

Expert Review:

Risk assessment of TBBPA: Updating the EU-Risk Assessment Reports

Reviewer:

Wolfgang Dekant, Dr. rer. nat.; Professor of Toxicology

Home address: Rhönstrasse 9, 97080 Würzburg

University address: Institut für Toxikologie, Universität Würzburg, Versbacherstr. 9, D-97078 Würzburg

e-mail: dekant@toxi.uni-wuerzburg.de

Sponsor:

This report was commissioned by the Bromine Science and Environmental Forum - BSEF - (www.bsef.com), the global association of the brominated flame retardant industry.

W. Dekant, Professor of Toxicology

Date

Table of contents

1. Introduction	3
2. Task	3
3. Methods	3
4. Health and environmental risk assessment of TBBPA	4
5. Evaluation of recent literature	6
5.1 Human health relevant endpoints	6
5.2 Environmentally relevant endpoints	10
5.3 Analytical determinations of TBBPA in biota or the environment	11
5.4 Are changes in the conclusions in risk assessment necessary based on the new studies ?	12
6. Comments on the Ökoinstitut Freiburg e.V. report and its conclusions regarding TBBPA .	15
6.1 General comments.....	15
6.2 Specific points regarding TBBPA.....	15
7. References.....	17

Summary conclusions

A study by the Ökoinstitut Freiburg e.V. commissioned by DG Environment identified TBBPA as “high priority hazard”. Ökoinstitut Freiburg e.V. proposed to restrict the use of TBBPA in electric and electronic equipment. This review examines the scientific basis and the reliability of the conclusions by Ökoinstitut Freiburg e.V. applying scientifically accepted procedures in risk assessment for human health and environmental effects of chemicals. When considering the available data on TBBPA evaluated in recent EU risk assessment reports and the information on the toxicity and environmental concentrations of TBBPA published between 2002 and February 2010, after the EU risk assessments, the scientific support for the conclusions of Ökoinstitut Freiburg e.V. is weak. TBBPA is not an “endocrine disruptor” and does not have a significant potential for bioaccumulation; the EU-RARs and their conclusions are based on established science and do not need to be changed due to new observations published over recent years.

1. Introduction

Tetrabromobisphenol A (TBBPA) is used as a highly efficient additive and reactive flame retardant in electric and electronic equipment. It is required to meet legal fire safety standards. The use of TBBPA in electric and electronic equipment is not regulated in the “Directive on the restriction of the use of certain hazardous substances in electrical and electronic equipment”. However, a study by the Ökoinstitut Freiburg e.V. requested by DG Environment identified TBBPA as “high priority hazard” and Ökoinstitut Freiburg e.V. proposed an inclusion of TBBPA in the directive and restrictions on the use of TBBPA in electric and electronic equipment.

2. Task

The scope of this review is to examine the scientific basis and the reliability of the conclusions by Ökoinstitut Freiburg e.V.. All decisions by the EU-commission and restrictions of the use of chemicals in products should be based on reliable and scientifically sound information. Therefore, information contained in the recent European Risk Assessment Report (EU-RAR-HH, 2006; EU-RAR-Env, 2008) and also more recently published information is assessed to see if the conclusions of the EU risk assessment still apply or if there is a need for revision. This assessment was performed using accepted procedures for health and environmental risk assessment.

3. Methods

Regarding the toxicity database and exposure assessment for TBBPA, the peer reviewed EU-RAR (EC-SCHER, 2005; EU-RAR-HH, 2006; EC-SCHER, 2008; EU-RAR-Env, 2008) are used as basis for an evaluation of the information available at the time of publication of the EU-RARs. Peer reviewed publications on TBBPA between the cutoff dates used in the EU RARs (end of 2002 for human health and Sept. of 2006 for environmental effects) to March 10, 2010 were evaluated using established principles for the assessment of human health and environmental risks of chemicals. The following basic concepts of risk assessment are applied:

In risk assessment, exposure and hazard (potential toxicity) are separately assessed (Henry, 2003; MacDonald, 2004). The results are then integrated into risk assessment using dose and species extrapolations. Detailed guidance to perform risk assessment for a variety of

endpoints is available from the US-EPA, the European Chemicals Agency and international organizations such as WHO and is practiced by EU-member state competent authorities and by EU advisory committees in health and environmental risk assessment. The approaches included in these guidance documents are integrated in this assessment.

Regarding exposure assessment to TBBPA, the EU-RARs developed a number of exposure scenarios using conservative default assumptions, modeling, or measured data. The potential toxicity of TBBPA has been intensively studied and studies performed along testing guidelines and following good laboratory practices (GLP) cover most of the major toxicity endpoints relevant for hazard assessment. Since TBBPA is not genotoxic (EU-RAR-HH, 2006), thresholded dose-responses with defined NOAELs or benchmark doses (BMDs) can be used as starting points for extrapolations to define human exposures without appreciable health risks. More recent references not covered by the EU-RARs were searched in PubMed using the search terms “tetrabromobisphenol A” or “TBBPA”, and toxicity“ and in Chemical Abstracts using the CAS-number of TBBPA and “toxicity” as keywords.

A comparison of the approach of the Ökoinstitut Freiburg e.V. with internationally accepted procedures in risk assessment and specific comments on the conclusions and their scientific validity is also included.

4. Health and environmental risk assessment of TBBPA

Regarding endpoints relevant to human health, a peer reviewed EU-RAR was published in 2006 (EU-RAR-HH, 2006). This comprehensive document evaluated all available toxicity studies on TBBPA including toxicity testing guideline compliant GLP studies and performed exposure assessments regarding a variety of likely exposure scenarios. Estimated exposures of humans in the different scenarios were then compared with “no-observed-adverse-effect-levels” (NOAELs) from animals toxicity studies using the “Margin-of-exposure” (MoE) approach. In the concept, risk reduction measures are not mandated when the MoE is > 100 ; i.e., the actual exposure is more than 100-fold below the NOAEL of the chemical in animal toxicity studies. Regarding risk management, the RAR on human health consistently proposed conclusion ii) “There is at present no need for further information and/or testing, or for risk reduction measures beyond those which are being applied already” for workers, consumers and indirect exposures both on a local and regional scale including infants due to high MoEs.

