crucial binding affinity to the Ah receptor (AhR), no associations were observed regarding PCDF TEQ (trend p = 0.78) and DL-PCBs TEQ (trend p = 0.73).
- The authors provided no justification why the age of 8-9 years was chosen for the exposure assessment, and why they think that exposure at this age has relevance for the effect on sperm concentrations measured about 10 years later.
- In contrast to studies of Mocarelli et al. (2008, 2011), at clear dose-response relationship based to the levels of PCDD/F TEQ seems to be observed. However, this interpretation would mean a very steep curve with a -41 % response between the levels of 7.0 pg/g fat (median Q1) and 10.9 pg/g fat (median Q2), reaching its maximum already at the Q2 dose level (no further decrease at higher dose levels).

2 Aspects of minor importance

Kinetic modelling

Time was too short for the BfR to evaluate all the assumptions made and to run the model used by the CONTAM Panel. However, the duration of breastfeeding (12 months) and the volume of mother's milk transferred (800 mL per day) are very high and lead to an over-conservative contribution of this exposure pathway. Realistically, the milk volume increases during the first weeks of life, and at latest with the beginning of the seventh month, meal by meal is replaced by complementary foods, commonly month by month. At the end of the first year of life, breastfed infants usually get mother's milk on one or two daily occasions only.

Relevance of furans and PCBs

From the three human studies dealing with the possible influence of dioxin exposure on sperm concentrations, only TCDD is addressed in the Seveso studies, and an association with TCDD as well as with PCDD TEQ but not with PCDF TEQ and DL-PCBs was observed in the Russian Children's Study. Therefore, no evidence from human studies is available that PCDFs and DL-PCBs contribute to an effect on sperm concentrations, and the TWI derived by the CONTAM Panel would be a HBGV for PCDDs only, but not for PCDF TEQ, DL-PCB TEQ and total TEQ. However, the latter groups of compounds are also considered for the exposure estimated done by the CONTAM Panel, and contributed to the exceedance of the TWI value.

Internal exposure

The CONTAM Panel considered data on internal exposure to dioxins and the other relevant compounds, however, they were not used for the risk characterization. In view of the BfR, the simultaneous consideration of external and internal exposure levels and their use for mutual plausibility checks would help to better take into account the respective uncertainties of each of both data sources.
In Germany, levels PCDD/Fs are continuously decreasing over time (Padberg et al. 2018). Using to current analytical data for human milk, only a part of the German population would exceed the level of 5.9 pg/g fat corresponding to the TWI, even if the total TEQ is considered.

3 Key questions on studies reporting decreased sperm concentrations in association with dioxin levels

(1) How robust is the parameter "sperm concentration"?

In view of the BfR, studies in humans on semen quality (focus in this document: sperm concentrations only) are difficult to control and have to deal with many methodological aspects and other factors potentially influencing the results. Technically, the duration between ejaculation and investigation, the transport as well as the experience of the technician may have an influence on the results. Individual factors influencing the results are age, smoking (prenatal and current exposure), and especially the duration of abstinence and the frequency of ejaculations. For the latter parameters, it may not be easy to get reliable answers from study participants (Cooper et al. 1993). Furthermore, many life-style factors like nutrition, obesity, physical activity or a warm environment are under discussion to contribute to the huge inter- and intraindividual variability. For example, in a Danish study (Priskorn et al. 2018) investigating yearly about 300 young men of the general population for 21 years (1996 to 2016,

median age 19 years), the concentrations varied between less than 10 million/mL and about 150 million/mL for the 5. and the 95. percentile, respectively. Despite the high number of participants and otherwise unchanged conditions, the yearly median concentrations varied between about 36 to 50 million/mL, as estimated from Figure 1A. Therefore, the number of subjects in the studies considered by the CONTAM Panel (Mocarelli et al. 2008, 2011; Minguez-Alarcon et al. 2017) seems to be too low, and adjustment during statistical evaluation may not handle the many possible confounders adequately.

Regarding the absolute sperm concentrations, the mean values were higher in Seveso controls of the first study (Mocarelli et al. 2008; 72.5 million/mL, age 22-31 years, n=82) and the second study (Mocarelli et al. 2011; 88.7 million/mL, age 24.6 ± 2.0 years, n=58) compared the first quartile of the Russian Children's Study (63.6 million/mL, age 18-19 years, n=33), despite the expected higher Seveso exposure of control men due to background dioxin levels in the 1970s/1980s. At first glance, this seems inconsistent. However, the sperm concentrations are obviously in part determined by unidentified factors. In the Danish study already mentioned (Priskorn et al. 2018), median sperm concentrations examined in 6,386 healthy participants of the general population were 44 million/mL only, corresponding to the level of the "affected" quartiles Q2 to Q4 in the Russian Children's Study, and are distinctly lower compared to the males exposed at the age of 1-9 years in the Seveso accident (53 million/mL, Mocarelli et al. 2008). This issue further underlines the complexity of investigating sperm concentrations and to compare results from different countries.

(2) What is the critical window of exposure?

In view of the BfR, the investigation of sperm concentrations gets even much more complex, as – according to the hypothesis of a sensitive window during pre- and/or postnatal development – exposure has to be measured during the relevant period far earlier than the investigation of sperm concentration possible in the adult age. But what is the age period of the relevant critical window? According to general knowledge of the development of the male reproductive system in humans, the "neonatal minipuberty" (e.g. Lanciotti et al. 2018) as well as birth and the "neonatal-infantile" period (ending with 12-18 months, Sharpe et al. 2003) are under discussion. This would be consistent with the study of Mocarelli et al. (2011), less consistent with the study of Mocarelli et al. (2008) due to the broad range of the age span with a mean age of 6.2 years, and not consistent with the Russian study of Minguez-Alarcon et al. (2017) with exposure assessment at the age of 8-9 years not reflecting the exposure in the first year of life. Currently, there is only inadequate evidence for the existence of a critical window in childhood in which TCDD/dioxin exposure leads to a persistent effect e.g. on Sertoli cells, measurable as decreased sperm concentrations in adulthood.

(3) What could be regarded as mode of action for the effect on sperm concentrations?

The CONTAM Panel noted on page 148: "*There is a large body of evidence attesting to the binding of PCDD/Fs and DL-PCBs to the AHR as a prerequisite for their toxic manifestations. AHR binding and its consequent activation can thus be considered the molecular initiating event. Various adverse effects may result from this initial event although the modes of action have not been clarified*." Unfortunately, no data are available on the possible mechanisms regarding of action of such consequent activations e.g. on Sertoli cells in humans.

(4) What strength of evidence is provided by data from the Seveso studies in relation to a causal effect of TCDD on sperm concentrations?

The first Seveso study (Mocarelli et al. 2008) was not able to demonstrate dose-dependency for TCDD, and in the second study (Mocarelli et al. 2011), a dose-response model did not reach statistical significance, maybe due to the low number of exposed men. A missing dose-dependency is important and rules out stronger evidence. The effect between exposed and control men may be due to the different levels of dioxin exposure, but may also be due to other factors, like co-exposure to chemicals other than TCDD in the exposed men, or the fact that control men were healthy blood donors, as also stated by the CONTAM Panel. In view of the BfR, the evidence for a causal effect of TCDD on sperm concentration is limited.

(5) Do the data from the "Russian Children's Study" provide an appropriate basis for a causally related dioxin effect to be used for the derivation of a HBGV?

In view of the BfR, the paper of Minguez-Alarcon et al. (2017) has several shortcomings and limitations. It is part of a large cohort study ("Russian Children's Study") in the vicinity of a chemical manufacturing plant which has led to an extensive contamination of the environment with special pattern of dioxins, but also with organochlorine pesticides, lead and probably other compounds with shorter half-lives not detected in the study. A couple of other effects related to different compounds has been published over the last years (Sergeyev et al. 2017). An association between sperm concentrations and TCDD/dioxins was observed in a very narrow dose range, but no associations were observed regarding PCDF TEQ and DL-PCBs TEQ. This is surprising and contradicts the theory of the crucial binding affinity at the AhR. Regarding the different quartiles based on PCDD/F TEQ, no description is available on the differences in other relevant factors possibly influencing the outcome (e.g. lifestyle characteristics or other chemicals). Therefore, the value of statistical adjustment and the possible residual confounding can't be adequately evaluated. Furthermore, more information about the shape of the association would be of interest. How is the distribution of PCDD in the study population? More information on linearity/non-linearity, on results of other percentiles

(e.g. tertiles, quintiles) as well as on sensitivity analyses (e.g. results restricted to non-smokers) could be provided to show the stability of the association.

More importantly, the question of quantification of the exposure during the critical window is crucial for this kind of studies. Minguez-Alarcon et al. (2017) provided no justification why the age of 8-9 years was chosen for the exposure assessment, and why they think that exposure at this age has relevance for the effect on sperm concentrations measured about 10 years later. The age of 8-9 years is within the period of 1-9 years chosen by Mocarelli et al. (2008), but – as discussed above – no reasonable justification was given for such a long period. Regarding the hypothesis of the "neonatal minipuberty" or the "neonatal-infantile" period ending with 12-18 months as sensitive window, the exposure during the first months and up to 1.5 years of life would be relevant. Therefore, data on maternal exposure and the duration of breastfeeding would be helpful to calculate an exposure equivalent for this period (e.g. as peak level or as area under the curve, AUC). Indeed, this data is available for the mothers of the study participants (for the same time their boys were included, Humblet et al. 2010), but in the paper by Minguez-Alarcon et al. (2017), the exposure related to breastfeeding is not mentioned at all. Instead, the study was evaluated using the serum levels measured at the age of 8-9 years in the boys' blood. However, at this age dioxin burden of breast-fed and formula-fed children have largely aligned (Kreuzer et al., 1997), so that both the peak exposure at the end of the breastfeeding period as well as the AUC were missed as potentially important surrogates of exposure.

