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A reproductive, developmental and neurobehavioral study following oral exposure of tetrabromobisphenol A on Sprague-Dawley rats

Rhian B. Cope [a], Sam Kacew [b], Michael Dourson [a,*]

[a] Toxicology Excellence in Risk Assessment, Cincinnati, OH, USA
[b] McLaughlin Centre for Risk assessment, University of Ottawa, Ottawa, Canada

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ABSTRACT

The objectives of these GLP US EPA OPPTS 970.3800 and 970.3700 studies were to examine the effects of tetrabromobisphenol A (TBBPA) at oral doses of 10, 100 or 1000 mg/kg BW/day over the course of 2 generations on growth as well as behavioral, neurological and neuropathologic functions in offspring. In a separate study the influence of oral TBBPA (0, 100, 300 or 1000 mg/kg BW/d) was examined on embryonic/fetal development from gestation days (GDs) 0–19. In the reproductive study, exposure to ≥100-mg/kg BW/d TBBPA resulted in a decrease in circulating, peripheral thyroxine (T_4) levels in rats that were not accompanied by any marked alterations in triiodothyronine (T_3) and thyroid stimulating hormone (TSH). These findings are explainable on the basis of induction of rat liver catabolism, a phenomenon that may be species-specific and not relevant for humans. TBBPA at up to 1000 mg/kg BW/d was not associated with any significant non-neurological effects on reproduction, growth and development. A subtle reduction, of unknown biological relevance, in the thickness of the parietal cortices of 11-day-old F2 pups in the 1000 mg/kg BW/d group was noted. This change was not accompanied by evidence of micro-anatomic changes. No estrogenic effects sufficient to affect macro and micro anatomy, fertility, reproduction, development, survival or behavior were detected in the embryofetal development study or in the multigenerational study. No other TBBPA-related effects on developmental neurotoxicity/neuropathology were detected. In the developmental study no TBBPA related change in mortality rate was observed in any of the dams. No other significant test article-related effects were noted. The no observed effect level (NOEL) for maternal and developmental toxicity was 1000 mg/kg BW/d, the highest dose evaluated.

1. Introduction

Because of their effectiveness and in order to meet fire safety standards, organobromide flame retardants, such as 2,2′,6,6′-tetrabromo-4,4′-isopropylidenediphenol (tetrabromobisphenol A, TBBPA), have been incorporated into a variety of consumer products including electrical equipment, and furniture (ARCADIS, 2011). TBBPA is an efficacious fire retardant, which partially accounts for its annual global production of more than 170 kt in 2004 (ECB, 2006), used primarily in epoxy resin printed circuit boards.

Despite its effectiveness as a fire retardant, concerns have been raised that TBBPA can leach into the environment, producing adverse effects on ecosystems and human health (Decherf and Demeneix 2011). In vitro studies reported binding of TBBPA to estrogen hormone receptors at high concentrations (Gosavi et al., 2013) or effects of TBBPA on hormone-sensitive parameters (Decherf and Demeneix, 2011). These concerns are tempered by the fact that 70–80% of TBBPA produced is not used as an additive flame retardant, but as a reactive flame retardant, which is covalently incorporated into electronic circuit board polymers, thus reducing its human and environmental bioavailability (ECB, 2006). Only 20–30% of the TBBPA produced is used as an additive flame retardant in applications and thus subject to environmental leaching (ECB, 2006). Although in vivo animal toxicity database on the effects of TBBPA is not indicative that this flame retardant mediates any substantial influence on systemic effects, including endocrine function, at practicable experimental dosages (NTP, 2014; Colnot et al., 2014; Osimitz et al., 2014), the potential effects of TBBPA on developmental and reproductive functions have not been examined in a comprehensive fashion.

In order to delineate whether TBBPA in vivo exerts any effects on reproductive and developmental systems, and to further

* Correspondence to: Toxicology Excellence in Risk Assessment, 2300 Montana Ave., Suite 409, Cincinnati, OH 45211, USA. Tel.: +1 513 542 7475x14.
E-mail address: dourson@tera.org (M. Dourson).

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determine whether the endocrine system including thyroid function and the nervous system are impacted, this investigation was undertaken to determine the consequences of oral administration of TBBPA onthese parameters in CD rats.

2. Materials and methods

2.1. Good laboratory practices

All studies were conducted in accordance with good laboratory practices (GLP) (United States 40 CFR parts 160 and 179, Organization for Economic Co-operation and Development C[81] 30 [final] Annex 2 and United States Title 21 CFR Part 11 computer compliance).

2.2. Study method

The studies were conducted in accordance with United States (US) Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) test methods 970.3700 (developmental toxicology study) and 970.3800 (reproductive toxicology study), and Organization for Economic Co-operation and Development (OECD) guidelines No. 414 (developmental study) and 416 (reproductive study).

2.3. Test article and vehicle control

The test articles (TBBPA) used in the GLP studies were mixtures of equal proportions of three different commercial TBBPA products produced by Albemarle Corporation, Great Lakes Chemical Corporation and the Dead Sea Bromine Group. Before the initiation and at completion of dosing, samples of test article were collected for analysis for identity, composition, purity, homogeneity, and stability.

In the case of examination for chemical identity,samples were taken from the middle center of the test article bulk container and examined by Fourier transform infrared spectroscopy (FTIRS) in comparison with a standard reference spectrum of TBBPA. The TBBPA reference spectrum was derived from a sample of Albemarle Corporation's CP 2000 brand of TBBPA, lot number 25948M-1.

Samples for homogeneity and purity testing were collected from the top, middle and bottom of the test article bulk container. Purity (% of TBBPA) was determined using high performance liquid chromatography (HPLC). Test article homogeneity was established by demonstrating that all 3 samples possessed the same purity (<5% difference of the TBBPA % purity for each sample compared with the average TBBPA area % of the three samples).

The samples were further characterized by measuring the concentration (area %) of tribromophenol, tribromobisphenol-A and o,p′-tetrabromobisphenol-A. Stability was confirmed by demonstrating that the composition of the test article before and after the study did not differ by more than 5%.

The control substance, corn oil, was the vehicle used to prepare test formulations. Corn oil was purchased from Spectrum Chemical Manufacturing Corporation (Gardena, California, USA), Spectrum Laboratories (New Brunswick, New Jersey) and BioServe (Frenchtown, New Jersey).

2.4. Animal care and maintenance

Male and female CD® [Crl: CD® (Sprague-Dawley) IGS BR] 6–8 week-old rats were obtained from Charles River Laboratories (Portage, MI) and allowed to acclimatize for at least 2 weeks prior to assignment to the studies. During acclimation, animals were clinically examined. Animals with any evidence of disease or physical abnormality were not assigned to the studies.

2.5. Experimental designs

In the multigenerational study, individual parental (P) generation rats were identified using an implanted microchip. Offspring F_1 and F_2 pups were identified by paw tattoo on Day 0 of lactation. Metal ear tag identification was used in the developmental toxicology study. The individual animal number plus the study number comprised a unique identification for each individual animal. In the reproductive study female rats were 10 weeks and male rats were 12 weeks of age at the start of mating in the developmental toxicology study. Rats assigned to the multigenerational study were 18 weeks old at the start of mating. Rats were assigned to the various study groups using a standard, by weight, block randomization procedure.