The EU-RAR evaluating potential environmental effects of TBBPA (EU-RAR-Env, 2008) used the PEC/PNEC approach. A chemical is judged to be environmentally compatible if the Predicted No Effect Concentration (PNEC), the concentration that causes no adverse effect to the environment, is higher than the Predicted Environmental Concentration (PEC), which is the measured or model predicted concentration of the chemical in the environment. Such an assessment is repeated for each relevant environmental compartment, such as wastewater treatment plants, rivers and soils. Using this concept, the EU-RAR also came to conclusion ii) regarding a variety of environmental exposure scenarios. Further information (conclusion i) was needed on the potential of a TBBPA metabolite and its environmental effects and on the reductive formation of bisphenol A from TBBPA. The EU-RAR identified only one scenario where conclusion iii) "There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account" was applicable. Risk reduction was considered necessary for TBBPA-use in compounding and conversion sites regarding surface water and, under certain limits, when sewage sludge from such sites is applied. Risk reduction measures have been performed to address this conclusion and it is considered as resolved.

Both RARs specifically considered the potential for endocrine system mediated toxic effects of TBBPA, often termed incorrectly as "endocrine disruption". For both human health and environmental effects, it was concluded that "endocrine disruption" is of low relevance for TBBPA toxicity and risk assessment (EU-RAR-HH, 2006; EU-RAR-Env, 2008). This conclusion was supported by the peer-review by the EU Scientific Committee on Health and Environmental Risks (EC-SCHER, 2005; EC-SCHER, 2008). In addition, TBBPA is not considered as a persistent and bioaccumulative toxicant in the RARs. The bioaccumulation assessment was properly performed and a bioconcentration factor (BCF) of 1,234 L/Kg was derived. This BCF was considered conservative by SCHER: *A conservative fish BCF of 1,234 l/kg is used in the environmental risk assessment representing a worst case BCF based on total radioactivity. Due to extensively metabolism, the BCFs based on tetrabromobisphenol-A itself are well below this value (range 160-485 l/kg for fish and around 148-160 for marine invertebrates). Excretion of tetrabromobisphenol-A and metabolites from aquatic organisms and mammals is very rapid. No accumulation in lipid tissues has been observed in toxicokinetic studies with mammals* (EC-SCHER, 2008).

Since the completion of the RARs, further studies on potential effects of TBBPA have been published. The information contained in these studies is evaluated in the following to assess if the conclusions of the RARs may require modifications due to the new information.

5. Evaluation of recent literature

5.1 Human health relevant endpoints

Regarding human health, *in vitro* studies using biochemical or simple cytotoxicity endpoints are of limited use for risk assessment since the adversity of the biochemical effect is usually unknown (except for genotoxicity testing) and concentrations applied *in vitro* need to be related to actual blood and tissue levels of TBBPA and its metabolites.

Several studies (Kibakaya *et al.*, 2009; Sun *et al.*, 2009; Li *et al.*, 2010; Shiizaki *et al.*, 2010) used *in vitro* methods to assess biochemical effects of TBBPA. In one study, TBBPA (2.5 and 5 microM) decreased the lytic function of human NK cells (Kibakaya *et al.*, 2009), but the relevance of such an effect regarding risk assessment is unknown. While the publication speculates on a possible effect of this inhibition on tumor development, the absence of tumor formation or pathological changes in animals given high doses of TBBPA for prolonged times does not suggest that this observation is relevant for risk assessment.

In other studies, mechanisms of toxicity of TBBPA were investigated using a variety of cell culture models to define mode-of-action for cell death or biochemical changes (Reistad *et al.*, 2005; Nakagawa *et al.*, 2007; Ogunbayo *et al.*, 2007; Reistad *et al.*, 2007; Strack *et al.*, 2007; Ogunbayo *et al.*, 2008). The concentrations of TBBPA used in these studies were much higher than those achieved in rodents after administration of TBBPA by a realistic exposure route. Many of the cell systems or endpoints investigated are only affected in experimental animals after very high doses of TBBPA, or are not reflected by *in vivo* toxicities of TBBPA at all. Therefore, the results of these studies do not have an impact on risk assessment for TBBPA regarding human health.

The ability of TBBPA, other brominated flame retardants, and several phenols to interact with the estrogen receptor (ER), androgen receptor (AR), progesterone receptor (PR), estrogen-related receptor (ERR), and thyroid endocrinology were determined in recombined yeast strains and in mammalian cell based reporter assays (Reistad *et al.*, 2005; Nakagawa *et al.*, 2007; Ogunbayo *et al.*, 2007; Reistad *et al.*, 2007; Strack *et al.*, 2007; Ogunbayo *et al.*, 2008). TBBPA was either not interacting with estrogen receptors (ERs) or was only a very weak ERalpha agonist/antagonist, with a potency orders of magnitude below that of natural ER-ligands. TBBPA also was not interacting with or was only a very weak PR antagonist,

assays for antiandrogenic activity were also negative and TBBPA did not interfere with CYP17 catalytic activity (Canton *et al.*, 2006). TBBPA did also not interact with the arylhydrocarbon (Ah)-receptor. Consistently, TBBPA showed a potential to interact with thyroid hormone signaling pathways. In vitro studies available at the time of publication of the EU-RAR already described a potential of TBBPA to interact with the thyroid axis; thus, the new results only confirm observations already evaluated in the RAR.

A number of studies have assessed effects of TBBPA in rodents after repeated administration, often during pregnancy, due to concerns for a potential reproductive or developmental toxicity of TBBPA. Such studies are more relevant for human health risk assessment when properly executed and analyzed.

