

## Connection between “chalky teeth” in children (molar-incisor hypomineralisation, MIH) and the uptake of Bisphenol A not likely

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Medical associations are reporting increased occurrences of disturbed dental mineralisation in children. The so-called “chalky teeth” show discolouration and can be extremely sensitive to pain. Furthermore they tend to react sensitively to heat, cold and brushing. Depending on symptomatic and phenotypic severity the condition of chalky teeth is categorised into three levels. The symptoms of chalky teeth were first described in 1978, with the term molar-incisor hypomineralisation (MIH) introduced in 2001. The condition is the consequence of a delineated defect in tooth enamel development which affects at least one of the permanent back teeth (molars) and, under certain circumstances, will also comprise the incisors. According to recent media coverage such tooth defects are claimed to be attributable to the uptake of Bisphenol A (BPA).

Amongst a wide range of various products BPA can also occur in food contact materials. Its use in the manufacture of baby bottles has been banned since 2011. Reports of a possible connection between MIH and BPA-exposure are based on a study by Jedeon *et al.* (2013) which examined the connection between BPA exposure and mineralisation defects of tooth enamel in rats. In subsequent publications the authors reported that the mineralisation disturbances occurred mainly in male (up to 71%) and less frequently in female rats (only up to 31%) (Jedeon *et al.*, 2016a; Jedeon *et al.*, 2014), and identified selected hormone-controlled signalling pathway as potential molecular targets (Houari *et al.*, 2016).

The German Federal Institute for Risk Assessment (BfR) has evaluated the study (Jedeon *et al.*, 2013) and concludes that there is currently no scientific reason to assume a connection between the uptake of BPA the occurrence of MIH in children. According to recent data from the Netherlands, oral uptake of BPA in highly-exposed children amounts to 0.14 micrograms ( $\mu\text{g}$ ) per kilogram (kg) body weight and day. This is 35 times lower than the dose used by Jedeon *et al.* (2013). In conjunction with the different toxicokinetic behaviour of BPA in humans a direct connection between BPA and MIH therefore appears unlikely in humans under conditions of expectable real-life exposure.

It should be noted that the study of Jedeon *et al.* is subject to several limitations, which limit its transferability. The examination in 2013 was conducted exclusively on male rats with only one dose of BPA being used. Later studies showed that the respective findings were considerably weaker or non-existent in females (Jedeon *et al.*, 2014). It also appears that missing effects on day 100 of postnatal development were not put sufficiently into context. The findings of other groups from multigenerational studies on rats and mice, some of which used very high BPA doses with no reported tooth damage, were not taken into consideration.

The condition of MIH occurs in Europe with a frequency of 3 – 22 %, with a worldwide occurrence of 2 – 40 % (Elhennawy *et al.*, 2017). Various reasons are assumed to contribute to this occurrence. Epidemiological studies point for example to maternal diseases during the last quarter of pregnancy, complications during birth or frequent illness in the first year of the born child (possibly also connected too high fever). Other reasons discussed are low blood levels of vitamin D as well as early intake of the antibiotic amoxicilli. Other studies report on a possible connection between MIH and increased exposure to dioxin, for under 5-year olds with high serum levels of tetrachlorodibenzo dioxin (TCDD) in Seveso later showed an increased prevalence of MIH.

Altogether it appears that MIH is caused by a variety of factors and thus has to be considered a multifactorial condition (Schneider and Silva, 2018).

### Description of the study

In the study of Jedeon *et al.* (2013) dams were exposed to Bisphenol A (BPA) throughout gestation with continued exposure of the offspring via the breastmilk until weaning followed by direct dosing thereafter from postnatal day 21 until the day of examination, that is day 30 or 100, respectively. Dams and the weaned offspring were given a dose of 5 micrograms ( $\mu\text{g}$ ) per kilogram (kg) body weight per day. Only male rats were examined. The group size per test day was 16 animals, eight of which had their lower jaw histologically examined and their incisors analysed by means of scanning electron microscopy and elementary analysis. The remaining group of animals was used to obtain samples comprising various cell types involved in tooth (enamel) formation, which were subjected to molecular analysis. In each instance a control group of equal size was examined for comparison.

### Results

Effects were only observed in the group examined on postnatal day 30. Whitish discolouration was observed on the incisors of 12 of the 16 male rats exposed to BPA. The entire surface of the tooth was affected in six of these animals and the tooth enamel was destroyed in three of them. There were no findings in 16 out of 16 animals in the control group. The affected teeth of 8 animals in the 30-day group were examined chemically and optically (scanning electron microscopy) and compared to human teeth which showed symptoms of molar-incisor hypomineralisation (MIH) (Knapp and Nies, 2009). The authors drew the conclusion that the observed symptoms in humans and rats were sufficiently similar. A decrease in the ratios of calcium to phosphorus and more importantly of calcium to carbon was observed in both species, along with typical changes in the tooth surface compared to non-affected teeth.

