Sundhedsstyrelsen

Dato 27. februar 2014 MDJ

Referat fra møde i Udvalg for Medicinsk Udstyr
Torsdag den 27. februar 2014 kl. 10-12

Deltager:
Gunnar Lose (Lægevidenskabelige Selskaber), Sine Jensen (Forbrugerrådet), Henrik Nielsen (Tandlægeforeningen), Birthe Oldenborg (Radiometer), Geert Amstrup (Lægeforeningen), Martin E. Bommersholdt (Patientombudet), Josefine Thrane Sletten (Dansk Erhverv), Annemarie Hellebek (Danske Regioner), Jesper Jerlang (Dansk Standard), Birgitte Gram Blenstrup (Ministeriet for Sundhed og Forebyggelse).

Fra Sundhedsstyrelsen deltog: Vagn Nielsen, Kristine Rasmussen, Mia Damgaard Sjøgren (referent).

Afbud:
Helle Jacobsgaard (Danmarks Apotekerforening), Mie Rasbech (DI), Anna Marie Høstgaard (Dansk Selskab for Medicinsk Informatik – udtræder af udvalget grundet konkurs). Gunilla Svensmark (Dansk Sygeplejeråd), Jens Oluf Bruun Pedersen (Danske Patienter), Peter Huntley (Medicoindustrien), Torben Mogensen (Dansk Selskab for Patientsikkerhed)

1. **Velkomst**
   Vagn Nielsen bød velkommen.

2. **Godkendelse af referat fra forrige møde**
   Referatet blev godkendt uden kommentarer.

3. **Sundhedsstyrelsens erfaring med rekommandationslister for lægemidler**
   Oplæg ved Enhedschef Søren Brostrøm (Sundhedsstyrelsens Enhed for Sygehus og Beredskab).

   Søren Brostrøm fortalte kort om Institut for Rationel Farmakoterapi (IRF) og præsenterede rekommandationslisterne på hjemmesiden www.irf.dk.
   Rekommandationslisterne udarbejdes for udvalgte områder, først og fremmest til almen praksis, men finder også anvendelse i sygehusvæsenet og i samarbejde mellem primær- og sekundærsektoren. Søren Brostrøm fortalte om formålet, udvælgelseskriterier og hvad der danner basis for anbefalingerne. Dette kan man læse mere om på IRFs hjemmeside.
   Der tages ikke hensyn til prisforhold og tilskud i de nationale rekommandationslister. Dette gøres i de regionale basislister, som udarbejdes af de regionale lægemiddelråd, se www.basislisten.dk

Udvalget spurgte til samarbejde med medicinhåndbogen og lægehåndbogen. Sundhedsstyrelsen forklarede, at Promedicin.dk linker til produkter fra IRF og Sundhedsstyrelsen, herunder 'Nyt Om Bivirkninger'. Lægehåndbogen har endnu ikke forespurgt et samarbejde. Udvalget diskuterede udarbejdelsen og anvendelsen af de nationale rekommandationslister. Der var enighed om, at åbenhed og transparens er vigtigt i forhold til metoden og vurderingen af lægemidlerne – og at der er et behov for at have lignende oversigter for medicinsk udstyr. Udvalget foreslog at teste det på et område, for eksempel tandimplantater.

Sundhedsstyrelsen vil se på mulighederne for en rekommandationsliste for medicinsk udstyr på udvalgte områder, udvalget var interesseret i at bistå i dette arbejde.

4. **Regionernes udbudsproces**
Oplæg ved Johanne Boelskov (Region Hovedstadens, Indkøbsafdeling). Præsentationen er sendt ud til Udvalgets medlemmer i forbindelse med referatet.

Udvalget fandt oplæget meget interessant og diskuterede blandt andet patientinddragelse i regionens brugergrupper og standardisering af operationer. Johanne Boelskov fortalte, at minimumskrav i udbuddet ofte afhænger af de standarder og retningslinjer, som findes på det givne områder - og de efterspurgte større inddragelse af input fra sundhedssektoren i udarbejdelsen af standarder.

Kristine Rasmussen fortalte, at udvalget vil gå videre med emnet omkring udbudsproces, da Peter Skjøt fra Region Hovedstadens enhed for kvalitet og patientsikkerhed kommer til næste møde og fortæller om de udfordringer der kan være, når et udbud er afsluttet.

5. **Status på DaVinci operationsrobot**
Punktet blev flyttet til næste møde, da undersøgelsen endnu ikke er afsluttet.

6. **Høring af SCENIHR udtalelse om " the safety of the use of bisphenol A in medical devices"**
Ifølge SCENIHR er de væsentligste kilder til Bisphenol A fra medicinsk udstyr dental materiale og udstyr af plast som eksempelvis katetre og hæmodialyse udstyr. Ifølge SCENIHR vurderes bisphenol A i dental materiale ikke at udgøre en risiko. For udstyr af plast er der en bekymring, især omkring længerevarende brug til børn, herunder neonatale. Risikoen skal dog opvejes mod behandlingsmulighederne i denne patientgruppe. Kristine Rasmussen opfordrede til at sende høringssvar til SCENIHR.

7. **Forslag af emner til kommende møder**
    - Det danske sprogkrav
    - Opsamling på arbejdet i udvalget, herunder hvad vi fremadrettet skal beskæftige os med
    - Oplæg ved Dansk Standard

8. **Datoer for kommende møder**
Mandag den 2. juni 2014 kl. 10 – 12

9. **Evt.**

Kristine Rasmussen vil udsende en påmindelse til de udvalgsmedlemmer, som mangler at indsende en habilitetserklæring.

Kristine Rasmussen fortalte, at projektet for lægers tilknytning til Medicoindustrien er udskudt til den 1. oktober 2014.

Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Preliminary Opinion on

The safety of the use of bisphenol A in medical devices

SCENIHR adopted this opinion by written procedure on 27 of January 2014

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR).

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

The SCENIHR

This Committee deals with questions related to emerging or newly identified health and environmental risks and to broad, complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies. Examples of potential areas of activity include potential risks associated with interaction of risk factors, synergic effects, cumulative effects, antimicrobial resistance, new technologies such as nanotechnologies, medical devices including those incorporating substances of animal and/or human origin, tissue engineering, blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields (from mobile phones, transmitters and electronically controlled home environments), and methodologies for assessing new risks. It may also be invited to address risks related to public health determinants and non-transmissible diseases.

Scientific Committee members

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http://ec.europa.eu/health/scientific_committees/policy/index_en.htm

ACKNOWLEDGMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

SCENIHR members:

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Prof. Eduardo Rodriguez-Farré - Barcelona Institute of Biomedical Research, Spain

Dr. Emanuela Testai - Istituto Superiore di Sanità, Environment & Primary Prevention Dept., Mechanisms of Toxicity Unit, Roma, Italy (chair of the Working Group since April 2013)

SCCS member:

Dr. Suresh Chandra Rastogi

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All Declarations of working group members and supporting experts are available at the following webpage:

http://ec.europa.eu/health/scientific_committees/emerging/members_wg/index_en.htm

ABSTRACT

Currently many scientific discussions are ongoing on the possible adverse effects of Bisphenol A (BPA). The BPA exposure of the population is mainly via food as a result of the use of BPA in food packaging. The vast majority of the population (91–99%) has detectable levels of BPA-conjugates in their urine. Medical devices are a specific product category in which BPA may also be present. Examples include implants, catheters, and most dental devices. BPA is a key building block of polycarbonate plastic and a precursor for the manufacturing of monomers of epoxy resins. Polycarbonate is used in a wide variety of products including medical devices for its balance of toughness, dimensional stability, optical clarity, high heat resistance and electrical resistance. In addition to polycarbonate medical devices, various dental materials are fabricated from monomers such as bisphenol A glycidyl methacrylate (Bis-GMA) and bisphenol A dimethacrylate (Bis-DMA), derived from BPA. This Opinion describes the risk assessment of exposure to BPA via medical devices, for which the exposure routes are not limited to oral applications.

