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Commentary and Reply

Endocrine disruptors in bottled mineral water: Estrogenic activity in the E-Screen

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To the Editor,

In *Endocrine disruptors in bottled mineral water: Estrogenic activity in the E-Screen*, Wagner and Oehlmann [M. Wagner, J. Oehlmann, Endocrine disruptors in bottled mineral water: Estrogenic activity in the E-Screen, J. Steroid Biochem. Mol. Biol. (2010), 127, 128-135] reach several conclusions that are very open to question.

Most notably, in concluding that PET bottles are a source of estrogenic activity, the authors focused only on the data pairs of products 5 through 10, ignoring products 1 through 4. It should be noted that product pairs 1 and 2 showed equal activity in glass and PET, and product pairs 3 and 4 showed higher activity in glass than in PET. Moreover, in both of these pairs, there was no detectable estrogenic activity (EA) in the PET. This alone would seem to negate their conclusion that PET is the source of the estrogenicity.

Second, there is no evidence that the observed activity is estrogenic. The MCF-7 assay is well known to be very sensitive, but is also well known to have false positives. Further, since the mechanisms of ER-mediated cell growth are not clear, many chemicals can act as mitogens in this system through non-estrogenic mediated routes. Without an estrogen receptor binding assay or co-exposure to an estrogen antagonist, it cannot be determined whether the MCF-7 cell growth was attributable to chemicals binding to the estrogen receptor, a non-specific growth factor present in mineral water, or both.

In addition, the actual biological significance of the level of the EA readings seems to have been lost in the authors' focus on arithmetic differences between the pooled data for the PET and glass bottles. The WHO-derived acceptable daily intake for estrogen is 3 μg/person/day based on a 60 kg adult body weight. The average estrogenic activity calculated from all of the analyzed samples in this study was 3.33 pg EEQ/L. This is approximately one million-fold below any level of concern, even with a 2-liter daily intake.

Finally, it should be remembered that this study, like the authors' 2009 paper [1], does not provide any qualitative or quantitative analysis identifying what was in the water to help determine if it came from the packaging, the water itself, or some other source. In light of the fact that other studies have shown both tap and mineral water to be a source of estrogenic activity at wide-ranging levels [2–4], it is curious that Wagner and Oehlmann have not investigated mineral water as the source of the EA. This exclusion of mineral water from their testing severely limits any conclusions that can be drawn.

In any case, nothing in the authors' data would indicate that the packaging, let alone PET, is the source of the estrogenic-like activity. On behalf of the PET Resin Association (PETRA) – the industry organization representing the North American producers of PET resin – we welcome dialogue with researchers and health-safety authorities regarding the chemistry and safety of polyethylene terephthalate.

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[1] M. Wagner, J. Oehlmann, Endocrine disruptors in bottled mineral water: total estrogenic burden and migration from plastic bottles, Environ. Sci. Pollut. Res., 16(3) (2009) 278–286.

[2] G. Boehmler, R. Kohnen, U. Borowski, A. Ruehe, Einsatz eines biologischen Testsystems (E-Screen) in der amtlichen Lebensmittelüberwachung zum Nachweis estrogen wirksamer Substanzen, J. Verbr. Lebensm., 1 (2006) 325–331, (in German).

[3] A. Misund, B. Frengstad, U. Siewers, C. Reimann, Variation of 66 elements in European bottled mineral waters, Sci. Total Environ., 243 (1999) 21–41.

[4] B. Pinto, D. Reali, Screening of estrogen-like activity of mineral water stored in PET bottles, Int. J. Hyg. Environ. Health, 212(2) (2009) 228–232.

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Reply

Reply By Martin Wagner, Jörg Oehlmann

Endocrine disruptors in bottled mineral water: Estrogenic activity in the E-Screen

We share the wish of Heinze regarding a constructive and multilateral dialogue on the chemistry and safety of polyethylene terephthalate (PET). In that sense, we take the opportunity to respond to the four points raised by Heinze.

1) PET as a source of estrogen-like compounds

Heinze expands on the fact that we did not detect significantly elevated estrogenic activity in two brands of water that were bottled at the same springs but packed in both glass and PET. Heinze takes this as an argument for excluding PET as a potential source of estrogen-like chemicals. In doing so, he neglects that different formulations and additives are used to produce PET with specific material properties (e.g. color, rigidity, wall thickness in case of water bottles). In accordance with our previous work [5] we documented quantitative differences in the estrogenic activity of different PET bottled waters, including few non-detects. This suggests that some PET formulations leach estrogen-like compounds while others do not. One might either interpret this repeated observation as an inconsistency or take it as a chance to identify "estrogen-free" PET materials.

