January 25, 2008

Dr. Michael D. Shelby
CERHR Director, NIEHS
P.O. Box 12233
MD ED-32
Research Triangle Park, NC 27709

Sent as e-mail attachment to: Shelby@niehs.nih.gov

Re: Comments on the final CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A

Dear Dr. Shelby:

I am pleased to provide these comments on behalf of the Polycarbonate/BPA Global Group in regard to the final CERHR report on the reproductive and developmental toxicity of bisphenol A. The Polycarbonate/BPA Global Group represents the leading global manufacturers of bisphenol A and polycarbonate plastic, who for many years have supported and conducted scientific research to understand whether bisphenol A has the potential to cause health or environmental effects and to support scientifically sound public policy.

We have also supported critical reviews by scientific experts of the many studies relevant to whether bisphenol A has the potential to cause health or environmental effects. When properly conducted, such reviews can be of high value to support public policy, guide future research and reduce controversy. Our comments are provided to support the preparation of a scientifically sound and defensible NTP Brief on the potential reproductive and developmental toxicity of bisphenol A.

Our comments on the final expert panel report are summarized in the following points, which are described in detail in the attachment to this letter.

1. **The Final CERHR Expert Panel Report is a Scientifically Sound Basis for the NTP Brief**
    a. The CERHR evaluation process is credible and reliable
    b. Recent credible evaluations of bisphenol A validate the CERHR conclusions
    c. The "Chapel Hill" evaluation should not be relied on in the NTP Brief as the evaluation process lacked transparency and failed to meet accepted standards for conflict of interest

2. **New Scientific Information Further Supports the Conclusions of the CERHR Expert Panel Report**
    a. The biological plausibility of low-dose endocrine effects is not supported by existing and newly published scientific evidence
    b. Reported low-dose effects are not replicated or corroborated
    c. Many low-dose studies are of limited relevance to human health

Please do not hesitate to contact me if I can be of further assistance to clarify any comments or if additional information is needed. I can be reached at (703) 741-5588 or by e-mail at steve_hentges@americanchemistry.com.

Regards,

Steven G. Hentges, Ph.D.
Executive Director
Polycarbonate/BPA Global Group

Attachment

Comments of the Polycarbonate/BPA Global Group
on the Final CERHR Expert Panel Report on the
Reproductive and Developmental Toxicity of Bisphenol A

January 25, 2008

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1. The Final CERHR Expert Panel Report is a Scientifically Sound Basis for the NTP Brief

a. The CERHR evaluation process is credible and reliable

The CERHR evaluation process, which has been in place for nearly ten years, has been successfully applied to evaluations of numerous substances. The key attributes of the evaluation process described below help ensure that CERHR evaluations are credible and reliable.

Rigorous Review Process

- Scientists are selected to represent a wide range of expertise.
- Rigorous process to gather relevant scientific literature, the integrity of which was confirmed by an independent audit for the bisphenol A evaluation.
- Rigorous process to evaluate literature according to explicit published guidelines that are uniformly applied. The guidelines were also subjected to public comment when being developed.
- Quality of each literature study is assessed and documented in the report, noting any methodological shortcomings or limits to interpretation, and establishing the utility of studies for the assessment of human reproductive and developmental risk.
- Conclusions are based on a weight-of-evidence approach that considers all of the pertinent evidence.

Transparency and Public Participation

- Nominations for qualified scientists to participate on the expert panel are publicly invited. Criteria for participation include:
  - Formal academic training and experience in a relevant scientific field
  - Publications in peer-reviewed journals
  - Membership in relevant professional societies
  - Certification by an appropriate scientific board or other entities
  - No conflict of interest
- Expert panel meets in a public forum to discuss their review of the literature, identify research needs, and develop conclusions. The meeting is publicly announced in the Federal Register and by other channels, and the public is invited to make oral comments at the meeting.
- Written public comments are invited at the beginning of the process before a draft report is prepared, when the draft report is available, and when the final report is available.

Independence and Control of Conflict of Interest

- The CERHR evaluation process is subject to Federal Advisory Committee Act guidelines as a "special emphasis panel." Panel members sign conflict of interest forms when they agree to participate and again at the end of the process.
- Scientists who have conducted significant amounts of research or have otherwise taken a position on the chemical of interest, either favorable or unfavorable, are generally excluded from participation on the panel to avoid conflicts of interest or bias.