In these studies, all rats were maintained in an environmentally controlled room. Temperature and relative humidity were maintained between 14.4 and 26.1 °C and 30–70% respectively. Twelve hour fluorescent light was provided per day. Water and a rodent diet (Certified Lab Diet® meal rodent Chow #5002, PMU Nutrition International, Inc. St. Louis, Missouri) were freely available to all animals. The rats were individually housed in suspended, stainless steel, wire-mesh cages except during parturition and lactation in the multigenerational study. Females in the multigenerational study were individually housed in solid plastic cages containing wood chip bedding from Day 18 of gestation (GSD18) through GSD21 of the lactation/postnatal period.

Parental males and females were treated with TBBPA prior to and during mating, and females through gestation, followed by treatment of pups in accordance with OECD guidelines. The developmental study was conducted at a separate time, and included exposure during the period of organogenesis.

2.6. Test formulations and dosing

The test and control formulations were administered in corn oil by oral gavage once per day, 7 days per week throughout the studies. Based upon data available from preliminary repeat-dose sub-chronic and chronic studies, dose levels selected for these studies were 0, 10, 100 or 1000 mg/kg body weight (BW)/day for the multigenerational study and 0, 100, 300 or 1000 mg/kg BW/day for the developmental investigation. The highest dose selected did not exceed the maximum tolerated repeated dose exposure for TBBPA for the current study durations. Fresh batches of test formulations were prepared weekly and analytically confirmed by KAR Laboratories Inc. (Kalamazoo, Michigan) according to GLP.

2.7. In-life examinations

2.7.1. Mortality and cage-side observations performed in all studies

All animals were observed at least twice per day, seven days per week for evidence of morbidity, mortality, injury, availability of food, availability of water and any overt evidence of toxicity. Animals showing signs of severe debility or toxicity were euthanized. All animals euthanized or found dead underwent full post mortem evaluation as described below.

2.7.2. Detailed clinical/physical examinations performed in all studies

Mated females in the developmental toxicity study were given a detailed clinical/physical examination on a daily basis from gestation day (GD) 0–20. Parental animals in the multigenerational study (P and F_1 generation) also had a weekly clinical/physical examination for the duration of the study. The clinical/physical investigation included, but was not limited to, examination of skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs, feet, respiration and palpation of soft tissues.

2.7.3. Neurological examination of the F_1 parental animals in the multigenerational study

Ten randomly selected females per dose group were administered a detailed neurological examination on GD 9 and 18 and lactational days (LDs) 4 and 14. All neurological examinations were performed blinded to treatment and used standardized procedures to minimize inter-observer variability and error. The neurological examinations included but were not limited to: assessment of markers of parasympathetic function (lacrimation, salivation, frequency of urination, frequency of defecation), the presence or absence of piloerection, pupillary light reflex, palpebral reflex, evidence of ptosis, the presence or absence of convulsions, the presence or absence of convulsions, abnormal movement, gait or posture, muscle tone, unusual/abnormal behaviors, the presence or absence of stereotypes, abnormal secretions, and forelimb and hind limb grip strength (Meyer et al., 1979).

2.7.4. Body weight

Individual body weights were measured on GD 0 and every 3 days during the gestational phase of the developmental toxicology study. Individual body weights were measured once pre-test and then at least weekly during over the course of the multigenerational study. In the multigenerational study, pregnant females were weighed on GDs 0, 7, 14 and 20 and LD 0, 4, 7, 14 and 21.

2.7.5. Food consumption

In the developmental toxicology study, food consumption was measured over GDs 0–3, 3–6, 6–9, 9–12, 12–15, 15–18, 18–20 and 0–20. Food consumption was recorded weekly in the multigenerational study except for the period of cohabitation/mating. Food consumption by mated/pregnant females was measured on GDs 0, 4, 7, 14 and 20 and LDs 0, 4, 7, 14 and 21.

2.7.6. Estrous cyclicity in the multigenerational study

Daily vaginal smears were performed three weeks prior to mating and until the mating was confirmed or the mating period ended in P and F_1 parental females.

2.7.7. P-generation breeding procedures in the multigenerational study

P generation animals were individually housed until mating. After a minimum of 10 weeks of dosing, males and females within each dosing group were randomly cohabitated with one male per female for 14 days. Mating occurred in the cage of the male. Vaginal smears were performed daily during the mating period and the presence of sperm or a vaginal plug was recorded. The day on which evidence of mating was first observed was designated as GD 0 and the female was removed from the cage.

2.7.8. P-generation parturition and F_1 litter observations in the multigenerational study

Parental animals were regularly observed for signs of parturition and allowed to give birth to the F_1 generation. The duration of gestation and any evidence of dystocia wererecorded and the day on which the F_1 pups were delivered was designated lactational day (LD) 0. Pups were clinically examined in the periparturient period and the following parameters were recorded: litter size, number of stillborn pups, number of live born pups, gross abnormalities, pup body weight and sex. On LD 4, litter size was reduced by random selection of 8 pups per litter of approximately equal sex distribution. Cull pups were submitted to post-mortem study evaluations. The remaining pups were housed with dams until weaning at LD 21. Dams and pups were observed for survival, nesting and nursing. Each pup was weighed and examined for abnormality on LDs 0, 4, 7, and 21. Any intact dead pups were subjected to post-mortem evaluations.

2.7.9. Selection of the F_1 generation and termination of the P generation in the multigenerational study

At LD21, at least one male and one female from each litter in each dose group was randomly selected to become F_1 parents (a total of 30 males and 30 females per dose group). All pups were available for selection and sibling matting amongst the F_1 parents were avoided. All non-selected F_1 animals and surviving P generation females were subjected to post mortem evaluations.

2.7.10. F_1 generation pre-mating observations in the multigenerational study

F_1 parents were individually caged and dosed for 10 weeks prior to mating. Indices of sexual maturation (vaginal opening, preputial separation) were assessed during this period.

2.7.11. F_1 generation mating, gestation and lactation in the multigenerational study

Following the 10-week pre-mating treatment period the F_1 parents were mated and observed as described for the P generation. Dosing continued through the mating period until termination. All F_1 females continued on treatment until F_2 pups were weaned and until an assessment was performed to determine if a second mating was required.

2.7.12. F_2 generation animal selections in the multigenerational study

Prior to weaning and after standardization of the F_2 litter size to 10 pups (5 per sex) on LD4, pups were selected for clinical examination, motor activity and neuropathology studies. Ten males and females (at least one male and/or one female per litter) per dose group were randomly selected and given detailed clinical examinations on LD 4, 11, and 21 and at 35, 45, 49 and 60 days of age and at termination of the study. Similarly, 10 males and females (at least one male and/or one female per litter) per dose group were randomly selected and evaluated for motor activity on LDs 13, 17 and 21 and at 60 ± 2 days of age. Milestones of sexual development (preputial separation or vaginal opening) were also evaluated in these animals. Similarly, 10 males and females (at least one male and/or one female per litter) per dose group were randomly selected on LD 11 for neuropathological examination.

After weaning (LD 21) F_2 pups were selected on day 22 and day 60 of for auditory startle habituation (10 males, 10 females per dose group), and learning and memory (10 males, 10 females per dose group) evaluation. Milestones of sexual development (preputial separation or vaginal opening) were also evaluated in all of these animals. On day 60 of life, F_2 pups (10 males, 10 females per dose group) were selected for detailed neuropathological examination. Any non-assigned F_2 pups were euthanized and subjected to anatomical pathology examination.