In contrast to studies of Mocarelli et al. (2008, 2011), a clear dose-response relationship based to the levels of PCDD/F TEQ seems to be observed. However, this interpretation would mean a very steep curve with a -41 % response between the levels of 7.0 pg/g fat (median Q1) and 10.9 pg/g fat (median Q2), reaching its maximum already at the Q2 dose level (no further decrease at higher dose levels). Theoretically, such a steep dose-response with levelling off at higher doses is possible. This, however, would question the results of the Seveso studies which observed the decrease of sperm concentrations roughly in the same magnitude (-25 to -45 %), but at about 10-fold higher levels (see above). The CONTAM Panel noted in this context that a comparison of the studies from Italy and Russia is difficult, as "*timing of exposure …. differed, and other co-exposures are likely to have differed as well"*. These considerations, however, are not convincing, as they question all the results. Furthermore, humans are known to be relatively insensitive to effects of dioxins, and yet no other effect has been reproducibly observed with convincing evidence at such a low dose level (representing common background levels in Europe in the 1990s). As a third argument, an effect of this magnitude at high background levels is not compatible with data on the global "sperm crisis", as discussed below. Taking all together, the observed association between PCDD/F levels and lower sperm concentrations does not seem to be plausible with regard to a causal relation.

In summary, the 133 participants of the Russian Children's Study were exposed to a complex chemical mixture with a special contamination pattern, with many compounds possibly

confounding the results on the sperm concentrations. The association observed between sperm concentrations and dioxins (but surprisingly not for furans, DL-PCBs and total TEQ) in the (high) background range suggests a very steep dose-response relation with a -40% effect between 7.0 and 10.9 pg/g fat, with no further decrease at higher levels. While theoretically possible, the interpretation of the results as causally related to dioxin exposure is not consistent with the results of the Seveso studies reporting an effect of roughly the same extent but at about 10-fold higher dose levels. A dioxin-related effect is also not plausible in the context of the period of exposure considered (age 8-9 years) which is chosen without reasonable explanation and which is not suitable to represent the exposure in early childhood. Maybe, a better surrogate of exposure during this period would be possible to calculate from data (exposure and duration of breastfeeding) of the boys' mothers, but such an attempt was not made by the authors.

In view of the BfR, the selection of the study of Minguez-Alarcon et al. (2017) as a key study does not seem justified. The described shortcomings and limitations of the study do not provide an appropriate basis for a casually related dioxin effect to be used as "Point of Departure" for the derivation of a HBGV. To more strengthen the issue, at least one additional study with high statistical power should to be performed to confirm the results.

(6) Does a dioxin effect on sperm concentrations in the (high) background range match with epidemiological data on sperm concentrations in developed countries (global "sperm crisis")?

A decrease of sperm concentrations in developed countries is under scientific and public debate since 1992 (Carlsen et al. 1992). Many life-style factors like smoking, nutrition, obesity, physical activity or a warm environment are discussed to be responsible for the effect. In a recent meta-regression analysis, a significant decline in sperm concentrations was reported between 1973 and 2011, driven by a 50-60 % decline among men unselected by fertility from North America, Europe, Australia and New Zealand (Levine et al. 2017).

With regard to dioxin, the peak of emissions was probably in the 1960s (Hagenmaier and Walczok 1996; Zennegg et al. 2004), and the peak of human exposure probably in the beginning of the 1970s; since then there has been a continuous decline of human exposure (by a factor of about ten from the end of the 1980s to 2016 in Germany: Padberg et al. 2018). Considering the delay between exposure in young childhood and the possibility to measure semen quality of young men, the sperm concentrations would be expected to show a low in the beginning of the 1990s and a subsequent increase, if dioxins would contribute substantially to the effect. However, sperm concentrations were found to further decrease in the meta-analysis of Levine et al. (2017).

An alternative view on the data is provided by a part of the experts in this field, questioning the comparability of results from different labs and different countries (Nieschlag and Lerchl 2013). They emphasize that other long-term studies in individual countries under controlled conditions in the same lab did not show any significant trend. The best example is the Danish study already mentioned (Priskorn et al. 2018) which yearly examined about 300 young men over a period of 21 years (from 1996 to 2016). No time trend of the sperm concentrations was observed. If dioxins would have an important effect on sperm concentrations at (high) background levels, at least a positive trend should have been observed in the Danish study during this period of strongly decreasing dioxin background levels.

In view of the BfR, a relevant negative effect of dioxins on sperm concentrations in the background range, as suggested from results of the Russian Children's Study, does not match with results of the global "sperm crisis" in developed countries, independently of the expert views on the global data (decreasing or missing time trend).

4 References

Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. BMJ 1992;305:609–613.

Cooper TG, Keck C, Oberdieck U, Nieschlag E. Effects of multiple ejaculations after extended periods of sexual abstinence on total, motile and normal sperm numbers, as well as accessory gland secretions, from healthy normal and oligozoospermic men. Human Reproduction Hum Reprod. 1993 Aug;8(8):1251-8.

Hagenmaier H and Walczok M. Time trends in levels, patterns and profiles for PCDD/PCDF in sediment cores of Lake Constance. Organohalogen Compounds 1996; 28: 101-104

Humblet O, Williams PL, Korrick SA, Sergeyev O, Emond C, Birnbaum LS, Burns JS, Altshul L, Patterson DG, Turner WE, Lee MM, Revich B, Hauser R. Predictors of serum dioxin, furan, and PCB concentrations among women from Chapaevsk, Russia. Environ Sci Technol. 2010 Jul 15;44(14):5633-40.

Kreuzer PE, Csanády GA, Baur C, Kessler W, Päpke O, Greim H, Filser JG. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and congeners in infants. A toxicokinetic model of human lifetime body burden by TCDD with special emphasis on its uptake by nutrition. Arch Toxicol. 1997;71(6):383-400.

Lanciotti L, Cofini M, Leonardi A, Penta L, Esposito S. Up-to-date review about minipuberty and overview on hypothalamic-pituitary-gonadal axis activation in fetal and neonatal Life. Front Endocrinol (Lausanne) 2018; 9: 410.

Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R, Swan SH. Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum Reprod Update. 2017 Nov 1;23(6):646-659. doi: 10.1093/humupd/dmx022.

Mínguez-Alarcón L, Sergeyev O, Burns JS, Williams PL, Lee MM, Korrick SA, Smigulina L, Revich B, Hauser R. A Longitudinal Study of Peripubertal Serum Organochlorine Concentrations and Semen Parameters in Young Men: The Russian Children's Study. Environ Health Perspect. 2017 Mar;125(3):460-466.

Mocarelli P, Gerthoux PM, Needham LL, Patterson DG Jr, Limonta G, Falbo R, Signorini S, Bertona M, Crespi C, Sarto C, Scott PK, Turner WE, Brambilla P. Perinatal exposure to low doses of dioxin can permanently impair human semen quality. Environ Health Perspect. 2011 May;119(5):713-8.

Mocarelli P, Gerthoux PM, Patterson DG Jr, Milani S, Limonta G, Bertona M, Signorini S, Tramacere P, Colombo L, Crespi C, Brambilla P, Sarto C, Carreri V, Sampson EJ, Turner WE, Needham LL. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environ Health Perspect. 2008 Jan;116(1):70-7.

Nieschlag E, Lerchl A. Sperm crisis: what crisis? Asian J Androl. 2013 Mar;15(2):184-6.

Padberg S, Bührer C, Menzel J, Weikert C, Schaefer C, Abraham K. [Xenobiotics and pathogens in breast milk : A risk for the child?] Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2018 Aug;61(8):960-970.

Priskorn L, Nordkap L, Bang AK, Krause M, Holmboe SA, Egeberg Palme DL, Winge SB, Mørup N, Carlsen E, Joensen UN, Blomberg Jensen M, Main KM, Juul A, Skakkebaek NE, Jensen TK, Jørgensen N. Average sperm count remains unchanged despite reduction in maternal smoking: results from a large cross-sectional study with annual investigations over 21 years. Hum Reprod. 2018 Jun 1;33(6):998-1008.

Sergeyev O, Burns JS, Williams PL, Korrick SA, Lee MM, Revich B, Hauser R. The association of peripubertal serum concentrations of organochlorine chemicals and blood lead with growth and pubertal development in a longitudinal cohort of boys: a review of published results from the Russian Children's Study. Rev Environ Health. 2017 Mar 1;32(1-2):83-92. doi: 10.1515/reveh-2016-0052.

Sharpe RM, McKinnell C, Kivlin C, Fisher JS. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. Reproduction 2003 Jun;125(6):769-84.

Zennegg M, Kohler M, Hartmann P, Sturm M, Gujer E, Schmid P, Gerecke AC, Heeb NV, Kohler HP, Giger W. The historical record of PCB and PCDD/F deposition at Greifensee between 1848 and 1999. Organohalogen Compounds 2004; 66: 1363-1366

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EFSA Opinion on Dioxins and DL-PCBs in Food and Feed

Remarks of the BfR

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Preface

The BfR thanks the CONTAM Panel for the outstanding work done for the preparation of the Opinion on Dioxins and DL-PCBs in Food and Feed.

In view of the BfR, the main issue for discussion is the critical effect chosen by the CONTAM Panel for the derivation of a HBGV: **lower sperm concentrations in association with TCDD/dioxin exposure during childhood**. Other effects, e.g. on sperm motility or hormone levels, are not discussed here.