Claims that the CERHR report is flawed due to a contractor's potential conflict of interest have been dispelled by a thorough audit conducted by NIEHS. The conclusion of the audit report states: "The audit provides assurance that the draft BPA expert panel reports include consideration of all relevant references and reliably include changes requested by the expert panel members. NTP concludes that the draft expert panel reports are useful for the CERHR evaluation of BPA."

The CERHR evaluation process is sound and the audit verifies the integrity of the final report. The final CERHR expert panel report on bisphenol A should be the primary basis from which NTP develops a Brief on bisphenol A.

b. Recent credible evaluations of bisphenol A validate the CERHR conclusions

In the last year, two other comprehensive human health evaluations of bisphenol A have been published by credible organizations.[1,2] Both evaluations are scientifically rigorous and, like the CERHR evaluation, both were conducted by independent organizations and scientists in a process that controls conflict of interest.

The European Food Safety Authority (EFSA) is a government organization that was established by the European Parliament in 2002 to provide the European Commission, the European Parliament and the European Member States with a sound scientific basis for legislation and policies related to food safety. Included in EFSA's work are assessments of the safety of food packaging and other materials, such as bisphenol A, that may contact food. The EFSA evaluation of bisphenol A was conducted by a panel of 21 independent scientists drawn from across the European Union.

NSF International is a not-for-profit non-governmental organization that is a world leader in standards development, product certification, education, and risk-management for public health and safety. The NSF evaluation of bisphenol A was lead by Dr. Calvin Willhite of the California Department of Toxic Substances Control. In addition to publication of the evaluation in the peer-reviewed scientific literature, the evaluation was reviewed by the NSF Health Advisory Board, including members from the US Environmental Protection Agency and Health Canada.

These evaluations are suitable for use by NTP to validate the assumptions, information and approaches used, and the conclusions reached by the CERHR expert panel. The NTP Brief, based primarily on the final CERHR expert panel report, will be significantly strengthened by careful consideration of these credible and comprehensive evaluations.

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[1] Willhite, C. C., Ball, G. L., and McLellan, C. J. 2008. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. Journal of Toxicology and Environmental Health, Part B. 11(2):69-146.
[2] European Food Safety Authority. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A). Adopted 29 November 2006.

c. The "Chapel Hill" evaluation should not be relied on in the NTP Brief as the evaluation process lacked transparency and failed to meet accepted standards for conflict of interest

In approximately the same timeframe as the CERHR expert panel evaluation of bisphenol A, a competing evaluation called the "Chapel Hill Consensus" was conducted and published. The process by which this evaluation was conducted is significantly deficient compared to the CERHR evaluation process described above. In comparison to the CERHR evaluation process, the most notable deficiencies of the Chapel Hill evaluation process are the lack of transparency and public participation, and the failure to control conflict of interest.

In contrast to the CERHR expert panel, the Chapel Hill panel included and was led by scientists who have conducted research on bisphenol A and, more importantly, have actively and broadly advocated that bisphenol A and products containing bisphenol A are not safe (including, in some cases, paid expert witness testimony regarding bisphenol A). Both the participation in research and the public advocacy positions represent serious conflicts of interest. Scientists who are necessarily reviewing their own research and who have already reached conclusions before participating on the panel are not likely to be without bias.

Unlike the CERHR process, the Chapel Hill process was not transparent and there was no public participation of any type. The criteria for participation on the panel have not been made public and there was no opportunity for either public nominations or for participation by other interested scientists. Consequently, the resulting Chapel Hill conclusions must be viewed in the context of what was apparently a carefully selected group of like-minded individuals.

The Chapel Hill panel was funded by NIEHS, US EPA, Commonweal and others; however, it is explicitly stated that "this manuscript does not reflect US-EPA or NIH agency policy" and is therefore explicitly not an official position of any US government agency.

For these reasons, the Chapel Hill evaluation cannot be considered to be an independent evaluation on a par with the CERHR expert panel evaluation. Overall, the Chapel Hill evaluation process does not meet NTP's standards for expert panel evaluations. The Chapel Hill evaluation should not be used by NTP as a primary basis for development of the Brief and should also not be considered to be even remotely equivalent to the final CERHR expert panel report.

2. New Scientific Information Further Supports the Conclusions of the CERHR Expert Panel Report

a. The biological plausibility of low-dose endocrine effects is not supported by existing and newly published scientific evidence

Underlying much of the controversy around potential health effects of bisphenol A is the so-called "low-dose hypothesis," which asserts that estrogenic substances may cause health effects at low doses that are not observed at higher doses. The hypothesis is that estrogenic substances exert their biological effects with non-monotonic dose-response.

