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Invited Review

Bisphenol A: Update on newly developed data and how they address NTP's 2008 finding of "Some Concern"

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ABSTRACT

Bisphenol A (BPA) is a component of polycarbonate plastics and epoxy resins used in many commercial products including coatings and liners of food containers. Low levels of BPA can be detected in over 90% of human urine samples in the US, indicating that exposure to BPA is widespread. In 2008, the US National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) expressed concerns regarding BPA's potential health effects, and suggested improved study designs and methodologies that they believed would address those concerns. This paper discusses some of the controversial issues surrounding BPA, summarizes the current regulatory status of BPA, reviews recent pharmacokinetic studies, and describes ongoing and planned research on the effects of BPA. In addition, we evaluate two papers studying BPA neurobehavioral effects, identified by the European Food Safety Authority and the German Federal Institute for Risk Assessment as being valid for use in risk assessment, to determine whether they address the NTP-CERHR methodological concerns. The data from these studies would likely be sufficient for NTP to lower its concern level for neurobehavioral effects of BPA. At this time, many regulatory agencies from around the world support the use of BPA in food contact materials.

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1. Introduction

In recent years, bisphenol A (BPA), a component of plastics and coatings used for food storage, has received considerable attention regarding the potential for adverse health effects, much of which stems from the fact that is has been shown to have weak estrogenic-like effects (Matthews et al., 2001). Studies using laboratory animal models, when well-conducted by well-accepted standards, can provide valuable information for use in safety assessments to predict potential toxicity in humans. However, discrepancies in outcomes among and between studies with BPA, in particular between standard guideline studies on one side and a large number of small scale *in vit*ro and *in vivo* research or experimental studies addressing novel endpoints on the other side, has often led to controversy.

In 2008, the National Toxicology Program's (NTP) Center for Evaluation of Risk to Human Reproduction (CERHR) reviewed the scientific literature on BPA and published their findings regarding health effects in a NTP-CERHR Monograph on BPA (NTP, 2008). NTP-CERHR scored their findings as "Negligible Concern", "Minimal Concern", "Some Concern", "Concern" and "Serious Concern". The NTP's report found that there was "Some Concern" for BPA effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures. The NTP report also found "Minimal Concern" for BPA effects on mammary gland, early age for puberty for females in fetuses, infants and children, or for workers exposed to higher levels in occupational settings; and "Negligible Concern" that BPA exposure to pregnant women will result in neonatal mortality, birth defects or reduced birth weight and growth in offspring, or will cause reproductive effects in non-occupationally exposed adults. NTP stated that for areas of "Some Concern", additional research is needed to fill scientific data gaps, and recommended improved study designs and methodologies to facilitate published research utility for future BPA health effects assessments.

Since the publication of the NTP-CERHR monograph, hundreds of scientific studies have been published on the health effects of BPA, including studies that address the additional research identified by NTP in the areas of "Some Concern". This paper discusses some of the controversial issues surrounding BPA, summarizes the current regulatory status of BPA, reviews recent pharmacokinetic studies, and describes ongoing and planned research on the effects of BPA. In addition, we evaluate two papers studying BPA neurobehavioral effects, identified by the European Food Safety Authority (EFSA) and the German Federal Institute for Risk Assessment (BfR) as being valid for use in risk assessment, to determine whether they address the NTP-CERHR suggestions for study designs and methodologies.

1.1. Uses of Bisphenol A

BPA monomer (aglycone) is an industrial chemical used to make polycarbonate plastic, a lightweight, high-performance plastic, and epoxy resins, some of which are used to coat surfaces of food containers. Examples include wine storage tanks, food transport vehicles, and metal cans containing processed foods and beverages. BPA-containing resin coatings have been used by the food and beverage industry for over 50 years; they provide an effective barrier preventing chemical reactions between the food and the metal, thereby enhancing shelf life and food safety. Manufactured products containing BPA have other specialized uses, such as in flame-retardant materials, special plastics used in medical devices and medical adhesives and dental prosthetics and sealants. There is a large scientific database assessing the safety of BPA, making it one of the most thoroughly studied materials approved for food contact use. While there is continued effort by the food and canning industry to find an effective and safe alternative, at this time there is no known practical and safe alternative to BPA-containing epoxy resin coatings for the myriad of food products packaged.

2. Bisphenol A exposure

BPA may be present in small quantities as unreactive monomer in some materials, and may leach from some food contact substances and polycarbonate products as the material ages or is exposed to harsh chemicals such as dish washing detergents or is exposed to UV radiation. The US Centers for Disease Control and Prevention (CDC) identified BPA (as the inactive conjugate metabolite) in 93% of urine samples obtained as part of the National Health and Nutrition Examination Survey (NHANES) from over 2460 people, suggesting that BPA exposure is widespread (Calafat, 2011). The geometric mean urine concentration was calculated to be 1.9 μg BPA/l, when normalized for creatinine concentration (CDC, 2012). In 2011, Lakind and Naiman estimated the mean daily BPA intake from all sources in the US population age 6–60 ranged from 0.03 to 0.05 μg/kg body weight (bw) (Lakind and Naiman, 2011). The World Health Organization (WHO) estimated the mean BPA exposure for adults ranged from less than 0.01 to 0.40 μg/kg bw/day (95th percentile 0.06–1.5 μg/kg bw/day) and for young children to teenagers was 0.1–0.5 μg/kg bw/day (95th percentile 0.3–1.1 μg/kg bw/day) (WHO, 2011).

Health Canada recently decreased its probabilistic estimate of dietary exposure to BPA, and determined that the general population has a mean BPA exposure of 0.055 μg/kg bw/day and that children age 0–18 months had exposures ranging from 0.083 to 0.164 μg/kg bw/day (Health Canada, 2012). The Canadian Total Diet Study estimated a BPA intake of 0.075 μg/kg bw/day for all Canadians and a maximum BPA intake in children less than 1 month of age of 0.33 μg/kg bw/day (Cao et al., 2011). The US Food and Drug Administration (FDA) estimates that BPA exposure from use in food contact materials in infants and adults is 2.42 μg/kg bw/day and 0.185 μg/kg bw/day, respectively. For comparison, the FDA has determined the appropriate No-Observed-Adverse-Effect Level (NOAEL) for BPA to be 5 mg/kg bw/day (5000 μg/kg bw/day); the NOAEL for systemic toxicity is derived from two multigenerational rat studies (FDA, 2008). This is approximately 2000 and 27,000 times higher than the FDA estimates of BPA intake from food contact materials for infants and adults, respectively.

The US Environmental Protection Agency (USEPA) has set a chronic oral Reference Dose (RfD) for BPA of 50 μg/kg bw/day (USEPA, 2012a). The RfD is defined as "An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects

during a lifetime." (USEPA, 2012b). The European Food Safety Authority (EFSA) has set a Tolerable Daily Intake (TDI) for BPA of 50 μg/kg bw/day (EFSA, 2010). The TDI is defined as "an estimate of the quantity of a chemical contaminant to which we may be exposed through environmental contamination, and which when found in food can be ingested daily over a lifetime without posing a significant risk to health" (EFSA, 2012). Both the RfD and the TDI are approximately 100 times higher than the 95th percentile BPA intake level for adults of 1.5 μg/kg bw/day, estimated by WHO (2011) and approximately 20 times the FDA estimation of the BPA intake in infants .

There is a general consensus that effects on children should be evaluated carefully, even when exposed to compounds that may pose little if any risk to adults, due to children' rapidly developing organ systems, and metabolic and digestive differences. FDA notes that children have unique health and developmental characteristics that may affect how they respond to various food and medical products. Through research both within and outside FDA, there are many studies being conducted that assess how therapeutics and food substances behave in adults and children (FDA, 2010). FDA's National Center for Toxicological Research (NCTR), in partnership with the National Institutes for Environmental Health Sciences (NIEHS) and NTP, has been investigating the potential differences between fetal, neonatal, juvenile and adult mice, rats and non-human primates (NHP) exposed to BPA. Recent pharmacokinetic studies in this area are summarized in Section 9.1.

3. Current regulatory status and assessments of authoritative scientific organizations

Numerous regulatory agencies and authoritative scientific bodies around the world have conducted and published health assessments regarding exposure to BPA from food packaging materials, baby bottles and sippy cups made of polycarbonate plastic and exposure from other sources. For example, the EFSA conducted a comprehensive review of over 800 new literature citations and published their findings in September, 2010; EFSA's conclusion was that there was no reason to change the established Tolerable Daily Intake (TDI) of 50 μg/kg bw/day lifetime (EFSA, 2010). A UN joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Meeting reviewed over 100 key literature citations and published their findings. They concluded that "Continued research into the toxicokinetics of BPA and its estrogenic and other mechanisms of action will be needed before it is possible to determine the appropriate points of departure (e.g. NOAEL, LOAEL, benchmark dose) for human risk assessment with confidence" (WHO, 2011).

