

20 February 2015

## Evaluation of EFSA's new Scientific Opinion on Bisphenol A

EFSA has on January 21, 2015 published the new evaluation of bisphenol A (BPA):

<http://www.efsa.europa.eu/en/efsajournal/pub/3978.htm>

(Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs).

The EFSA evaluation of BPA consist of a summary (22 pages), Part 1: Exposure assessment (396 pages) and part 2: Toxicological assessment and risk characterisation (621 pages).

Thus, it is a very large EFSA document with a total of 1039 sider.

The National Food Institute, Technical University of Denmark (DTU) has evaluated this EFSA opinion and the following is addressed:

1. The most important conclusions in the EFSA report
2. Proposal for an alternative TDI.

DTU has focused on Part 2 that gives the background for the new EFSA temporary TDI of 4 µg/kg bw/day. In addition, the overall conclusion with regards to risk assessment has been considered. DTU has not made a specific evaluation of the EFSA exposure assessment (i.e. Part 1). DTU has in September 2013 evaluated the EFSA draft exposure assessment and concluded the draft was of high quality and based on high quality data. This is still the opinion of DTU.

### DTU conclusions

EFSA has in the 2015 evaluation included uncertainty evaluations of the likelihood for effects of BPA on the mammary gland, and the reproductive, neurobehavioural, immune and metabolic systems.

The EFSA uncertainty evaluation is considered as insufficient by DTU. Thus, DTU does not support the extra factor of 6 chosen by EFSA leading to the use of 100 µg/kg bw/day as basis for deriving the new EFSA t-TDI of 4 µg/kg bw/day.

DTU evaluates that 4 µg/kg bw/day is not sufficiently protective with regards to endocrine disrupting effects of BPA. DTU finds that a TDI for BPA has to be 0.7 µg/kg bw/dag or lower to be sufficiently protective with regards to endocrine disrupting effects of BPA.

Highly exposed humans incl. pregnant women and children can according to EFSA's exposure assessment be exposed to more than 0.7 µg/kg bw/day. DTU finds that this gives rise to concern with regards to risk for health effects of BPA for highly exposed persons.

## Ad. 1) Evaluation of the most important EFSA conclusions

The most important EFSA conclusions are considered to be:

- EFSA establishes a new temporary TDI for BPA on 4 µg/kg bw/day. This value is lower than the TDI of 5 µg/kg bw/day proposed by EFSA in January 2014.
- EFSA concludes that based on the TDI of 4 µg/kg bw/day, and using the EFSA estimates of the total exposure to BPA, there is no health concern.

To evaluate these conclusions DTU has evaluated the background for the new TDI for BPA of 4 µg/kg bw/day with regards to the most important issues.

For the hazard identification, EFSA assessed the likelihood of the association between BPA exposure and the relevant toxicological endpoint, taking the degree of evidence into consideration. Based on that, the likelihood was evaluated as "very likely", "likely", "as likely as not", "unlikely to as likely as not", "unlikely" eller "very unlikely". Adverse effects on kidneys in adult animals and on mammary gland development were evaluated as "likely" and only these effects are taken forward to the EFSA hazard characterization.

The EFSA hazard characterization is based on benchmark dose (BMD) response modelling, which is generally regarded as the best method if data allow this.

For kidney effects in adult animals a BMDL10 (Benchmark dose low for 10% effect) of 8.960 µg/kg bw/day was reached, based on effects on decreased mean relative kidney weight in a two-generation study in mice. The human equivalent dose factor (HEDF) of 0.068 for oral exposure of adult mice was applied to the BMDL10 of 8 960 µg/kg bw per day, which results in a HED of 609 µg/kg bw per day. In the EFSA draft evaluation in 2014, the HED was 113 µg/kg bw/day for kidney effects in adult animals and DTU found that this value appeared conservative (very cautious). It is DTU's impression that the change in the HED from 113 µg/kg bw/day to 609 µg/kg bw/day is due to EFSA using relative kidney weight instead of absolute kidney weight. DTU evaluates that as toxicologically relevant and therefore has no scientific disagreement with that change.

For the other type of effect considered "likely" by EFSA, i.e. effect on mammary gland development, a BMDL10 could not be derived due to very large confidence intervals on the BMD estimated from the models used.