Behavioral effects of TBBPA were studied by Nakajima *et al.*, 2009. TBBPA (0.1, 5 and 250 mg/kg body weight) administered orally three hours before the open-field test induced dose-unrelated small changes in some of the behavioral parameters assessed (Nakajima *et al.*, 2009). The absence of a dose response, the use of a single dose, the short time between administration of TBBPA and testing, and lack of a positive control to evaluate the performance of the test battery severely limit the utility of the study for risk assessment.

After a high intraperitoneal doses of 100 or 600 mg/kg bw, TBBPA was shown to induce radical formation in rat liver (Chignell *et al.*, 2008). The results were claimed to explain the high dose hepatotoxicity of TBBPA in rats (Szymanska *et al.*, 2000) already considered in the RAR. A very low potential for nephrotoxicity of TBBPA, even after daily doses of 1,000 mg/kg bw for two weeks, was derived (Kang *et al.*, 2009). This study also did not observe bioaccumulation of TBBPA and determined a short half-life in the range of 7 – 9 hours for TBBPA in rats.

In a study to assess the capacity of TBBPA to modify tumor incidence induced by N-bis-hydroxypropyl(nitrosamine) and by 7,12-dimethylbenz(a)anthracene, TBBPA was administered with diet to dams in concentrations of up to 1 % in diet for three weeks after parturition (Imai *et al.*, 2009). Daily intake of TBBPA in the dams at the high dose was calculated as 1,249 mg/kg bw. Offspring were then treated with the same dietary concentrations of TBBPA for two weeks (highest daily dose of TBBPA was 2,359 mg/kg bw in male offspring). Males and females were then exposed to concentrations of up to 0.2 of the nitrosamine with drinking water for 4 weeks, female offspring also were gavaged with a single dose of dimethylbenzanthracene (50 mg/kg bw) after week seven and all animals were terminated after 39 weeks (males) and 49 weeks (females). TBBPA did not cause changes in body weight gain, relative organ weights, and histologic lesions in any organ except the

thyroid and the urinary bladder. In the high dose TBBPA females, the incidence of thyroid tumors was slightly increased; increased incidences of transitional cell papilloma of the urinary bladder was observed at all TBBPA doses in females, but not in males. Due to the very high doses used, the absence of genotoxicity of TBBPA, and the apparently very low exposure of the urinary bladder to TBBPA due to the major role of fecal excretion of TBBPA, the relevance of these results for risk assessment of human TBBPA-exposures is very limited.

The results of more relevant studies on the potential for reproductive effects of TBBPA are summarized in the following text.

TBBPA was administered at concentrations of 100, 1000 or 10,000 ppm in diet (TBBPA-intake of up to 2,129 mg/kg bw/day, calculated from food consumption) from gestation day (GD) 10 to postnatal day (PND) 20 to rats and offspring was evaluated. Offspring exposed to TBBPA showed slight dose-unrelated decreases of serum triiodothyronine, but no effects on hypothyroidism-related parameters in the TBBPA-exposed animals. No changes in relative organ weights (including testes) and many other parameters relevant to reproductive outcome or potentially related to “endocrine disruption” were observed (Saegusa *et al.*, 2009). Reproductive effects of TBBPA were also not observed in mice administered daily doses of TBBPA up to 1,640 mg/kg bw (GD 0-17) and 4,156 mg/kg bw (PND 0 – 21) to mice (Tada *et al.*, 2006) and in a comprehensive 2-generation reproductive toxicity study using doses of up to 1,000 mg/kg bw (MPI-Research, 2002; MPI-Research, 2003) evaluated in the EU-RAR.

A comprehensive assessment of TBBPA-effects in rodents was performed in the EU-sponsored FIRE-Project which included a toxicokinetic study in human subjects (Schauer *et al.*, 2006) and an additional detailed assessment of reproductive toxicity of TBBPA in rodents (Lilienthal *et al.*, 2008; Van der Ven *et al.*, 2008). Potential reproductive effects of TBBPA were studied in a one-generation reproduction assay in rats using dietary exposure applying targeted doses of 0, 3, 10, 30, 100, 300, 1,000, and 3,000 mg/kg bw/day and in a 28-day subacute toxicity study with targeted doses of 0, 30, 100, and 300 mg/kg/day. The large number of dose groups permitted analysis of dose response and calculation of a benchmark dose (BMD). Offspring were also subjected to neurobehavioral testing using specific endpoints.

In the reproduction study, decreased levels of circulating thyroxine (BMDLs of 31 mg/kg bw/day in males and 16 mg/kg bw/day in females), increased testes weights (BMDL of 0.5 mg/kg bw/day), and pituitary gland weights in males (BMDL of 0.6 mg/kg bw/day) were observed.

However, the increases were small (e.g. a maximal response of 15.5 % for testes weight), and the weight changes were not accompanied by histological or functional changes. Effects on the many other reproductive and developmental parameters determined in the study (including immunotoxicology, clinical chemistry, and endocrine parameters) in the offspring were only seen with BMDLs > 100 mg/kg bw/day, and histopathological changes were not observed in the many analyzed tissues including the thyroid and the testes. The only effects in the subacute study were decreased circulating T4 and increased T3 levels in males (BMDL of 48 and 124 mg/kg bw/day), again without any histopathological changes (Van der Ven *et al.*, 2008). The neurobehavioral analysis of the F1 animals showed alterations in some brainstem auditory evoked potentials, some of them with BMDLs in the range of the observed changes in thyroid hormones (Lilienthal *et al.*, 2008). However, the reported neurobehavioral alterations were criticized as inconsistent with the anatomy and physiology of the auditory system and were claimed as based on an insufficient control of confounders ((Strain *et al.*, 2009).