The FDA has conducted several in depth reviews of BPA exposure and risk as a result of exposure from food contact materials. FDA has determined the appropriate NOAEL for its assessment of BPA to be the NOAEL for systemic toxicity of 5 mg/kg bw/day (5000 μg/kg bw/day) derived from two multigenerational rodent studies. FDA states "FDA is not recommending that families change the use of infant formula or foods, as the benefit of a stable source of good nutrition outweighs the potential risk from BPA exposure" (FDA, 2012a). However, FDA is continuing its research and monitoring of studies to address uncertainties raised about BPA.

The Advisory Committee of the German Society of Toxicology evaluated the scientific literature and concluded that the "current Tolerable Daily Intake (TDI) [50 μg/kg bw/day] for BPA is adequately justified and that the available evidence indicates that BPA exposure represents no noteworthy risk to the health of the human population, including newborns and babies" (Hengstler et al., 2011). In addition, the Japanese National Institute of Advanced Industrial Science and Technology (AIST) concluded that the risk of BPA to human health is believed to "be very small" (Japan AIST, 2011), and Health Canada recently concluded "Therefore, based on the overall weight of evidence, the findings of the previous assessment remain unchanged and Health Canada's Food Directorate continues to conclude that current dietary exposure to BPA through food packaging uses is not expected to pose a health risk to the general population, including newborns and young children. This conclusion is consistent with those of other food regulatory agencies in other countries, including notably the United States, the European Union and Japan".

Consumers remain concerned about exposures in infants and children, due to the suggestion of developmental effects in some animal studies. In 2011, the American Chemistry Council requested that the FDA "remove infant feeding bottles ("baby bottles") and spill-proof cups designed to help train babies to drink from cups ("sippy cups") from the scope of permitted food contact applications for polycarbonate resins. This request was "based solely on the grounds that these uses have been intentionally and permanently abandoned by all major product manufacturers" (ACC, 2011). In 2012, the FDA responded by amending the food contact regulations, based solely on abandonment, by removing BPA manufactured baby bottles and sippy cups from approved food contact substances (FDA, 2012b). In early 2011 the European Commission issued a new Directive setting a temporary ban on the use of BPA in the manufacture and placing on the market of polycarbonate infant feeding bottles in Europe (European Commission, 2011). France has a ban on BPA in baby bottles, and has introduced legislation that would ban use of BPA for materials in contact with food for children 0–3 years of age in 2013. The Danish Veterinary and Food Administration banned BPA in food containers and products for very young children effective July 1, 2010. The Australian government has instituted a voluntary phase-out of polycarbonate baby bottles (FSANZ, 2012).

See Supplemental Table 1 for statements and conclusions from several regulatory agencies and authoritative scientific organizations regarding BPA in food packaging materials. At this time, USFDA, EFSA, BfR, FDA, German Society of Toxicology, Health Canada and the Japanese AIST Australia/New Zealand support the use of BPA in food contact materials.

4. Overview of the Weight of Evidence approach in evaluating the scientific literature

Generally, regulatory agencies and risk assessment scientists evaluate scientific studies using a Weight of Evidence (WOE) approach. This approach uses the results found in multiple, high quality studies as the basis for determining effects. Single studies, or studies with methodological or study design deficiencies, are considered, but the results are usually given less weight.

As an example, a WOE methodology was published by Klimisch et al. (1997), in which studies are assigned to one of four categories:

1. Reliable without restriction (i.e., conforming to good laboratory practices (GLP) or some other set of quality criteria)
2. Reliable with restriction (i.e., well documented and scientifically acceptable, but falling short of GLP)
3. Not reliable (not well documented or used unacceptable methods)
4. Not assignable (e.g., abstracts)

Evidence that is considered "not reliable" may be used to an extent on a case-by-case basis, depending on the expertise of the evaluators. McCarty et al. (2012) have proposed a six step scheme for regulatory peer review that derives a numerical score and

narrative describing all judgments and conclusions derived from the WOE evaluation process, including key assumptions, uncertainties, and any adjustments or refinements in weighting factors required subsequent to their initial formulation.

WOE is widely used by scientists in the evaluation of scientific data and in setting regulations and establishing guidelines in the public health policy sphere. For example, the USEPA applies a WOE approach to carcinogen risk assessment (USEPA, 2005). Such evaluations rely on scientific consensus, which is essential for regulatory integrity and expert judgment when reaching final conclusions (Lorentzen and Hattan, 2010). Regulators can then determine if the WOE does or does not support the safety of an agent, determine if more research is required, and establish a set of uncertainties around a decision.

5. Comparison of research and guideline-compliant studies

Studies used to evaluate safety, or the probability of harm to humans, must be of high quality; often this means that studies must be guideline-compliant; i.e., follow FDA or Organisation for Economic Co-operation and Development (OECD) Good laboratory Practice guidelines (GLP). The US Code of Federal Regulations (CFR) Title 21, Subchapter A, Part 58: *Good Laboratory Practice for Nonclinical Laboratory Studies*, was established in 1978 by the US Food and Drug Administration. Its creation was driven by in-depth investigations of 40 contract and/or research laboratories across the US that identified fraudulent activities in some laboratories and very poor laboratory practices in most. "GLP embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, reported and archived."(MHRA, 2012). GLP assures that the data submitted to FDA are a true reflection of the study outcome and that the data are traceable and can be reviewed by the regulatory evaluators. Moreover, it assures that protocols are designed and data interpreted such that the chance for investigator bias is minimized. GLP is the international set of standards required by global regulatory bodies reviewing a sponsor's non-clinical laboratory study submissions or in establishing product safety.

Regulatory agencies thus generally expect that most animal studies that are used in support of a sponsor's submission to regulatory authorities should follow GLP guidelines; even most analytical procedures and *in vitro* studies used in support of animal studies or conducted independently should follow GLP. However, as previously noted, a balanced and objective risk assessment is a comprehensive process that relies on WOE to consider all the available data. While FDA/OECD guideline compliance plays a major role when considering data for regulatory decision making, other criteria are considered when judging the validity and regulatory utility of data (e.g. sample size, statistical analysis, reproducibility, dose–response, potential for background contamination, adequate reporting, availability of raw data, etc.) and that non-compliance with GLP criteria alone is not sufficient for a risk assessor to invalidate and dismiss a study (see Table 1).

There remains significant controversy regarding protocol design and the research environment where BPA studies are conducted. Many small scale research and exploratory studies designed to show potential adverse outcomes for BPA are conducted in academic institutions, which generally do not have the physical or financial resources to conduct GLP or guideline compliant studies. Studies conducted following FDA or OECD Good Laboratory Practice (GLP) guidelines generally take years to conduct and cost millions of dollars. In contrast, academic or experimental research studies are generally conducted in months, do not follow GLP guidelines, and may use unique or novel protocol designs. Also, the studies may use a limited number of animals and use doses or routes of administration not applicable to human exposure. Rarely are raw data made available for review.

Table 1
NTP concerns regarding study design and methodology.

Appropriate route of exposure (i.e., one that is relevant to human oral exposure)
Animals studied for an appropriate length of time
Adequate number of animals
Use of appropriate or recognized animal model
Appropriate statistical analysis (including use of the litter as the experimental unit, rather than the pup, to control for litter effects)
Were differences in metabolism, pharmacokinetics and pharmacodynamics between humans and animals taken into account?
Did the researchers take steps to eliminate BPA from their analytical equipment?
Did the study evaluate dose–response?
Are the conclusions appropriate to the study design (i.e., are conclusions overly broad?)

NTP (2008).

6. Laboratory analytical concerns

BPA has numerous uses and can be found in products used in virtually every room in homes, hospitals, schools, and office buildings. It is also widespread in research laboratories and animal rooms (Markham et al., 2010; Twaddle et al., 2010). Many laboratories use polycarbonate animal cages, and plastic items are used extensively in research laboratories and laboratory equipment, even in equipment that is utilized to measure BPA. Therefore, researchers must take deliberate and extensive measures to control for BPA contamination. An alternative approach is to use stable-isotope labeled BPA (e.g., d6-BPA) to control for BPA contamination (Doerge et al., 2011a; Twaddle et al., 2010; Ye et al., in press). BPA analysis requires the use of highly sensitive equipment (e.g., LC/MS/MS system) and methodology that has been validated (internal standards) to measure both the conjugated and aglycone BPA. Adherence to these methodologies is required to produce valid and reproducible results when analyzing BPA in biological samples.

