

BfR evaluates study results for mineral water samples with hormone-like activity

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Media reports on the study results of scientists at Goethe University Frankfurt on mineral water samples with hormone-like activity from plastic bottles have caused uncertainty amongst consumers. An oestrogenic activity was observed in 12 out of 20 mineral waters examined in the study. The scientists suspect that this activity is caused by substances leaching from the plastic bottles into the mineral waters. In principle, the Federal Institute for Risk Assessment (BfR) is of the opinion that mineral water should not have any hormone-like activity. It is known that substances like the plasticiser DEHP, bisphenol A or nonylphenol may have negative effects on offspring because of their hormone-like properties. However, when evaluating the study, BfR concluded that the results do not permit any conclusions about the origin of substances with hormone-like activity from PET bottles.

In this study two assays were used to screen for the presence of substances with hormone-like activity in mineral waters: With the first assay mineral waters of the same brand from glass and plastic bottles were compared in a recognised cell culture system. The samples did not reveal any significant differences in the occurrence of hormonal effects in glass or PET bottles. In fact there was a clear difference between the various brands of mineral water. BfR is of the opinion that this test does not prove that the hormone-like activity was caused by PET bottles; nor does it permit any statements about which substances are possibly involved. As a second assay a snail test model was used: Fresh water snails were kept in glass and plastic bottles and their reproductive behaviour was examined. It was shown that the snails in plastic bottles produced more embryos than those kept in glass bottles. From the published data, it is not possible to determine whether this was due to a contamination with hormone-like substances from the PET bottles or different living conditions of the snails in the plastic and glass bottles. In the opinion of BfR, no scientifically sound conclusions regarding health risks for consumers can be drawn from these data.

Neither of the tests permits any conclusions about the substances that cause the hormone-like effect in water. BfR is of the opinion that consumers need not refrain from consuming mineral water from PET bottles on the basis of the above study results. BfR has no information on any substances used in PET production with corresponding hormonal activity that could migrate to the mineral water. Thus, the results raise questions about the relevant substances and their sources.

1 Subject matter of the evaluation

The Federal Institute for Risk Assessment (BfR) has voiced its opinion on the contamination of mineral waters with hormonally active substances. A study was recently published on this subject by Wagner and Oehlmann entitled “Endocrine disruptors in bottled mineral water: total oestrogenic burden and migration from plastic bottles” [1]. BfR already published its initial provisional evaluation of the results of this study in its Information No. 006/2009 [2].

2 Results

In principle, BfR is of the opinion that mineral waters should not have any hormone-like activity. Hence BfR believes it is important to identify the possible entry pathways and to analyse the substances that trigger the hormonal activity and then reduce or remove them. Based on the available data, it is highly unlikely that the substances come from PET bottles.

Hence, BfR believes that the results of the study do not lead to any need for consumers to stop consuming mineral water from PET bottles or switch to mineral water in glass bottles.

3 Reasons

The study mentioned above points to the presence of contamination with oestrogen-like activity in some of the mineral water samples examined that were taken from plastic bottles and packaging. Proof of activity was undertaken in two test systems: an *in vitro* system established in various laboratories with genetically modified yeast cells (YES test) and a snail model used in environmental toxicology.

The modified yeast cells contain parts of the human hormone signalling cascade and are very sensitive to the natural hormone 17β oestradiol and other substances that act on the human oestrogen receptor α (e.g. phytoestrogens, xenoestrogens). In this system 20 samples of bottled mineral waters were tested for oestrogen-like activity. 14 different brands of mineral water were examined. The mineral waters had been bottled in various packaging materials (glass bottles, PET bottles, beverage cartons). For four brands water bottled in glass and in PET bottles was examined. For the other brands only one type of packaging material was examined. The data for water in Tetra Pak are only of limited value because of the low number of samples taken (only two brands).

The authors interpreted their results in such a way that there must be substances with an effective oestrogenic concentration in the mineral waters tested positive, and these substances were probably released from the plastic packaging materials. However, it should be considered that in the used test system known xenoestrogens only have a relatively weak oestrogen-like potency, (e.g. nonylphenol was approximately 5,000 and bisphenol A was 15,000-fold less active than the natural hormone 17β -oestradiol) [3]. Also other possible contaminants like phthalates or antimony compounds, which were discussed by the authors, have oestrogen-like potencies that are several orders of magnitude lower than the natural hormone. This means that correspondingly high substance concentrations would have to be present in order to trigger the oestrogen activities mentioned in the publication. In terms of what is known about the migration behaviour of substances from the plastic PET, this does not seem plausible, even when assuming the migration of substances with additive effects.

Samples from different mineral water brands showed major differences in oestrogen activity. In contrast, the test results of mineral waters of the same brand, filled into glass and PET bottles, only differed to a minor degree in the yeast test system. From the data given in the publication, in contrast to the statement by the authors, no significant differences can be established regarding the packaging (glass versus PET). The possible origin of the oestrogen-like substances from PET bottles discussed by the authors is not confirmed in their comparative tests of samples of the same mineral water brand.