In the context, the BfR gives rise to 6 key questions:

BfR Key questions

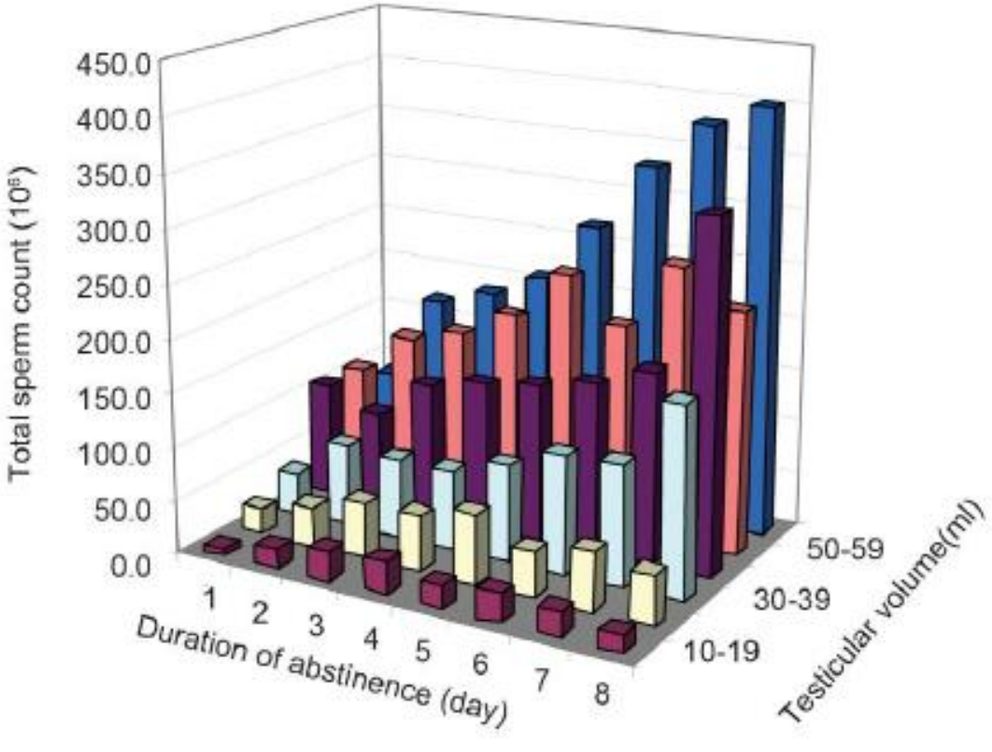
(1) How robust is the parameter “sperm concentration”?

Many factors potentially influence the result. They are difficult to control.
⟶ Huge variability far above that of most other biological parameters

Individual factors
age
smoking (prenatal + current exposure)
duration of abstinence
frequency of ejaculations
onset of puberty
testicular volume

Life-style factors under discussion:
nutrition, obesity, physical activity
warm environment

Technical factors
transport (temperature, duration)
experience of the technician



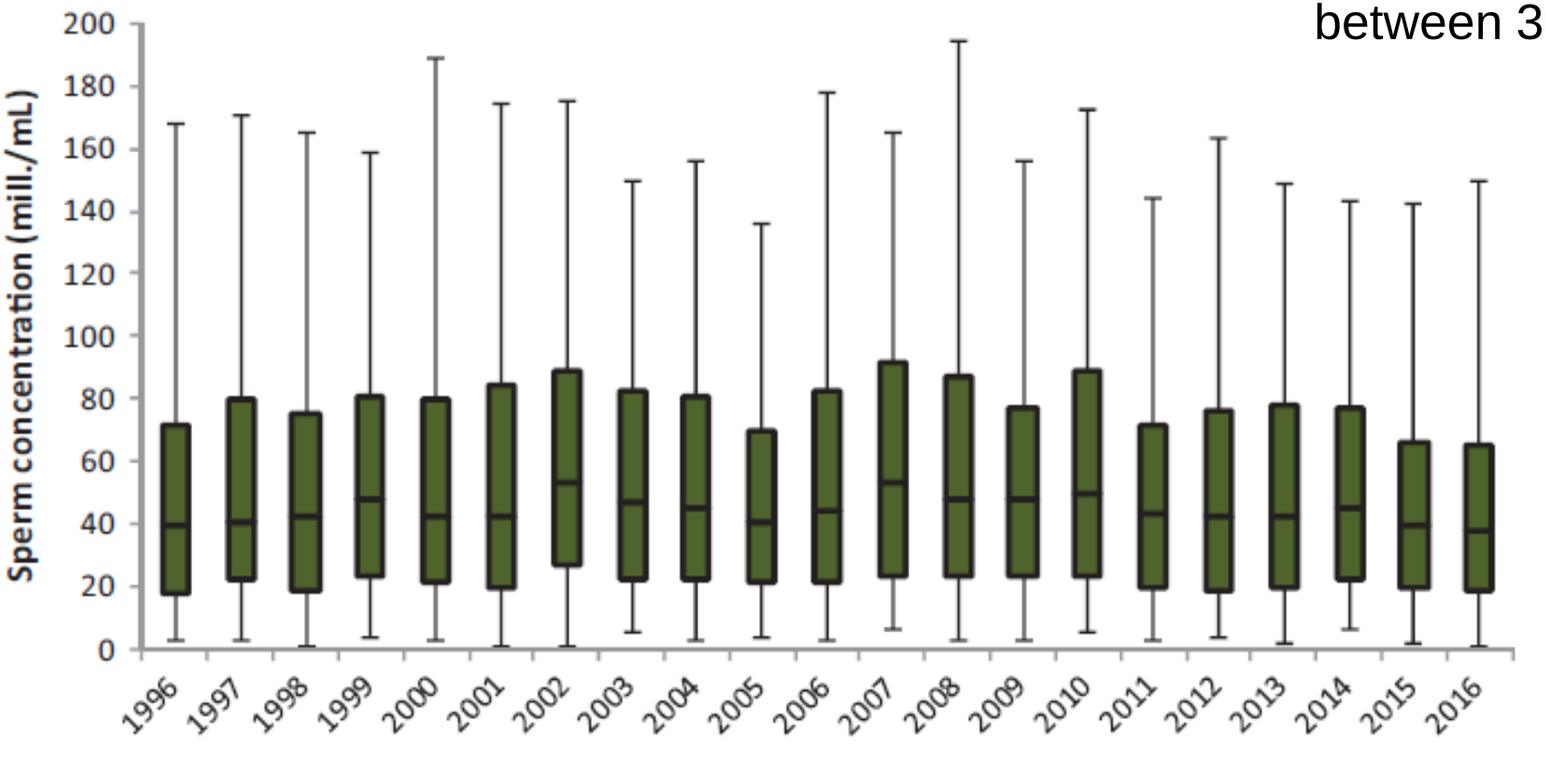
Nieschlag and Lerchl 2013

BfR Key questions

(1) How robust is the parameter “sperm concentration”?

General population of Denmark: about 300 recruits investigated each year

Annual median values
between 36 and 50 million/mL



(n=6386)

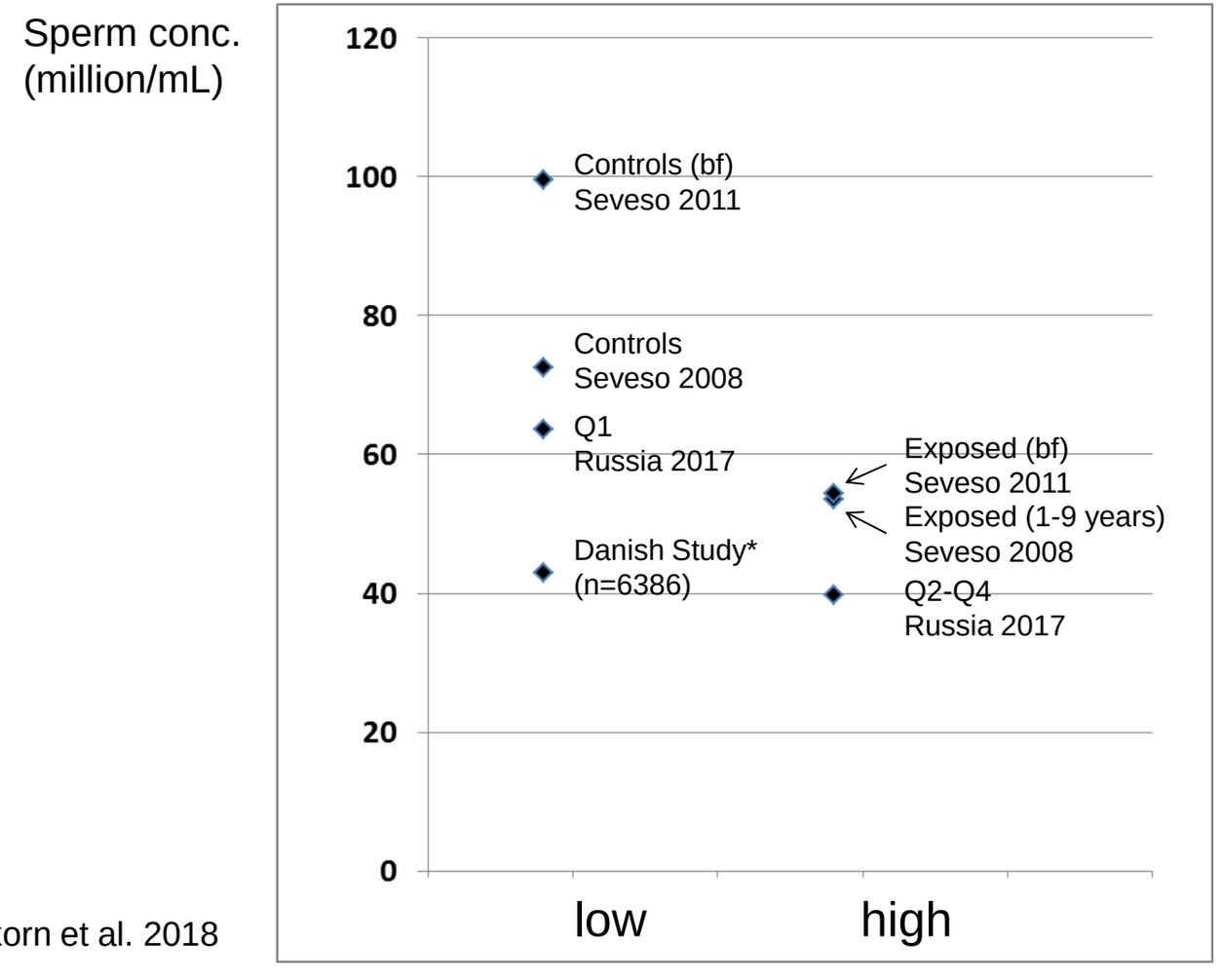
Priskorn et al. 2018

BfR Key questions

(1) How robust is the parameter “sperm concentration”?