For bisphenol A, which is weakly estrogenic, the low-dose hypothesis is just that, a hypothesis that has not been proven or reproducibly demonstrated. In fact, as recognized in the CERHR report, studies that report low-dose effects for bisphenol A have consistently not been replicated or corroborated in multiple studies employing internationally approved test protocols, significant sample size, a wide range of doses, appropriate experimental design and route of exposure, and conducted under Good Laboratory Practices.

Subsequent to the completion of the final CERHR expert panel report, two new studies on other estrogenic substances have been published that further cast serious doubt on the validity of the low-dose hypothesis. These studies were conducted on the synthetic estrogen ethinylestradiol (EE)[3] and the prototypical natural estrogen estradiol (E2).[4]

Howdeshell et al. (2007)
The EE study was designed to assess the effect of a wide range of six EE doses (0.05 to 50 µg/kg bw/day; administered by gavage in corn oil) on the reproductive morphology and associated hormone levels of male Long Evans hooded rats. The exposure period covered pregnancy through lactation from gestational day 7 to postnatal day (PND) 18 and a wide range of endpoints were measured at study termination (PND 150) or earlier as appropriate (e.g., anogenital distance at PND 2; nipple retention at PND 14).

Treatment with EE demonstrated estrogenic action at doses of 5 to 50 µg/kg bw/day and an absence of effects at the lower doses. There was no evidence for non-monotonic dose-response in any endpoint examined.

This study also included treatment with three low doses of bisphenol A (2, 20, 200 µg/kg bw/day). No significant effects on any male endpoint examined were found. Although the study was not designed to identify high dose effects from bisphenol A, the lack of effects at the three low doses, which have been claimed to cause effects in other studies, provides no support for the low-dose hypothesis for bisphenol A. These results are particularly powerful since the test

[3] Howdeshell, K. L., Furr, J., Lambright, C. R., Wilson, V. S., Ryan, B. C., and Gray, L. E. 2007. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male Long Evans hooded rat. Toxicological Sciences. In Press.
[4] Tyl, R. W., Myers, C. B., Marr, M. C., Sloan, C. S., Castillo, N. P., Veselica, M. M., Seely, J. C., Dimond, S. S., Van Miller, J. P., Shiotsuka, R. S., Stropp, G. D., Waechter, J. M., and Hentges, S. G. 2008. Two-generation reproductive toxicity evaluation of dietary 17β-estradiol (E2; CAS No. 50-28-2) in CD-1 (Swiss) mice. In Press.

system was demonstrated to be sensitive to estrogenic effects at the high end of the wide range of EE doses.

Female offspring reproductive function and morphology as well as assays of sexually dimorphic behaviors will be published elsewhere, but preliminary results have been presented at a scientific conference.[5] Effects on females were reported at the two highest doses of EE but developmental exposure to bisphenol A caused no consistent effects in any of the endpoints that had been measured.

Tyl et al. (2008)

The E2 study was designed to develop information on reproductive and developmental effects in mice exposed to dietary E2 over multiple generations. The study was conducted with CD-1 mice under OECD Test Guideline 416 with enhancements and included five dietary exposure levels ranging from 0.001 to 0.5 ppm (~0.2 to 100 µg/kg bw/day). For the F0 generation, the exposure period covered 8 weeks prebreed, 2 weeks mating, ~3 weeks gestation, and 3 weeks lactation. Selected F1 offspring were exposed after weaning to the same dietary concentrations and durations as the F0 generation with study termination occurring at F2 weaning. A wide range of endpoints were examined at the appropriate time points in the multi-generation study.

Estrogenic effects were found at the three highest doses and there was no evidence for non-monotonic dose-response in any endpoint examined.

This study was used as the basis for selection of a positive control dose (0.5 ppm) that was included in a subsequent multi-generation study of the same design on bisphenol A.[6] The six dietary exposure levels for bisphenol A ranged from 0.018 to 3500 ppm (~0.003 to 600 mg/kg bw/day). Parental systemic effects were found at the two highest doses and the NOEL for bisphenol A was determined to be 30 ppm (~5 mg/kg bw/day). At lower doses there were no treatment related effects and no evidence for non-monotonic dose response for any parameter. The bisphenol A study was reviewed by the CERHR expert panel and is included in the final report (CERHR citation 436).

Combined, these studies provide no support for the validity of the low-dose hypothesis, in particular the existence of non-monotonic dose-response for estrogenic substances, and the bisphenol A study provides strong evidence for the lack of low-dose effects for bisphenol A.