Several studies published after completion of the EU-RAR on human health effects further support a low bioavailability and a rapid excretion of TBBPA in mammals. The toxicokinetics of TBBPA were studied in rodents and in humans. After a single oral dose of 0.1 mg/kg TBBPA in human subjects and a single oral dose of 300 mg TBBPA/kg body weight in rats. TBBPA-glucuronide and TBBPA-sulfate were identified as metabolites of TBBPA in blood and urine of the human subjects and rats. In humans, maximum plasma concentrations of TBBPA-glucuronide (16 nmol/l) were obtained within 4 h after administration and TBBPA-glucuronide was slowly eliminated in urine. Parent TBBPA was not detected in the human plasma samples. In rats, TBBPA-glucuronide and TBBPA-sulfate were major metabolites of TBBPA present in blood; in addition, a diglucuronide of TBBPA, a mixed glucuronide-sulfate conjugate of TBBPA, tribromobisphenol A, and a glucuronide of tribromobisphenol A were present in low concentrations. TBBPA (103 µmol/L) and TBBPA-glucuronide (25 µmol/l) plasma concentrations peaked at 3 h after administration and TBBPA-sulfate (694 micromol/l) peaked 6 h after administration.

A detailed study on the toxicokinetics of TBBPA in rats confirmed the intensive first-pass metabolism of TBBPA in the liver after oral administration and a rapid elimination of TBBPA-metabolites mainly with feces. After intravenous injection, TBBPA was also rapidly cleared from the organism and the majority of a iv dose was also recovered in feces (Kuester *et al.*, 2007).

The results obtained in the kinetic studies in rodents and in human suggest absorption of TBBPA from the gastrointestinal tract and rapid metabolism of the absorbed TBBPA by conjugation (Schauer *et al.*, 2006; Kuester *et al.*, 2007). Formed conjugates are rapidly excreted thus confirming previous results in rats considered in detail in the EU-RAR. These results further support the absence of a potential of TBBPA for bioaccumulation in mammals.

5.2 Environmentally relevant endpoints

In two populations of adult zebrafish (*Danio rerio*) exposed to 0.75 µM and 1.5 µM TBBPA for 14 days, a combined transcriptomic and proteomic approach was applied to evaluate effects of TBBPA on liver. Based on the analysis, an interference of TBBPA with thyroid and vitamin A homeostasis in zebrafish was concluded, TBBPA also elicited responses indicating onset of oxidative stress and general stress responses. Additionally, numerous differentially expressed transcripts could be associated with defense mechanisms or corresponded to metabolizing enzymes (De Wit *et al.*, 2008).

TBBPA-induced effects on superoxide dismutase activity, lipid peroxidation, and heat shock protein expression were evaluated in zebrafish embryos. At higher concentrations, TBBPA (> 0.75 mg/L) caused lethality. Furthermore, TBBPA caused a concentration-dependent increase in superoxide dismutase activity, heat shock expression, and lipid peroxidation (Hu *et al.*, 2009). Since a high toxicity of TBBPA to aquatic organisms was known at the time of development of the EU-RAR and integrated in the conclusions, these newer results do not influence the conclusions of the EU-RAR.

One study also confirmed the O-methylation of TBBPA to its mono- and dimethyl ether derivatives by microorganisms present in different sediments. This metabolite of TBBPA has also been detected in environmental samples. Due to the addition of hydrophobic methyl groups, O-methylated derivatives are more lipophilic (George and Haggblom, 2008). However, information on the demethylation of this TBBPA-metabolite in the environment or in animals is not available, therefore, its potential relevance regarding bioaccumulation cannot be assessed. Moreover, this metabolite was detected as a minor product of TBBPA-degradation in soil in studies integrated in the EU-RAR and is present in very low concentrations in human blood and milk as described in the EU-RAR. Therefore, the results from this study do not change the conclusions from the EU-RAR.

5.3 Analytical determinations of TBBPA in biota or the environment

In a monitoring study, TBBPA was determined in maternal and cord serum, adipose tissue and breast milk collected from 93 female subjects at delivery. TBBPA was detected in 44% of the analyzed breast milk samples at levels varying from 0.06 to 37.34 ng/g lipid and in 30% of the serum samples, with similar average values in maternal and cord serum (154 pg/g and 199 pg/g fresh weight). TBBPA was not detected in adipose tissue (Cariou *et al.*, 2008).

TBBPA-concentrations were analyzed in indoor dust samples from 18 houses and two offices in Belgium. Median concentrations of TBBPA in the 18 domestic dust samples were 10 ng/g dust with concentrations in the dust samples from offices being 5-10 times higher. It was concluded that dust is a minor contributor (<10% of total exposure) to the total daily exposure to TBBPA (Geens *et al.*, 2009). On the other side, a study in the UK determined concentrations of TBBPA in house dust of up to 62 ng/g and concluded that dust ingestion may be a relevant exposure pathway to TBBPA for infants due to dust ingestion (Abdallah *et al.*, 2008).

Concentrations of TBBPA were analyzed in water (n = 27), sediment (n = 9), and fish samples (n = 30) from nine English lakes. Concentrations of TBBP-A ranged from 140 to 3,200 pg/L in water, 330 to 3,800 pg/g in dry sediment and up to 1.7 ng/g lipid weight in fish. ((Harrad *et al.*, 2009). Another study analyzed TBBPA in water and sediments from French rivers. TBBPA was detected in the dissolved phase only (< 35-68 pg/L) and from 70 to 280 pg/g (dry weight) in sediments (Labadie *et al.*, 2010). One study determined air concentrations of TBBPA in a circuit board factory, a furniture workshop, and two electronics dismantling facilities (Makinen *et al.*, 2009) TBBPA was detected in the air of the electronics dismantling facilities with a geometric means of 1,050, resp. 970 ng/m³. TBBPA was below LOD in the circuit board factory and the furniture workshop. High air concentrations of TBBPA in electronics dismantling had been taking in account in the EU-RAR.