After 30 days the treated group showed increased levels of enamelin, a protein involved in the structuring and mineralisation of tooth enamel as well as exogenous albumin. Moreover, messenger RNA levels were increased for enamelin and decreased for the kallikrein-related peptidase 4 (klk-4). KLK4 is involved in the removal of enamel proteins such as enamelin in the maturation phase of the tooth enamel (hardening phase). This result was reproduced *in vitro* in HAT-7 ameloblasts. Concomitantly promoter activities for enamelin and klk-4 were increased and decreased, respectively. These molecular differences were exclusively observed in male rats and were later reported to be absent in female animals (Jedeon *et al.*, 2016a).

Overall, the authors deduced from the results that the resulting too high levels of enamelin and albumin during the first (secretory) phase of tooth enamel formation (amelogenesis), cannot be degraded sufficiently enough during the second phase of mineralisation (maturation) as to warrant healthy enamel formation.

No differences to the control group were established in the group examined on postnatal day 100. The authors explain the occurrence of effects on postnatal day 30 by the considerably less well pronounced ability of embryonic and neonatal rats to Phase II metabolism (and subsequent excretion) of BPA compared to adult animals. Around the time window of birth, this leads to significantly higher BPA concentrations in the blood compared to adolescent and adult animals. Consequently, no effects of BPA on the enamel formation process, which lasts a lifetime in rodents, can be observed in adolescent and adult rats. Unlike rats, the for-

mation of enamel is completed in humans during childhood. The authors argue that mineralisation disturbances which can no longer be corrected through further, natural tooth development, only occur after the remaining teeth have broken through.

### **Strengths of the study by Jedeon *et al.* (2013)**

- Phytoestrogen-free animal diet
- BPA-free cages and drinking bottles
- Comparison to human teeth (disease pattern similar to MIH)
- Experimental approaches to explain the mechanism

### **Weaknesses of the study**

- Examination only on male animals
- Use of only one BPA dose
- Insufficient interpretation of the absence of mineralisation disturbances on postnatal day 30; in particular no consideration of findings from multigenerational studies on rats (Tyl *et al.*, 2002) and mice (Tyl *et al.*, 2008), some of which used very high BPA doses with no tooth damage being observed
- Treatment of rats over the pre- and postnatal phase, so that the critical time frame during which BPA has an effect on mineralisation could not be identified in the rats
- No determination of the ingested BPA dose during breastfeeding

### **Evaluation of the study**

The study by Jedeon *et al.* (2013) includes a large number of methods for the examination of teeth (of rats and humans) which go way beyond those routinely prescribed in the respective OECD test guidelines for repeated dose testing (28- or 90-day study). By doing so, various effects on tooth enamel can be described on postnatal day 30 in rats treated with BPA. An attempt is made to explain the lack of effects on postnatal day 100 through possible toxicokinetic phenomena, as the neonatal rat has a very low capacity for the enzymatic coupling of BPA with glucuronic acid. In the view of the BfR, there is a lack of deliberation on the dose-response relationship as only one dose was used. The authors postulate that due to the improved BPA elimination in adolescent rats no further effects on tooth enamel for the continuously growing incisors were to be observed on postnatal day 100. It is assumed that the defective areas that appear on rat incisors after 30 days have disappeared after 100 days through the wear of the teeth. A dose of 5 µg per kg body weight was administered in the study. To the knowledge of the BfR, doses of 2.5 µg per kg body weight per day up to 500 mg per kg body weight per day were used in multigenerational studies and (sub) chronic studies with BPA on rats and mice without any reports of tooth damage, (Delclos *et al.*, 2014; NTP, 2018; Tyl *et al.*, 2008; Tyl *et al.*, 2002). It was shown in a later study (Jedeon *et al.*, 2016b) that the male sexual hormone testosterone also has an effect on the mineralisation of the teeth and it is postulated that BPA can impair this effect.

### **Significance for humans**

With the reference to toxicokinetic modelling, the authors emphasize the sensitivity of the human infant to BPA. In humans, mineralisation of the 6-year molars begins with the ninth month of fetal development. The 6-year molars are the permanent teeth which break through first. Mineralisation ends with all permanent teeth in postnatal year 6 (Knapp and Nies, 2009). Based on the study results produced by Jedeon this would mean that there is a possibility that BPA could damage the dental germ of the permanent teeth to such an extent that

MIH symptoms appear. The authors emphasize further that because metabolism is not yet sufficiently developed, rats in the perinatal phase are susceptible to the BPA effect on the teeth, whereas adolescent rats glucuronidate to a higher extent, thus allowing them to eliminate BPA. In later studies, the authors show that the effect of BPA varies in strength in male and female rats (Jedeon et al., 2014) and propose differences in the testosterone level together with its effect on tooth mineralisation as possible reason (Houari et al., 2016) (Jedeon et al., 2016b).