In the existing evaluations for the oral route of exposure to BPA a No Observed Adverse Effect Level (NOAEL) of 5 mg/kg body weight/day (b.w./day) in rats was established, from which a Tolerable Daily Intake (TDI) of 50 µg/kg b.w./day was derived by using default inter- and intra-species uncertainty factors in the risk assessment. More recently, based on a different approach, EFSA has established a temporary TDI of 5 µg/kg b.w./day. The main focus of these evaluations was on the oral route of exposure as this is the main exposure route for the general population. However, there are still unresolved issues in the risk assessment of BPA after oral uptake. In addition, especially for medical devices manufactured from polycarbonate plastics, other exposure routes such as subcutaneous and intravenous (e.g. during heamodialysis) are important.

After oral exposure BPA is readily absorbed from the gastro-intestinal (GI) tract and due to the first pass effect in the liver is rapidly conjugated to BPA-glucuronide and to a lesser extent to BPA-sulphate. BPA has a low systemic bioavailability (around 1%) and BPA and its conjugates are eliminated in humans in a half life time of a few hours. At variance for all the parenteral routes of exposure (including intravenous, intraperitoneal, or subcutaneous), that may be relevant for medical devices, the chemical can be considered 100% systemically bioavailable. However, BPA will also be conjugated in the liver and the clearance of free BPA from the circulation appears to be relatively fast.

There are several indications that BPA might have biological effects below the current NOAEL of 5 mg/kg b.w./day and the recently determined bench mark dose low$_{10}$ (BMDL$_{10}$) of 3.76 mg/kg b.w./day. However, the evidence has been mainly obtained in dedicated studies focussing on specific outcome parameters like adiposity and hormone levels, and not in general toxicity studies. Some of those parameters resulted in contradicting results in various studies like a decrease, no increase or increase in weight. Furthermore, dose-response relationships could not be established. Regarding possible low dose effects the studies raise some concern for prenatal BPA exposure and an effect on mammary gland development and on behaviour/anxiety, the relevance of which for human health is not clear. In addition, the possible effects on metabolism and adiposity also need further investigations in large scale studies with a wide dose range of BPA. So far, the epidemiological studies performed do not provide consistent outcomes to conclude on possible human health effects. Further extensive dose-response and/or epidemiological studies are needed to confirm or negate these observations and their relevance for human health effects, Therefore, SCENIHR considered that the recently derived temporary oral TDI (t-TDI) based on general toxicity studies still represents a solid base for carrying out a BPA risk assessment for the use of BPA in medical devices.

For medical devices, several exposure scenarios were evaluated such as external short-term contact with a medical device, short and long-term contact with dental materials, medium and long-term contact with an implanted medical device, long-term contact via hemodialyzers and medium-term contact in intensive care units with various medical devices. The highest estimated BPA exposures occurred during prolonged medical procedures in infants (685 ng/kg b.w./day), and for prematurely born infants in neonatal intensive care units (3000 ng/kg b.w./day, 3 µg/kg b.w./day). Contact with dental materials gave an estimated short-term (<24 hours) exposure of 140 to 200 ng/kg b.w./day for respectively children and adults, whereas long-term exposure ranges from 2 to 12 ng/kg b.w./day. Some of the estimated BPA exposures due to medical devices are in the same range as exposure to BPA via the food (EFSA 2013). However, exposure due to medical devices generally occurs for a limited period of time, resulting in a lower overall exposure.

In general, it can be concluded that the long-term oral exposure via dental material is far below the recently derived temporary oral external TDI of 5 µg/kg b.w./day derived from animal studies and poses no risk for human health. In addition, short-term (relatively high) exposures to dental materials are below this recently established temporary TDI (t-TDI).

The parenteral exposure via medical devices, taking treatment of neonates in intensive care units as the worst case scenario, is before adjustment for route specific systemic availability, below the oral t-TDI of 5 µg/kg b.w./day derived from the $BMDL_{10}$ of 3.76 mg/kg b.w./day in animal studies. However, the kinetic differences between routes of exposure indicate that the bioavailability after oral route of exposure is significantly lower when compared to the parenteral one. Based on the analysis of oral versus intravenous toxicokinetic data, the oral systemic bioavailability of unconjugated BPA is 2.8%, 0.2%, 0.9% and less than 1% in rats, mice, monkeys, and dogs, respectively. The systemic availability of unconjugated BPA in humans has not been evaluated experimentally, however, modelled data as well as controlled biomonitoring studies indicated that internal exposure in humans to unconjugated BPA is very low (1-10%). Therefore, the SCENIHR considered it appropriate to make the comparison using the internal dose rather than the external one. Considering the internal BPA exposure for the worst case scenario (the estimated exposure in neonatal intensive care units of 3 µg/kg b.w./day), and using a 100% bioavailability of BPA for the exposure via medical devices, the internal exposure is higher than the internal exposure based on the t-TDI (being 0.05 µg/kg b.w./day as 1% bioavailability – taken as worst case - of 5 µg/kg b.w./day). However, when comparing this systemic exposure due to medical devices (3 µg/kg b.w./day) against the internal exposure of a dose at the $BMDL_{10}$ in rats of 3.76 mg/kg b.w./day (37.6 µg/kg b.w./day), the highest internal exposure of BPA via medical devices is about 12-fold lower than the internal dose of the $BMDL_{10}$ observed in rats. The factor of 12 is lower than the usual safety factor of 100 for assessing a margin of safety (MOS) when extrapolating low to no risk exposure doses for humans based on results obtained in animal studies. For prolonged medical procedures in infants with an estimated exposure of 685 ng/kg b.w./day, the margin of safety is 55, while for the other long and short-term exposure scenarios estimated for medical devices, the MOS is well above 100.

Based on these data. it is concluded that there may exist some risk for adverse effects of BPA, when the BPA is directly available for systemic exposure after non-oral exposure routes especially when neonates in intensive care units are concerned. However, better data on exposure would be beneficial for the refinement of this risk assessment. In addition, the controversial issues regarding possible effects at low dose and their relevance for human health, especially after prenatal and/or perinatal exposure, do raise some concern for exposure to BPA via medical devices especially in prematurely born infants. Further research under well controlled exposure conditions is warranted to confirm or negate these possible low dose effects in animal models and their relevance for human health. The currently performed studies by the FDA's National Center for

Toxicological Research in the USA, with animals under a strict exposure regimen, may clarify some of these controversial issues.