Determinations of TBBPA in tissues of top predators collected in the southeast of the US gave a maximum concentration of 35.6 µg TBBPA/kg lipid. The study also determined TBBPA in human adipose tissue with a maximum determined concentration of 0.46 µg TBBPA/kg lipid. In aquatic animals, the concentrations determined were similar to those already considered in the EU-RAR (see table 13 of Ökoinstitut Freiburg eV Report); therefore, the results have to be considered as confirmatory and do not indicate a need to change the conclusions of the RAR (Johnson-Restrepo *et al.*, 2008). In other studies in deepwater fish and shellfish collected off the coast of Scotland, TBBPA-concentrations were below the limit of detection (Fernandes *et al.*, 2008; Webster *et al.*, 2009). Low average daily intakes in the

range of 0.03 ng TBBPA/kg bw were calculated from measured concentrations of TBBPA in fish collected in Japan (Ashizuka *et al.*, 2008).

5.4 Are changes in the conclusions in risk assessment necessary based on the results of new studies ?

New risk assessment-relevant results for TBBPA are described in studies on the reproductive toxicity of TBBPA in animals (Lilienthal *et al.*, 2008; Van der Ven *et al.*, 2008; Saegusa *et al.*, 2009). In the one-generation reproductive toxicity study using the standard OECD-415 protocol, small changes in testes and pituitary gland weights, in circulating thyroid hormones, and in some neurobehavioural parameters were observed in F1-generation animals. Regarding the neurobehavioural effects, these occurred with benchmark doses (BMDLs) similar to observed changes in circulating thyroid hormones and may be related to effects of TBBPA on thyroid hormone homeostasis (Lilienthal *et al.*, 2008). Due to the low BMDLs derived for testes and pituitary gland weights, the authors concluded a low margin of exposure (MoE) to estimates of human exposures made in the EU-RAR. Based on model calculations, the EU-RAR predicted a maximum daily dose of TBBPA of 1.9 mg/kg/day from “indirect” exposure, which gives a MoE to the lowest BMDL in these studies of less than 5. In contrast, a second one-generation reproductive toxicity study exposing rats to TBBPA from mid-gestation through lactation did not report effects on organ weights and many other parameters.

However, conclusions based on the BMDLs observed in this one-generation reproductive toxicity study (Lilienthal *et al.*, 2008; Van der Ven *et al.*, 2008) and the exposure assessment of the EU-RAR for risk characterization needs to consider a number of additional issues:

- The reported small increase in testes weights in F1 males is of questionable adversity in the absence of histopathological and functional changes in testes, which were not observed in the detailed evaluation. Only effects accompanied by histopathological and functional changes should be considered as “adverse” and used as pivotal points in risk assessment. Moreover, the data on testes weights are inconsistent over a number of studies. A guideline compliant two-generation reproductive toxicity study including a neurobehavioral component using daily oral doses of 0, 10, 100, and 1,000 mg/kg and high statistical power (n = 28-29/dose group), a second recent developmental toxicity study (Saegusa *et al.*, 2009), and several studies with varying reliability discussed in the RAR or published recently did not observe effects on offspring testes weights.

- While the different studies on developmental neurotoxicity have not addressed the same neurobehavioral endpoints, the overall database on neurodevelopmental effects is somewhat inconsistent and one or two member states proposed a NOAEL for TBBPA-induced developmental neurotoxicity (50 mg/kg bw/day). Changes in neurobehavioral parameters or neurohistopathology were absent in the two-generation reproductive study and two other well-conducted developmental neurotoxicity studies in the rat and a postnatal developmental study in the mouse. These were evaluated in the EU-RAR. Moreover, toxicokinetic studies do not show a significant transfer of TBBPA or TBBPA-metabolites to fetus due to a very low systemic bioavailability of TBBPA (see above). These considerations further question the relevance of the results of the Lilienthal study for assessment of effects of TBBPA at low doses as expected in humans. Therefore, the overall evaluation of the database on developmental neurotoxicity using a weight-of-evidence approach remains as outlined in the EU-RAR *“Overall, the available data do not provide strong evidence of the potential of TBBPA to act as a developmental toxicant or neurotoxicant”*.
- Observations made regarding thyroid hormone homeostasis were already taken into account in the EU-RAR. Effects on circulating thyroid hormones were observed in a 90-day oral toxicity study evaluated in the EU-RAR (daily doses of 0, 100, 300, or 1,000 mg/kg bw) and were not considered as adverse. Effects on thyroid-related parameters were not observed in the 2-generation reproductive toxicity study.
- Regarding the high human intake estimate for TBBPA cited as the basis for concern (Van der Ven *et al.*, 2008) due to a low MoE, the EU-RAR clearly states that *“ it should be recognised that the calculation methods used are uncertain and their general applicability to a substance such as tetrabromobisphenol-A ... is unknown. The estimates (of exposure) should be treated as representing a worst case and uncertain estimate”*. The estimates in the EU-RAR were generated by calculation with a model based on partition coefficients.

They are inconsistent with exposures derived from measured concentrations of TBBPA in food, the major human exposure source for TBBPA (Geens *et al.*, 2009). A high intake of TBBPA predicted by the model should be supported by high concentrations of TBBPA in food. However, available data from a total diet survey in the United Kingdom do not support a high intake of TBBPA with food since concentrations of TBBPA were below LOD in all main food groups.

