Comments to the

Annex XV report: PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

for bis(2-ethylhexyl) phthalate (DEHP)

by the Danish Environmental Protection Agency, Denmark (dated 26 August 2014)

Introductory remarks

As an introductory remark to our comments on the Annex XV report by the Danish EPA we would like to highlight that anti-androgenic activities of DEHP are discussed since long as a possible or likely mechanism for reproductive toxic effect of DEHP and were as such subject to the detailed evaluation carried out by RAC in relation to the authorization procedure just completed. No additional relevant human health data were presented in this Annex XV report. No justification can be envisaged to subject a substance twice to the authorization regimen on basis of the same data.

When setting DNELs for DEHP in their opinion on the Danish restriction proposal in (2012) and again in 2013 for the purpose of authorisation RAC evaluated in detail the reproductive toxicity data available for DEHP and adopted the concept of a threshold for its evaluations. No relevant new data emerged since then to justify a re-evaluation of the same database.

Comments on the human health part

In the absence of harmonised criteria on the identification of endocrine disrupting chemicals the Danish EPA follows the proposal of the ED EAG (JRC, 2013) which suggested that the elements for identification of an ED are

1) Adverse health effects
2) Endocrine mode of action
3) Plausible link between adverse effects and endocrine mode of action
4) Human relevance

Criterion 1) Demonstration of adverse health effect

The Danish EPA summarized information from rodent studies on adverse effects. They mainly refer to studies already discussed in the EU RAR and documented in Annex 1 of the Annex XV report. Further in Table 3 of the Annex XV report studies documenting effects on male reproductive system published after data collection for the EU RAR – according to the Danish EPA – were documented. But some of these Table 3 studies have already been discussed in the EU RAR (Akingbemi et al., 2001; Arcadi et al., 1998; Li et al., 2000; Parks et al., 2000). Effects on the male reproductive system are e.g. reduced anogenital distance, increased nipple retention, cryptorchidism, effects on Leydig cell steroidogenesis and testicular changes observed in adult and developing males. All these adverse effects have already been addressed in the evaluation carried out by RAC in relation to the authorisation procedure just completed.

Criterion 2) Endocrine mode of action

The Danish EPA argues that some of the in vivo studies indicate an endocrine mode of action of DEHP by showing effects on steroidogenesis like effects on testosterone production additionally supported by in vivo studies showing changes in activity of steroidogenic enzymes and effects on gene pathways of steroidogenesis. They further argue that "studies on testosterone production and steroidogenesis in fetal male rats indicate an endocrine disrupting mode of action of DEHP and its monoester metabolite methylhexyl phthalate (MEHP) in vivo". In fact, the studies documented in Table 3 of the Annex XV report do not document an effect of MEHP on testosterone synthesis in vivo. There was only one study included in Table 3 using MEHP as test item (Li et al., 2000) where histological changes of the gonocytes in the presence of MEHP were described, but no information on the effects of MEHP on testosterone synthesis was presented. Only the in vitro data on MEHP referred to by the Danish EPA in Annex 1 show that MEHP influences testosterone synthesis in Leydig cells (Jones et al., 1993). These data are not sufficient to argument that

- the in vivo data on MEHP provide information on an endocrine mode of action of MEHP, because there are no in vivo data on MEHP effects on sex hormones in the Annex XV report;
- "DEHP and MEHP produce similar changes both in vivo and in vitro both in Leydig cells and in Sertoli cells" (argumentation in Annex 1 with reference to Jones et al., 1993), because, in this study no in vivo investigations have been performed with MEHP and in vitro experiments were only performed in Leydig cells, but not in Sertoli cells.

Contradictory results e.g. on the binding of DEHP in different assays and on the binding of DEHP and MEHP on the androgen receptor were not discussed. Moreover it is implied that there is a logical association between DEHP exposure, its metabolism to MEHP and the observed anti-androgenic effects. But the data provided do not strengthen this conclusion, but leave several unsolved questions: no additional information was provided to indicate how MEHP might inhibit testosterone synthesis (apart from referring to Jones et al., 1993).

Additional information on possible interactions with estrogens and the thyroid system were provided in an inconsistent and rudimentary way, but convincing data are missing that DEHP affects the thyroid.

In conclusion, no new data or aspects were added to the discussion on the possible anti-androgenic mode of action of DEHP, but the studies selected for the argumentation are regarded as partially inappropriate/insufficient to support the argumentation.