It should be realised that the benefit of medical devices has also to be considered: the survival of these premature infants often depends on the availability of the same medical devices which result in a relatively high BPA exposure due to treatment. The possibility to replace BPA in these products should be considered against their efficiency in the treatment, as well as the toxicological profile of the alternative materials.

Keywords:

Opinion to be cited as:

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), **Safety of the use of bisphenol A in medical devices**, Date of adoption

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EXECUTIVE SUMMARY

Background

Currently, many scientific discussions are ongoing on possible adverse effects of BPA. The BPA exposure of the population is mainly via food as a result of the use of BPA in food packaging. Medical devices are a specific product category in which BPA may also be present. Examples include implants, catheters, and most dental devices. Some BPA-containing medical devices may have direct and/or indirect contact with patients (e.g. hemodialyzer apparatus, filters, bypasses, tubing, pumps, instruments, surgical equipment, blood pathway circuits and respiratory tubing circuits). This Opinion describes the risk assessment of exposure to BPA via medical devices, for which the exposure routes are not limited to oral applications.

What is BPA?

Bisphenol A [Bis(4-hydroxyphenyl)propane], BPA is a high-production volume industrial chemical. BPA is a key building block of polycarbonate (PC) plastic and a precursor for the manufacturing of monomers of epoxy resins. Polycarbonate is used in a wide variety of products including medical devices for its balance of toughness, dimensional stability optical clarity, high heat resistance and electrical resistance. In addition to polycarbonate medical devices, various dental materials are fabricated from monomers such as bisphenol A glycidyl methacrylate (Bis-GMA) and bisphenol A dimethacrylate (Bis-DMA), derived from BPA. BPA-resins are also used in inks and adhesives. In addition to BPA itself, polymers produced using BPA like polysulfone (PSU) used in medical devices are considered because they can release BPA. For example, the BPA derived polysulfone is used as membrane in hemolysis dialyzers.

Previous risk assessments

In the existing evaluations, the following conclusions have been drawn for the oral route of exposure to BPA:

- No Observed Adverse Effect Level (NOAEL) of 5 mg/kg b.w./day in rats
- Tolerable Daily Intake (TDI) of 50 µg/kg body weight
- developmental toxic effects only observed at doses with severe maternal toxicity in rats and mice
- an overall NOAEL for reproductive toxicity of 50 mg/kg b.w./day in rats and mice
- in terms of toxicokinetics, there is a difference between rats and humans (the latter presenting a shorter half-life) as well as between the oral and the parenteral route of exposure.
- due to the first pass effect, after oral uptake, the systemic exposure to free BPA is a small fraction of the external dose in all species.
- there are still unresolved issues in the risk assessment of BPA after oral uptake.

More recently, EFSA established a temporary (t)-TDI of 5 µg/kg b.w./day for oral exposure to BPA based on kidney alterations as the critical effect (EFSA 2014).

The main focus of these evaluations was on the oral route of exposure. Especially for medical devices manufactured from polycarbonate plastics, other exposure routes such as subcutaneous and intravenous (e.g. during haemodialysis) are important.

General exposure

The human population is exposed to BPA mainly through the diet, while air, dust, and water, including skin contact with thermal paper, are other possible sources of exposure. Bisphenol A in food and beverages accounts for the majority of daily human exposure. BPA exposure results from either the release of non-polymerized monomers or from the slow decay of polymer bonds in polycarbonate leading to monomer release into foods and liquids. A number of studies in various countries have indicated that the vast majority of the population (91–99%) has detectable levels of BPA-conjugates in their urine. The measured BPA levels reflect the recent exposure of the past several hours just before the

sample collection in view of the rapid conjugation and elimination half-time of BPA in blood of a few hours. Free and conjugated BPA levels in blood are typically ≤ 1 µg/L, which is consistent with the known rapid plasma clearance of BPA and its metabolites and kinetic studies conducted in humans.

Notably, regarding BPA determination, the analytical method used to detect both the parent compound and its metabolites is crucial especially at the low levels expected in biological samples. Therefore, the sampling and analytical methods used represent a relevant source of differences among available studies. A potential artifact in BPA measurements is the leakage of BPA from the labware used, which results in sample contamination.

In urine, BPA is present mainly in its conjugated form. Urinary biomonitoring data provide information on the internal dose, which is the result of total BPA exposure, independently from the sources. Therefore, biomonitoring data in urine account not only for dietary exposure, but also for non-food sources (e.g. medical devices, dust, thermal and other kinds of papers). Since BPA urinary excretion is almost complete within 24 hours after exposure and due to the less invasive sampling, urine is the matrix of choice for assessing daily exposure to BPA in humans. Urine BPA levels depend on frequency of exposure (e.g. food intake, treatment with medical devices), time of sampling after exposure, the last urination and urine production rate. Blood concentrations of total BPA (free plus conjugates) determined at one time point are not representative of an average exposure, because it is strongly dependent on the time of blood sampling with respect to the exposure time.

On the basis of available biomonitoring and exposure data, it was recently concluded that the exposure to BPA from non-food sources that by some authors was hypothesized as potentially relevant sources, is generally lower than that from exposure from food by at least one order of magnitude for most studied general population subgroups. Dietary exposure was indeed estimated to contribute more than 90% to the overall BPA-exposure for non-occupationally exposed individuals. However, few data are available for patients treated with BPA-containing medical devices.

For risk assessment purposes, the bioavailability of free BPA is crucial as this is the active compound. However, data on both free and conjugated BPA are required to assess the exposure and fate of BPA.

Exposure from medical devices

Medical devices based on polycarbonate and polysulfone due to their chemistry can contain BPA residues, whereas others like PVC based medical devices may or may not contain BPA residues depending on their production method. In addition, some other BPA-derivatives (such as epoxy resins) are used specifically in dental materials. The major factor influencing the residual amount of BPA levels is the employment of incorrect operating conditions during the processing step. Moreover, breakdown or hydrolysis of the polycarbonate polymer after manufacturing can occur, thus giving rise to the free monomer from the polymer available for exposure. In polycarbonate articles used for food contact, the residual content is usually less than 10 µg/g of polycarbonate (ECB, 2003).

Exposure can be estimated by either measuring the BPA content of the medical devices or by extraction assays for potential release. Extraction of BPA was much more prominent in aqueous ethanol (17.2% v/v) and bovine serum (mimicking human serum) than in water. For PC casings of hemodialyzers and hollow fibres used in hemodialyzers, extracted amounts of BPA were reported up to 12.2 mg/kg material. Under simulated use conditions, release in bovine serum was up to 2090 ng/dialyzer, and in aqueous ethanol (17.2% v/v) up to 4300 ng/dialyzer. For dental materials, the leakage is limited to resins composed of Bis-DMA (Bisphenol A dimethylacrylate) which has an ester linkage that can be hydrolysed to BPA, whereas the ether linkage in Bis-GMA (Bisphenol A glycidyl methacrylate) was found to be stable.