- High daily intakes of TBBPA should give high blood levels of TBBPA in non-occupationally exposed human populations. Administration of TBBPA as a single dose of 0.1 mg/kg bw TBBPA to human subjects resulted in peak plasma levels of 16 nmol/L of TBBPA-glucuronide (equivalent to 8.6 $\mu\text{g/L}$ of TBBPA). However, blood concentrations of TBBPA in non-occupationally exposed humans are below 100 ng/L. Therefore, these observations further support the conclusion that the model predicted high intake of TBBPA by indirect exposure is unrealistically high and that the margin of safety therefore is widely underestimated.
- Blood levels of TBBPA in the F1-animals from the one-generation study at doses with observed effects were between 1 – 10 $\mu\text{moles/L}$ (540 to 5,400 $\mu\text{g/L}$), at least three orders of magnitude above realistic human exposures (see above).
- Toxicokinetics of TBBPA in humans show a pronounced first-pass metabolism to conjugates, which are unlikely to interfere with hormone function due to the major structural change in the molecule and increased polarity enabling rapid excretion. This has been conclusively demonstrated for the structurally related bisphenol A (Snyder *et al.*, 2000; Matthews *et al.*, 2001) and conjugates of estrogens. In rodents, after much higher doses as compared to humans, some of the administered TBBPA may escape first-pass metabolism and thus be transferred to the systemic circulation to cause effects in target organs. Disproportionally high concentrations of free TBBPA are present in the high dose animals in the one-generation study and this therefore represents a worst-case situation.

In conclusion, the results of these two studies do not change the overall conclusion of the EU-RAR regarding human health effects. MoEs based on actual human exposures are still well above 100 even when considering the BMDLs (Lilienthal *et al.*, 2008; Van der Ven *et al.*, 2008) for effects with questionable adversity as pivotal points in risk assessment. The other new results published since the publication of the EU-RAR confirm observations already available at the time when the EU-RARs were prepared.

6. Comments on the Ökoinstitut Freiburg e.V. report and its conclusions regarding TBBPA

6.1 General comments

Selection criteria applied. The Ökoinstitut Freiburg e.V. applied the following selection criteria on hazardous substances listed in the inventory:

1. Substances meeting the criteria for classification as dangerous in accordance with Directive 67/548/EEC
2. Substances meeting the criteria for classification as substances of very high concern (SVHC) in accordance with REACH
3. Substances which have been found as contaminants in humans and biota.

In general, this selection process is hazard based and not consistent with present risk assessment procedures. Hazard assessment alone does not permit conclusions on potential risks since exposure is of equal importance. If exposure is sufficiently low, an exposure to chemicals with a very high potential for toxicity (hazard) may be without risk. A selection based on detection of a chemical in human tissue samples or in biota does also not permit conclusions on potential risks. Analytical chemistry is highly sensitive and detects traces of almost any manmade chemical that is looked for. Also, exposure again has to be connected with the toxicological data of the chemical. Therefore, the Ökoinstitut Freiburg e.V. selection process is not based on the principles of risk assessment and thus does not have scientific validity.

6.2 Specific points regarding TBBPA

Endocrine disruption. Both EU-RARs conclude that “endocrine disruption” is of low relevance regarding TBBPA toxicity (EU-RAR-HH, 2006; EU-RAR-Env, 2008). This conclusion was confirmed by peer review (EC-SCHER, 2005; EC-SCHER, 2008). These conclusions regarding risk assessment have not been changed by newer results on TBBPA since the affinity of TBBPA to a variety of hormone receptors is very low and the low bioavailability of TBBPA indicate that an interaction with hormone receptors is highly unlikely. Regarding the interference of TBBPA with the thyroid hormone axis, the doses required for a measurable biochemical change are well above doses encountered by the human population. Therefore, the Ökoinstitut Freiburg e.V. text is speculative and not supported by the available science.

PBT and vPvB evaluation. As indicated above, the bioconcentration factor derived for TBBPA in the EU-RAR is highly conservative and the available data do not support a significant potential for bioaccumulation. The conclusions regarding monitoring data are not indicative of a bioconcentration as outlined in the EU-RARs and the detailed studies on the toxicokinetics of TBBPA do not show a potential for bioaccumulation. Again, the conclusions of Ökoinstitut Freiburg e.V. are not supported by the available science.

Formation of brominated dibenzo-p-dioxins. The EU-RAR concludes that the additional formation of halogenated dibenzo-p-dioxins and dibenzofurans due to the presence of bromine in TBBPA will only be a minor contribution to total polyhalogenated dioxin and furan formation when waste materials are incinerated or burned. In contrast to the statement of Ökoinstitut Freiburg e.V., risk assessment can be performed on persistent contaminants when appropriate data are available and risk assessment have been performed for a variety of persistent agents such as chlorinated dibenzodioxins or ochratoxin A. The concerns expressed by Ökoinstitut Freiburg e.V. are therefore not well supported.

Concentrations of TBBPA in biota. The data by Johnson-Restrepo presented in table 14 of the Ökoinstitut Freiburg e.V. report are similar to those already considered in the EU-RAR and do not influence the conclusions made there.

Overall conclusion on TBBPA:

- As indicated above, conclusions regarding bioaccumulation of TBBPA and “endocrine disrupting” potential have been performed in the EU-RARs and integrated in the final conclusions. The conclusions in the RARs are clear and well supported by the data available. Integration of the more recently published data show that the conclusions of the EU-RAR remain valid. The database therefore does not support the opinion of Ökoinstitut Freiburg e.V.