### *DTU's evaluation of effects on mammary gland development in the new FDA study*

A very important study for the EFSA's hazard evaluation of effects on mammary gland is a new study from FDA. In this study, Sprague-Dawley rat dams were dosed daily from gestation day 6 until the start of labour, and their pups were directly dosed from one day after birth to termination. The study included seven equally spaced low doses of BPA (2.5, 8, 25, 80, 260, 840 and 2,700 µg/kg bwt/day) and two high BPA doses (100,000 and 300,000 µg/kg bw/day). Also included were a naïve control, vehicle control and two doses of ethinyl estradiol (EE2). There were around 20 litters per group. As this is a very large study, DTU has also evaluated this study.

EFSA refers both to the study report (U.S. FDA/NCTR, 2013) and to a published paper on the study (Delclos et. al. 2014), but DTU has not been able to obtain the study report after contact to EFSA and FDA. The DTU evaluation is therefore based on Delclos et. al. 2014.

In female pups before sexual maturation (postnatal day 21), a significantly increased frequency of mammary gland ductal hyperplasia was found at 2700 µg/kg bw/day and higher doses by histopathological studies. However, at this pup age there will only be a limited amount of mammary tissue on the histological slides, which limits the sensitivity for detecting effects. Assessment of all of the mammary tissue using "whole mounts" is expected to be more sensitive and is therefore the preferred by DTU when investigating mammary gland effects before puberty. The results of such studies are presently not available from the FDA study.

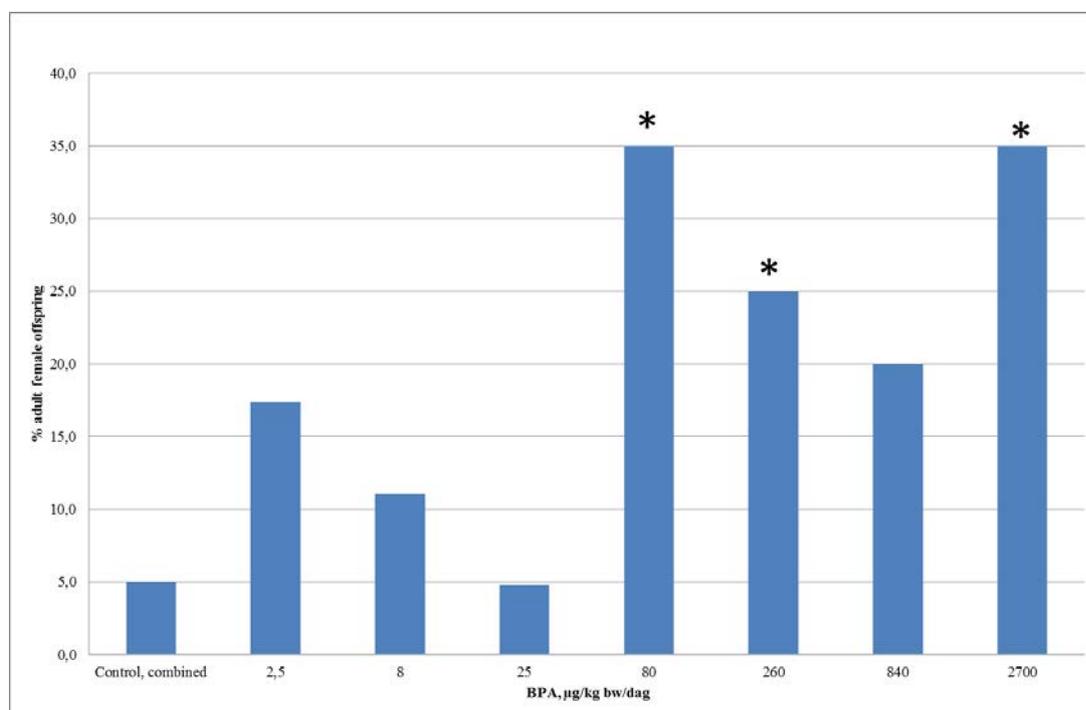
Significantly increased frequency of hyperplasia was found at 300 mg/kg bw/day after histopathological studies of the mammy tissue from the adult female offspring. Taken together with the effects observed at 2700 µg/kg bw/day in female pups before puberty, EFSA concludes that 2700 µg/kg bw/dag is a LOAEL for BPA in this study.