For BPA exposure resulting from the use of medical devices, little information is available. For the placing of dental composite resin restorations, measurements have shown that the release of BPA mainly occurs during the few hours directly after application while the BPA level is back to pretreatment levels at 24 hours. Values measured were up to 30 µg/mL saliva, and 931 µg in total saliva volume produced in one hour. Calculations based on the actual amount of material used in clinical practice and a median 4-year life-time of a composite restoration, suggests a maximum exposure of 0.06 µg BPA/day from fissure sealants, and a maximum exposure of 0.36 µg BPA/day from composite restorations. Contact with dental materials gave an estimated short-term (<24 hours) exposure of 140 to 200 ng/kg body weight per day for children and adults, respectively, whereas long-term exposure ranges from 2 to 12 ng/kg b.w./day. These BPA releases contribute to the oral exposure to BPA.

Measurements in dialysis patients found total BPA values up to 6.6 ng/mL blood. In prematurely born infants undergoing intensive therapeutic medical interventions, for BPA a geometric mean urinary concentration of 30.3 µg/L (ng/mL) was observed with the highest value measured 946 µg/L (ng/mL), which was about ten times higher than that among children 6-11 years old. More than 90% of the BPA detected in the urine of the prematurely born infants was in its conjugated (e.g. glucuronide, sulphate) form.

The highest exposures estimated occurred during i) prolonged medical procedures in infants (685 ng/kg body weight per day), ii) the treatment of adults with medical devices consisting of BPA containing PVC (1000 ng/kg b.w./day), iii) treatment of prematurely born infants in neonatal intensive care units (NICU) (3000 ng/kg b.w./day), and iv) treatment of prematurely born infants with medical devices consisting of BPA containing PVC (7000 ng/kg body weight per day, 7 µg/kg body weight per day). Short-term exposure via medical devices consisting of BPA containing PVC might even be higher (adults up to 5000 ng/kg b.w./day, infants up to 12000 ng/kg b.w./day). However, it is worth noting that exposure to BPA via BPA-containing PVC has been estimated based on extrapolation from data on phthalate leakage from PVC and are, therefore, affected by a high degree of uncertainties.

Contact with dental materials gave an estimated short-term (<24 hours) exposure of 140 to 200 ng/kg body weight per day for respectively an infant and an adult.

Taking into account the many possible sources of exposure of patients during hospital care and the scarcity of information related to release of BPA from medical devices, six critical exposure scenarios were evaluated to estimate potential exposure to BPA from medical devices. Some of the estimated BPA exposures due to medical devices are in the same range as exposure to BPA via food (high exposure for population older than 6 months 857 ng/kg b.w./day, high exposure for infant day 1-5 after birth 495 ng/kg b.w/day) (EFSA 2013). However, exposure associated to medical devices use generally occurs for a limted period of time with the exception of haemodialysis practices.

BPA metabolism and toxicokinetics in humans

The major BPA metabolite in humans is BPA-glucuronide, which is quantified in plasma and rapidly excreted in the urine; BPA-sulphate has been also detected after oral exposure as a minor metabolite. After oral exposure, there is a very high first pass effect in the liver that results in very small amounts of unchanged parent BPA. In humans, a polymorphism exists for the conjugation of BPA. However, the polymorphism was found to result in a 4-5-fold variability of plasma BPA. Therefore, it can be considered that the default value used in the risk assessment to account for kinetic interindividual variability within the general population can cover the differences due to polymorphically expressed enzyme activity involved in BPA metabolism.

Studies on toxicokinetics of BPA available to date have demonstrated a significantly lower internal exposure to free BPA after oral intake as compared to parenteral exposure. This is essentially due to the highly efficient pre-systemic conjugation to glucuronide and sulphate, which occurs mainly in the liver and partially in the gut after oral administration

independently on the species. Thus, the internal exposure to free BPA after oral intake is lower as compared to dermal or parenteral exposure, although for the latter routes of exposure, the biotransformation (mainly in the liver) quickly diminishes free circulating BPA.

After oral exposure, both low and high single oral doses of BPA are well absorbed (>90%), but the systemic bioavailability of free BPA after oral exposure is reduced by the first pass effect. Based on the analysis of oral versus intravenous toxicokinetic data (Doerge et al., 2010a, 2010b; 2011; 2012), the oral systemic bioavailability of unconjugated BPA is 2.8%, 0.2%, 0.9% and less than 1% in rats, mice, monkeys, and dogs, respectively. The systemic availability of unconjugated BPA in humans has not been evaluated experimentally, however, modelled data as well as controlled biomonitoring studies indicated that internal exposure in humans to unconjugated BPA is very low (1-10%) (Mielke and Gundert-Remy, 2012; ANSES, 2013). The conjugates are readily excreted in the urine, as a consequence the half-life of BPA in humans is very short, ranging from 1 to 3.5 hours. After dermal exposure, the absorption fraction can be considered approximately 25-30% of the applied dose, which is directly systemically bioavailable.

For all the parenteral routes of exposure (including intravenous, intraperitoneal, transdermal or subcutaneous), the chemical is 100% systemically bioavailable: however, the clearance of free BPA from the circulation appeared to be quite fast, as indicated by controlled studies in non human primates and rats with >50% of circulating BPA already conjugated 5 min after intravenous injection, and showing a half-life of 0.66 h in rats.

The available modeled data, obtained after oral exposure, indicate that newborns and babies up to 6 months constitute a potentially susceptible subpopulation due to potential immature BPA metabolism. However, the default uncertainty factor which is used to account for the toxicokinetic variability in the general population seems to be large enough to cover the variability in the newborn population exposed via the oral route.

Pharmacokinetics in animals

Rats show a prolonged clearance of BPA due to the existence of the so-called entero-hepatic recirculation. After uptake from the GI-tract in rats there is a high degree of conjugation of BPA in the liver. However, BPA is excreted from the liver via bile into the GI-tract where it can be cleaved again resulting in free BPA that can be recirculated or excreted via the faeces. Thus, there may be a higher exposure to free BPA especially in neonatal animals after a specific oral dose when compared to humans. It may be considered that neonatal effects studies in mice and rats may over-predict adverse outcomes in humans.

Toxicity of BPA

Several toxicity studies have been performed in rodents and dogs. BPA was found to be of low acute toxicity for all routes of exposure relevant to human health. The lowest NOAEL after oral repeated exposure was indicated in the previous evaluations as approximately 5 mg/kg b.w./day, based on effects on the liver as target organ, as identified in several studies, including multigeneration reproductive toxicity studies. The next lowest NOAEL for oral exposure was 50 mg/kg b.w./day, based on toxic effects on the kidney and reproductive toxicity.

By applying the benchmark dose (BMD) approach, EFSA recently calculated a BMDL10 of 3.76 mg/kg b.w./day using data from the same multigeneration reproductive toxicity study in mice from which the NOAEL of 5 mg/kg b.w./day was previously derived (EFSA 2014). The $BMDL_{10}$ represents the lower level of the confidence interval of the effect resulting in a 10% deviation from vehicle-treated control animals. The critical endpoint (i.e. appearing at the lowest dose) for this $BMDL_{10}$ was the alteration in kidney weight.

BPA is not a mutagen in *in vitro* test systems, nor does it induce cell transformation. BPA has been shown to affect chromosomal structure in dividing cells in *in vitro* studies, but evidence for this effect in *in vivo* studies is inconsistent and inconclusive. In addition, BPA

was found to be genotoxic in *in vitro* micronucleus assays. These findings were not confirmed by *in vivo* studies. Therefore, BPA is not likely to pose a genotoxic hazard to humans.

