Comment on "Toxicokinetics of bisphenol A, bisphenol S, and bisphenol F in a pregnancy sheep model".

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Gingrich et al. (2019) report a comparative toxicokinetic (TK) study of Bisphenol A (BPA), Bisphenol S (BPS) and Bisphenol F (BPF) in the pregnant ewe model after a single subcutaneous administration of BPS or a mixture of BPA, BPS and BPF.

The topic is highly relevant. Unfortunately, many errors were noted in the calculation of TK parameters. In the results reported, the disposition of aglycone is systematically confused with that of the corresponding glucuronide, and the interpretation of computed or observed data is therefore erroneous. This makes the paper misleading in a field that does not need these kinds of error to fuel debates that are already heated and confusing. Inspection of Table 2 immediately indicates that the computed clearances (CL) of 45.4 mL/h for BPA (but actually BPA plus BPAG) or the clearance of 5.2 ml/h for BPS (but actually BPS and BPSG) are impossible from a biological point of view. Indeed, this is clearly illustrated by the estimation of Volume of Distribution (Vss) from the product of Mean Residence Time (MRT) and Clearance (CL) (Toutain and Bousquet-Melou, 2004). The computed MRTs of 8.3h for BPA and 6.7h for BPS produce an estimated Vss of 4.94 and 0.457 ml/kg for BPA and BPS, respectively. However, these values are not plausible, given that the minimal possible Vss for any substance is the plasma volume (i.e. about 40mL/kg in sheep). This error in the computation of plasma clearance is rooted in the flawed computation of the corresponding AUC (0.81 and 7.5 mg*h/mL for BPA and BPS, respectively). The order of magnitude of these AUCs can be readily obtained by dividing the maximal observed plasma concentration (C_{max}

of 66.7 and 643 ng/mL for BPA and BPS, respectively) by the slope of the terminal phase (that is equal to 0.693/half-life) (Toutain and Bousquet-Mélou, 2004). This leads to computed AUCs of 0.000510 and 0.00343 mg*h/mL for BPA and BPS, respectively. These roughly estimated AUCs are 1588 to 2185 fold lower than those reported in the present article and we suspect that the authors, at the very least, made an error in handling their concentration units. Using correctly computed AUCs would lead to estimates of total BPA and BPS plasma clearance (actually CL/F, with F the bioavailability factor associated with the subcutaneous administration) of about 980 and 146 mL/Kg/h. These values would be of the same order of magnitude as the plasma clearance that we directly measured using an IV administration of BPAG and BPSG in sheep (333 and 49 mL/kg/h) for BPAG and BPSG (Gauderat et al., 2016; Grandin et al., 2018). The remaining difference is probably due in part to incomplete bioavailability following the subcutaneous administration of BPA and BPS.

Moreover, the authors wrote in their “Toxicokinetic analysis” section that the TK parameters were calculated using the plasma concentrations of total BPA, BPS, or BPF (i.e. unconjugated and conjugated forms of bisphenols). Bisphenol metabolites and bisphenol aglycone are distinct substances with their own physico-chemical properties and their own dispositions. Therefore, total bisphenol concentrations cannot be considered for a meaningful TK analysis. Given that the ratio of the bisphenol metabolite over bisphenol aglycone is about 100 for BPA (Gauderat et al., 2016) and 10 for BPS (Grandin et al., 2018), the results published by Gingrich et al. describe the disposition of the bisphenol metabolites i.e. BPAG and BPSG rather than the disposition of bisphenol aglycone, at least for BPA and BPS. Hence the main message conveyed by the present paper is not related to BPA and BPS but to their mainly inactive metabolite.

Other surprising results are the urinary concentrations. It is claimed that 9h after bisphenol administration, the *peak maternal urine concentrations for BPA and BPS* were 1300 and 3870 ng/ml, respectively. Again, this is very unlikely as the daily urine output in sheep is similar to that of humans (i.e. 1.5L for sheep of 76.3Kg BW). If, as a worst-case scenario, we consider that these so-called maximal urine concentrations are similar to the average urine concentration, the total amounts of BPA/BPAG and BPS/BPSG that are eliminated by urine over 24h are no more than 2 and 6 mg in total, i.e. about 5 and 16% of the administered dose of 36.7 mg, for BPA/BPAG and BPS/BPSG, respectively. This is at variance with the literature reports that most of the BPA/BPAG is eliminated in urine over 24 h (Gauderat et al., 2016). The discrepancy of the authors' result may be due to the low bioavailability of the two tested compounds or, more likely, to the fact that most of the dose had already been eliminated during the first hours following subcutaneous administration and was simply missed by the sampling design because the first urine sampling at 9h post-administration was too late.

The interpretation of the results is also questionable. For instance, the authors suggest that a first-pass effect may reduce the terminal half-life of a substance or compare MRTs (as computed for an extravascular route with a mixture of the aglycone and its conjugate) with a half-life, as obtained after an IV administration. The authors may not have realized that MRT are additives, not half-lives, and that what they actually measured were not the genuine MRTs of the different substances under investigation (only possible by an IV administration) but an overall MRT that includes a Mean Absorption time (MAT) plus a Mean Time of

conjugation of bisphenol aglycone into the bisphenol metabolite. This misunderstanding makes any comparison with the results obtained for Bisphenols by IV route meaningless.

Finally, the authors indicate in their conclusion that "this is the first report on the toxicokinetic of either BPS and BPF in a pregnancy model". This statement is, to our knowledge, correct for BPF but, for BPS, the first toxicokinetic parameters were published in Environment International and have been available since August 2018 (Grandin et al., 2018).

The authors declare they have no competing financial interests.

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