2.7.13. Thyroid hormone analyses in the multigenerational study

At the termination of the P and F_1 generations blood was collected from 10 male and 10 female per dose group (randomly selected). Serum analyses for thyroid stimulating hormone (TSH), 3,5,3′-triiodothyronine (T_3) and 3,5,3′5′-tetraiodothyronine (T_4) were performed. All thyroid hormone analyses were performed by Laboratory Corporation of America (Burlington, NC USA) according to GLP. Serum TSH and T_4 measurements were performed using electro-chemiluminescence immunoassay (ECLIA). Serum T_3 measurements were performed using cloned enzyme donor immunoassay (CEDIA).

2.7.14. F_2 generation neurobehavioral evaluations in the multigenerational study

Selected F_2 pups underwent detailed neurobehavioral (Functional Observation Battery; FOB) examination according to US EPA guidelines.

2.7.15. Postmortem examinations in the multigenerational study

Complete necropsies were performed on: P, F_1 and F_2 animals that died spontaneously or were euthanized *in extremis*, P and F_1 generation females that displayed evidence of mating but failed to deliver, P and F_1 generation females that demonstrated no evidence of mating and failed to deliver, P and F_1 that survived to scheduled termination, F_1 and F_2 generation pups culled on LD 4, F_1 pups not selected to continue on study, all selected F_2 pups at weaning, all F_2 animals used in developmental and behavioral evaluations, all F_2 animals that survived until study termination or that died.

The following tissues were always collected during necropsies for subsequent histological examination: adrenal glands, brain, ovaries or testes, epididymis, grossly discernable lesions, kidneys, liver, pituitary, prostate glands, seminal vesicles with coagulating glands, spleen, thymus and uteri (both horns) and cervix, and vagina. Representative samples of any grossly discernable lesions and any tissue masses were also collected.

Specific reproduction parameters examined in the parental (P and F_1 generations) included: number of implantation sites (when present) for all cohabitated females, stage of estrus, right and left epididymal weights, right cauda epididymis weight, right testicular weight and left testicular weight. The left testis and epididymis were processed for microscopic evaluation using hematoxylin and periodic acid Schiff staining. The right testis and epididymis were used for sperm analysis.

The following analyses of the spermatogenic tissues of the parental (P and F_1 generations) were performed: daily sperm production in the right testis as per the method of (Blazak et al., 1993), sperm motility in the right vas deferens using the Hamilton–Thorne Computer Assisted Semen Analysis System (percent motile and progressively motile sperm), right epididymal sperm count (sperm concentration per unit mass using hemocytometer counts) and morphology.

Specialized neuropathological examinations were performed on selected F_2 generation animals. Samples of all major brain regions (olfactory bulbs, cerebral cortices, hippocampus, basal ganglia, thalamus, hypothalamus, tectum, tegmentum, cerebral peduncles, brainstem and cerebellum were collected from the brains from F_2 generation animals that were selected for neuropathology examination on day 11 of life. Brain tissues were plastic embedded and process for microscopic examination using hematoxylin and eosin (H&E) staining. Neuropathological examinations included qualitative, semiquantitative as well as simple morphometric analyses.

Brain morphometric analyses of plastic embedded H&E stained neurological tissues were performed using digital imaging captured using a Nikon microscope and SPOT RT Color image-capturing device (Diagnostic Instruments, Sterling Heights, MI) and analyzed using SPOT version 3.4.4 for Microsoft Windows (Diagnostic Instruments, Sterling Heights, MI). The thickness of various neurological layers (in mm or μm) was determined linearly by measuring the distance between 2 points on histologic sections of the brain. Parietal cortex and hippocampal morphometric analyses were performed using one captured image at the level of the infundibulum from each tissue block at 2× magnification. The parietal cortex thickness (in mm) was measured by drawing a line from the meningeal surface to the layer of neurons that borders the external capsule. The thickness of the hippocampus (in mm) was measured by drawing a line from the CA1 region to the ventral line of neurons of the dentate gyrus. Cerebellar morphometric analyses were performed using one captured image from one cerebellar lobe from each tissue block at 40× magnification. The external granular layer, the molecular layer and the combined Purkinje cell and internal granular cell layer were measured (in μm). Morphometric analyses of the thalamus were performed using one captured image at the level of the pontine nuclei at 2× magnification. The width of the thalamus was measured by drawing a line from the lateral edge of the thalamus to the center of the mesencephalic aqueducts (i.e. half the overall width since the entire thalamus did not fit in a single photographic frame).

Neurological tissues collected from selected F_2 generation animals at 60 days of age included the brain (10 animals per sex per dose for 10% neutral buffered formalin immersion fixation, and 10 animals per sex per dose for in situ perfusion with 3% paraformaldehyde and 3% glutaraldehyde in 0.1 M phosphate buffer), both sciatic nerves with tibial, fibular and sural extensions to the level of the hock, cervical spinal cord and lumbar spinal cord/proximal sciatic nerves. Neurological tissues were subject to qualitative microscopic examination (paraffin embedded, H&E stained) and subject to morphometric measurement (plastic embedded, H&E stained). Particular attention was paid to regions of the nervous system known to be sensitive to neurotoxic insult or to those regions likely to be affected, based on the results of the functional testing. Representative sections of ganglia, dorsal and ventral root fibers, tibial nerves, sural nerves and fibular nerves were also evaluated (plastic embedded, H&E stained).

2.7.16. Postmortem evaluations in the developmental toxicity study

All evaluations were performed blinded to treatment. Ovarian and uterine examinations were performed following post-euthanasia laparohysterectomy. Gravid uterine weight was recorded and, beginning at the distal end of the left uterine horn, the location of viable and nonviable fetuses and early and late resorptions along each uterine horn and the total number of implantations were recorded. Following opening of the uterine horns, placentae were examined grossly in situ and fetuses collected. The numbers of ovarian corpora lutea were counted. Uteri from females that appeared non-gravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites (Kopf et al., 1964).

A necropsy was conducted on each dam. Special emphasis was placed on structural abnormalities or pathologic changes that may have influenced pregnancy. The livers of all maternal animals were weighed.

Approximately half of the fetuses per litter were fixed in Bouin's solution for subsequent soft tissue examination using the Wilson razor–blade sectioning technique (Wilson, 1965). The remaining fetuses in each litter were fixed in ethanol and processed for staining of mineralized and cartilaginous tissues using Alizarin Red S and Alcian Blue and glycerin clearance (Kimmel and Trammell 1981). Fetal findings were classified as malformations or developmental variations. In the distribution of fetuses for visceral and skeletal examinations, every attempt was made to equalize the ratio of male and female fetuses per litter processed for each evaluation.

2.8. Statistical analyses

Group pair-wise comparisons were used for the following endpoints in the multigenerational study: gestation length, copulatory interval, estrous cycle length, number of estrous cycles, absolute and relative organ weights (parental and pups; for pups, the litter was the experimental unit), sperm concentration, daily sperm production, primordial follicle count, TSH levels, T_3 levels, T_4 levels, total uterine implantations (scars), litter size (F_1 and F_2), live pups (F_1 and F_2), age and body weight at preputial separation (F_1), age and body weight at vaginal opening (F_1), motor activity, forelimb and hindlimb grip strength (F_2), emotionality (defecation, grooming, rearing, backing; F_2), learning and memory, F_2neuropathological observations, and F_2 brain morphometry.

Table 1
Effect of TBBPA on various reproductive parameters parental generation.