[5] Ryan, B. C., Howdeshell, K. L., Vandenbergh, J. G., Crofton, K. M., and Gray, L. 2006. An assessment of the effects of gestational and lactational exposure to ethinylestradiol (EE) and bisphenol A (BPA) on reproductive morphology and behavior in female and male Long Evans hooded rats. Society of Toxicology Annual Meeting. Abstract No. 405.

[6] Tyl, R. W., Myers, C. B.., Marr, M. C., Sloan, C. S., Castillo, N. P., Veselica, M. M., Seely, J. C., Dimond, S. S., Van Miller, J. P., Shiotsuka, R. N., Beyer, D., Hentges, S. G., and Waechter, J. M. 2008. Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1 (Swiss) mice. Submitted for publication.

b. Reported low-dose effects are not replicated or corroborated

The final CERHR report includes several notable examples of studies reporting low-dose effects (CERHR citations 275, 320, 498)[7,8,9] that could not be replicated in subsequent, often larger scale studies (CERHR citations 393, 394, 321, 499).[10,11,12,13]

Another recent example of a study reporting low-dose effects (CERHR citation 299)[14] that could not be replicated or corroborated was partially included in the final CERHR report. The originating study reported that exposure of female mice to low doses of bisphenol A caused a chromosomal abnormality in oocytes known as congression failure. Based on this, the authors suggested that bisphenol A causes aneuploidy, which in humans might result in miscarriage, Down syndrome and birth defects. However, this finding has not been corroborated by comprehensive studies in both rats and mice, all of which find no effects on fertility or birth defects, the expected outcome of aneuploidy, at doses far above those used in the original study.

Two new studies have now been published that report that the original findings can not be replicated. One of these studies, which appeared on-line in time for inclusion in the final CERHR report (CERHR citation 301),[15] investigated the aneugenic effects of bisphenol A in mouse somatic and germ cells. In a series of experiments, these researchers found no evidence to support the original findings and concluded "our results do not add evidence to the suspected aneugenic activity of BPA and suggest that other factors or co-factors should be considered to explain the unexpected burst of meiotic abnormalities previously attributed to accidental BPA exposure."

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[7] Nagel, S. C., vom Saal, F. S., Thayer, K. A., Dhar, M. G., Boechler, M., and Welshons, W. V. 1997. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environmental Health Perspectives. 105(1):70-76.

[8] Talsness, C., Fialkowski, O., Gericke, C., Merker, H.-J., and Chahoud, I. 2000. The effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring. Congenital Anomalies. 40:S94-S107.

[9] Sakaue, M., Ohsako, S., Ishimura, R., Kurosawa, S., Kurohmaru, M., Hayashi, Y., Aoki, Y., Yonemoto, J., and Tohyama, C. 2001. Bisphenol A affects spermatogenesis in the adult rat even at a low dose. Journal of Occupational Health. 43:185-190.

[10] Cagen, S.Z., Waechter, J.M., Dimond, S.S., Breslin, W.J., Butala, J.H., Jekat, F.W., Joiner, R.L., Shiotsuka, R.N., Veenstra, G.E., and Harris, L.R. 1999. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. Toxicological Sciences. 50:36-44.

[11] Ashby, J., Tinwell, H., and Haseman, J. 1999. Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. Regulatory Toxicology and Pharmacology. 30:156-166.

[12] Tinwell, H., Haseman, J., Lefevre, P.A., Wallis, N., and Asbhy, J. 2002. Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. Toxicological Sciences. 68:339-348.

[13] Ashby, J., Tinwell, H., Lefevre, P. A., Joiner, R., and Haseman, J. 2003. The effect on sperm production in adult Sprague-Dawley rats exposed by gavage to bisphenol A between postnatal days 91-97. Toxicological Sciences. 74:129-138.

[14] Hunt, P. A., Koehler, K. E., Susiarjo, M., Hodges, C. A., Ilagan, A., Voigt, R. C., Thomas, S., Thomas, B. F., and Hassold, T. J. 2003. Bisphenol A exposure causes meiotic aneuploidy in the female mouse. Current Biology. 13:546-553.

[15] Pacchierotti, F., Ranaldi, R., Eichenlaub-Ritter, U., Attia, S., and Adler, I.-D. 2007. Evaluation of aneugenic effects of bisphenol A in somatic and germ cells of the mouse. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. In Press.