Criterion 3) Demonstration of a biologically plausible causal link between the adverse effect and the endocrine MoA

The Annex XV report claims that the documentation provided of adverse effects on reproductive organs in combination with effects on the hormonal system by DEHP in vivo support the argumentation that there is an association between adverse effects and endocrine mode of action.

But several contradictory experimental results were not addressed: The Danish EPA refers to the inhibition of the testosterone synthesis by DEHP and MEHP in vivo and in vitro, respectively. By reporting studies which observed a binding of DEHP to the androgen-receptor it is implied that this

could be the MoA. The Danish EPA further argues that DEHP is absorbed as monoester and/or rapidly metabolized to the monoester which is transported across the placenta and is mainly responsible for the observed effects. But the information that MEHP does not interact with the receptor (David, 2006) leaves the question open by which MoA MEHP might influence the testosterone synthesis.

The conclusion “it is biologically plausible that the observed adverse effects are linked to the endocrine disrupting mode of action of DEHP and the metabolite MEHP” is not obvious from the presented data, because no data were presented which revealed effects of MEHP on apical endpoints and endocrine endpoints in vivo.

Criterion 4) Demonstration of the relevance of the data to humans

In this section the data used for the documentation that DEHP causes adverse effects causally linked to an endocrine mode of action were discussed in a broader context. Especially, the contradictory observations in non-human primates (marmosets) were discussed in light of the findings in rats. The authors refer inter alia to the study by Tomonari et al. (2006), which did not reveal reproductive effects of DEHP in concentrations up to 2500 mg/kg bw/d in male marmosets. Additionally the negative findings described by Kurata et al. (1998) in male marmosets receiving up to 2500 mg/kg bw/d were reported. Also the mechanistic discussions of Johnson et al. (2012) were cited who concluded that “it appears the human fetal testis responds more like a mouse than a rat”, i.e. the most sensitive animal model (rat) does not seem to be a good model for the human situation. Despite this experimental evidence of obvious species differences the Danish EPA concluded that the available adverse, endocrine related effects observed in rats are relevant for humans. The authors interpreted the effects seen on germ cells in marmosets exposed during gestation (“unusual clusters of undifferentiated germ cells”) (McKinnell et al., 2009) in favour of their argumentation, although they are of uncertain significance. Due to the observed interspecies differences the rat data should be interpreted more carefully with respect to their relevance to humans.

Comments on the environmental part

1. Reliability of reported new data

DEHP is an organic substance of low water solubility. In the EU Risk Assessment Report (ECB, 2008) the data are summarised and discussed, with the conclusion that the true water solubility is 3 µg/L. Higher concentrations measured consist most certainly of colloidal forms of DEHP in water. All new ecotoxicity studies presented in the Danish Annex XV report use solvents such as DMSO or ethanol to prepare stock solutions, which are further diluted for the experiments. As concentrations were not analytically confirmed the true concentrations used in the experiments are unknown. Inhomogeneous distributions of colloidal material might have occurred.

In addition to the lacking analytical verification of exposure concentrations, several reported studies are hampered by a study design not adequate for the assessment of DEHP’s aquatic toxicity. For example, a study by Uren-Webster et al. (2010) is reported in the Annex XV report. The authors investigated the reproductive toxicity of DEHP to male zebrafish by applying the substance at doses up to 5 g/kg body weight (!) for a period of 10 days via intraperitoneal injection. This study received a reliability score of 2 (according to Klimisch) by the authors of the Annex XV report.

Other deficiencies relate to the proper documentation of basic and relevant information to assess the study outcomes. For example, in the study by Zanotelli et al. (2010) (see below) effects of DEHP on growth (size and weight) of fish larvae were assessed. Apart from the lacking analytical monitoring, no measurements of dissolved oxygen concentration, no data on the origin of fish and especially neither weight distribution nor length distribution of fish at the start of the test was provided.