Absolute values?

Median values



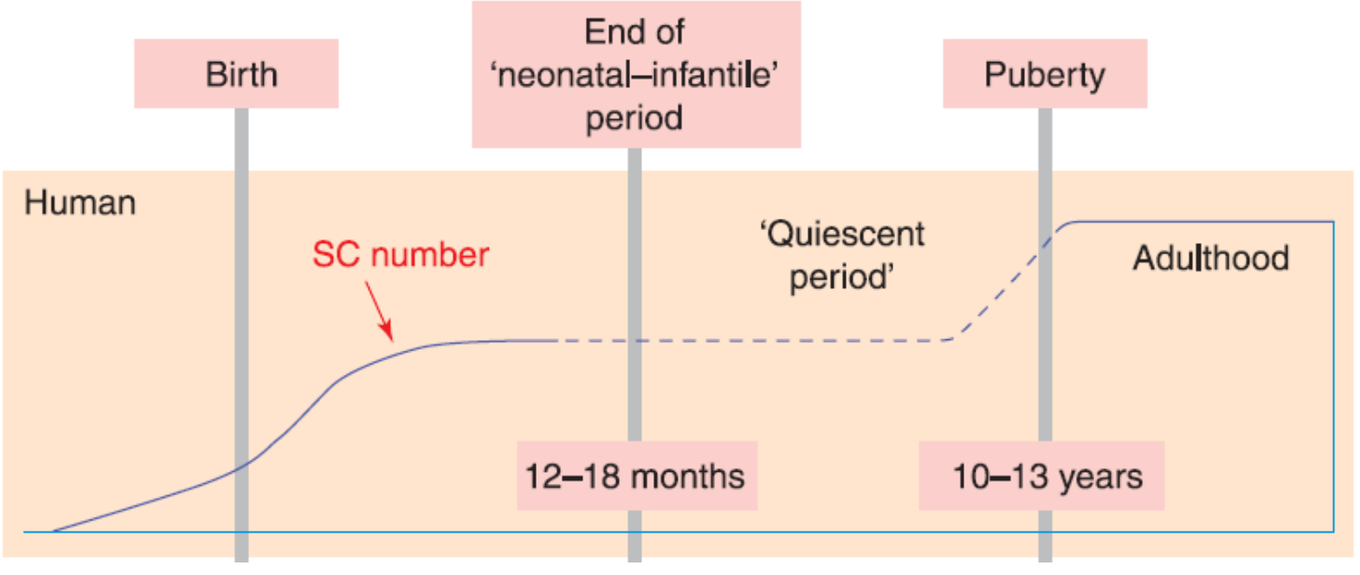
*Priskorn et al. 2018

BfR Key questions

(2) What is the critical window of exposure?

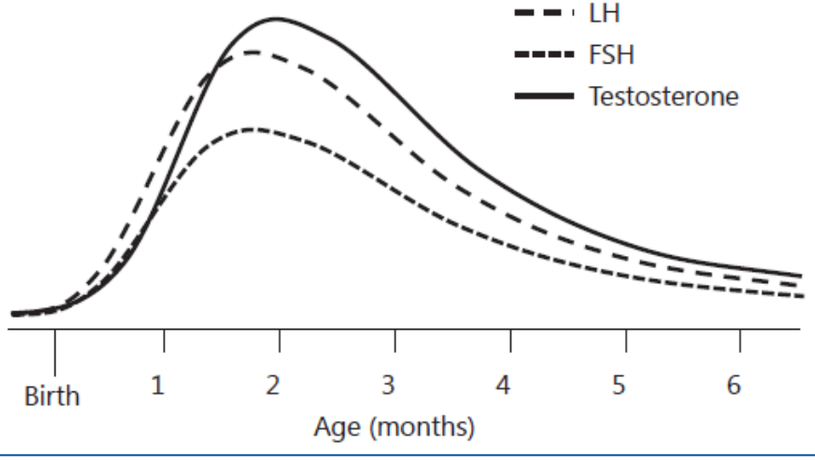
Hypothesis: exposure during a critical window in (early) childhood leads a permanent effect on sperm concentrations measurable in adulthood.

Timing of maturation of Sertoli cells



Sharpe et al. 2003

„Minipuberty“



Kuiri-Hänninen et al. 2014

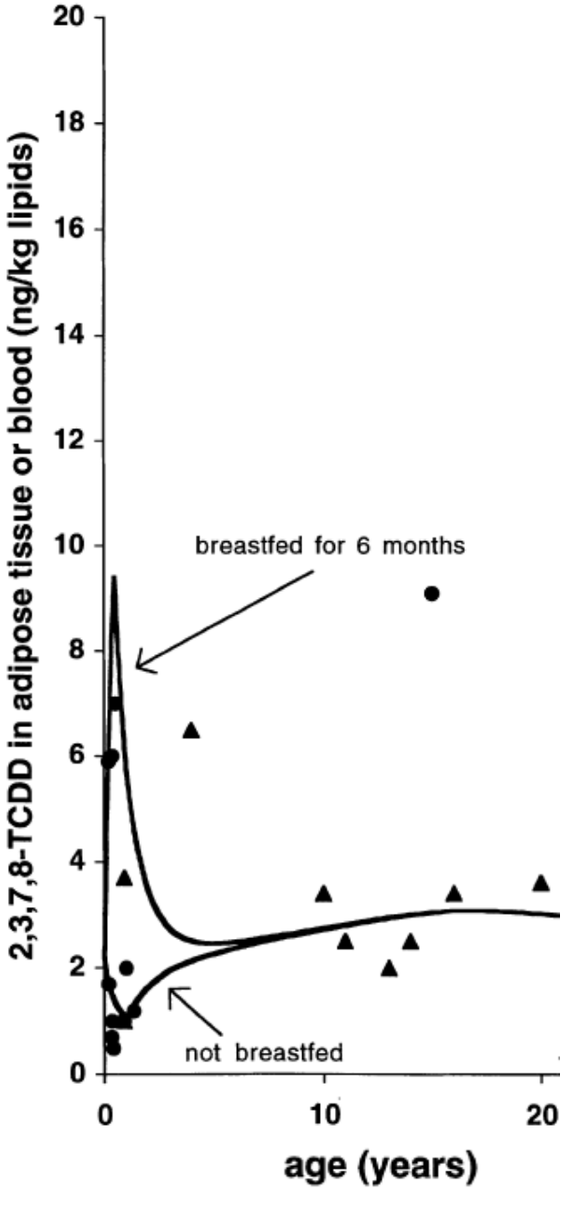
BfR Key questions

(2) What is the critical window of exposure?

This would be consistent with the study of Mocarelli et al. (2011),

but less consistent with the study of Mocarelli et al. (2008) due to the broad range of the age span with a mean age of 6.2 years,

and not consistent with the Russian study of Minguez-Alarcon et al. (2017) with exposure assessment at the age of 8-9 years not reflecting the exposure in the first year of life.



Kreuzer et al. 1997

BfR Key questions

(3) What could be regarded as mode of action for the effect on sperm concentrations?

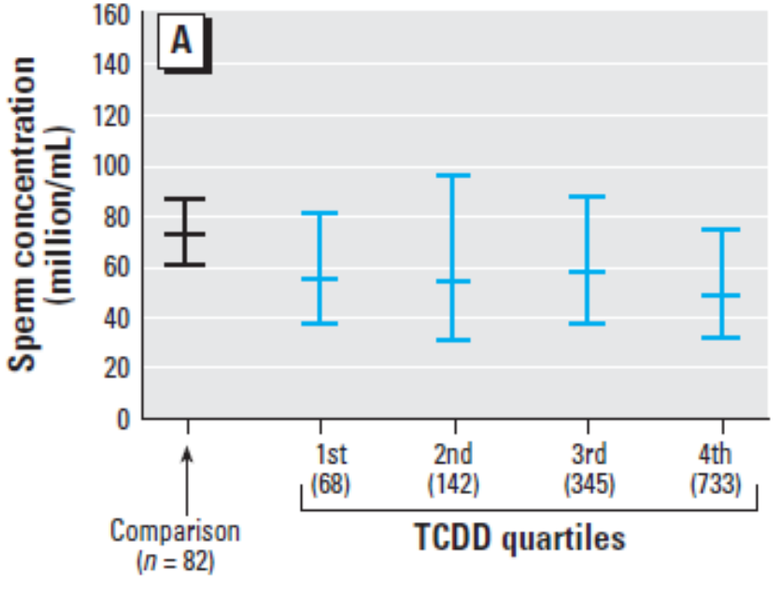
The CONTAM Panel noted on page 148: "*There is a large body of evidence attesting to the binding of PCDD/Fs and DL-PCBs to the AHR as a prerequisite for their toxic manifestations.*
AHR binding and its consequent activation can thus be considered the molecular initiating event. Various adverse effects may result from this initial event although the modes of action have not been clarified."