Delclos et al. (2014) grades the severity of the hyperplasia findings as minimal, mild, moderate or marked. Moderate or marked hyperplasia was not found in any of the adult females. Minimal hyperplasia was found in vehicle control females, naive (un-dosed) control females and BPA-dosed females and there was no sign of effect of BPA exposure on the frequency of this finding. However, at BPA doses from 80-2700 µg/kg bw/dag, there appears to be more females with the next severity grade for hyperplasia (score mild, see figure 1). Delclos has analysed the data taking the severity grade into account and finds no significant effects at these dose levels. Delclos et al. (2014) has analysed the BPA data in two subgroups consisting of those exposed to low doses of BPA (2,5-2700 µg/kg bw/day) and high doses of BPA (100.000 and 300.000 µg/kg bw/day). In both cases the vehicle control was used for the analysis. Delclos et al. (2014) states that these subgroups were analysed to avoid over correction for many dose groups analysed together and to minimize the risk false-negatives. DTU agrees that it is relevant to analyse the very large data material in subgroups, but finds the subgroups unbalanced as there are 7 dosed groups in the low dose subgroup and only 2 in the high dose subgroup. DTU finds that more balanced subgroups should have been used, for instance by using 3 or 4 subgroups instead of 2, and also that the analysis of 7 dose groups together does not sufficiently minimize the risk for false-negatives.

DTU has made new statistical analysis of the data. Comparing dosed animals to the vehicle controls as done by Delclos et al. (2014) is generally the most relevant as the animals are treated in the same way apart from the BPA dosing. DTU has, however, chosen also to analyse the BPA exposed groups compared to a combined control group consisting of both vehicle controls and un-dosed controls. This is chosen, because there are no females with hyperplasia score mild in the vehicle controls, whereas there are 2 of 20 in the un-dosed control females. Comparison only to the "clean" vehicle control could increase the likelihood for false-positive findings and DTU has therefore analysed the data compared to all control animals.

**Figure 1 Adult female offspring with mammary gland hyperplasia (Score 2, mild) in the FDA study, shown as % of animals in each low dose group.**

\* = significantly different from vehicle control and from combined control (control, combined)



Significantly more females with hyperplasia (score mild) was found at 80 µg/kg bw/day ( $p < 0.01$ ) and at higher doses, both when comparing to vehicle control and to all controls (see figure 1). The statistical analysis used by DTU is "Fisher exact 2x2 test", where each dosed group, one at a time, is compared to control. This analysis does not correct for multiple comparisons and may therefore lead to false-positives. However, the p-value at 80 µg/kg bw/day is quite low, i.e. 0.004 and DTU therefore regards it as unlikely that the significant finding is a false-positive. The dose-response on figure 1 does not show a clear increase in animals with hyperplasia with increasing dose of BPA. This may, however, be due to a limited sensitive for detecting effects of low doses of BPA. DTU evaluates that these data suggest that increased number of female animals with hyperplasia of mammary tissue are found at 80 µg/kg bw/day BPA and higher doses. Such changes indicate an increased risk for breast cancer later in life.

As already mentioned above EFSA concludes that 2700 µg/kg bw/day is a LOAEL for BPA in this study. DTU does not agree with that conclusion. DTU finds that the study leads to a tentative LOAEL of 80 µg/kg bw/dag and a NOAEL of 25 µg/kg bw/day. However, these values should be re-evaluated, when all data on mammary gland effects in female pups incl. whole mount data are available.

#### *EFSA's evaluation of uncertainty*

EFSA has in 2015 introduced uncertainty evaluations of the likelihood for effects of BPA at low and high doses. For a number of effect areas including the mammary gland, and the reproductive, neurobehavioural, immune and metabolic systems the individual EFSA experts have evaluated the probability for effect within dose intervals based on a logarithmic scale.

Starting from the HED of 609 µg/kg bw/day for effects on adult kidney weight, EFSA uses an uncertainty factor of around 6 which EFSA evaluates to cover the additional uncertainty regarding possible low dose effects on reproductive, neurobehavioural, immune and metabolic systems. They therefore end up with a value of 100 µg/kg bw/day, which is used to derive a t-TDI of 4 µg/kg bw/day by using a factor 25 (factor 10 for intraspecies differences, 2.5 for toxicodynamics and 1 for toxicokinetic, reflecting that toxicokinetic intraspecies differences have been addressed when using the HED approach).