In its opinion, the European Food Safety Authority (EFSA) demonstrates that due to the higher rate of metabolism human infants can metabolise BPA much better than neonatal rats. Hence any damaging effects would occur at considerably lower doses in neonatal rats than they would in humans (EFSA CEF Panel, 2015). Considering data regarding human BPA exposure, (which has continued to decline in recent years) the data of Jedeon *et al.* (2013) therefore are most likely not transferable to the human situation.

### Uncertainties

- Only male rats were examined in the study by Jedeon *et al.* (2013). Female animals react less pronounced as shown in subsequent studies (Jedeon et al., 2016a; Jedeon et al., 2014).
- The effects observed in the rat model could possibly occur with doses lower than 5 µg per kg body weight. Without this dose-response relationship, an assessment of the study is only possible to a limited extent.
- The possibly critical time frame (pre-, peri- or postnatal) for the effect of BPA on mineralisation is not known.
- The actual BPA exposure of the rat offspring is not known. According to other studies, the dose ingested via breastmilk is much lower for the young animals than the dose administered to the dams (EFSA CEF Panel, 2015). Whether a damaging effect could be triggered in this phase of development by such low doses of BPA could be assessed.
- The effect of BPA on enamel formation in rats is only described by Jedeon *et al.* (2013). Other studies using higher doses do not observe this effect.

### Risk assessment

The EFSA derived a temporary value for the tolerable daily intake (t-TDI) of BPA of 4 µg per kg body weight per day based on renal effects, with consideration being paid to uncertainties regarding reproduction toxicity, changes to mammary gland tissue and other effects.

BPA exposure has been in constant decline in recent years (EFSA CEF Panel, 2015). The BfR has no more detailed information on changes of MIH case numbers over the last few years. A high exposure scenario (conservative due to overestimation of BPA contamination of canned foods) in 2015 estimated oral BPA exposure of small children (6 months to 3 years of age) to amount to 0.857 µg BPA per kg body weight per day (EFSA CEF Panel, 2015). According to a current study conducted by the *Rijksinstituut voor Volksgezondheid en Milieu* (RIVM) (Boon *et al.*, 2018) food-related exposure of 2-6 year-olds amounted to 0.14 µg BPA per kg body weight per day (95<sup>th</sup> percentile, upper bound). It is assumed that this value can be transferred to children in Germany. In this scenario, adults including women of child-bearing age, have a daily uptake of up to 0.388 µg BPA per kg body weight. This value is roughly 12.8 times lower than the dose administered by Jedeon *et al.* (2013) before and after birth. From the study data, however, it is not possible to compare the quantity of BPA ingested via breastmilk, which means that no conclusive assessment can be made for this possibly sensitive period of tooth development in rats. With 0.857 µg per kg body weight, the daily oral

BPA exposure of severely exposed children (6 months to 3 years of age) calculated by EFSA (EFSA CEF Panel, 2015) is at least 5.8 times lower than the dose administered by Jedeon *et al.* (2013) in an animal experiment with weaned rat offspring. With 0.14 µg per kg body weight per day, current oral exposure of children to BPA (Boon *et al.*, 2018) is even 35.7 times lower ( $5 / 0.14$ ) than the dose administered by Jedeon *et al.* to weaned rat offspring.

Due to the considerably lower BPA exposure of humans compared to the study conducted by Jedeon *et al.* (2013) and its continuous decline in recent years, as well as the toxicokinetic differences between rats and humans during the neonatal phase (EFSA CEF Panel, 2015), a direct connection between BPA and MIH appears unlikely in humans.

#### More information on the subject of Bisphenol A at the BfR website

A-Z Index “Bisphenol A”: [https://www.bfr.bund.de/en/a-z\\_index/bisphenol\\_a-129760.html](https://www.bfr.bund.de/en/a-z_index/bisphenol_a-129760.html)

BfR FAQs of 12 October 2017 on bisphenol A in consumer products:  
[http://www.bfr.bund.de/en/faqs\\_on\\_bisphenol\\_a\\_in\\_consumer\\_products-60837.html](http://www.bfr.bund.de/en/faqs_on_bisphenol_a_in_consumer_products-60837.html)

BfR Communication of 19 February 2015 “No health risk for consumers through Bisphenol A exposure – the BfR endorses the conclusion of the new EFSA assessment”:  
<https://www.bfr.bund.de/cm/349/no-health-risk-for-consumers-from-bisphenol-a-exposure-the-bfr-endorses-the-conclusion-of-the-new-efsa-assessment.pdf>

BfR “Opinions App”



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## About the BfR

The Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. It advises the Federal Government and Federal Laender on questions of food, chemical and

product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.

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