Unfortunately, no data are available on the possible mechanisms regarding of action of such consequent activations e.g. on Sertoli cells in humans.

BfR Key questions

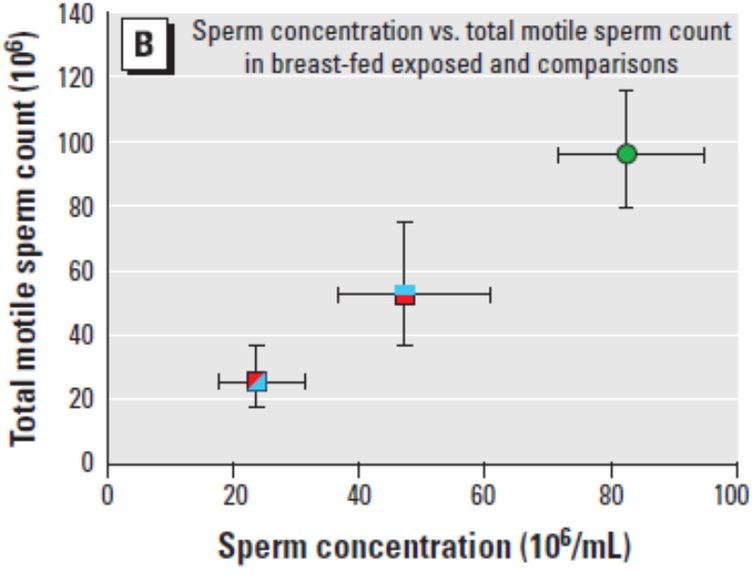
(4) What strength of evidence is provided by data from the Seveso studies in relation to a causal effect of TCDD on sperm concentrations?

Mocarelli et al. 2008
Age at exposure: 1-9 years
Controls: age-matched blood donors



No dose-dependency for TCDD

Mocarelli et al. 2011
Maternal exposure estimated
breast-feeding vs. formala-feeding
Controls: age-matched blood donors



Dose-response model: no statistical significance

→ Limited Evidence for a causal effect of TCDD on sperm concentrations in the Seveso studies

BfR Key questions

(5) Do the data from the "Russian Children's Study" provide an appropriate basis for a causally related dioxin effect to be used for the derivation of a HBGV?

Co-exposure to other chemicals: Extensive contamination of the environment in the vicinity of a chemical manufacturing plant with unusual dioxin pattern.
Consideration as confounders possible only for very few compounds.

Why quartiles used for the evaluation? No detailed description of the quartile differences in other relevant factors. Therefore, the value of statistical adjustment and of possible residual confounding can't be adequately evaluated.

No association observed for furans and DL-PCBs, contrary to the concept of AhR binding as key event.

Investigation of exposure at the age of 8-9 years:

- no justification given
- not reflecting differences in exposure in the first year of life
- no attempt made to used other surrogates of exposure

Implausible dose-response relationship:

BfR Key questions

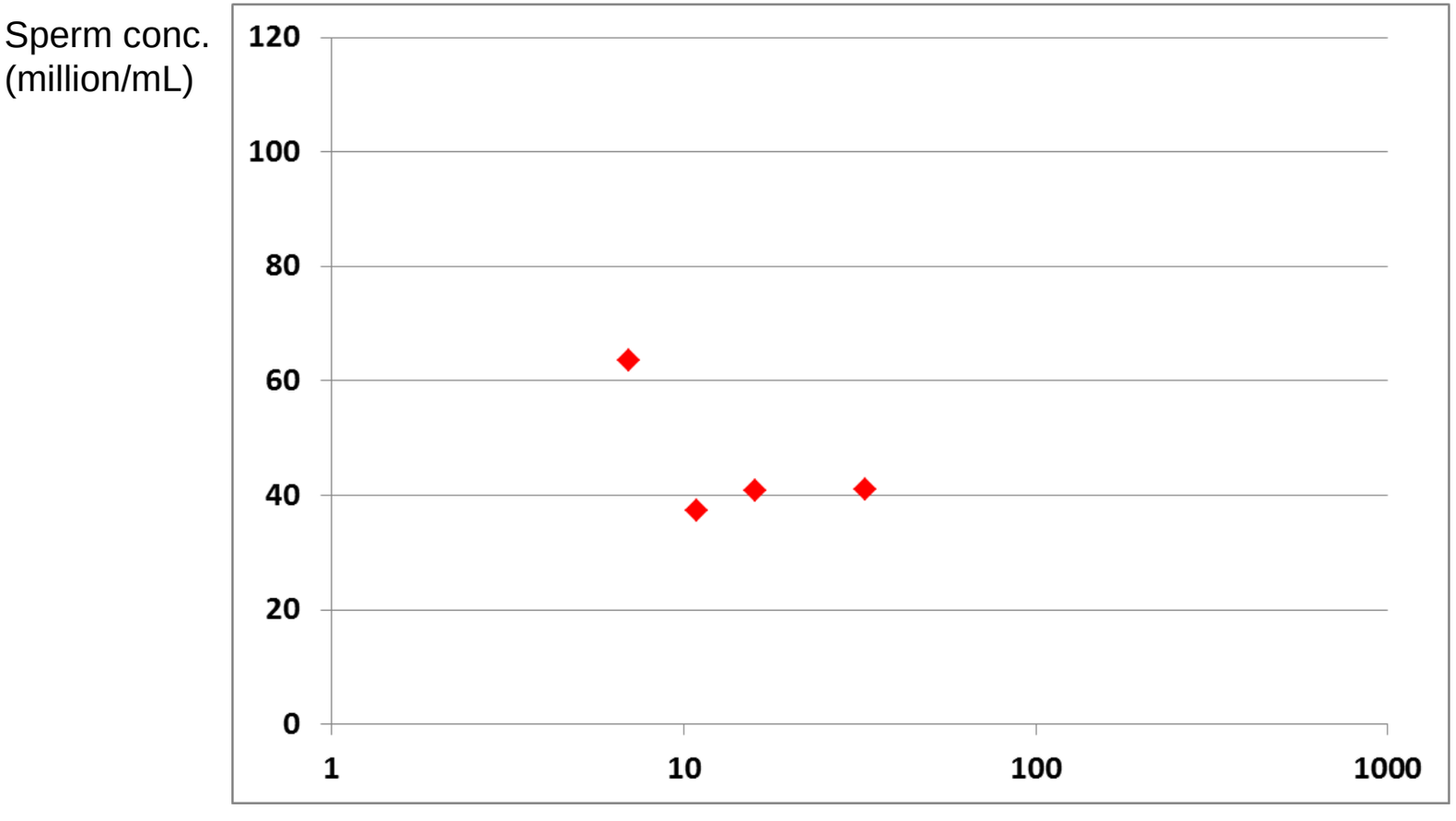
(5) Do the data from the "Russian Children's Study" provide an appropriate basis for a causally related dioxin effect to be used for the derivation of a HBGV?

Dose-response relationship and overall consistency ?

Median values

Russian Study 2017

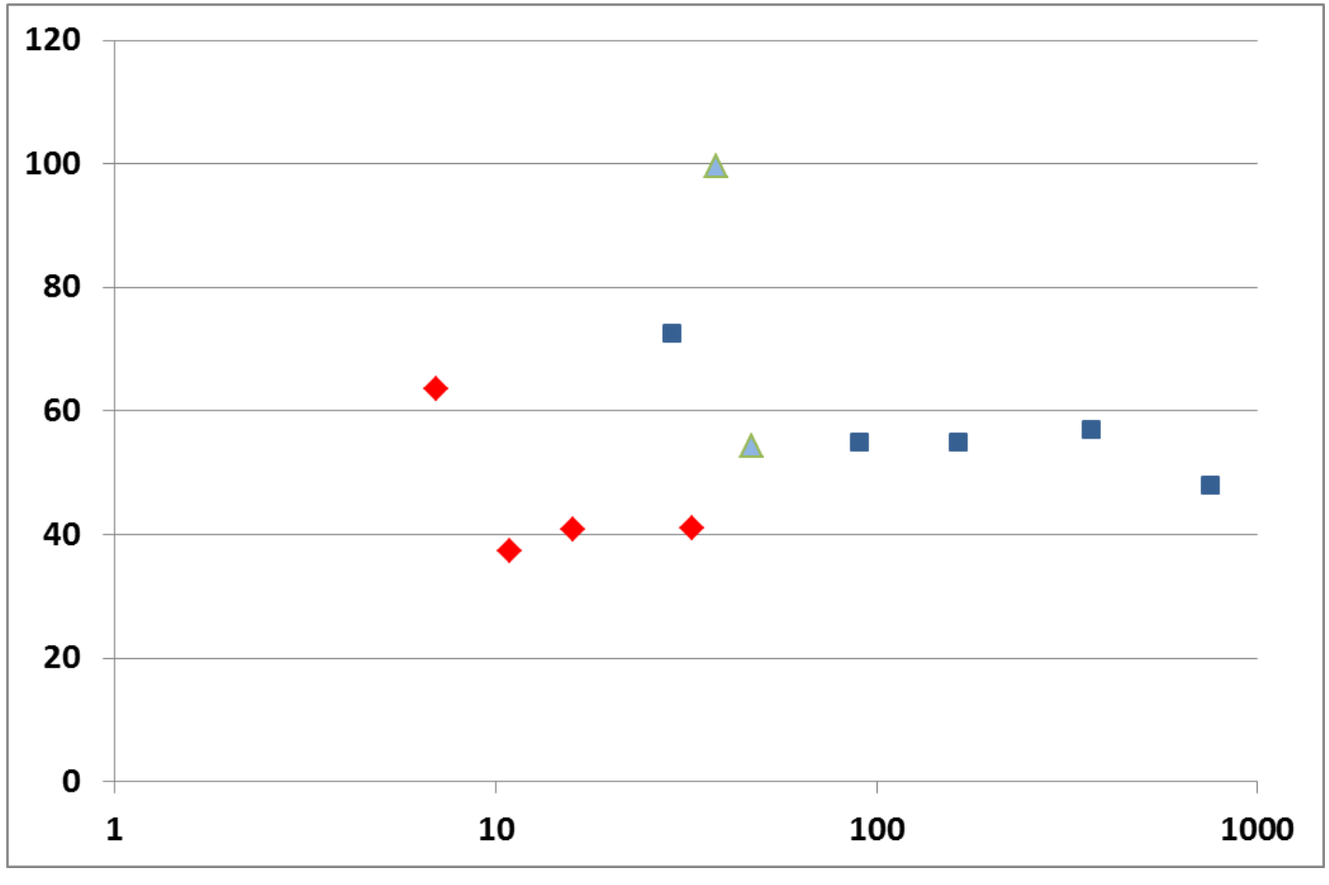
Q1 Q2 Q3 Q4



BfR Key questions

(5) Do the data from the "Russian Children's Study" provide an appropriate basis for a causally related dioxin effect to be used for the derivation of a HBGV?