7. Overview of cooperative studies between NTP/FDA and NIEHS extramural grantees

To help resolve differences between studies following traditional GLP guidelines and studies conducted in research laboratories using new (non-traditional) endpoints and methods, the NTP and the FDA's National Center for Toxicological Research (NCTR) are collaborating with the National Institute of Environmental Health Sciences (NIEHS) Division of Extramural Research and Training (DERT) to foster collaboration between federal and academic scientists. These studies are being conducted at NCTR with an FDA scientist serving as the Principal Investigator. The goal of the collaboration is to use a two year chronic study design for BPA, conducted under FDA's GLP guidelines, as the core study, and then at various times, designated animals or animal tissues will be distributed to grantees from research laboratories for further testing and analysis.

Twelve academic researchers have received NIEHS funding for this BPA project. The core study will assess traditional toxicology endpoints, such as cancer; other arms of the study will assess additional endpoints, typically not measured in GLP guideline studies, with the intent to provide the grantees with animals and tissues from a GLP guideline study. The investigators are: Nira Ben-Jonathan, Ph.D., University of Cincinnati – Metabolism and heart disease, Kim Boekelheide, M.D., Ph.D., Brown University – Male reproduction, Jodi Flaws, Ph.D., University of Illinois at

Urbana-Champaign – Female reproduction and fertility, Nestor Gonzalez-Cadavid, Ph.D., Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center – Male reproduction, Andrew Greenberg, Ph.D., Tufts University – Metabolic disease and diabetes, Shuk-Mei Ho, Ph.D., University of Cincinnati – Cancer, Norbert Kaminski, Ph.D., Michigan State University – Immunity, Heather Patisaul, Ph.D., North Carolina State University – Neurobehavior, Gail Prins, Ph.D., University of Illinois at Chicago – Prostate cancer, Ana Soto, M.D., Tufts University – Mammary cancer, Frederick vom Saal, Ph.D., University of Missouri – Reproductive development and Robert Zoeller, Ph.D., University of Massachusetts, Amherst – Brain and thyroid function.

Animals were allocated to the study in August, 2012 and dosing has begun. The core study will include a one year interim sacrifice and a two year sacrifice with complete gross and micropathology assessment. The test system includes Sprague–Dawley rats from NCTR's animal colony, a soy-free diet, gavage of dams from gestation day 6 (GD6) until parturition, then direct dosing of pups from post natal day 1 (PND1). Half of the animals will be dosed continuously for two years and half will have dosing stopped at PND 21. The dose groups will be vehicle, 2.5, 25, 250, 2500, and 25,000 μg/kg bw/day BPA. There will be two ethinyl estradiol "positive" control groups, 0.05 and 0.5 μg/kg bw/day. Littermates from the core study will provide animals and tissues for the NIEHS-funded grantees, including a behavior study. Not all of the grantees will use all dose groups or both the continuous and stop dose animals. Animals and tissues provided to the grantees will be blinded as to dose groups. Once the grantees studies are complete and the code unlocked, they are free to publish the results without further NTP or FDA review. The core study is expected to take 4–5 years to complete; grantee studies may be completed in a shorter time (Birnbaum et al., 2012).

8. Overview of NTP-CERHR findings and requests for further research

The NTP is a Department of Health and Human Services (DHHSs) Interagency Program established to coordinate toxicology research and testing, strengthen the science base in toxicology, develop and validate improved testing methods, and provide information about potentially toxic chemicals to health regulatory and research agencies, scientific and medical communities and the public. The Center for Evaluation of Risk for Human Reproduction (CERHR, currently called Office of Health Assessment and Translation (OHAT) is part of the NTP. CERHR/OHAT expert scientific panels evaluate evidence regarding chemicals of public health concern and provide assessments of potential health effects.

Concerns for the safety of BPA and the contradictory findings regarding BPA health effects published in the scientific literature prompted NTP-CERHR to establish an Expert Scientific Panel (Panel) in 2007 to conduct a comprehensive review of BPA using all resources available, including the scientific literature to date (NTP-CERHR, 2007). In 2008, the CERHR Expert Scientific Panel reported that there were no data on the effects of human developmental exposure to BPA available (at the time of the Panel's review) and thus no conclusions were made about risk to humans (Chapin et al., 2008). The Panel went onto summarize their findings, based only on animal studies, as: For pregnant women and fetuses: Some Concern for neural and behavioral effects; Minimal Concern for prostate effects; Minimal Concern for accelerated puberty; Negligible Concern for birth defects; For Infants and Children: Some Concern for neural and behavioral effects; Minimal Concern for accelerated puberty effects; For Adults (based on animal studies): Negligible Concern for adverse reproductive effects in the general population; and Minimal Concern for occupationally exposed populations (Chapin et al., 2008).

It is important to note that in the Panel's written conclusions, they stated "while the panel did not necessarily expect a specific effect to display a monotonic dose response (e.g. consistently increasing organ size), many members of the panel expected the high dose studies with bisphenol A to detect some manifestation of toxicity (e.g., altered weight, histopathology) in tissues reported to be affected at low doses even if the study could not replicate the reported low dose effect". The Panel also noted "There are several large, robust, well designed studies with multiple dose groups using several strains of rats and mice and none of these detected any adverse reproductive effects at low to moderate dosage levels of BPA administered via the relevant route of human exposures. Further, none of these studies detected changes in prostate weight, age at puberty (rat), pathology or tumors in any tissue, or reproductive tract malformations. For this reason, Panel members gave more weight to studies that evaluated both low-and high-doses of bisphenol A compared to low-dose-only studies in cases where the target tissues were comparably assessed". The written conclusions also note "Hence, the failure of BPA to produce reproducible adverse effects via a relevant route of exposure, coupled with the lack of robustness of the many of the low dose studies (sample size, dose range, statistical analyses and experimental design, GLP) and the inability to reproduce many of these effects of any adverse effect, strains the credibility of some of these study results" (Chapin et al., 2008).

Also in 2008, NTP published the NTP-CERHR Monograph on BPA, which provided the NTP's scientific basis for its conclusion of (1) "Some Concern" for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to BPA; and (2) "minimal concern" for effects on the mammary gland and an earlier age for puberty for females in fetuses, infants, and children at current human exposures to BPA. There are distinct differences in the Panel's report and the NTP Monograph on BPA (Expert Scientific Panels and other advisory groups act only in an "advisory capacity" to US federal agencies). The NTP-CERHR monograph discussed deficiencies in the published literature and identified areas requiring more research, noting study designs and methodologies considered appropriate to address these areas. Specifically, NTP-CERHR suggested conducting "low dose" animal studies (<5 mg BPA/kg bw/day) using oral dosing that evaluate developmental effects in the areas of neural and behavior alterations, lesions in the prostate and mammary glands, altered prostate and urinary tract development, and early onset of puberty. NTP-CERHR noted that many scientific studies "were designed not as toxicology studies but rather to probe very specific experimental questions, and their results are not always easily interpreted with regard to how they contribute to the weight-of-evidence for human health risks" (NTP, 2008). NTP also noted study design and analysis factors, such as animal models, routes of administration, and statistical data analysis methods that they would like to see used in future studies to address to their concerns. The impact of the NTP-CERHR Monograph on BPA research has been significant, with NIEHS itself funding $30 million dollars of additional BPA research through extramural granting programs, and the FDA committing to conducting additional risk assessments and supporting additional BPA research. The NTP-CERHR concerns regarding study design and methodology are listed in Table 1.

Finally, the NTP discussed areas of difficulty in interpreting study results. These are listed in Table 2.

9. Evaluation of recent studies regarding pharmacokinetics, and brain and behavioral effects

Since the NTP-CERHR report (NTP, 2008), hundreds of studies evaluating the health effects of BPA have been published. We summarize the recently published scientific literature regarding

Table 2
NTP concerns regarding interpretation of study results.

Insufficient replication of studies by independent investigators
Suitability of various experimental approaches
Relevance of the specific animal model
Incomplete understanding of whether a reported effect is adverse
Does the effect persist in life or does it manifest as a clear health effect later in time?
Incomplete understanding of the metabolism and pharmacokinetics of BPA in humans and animals
Incomplete understanding of the shape of the dose response curve
Are the results biologically plausible?
Are results across studies consistent?

NTP (2008).

pharmacokinetics in humans and laboratory animals, and two papers studying BPA neurobehavioral effects that were identified by EFSA and BfR as being valid for use in risk assessment, to determine whether they address the NTP-CERHR suggestions for study designs and methodologies (BfR, 2008; EFSA, 2012).