Parameter	TBBPA dose mg/kg BW			
	Control Parental generation	10	100	1000
Estrus cycle length (mean ± SD)	4.8 ± 0.69	4.6 ± 0.63	4.6 ± 0.69	4.4 ± 0.68
Female mating index	96.7	93.3	93.3	100.0
Female fertility index	80.0	86.7	83.3	96.7
Male mating index	96.7	93.3	93.3	100.0
Male fertility index	80.0	86.7	83.3	96.4
Sperm percent motility (mean ± SD)	97.4 ± 2.01	98.4 ± 1.25	97.2 ± 2.29	97.4 ± 2.21
Sperm percent progressive motility (mean ± SD)	77.4 ± 6.21	79.1 ± 6.70	73.9 ± 6.10	71.3 ± 8.68
Total sperm concentration/caudaepididymus × 10^8 (mean ± SD)	3.262 ± 0.5391	3.467 ± 0.4970	3.195 ± 0.5959	3.256 ± 0.5856
Percent abnormal sperm (mean ± SD)	0.70 ± 0.934	0.45 ± 0.562	1.07 ± 1.187	1.20 ± 1.595

Group pair-wise comparisons were also used for the following endpoints in the prenatal developmental toxicity study: gestation body weights, gestation food consumption, absolute and relative liver weight, gravid uterine weights, corpora lutea counts, total implantation counts, viable fetus counts, and early resorption counts

Group pair-wise comparisons involved examination for homogeneity of group variances using Levene's test (Milliken and Johnson 1992). If homoscedasticity was established by Levene's test ($p \leq 0.01$), Dunnett's test was used to compare each treatment group with the control group (Dunnett 1955). If Levene's test was significant ($p < 0.01$), comparisons with the control group were made using Welch's *t*-test with a Bonferroni correction (Welch 1937). Two tailed tests were used when relevant.

Arcsin-square-root transformation followed by group pair-wise comparisons was used for the following endpoints in the multigenerational study: % abnormal sperm, % motile sperm, pup sex ratios, live birth index stillborn index, and pup survival indices. This analysis was also used for the following endpoints in the prenatal developmental toxicity study: fetal sex ratio, % viable fetuses/implants, % early resorptions/implants, preimplantation loss, and postimplantation loss. Data comprised % values were transformed using the arcsin of the square root (Steel and Torrie 1980). Data were then subjected to the group pair-wise comparison techniques described above.

Fisher's exact test was used for the following endpoints in the multigenerational study: mating indices, fertility indices, fecundity indices, gestation index, and emotionality (urination; F_2). This statistical method was also used for the following endpoints in the prenatal development study: malformation by type and total (litter was the experimental unit), developmental variations by type and total (litter was the experimental unit), and pregnancy index. For binomial endpoints (excluding sex ratios), each treatment group was compared with the control using a Fisher's exact test with a Bonferroni correction. Two-tailed tests were used when relevant.

For fetal body weights, litter size was included as a covariate in the model used to conduct the Dunnett's test. Each treatment group was compared with the control group. Two-tailed tests were used as appropriate.

Auditory startle habituation data from the multigenerational study were analyzed using repeated measures (mixed model) analysis of variance using time as a regression variable (Littell et al., 1996). If there was no significant group-period interaction ($p > 0.05$), Dunnett's test was used to compare the control group mean averaged over all time periods to each treatment group mean averaged over all time points. If the interaction was significant ($p < 0.05$), each time period was analyzed separately using the group pairwise comparisons methods described above.

Water M-maze endpoints (number of errors and time to completion) from the multigenerational study were analyzed using rank transformation followed by analysis of variance analysis (ANOVA) (split–split plot design). Historical data on water M-maze data indicated that these endpoints have unpredictable distribution characteristics, thus analysis would be enhanced by use of a non-parametric test. Rank transformations were performed for both of these endpoints (number of errors and time to completion) for all collection intervals. During ANOVA, data for each sex were analyzed separately and a split-split plot design was assumed (Hicks 1982). The model tested for the effects of treatment, days, trials and all associated interactions. If there were no significant treatment effects ($p > 0.05$) or no significant treatment interaction effects ($p > 0.10$), no further analysis was conducted. If there was a significant treatment effect or interaction effect involving treatment, the follow-up analyses were performed using the group pair-wise comparison methods described above.

Table 2
Influence of TBBPA on various reproductive parameters F1 generation.

Parameter	TBBPA dose mg/kg BW			
	Control F1 Generation	10	100	1000
Female mating index	100	92.9	86.2	89.3
Female fertility index	86.7	92.9	69.0	75.0
Male mating index	100.0	92.9	86.2	89.3
Male fertility index	86.2	92.9	69.0	75.0
Sperm percent motility (mean ± SD)	96.5 ± 3.29	88.1 ± 20.87*	96.3 ± 2.85	95.7 ± 4.92
Sperm percent progressive motility (mean ± SD)	78.8 ± 5.63	73.5 ± 18.73	78.9 ± 6.30	75.9 ± 9.54
Total sperm concentration/cauda epididymus × 10^8 (mean ± SD)	3.365 ± 0.5380	3.350 ± 0.7549	3.308 ± 0.6111	2.941 ± 0.6338*
Percent abnormal sperm (mean ± SD)	0.19 ± 0.489	0.39 ± 0.550	1.31 ± 1.064	2.24 ± 1.935

* Significantly different from control $p < 0.05$.

3. Results

3.1. Parental generation evaluations in the multigenerational study

In the multigenerational study there were no significant effects on mortality, body and tissue weights, clinical observations and tissue histopathology. As shown in Table 1 (parental) and Table 2 (F1 generation) there were no marked effects on various reproductive parameters including time to vaginal opening and anogenital distance. The parental no observed adverse effect level (NOAEL) was ≥1000 mg/kg BW/day.

Mean serum T_3 levels were significantly reduced in the high dose (1000 mg/kg BW/day) males (Fig. 1) at the termination of the P generation. No significant effect was noted in the serum T_3:T_4