References

- Abdallah, M. A., Harrad, S., and Covaci, A. (2008). Hexabromocyclododecanes and tetrabromobisphenol-A in indoor air and dust in Birmingham, U.K: implications for human exposure. *Environ Sci Technol* **42**, 6855-6861.
- Ashizuka, Y., Nakagawa, R., Hori, T., Yasutake, D., Tobiishi, K., and Sasaki, K. (2008). Determination of brominated flame retardants and brominated dioxins in fish collected from three regions of Japan. *Molecular Nutrition & Food Research* **52**, 273-283.
- Canton, R. F., Sanderson, J. T., Nijmeijer, S., Bergman, A., Letcher, R. J., and van den Berg, M. (2006). In vitro effects of brominated flame retardants and metabolites on CYP17 catalytic activity: a novel mechanism of action? *Toxicol Appl Pharmacol* **216**, 274-281.
- Cariou, R., Antignac, J. P., Zalko, D., Berrebi, A., Cravedi, J. P., Maume, D., Marchand, P., Monteau, F., Riu, A., Andre, F., and Le Bizec, B. (2008). Exposure assessment of French women and their newborns to tetrabromobisphenol-A: occurrence measurements in maternal adipose tissue, serum, breast milk and cord serum. *Chemosphere* **73**, 1036-1041.
- Chignell, C. F., Han, S. K., Mouithys-Mickalad, A., Sik, R. H., Stadler, K., and Kadiiska, M. B. (2008). EPR studies of in vivo radical production by 3,3',5,5'-tetrabromobisphenol A (TBBPA) in the Sprague-Dawley rat. *Toxicol Appl Pharmacol* **230**, 17-22.
- De Wit, M., Keil, D., Remmerie, N., van der Ven, K., van den Brandhof, E. J., Knapen, D., Witters, E., and De Coen, W. (2008). Molecular targets of TBBPA in zebrafish analysed through integration of genomic and proteomic approaches. *Chemosphere* **74**, 96-105.
- EC-SCHER (2005). Scientific Committee on Health and Environmental Risks opinion on: Risk Assessment Report on Tetrabromobisphenol-A Human Health Part CAS No.: 79-94-7 EINECS No.: 201-236-9 Adopted by the SCHER during the 7th plenary of 23 September 2005.
- EC-SCHER (2008). Scientific Committee on Health and Environmental Risks opinion on: 2,2',6,6'-TETRABROMO-4,4'-ISOPROPYLIDENE DIPHENOL (TETRABROMOBISPHENOL-A) Environmental Part Adopted by the SCHER during the 21st plenary of 15 January 2008.
- EU-RAR-Env (2008). *European Union Risk Assessment Report. 2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol (tetrabromobisphenol-A or TBBP-A) (CAS: 79-94-7) Part I – environment (final draft).*
- EU-RAR-HH (2006). *European Union Risk Assessment Report. 2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol (tetrabromobisphenol-A or TBBP-A) (CAS: 79-94-7) Part II – human health.* Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, 4th Priority List, Luxembourg: Office for Official Publications of the European Communities.
- Fernandes, A., Dicks, P., Mortimer, D., Gem, M., Smith, F., Driffield, M., White, S., and Rose, M. (2008). Brominated and chlorinated dioxins, PCBs and brominated flame retardants in Scottish shellfish: methodology, occurrence and human dietary exposure. *Mol Nutr Food Res* **52**, 238-249.
- Geens, T., Roosens, L., Neels, H., and Covaci, A. (2009). Assessment of human exposure to Bisphenol-A, Triclosan and Tetrabromobisphenol-A through indoor dust intake in Belgium. *Chemosphere* **76**, 755-760.

- George, K. W., and Haggblom, M. M. (2008). Microbial O-methylation of the flame retardant tetrabromobisphenol-A. *Environ Sci Technol* **42**, 5555-5561.
- Harrad, S., Abdallah, M. A., Rose, N. L., Turner, S. D., and Davidson, T. A. (2009). Current-use brominated flame retardants in water, sediment, and fish from English lakes. *Environ Sci Technol* **43**, 9077-9083.
- Henry, C. J. (2003). Evolution of toxicology for risk assessment. *Int J Toxicol* **22**, 3-7.
- Hu, J., Liang, Y., Chen, M., and Wang, X. (2009). Assessing the toxicity of TBBPA and HBCD by zebrafish embryo toxicity assay and biomarker analysis. *Environ Toxicol* **24**, 334-342.
- Imai, T., Takami, S., Cho, Y. M., Hirose, M., and Nishikawa, A. (2009). Modifying effects of prepubertal exposure to potassium perchlorate and tetrabromobisphenol A on susceptibility to N-bis(2-hydroxypropyl)nitrosamine- and 7,12-dimethylbenz(a)anthracene-induced carcinogenesis in rats. *Toxicol Lett* **185**, 160-167.
- Johnson-Restrepo, B., Adams, D. H., and Kannan, K. (2008). Tetrabromobisphenol A (TBBPA) and hexabromocyclododecanes (HBCDs) in tissues of humans, dolphins, and sharks from the United States. *Chemosphere* **70**, 1935-1944.
- Kang, M. J., Kim, J. H., Shin, S., Choi, J. H., Lee, S. K., Kim, H. S., Kim, N. D., Kang, G. W., Jeong, H. G., Kang, W., Chun, Y. J., and Jeong, T. C. (2009). Nephrotoxic potential and toxicokinetics of tetrabromobisphenol A in rat for risk assessment. *J Toxicol Environ Health A* **72**, 1439-1445.
- Kibakaya, E. C., Stephen, K., and Whalen, M. M. (2009). Tetrabromobisphenol A has immunosuppressive effects on human natural killer cells. *J Immunotoxicol* **6**, 285-292.
- Kuester, R. K., Solyom, A. M., Rodriguez, V. P., and Sipes, I. G. (2007). The effects of dose, route, and repeated dosing on the disposition and kinetics of tetrabromobisphenol A in male F-344 rats. *Toxicol Sci* **96**, 237-245.
- Labadie, P., Tlili, K., Alliot, F., Bourges, C., Desportes, A., and Chevreuil, M. (2010). Development of analytical procedures for trace-level determination of polybrominated diphenyl ethers and tetrabromobisphenol A in river water and sediment. *Anal Bioanal Chem* **396**, 865-875.
- Li, J., Ma, M., and Wang, Z. (2010). In vitro profiling of endocrine disrupting effects of phenols. *Toxicol In Vitro* **24**, 201-207.
- Lilienthal, H., Verwer, C. M., van der Ven, L. T., Piersma, A. H., and Vos, J. G. (2008). Exposure to tetrabromobisphenol A (TBBPA) in Wistar rats: neurobehavioral effects in offspring from a one-generation reproduction study. *Toxicology* **246**, 45-54.
- MacDonald, J. S. (2004). Human carcinogenic risk evaluation, part IV: assessment of human risk of cancer from chemical exposure using a global weight-of-evidence approach. *Toxicol Sci* **82**, 3-8.
- Makinen, M. S., Makinen, M. R., Koistinen, J. T., Pasanen, A. L., Pasanen, P. O., Kalliokoski, P. J., and Korpi, A. M. (2009). Respiratory and dermal exposure to organophosphorus flame retardants and tetrabromobisphenol A at five work environments. *Environ Sci Technol* **43**, 941-947.
- Matthews, J. B., Twomey, K., and 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.
- MPI-Research (2002). An oral two generation reproductive, fertility and developmental neurobehavioural study of tetrabromobisphenol-A in rats (unpublished).