In standard carcinogenic OECD testing protocols, BPA has no carcinogenic activity. In addition, in multigeneration studies, no indication of increased cancerogenicity was observed. Studies using subcutaneous administration of BPA indicated that BPA may have the ability to increase the effects of well-known carcinogens even at very low BPA levels. These studies had limitations which render them unsuitable to assess whether BPA itself has a carcinogenic potential by prenatal or perinatal exposure.

Prenatal exposure to BPA by subcutaneous injection in pregnant rats induced mammary gland alterations in the offspring including cell proliferation, some described as pre-neoplastic and neoplastic lesions. Similar results were obtained in mouse studies and results observed in rhesus monkeys also indicated alterations of glandular tissue in the mammary gland after prenatal exposure. However, the variability in mammary gland development in this species makes it difficult to draw clear conclusions for the risk assessment. In contrast, similar alterations were not observed in the pups of mouse multigeneration studies with continuous oral BPA exposure.

In summary, at present there are no indications for carcinogenic effects of BPA in OECD guideline studies, but there are some effects observed in the mammary gland. The observed effects on mammary gland development raise some concern and do need further investigation, as the biological significance of such alterations is currently unknown.

The interpretation on neurological effects of BPA is uncertain. Studies on anxiety (rodent and non-human primate) have a behavioural endpoint which is highly dependent on study design, testing apparatus, inclusion of only one sex, and age at examination. In several studies, increased anxiety was observed. However, there is uncertainty with regard to the interpretation of the data. Recent data indicate sex-dimorphic effects of BPA on social behaviour. However, it is uncertain whether such an effect could be considered adverse for humans. Additionally, gene expression in the brain was altered after both prenatal BPA exposure and BPA exposure in adult mice. Other effects described in the recent studies may indicate that the effects observed with BPA on hypothalamic organization involves mechanisms different from its estrogenic action because they are very different from those of oestradiol which was used as positive control. The variety of read-out parameters and the effects observed warrant further investigation of the possible neurological and behavioural effects of BPA, as well as their relevance for humans, displaying a different pattern of brain development when compared to rodents.

BPA is able to elicit skin sensitization in humans, probably because it is a weak sensitiser. Studies on a possible relationship between prenatal and/or postnatal BPA exposure and allergic responses are not consistent. Although effects on the immune system are suggested, there is uncertainty on the immunotoxicity of BPA. In view of the suggested effects of BPA on the immune system, further investigation to determine potential immunotoxicity of BPA is warranted.

The toxicological data do not indicate a clear effect of BPA on cardiovascular function.

Several published studies have directly addressed the issue of whether developmental exposure to BPA can affect adiposity, glucose or insulin regulation, lipid profiles or other endpoints related to diabetes or metabolic syndrome. Animal studies, however, have shown an increase, a decrease and no effect on body weight. The discrepancy among the various animal studies may arise from variation in experimental conditions, such as the dosing regimen, animal species and strains, and the timing of evaluation of effects. A number of studies in prenatally- and postnatally- exposed rats and mice suggest that BPA exposure has an effect on metabolic function. In some of the studies the findings have been claimed as evidence of a non-monotonic dose-response, as effects seen at a lower dose were not observed at higher doses. However, these effects were only seen at

one dose level. There are no studies which demonstrate effects of different effect size at two dose levels and no or a reduced effect at a higher dose, thus corroborating the existence of non-monotonicity. There is, however, no convincing evidence that BPA is obesogenic later in life after intrauterine exposure or in longer-term studies. Thus, regarding a metabolic effect of BPA. no clear conclusions can be drawn at present due to a lack of consistent evidence. Additionally, in epidemiological studies, inconsistent results were obtained. Therefore, this issue still warrants further investigation.

A large number of studies is available on the effects of BPA on reproduction and prenatal development, some of which performed according to internationally agreed guidelines and compliant to GLP principles. A wealth of *in vitro* results and studies on non-intact animals (such as ovariectomised rodents) is available, but their value for risk assessment is questionable. There are also uncertainties as to reproducibility of several individual studies. These studies were conducted in rats and mice. Female reproductive toxicity after oral exposure occurred with an overall NOAEL of 50 mg/kg b.w./day and a LOAEL of 500 mg/kg b.w./day. However, at the LOAEL for female reproductive effects, significant body (or organ) weight reduction and hepatic toxicity occurred (i.e. the effects used as Point of Departure for the TDI derivation). As to developmental toxicity, BPA does not cause malformations or birth defects in rats or mice at levels up to the highest doses evaluated: 640 mg/kg/day (rats) and 1250 mg/kg/day (mice). Therefore, it can be concluded that BPA is essentially not a specific reproductive or developmental toxicant. General toxicity effects such as body and organ weights, liver histopathology and nephropathy often occur simultaneously with reproductive or developmental effects, which are only observed at high dosages.

In conclusion, there are several indications that BPA does have biological effects below the current NOAEL of 5 mg/kg b.w./day (oral exposure) and the recently calculated $BMDL_{10}$ of 3.76 mg/kg b.w./day (oral exposure). However, the evidence has not been obtained in general toxicity studies, but mainly in dedicated studies focussing on specific outcome parameters like adiposity and hormone levels. The effect on some of those parameters resulted in contrasting results in various studies (e.g. decrease, no effect or increase in body weight). In addition, dose-response relationships could not be established.

Regarding possible low dose effects, the studies raise some concern for prenatal BPA exposure. In addition, the possible effects on metabolism and adiposity need further investigation in large scale studies with a wide dose range of BPA.

Epidemiological studies

There are a limited but increasing number of epidemiological studies that investigated an association between BPA exposure and health outcomes. Studies have also been performed to determine potential altered behavior after dental BPA exposure. Epidemiological studies regarding effects of BPA on metabolic disorders and/or obesity show inconsistent results. Most of them use cross-sectional designs which are not suitable for establishing a cause-effect relationship, especially for outcomes that have long latency periods (e.g. cardiovascular disease, diabetes). Many of these cross-sectional analyses have several important weaknesses that limit their interpretation and relevance. For instance, a major limitation is the use of a single spot urine sample that reflects recent BPA exposure only (past several hours) and may not adequately measure BPA exposure during the relevant etiological window for health outcomes like cardiovascular disease and diabetes, which might be years or decades earlier.

For further clarification and interpretation of the relationship between BPA exposure and adverse health effects, additional studies are needed. These should include prospective studies with serial exposures to BPA assessed during etiologically relevant windows, years before development of disease, and thus prolonged follow up periods. Specific recommendations for use of existing data include the development of conversion models for the different media used for measurement of persistent chemicals, and inter-laboratory comparisons and calibrations. Recommendations for further data collection on

BPA include: i) better evaluation of exposure, especially in children; ii) repeated measurements over time; iii) validation and harmonization of questionnaires; and iv) adequate detection methods.