2. Discrepancies between studies

The Danish EPA divided their review of ecotoxicological data between data already presented in the EU Risk Assessment Report (ECB, 2008) and new data emerged since 2008. But the authors didn't to consolidate or explain contradictory results between studies, thus leaving important questions open:

Examples:

Zanotelli et al. (2010) (a study first-authored by a pupil of a Zurich grammar school) reported reduced growth of guppies already starting from concentrations of 0.1 µg/L (!). This is in striking contrast to publications reviewed in the EU Risk Assessment Report. For example, Mayer et al. (1977) (reference see (ECB, 2008) investigated the effect on growth in three different fish species (brook trout, fathead minnow and rainbow trout) of various stages of development with long-term exposure to DEHP in concentrations up to 100 mg/L. There was neither mortality nor significant effects on growth observed in this study. Adema et al. (1981) (reference see (ECB, 2008)) observed no effect on mortality or growth in guppies (the same species as used by Zanotelli et al (2010)) at concentrations up to 320 µg/L.

Corradetti et al. (2013) observed a dramatic decrease in fecundity of zebrafish at concentrations as low as 0.2 µg/L. Embryo production and hatching rates were reduced by more than 90% at 0.2 and 20 µ/L (without major differences between these two exposure concentrations despite the 100fold difference). Using the same species as well as guppies in their reproduction study Mayer and Sanders (1973)) (reference see (ECB, 2008) did not find any significant effect on reproduction at DEHP concentrations in food up to 100 µg/g food.

In conclusion, the Danish EPA did not critically assess discrepancies and caveats in the newly emerged data, but rather focussed on individual observations of high uncertainty.

3. EDC criteria

As already mentioned above, in the Annex XV report the following criteria are listed, against which DEHP as an EDC substance has to be evaluated:

1) Adverse health effects
2) Endocrine mode of action
3) Plausible link between adverse effects and endocrine mode of action
4) Human relevance respectively relevance for the environment

Criteria 1:

Due to the lacking critical assessment of the adverse outcome of the new studies reported in the Annex XV report (see above) confirmation of the first criterion (at least in a relevant concentration range close to the water solubility limit, thus avoiding artificial exposure situations) must be questioned.

Criteria 3:

The Annex XV report states that two studies are considered fulfilling this criteria (i.e. demonstration of adverse effects clearly linked to an endocrine mode of action: the study by Norrgren et al. (1999) (reference see (ECB, 2008) and Corradetti et al. (2013).

Norrgren et al. (1999) reported differences in the sex ratio (higher percentage of females in the high DEHP group) after administering DEHP at a low and a high dose of 300 and 1,500 mg/kg food to Atlantic salmon. In a follow-up of this study the impact on the sex ratio could not be confirmed (Norman et al., 2007). It was speculated that the actual concentration in the first study was higher than the nominal concentration, which could not be substantiated, as no analytical monitoring of the exposure took place in the Norrgren et al. study.

The striking discrepancy between the Corradetti study and other studies reported in ECB (2008) is already discussed above.

Criteria 4:

Environmental relevance, the fourth criterion, was not addressed in the Annex XV report. When doing so, the following information has to be taken into account:

- serious adverse effects are reported in reliable studies only in concentration ranges far above the water solubility limit (see ECB (2008))
- DEHP concentrations in surface water in the European Union are reported in INERIS (2014)[1]: according to this evaluation of data reported until 2008 by 16 EU member states for the Water Framework Directive (WFD) the mean of all measured concentrations (in the whole water body including suspended matter) is 1.04 µg/L (median: 0.25 µg/L;) with a 90th percentile value of 1.41 µg/ (n=4377) (DEHP in water bodies is regulated under the WFD; an Environmental Quality Standard for DEHP of 1.3 µg/L was derived under Directive 2008/105/EC)

Investigations with solvent-mediated high concentrations DEHP in colloidal form, without analytical control of real exposure conditions, are not of relevance for the real environmental situation. It should be noted that environmental DEHP concentrations are expected to decrease with further decreasing production volumes.

In conclusion, the Annex XV report does not conclusively provide evidence for fulfilment of criterion 3 and does not discuss or explain how the reported studies with questionable effect concentrations or concentrations orders of magnitude above the water solubility limit of DEHP and above measured

[1] Substances factsheet of chemical pollutants: DEHP; http://www.priority.substances.wfd.oieau.fr/fiche_pdfRank.php?determinandID=452

environmental concentrations should be able to prove the relevance for the environment (criterion 4).

Summary

We ask Member States Committee to reject Annex XV SVHC proposal made by Denmark identifying DEHP as endocrine disrupting chemical. The document does not provide sufficient evidence that DEHP fulfils criteria set out in Article 57f.

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