9.1. Pharmacokinetics and metabolism

Understanding the serum levels associated with typical dietary exposure and the differences in pharmacokinetics and metabolism between humans and laboratory animal species is critical for proper interpretation of toxicology studies. BPA is metabolized mainly through conjugation to form the glucuronide metabolite (BPA glucuronide), which lacks estrogenic activity (Calafat, 2011; Matthews et al., 2001). Humans and laboratory animals (rats, mice, and NHP) form the glucuronide as the primary metabolite. Adult rats or mice rapidly metabolize orally-administered BPA to the glucuronide, which is then eliminated primarily via the bile (Doerge et al., 2010b). NHP and humans also rapidly metabolize orally-administered BPA to the glucuronide; however, it is eliminated primarily via the urine (Doerge et al., 2010a). Recent metabolism and pharmacokinetic studies are discussed in detail below.

9.1.1. Teeguarden et al., 2011; Völkel et al., 2002

Two pharmacokinetic studies (Teeguarden et al., 2011; Völkel et al., 2002) have been conducted in humans using doses of approximately 70 μg/kg bw and 0.27 μg/kg bw (estimated dose), respectively. The Völkel study had a 10 nM (2.3 ng/mL) detection limit and the Teeguarden study had a detection limit of 1.3 nM (0.3 ng/mL). The Teeguarden study was conducted in volunteers who consumed a diet "enriched in canned food items likely to be significant dietary sources of BPA" (Teeguarden et al., 2011), and was intended to determine BPA aglycone serum levels in people with a high dietary BPA intake. Neither the Völkel nor the Teeguarden studies were able to detect BPA aglycone in serum, indicating that BPA aglycone was either not present or that concentrations were below their respective detection limits.

In addition, there are several published reports of serum BPA aglycone levels from single samples obtained from various populations. Many of these have found that levels are below detectable limits; however some have detected levels up to 1–2 ng/mL (information compiled from the scientific literature by Vandenberg et al., 2010). Since these serum levels are higher than what would be expected based on the studies by the Völkel and Teeguarden studies, some scientists have suggested that these reported levels may be due to undetected contamination of laboratory equipment with BPA, since BPA is present in common materials used during sample processing and analysis (Ye et al., in press).

9.1.2. Taylor et al., 2011

Taylor et al. (2011) conducted pharmacokinetic studies in adult female CD-1 mice and adult female rhesus monkeys. Monkeys were fed deuterated BPA (dBPA) at a dose of 400 μg/kg bw/day in food for 7 days. Blood samples were collected on the first and seventh days for 24 h. Mice were administered 400 μg/kg bw/day of tritium-labeled BPA (^{3}H-BPA) orally, and blood was collected for 24 h. In monkeys, the maximum BPA aglycone level (Cmax) occurred at 1 h after dosing and was 4 ng/mL. This level declined over 24 h, and there was no bioaccumulation of BPA. In mice, the Cmax also occurred at 1 h and was 3.2 ng/mL.

The authors then compared the levels of conjugated BPA in female mice and monkeys to those found in human females who had a single oral dose of 5 mg dBPA (which, based on the body weight of the subjects, averaged 69.3 μg/kg bw) in Völkel et al. study (2002). The authors found that BPA glucuronide conjugate pharmacokinetics were similar in female monkeys, mice and humans. Since Völkel et al. were unable to detect BPA aglycone in humans with a detection limit of 10 nM (2.3 ng/ml), no comparison could be made by Taylor et al. between human female BPA aglycone levels and those in mice and monkeys.

The authors also compared the 24-h average BPA aglycone levels in mice and monkeys of 0.5 ng/ml after a 400 μg/kg bw oral dose with studies that have reported levels in humans up to approximately 2 ng/ml (see compilation by Vandenberg et al., 2010) and concluded that "total daily human exposure…is much higher than previously assumed." This conclusion relies heavily on the reports of human BPA aglycone levels from single samples in various population groups. Since these reports are not studies in which humans were dosed with dBPA, the accuracy of these levels is unclear. An ongoing human pharmacokinetic study by NIEHS/FDA using dBPA, described below, should provide further information to clarify the blood levels of BPA aglycone achieved in humans following oral dosing.

9.1.3. Doerge et al. (2010a,b, 2011b)

Doerge et al. conducted a series of pharmacokinetic studies in neonatal and adult CD-1 mice, Sprague–Dawley rats and rhesus monkeys (Doerge et al., 2010a,b, and 2011b). The authors administered 100 μg BPA/kg bw in DMSO and water using both the intravenous (i.v.) and oral routes of exposure to allow comparison between species and routes of exposure. The results show that serum BPA aglycone levels are approximately an order of magnitude lower in newborn rhesus monkeys compared to CD-1 mice and Sprague–Dawley rats following the equal oral doses. In addition, for monkeys, the difference between the Cmax concentration for newborns and adults is about 2-fold, whereas, for mice and rats, the difference is about 10-fold (Fig. 6 in Doerge et al., 2011b), with the newborns having the higher levels in both species.

Furthermore, the percentage of the unconjugated BPA (aglycone) in the serum of monkeys following oral dosing is less than 1% of the total BPA for both neonates and adults (Doerge et al., 2010a). In comparison, the unconjugated BPA in neonatal mice and rats is substantially higher than the adult (8–20 times higher in rats and mice, respectively; Fig. 7 in Doerge et al., 2011b).

Doerge et al. (2011b) note: "[M]any of the human UGT 1A and 2B isoforms catalyzing glucuronidation of BPA…are homologous with those in monkeys…and the known ontogeny of human UGT isoforms…predicts that glucuronidation of BPA is well-developed at birth in primates vs. the overall immaturity of rodents at birth reflected by reduced UGT activity…These observations are consistent with the approximately order of magnitude lower internal exposures to the unconjugated form of BPA following oral administration seen in neonatal monkeys relative to neonatal rodents."

9.1.4. Fisher et al., 2011

Fisher et al. (2011) developed physiologically-based pharmacokinetic (PBPK) models for i.v. and oral administration of BPA to

adult monkeys based on the pharmacokinetic studies of Doerge et al. (2010a,b, 2011b). The authors also fit their model to the monkey data published by Taylor et al. (2011), and created a separate set of "revised model parameters" for this data.

In order to fit the model to the published monkey data, the authors assumed some renal tubular reabsorption of BPA conjugates occurs (based on the studies of Gotoh et al., 2002 and Jemnitz et al., 2010) and that metabolism of BPA to conjugates occurs in the small intestine (based on the studies of Audebert et al., 2011 and Mazur et al., 2010) as well as the liver. According to the authors, these two assumptions allowed for a better fit of the model to the data, compared to including enterohepatic recirculation in the model.

The authors then simulated BPA aglycone and conjugate pharmacokinetics for adult humans using human physiological parameters and a dose of 5 mg of BPA in their original model and the model with the "revised model parameters" obtained by using data from Taylor et al. (2011) study. The resulting plasma levels were compared to the human BPA pharmacokinetic data obtained by Völkel et al. (2002) following administration of 5 mg BPA to humans. (Völkel and colleagues were unable to detect BPA aglycone in plasma samples with a detection limit of 10 nM [2.3 ng/ml], however, they were able to detect BPA conjugates). Both the original model and the model with revised parameters predicted that BPA aglycone levels in humans following a dose of 5 mg BPA (Völkel et al. 2002) would be below a 10 nM detection limit, as was found in the Völkel et al. (2002) study.

9.1.5. Doerge et al., 2011a

Doerge et al. (2011a) administered dBPA at a dose of 100 μg/kg bw to pregnant SD rats by either the oral or i.v. routes of exposure. Blood samples, fetal tissues and amniotic fluid were collected for analysis. Whereas BPA administered i.v. to Sprague–Dawley rats resulted in measurable amounts of BPA aglycone in the fetus, orally administered BPA at the same dose level did not produce measurable levels of BPA in the fetus, with detection limits of 0.2 nM (0.045 ng/ml in serum, and 0.4 pmol/g (0.00009 ng/g) tissue (Doerge et al., 2011a). This is an important observation since the vast majority of humans are exposed to BPA via the oral route (WHO, 2011). In addition, amniotic fluid levels of BPA aglycone were at or below maternal serum levels.

9.1.6. Summary of pharmacokinetic studies

Table 3 below compares the maximum serum concentration of BPA aglycone (Cmax), the percentage unconjugated in the serum, and the elimination half-life reported in studies using various animal species. The table reports the dose, the Cmax as listed in the paper, and the Cmax corrected to a 100 μg/kg bw dose, for purposes of comparison. Cmax values corrected to a 100 μg/kg bw dose ranged from 0.041 ng/mL in adult CD-1 mice to 7.7 ng/mL in post-natal day (PND) 3 CD-1 mice. It is clear from the table that neonatal rats and mice have much higher Cmax values, when corrected for dose, than adults of the same species. In addition, neonatal rats and mice have much higher Cmax values than neonatal and adult monkeys, when corrected for dose.