Conclusions on health effects

Regarding potential health effects of BPA exposure and the level of exposure, several controversial issues remain, as there are indications, although not fully convincing, that BPA has biological effects below the NOAEL of 5 mg/kg b.w./day (oral repeated exposure) and the recently calculated $BMDL_{10}$ of 3.76 mg/kg b.w./day (oral repeated exposure). Regarding possible low dose effects, the studies raise some concern for prenatal BPA exposure and an effect on mammary gland development and altered behaviour/anxiety, although the results are not sufficiently robust to be used in risk assessment. In addition, the possible effects on metabolism and adiposity need further investigation in large scale studies with a wide dose range of BPA. Also effects of BPA on behaviour/anxiety need further investigations. To date, available epidemiological studies performed do not provide consistent outcomes to conclude on possible human health effects. The currently performed study by the FDA's National Center for Toxicological Research (NCTR) in the USA with animals under a strict exposure regimen as well as the studies to be conducted on these animals by various research groups may clarify some of these controversial issues.

Alternatives for BPA

Several alternatives for BPA exist and are increasingly used, notably Bisphenol S and Bisphenol F and some halogenated bisphenol A derivatives (e.g. tetrachlorobisphenol A and tetrabromobisphenol A). For some of the alternatives, similar effects as for BPA were reported regarding endocrine activity in *in vitro* assays, although with reduced activity/potency when compared to BPA. The general toxicological profile of alternatives is much less known.

Recommendations for research

The risk of BPA exposure from medical devices is associated with the release of BPA from these devices, although the actual level of exposure is poorly characterised and deserves further investigation. For sterilization of medical devices, it is known that steam sterilization may result in release of BPA from PC medical devices. Whether ethylene oxide (EtO) sterilization induces release of BPA from PC or PSU medical devices is yet unknown. Therefore, research into the use and consequences of EtO sterilization with regard to BPA release is also recommended.

At present, there are no indications for carcinogenic effects of BPA in OECD guideline studies. However, some studies investigating possible low dose effects raise some concern for prenatal BPA exposure and an effect on mammary gland development. The observed effects on mammary gland development need further investigation because the biological significance of these alterations is currently unknown.

Although effects on the immune system are suggested, the data are insufficient to draw final conclusions on immunotoxicity of BPA. In view of these suggested effects of BPA on the immune system, further investigation of potential immunotoxicity of BPA is warranted.

There is no convincing evidence that BPA effects metabolism and adiposity, or is obesogenic later in life after intrauterine exposure or in longer-term studies. Thus, regarding a metabolic effect of BPA, no clear conclusions can be drawn at present due to a lack of consistent evidence. Additionally, epidemiological study results were inconsistent . Therefore, the effects of BPA on metabolism warrants further investigation.

The currently performed study by the FDA's National Center for Toxicological Research (NCTR) in the USA with animals under a strict exposure regimen and the studies to be conducted on these animals by various research groups may clarify some of these controversial issues and give indications for specific research priorities.

Human studies should include prospective epidemiological studies with serial exposures to BPA assessed during etiologically relevant windows, years before development of disease, and prolonged follow up periods. Specific recommendations for use of existing data include the development of conversion models for the different media used for measurement of persistent chemicals, inter-laboratory comparisons and calibrations. Recommendations for further data collection on BPA include: i) better evaluation of exposure, especially in children; ii) repeated measurements over time; iii) validation and harmonization of questionnaires; and iv) adequate methods of detection.

Conclusions on medical devices

For medical devices, several exposure scenarios were evaluated such as external short-term contact with a medical device, short and long-term contact with dental materials, medium and long-term contact with an implanted medical device, long-term contact via hemodialyzers and medium-term contact in intensive care units with various medical devices. Exposure due to medical devices generally occurs for a limited period of time, with the exception of haemodialysis patients. Indeed, for implanted medical devices, the release of BPA is higher after the implantation and rapidly decreases with time to zero levels (kinetic being determined by the BPA initial content). BPA may be 100% systemically bioavailable following a parenteral exposure route (depending on the type of medical device); however, the bioavailability of free BPA after oral exposure is much lower (1-10% of the ingested dose).

The highest exposures estimated occurred during prolonged medical procedures in infants (685 ng/kg body weight per day) and prolonged exposure in the neonatal intensive care unit (NICU) for treatment of prematurely born infants (3000 ng/kg body weight per day). The latter exposure is about 6-fold the dietary exposure of infants at days 1-5 after birth (high exposure for infants day 1-5 after birth 495 ng/kg b.w/day).

Contact with dental materials gave an estimated short-term (<24 hours) oral exposure of 140 to 200 ng/kg body weight per day for children and adults, respectively, whereas long -term exposure ranges from 2 to 12 ng/kg b.w./day. Some of the estimated BPA exposures due to medical devices are in the same range as exposure to BPA via food (EFSA 2013).

It can be concluded that the oral long-term exposure via dental material is far below the recently determined temporary oral TDI of 5 µg/kg b.w./day derived from animal studies (EFSA 2014) and pose no risk for human health. The same applies to short-term (relatively high) exposure to BPA released from dental materials that is still below the recently established t-TDI, also considering that the peak of release is limited to few hours after application.

For the risk assessment, the exposure data of prematurely born infants in a NICU are used (3000 ng/kg b.w/day) as the worst case. The scenario for exposure to BPA via use of medical devices consisting of BPA containing PVC would result in a potential estimated higher exposure (up to 7000 ng/kg b.w/day) for these prematurely born infants. However, it is worth noting that in the absence of data, exposure to BPA via BPA-containing PVC has been estimated based on extrapolation from data on phthalate leakage from PVC and are, therefore, affected by a high degree of uncertainty. In addition, European PVC manufacturers do not use BPA in their PVC production. Hence, it is unlikely that such a high BPA exposure will be reached due to the use of medical devices consisting of BPA containing PVC.

Considering possible internal exposures and bioavailability of free BPA for the worst case scenario as estimated exposure via medical devices (3 µg/kg b.w./day with 100% systemic bioavailability), the systemic exposure is about 60-fold higher when compared to the internal exposure to free BPA using the oral t-TDI (being 0.05 µg/kg b.w./day based on a t-TDI of 5 µg/kg b.w./day with 1% systemic bioavailability).

When this worst case scenario estimated systemic exposure due to medical devices is compared against the oral $BMDL_{10}$ in rats of 3.76 mg/kg b.w./day, the internal exposure via medical devices (3 µg/kg b.w./day) is about 12-fold lower than the internal exposure of the oral $BMDL_{10}$ observed in rats (37.6 µg/kg b.w./day). The factor of 12 is lower than the usual safety factor of 100 for assessing a margin of safety (MOS) when extrapolating low to no risk exposure doses for humans from results obtained in animal studies. For prolonged medical procedures in infants with an estimated exposure of 685 ng/kg b.w./day, the margin of safety is 55, while for the other exposure scenarios estimated, the MOS is well above 100.

Based on these data, it is concluded that there may exist some risk for adverse effects of BPA when the BPA is directly available for systemic exposure after non-oral exposure routes especially in neonates. However, better data on exposure would be beneficial for the refinement of this risk assessment. In addition, the controversial issues regarding possible low dose effects and their relevance for human health, especially after prenatal and/or perinatal exposure do raise some concern for exposure to BPA via medical devices, especially in prematurely born infants. Further research under well controlled exposure conditions is warranted to confirm or negate these possible low dose effects in animal models and their relevance for human health.