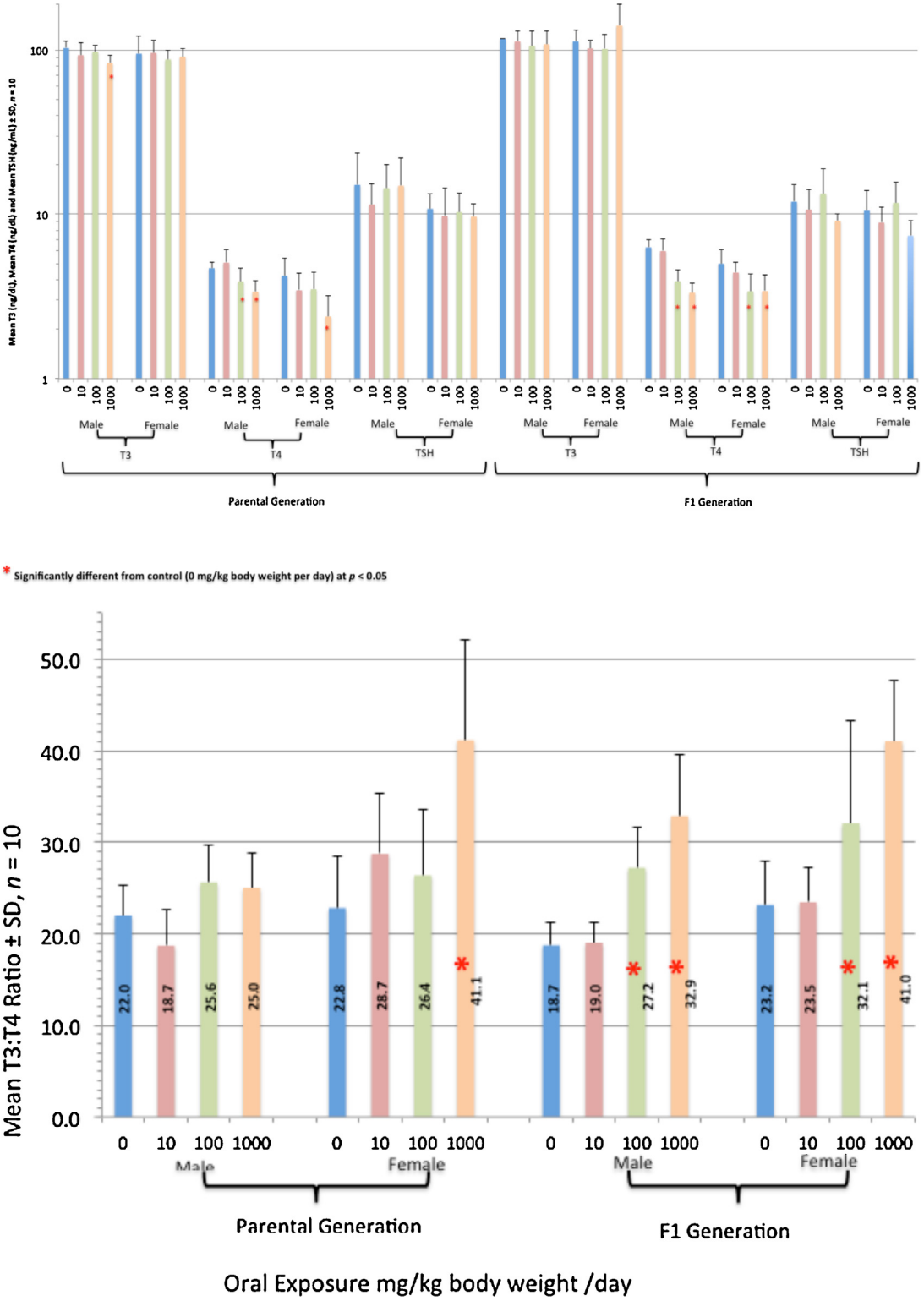

Fig. 1. Serum thyroid hormone parameters (*statistically different from controls, $p < 0.05$) in parental and F1 generation animals at experimental termination. A small reduction in T_3 was present in the high dose parental females. However the greatest effects were reduction of T_4 levels in both sexes of the parental and F1 generations at the 100 and 1000 mg/kg BW/d exposure levels, and associated increases in the T_3:T_4 ratio. These changes were not associated with increases in plasma TSH.

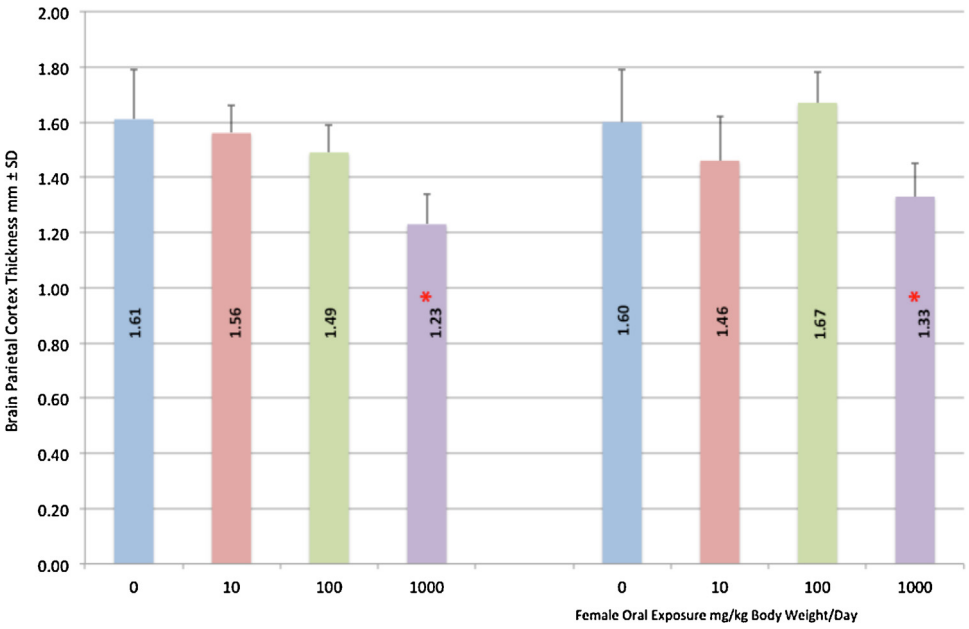

Fig. 2. Brain parietal thickness (*statistically different from controls, $p < 0.05$) in F_2 generation 11-day old pups. Oral exposure to 1000 mg/kg BW/day TBBPA was associated with significant reduction of thickness of the brain parietal cortex at the level of the infundibulum. This change was not associated with histologic changes in the parietal cortex and was not associated with significant test-article related changes in pre-weaning motor activity, step-through passive avoidance test performance, auditory startle test performance, forelimb and hind grip strength, emotionality, grooming behaviors, rearing, backing or water M-maze performance. Due to the limitations of the image analysis examinations, these results need to be interpreted with caution.

ratio. Serum T_4 levels were reduced in the mid (100 mg/kg BW/day) and high dose males and in the high dose females (Fig. 1). This was associated with a significantly increased serum T_3:T_4 ratio only in the high dose female group. Serum TSH and T_3 levels were not significantly affected by TBBPA treatment. The no observed effect level (NOEL) for the effects of TBBPA on serum thyroid hormone levels in the parental generation was 10 mg/kg BW/day.It is noteworthy that thyroid gland histopathology is a more sensitive indicator of thyroid status compared to T_3 or T_4 serum concentrations (Choksi et al., 2003; Jahnke et al., 2004). There were no marked histopathological alterations in thyroid or any other tissues. No estrogenic effects sufficient to affect macro and micro anatomy, fertility, reproduction, development, survival or behavior were detected in the embryofetal development study or in the multigenerational study. No other toxicologically meaningful test article-related findings were observed in the P generation.

3.2. F_1 generation evaluations in the multigenerational study

Mean serum T_4 level was significantly reduced in the mid (100 mg/kg BW/day) and high (1000 mg/kg BW/day) dose animals (both males and females; Fig. 1.) at the termination of F_1 generation. This was associated with a significantly increased serum T_3:T_4 ratios in the mid and high dose groups (both male and female). Serum T_3 and TSH levels were not significantly affected by TBBPA treatment. The NOEL for the effects of TBBPA on serum thyroid hormone levels in the F_1 generation was also 10 mg/kg BW/day. No other toxicologically meaningful test article-related findings were observed in the F_1 generation.

3.3. F_2 generation evaluations in the multigenerational study

Eleven day-old F_2 pups derived from F_1 dams in the high dose group (1000 mg/kg BW/day) had significant thinning of the brain parietal cortex (at the level of the infundibulum) at day 11 of life (Fig. 2). Due to the limitations of the image analysis examinations, these results need to be interpreted with caution. This change was not associated with histological changes in the parietal cortex and was not associated with significant test-article related changes in pre-weaning motor activity, step-through passive avoidance test performance, auditory startle test performance, forelimb and hind grip strength, emotionality, grooming behaviors, rearing, backing or

Table 3
Benchmark dose (BMD) continuous modeling of the female rat parietal cortex thinning[a].