Of note, a study of human BPA pharmacokinetics using a dose of 100 μg/kg bw deuterated BPA is expected to be completed in the near future by NIEHS/FDA (Patterson et al., 2013). The results of this study, utilizing deuterated BPA, will provide insight regarding BPA metabolism and distribution in humans after oral exposure. Upon completion of this study, pharmacokinetic data will be available for mice, rats, NHP and humans for comparative modeling,

Table 3
Comparison of BPA aglycone maximum serum levels, percentage unconjugated and elimination half-life reported in the scientific literature.

Reference	Dose (μg/kg BW)	Route	Species, sex	Age of animal (N)	Cmax[a] (nM)	Cmax (ng/mL)	Cmax (ng/mL) corrected to 100 μg/kg bw dose	Unconjugated Elimination half-life (h)	% Unconjugated at Cmax	Comments
Taylor et al. (2011)	400	Oral	CD-1 mice, female	Adult, N approx. 40[b]		3.28	0.82		0.8	% Unconjugated determined at a 100,000 μg/kg bw dose
			Rhesus monkey, female	Adult, *N* = 11		3.95	0.9875		2.6	
Doerge et al. (2011b)	100	Oral	CD-1 mice, males and females	Adult, *N* = 84	0.18	0.041	0.041	0.63	0.45	
				PND 3, *N* = 24	34	7.7	7.7	5.9	19.0	
				PND 10, *N* = 24	7.4	1.7	1.7	3	5.3	
				PND 21, *N* = 24	1.1	0.25	0.25	0.2	3.9	
Doerge et al. (2010a)	100	Oral	Rhesus monkeys, female	Adult, *N* = 4	0.84	0.19	0.19	0.39	0.14	
			Rhesus monkeys, males and females	PND 5, *N* = 6	2	0.46	0.46	2	0.29	
			Rhesus monkeys, males and females	PND 35, *N* = 6	1.1	0.25	0.25	1.7	0.20	
			Rhesus monkeys, males and females	PND 70, *N* = 6	1.5	0.34	0.34	1.5	0.60	
Doerge et al. (2010b)	100	Oral	S–D rats, female	Adult, *N* = 5	0.39	0.089	0.089	3	0.53	
			S–D rats, males and females	PND 3, *N* = 24	29	6.6	6.6	8.5	6.52	
				PND 10, *N* = 24	6.7	1.5	1.5	4	3.06	
				PND 21, *N* = 24	0.7	0.159	0.159	1.9	0.38	
Prins et al. (2011)	10	Oral	S–D rats, males	PND 3, *N* = 90	1.13	0.26	2.6	Not determined	27	Prins et al. (2011) reported % unconjugated as 29%, 21% and 31% at 0.5, 1 and 2 h, respectively. 27 is the average of these values

[a] Cmax = Maximum serum concentration.
[b] Five or six animals used per blood collection time point, with blood collected seven times.

Table 4
NTP-CERHR concerns regarding neural and behavioral studies in animals.

Neural and behavioral alterations
Use of appropriate route of exposure
Are the findings relevant to humans given the brain differences between humans and animals?
Is the body of literature consistent in the findings?
Did the authors assess the functional impacts of structural changes?
Were the changes transitory or permanent?
Did the authors control for the differences in circulating levels of sex hormones at the time of testing?

and should provide regulatory authorities with the type of information that may be able to reduce uncertainties in future safety analysis. In addition, a dBPA pharmacokinetic study in pregnant NHPs will be published in the near future (Patterson et al., 2013).

9.2. Neural and behavioral alterations

The NTP-CERHR monograph (2008) noted that studies assessing the neurological effects of BPA to date were difficult to interpret for health assessment purposes. For example, some studies administered BPA directly into the brain, rather than using the oral route of exposure, the relevant route for humans from food consumption, and some have found changes in the cellular composition of animal brain structures that humans do not have, making extrapolation to humans difficult or impossible. In addition, the NTP-CERHR had other methodological concerns, which are listed in Table 4 below.

Regarding studies of neural and behavioral alterations, there are two studies using the oral route of exposure that address NTP's areas of concern, Ryan et al. (2010) and Stump et al. (2010). Both of these were published since the NTP (2008) report.

9.2.1. Ryan et al. 2010

Ryan et al. (2010) orally dosed adult female Long Evans (LE) rats with BPA at doses of 0, 2, 20 or 200 μg/kg bw/day on day 7 of gestation through PND 18. As a positive control, the authors also orally dosed groups of female rats with ethinyl estradiol (EE) at doses ranging from 0.05 to 50 μg/kg bw/day during the same timeframe of pregnancy and lactation.

The authors evaluated onset of puberty, fertility, and anatomy of the female offspring. They also examined sexually dimorphic behavior using behavioral tests in rats specific for hormonally-mediated effects: sweet (saccharin) preference and lordosis (BfR, 2010).

BPA-dosed rats were not significantly different in any study endpoint from vehicle-treated controls. In contrast, EE-dosed rats had increased anogenital distance, decreased body weight of pups on PND 2, reduced F1 fertility and F2 litter sizes, and malformations of the external genitalia at a dose of 5 μg/kg bw/day. In addition, the female EE-dosed pups had an accelerated time of vaginal opening. The F1 females had indications of defeminization of the CNS, as evidenced by a reduced (male-like) saccharin preference at a dose of 5 μg/kg bw/day, and an absence of lordosis at a dose of 15 μg/kg bw/day.

This study addressed the following NTP concerns: (1) it used an oral exposure route with "low" doses of BPA (2–200 μg/kg bw/day); (2) animals were dosed at the gestational time of reproductive organ development and the time during lactation when hormonal imprinting of behaviors occurs; (3) an adequate number of dams were used (6–38 per group); (3) an appropriate animal model was used, as evidenced by the effects seen in the positive control group; (4) statistical analysis used the litter as the experimental unit; (5) the authors used a range of BPA doses that were relevant to estimated human exposures; (6) the positive controls exhibited a dose–response for estrogenic effects; (7) the authors controlled for variations in the circulating levels of sex hormones at times of testing by ovarectomizing the females and administering replacement hormones at a controlled dose; and (8) the animals were tested for behavioral (functional) impacts using tests (sweetness preference and lordosis) that the authors validated in LE rats in a pilot study.

EFSA (2010) evaluated the Ryan study and found the study valid. They stated "The size of the experimental groups was adequate and the statistical analysis of the data was appropriate. The inclusion of the estrogenic reference compound EE at several doses demonstrates the sensitivity of the test animals ($\geqslant$1.5 μg/kg bw/day) and of the methods to detect estrogen-related toxicity... The dosage levels of BPA used by Howdeshell et al. (2008) and Ryan et al. (2010a) are in line with those used in "low-dose" BPA studies with rats, with the lowest dose (2 μg/kg bw/day) being in the same range or below the estimated average intake of the European population including infants, children and adults (1.5–13 μg/kg bw/day) (EFSA, 2006).... Overall, the [EFSA] Panel considered the experimental design and the performance of the study as valid...The latter study (Ryan et al., 2010) also addressed specifically the impact of BPA on the sexually dimorphic behavior of female rats, i.e. sweet preference and lordosis behavior, which were clearly affected by ethinylestradiol treatment, but not by any BPA dose. Therefore, the Panel concludes that the study results did not indicate any low-dose effects of BPA on the development of sexually dimorphic behavior in female rats."

9.2.2. Stump et al. 2010

Stump et al. (2010) is a GLP-compliant study that evaluated functional and morphological nervous system effects following exposure to BPA during gestation and lactation. Female Sprague Dawley rats were fed chow to which BPA had been added at levels of 0, 0.15, 1.5, 75, 750 and 2250 ppm. This resulted in estimated doses (based on feed consumption) of 0, 0.01, 0.12, 5.85, 56.4 and 164 mg BPA/kg bw/d during gestation, and to 0, 0.03, 0.25, 13.1, 129 and 410 mg BPA/kg bw/d during lactation. Thus, this study included groups with "low" BPA doses. Females were fed BPA from gestational day 0 until PND 21, when the pups were weaned, and the dams sacrificed. Pups were assigned to subset groups A, B, C (1 pup per sex per liter). Group A was given detailed clinical examinations, evaluated for developmental landmarks (balanopreputial separation and vaginal patency), auditory startle response, motor activity, and, starting on PND 62, for learning and memory. Group B was evaluated for learning and memory starting on PND 22. Group C was necropsied on PND 21, and brain weight and morphometric and neuropathological evaluations were determined.