Moreover, a recently published study investigates the leaching of estrogenic activity (EA) from plastic packaging materials in the E-Screen. Yang et al. [6] performed extensive migration experiments and conclude that "*Almost all commercially available plastic products we sampled* [. . .] *leached chemicals having reliably-detectable EA.*" This includes PET materials: depending on the migration conditions 47–94% of the PET materials released significant levels of estrogenicity. This supports our hypothesis that PET is a source of estrogen-like chemicals.

2) Chemicals in bottled water act via estrogen receptor

In our recent study bottled water induced proliferation of MCF-7 cells in the E-Screen, an endpoint that is well-recognized to be estrogenic. Heinze doubts that this effect is estrogen receptor-mediated and calls for additional experiments to elucidate the mechanism of action. Indeed, we already reported that chemicals present in bottled water bind and activate human estrogen receptor alpha in our previous paper [5]. In addition, Yang et al. [6] co-exposed PET extracts to an estrogen receptor antagonist and confirmed that the proliferative effect of PET in MCF-7 cells is estrogen receptor-mediated. Using an identical experimental approach Boehmler et al. [7] demonstrated that the proliferative effect of bottled water in the E-Screen is receptor-mediated. Therefore, we believe there is sufficient evidence supporting an estrogen receptor-mediated mechanism of action of chemicals present in bottled water.

3) Biological significance

We repeatedly emphasized that our data on the estrogenicity of bottled water cannot be used to predict actual human health effects. Still, Heinze questions the biological significance of our findings by comparing the acceptable daily intake (ADI) for 17β-estradiol derived from human health risk assessment and estradiol equivalent concentration (EEQ) used to quantify estrogenic effects. The numerous flaws inherent in such a comparison seems to have been lost in the author's focus on arithmetic differences.

First of all, the ADI denotes a chemical concentration of one substance. In contrast, an EEQ expresses a biological effect level observed in a specific bioassay that is caused by an unidentified (mixture of) chemical(s). Here, the estrogenic effect of a sample is translated into the concentration of 17β-estradiol *theoretically* needed to induce the same magnitude of effect. Therefore, comparing both values is only valid if 17β-estradiol was the only active compound present in the sample (the EEQ would then equal the chemical concentration of 17β-estradiol). For bottled water such scenario is very unrealistic. Second, many estrogen-like chemicals induce multiple effects *in vivo* that cannot be predicted by estrogenic activity solely. One well-documented example is the synthetic estrogen diethylstilbestrol (DES) prescribed to pregnant women to prevent miscarriage in the mid of the last century. Much later, DES has been recognized to be a trans-generational carcinogen [8], an effect that cannot be attributed to the compound's estrogenicity alone. Third and above all, it is highly disputable whether the current risk assessment paradigm (and hence ADIs based thereon) is applicable to endocrine disruptors because it does not cover effects of low-dose and long-term exposure [9].

Finally, with regard to the present data we want to point out that a 50% increase in proliferation of a human breast cancer cell line is a biologically significant effect, especially when remembering that it is caused by chemicals present in 7.5 mL of commercially available bottled water. These findings need to be interpreted taking the limitations of *in vitro* bioassays into account. However, *in vitro* estrogenicity is recognized as tier 1 indicator for endocrine disrupting properties by many regulatory agencies. Insofar, our *in vitro* data can serve as a starting point for further investigations.

4) Chemical identity of estrogenic contamination

Heinze criticizes the lack of chemical analysis in our study and claims that three other studies have identified both tap and mineral water as source of estrogenic activity. As a matter of fact, in one of these studies [7] tap water was free of estrogenicity while a second [10] did not investigate estrogenic activity at all. Nonetheless, in our paper we have readily addressed that a contamination of the aquifer is one potential source of estrogen-like compounds that needs further investigation.

We agree that the identification of endocrine disrupting chemicals in bottled water is crucial to assess the source(s) of contamination and potential health implications. However, a conclusive chemical elucidation is extremely resource-consuming since many compounds present in bottled water are so-far unidentified. Similar experiences are made by Bradley and Coulier [11] who investigated non-intentionally added substances (NIAS) in plastic food contact materials. They note that "[. . .] *a larger number of substances remain either unidentified or with an ambiguous identification only*" and that "[. . .] *within a reasonable resource and time allocation* [*it*] *is unlikely to be capable of detecting and identifying every non-intentionally added substance in food contact plastics*." The inability to completely disclose what is migrating from plastic food packaging is more than just a scientific puzzle: It poses a problem for consumers, producers, and regulators, as well. An approach that combines the toxicological assessment of whole migrates with effect-directed chemical analysis will help to solve this issue. An approach based on denying the problem would not.

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