Continuous Model[b]	Specified effect	BMD	BMDL	*p*-value test 1: lack dose response?	*p*-value test 2: constant variance?	*p*-value test 3: good variance model?	*p*-value for fit: does the model for the mean fit?	Visual fit	Scaled residual of interest	AIC
Exponential 2	0.1	7.0E + 02	5.1E + 02	0.002	0.279	0.253	0.108	Moderate	−0.04	−105
Exponential 3	0.1	7.0E + 02	5.1E + 02	0.002	0.279	0.253	0.108	Moderate	−0.04	−105
Exponential 4	0.1	7.0E + 02	1.6E + 02	0.002	0.279	0.253	0.108	Moderate	−0.04	−105
Exponential 5	0.1	7.0E + 02	1.0E + 02	0.002	0.279	0.253	0.035	Moderate	−0.04	−103
Hill	0.1	7.2E + 02	5.3E + 02	0.002	0.279	0.253	0.035	Moderate	−0.04	−103
Linear	0.1	7.2E + 02	5.3E + 02	0.002	0.279	0.253	0.109	Moderate	−0.04	−105
Polynomial	0.1	8.2E + 02	5.3E + 02	0.002	0.279	0.253	0.035	Moderate	−0.02	−103
Polynomial	0.1	7.8E + 02	5.3E + 02	0.002	0.279	0.253	0.035	Moderate	−0.02	−103
Power	0.1	7.2E + 02	5.3E + 02	0.002	0.279	0.253	0.109	Moderate	−0.04	−105

[a] One standard deviation of the mean is assumed for the Benchmark response (BMR).
[b] Complete information on this modeling effort is available upon request.

Table 4
Benchmark dose (BMD) continuous modeling of the male rat parietal cortex thinning.[a]

Continuous model[b]	Specified effect	BMD	BMDL	*p*-value test 1: lack dose response?	*p*-value test 2: constant variance?	*p*-value test 3: good variance model?	*p*-value for fit: does the model for the mean fit?	Visual fit	Scaled residual of interest	AIC
Exponential 2	0.1	4.3E + 02	3.4E + 02	<0.0001	0.15	0.15	0.33	Moderate	−1.04	−118
Exponential 3	0.1	4.3E + 02	3.4E + 02	<0.0001	0.15	0.15	0.33	Moderate	−1.04	−118
Exponential 4	0.1	1.7E + 02	8.0E + 01	<0.0001	0.15	0.15	0.50	Good	0.07	−117
Exponential 5	0.1	1.7E + 02	8.0E + 01	<0.0001	0.15	0.15	0.50	Good	0.07	−117
Hill	0.1	1.7E + 02	7.3E + 01	<0.0001	0.15	0.15	0.51	Good	0.08	−117
Linear	0.1	4.6E + 02	3.8E + 02	<0.0001	0.15	0.15	0.29	Moderate	−1.10	−117
Polynomial	0.1	4.6E + 02	3.8E + 02	<0.0001	0.15	0.15	0.29	Moderate	−1.10	−117
Polynomial	0.1	4.6E + 02	3.8E + 02	<0.0001	0.15	0.15	0.29	Moderate	−1.10	−117
Power	0.1	4.6E + 02	3.8E + 02	<0.0001	0.15	0.15	0.29	Moderate	−1.10	−117

[a] One standard deviation of the mean is assumed for the Benchmark response (BMR).
[b] Complete information on this modeling effort is available upon request.

water M-maze performance. The effect on brain parietal cortex thickness was also not observed at later stages of development. The NOEL for brain parietal cortex thinning at day 11 of life in the F_2 generation was 100 mg/kg BW/day. Benchmark doses (BMDs) were developed from these data using software freely available from the U. S. Environmental Protection Agency (http://www.epa.gov/ncea/bmds/dwnldu.html). BMDs of approximately 700 mg/kg-day (lower 95% limit of 160 mg/kg-day) for females, and 170 mg/kg-day (lower 95% limit of 73 mg/kg-day) for males were determined (Tables 3 and 4, respectively). No other toxicologically meaningful test article-related findings were observed in the F_2 generation. Specific details of these findings can be found in the Supplemental materials.

3.4. Fetal evaluations in the prenatal development study

This study was done according to GLP and monitored a sufficient number of parameters to afford confidence to these results. Data in Table 5 clearly demonstrate that none of the maternal and fetal parameters were markedly affected. Thus, the NOEL for prenatal effects was ≥1000 mg/kg BW/day.

4. Discussion

Both prenatal development and multigenerational toxicology studies demonstrated that TBBPA is not an adult maternal toxicant nor an overt teratogen at oral doses of up to 1000 mg/kg BW/day in CD® [Crl: CD® (Sprague-Dawley) IGS BR] rats. In brief, TBBPA is thus not considered a developmental toxicant in rats. Similarly, the multigenerational study was conducted according to GLP and also produced negative results for numerous parameters. However, this study did show thinning of the brain parietal cortex in F_2 generation animals at day 11 of life at the maternal oral dose of 1000 mg/kg BW/day. The NOEL for this effect was thus the maternal oral dose of 100 mg/kg BW/day. The multigenerational

Table 5
Influence of TBBPA on reproductive and pre-natal developmental parameters.

Endpoint evaluated	TBBPA dose			
	Control	100 mg/kg BW/day	300 mg/kg BW/day	1000 mg/kg BW/day
Number of females on study	25	25	25	25
Number not pregnant	0	1	0	1
Number pregnant	25	24	25	24
Pregnancy index	100.0	96.0	100.0	96.0
Number died during pregnancy	0	0	1	0
Number of abortions	0	0	0	0
Number of early deliveries	0	0	0	0
Number of females with viable fetuses on day 20 gestation	25	24	24	23
Number of coprora lutea per animal (mean ± SD)	16.8 ± 2.72	16.6 ± 2.21	16.8 ± 3.60	18.0 ± 2.57
Number of implantation sites per animal (mean ± SD)	15.4 ± 1.76	15.3 ± 2.48	15.4 ± 3.52	15.6 ± 8.059
Preimplantation loss %/animal (mean ± SD)	7.25 ± 7.540	7.77 ± 9.699	10.18 ± 15.696	10.65 ± 8.059
Viable fetuses number/animal (mean ± SD)	14.6 ± 1.68	14.5 ± 2.64	14.1 ± 3.71	14.3 ± 3.43
Viable fetuses/implant %/implant (mean ± SD)	95.05 ± 6.636	94.63 ± 7.523	92.34 ± 12.560	90.25 ± 20.023
Fetal sex ratio; % males/animal (mean ± SD)	52.9 ± 12.20	50.7 ± 15.95	47.5 ± 16.58	52.5 ± 13.64
Postimplantation loss; %implants/animal (mean ± SD)	4.95 ± 6.636	5.37 ± 7.523	7.66 ± 12.560	9.75 ± 20.023
Non-viable fetuses; number/animal (mean ± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Early resporptions; number/animal (mean ± SD)	0.8 ± 1.12	0.8 ± 1.13	1.3 ± 2.01	1.3 ± 1.73
Early resoprtions/implant; %/impant (mean ± SD)	4.95 ± 6.636	5.37 ± 7.523	7.68 ± 12.560	9.75 ± 20.023
Late resorptions; number/animal	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Gravid uterine weight (g; mean ± SD)	83.0 ± 8.21	81.3 ± 14.17	77.5 ± 19.54	83.7 ± 9.71
Male fetal weight (mean ± SD)	3.81 ± 0.258	3.81 ± 0.319	3.67 ± 0.240	3.75 ± 0.357
Female fetal weight (mean ± SD)	3.62 ± 0.262	3.63 ± 0.276	3.53 ± 0.208	3.56 ± 0.293
Male + female fetal weight (mean ± SD)	3.72 ± 0.254	3.72 ± 0.296	3.59 ± 0.221	3.66 ± 0.322
Forelimb external observations				
Digits, ectrodactyly malformations Number of litters (%)	0	0	0	0
Digits, ectrodactyly malformations Number of foetuses (%)	0	0	0	0
Abnormal forelimb flexure variations Number of litters (%)	0	0	1	0
Abnormal forelimb flexure variations Number of foetuses (%)	0	0	1	0