The BPA-treated dams in the two highest dose groups had decreased maternal body weight gain (9.5% for the 56.4 mg/kg bw/day group and 22.4% for the 164 mg/kg bw/day group). There were no effects on gestation length or parturition, number of pups born, live litter size, sex ratio, postnatal viability, and the BPA treated pups had no differences in surface righting response compared to untreated controls. The authors found that the NOAEL for developmental neurotoxicity was 2250 ppm BPA added to food (the highest dose tested), which corresponds to a dose of approximately 164 and 410 mg/kg bw/day during the periods of gestation and lactation, respectively.

For evaluation of motor activity, the authors provided historical control data and used haloperidol, nicotine and amphetamine as a positive control. The results showed that BPA exposed offspring had no differences in the pattern of activity counts. Regarding the developmental landmarks of balanopreputial separation and vaginal patency, there was no difference between the BPA-treated pups and untreated controls. For evaluation of the auditory startle

response, the authors also provided historical control data and used chlorpromazine, nicotine, amphetamine or 8-hydroxy-N,N-dipropyl-2-aminotetralin as positive controls. No differences were found in the BPA-exposed offspring compared to untreated controls.

For evaluation of memory and learning, the authors used the Biel maze test (swimming test). The authors reported some sporadic differences between groups and stated "all statistically significant differences noted were considered spurious and not BPA related because they did not occur consistently between or within testing periods, did not demonstrate any evidence of a dose-related trend, and/or were associated with atypical control responses" (Stump et al., 2010).

The authors concluded that there was no evidence for BPA neurotoxicity, and established a No Observable Adverse Effect Level (NOAEL) for developmental neurotoxicity of 2250 ppm in feed (the highest dose tested), which corresponds to 164 and 410 mg/kg bw/day during gestation and lactation, respectively.

The German Federal Institute for Risk Assessment (BfR) evaluated this study and stated "for the tests on *learning and memory* historical control data as well as data on the test performance and validity – as normally required by the test guideline – were provided with the test report. Data obtained with scopolamine served as a positive control for prolonging latency and increasing erroneous trials in the Biel maze. The swimming ability on the first day of testing was similar across all groups (BPA exposed and non-exposed) for either age. Also, the number of errors in escaping the maze via either path as well as after reverse sequence did not reveal significant differences across groups at either age tested."(BfR, 2010)

However EFSA's assessment found "that the Biel maze test as performed by Stump et al. does not have the potential to demonstrate equivalence of BPA compared to a control.... Based on the re-analysis the Panel considered that no conclusion can be drawn from this study on the effect of BPA on learning and memory behavior due to large variability in the data." (EFSA, 2010)

Stump et al. also noted some findings that they state are "incidental". They report: "Incidental findings in the BPA-exposed groups included the following: on PND 11 only, a total of six pups (two animals from the 750-ppm group and four from the 2250-ppm group) exhibited irregular jerking movements of limbs, head, and/or body and/or jumping with all four feet in the air. These clonic movements were recorded as "convulsions" and/or "popcorn seizures." The incidence of these findings was not statistically significant and occurred during the same period where statistically significantly reduced mean pup body weight gains were noted (PNDs 7–14) in the 750- and 2250-ppm groups. These findings were not observed at any other age. For these six animals, there were no remarkable findings in the other end points examined as part of the detailed clinical observations. The incidence of these findings in the historical control database at PND 11 is 2 of 244 females and 0 of 243 males. Since the incidence of these findings in the present study was greater than the historical control incidence, a focused follow up study was conducted at 2250 ppm BPA to determine reproducibility and, if appropriate, to further characterize these findings. Similar effects were noted on body weight in the dams and offspring; however, no clonic movements were observed on PND 11 in this robust follow-up study; therefore, the initial observations were not considered being treatment related" (Stump et al., 2010).

EFSA's evaluation of this finding states "the Panel considered the toxicological significance of this observation as being very limited because convulsions and seizures were not seen in any other occasion during the study, and could not be reproduced in a follow-up study with animals exposed to 0 or 2250 mg/kg feed according to the same study design... Also, convulsions or "popcorn seizures" or similar clonic movements have not been reported in any other study on BPA, including the available reproductive toxicity and developmental neurotoxicity studies, as summarized in EFSA previous evaluations, in the EU-RAR and its addendum or the NTP-CERHR Monograph."

BfR reviewed the incidental finding and determined that since "the findings from the main study had not been reproduced in the follow-up study, the behavioral abnormalities as observed in six animals on PND 11 in the study were not considered to be substance related" (BfR, 2010).

This study addressed the following NTP concerns: (1) it used an oral exposure route with "low" doses of BPA; (2) animals were dosed at the gestational time of reproductive organ development and the time during lactation when hormonal imprinting of behaviors occurs; (3) an adequate number of dams were used (12 per group); (4) an appropriate animal model was used, as there is evidence that SD rats are responsive to the effects of estrogen (EFSA, 2010); (5) statistical analysis used the litter as the experimental unit; (6) the authors used "low" doses of BPA that are relevant to estimated human exposures; and (7) the animals were tested for behavioral (functional) impacts using tests (auditory startle response and learning and memory) with positive controls.

Regarding their overall conclusions for Stump et al., 2010 study, EFSA states "The CEF Panel considers this treatment schedule as relevant to human exposure *in utero* and via either breastfeeding or infant bottle feeding (in this study the estimated exposure of rat pups to BPA is ca. 30 times higher than that of bottle-fed infants)." EFSA notes "The study by Stump covers motor activity, learning and memory (spatial behavior), auditory startle response, brain histopathology and morphology. The study does not cover some specific aspects of learning and memory (i.e. avoidance learning, schedule-controlled behavior, and impulsiveness), anxiety-related behavior or sexual dimorphic behavior, but this does not invalidate the study. No treatment-related changes were observed in motor activity tests, auditory startle response or brain histopathology and morphology."

BfR states "The study of Stump et al. (2010) meets the requirements of the neurodevelopmental toxicity study as requested by the Norwegian Food Safety Authority consequently to their re-evaluation of the four crucial non-regulatory studies that had been considered as valid and relevant for quantitative risk assessment by three Nordic EU countries during the EU risk assessment of BPA. The test followed a guideline-conform standard protocol using oral administration via diet (an application route of human relevance) and addressed relevant end-points for the detection of a specific toxic potential harmful to brain and/or neurobehavioural development. With this protocol (OECD 426, adopted in 2007), which is the counterpart of the US EPA guideline OPPTS 870.6300, used in the US and in Canada for the approval of pesticide active substances, more than 130 test reports and a comprehensive retrospective evaluation are available... Thus, this test protocol has already been applied routinely and broad experience has been gained with it."

BfR (2010) states "Except the effects observed on body weight gain at the two higher dietary concentrations no further effects had been observed in this test. The testing of standard endpoints able to detect effects on the brain and/or neurological and behavioral development, however, did not reveal any impairment at very low exposure levels and no impairments were seen at exposure levels that had been associated with effects on body weight. Thus the results of this study do not provide evidence for neurodevelopmental toxicity of BPA at the exposure levels that had been tested." Despite some critiques pointing to study limitations; e.g., low doses and issues with caging materials and phytoestrogens in the diet – (Ryan et al.); no reference estrogenic standard to measure test sensitivity - (Stump et al.), there is a general regulatory

consensus that these studies were well conducted and have regulatory merit (FSANZ, 2012; EFSA, 2012).

10. Discussion

BPA is widespread worldwide and has been used for over 50 years as a component of polycarbonate plastic epoxy resins that are, among other uses, used to coat metal food and beverage cans. Low levels of BPA can leach from the food containers into foods, and BPA can be found in trace levels in human urine (Lakind and Naiman, 2011). Both the RfD and the TDI of 50 μg/kg bw/day are approximately 33 times higher than the 95th percentile BPA intake level for adults of 1.5 μg/kg bw/day, estimated by WHO (2011), 225 times greater than the 95th percentile daily BPA intake of 0.223 μg/kg bw/day for individuals aged 6–60 years estimated by Lakind and Naiman (2011), and approximately 20 times the FDA estimation of the BPA intake in infants of 2.42 μg/kg bw/day FDA (2008).