Dose-response relationship and overall consistency ?



Median values

Russian Study 2017
Q1 Q2 Q3 Q4

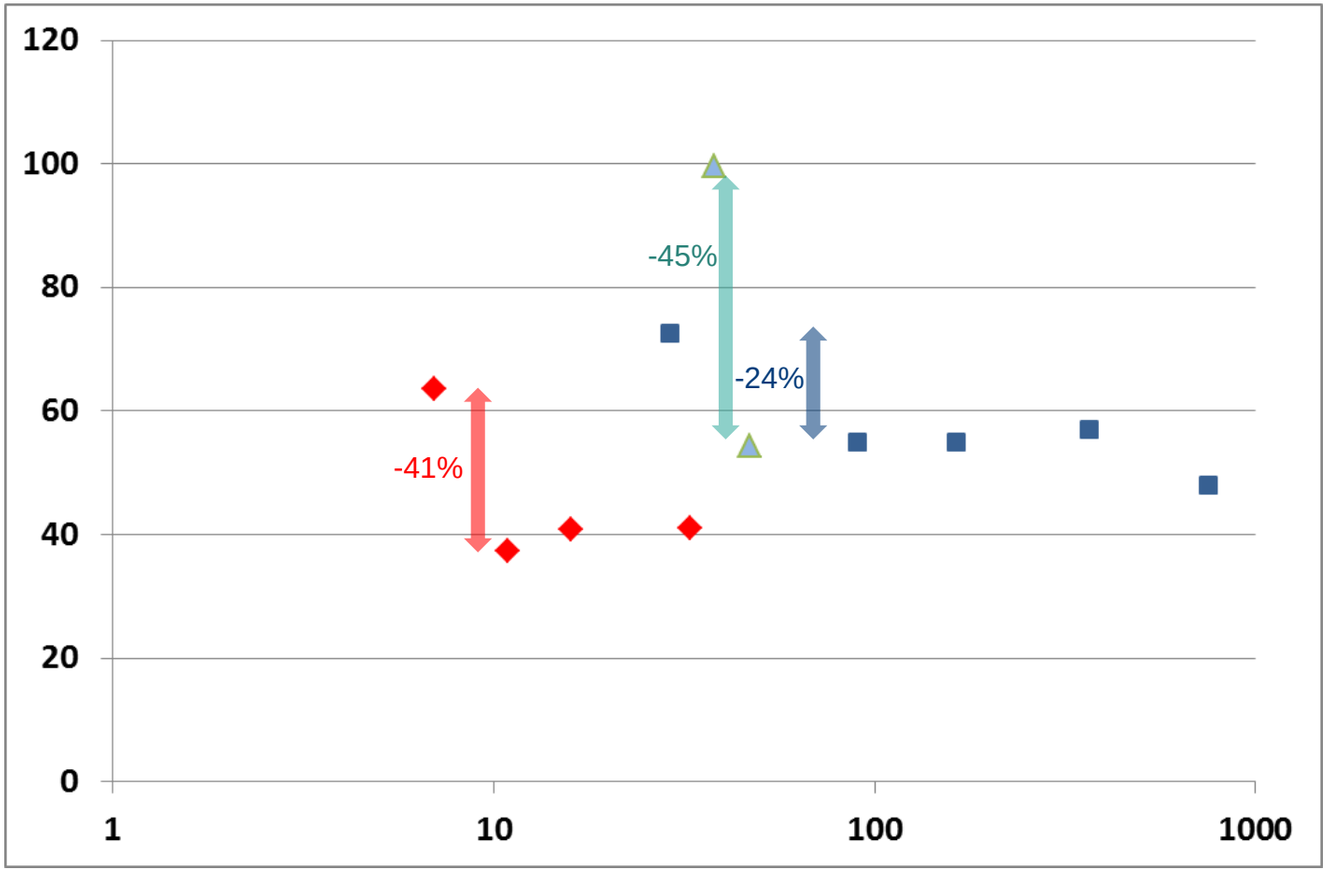
Seveso Study 2008
CG Q1 Q2 Q3 Q4

Seveso Study 2011
CG(bf) Exposed(bf)

BfR Key questions

(5) Do the data from the "Russian Children's Study" provide an appropriate basis for a causally related dioxin effect to be used for the derivation of a HBGV?

Dose-response relationship and overall consistency ?



Median values

Russian Study 2017
Q1 Q2 Q3 Q4

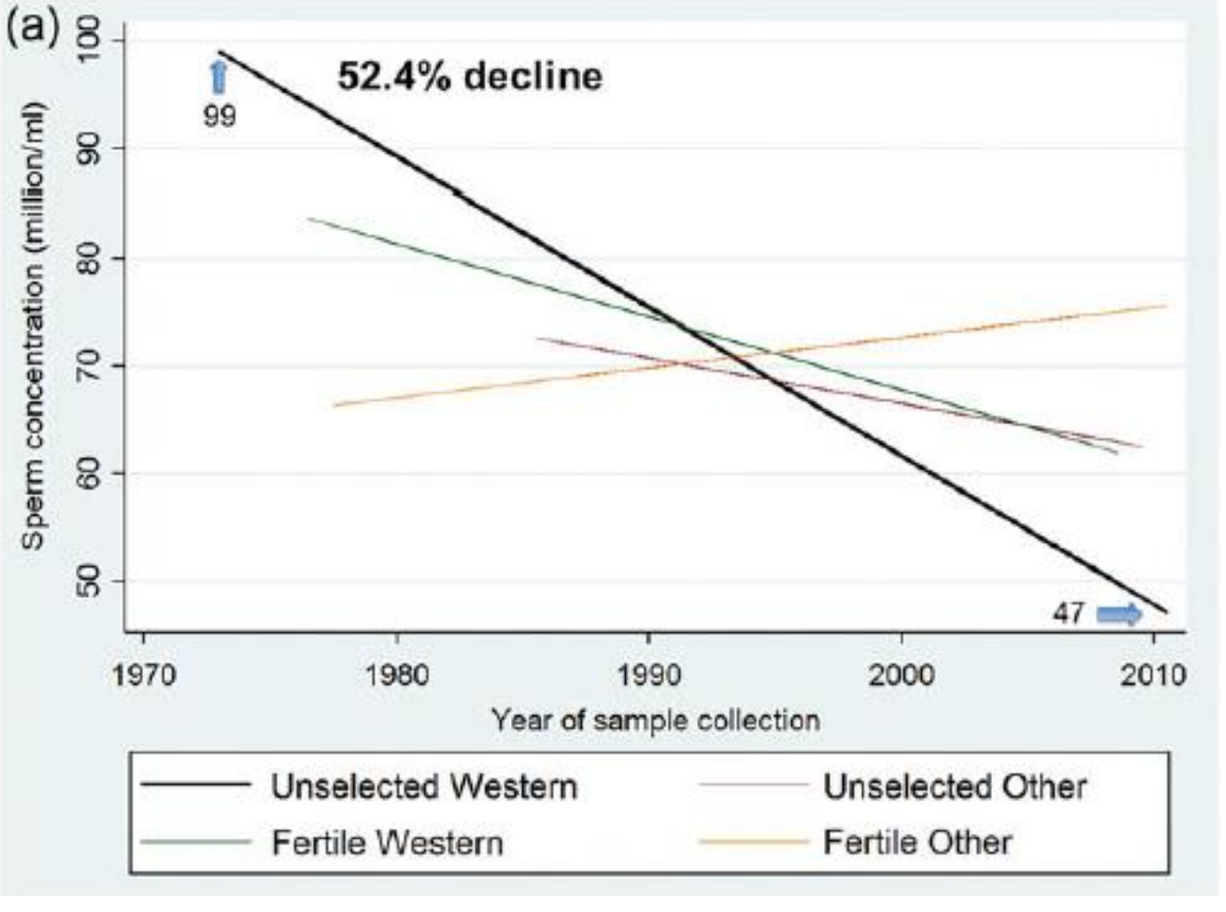
Seveso Study 2008
CG Q1 Q2 Q3 Q4

Seveso Study 2011
CG(bf) Exposed(bf)

BfR Key questions

(6) Does a dioxin effect on sperm concentrations in the (high) background range match with epidemiological data on sperm concentrations in developed countries (global “sperm crisis”)?