study also demonstrated changes in thyroid T_4 hormones levels in both the parental and F1 generations. The NOEL for these changes is 10 mg/kg BW/day. These findings are consistent with the observations of an extensive review of currently available TBBPA neurological data by Williams and DeSesso (2010) that demonstrated no adverse effects attributable to TBBPA exposures up to and including 1000 mg/kg BW/day on neurodevelopment, neuromotor functions, learning, memory, and neurobehavioral endpoints.

It is noteworthy that thinning of the brain parietal cortex in the F_2 generation at post-natal day (PND) 11 was not associated with lesions or changes that were detectable by H&E histological examination. Further, morphometric alterations in the brain parietal cortex were not associated with any significant effects on forelimb and hind limb grip strength between PND4–60, motor activity PND13–60, step through passive avoidance behavior at PND 22 and 60, water M-maze performance at PND day 110 or auditory startle habituation at PND 22 and 60. Thus the NOEL for all neuro-functional examinations is $\geq$1000 mg/kg BW/day.

However, transient (reversible) effects on brain neuronal development in the hippocampal dentate gyrus have been observed in Sprague-Dawley rats exposed to 1000 mg/kg BW/day of TBBPA (Saegusa et al., 2012). Thus, it could be that the thinning of the brain parietal cortex in the F_2 generation observed in this study at postnatal day 11 might be a transient effect that resulted in no subsequent detectable functional and pathological deficits. It is also conceivable that this finding was simply due to chance. In any case the biological relevance is not clear as there were no consequent associated neurological and functional alterations. In the absence of detectable lesions or change and no apparent effects on function, the finding of brain parietal cortex thinning on day 11 of life in the F_2 generation needs to be interpreted with caution, particularly because of the limitations of the morphometric technique used. Morphometric measurements were only taken from one section of the parietal cortex per animal, at only one time point during development and by using single line transect sampling.

At the termination of the P and F_1 generations, decreased serum total T_4 levels and elevated T_3:T_4 ratios were evident at doses of $\geq$100 mg/kg BW/day. Notably, blood TSH levels were not markedly altered despite the presence T_3:T_4 ratios >27. It should be noted that TBBPA exposure did not significantly affect serum T_3 levels and that elevations in the T_3:T_4 ratios were due simply to a lower serum T_4 concentration. Similar findings were reported from a one generation reproduction study in Wistar rats (Van der Ven et al., 2008) and studies in male Sprague-Dawley rats (Choi et al., 2011).

A comprehensive National Toxicology Program investigation on the effects of TBBPA was recently conducted. In a 3-month daily oral gavage study at doses of 0, 10, 50, 500, or 1000 mg/kg administered to F344/NTAC rats for 14 weeks, a decrease was noted in T_4 levels at day 4 and week 14 at only 500 and 1000 mg/kg/d in both genders. In agreement with our findings there were no treatment-related histopathological lesions and no marked changes reported in TSH and T_3 levels. Further, in an oral gavage study in B6C3F1/N mice daily administration of 0, 10, 50, 100, 500, or 1000 mg/kg/day TBBPA did not markedly affect T_4, T_3 and TSH levels or histopathology. Recently Osimitz et al. (2014) in a 90 day oral gavage administration of 0, 100, 300 or 1000 mg/kg/d TBBPA also found this flame retardant at 100 mg/kg and higher there was reduced T_4 but no effect on the levels of T_3 and TSH and thyroid tissue histopathology in CD rats. It is of interest that in rats given 1000 mg/kg/d for 90 days and then allowed to recover with no TBBPA for 6 weeks the levels of T_4 were similar to control indicating that the observed change in T_4 was transient and not related to any functional alterations. In a 2-year carcinogenesis study using Wistar Han rats or B6C3F1/N mice administration of 0, 250, 500 or 1000 mg/kg/day TBBPA there was no evidence of increased pathological alterations in thyroid tissue such as cyst, C-cell hyperplasia, follicle hyperplasia, inflammation, and follicular adenoma in thyroidal tissue in both species and genders. Data on thyroid hormone levels were not reported.The NTP study reaffirmed that the thyroid gland is not a target tissue following sub-chronic and chronic exposure in mice and rats. In agreement with the NTP 2-year study, the relevance of the T_4 changes observed in our investigation remain unclear but it is evident there were no associated physiological functional alterations.

Serum T_4 represents a circulating thyroid hormone reserve since it is a pro-hormone, with T_3 being the ultimate hormone (Larsen and Zavacki 2012; Marsili et al., 2011). Thus, the changes observed in the current multigenerational study, primarily T_4, represent a reduction in the circulating thyroid hormone functional reserve pool rather than a decrease in circulating pool of the ultimate T_3 hormone (Larsen and Zavacki 2012; Marsili et al., 2011). This interpretation is consistent with the lack of any evidence of overt thyroid disease such asovert hypothyroidism as evidenced by lack of histopathology and changes in circulating TSH and T_3. Overt hypothyroidism did not occur in the multigenerational study because the circulating ultimate hormone T_3 pool was not depleted to a biologically significant level (i.e., T_3 was only statistically significantly lower in males of the parent generation). Further, measurements of serum thyroid hormone levels are essentially screening-level assessments of thyroid function. Thyroid histopathology and scintigraphic techniques provide greater sensitivity and substantially more comprehensive information regarding thyroid function and dysfunction (Choksi et al., 2003). It should be clearly noted that no anatomical thyroid abnormality was detected in any of the animals in the multigenerational study. This finding was reaffirmed in several sub-chronic and chronic NTP (2014) studies in mice and rats.

In addition, there are numerous investigations that demonstrate that in humans functionally significant changes in T_4 production during development are accompanied by developmental delay, low body mass, brain developmental abnormalities and neurobehavioral developmental disorders (Di Liegro 2008; Forhead and Fowden 2014; Koibuchi 2013; Negro et al., 2011). However, there was clearly no evidence of prenatal or postnatal developmental delay, reduced body mass or reduced weight gain in either the prenatal development or multigenerational studyin rats administered TBBPA. Apart from limited evidence of thinning of the parietal cortex at postnatal day 11 using a crude screening-level morphometric method that resulted in no apparent neurological, neurodevelopmental or neuroperformance effects, there is clearly no evidence of brain developmental abnormality or neurobehavioral developmental disorders in this multigenerational study. Thus, while circulating T_4 functional thyroid reserve pool may have been reduced in the P and F_1 generations in the multigenerational study, the effects were clearly not adverse as measured by other parameters in this study.