DTU has evaluated the EFSA's uncertainty evaluations to elucidate whether 100 µg/kg bw/day takes the uncertainty sufficiently into account for mammary gland effects, because this type of effects is considered "likely" by EFSA. Additionally, the EFSA experts find that effects on mammary glands in experimental animals is relevant for humans and most of the experts (7 of 10) also find that effects on mammary gland development in humans is an adverse effect.

The precise question each EFSA expert considered for each dose interval is as follows:

*"What is the likelihood that BPA has the capability to cause proliferative changes in mammary gland (of one or more of the types listed in the summary graph) in this dose interval, for one or more combinations of animal species, exposure period and measurement time. In other words, if large, well-conducted experiments were done for the same species with a range of combinations of exposure period and time, what is the likelihood that one or more of the types of proliferative changes in mammary gland listed in the summary graph would be found in this dose interval?"*

The terms and abbreviations used are shown in table 1 (Table 58 in EFSA report). Table 2 (table 71 in EFSA report) shows the evaluations from the 6 EFSA mammary gland experts and the overall group assessment for the likelihood that BPA causes proliferative changes in mammary gland. The 6 EFSA experts appear equally distributed into two subgroups with different evaluations. Based on this EFSA decides that 100 µg/kg bw/day can be used as starting point for deriving the TDI.

**Table 1: Terms and abbreviations used by EFSA to express likelihood in the uncertainty evaluation for hazard characterisation (table 58 i EFSA report)**

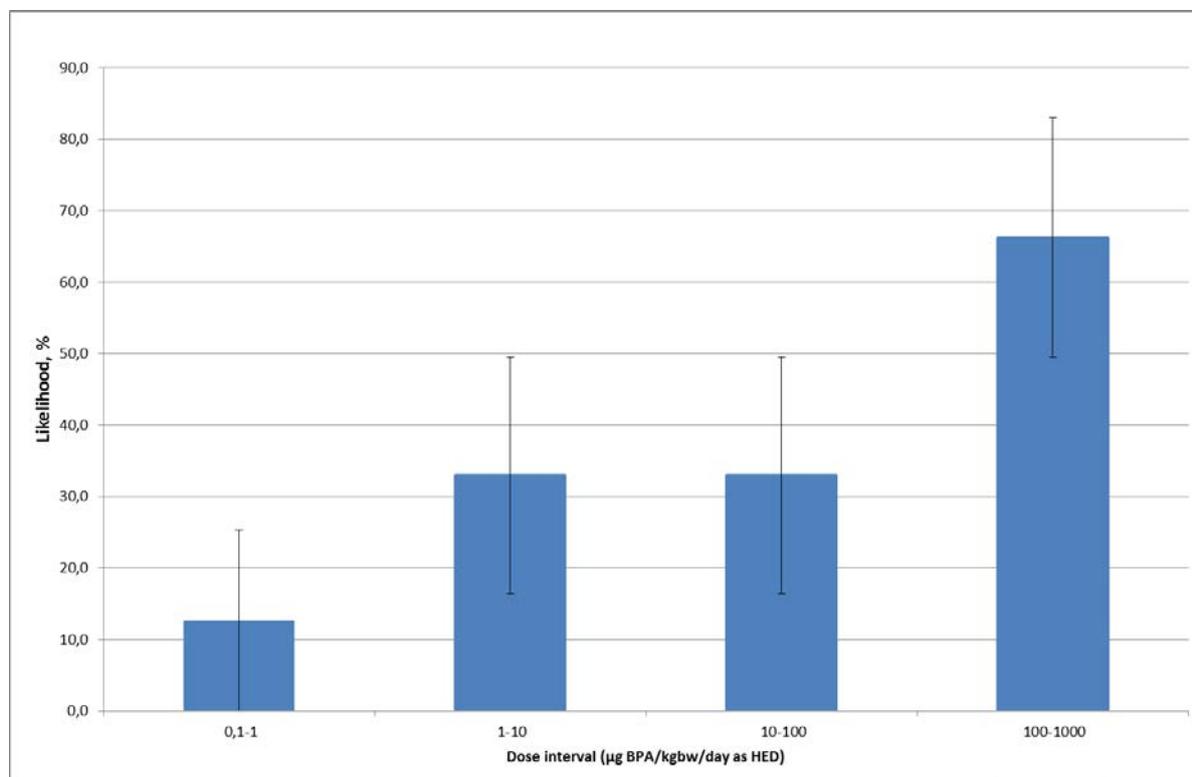
Virtually certain (VC)	99 - 100% probability
Very likely (VL)	90 - 100% probability
Likely (L)	66 - 100% probability
About as likely as not (ALAN)	33 to 66% probability
Unlikely (U)	0 - 33% probability
Very unlikely (VU)	0 - 10% probability
Exceptionally unlikely (EU)	0 - 1% probability