Temporal trends in sperm count: a systematic review and meta-regression analysis

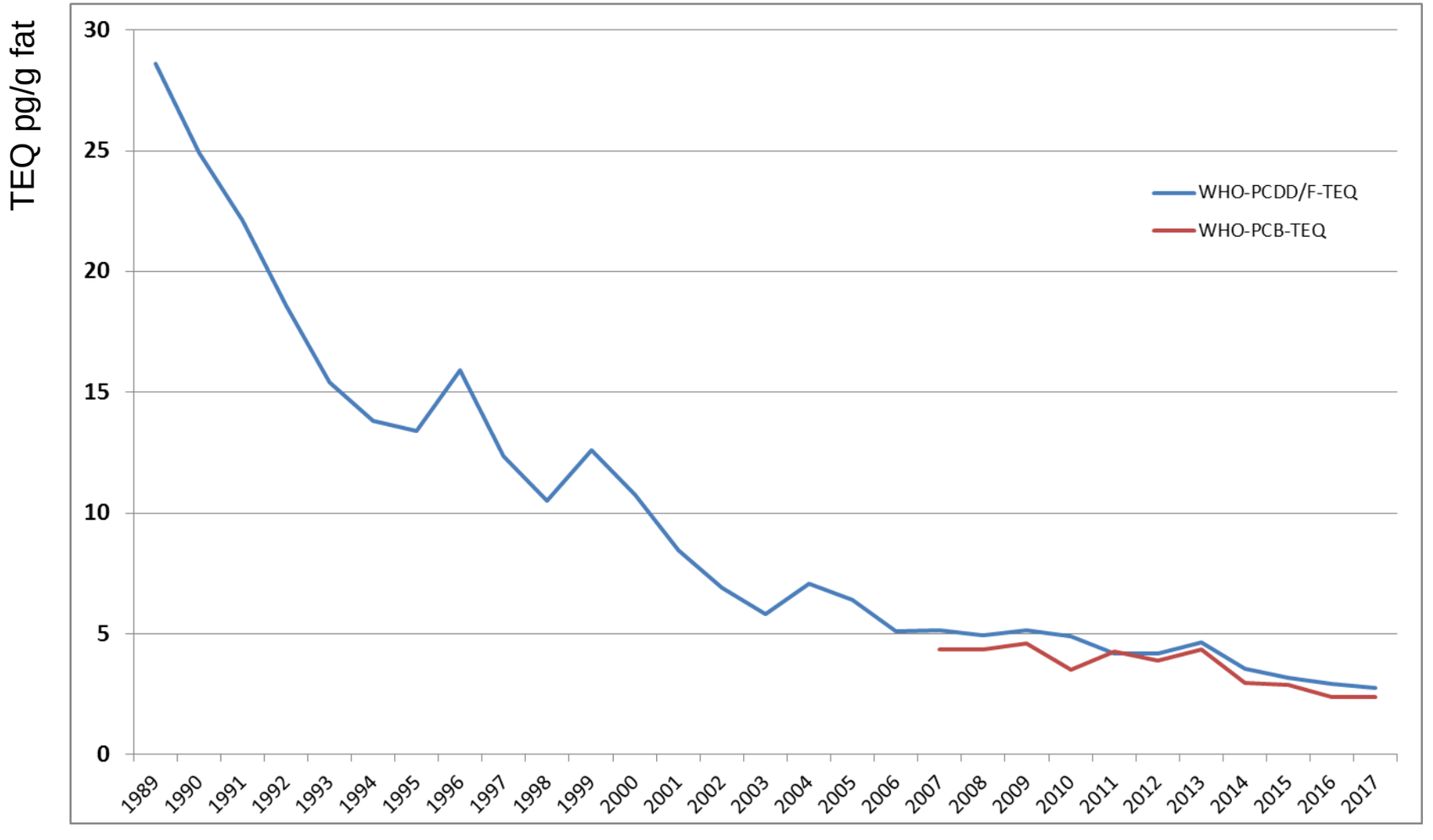


Levine et al. 2017

BfR Key questions

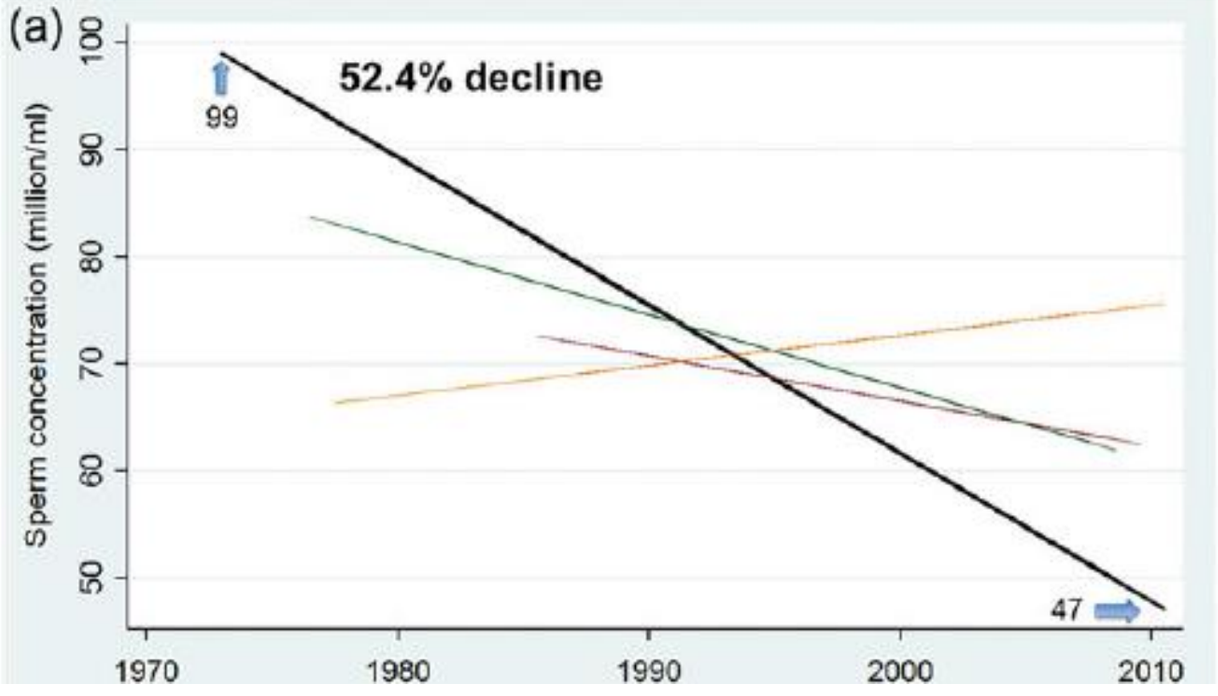
(6) Does a dioxin effect on sperm concentrations in the (high) background range match with epidemiological data on sperm concentrations in developed countries (global “sperm crisis”)?

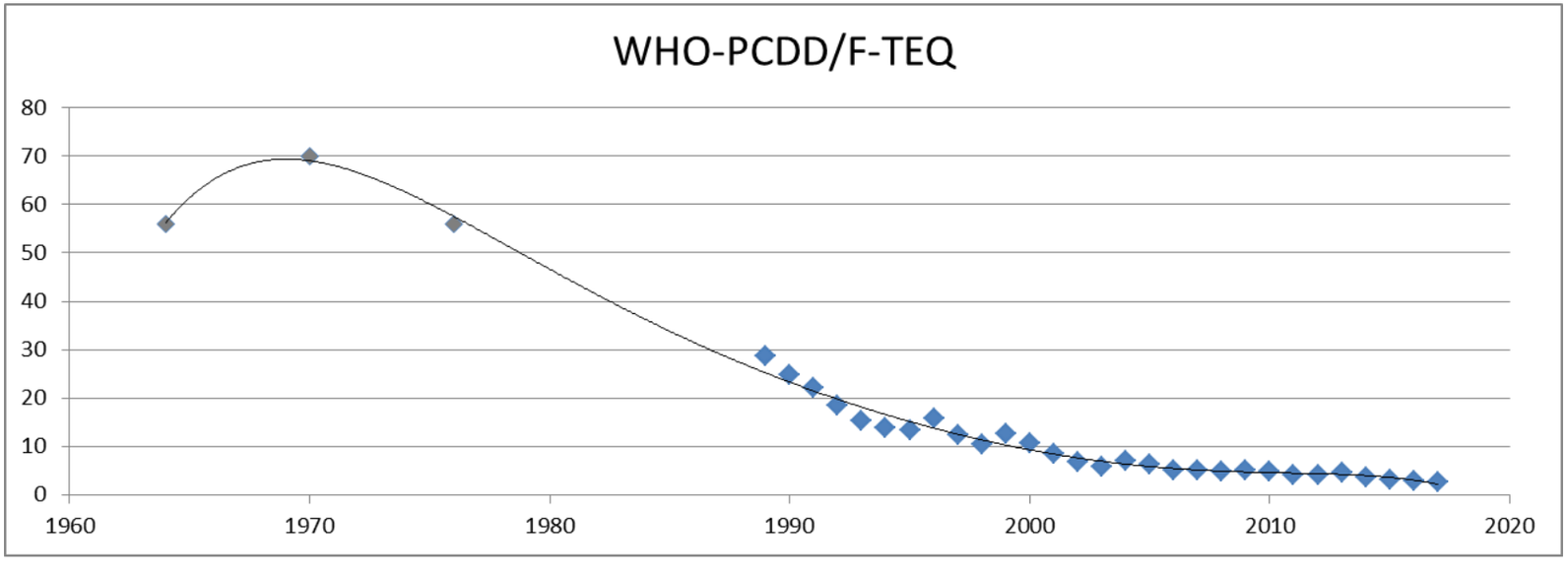
Time trend Dioxins/Furans and DL-PCBs in mothers‘ milk