The combination of lowered serum T_4, increased serum T_3:T_4 ratios, normal serum T_3 and normal serum TSH levels (i.e. no compensatory increase in TSH in response to lowered T_4) was previously observed in several rat strains following xenobiotic stimulated induction of hepatic microsomal enzymes (Richardson and Klaassen, 2010). T_4 is selectively inactivated by glucuronidation via hepatic microsomal UDP glucuronyltransferase (UGT) 1A and other 3-methylcholanthrene inducible UGTs in several rat strains (Beetstra et al., 1991; Richardson and Klaassen, 2010; Visser et al., 1991, 1993). T_3 is selectively inactivated by pregnane X receptor (PXR) inducible hepatic microsomal UGT2B2 rather than UGT1A in several rat strains (Beetstra et al., 1991; Hood and Klaassen, 2000; Richardson and Klaassen, 2010; van Raaij et al., 1993; Visser et al., 1993). Selective induction of UGT1A and other

3-methylcholanthrene inducible UGTs in the absence of induction of UGT2B2 results in a fall in serum T_4 levels without concurrent decreases in serum T_3 and without concurrent compensatory increases in serum TSH in rats (Hood et al., 1999; Hood and Klaassen, 2000; Richardson and Klaassen, 2010). However, selective induction of UGT2B2 by PXR agonists is followed by a fall in serum T_3 levels and a marked compensatory increase in TSH (Hood et al., 1999; Hood and Klaassen, 2000; Richardson and Klaassen, 2010). Thus, it is apparent that, at least in some rat strains, selective hepatic microsomal UGT1A induction by xenobiotics (notably3-methylcholanthrene) results in reduction of serum T_4 without concurrent decreases in serum T_3 and without concurrent compensatory increases in serum TSH (Richardson and Klaassen, 2010). It should be noted that these effects have only been observed in rodents and are yet to be demonstrated in humans.

In this study, decreases in serum T_4 in the general absence of concurrent changes in serum T_3 and in complete absence of concurrent compensatory increases in serum TSH observed in the multigenerational study appear to be due to TBBPA selective induction of rat hepatic microsomal UGT1A and other 3-methylcholanthrene inducible UGTs (i.e., hypothesis #2). The implications of this hypothesis and the findings of the multigenerational study are that CD® [Crl: CD® (Sprague-Dawley) IGS BR] rats should be resistant to TBBPA-induced thyroid follicular changes, including carcinogenesis, since such changes and tumors are largely driven by excessive and sustained TSH stimulation in this species (IARC, 1997; Parkinson et al., 2013). Further, the NTP (2014) study described above showed that TBBPA did not produce thyroid follicular hyperplasia in a 2-year carcinogenesis studies in rat and mouse. These observations are consistent with the known threshold effects of other possible thyroid toxicants.

A recent human health risk assessment for TBBPA demonstrated a worst-case scenario, highest adult European human exposure level of 0.19 mg/kg BW/day (ECB, 2006). Accordingly, this implies a worst-case margin of exposure (MOE) of $10/0.19 \cong 50$ or $100/0.19 \cong 500$ depending on whether the dose of 10 or 100 mg/kg BW-day is considered to be a no observed adverse effect level (NOAEL) based upon changes in thyroid hormone levels in the absence of other indications of thyroid insufficiency. A worst-case scenario, based on defining the thinning of the parietal cortex at postnatal day 11 at 1000 mg/kg BW-day in the 2-generation study as a lowest observed adverse effect level (LOAEL), would be 5000 (i.e., $MOE = 100/0.19 \cong 500$). A recent risk assessment by Colnot et al. (2014) demonstrated adult human exposure levels to be only a few ng/kg BW/day. Based on the Colnot et al. (2014) assessment, the MOE is in the 10^6 to 10^5 range and the LOAEL:exposure ratio is in the 10^7 range. In general, since a MOE of ≥ 100 is desirable, these results indicate a more than acceptable MOE for adult humans, even in the presence of an absolute, worst-case exposure scenario. Benchmark doses (BMDs) that were developed for thinning of the parietal cortex could also be used in this evaluation of MOE (Tables 3 and 4).

Based on the same European risk assessment, the average daily uptake by a breast feeding human infant (ADU_{Infant}) for the 0–3 month and 3–12 month periods of development were 0.044×10^{-3} and 0.017×10^{-3} mg/kg BW/day respectively (ECB, 2006). Thus the NOEL (maternal exposure):ADU_{Infant} ratios (also referred to as a MOEs by some regulatory agencies) are $>2 \times 10^6$. A recent Canadian human health risk assessment reported higher human infant exposure levels than those reported in Europe with the highest upper bound estimate of ADU_{Infant} being 1.95×10^{-4} mg/kg BW per day (Health-Canada, 2013). This would still result in a NOEL (maternal exposure):ADU_{Infant} ratio of $>5 \times 10^5$. These estimates are consistent with other published risk assessments (Colnot et al., 2014).

It should be noted that these simple screening-level approaches do not take into account the lower sensitivity of humans compared with rats to the thyroid effects, due to the rat lower "thyroid efficiency" (Parkinson et al., 2013). Specifically, the plasma $T_1/2$ of T_3 and T_4 in the rat is considerably shorter than in humans due to protection of circulating thyroid hormones by the protein chaperone thyroxine binding globulin (TBG) in humans (Parkinson et al., 2013). TBG is not present in rats. Accordingly, rats compensate for the more rapid turnover circulating thyroid hormones by secreting more TSH resulting in more T_4 production (Parkinson et al., 2013). In effect, rats need to have a 10-fold higher rate of T_4 production on a per unit body mass basis than humans in order to maintain physiological homeostasis (Parkinson et al., 2013). Accordingly it is probable that humans are resistant to the effects of TBBPA on T_4 compared with CD® [Crl: CD® (Sprague-Dawley) IGS BR] rats, implying that the actual MOE for adult humans may be substantially larger than 50–500.

Consistent with the results of other published rodent studies (Kang et al., 2009; Saegusa et al., 2009; Tada et al., 2006; Van der Ven et al., 2008), TBBPA was not nephrotoxic at oral exposures of up to 1000 mg/kg BW/day in the current rat prenatal development and multigenerational studies. Notably, this is not consistent with the findings of Fukuda et al. (2004) that demonstrated persistent TBBPA-induced nephrotoxicity in rats dosed at 200 or 600 mg/kg BW/day between days 4 and 21 of life.

5. Conclusions

This developmental toxicology study demonstrated that TBBPA exerted nomarked developmental effects at oral gavage doses of up to and including 1000 mg/kg BW/day, the highest dose tested. The multigenerational study also was without any significant reproductive effects and was without all but one histopathological alteration at doses of $\leq$1000 mg/kg BW/day. The sole histopathology lesion was thinning of the brain parietal cortex in F_2 generation animals at PND 11 at the maternal oral dose of 1000 mg/kg BW/day. This effect was not accompanied by any detectable neurodevelopment, neurofunctional or neurobehavioral deficits and needs to be interpreted with caution given the limitations of brain morphometric techniques used. The multigenerational study also showed decreases in serum T_4 in the general absence of concurrent changes in serum T_3 and in the complete absence of concurrent compensatory increases in serum TSH as well as lack of histopathological alterations. This pattern of change in serum thyroid hormones has been observed with other xenobiotics in rodents and is suggestive of selective induction of hepatic microsomal UGT1A in the absence of induction of UGT2B2, a pathway not yet observed in humans.

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The text of this article reflects the personal opinions of the authors and does not indicate any regulatory decision or opinion by the Therapeutic Goods Administration of Australia, the Department of Health Australia or the Federal Government of Australia.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tox.2014.12.013.

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