**Table 2 Likelihood that BPA causes proliferative changes of mammary gland, individual assessment by the 6 EFSA experts and overall group assessment (table 71 in EFSA report)**

Dose interval ( $\mu\text{g}$ BPA/kg bw/day)	$<10^{-1}$	$10^{-1}-10^0$	$10^0-10^1$	$10^1-10^2$	$10^2-10^3$	$10^3-10^4$	$10^4-10^5$	$10^5-10^6$
Expert 1	VU	VU	U	U	ALAN	ALAN	ALAN	ALAN
Expert 2	VU	VU	U	U	ALAN	ALAN	ALAN	ALAN
Expert 3	VU	U	ALAN	ALAN	L	L	L	L
Expert 4	VU	U	ALAN	ALAN	L	L	ALAN	ALAN
Expert 5	VU	U	ALAN	ALAN	L	L	ALAN	ALAN
Expert 6	VU	U	U	U	ALAN	ALAN	ALAN	ALAN
<b>Overall group assessment</b>	<b>VU</b>	<b>VU*</b>	<b>U/ALAN</b>	<b>U/ALAN</b>	<b>ALAN/L</b>	<b>ALAN/L</b>	<b>ALAN/L</b>	<b>ALAN/L</b>

\* DTU note: Should most likely be VU/U, but only VU in EFSA report.

**Figur 2 Likelihood that BPA causes proliferative changes of mammary gland in experimental animals. Figur is based on table 1 and 2 (table 58 and 70 in EFSA report), mean and standard deviation for the 6 EFSA experts**



In figure 2, DTU shows the likelihood that BPA causes proliferative changes of mammary gland. The figure is based on table 1 and 2 (table 58 and 71 in EFSA report) and includes a calculated mean and standard deviation. The figure shows a dose-response curve with increasing likelihood for effect with increasing doses of BPA. A low likelihood where the interval includes 0% likelihood is only seen at doses below 1 µg/kg bw/day.

DTU finds that there are a number of problems with regard to the EFSA evaluation of uncertainty:

- It appears unusual and unsuitable that EFSA uses a logarithmic scale for defining the dose intervals, as e.g. the dose interval 100-1000 µg/kg bw/day is a much larger dose span than 10-100 µg/kg bw/day. Also DTU finds it important for the uncertainty evaluation whether there is likelihood for effects at e.g. 200 eller 800 µg/kg bw/day.
- The dose of 100 µg/kg bw/day, chosen by EFSA to derive the TDI is included both in the dose interval 10-100 and 100-1000 µg/kg bw/day. The likelihood for effects in the dose interval 100-1000 µg/kg bw/day is by the EFSA experts evaluated to be 33-66% (3 experts) or 66-100% (3 experts). This indicates that the EFSA experts evaluates 100-1000 µg/kg bw/day as a dose span where it is likely that BPA cause effect, i.e. somewhat similar to a LOAEL dose span. DTU also finds that this is the case. When deriving a TDI the starting point is normally a NOAEL and an additional uncertainty factor is applied if only a LOAEL is available. Thus DTU finds it insufficient that EFSA when deriving the TDI use the lower end of a LOAEL-like dose interval without using an additional uncertainty factor to estimate a NOAEL-like dose.
- For the two dose intervals 1-10 and 10-100 µg/kg bw/day the EFSA experts evaluate the likelihood for effect as 33-66% (3 experts) or 0-33% (3 experts). Based on this DTU finds that these dose intervals do not point to a clear NOAEL for BPA, as there is some uncertainty with regards to effects of BPA at these doses.

Overall, DTU finds that the EFSA uncertainty analysis is insufficient. Therefore, DTU does not support the additional uncertainty factor of 6 used by EFSA and the use of 100 µg/kg bw/day as starting point for deriving the for TDI of 4 µg/kg bw/day for BPA.