While numerous animal studies have been conducted in the last few years, due to study designs and methodological problems, it is not clear how some of those studies apply to human risk assessment. In addition, there are pharmacokinetic differences between neonatal and adult laboratory animals and humans, which must be accounted for when evaluating potential health effects of BPA. For example, neonatal rats or mice do not metabolize BPA as efficiently as adult animals, resulting in higher blood levels of BPA in neonates compared to adults, and due to its rapid metabolism and excretion, BPA does not bio-accumulate (Doerge et al., 2010a; 2011a,b). In contrast to rats and mice, minimal pharmacokinetic differences were seen comparing fetal, neonatal and adult NHPs (Doerge et al., 2010a; 2011a,b). Also, in contrast to mice and rats, BPA-glucuronide is excreted predominantly via the urine rather than the feces in NHP and humans, does not undergo significant enterohepatic recirculation, and is rapidly eliminated from the body (Doerge et al., 2010a; 2011a,b). Furthermore, in rats and mice, BPA glucuronide is excreted predominantly via the bile into the feces, where there is evidence that it undergoes enterohepatic re-circulation. This results in re-generation of the BPA aglycone, which is reabsorbed into the blood stream, and prolongs the exposure to BPA. Thus, when administered the same dose, rats will have a longer exposure compared to NHPs.

In pregnant Sprague–Dawley rats administered BPA orally, maternal gut and liver metabolism rapidly converts BPA aglycone (active) to the inactive BPA-glucuronide via first pass phase II metabolism. This significantly reduces the amount of BPA aglycone that enters the circulation and decreases that amount that could reach the fetus (Doerge et al., 2011a), although the maternal rat also showed evidence of enterohepatic recirculation through reabsorption of BPA from bile, which would prolong the exposure to BPA (Doerge et al., 2011a). Of note, when rats are dosed intravenously, BPA aglycone concentrations in the blood stream are greater than with oral dosing since it does not go through first-pass metabolism.

The rat fetus also has the capacity to convert BPA aglycone to BPA glucuronide; this capacity increases as the fetus matures *in utero* achieving near maternal capacity at term (Doerge et al., 2011a). Pharmacokinetic and metabolism research conducted in pregnant NHP is expected to be published and this will provide additional information (Patterson et al., 2013).

Due to pharmacokinetic and metabolic differences, there is the potential for rats and mice, especially neonatal rats and mice, to have a higher exposure to BPA aglycone than NHP and humans; thus, studies in postnatal rats or mice may over-predict adverse outcomes in NHP and humans (Doerge et al., 2010a). Extrapolation of adverse findings from rats and/or mice to humans may not be appropriate or straightforward.

There are two recent studies assessing BPA's effect on the brain and behavior after oral administration; both of these studies appear to meet NTP-CERHR concerns regarding study design and methodology. Ryan et al. (2010) evaluated sexually dimorphic behavior, onset of puberty, fertility, and anatomy of female LE rats following *in utero* and lactational exposure and concluded that BPA (up to 200 μg/kg bw/day) had no effects. Stump et al. (2010) evaluated functional and morphological nervous system effects following exposure to BPA during gestation and lactation in SD rats, and found no evidence for BPA developmental neurotoxicity.

EFSA and BfR evaluated both Ryan et al. (2010) and Stump et al. (2010) studies and found they were both valid for use in risk assessments. EFSA concluded: "Overall, based on the comprehensive evaluation of recent human and animal toxicity data, the Panel concluded that no new study could be identified, which would call for a revision of the current TDI of 0.05 mg/kg bw/day. This TDI is based on the NOAEL of 5 mg/kg bw/day from a multi-generation reproductive toxicity study in rats, and the application of an uncertainty factor of 100, which is regarded as conservative based on all information on BPA toxicokinetics... The Panel considers that the valid studies do not raise concern regarding reproductive and developmental toxicity of BPA at doses lower than 5 mg/kg bw/day... The Panel does not consider the currently available data as convincing evidence of neurobehavioural toxicity of BPA" (EFSA, 2010). BfR concluded "[T]he results of the two studies do not substantiate the concerns for a specific toxic potential of bisphenol A adverse to neurological and behavioral development" (BfR, 2010).

11. Conclusions

This paper discusses some of the controversial issues surrounding BPA, summarizes the current regulatory status of BPA, reviews recent pharmacokinetic studies, and describes ongoing and planned research on the effects of BPA. In addition, we evaluate two papers studying BPA neurobehavioral effects, identified by EFSA and BfR as being valid for use in risk assessment, to determine whether they address the NTP-CERHR suggestions for study designs and methodologies.

Significant progress has been made in evaluating the pharmacokinetic similarities and differences in rats, mice and NHP. A recent human study has assessed the fate of BPA after consumption of BPA-containing meals, and a currently-ongoing human pharmacokinetic study using deuterated BPA will facilitate direct comparisons of BPA metabolism and pharmacokinetic parameters to that of mice, rats and NHP at doses consistent with known human BPA exposures. The results are anticipated to provide information regarding the potential for rats and mice, especially neonatal rats and mice, to have greater exposure to BPA aglycone compared to NHP and humans. Two studies referenced in this paper assessing BPA's neurobehavioral effects, which incorporated NTP-CERHR suggestions for study design and methodologies, did not find neurological or behavioral effects at low dose BPA exposures. At this time, EFSA, BfR, FDA, Health Canada, German Society of Toxicology, and the Japanese National Institute of Advanced Industrial Science and Technology (AIST) all support the use of BPA in food contact materials.

12. Conflict of Interest

William Allaben was previously employed by the US FDA for 33 years. The authors received funding for the review of the scientific literature and preparation of this manuscript from the North American Metal Packaging Alliance (NAMPA). The statements and conclusions in this work are solely those of the authors.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fct.2013.03.027.

References

ACC, 2011. Petition Seeking Amendment of Food Additive Regulation 21 C.F.R. § 177.1580 to remove the approval for polycarbonate resins in infant feeding bottles and certain spill-proof cups due to the abandonment of these uses. American Chemistry Council, Washington, DC. <http://plastics.americanchemistry.com/Product-Groups-and-Stats/PolycarbonateBPA-Global-Group/FDA-Petition-Letter-ACC.pdf.>

Audebert, M., Dolo, L., Perdu, E., Cravedi, J.P., Zalko, D., 2011. Use of the gammaH2AX assay for assessing the genotoxicity of bisphenol A and bisphenol F in human cell lines. Arch. Toxicol. 85, 1463–1473.

BfR, 2008. New studies on bisphenol A do not challenge earlier risk assessment. German Federal Institute for Risk Management, Bundesinstitut für Risikobewertung (BfR), Berlin.

BfR, 2010. Bisphenol A: studies by Stump et al. (2010) and Ryan et al. (2010) provide no indications for adverse effects on neurological development and behaviour. German Federal Institute for Risk Management, Bundesinstitut für Risikobewertung (BfR), Berlin.

Birnbaum, L.S., Bucher, J.R., Collman, G.W., Zeldin, D.C., Johnson, A.F., Schug, T.T., Heindel, J.J., 2012. Consortium-based science: The NIEHS's multipronged, collaborative approach to assessing the health effects of bisphenol A. Environ. Health Perspect. 120, 1640–1644.

Calafat, A., 2011. Background Paper on BPA Biomonitoring and Biomarker Studies. World Health Organization, Geneva.

Cao, X.L., Perez-Locas, C., Dufresne, G., Clement, G., Popovic, S., Beraldin, F., Dabeka, R.W., Feeley, M., 2011. Concentrations of bisphenol A in the composite food samples from the 2008 Canadian total diet study in Quebec City and dietary intake estimates. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 28, 791–798.

CDC, 2012. Fourth National Report on human exposure to environmental chemicals. Updated Tables. Centers for Disease Control, Atlanta, GA.

Chapin, R.E., Adams, J., Boekelheide, K., Gray Jr., L.E., Hayward, S.W., Lees, P.S., McIntyre, B.S., Portier, K.M., Schnorr, T.M., Selevan, S.G., Vandenbergh, J.G., Woskie, S.R., 2008. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res. B Dev. Reprod. Toxicol. 83, 157–395.

Doerge, D.R., Twaddle, N.C., Woodling, K.A., Fisher, J.W., 2010a. Pharmacokinetics of bisphenol A in neonatal and adult rhesus monkeys. Toxicol. Appl. Pharmacol. 248, 1–11.

Doerge, D.R., Twaddle, N.C., Vanlandingham, M., Fisher, J.W., 2010b. Pharmacokinetics of bisphenol A in neonatal and adult Sprague–Dawley rats. Toxicol. Appl. Pharmacol. 247, 158–165.

Doerge, D.R., Twaddle, N.C., Vanlandingham, M., Brown, R.P., Fisher, J.W., 2011a. Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague–Dawley rats. Toxicol. Appl. Pharmacol. 255, 261–270.

Doerge, D.R., Twaddle, N.C., Vanlandingham, M., Fisher, J.W., 2011b. Pharmacokinetics of bisphenol A in neonatal and adult CD-1 mice: inter-species comparisons with Sprague–Dawley rats and rhesus monkeys. Toxicol. Lett. 207, 298–305.