Subsequent to finalization of the CERHR report, a related study was published that reported an attempt to exactly replicate the original study.[16] The study was unable to replicate the original findings and the authors concluded "there was no evidence that low BPA doses increased hyperploidy at meiosis II." The negative results in the studies above are consistent with the negative results for bisphenol A in a previous mouse micronucleus study, a design that examines the potential for a chemical to cause either aneuploidy or clastogenicity.[17]

These studies collectively reinforce the need for a rigorous weight of evidence assessment to reach sound conclusions based on all of the relevant scientific evidence. In particular, all relevant negative data must be included to thoroughly test for replication or corroboration of positive data. The weight of evidence, as evaluated by the CERHR expert panel and other recent evaluations, supports the conclusion that bisphenol A is not a risk to human health at the extremely low levels to which people might be exposed.

c. Many low-dose studies are of limited relevance to human health

Human Exposure

The final CERHR report contains extensive information on human exposure to bisphenol A. Of particular importance is information derived from analysis of urine, which is the preferred biological sample for direct measurement of bisphenol A in humans.

Urine biomonitoring is preferred for both analytical chemistry and biological reasons. Sensitive and reliable mass spectroscopy based analytical methodology is readily available to measure the amount of bisphenol A in urine, where it is concentrated. In addition, urine is a less challenging matrix for these analytical methods. Human volunteer studies, which are reviewed in the final CERHR report (CERHR citations 6, 7, 109),[18,19,20] have shown that bisphenol A is efficiently metabolized after oral exposure to a glucuronide conjugate, which is then rapidly excreted from the body. Excretion is entirely into urine with an elimination half-life for the glucuronide of approximately 4 hours. Measurement of bisphenol A (as the conjugate) in urine is thus an integrated measure of total short-term exposure.

The final CERHR report includes numerous small scale studies that report the concentration of bisphenol A in human urine. Because bisphenol A is so rapidly excreted, it is possible to

[16] Eichenlaub-Ritter, U., Vogt, E., Cukurcam, S., Sun, F., Pacchierotti, F., and Parry, J. 2007. Exposure of mouse oocytes to bisphenol A causes meiotic arrest but not aneuploidy. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. In Press.

[17] Gudi, R., and Krsmanovic, L. 1999. Mammalian erythrocyte micronucleus test, BioReliance, AA12WJ.123.BTL (reviewed in Haighton, L.A., Hlywka, J.J., Doull, J., Kroes, R., Lynch, B.S., and Munro, I.C. 2002. An evaluation of the possible carcinogenicity of bisphenol A to humans. Regulatory Toxicology and Pharmacology. 35:238-254).

[18] Tsukioka, T., Terasawa, J., Sato, S., Hatayama, Y., Makino, T., and Nakazawa, H. 2004. Development of analytical method for determining trace amounts of BPA in urine samples and estimation of exposure to BPA. Journal of Environmental Chemistry. 14(1):57-63.

[19] Völkel, W., Bittner, N., and Dekant, W. 2005. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by HPLC-MS/MS. Drug Metabolism and Disposition. 33(11):1748-1757.

[20] Völkel, W., Colnot, T., Csanady, G.A., Filser, J.G., and Dekant, W. 2002. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chemical Research in Toxicology. 15(10):1281-1287.

estimate daily intake of bisphenol A from the measured urinary concentrations.[21] Typical urinary concentrations of bisphenol A (in the form of the glucuronide) are in the range of 1-3 µg/L (ppb). These studies consistently indicate that typical daily intake of bisphenol A is less than 0.1 µg/kg bw/day.

Subsequent to finalization of the CERHR report, the US Centers for Disease Control (CDC) published their analysis of urine biomonitoring data on bisphenol A from the NHANES 2003-2004 survey, which generated data on 2,517 individuals ranging in age from 6-85.[22] By design, the sample analyzed is representative of the US population. For the population as a whole, the median concentration of bisphenol A in urine was 2.7 µg/L.

The median urinary concentration for the US population corresponds to a median daily intake of 0.051 µg/kg bw/day.[23] Consistent with the slight differences in urinary concentration reported by CDC, only slight differences in estimated daily intake are found between different subgroups. Median daily intakes for males and females are 0.057 µg/kg bw/day and 0.044 µg/kg bw/day, respectively. For different age groups, daily intakes ranged from a high of 0.077 µg/kg bw/day for ages 12-19 to a low of 0.033 µg/kg bw/day for ages 60 and higher.