## **Ad. 2) DTU proposal for TDI for BPA**

DTU finds the EFSA TDI of 4 µg/kg bw/day as insufficiently protective with regards to protection against endocrine disrupting effects of BPA. DTU therefore finds it relevant to do a "classical" hazard characterization of BPA using estimated NOAELs or LOAEL to estimate a sufficiently protective TDI.

For effect on mammary gland development, DTU has above evaluated that the results in Delclos et al (2014) leads to an estimated LOAEL of 80 µg/kg bw/day and a NOAEL of 25 µg/kg bw/dag. DTU finds that the TDI should also take into account 3 other studies in rats showing mammary gland effect at 250 µg/kg bw/day as well as a study in Rhesus monkeys where effects are seen at 400 µg/kg bw/day (Betancourt et al. 2010, Jenkins et al. 2009, Horal et al. 2008, Tharp et al. 2012). These four studies have some limitations (e.g. only one dose level, small groups), but also appears supportive when seen together, because the HEDs are rather similar in rats and monkeys (180 µg/kg bw/day in rats and 168 µg/kg bw/day in monkeys). Two of the studies in rats and the monkey study did not include lower

doses pointing towards a NOAEL. The third study, however, does not find effects at 25 µg/kg bw/day, pointing towards a similar NOAEL as estimated by DTU for the Delclos-study.

Using this estimated NOAEL of 25 µg/kg bw/day for effects on mammary gland development, the factor of 0.72 (HEDF) used by EFSA to calculate rat doses into HED, and the factor 25 used by EFSA for effects on the kidneys leads to 0.7 µg/kg bw/day. DTU therefore concludes that TDI for BPA should be 0.7 µg/kg bw/day or lower to be sufficiently protective with regards to endocrine disrupting effects of BPA on mammary gland development.

In addition, DTU finds that EFSA has not taken sufficiently account of results from oral rat studies pointing to effects on male sexual development at 250 or 260 µg/kg bw/day (decreased anogenital distance in Christiansen et al. 2014, delayed testes descent in U.S. FDA/NTCR 2013). Using these potential LOAELs of 250-260 µg/kg bw/day, a factor 5 to compensate for the use of a LOAEL instead of a NOAEL<sup>i</sup>, the factor of 0.72 (HEDF) used by EFSA to calculate rat doses to HED and the factor 25 used by EFSA when evaluating effects on the kidneys leads to 1.4-1.5 µg/kg bw/day, i.e. a lower value than the EFSA TDI of 4 µg/kg bw/day.

Studies indicate a possible risk for developmental neurotoxicity effects after exposure to 100-250 µg/kg bw/day BPA. Using these potential LOAELs of 100-250 µg/kg bw/day, a factor 5 to compensate for the use of a LOAEL<sup>1</sup> instead of a NOAEL, the factor of 0.72 (HEDF) used by EFSA to calculate rat doses to HED and the factor 25 used by EFSA when evaluating effects on the kidneys leads to 0.6-1.4 µg/kg bw/day, i.e. a clearly lower value than the EFSA TDI of 4 µg/kg bw/day.

Based on the above DTU finds that TDI for BPA should be 0.7 µg/kg bw/day or lower to be sufficiently protective with regards to endocrine disrupting effects of BPA.

## **Risk assessment**

Highly exposed humans are according to EFSA's exposure assessment exposed to 1.01-1.06 µg/kg bw/day for men and women and 1.26-1.45 µg/kg bw/day for children (3-10 years) and teenagers. These exposures are around 3-4 times lower than the EFSA t-TDI of 4 µg/kg bw/day and EFSA concludes that the aggregated exposure to BPA indicates no health concern for BPA.

DTU finds that the TDI for BPA should be 0.7 µg/kg bw/day to be sufficiently protective with regards to endocrine disrupting effects of BPA. Highly exposed humans incl. pregnant women and children are according to EFSA's exposure assessment exposed to around 1.4-2 times more than 0.7 µg/kg bw/day BPA. DTU concludes that this gives rise to health concern for highly exposed humans.

The risk assessment from both EFSA and DTU has not taken potential mixture effects due to exposure to other chemicals with similar types of effects as BPA into account. This means that the risk can be underestimated.

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<sup>i</sup> An additional factor of 3-10 is generally used when using a LOAEL instead of NOAEL. The specific value is chosen based on the quality and sensitivity of the studies and DTU finds that a factor 5 is most appropriate based on this.

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