EFSA, 2006. 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) Question number EFSA-Q-2005-100. EFSA J. 428, 1–75.

EFSA, 2010. Scientific opinion on bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of bisphenol A. EFSA J. 8, 1829.

EFSA, 2012. FAQ on chemicals in food. European Food Safety Authority, Parma, Italy. <http://www.efsa.europa.eu/en/faqs/faqchemicalsinfood.htm#5.>

European Commission, 2011. Commission Directive 2008/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards to the restriction of the use of Bisphenol A in plastic infant feeding bottles. Official Journal of the European Union 26, 11-14.

FDA, 2008. Draft assessment of bisphenol A for use in food contact applications. US Food and Drug Administration, Silver Spring, MD.

FDA, 2010. Advancing regulatory science for public health. US Food and Drug Administration, Silver Spring, MD.

FDA, 2012a. Bisphenol A (BPA): Use in food contact application. US Food and Drug Administration, Silver Spring, MD. <http://www.fda.gov/NewsEvents/PublicHealthFocus/ucm064437.htm.>

FDA, 2012b. Indirect food additives: polymers final rule. Federal Register 77, 41899–41902.

Fisher, J.W., Twaddle, N.C., Vanlandingham, M., Doerge, D.R., 2011. Pharmacokinetic modeling: prediction and evaluation of route dependent dosimetry of bisphenol A in monkeys with extrapolation to humans. Toxicol. Appl. Pharmacol. 257, 122–136.

FSANZ, 2012. Regulation and monitoring of BPA. Food Standards Agency of Australia and New Zealand, Canberra, Australia. <http://www.foodstandards.gov.au/consumerinformation/bisphenolabpa/regulationandmonitor5377.cfm.>

Gotoh, Y., Kato, Y., Stieger, B., Meier, P.J., Sugiyama, Y., 2002. Gender difference in the Oatp1-mediated tubular reabsorption of estradiol 17beta-D-glucuronide in rats. Am. J. Physiol. Endocrinol. Metab. 282, E1245–E1254.

Health Canada, 2012. Updated Assessment of Bisphenol A (BPA) Exposure from Food Sources Health Canada, Bureau of Chemical Safety, Ottawa, Ontario, Canada. <http://www.hc-sc.gc.ca/fn-an/alt_formats/pdf/securit/packag-emball/bpa/bpa_hra-ers-2012-09-eng.pdf>.

Hengstler, J.G., Foth, H., Gebel, T., Kramer, P.J., Lilienblum, W., Schweinfurth, H., Völkel, W., Wollin, K.M., Gundert-Remy, U., 2011. Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A. Crit. Rev. Toxicol. 41, 263–291.

Howdeshell, K.L., Furr, J., Lambright, C.R., Wilson, V.S., Ryan, B.C., Gray Jr., L.E., 2008. 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. Toxicol. Sci. 102, 371–382.

Japan AIST, 2011. Updated hazard assessment of bisphenol A. National Institute of Advanced Industrial Science and Technology, Tokyo, Japan.

Jemnitz, K., Heredi-Szabo, K., Janossy, J., Ioja, E., Vereczkey, L., Krajcsi, P., 2010. ABCC2/Abcc2: a multispecific transporter with dominant excretory functions. Drug Metab. Rev. 42, 402–436.

Klimisch, H.J., Andreae, M., Tillmann, U., 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul. Toxicol. Pharmacol. 25, 1–5.

Lakind, J.S., Naiman, D.Q., 2011. Daily intake of bisphenol A and potential sources of exposure: 2005–2006 national health and nutrition examination survey. J. Expo. Sci. Environ. Epidemiol. 21, 272–279.

Lorentzen, R., Hattan, D., 2010. Online comment on "The weight of evidence". Nature 464, 1103–1104, <http://www.nature.com/nature/journal/v464/n7292/full/4641103b.html#comment-12729>.

Markham, D.A., Waechter Jr., J.M., Wimber, M., Rao, N., Connolly, P., Chuang, J.C., Hentges, S., Shiotsuka, R.N., Dimond, S., Chappelle, A.H., 2010. Development of a method for the determination of bisphenol A at trace concentrations in human blood and urine and elucidation of factors influencing method accuracy and sensitivity. J. Anal. Toxicol. 34, 293–303.

Matthews, J.B., Twomey, K., Zacharewski, T.R., 2001. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. Chem. Res. Toxicol. 14, 149–157.

Mazur, C.S., Kenneke, J.F., Hess-Wilson, J.K., Lipscomb, J.C., 2010. Differences between human and rat intestinal and hepatic bisphenol A glucuronidation and the influence of alamethicin on in vitro kinetic measurements. Drug Metab. Dispos. 38, 2232–2238.

McCarty, L.S., Borgert, C.J., Mihaich, E.M., 2012. Information quality in regulatory decision making: peer review versus good laboratory practice. Environ. Health Perspect. 120, 927–934.

MHRA, 2012. Good Laboratory Practice: Background and Structure. Medicines and Healthcare Products Regulatory Agency, London, UK. <http://www.mhra.gov.uk/Howweregulate/Medicines/Inspectionandstandards/GoodLaboratoryPractice/Structure/index.htm>.

NTP, 2008. Monograph on the potential human reproductive and developmental effects of bisphenol A. National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction, Research Triangle Park, NC.

NTP-CERHR, 2007. Expert panel report on the reproductive and developmental toxicity of bisphenol A. National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction, Research Triangle Park NC.

Patterson, T.A., Twaddle, N.C., Roegge, C.S., Callicott, R.J., Fisher, J.W., Doerge, D.R., 2013. Concurrent determination of bisphenol A pharmacokinetics in maternal and fetal rhesus monkeys. Toxicol. Appl. Pharmacol. 267, 41–48.

Prins, G.S., Ye, S.H., Birch, L., Ho, S.M., Kannan, K., 2011. Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague–Dawley rats. Reprod. Toxicol. 31, 1–9.

Ryan, B.C., Hotchkiss, A.K., Crofton, K.M., Gray Jr., L.E., 2010. In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. Toxicol. Sci. 114, 133–148.

Stump, D.G., Beck, M.J., Radovsky, A., Garman, R.H., Freshwater, L.L., Sheets, L.P., Marty, M.S., Waechter Jr., J.M., Dimond, S.S., van Miller, J.P., Shiotsuka, R.N., Beyer, D., Chappelle, A.H., Hentges, S.G., 2010. Developmental neurotoxicity study of dietary bisphenol A in Sprague–Dawley rats. Toxicol. Sci. 115, 167–182.

Taylor, J.A., Vom Saal, F.S., Welshons, W.V., Drury, B., Rottinghaus, G., Hunt, P.A., Toutain, P.L., Laffont, C.M., VandeVoort, C.A., 2011. Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. Environ. Health Perspect. 119, 422–430.

Teeguarden, J.G., Calafat, A.M., Ye, X., Doerge, D.R., Churchwell, M.I., Gunawan, R., Graham, M.K., 2011. Twenty-four hour human urine and serum profiles of bisphenol a during high-dietary exposure. Toxicol. Sci. 123, 48–57.

Twaddle, N.C., Churchwell, M.I., Vanlandingham, M., Doerge, D.R., 2010. Quantification of deuterated bisphenol A in serum, tissues, and excreta from adult Sprague–Dawley rats using liquid chromatography with tandem mass spectrometry. Rapid Commun. Mass Spectrom. 24, 3011–3020.

USEPA, 2005. Guidelines for carcinogen risk assessment. US Environmental Protection Agency, Washington, DC.

USEPA, 2012a. IRIS (Integrated Risk Information System). US Environmental Protection Agency, Washington, DC. <http://www.epa.gov/iris/index.html>.

USEPA, 2012b. Glossary of IRIS Terms. U.S. Environmental Protection Agency, Washington, DC. <http://www.epa.gov/iris/help_gloss.htm.>

Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118, 1055–1070.

Völkel, W., Colnot, T., Csanady, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chem. Res. Toxicol. 15, 1281–1287.

WHO, 2011. Toxicological and health aspects of bisphenol A: report of joint FAO/WHO expert meeting and report of stakeholder meeting on bisphenol A, November 1–5, 2010, Ottawa, Canada. World Health Organization and Food and Agriculture Organization of the United Nations, Geneva, Switzerland.

Ye, X., Zhou, X., Hennings, R., Kramer, J., Calafat, A.M., in press. Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomonitoring analysis: an elusive laboratory challenge. Environ. Health Perspect. doi: 10.1289/ehp.1206093.