Consistent with the many previous smaller scale studies, these CDC data confirm that typical daily intake of bisphenol A in the US population is less than 0.1 µg/kg bw/day. These intakes are well below the Tolerable Daily Intake for bisphenol A of 50 µg/kg bw/day that was recently set by the European Food Safety Authority based on a comprehensive evaluation of bisphenol A,[24] and two orders of magnitude further below the NOEL of 5 mg/kg bw/day established in comprehensive multi-generation studies in both mice and rats.

A comprehensive review of bisphenol A biomonitoring and assessment of human exposure based on biomonitoring data has recently been published.[25] This paper reviews available analytical methods and the human metabolism and pharmacokinetic basis for estimation of daily intake from biomonitoring data, and concludes with daily intake estimates. Consistent with other sources, typical human daily intake of bisphenol A is reported to be less than 0.1 µg/kg bw/day.

Metabolism and Pharmacokinetics

The CERHR expert panel considered studies with oral routes of exposure to be most appropriate and that studies with non-oral routes of exposure are of limited utility as supplemental information.[26] As noted by the panel, the basis for this decision is that "human exposure is

[21] Daily intakes are estimated by multiplying the measured concentrations of bisphenol A with a typical urine output per day, and dividing by measured or typical bodyweights, resulting in daily intakes with units of µg/kg bw/day.
[22] Calafat, A. M., Ye, X., Wong, L.-Y., Reidy, J. A., and Needham, L. L. 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environmental Health Perspectives. 116(1):39-44.
[23] LaKind, J. S. and Naiman, D. Q. 2008. Bisphenol A (BPA) daily intakes in the United States: Estimates from the 2003-2004 NHANES urinary BPA data. Submitted for publication.
[24] European Food Safety Authority. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A). Adopted 29 November 2006.
[25] Dekant, W. and Völkel, W. 2007. Human exposure to bisphenol A by biomonitoring: Methods, results and assessment of environmental exposures. Toxicology and Applied Pharmacology. In Press.
[26] See page 122 of the final CERHR expert panel report, lines 17-30.

overwhelmingly oral, and oral exposure produces an internal metabolite profile which is overwhelmingly dominated by the (inactive) glucuronide in both rats and humans. Subcutaneous or parenteral injections result in blood levels of active parent compound which are much higher than those seen after oral exposure." As summarized in Chapter 2 of the CERHR final report, this decision is supported by numerous studies that document the metabolism and pharmacokinetics of bisphenol A in rodents, non-human primates, and humans.

Subsequent to the finalization of the CERHR expert panel report, a new study was published on the pharmacokinetics of bisphenol A administered to neonatal mice by subcutaneous injection or oral exposure.[27] Consistent with the views of the CERHR expert panel, the authors concede that route of exposure is of critical importance in adults,[28] but report that oral and subcutaneous injection exposures result in essentially identical plasma levels of unconjugated bisphenol A in neonatal mice. Although only neonatal mice were studied, this finding was the basis for the overall conclusion that route of administration is irrelevant for pregnant mice and rats as well as neonatal mice and rats, and thus animal studies with non-oral routes of exposure during early development are as valid as studies with oral routes of exposure for assessing human health impacts.

The far-reaching conclusions presented in this paper are not well supported by the experimental data. The study was conducted to test the hypothesis that the known rapid metabolism of bisphenol A to the corresponding glucuronide in adults would not occur in neonates due to lower UDP-glucuronosyltransferase activity at early stages of development. However, due to several significant flaws in the design and execution of this study, that hypothesis is not tested in this study at all.

- Conjugated metabolites are neither directly not indirectly measured

  The procedure to extract plasma (with tert-butyl methyl ether) is reported to extract bisphenol A but not water soluble conjugates. Consequently, since the extraction procedure excludes conjugated metabolites entirely from the analysis, the study provides no direct or indirect measure of whether or to what extent bisphenol A was metabolized to the glucuronide or to any other metabolite. In particular, if sulfotransferase is expressed in the neonatal mouse as expected (see below), the analysis is incapable of measuring the extent of sulfonation of bisphenol A to the corresponding sulfate. The study is thus incapable of testing the hypothesis that conjugative metabolism of bisphenol A in neonates does not occur.

[27] Taylor, J. A., Welshons, W. V., and vom Saal, F. S. 2008. No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24 hr after administration in neonatal female mice. Reproductive Toxicology. In Press.

[28] In regard to rodent studies comparing oral and non-oral routes of exposure, the authors state "We view these prior findings as providing conclusive evidence that in the adult rodent, route of administration of BPA has a substantial effect on the proportion of the BPA dose available to reach receptors in target tissues due to differences in pharmacokinetics."