A snail model (*Potamopyrgus antipodarum*) was used as the second test system to detect oestrogen-like substances. With this test organism Oehlmann *et al.* [4] were able to identify the stimulation of embryo production with bisphenol A concentrations of 5 $\mu\text{g/l}$ water and higher. Wagner and Oehlmann are of the opinion that their latest study prove that the results obtained in the yeast test can be attributed solely to the packaging material. The snails were kept in four glass and six PET bottles (into which examined mineral water samples were also stored) with special mixed water as the culture medium (i.e. no mineral water). After 56 days the number of embryos produced by the snails were determined. The test time points after 14 and 28 days mentioned in the methodology section are not mentioned in the results section. It was shown that the reproduction rate of the snails in the PET bottles was higher than the reproduction rate of the snails in the glass bottles. It is not possible to conclude

whether this test system does react exclusively to oestrogen-like substances or might also be influenced by adsorption effects to the matrix (glass, PET). Based on the data from the snail model, no scientifically sound conclusions can be drawn concerning the health risk for consumers. In order to proof these conclusions the chemical identification of possible migrates from the plastic would be necessary.

When interpreting the results from the two test systems, it should also be considered that there is apparently no correlation between the oestrogen-like activity in the mineral waters in the YES test and the effects on the reproduction of the snails. For instance, the PET bottles of one mineral water brand in the snail test showed the highest reproduction rates compared to the other brands whereas the oestrogen-like activity of the mineral water in this PET bottle in the YES test was very low (2.65 ng EEQ/l). By contrast, the PET bottles of another mineral water brand, whose mineral water showed particularly high activity in the YES test (75.2ng EEQ/l), only produced a mean stimulation of the snail reproduction rate. This comparison also shows that further data are needed to confirm the validity of the results from the snail test.

Both test methods are screening tests which do not permit any conclusions about the substances that caused the observed effects. BfR has no information on any substances used in PET production that migrate to the mineral water and that could be responsible for the oestrogen-like activity in the samples from the PET bottles. It is common knowledge that certain plasticisers (e.g. phthalates like DEHP), which have proved to be endocrine modulators, are used in plastics like PVC. However, plasticisers are not used for PET production. Other oestrogen-active substances like bisphenol A or nonylphenol are not used for PET production either. Hence the results of the study primarily throw up questions about the active substances themselves and their origin. However, these questions cannot be answered on the basis of the available study.

3.1 Further studies on substances with hormonal activity in mineral waters

BfR has the following additional knowledge about the occurrence of substances with hormonal activity in mineral waters:

Studies by the Lower Saxony *Land* Office for Consumer Protection of Food Safety have shown that some mineral and table waters already collected at the wellhead show oestrogen activity [5]. Humic substances in spring water are under discussion as one possible cause of these activities. Based on the comparative data on spring water and packaged samples, the authors assume that the migration of oestrogen-active substances from the packaging to the water is unlikely. However, leaching of correspondingly active substances from materials during processing and bottling cannot be ruled out.

In the Rapid Alert System for Food and Feed individual findings on the occurrence of 8 - 188 mg DEHP/l were notified in a special water imported from the USA. However, this cannot be deemed to be proof of regular contamination of mineral waters with oestrogen-active substances. This is confirmed by a study on phthalates in Italian mineral waters involving 71 brands in PET bottles. It identified a maximum value for DEHP of 0.17 µg/l and of 0.02 µg/l for the highest contaminated quartile [6]. A comparison by the authors based on data from literature with tap water did not reveal any elevated phthalate concentrations in the bottled water. Also bearing in mind the weak oestrogenic activity of phthalates (Jobbling *et al.*, 1995: Environ. Health Perspect. 103, 582-587), particularly of DEHP, a general oestrogenic contamination of mineral waters by this substance group is highly unlikely.

Furthermore, in the past there have been problems with the migration of nonylphenol, a chemical that manifests oestrogen-like activity, from the closure materials used in mineral water bottles. The former Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV) issued its opinion in 1996 on a finding of 6 µg nonylphenol/l. The source given for nonylphenol was the plastic insert in the screw top of a mineral water bottle. In recent years BfR has received no further reports on the detection of nonylphenol in bottle closures.

In the yeast test system 4-nonylphenol has a 5,000-fold lower oestrogen potency than 17β-oestradiol as indicated above. According to the Fraunhofer Institute for Process Engineering and Packaging, no nonylphenol was found within the framework of numerous studies conducted in recent years concerning the composition of bottle closures for mineral waters. It is, therefore, highly unlikely that this substance can cause the general oestrogen-like contamination of mineral waters reported by Wagner and Oehlmann.

4 Recommendations / Management options

In principle, BfR is of the opinion that oestrogen-like effects of mineral water are problematic. Nonetheless, BfR is also of the opinion that there is need for confirmation of these test results. For the purposes of a rational evaluation of the observed effects, priority should be given above all to identifying the responsible contaminants and to determining the concentrations present.

Furthermore, BfR believes it is important to identify the possible entry pathways. To this end, multi-stage checks would be required starting with water taken directly from the springs, then water that has gone through treatment processes at the bottlers down to bottled mineral water that reaches the retail trade. During these checks the findings of the oestrogenicity tests could be compared with chemical-analytical data.

An estimation of the health risk for consumers would require further *in vivo* studies with robust end points.

5 References

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[6] Montuori et al. 2008: Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles. Food Add. Contam. 25, 511-518