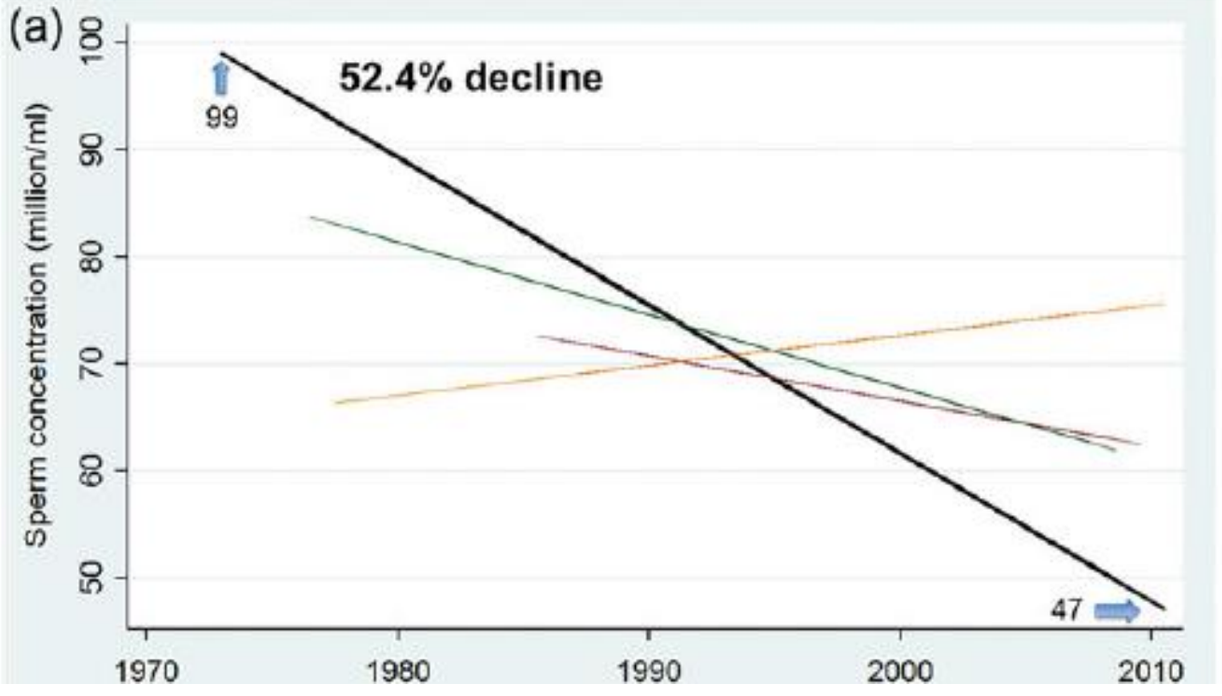
Data: LAVES Oldenburg, Germany

BfR Key questions



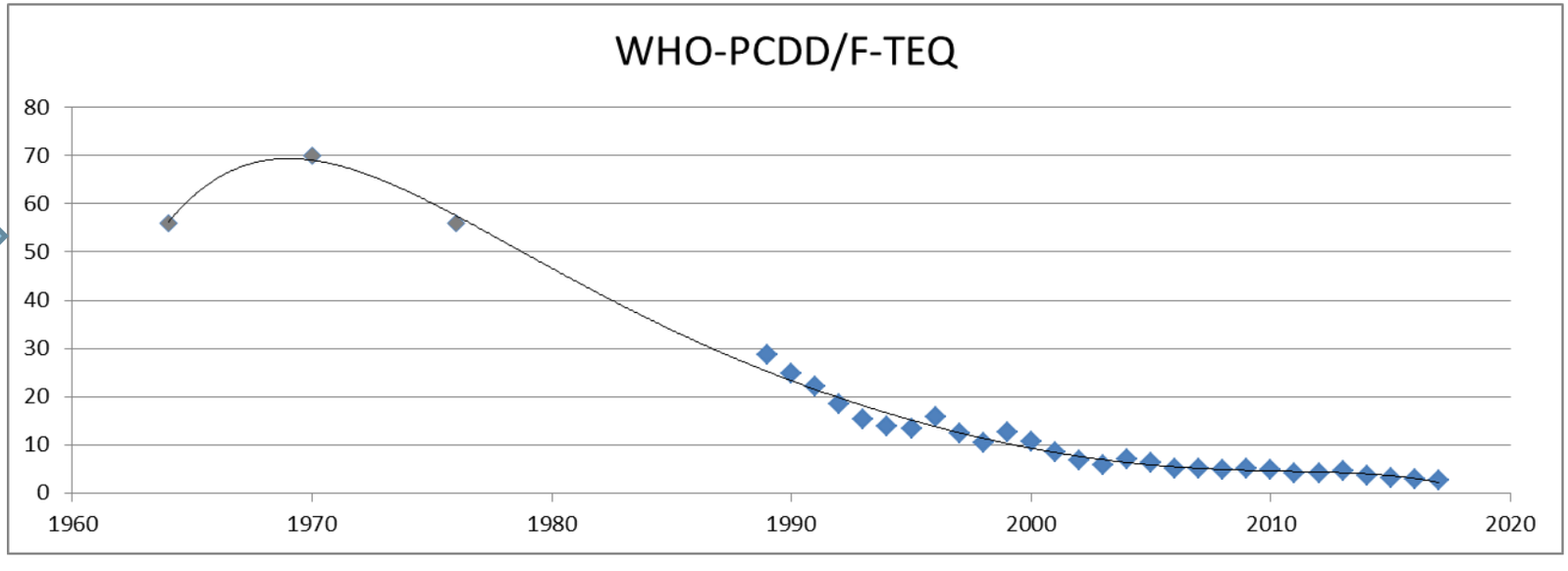


BfR Key questions



If PCDD/Fs would have an important impact on sperm concentrations in the background range, at least no further decrease would be expected since about 1990

+ 20 years



BfR Key questions

(6) Does a dioxin effect on sperm concentrations in the (high) background range match with epidemiological data on sperm concentrations in developed countries (global “sperm crisis”)?

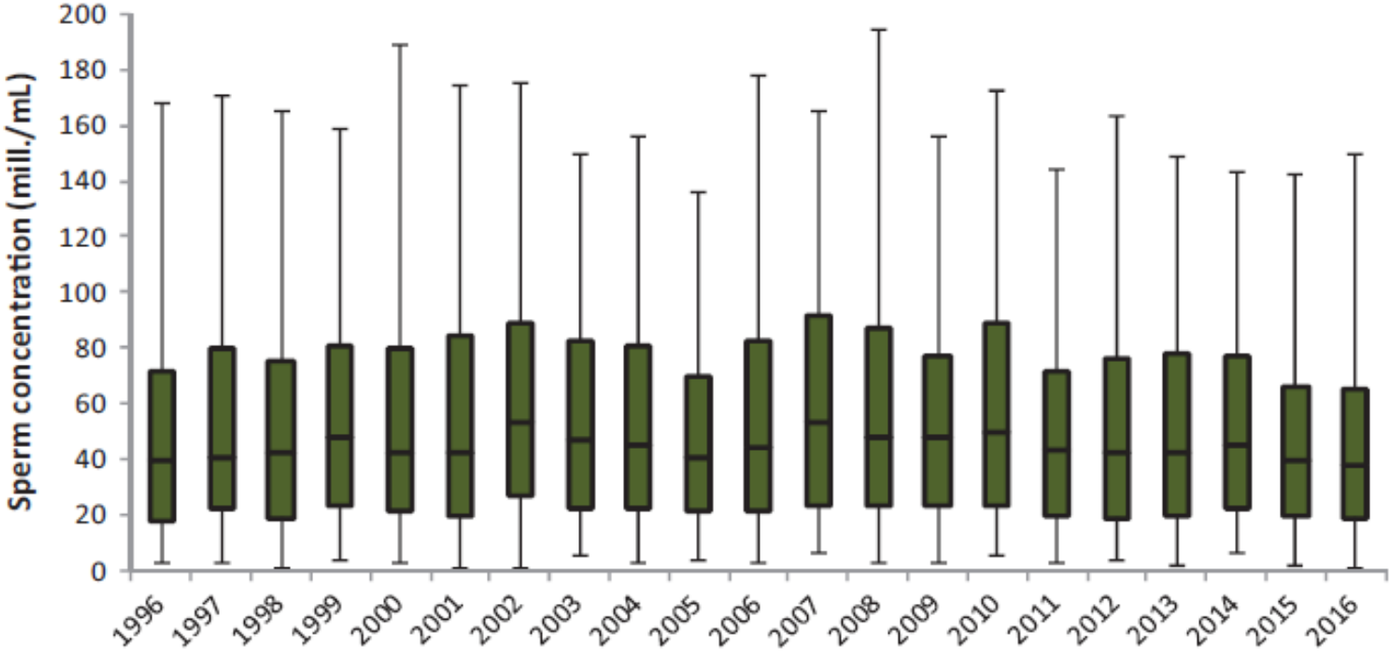
Alternative view on data: Some expert doubt the comparability of different studies from different countries.

Sperm crisis: what crisis?

Eberhard Nieschlag[1] and Alexander Lerchl[2]

Asian Journal of Andrology (2013) **15**, 184

General population of Denmark: about 300 recruits investigated each year



during a period of strongly decreasing dioxin background levels

Priskorn et al. 2018

In summary …

The parameter “sperm concentration” is very complex, and in relation to the huge variability observed, the number of participants in the 3 studies seems relatively low.

A critical window of exposure in (early) childhood is assumed, but in these studies, exposure was measured in different periods of age, and the mode of action is unknown.

Regarding the two Seveso studies, evidence for a causally related effect of TCDD is limited, especially due to missing dose-dependency.

The “Russian Children’s Study” has several limitations and provides no adequate evidence for an effect in the background range. Therefore, the selection as a key study for the derivation of a HBGV does not seem justified. At least one additional study should be performed to confirm the results.

Data on the global “sperm crisis” do not match with a relevant negative effect of dioxins.

GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

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Thank you for your attention

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