In addition, it has been reported that non-oral routes of exposure can produce a different profile of metabolites compared to oral exposure.[29,30] The extent to which additional metabolites formed with non-oral routes of exposure contribute to toxicity observed in non-oral studies is not known. An assessment of the presence or significance of additional metabolites formed by non-oral exposure in neonates is precluded in this study since the extraction and analytical procedure does not measure any metabolites.

- No mass balance was conducted to determine the fate of the administered dose

  Based on data presented in the paper, only a very small fraction of the dose administered was recovered in the HPLC fractions reported to be bisphenol A. At C_{max}, approximately 0.04% of the administered radioactivity was recovered in these fractions. No information is provided to account for the remaining 99.96% of the administered dose. In particular, no information is provided to determine whether or how much of the administered bisphenol A has been metabolized or to what metabolite(s). The conclusions of the study are thus based on analysis of only a tiny fraction of the dose administered.

- Bisphenol A itself is not positively identified in the analytical procedure

  A further limitation is that quantitation of parent bisphenol A in plasma was based only on scintillation counting of HPLC fractions. The fractions were collected over a very broad peak with a base peak width of over three minutes that elutes very close to the solvent front. No structural confirmation was conducted (e.g., mass spectrometry) to confirm that the radiolabeled peak consists partly or entirely of bisphenol A.

  Without positive identification of structure, it cannot be certain whether the fractions contain bisphenol A, an impurity originating with the ^{3}H-bisphenol A sample,[31] or other substances resulting from radiolabel exchange.[32] In addition to the lack of positive structural identification, the chromatographic procedure has essentially no capability of separating individual components that may be co-eluting over the three minute retention window assigned to bisphenol A.[33]

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[29] Pottenger, L.H., Domoradzki, J.Y., Markham, D.A., Hansen, S.C., Cagen, S.Z., and Waechter, J.M. 2000. The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicological Sciences. 54:3-18.

[30] Zalko, D., Soto, A.M., Dolo, L., Dorio, C., Rathahao, E., Debrauwer, L., Faure, R., and Cravedi, J.P. 2003. Biotransformations of bisphenol A in a mammalian model: Answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. Environmental Health Perspectives. 111(3):309-319.

[31] According to the supplier, ^{3}H-bisphenol A is provided as an ethanol solution with purity ≥97%. Some or all of the peak reported to be bisphenol A, at only 0.04% of the administered dose, could easily be an impurity.

[32] Radiolabel exchange of tritium from metabolism or during processing/analysis has been reported in other cases. For example, see Marcus, S. N. and Heaton, K. W. 1988. Evidence for loss of tritium from 3β-tritiated deoxycholic acid during enterohepatic cycling in man. Lipids. 23(6):629-630, and Raeside, J. I., Christie, H. L., Renaud, R. L., Waelchli, R. O., and Betteridge, K. J. 2004. Estrogen metabolism in the equine conceptus and endometrium during early pregnancy in relation to estrogen concentrations in the yolk-sac fluid. Biology of Reproduction. 71:1120-1127.

[33] Given the HPLC column (4.6 X 100 mm) void volume of 1.16 mL, it requires 1.4 minutes for an unretained component to elute from the column at the stated flow rate of 0.82 mL/min. A standard column efficiency calculation [$N = 16\ (t_r / W_b)^2$] yields a value of 36 which is much lower than what is typical for this column (a one-minute wide peak yields N = 324).

> To the extent that the detected peak represents bisphenol A, no analytical controls were included to be sure that the bisphenol A was truly present in plasma rather that forming adventitiously by hydrolysis of small amounts of conjugated metabolites during the extraction and analytical procedure.

More generally, the assertion that rodent or human neonates are incapable of metabolizing bisphenol A is not supported by the available scientific evidence. The operative question is not whether neonates have the same metabolic capability and capacity as adults, but rather whether neonates have adequate capability and capacity to metabolize very low doses of bisphenol A resulting from environmental exposures.

Bisphenol A can be metabolized in both rodents and humans to the glucuronide and sulfate conjugates[34,35] by UDP-glucuronosyltransferase (UDP-GT) and sulfotransferase (SULT) enzymes, respectively. Both enzymes exist in multiple isoforms and are distributed at differing levels in multiple tissues in both rodents and humans. In addition, the ontogeny of both families of enzymes varies by isoform, tissue and species, which precludes the generalization of a simple developmental pattern for UDP-GT or SULT activity.