It should be realised that the benefit of medical devices should also be considered: the survival specifically of these prematurely born infants often depends on the availability of the same medical devices which result in a relatively high BPA exposure due to treatment. The possibility to replace BPA in these products should be considered against their efficiency in the treatment, as well as the toxicological profile of alternatives.

1. BACKGROUND

Bisphenol A (BPA) is an intermediate that is mainly used in combination with other chemicals to manufacture plastics and resins. For example, BPA is used in polycarbonate, a high performance transparent, rigid plastic used to make food containers, such as returnable beverage bottles, tableware (plates and mugs) and storage containers. Residues of BPA are also present in epoxy resins used to make protective coatings and linings for food and beverage cans and vats. BPA can migrate in small amounts into food and beverages stored in materials containing the substance.

BPA is a weak oestrogen, as demonstrated by *in vitro* studies. Many *in vivo* studies have been performed to examine its potential effects on reproduction and development. The safety of BPA in food contact materials has been evaluated by the US Food and Drug Administration[1] and by the European Food Safety Authority[2]. Although these evaluations did not identify outright reasons for concern, a number of uncertainties in the current scientific knowledge concerning the safe use of BPA remain. Considering these remaining uncertainties, especially with regard to the potential adverse health effects of BPA exposure to infants through polycarbonate baby bottles, the European Commission decided on the basis of the precautionary principle that all baby bottles on the EU market containing BPA should be replaced by the middle of 2011.

Recently, safety concerns have been expressed for vulnerable groups such as infants, pregnant and breast-feeding women exposed to BPA through other products. Medical devices are a particular product category in which BPA is often found. Examples include implants, catheters, and most dental devices. Some BPA-containing medical devices may have direct and/or indirect contact with the patients (e.g. autotransfusion apparatus, filters, bypasses, tubing, pumps, instruments, surgical equipment, blood pathway circuits and respiratory tubing circuits). These products are used on all types of patients e.g. adults, children etc.

Due to the common use of polycarbonate plastic and epoxy resins in such a wide range of products, low level human exposure to BPA occurs, but the health significance of the exposure levels has been controversial.

According to Council Directive 93/42/EEC, medical devices may only be placed on the market if they meet the essential requirements laid down in its Annex I. The devices must be designed and manufactured in such a way that, when used under the conditions and for the purposes intended, they will not compromise the clinical condition or the safety of patients, or the safety and health of users or, where applicable, other persons, provided that any risks which may be associated with their use constitute acceptable risks when weighed against the benefits to the patient and are compatible with a high level of protection of health and safety.

2. TERMS OF REFERENCE

In the light of the above considerations, on the basis of the available scientific evidence and taking into account the previous safety evaluations of BPA, the Scientific Committee on Emerging and Newly Identified Health Risks is requested to provide a scientific opinion on 'The safety of the use of bisphenol A in medical devices'.

In particular, the SCENIHR is asked:

1. To determine whether levels of exposure to BPA from the use of the various medical devices containing BPA could give reasons for concern from the health point of view and, if possible, to provide indications on limit values for BPA release from medical devices.

[1] http://www.fda.gov/newsevents/publichealthfocus/ucm064437.htm

[2] http://www.efsa.europa.eu/en/topics/topic/bisphenol.htm

2. To identify whether any particular medical devices containing BPA could result in human exposures which will give reasons for concern under their normal use patterns or other foreseeable circumstances (e.g. high release of BPA due to the nature of the material of the medical device or to particular contact conditions).

3. To identify, any patient group e.g. infants, pregnant and breastfeeding women who would be particularly at risk in light of the answer to the above questions.

4. In case reasons for concern related to BPA are identified, to propose possible alternative approaches that could reduce potential risks either by identifying alternative practices or by identifying alternatives to the use of BPA in medical devices. If no clear answer can be provided on this point, the SCENIHR is asked to formulate recommendations for research that could help provide scientific evidence to that end.

3.SCIENTIFIC RATIONALE

3.1. Introduction

Bisphenol A [Bis(4-hydroxyphenyl)propane], BPA is a high-production volume industrial chemical. According to industry, about 3.8 million tons BPA were produced worldwide in 2006 (Plastic Europe 2007, WHO/FAO 2010). More than 95% of the BPA produced is used to manufacture polycarbonate plastic and as a precursor of the manufacturing of monomers of epoxy resins (Plastic Europe 2007, WHO/FAO 2010, Beronius and Hanberg 2011, Genuis *et al.*, 2011). Other uses of BPA include production of the flame retardant tetrabromobisphenol A, production of thermal paper, and as an antioxidant in plasticizers and for inhibiting the polymerization in polyvinyl chloride (PVC). The European Council of Vinyl Manufacturers informed that the use of BPA for polymerisation and stabilisator for storage of vinyl chloride in Europe was discontinued from December 2001 (KEMI 2011). However, PVC as a source of BPA exposure cannot be completely excluded because BPA-containing PVC may still be used in the EU due to the global market for medical devices.

BPA is a key building block of polycarbonate plastic. Polycarbonate plastic is a lightweight, high-performance plastic that possesses a balance of toughness, dimensional stability, optical clarity, high heat resistance and electrical resistance. Because of these attributes, polycarbonate is used in a wide variety of common products such as food and drink packaging materials, plastic water bottles and infant feeding bottles, digital media (e.g. CDs, DVDs), electrical and electronic equipment, construction glazing, sports safety equipment and medical devices. The durability, shatter-resistance and heat-resistance of polycarbonate make it a good choice for tableware as well as reusable bottles and food storage containers that can be conveniently used in the refrigerator and microwave. As an alternative to polycarbonate (PC), polysulfone (PSU) is also used in some medical devices. As polysulfones are polymers that can be obtained by a reaction between Bisphenol A and bis(4-chlorophenylsulfone), producing an ether-oxide, leaching of BPA from PSU is possible. BPA-resins (such as BADGE: Bisphenol A diglycidyl ether, Figure 1) are used as lacquers to coat metal products such as food cans, bottle lids, coatings inside drinking water and waste water tanks, large wine storage tanks and water supply pipes.

In addition to polycarbonate medical devices, some dental materials are fabricated from monomers such as bisphenol A glycidyl methacrylate (Bis-GMA, Figure 1) and bisphenol A dimethacrylate (Bis-DMA, Figure 1) derived from BPA (Fleisch *et al.*, 2010). BPA-resins are also used in inks and adhesives. Polymers produced using BPA (e.g.polysulfone) are used as membranes in hemolysis dialysers. A detailed description of use of BPA, polycarbonate, and epoxy resins is listed elsewhere (Beronius and Hanberg, 2011). Trace amounts of BPA are present, as residues of polymerisation process, in polycarbonate, epoxy resins and dental sealants.

About 3% of total polycarbonate production is used for the manufacture of medical devices (Beronius and Hanberg, 2011). Various medical devices produced with BPA derived materials (polycarbonate, polysulfone and BPA-resins) are mentioned in Annex 1.

Several health risk assessments of BPA have been conducted by regulatory authorities as well as expert groups based on oral exposure (ECB, 2003, EC 2010b; EFSA 2006 and 2010, NTP-CERHR 2008, Environment Canada/Health Canada 2008, WHO/FAO 2010; US-FDA, 2013). ANSES (2011) provided a report on the hazard identification of BPA. The risk assessment methodology used in this opinion includes the conclusions of the earlier risk assessments updated with the recent relevant data on BPA.