- MPI-Research (2003). Amendment to the final report. An oral two generation reproductive, fertility and developmental neurobehavioural study of tetrabromobisphenol-A in rats (unpublished report).
- Nakagawa, Y., Suzuki, T., Ishii, H., and Ogata, A. (2007). Biotransformation and cytotoxicity of a brominated flame retardant, tetrabromobisphenol A, and its analogues in rat hepatocytes. *Xenobiotica* **37**, 693-708.
- Nakajima, A., Saigusa, D., Tetsu, N., Yamakuni, T., Tomioka, Y., and Hishinuma, T. (2009). Neurobehavioral effects of tetrabromobisphenol A, a brominated flame retardant, in mice. *Toxicol Lett* **189**, 78-83.
- Ogunbayo, O. A., Jensen, K. T., and Michelangeli, F. (2007). The interaction of the brominated flame retardant: tetrabromobisphenol A with phospholipid membranes. *Biochim Biophys Acta* **1768**, 1559-1566.
- Ogunbayo, O. A., Lai, P. F., Connolly, T. J., and Michelangeli, F. (2008). Tetrabromobisphenol A (TBBPA), induces cell death in TM4 Sertoli cells by modulating Ca²⁺ transport proteins and causing dysregulation of Ca²⁺ homeostasis. *Toxicol In Vitro* **22**, 943-952.
- Reistad, T., Mariussen, E., and Fonnum, F. (2005). The effect of a brominated flame retardant, tetrabromobisphenol-A, on free radical formation in human neutrophil granulocytes: the involvement of the MAP kinase pathway and protein kinase C. *Toxicol Sci* **83**, 89-100.
- Reistad, T., Mariussen, E., Ring, A., and Fonnum, F. (2007). In vitro toxicity of tetrabromobisphenol-A on cerebellar granule cells: cell death, free radical formation, calcium influx and extracellular glutamate. *Toxicol Sci* **96**, 268-278.
- Saegusa, Y., Fujimoto, H., Woo, G. H., Inoue, K., Takahashi, M., Mitsumori, K., Hirose, M., Nishikawa, A., and Shibutani, M. (2009). Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. *Reprod Toxicol* **28**, 456-467.
- Schauer, U. M., Volkel, W., and Dekant, W. (2006). Toxicokinetics of tetrabromobisphenol a in humans and rats after oral administration. *Toxicol Sci* **91**, 49-58.
- Shiizaki, K., Asai, S., Ebata, S., Kawanishi, M., and Yagi, T. (2010). Establishment of yeast reporter assay systems to detect ligands of thyroid hormone receptors alpha and beta. *Toxicol In Vitro* **24**, 638-644.
- Snyder, R. W., Maness, S. C., Gaido, K. W., Welsch, F., Sumner, S. C., and Fennell, T. R. (2000). Metabolism and disposition of bisphenol A in female rats. *Toxicol Appl Pharmacol* **168**, 225-234.
- Strack, S., Detzel, T., Wahl, M., Kuch, B., and Krug, H. F. (2007). Cytotoxicity of TBBPA and effects on proliferation, cell cycle and MAPK pathways in mammalian cells. *Chemosphere* **67**, S405-411.
- Strain, G. M., Banasik, M., Hardy, M., and Stedeford, T. (2009). Tetrabromobisphenol A (TBBPA) and model-derived risks for neurobehavioral effects in offspring from a one-generation reproduction study. *Toxicology* **260**, 155-157; author reply 158-161.
- Sun, H., Shen, O. X., Wang, X. R., Zhou, L., Zhen, S. Q., and Chen, X. D. (2009). Anti-thyroid hormone activity of bisphenol A, tetrabromobisphenol A and tetrachlorobisphenol A in an improved reporter gene assay. *Toxicol In Vitro* **23**, 950-954.

- Szymanska, J. A., Piotrowski, J. K., and Frydrych, B. (2000). Hepatotoxicity of tetrabromobisphenol-A: effects of repeated dosage in rats. *Toxicology* **142**, 87-95.
- Tada, Y., Fujitani, T., Yano, N., Takahashi, H., Yuzawa, K., Ando, H., Kubo, Y., Nagasawa, A., Ogata, A., and Kamimura, H. (2006). Effects of tetrabromobisphenol A, brominated flame retardant, in ICR mice after prenatal and postnatal exposure. *Food Chem Toxicol* **44**, 1408-1413.
- Van der Ven, L. T., Van de Kuil, T., Verhoef, A., Verwer, C. M., Lilienthal, H., Leonards, P. E., Schauer, U. M., Canton, R. F., Litens, S., De Jong, F. H., Visser, T. J., Dekant, W., Stern, N., Hakansson, H., Slob, W., Van den Berg, M., Vos, J. G., and Piersma, A. H. (2008). Endocrine effects of tetrabromobisphenol-A (TBBPA) in Wistar rats as tested in a one-generation reproduction study and a subacute toxicity study. *Toxicology* **245**, 76-89.
- Webster, L., Walsham, P., Russell, M., Neat, F., Phillips, L., Dalgarno, E., Packer, G., Scurfield, J. A., and Moffat, C. F. (2009). Halogenated persistent organic pollutants in Scottish deep water fish. *J Environ Monit* **11**, 406-417.