Both families of enzymes exhibit extensive overlapping substrate activities (i.e., a substrate is metabolized by more than one isoform) and broad substrate specificity (i.e., an isoform metabolizes a wide range of substrates). In addition, many substrates metabolized by UDP-GT isoforms are also metabolized by SULT isoforms.

It is known that one UDP-GT isoform (UDP-GT2B1) is capable of glucuronidating bisphenol A in rats,[36] but the specific identity of other isoforms that glucuronidate bisphenol A is not known. It is widely known, as noted in the final CERHR report, that UDP-GT activity is lower in the fetus and neonates compared to adults. However, UDP-GT activity toward bisphenol A in rats at PND 3 was significant and slight activity was observed in the liver of prenatal rats just before delivery.[37] Although bisphenol A glucuronide was not measured by Taylor et al. (2008), it is likely that bisphenol A is glucuronidated to some extent in mice at PND 3, in particular by the oral route of exposure.

Similarly, UDP-GT activity in humans was found early in gestation in liver, spleen and kidney tissue, as well as in red blood cells. It has been suggested that UDP-GTs in circulating red blood

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[34] Pritchett, J.J., Kuester, R.K., and Sipes, I.G. 2002. Metabolism of bisphenol A in primary cultured hepatocytes from mice, rats, and humans. Drug Metabolism and Disposition. 30(11):1180-1185.

[35] Kim, Y., Kim, C., Park, S., Han, S. Y., Pyo, M., and Yang, M. 2003. Gender differences in the levels of bisphenol A metabolites in urine. Biochemical and Biophysical Research Communications. 312:441-448.

[36] Yokota, H., Iwano, H., Endo, M., Kobayashi, T., Inoue, H., Ikushiro, S., and Yuasa, A. 1999. Glucuronidation of the environmental oestrogen bisphenol A by an isoform of UDP-glucuronosyltransferase, UGT2B1, in the rat liver. Biochemical Journal. 340(2):405-409.

[37] Matsumoto, J., Yokota, H., and Yuasa, A. 2002. Developmental increases in rat hepatic microsomal UDP-glucuronosyltransferase activities toward xenoestrogens and decreases during pregnancy. Environmental Health Perspectives. 110(2):193-196.

cells "prevent the fetus from possible detrimental effects of xenobiotics at a time when the liver and kidney are not fully developed to perform this task."[38]

Although the specific identify of SULT isoforms that sulfonate bisphenol A have not been investigated, it is likely that the SULT1 family of phenol sulfotransferases are responsible. For example, SULT 1A1 has a broad substrate specificity and preferentially sulfonates simple planar phenols.

It is widely known that SULT activity is relatively high in the fetus and neonates. For example, SULT 1A1 mRNA was expressed at high levels in the liver of fetuses of mice, and mRNA levels gradually increased until 22 days of age.[39] Although bisphenol A sulfate was also not measured by Taylor et al. (2008), it is likely that bisphenol A is sulfonated in mice at PND 3, in particular by the oral route of exposure.

Likewise, SULT1A1 (and SULT1A3) are abundantly expressed in human fetal liver. The very broad expression pattern of the SULT1A isoforms suggests that these enzymes play an important role in protecting the human fetus from exogenous toxins. In addition, the presence of the SULT1A isoforms in the placenta indicate they may have a role in the metabolism of xenobiotics entering the fetal circulation from the maternal side, in particular since placental UDP-GT activity is relatively low in humans.[40]

Based on what is known about the ontogeny of UDP-GT and SULT enzymes, it is likely that the human fetus and infants do have adequate capability and capacity to metabolize low levels of bisphenol A to either the sulfate or glucuronide, starting at an early stage of development.

[38] The development of glucuronidation in humans is reviewed in de Wildt, S. N., Kearns, G. L., Leeder, J. S., and van den Anker, J. N. 1999. Glucuronidation in humans: Pharmacogenetic and developmental aspects. Clinical Pharmacokinetics. 36(6):439-452.

[39] For a review of sulfotransferase enzymes in mice, see Alnouti, Y. and Klaassen, C. D. 2006. Tissue distribution and ontogeny of sulfotransferase enzymes in mice. Toxicological Sciences. 93(2):242-255.

[40] For a review of sulfotransferase enzymes in humand, see Gamage, N., Barnett, A., Hempel, N., Duggleby, R. G., Windmill, K. F., Martin, J. L., and McManus, M. E. 2006. Human sulfotransferases and their role in chemical metabolism. Toxicological Sciences. 90(1):5-22.