Other bisphenols [for example Bisphenol B:Bis(4-hydroxypheny)butane, BPE: Bis(4-hydroxyphenyl)ethane], Bisphenol F: Bis(4-hydroxyphenyl)methane, and resins derived from it (BFDGE : Bisphenol F diglycide ether) and bisphenol-S [bis(4-hydroxyphenyl)sulfone, (BPSU)] are also used for similar purposes as BPA as well as resins derived from it.

Finally, halogenated derivatives of BPA, such as tetrabromobisphenol-A [2,2-bis(4-hydroxy-3,5-dibromophenyl)propane, (TBBPA)] and tetrachlorobisphenol-A [2,2-bis(4-hydroxy-3,5-dichlorophenyl)propane, (TCBPA)] are both widely used as flame-retardants for building material, paints, plastic products including epoxy resin, electronic circuit boards, and other electronic equipment.

This opinion does not include risk assessment of these BPA derivatives and the alternative substances. However, BPA-derivatives are considered, as they can release BPA.

The main focus of this opinion is adverse effects and risk assessment of exposure to BPA via medical devices, for which the exposure routes are not limited to oral applications. Information on adverse effects after oral exposure to BPA is included only regarding the additional literature published after the existing evaluations between 2010 and 2013. For other routes of exposure a more comprehensive overview is presented including literature before 2010.

3.2. Methodology

This Opinion of SCENIHR is concerned with the analysis of the evidence for the potential for BPA exposure due to the use of medical devices to have adverse effects on human health, from the perspectives of both scientific plausibility as well as experimental, clinical and epidemiological data. Recent scientific evidence is reviewed to determine whether it justifies any reason for concern with regard to health risks associated with the use of BPA based polycarbonates, resins and/or BPA containing PVC.

The SCENIHR has considered evidence derived from a wide variety of sources, including peer-reviewed scientific and medical literature and published reports of institutional, professional, governmental and non-governmental organisations. In common with the usual practice of SCENIHR, no reliance has been made on unpublished work or publicly available opinions that are not scientifically based. Due to the availability of extensive peer reviewed scientific publications with respect to BPA, it has not been necessary to rely on single case or anecdotal reports in establishing this Opinion.

The SCENIHR has reviewed as much evidence as possible and evaluated potential risk of the use of BPA in medical devices against the clinical benefit of the use of such medical devices. In a weight of evidence approach, lines of evidence or hypothesis for causality are evaluated based on the supportive studies. When a line of evidence is consistently supported by various studies (i.e. evidence is independently reproduced in different studies) causality is likely between the observed effect and exposure to the substance. Relevance, strength and weaknesses of the studies evaluated are considered. The weight of evidence (SCENIHR, 2012) can be categorized as follows:

Strong overall weight of evidence: Coherent evidence from human and one or more other lines of evidence (animal or mechanistic studies) in the absence of conflicting evidence from one of the other lines of evidence (no important data gaps).

Moderate overall weight of evidence: good evidence from a primary line of evidence but missing evidence from several other lines (important data gaps).

Weak overall weight of evidence: weak evidence from the primary lines of evidence (severe data gaps).

Uncertain overall weight of evidence: due to conflicting information from different lines of evidence that cannot be explained in scientific terms.

Weighing of evidence not possible. No suitable evidence available.

The evidence for the presence of a causal relationship between exposure to BPA due to the use of medical devices and adverse effects are discussed in the chapters below. In

addition, the risk will be evaluated against the potential benefit of the use of the various medical devices.

3.3. Chemistry of BPA

Identification of the Substance

CAS-No: 80-05-7
EINECS No: 201-245-8
IUPAC name: 2,2-bis(4-hydroxyphenyl)propane
Molecular weight: 228.29
Molecular formula: $C_{15}H_{16}O_2$
Structural formula: _

Smiles notation: Oc(ccc(c1)C(c(ccc(O)c2)c2)(C)C)c1

Synonyms:
BPA (Common abbreviation)
2,2-Bis(4-hydroxyphenyl)propane
2,2-Bis(p-hydroxyphenyl)propane
p,p'-Isopropylidene-bisphenol
p,p'-Isopropylidene-di-phenol
Phenol, 4,4'-Isopropylidene-di
Diphenylol Propane
Parabis (Trademark)
Bis (4-hydroxyphenyl) dimethyl methane
Bis (4-hydroxyphenyl)propane
Dian (Trademark)
Dimethylmethylene-p,p'-di-phenol
Dimethyl Bis(p-hydroxyphenyl)methane
4,4'-Dihydroxy-2,2'-diphenyl propane
4,4'-Dihydroxydiphenyldimethyl methane
4,4'-Dihydroxydiphenyl propane
ß-Di-p-Hydroxyphenyl propane
p,p'-Dihydroxydiphenyldimethyl methane
p,p'-Dihydroxydiphenyl propane
2,2'-(4,4'-Dihdroxydiphenyl) propane
4,4'-Dihydroxydiphenyl-2,2'-propane
2,2'-Di(4-hydroxyphenyl) propane

2,2'-Di(4-phenylol) propane

4,4'-Isopropylidene bisphenol

4,4'-(1-methylethylidene)bisphenol

Purity

The EU Risk Assessment Report of 2003 states a purity of BPA as being 99-99.8% with the impurities typically including phenol (<0.06%), other isomers of bisphenol-A (<0.2%) and water (<0.2%) (ECB 2003). Terasaki *et al.* (2004) examined four samples of industrial BPA with stated purities of 97% to 98% and a sample of laboratory grade BPA (99+% purity) and they found fifteen trace impurities. However, it is presently unknown whether such impurities are also present as such in medical devices, or if they participate in the polymerization reactions, and, therefore, whether or not they may leach out from the manufactured medical devices.

Additives

There are no stated additives used with BPA.

3.4. Physico-Chemical Properties

The physical-chemical properties of BPA are described below (Staples *et al.*, 1998; Cousins *et al.*, 2002; ECB 2008). Bisphenol A is a moderately polar substance (log Kow 3.3-3.5) with good solubility in most organic solvents and moderate solubility in water (300 mg/L at 25°C). It has a high boiling point and a low vapour pressure at ambient temperatures. BPA has two unhindered phenolic hydroxyl groups and hence, it exhibits chemical properties typical of simple phenols, such as a slightly acid character and susceptibility to oxidation. At physiological pH the BPA molecule is predominantly in the non-ionised form. The two phenolic hydroxyls are the main reaction centres of BPA and products derived from BPA involve reactions via these groups.

Main physical-chemical properties of BPA.

Physical State at STP:	White solid flakes or powder
Melting Point:	155-157°C
Boiling point:	360°C at 1013 kPa
Vapour pressure:	5.3×10^{-9} kPa at 25°C
Solubility in water:	300 mg/L at 25°C
Octanol-water partition coefficient:	Log Kow 3.3-3.5
Acid dissociation constant:	pK_a 9.6 - 11.3

3.5. Overview of existing assessments on BPA

3.5.1. Existing assessments

The toxicological profile of BPA has been described in several reports of EFSA (2006, 2010), USA National Toxicology Program (NTP-CERHR, 2008), FAO/WHO (2010), ANSES (2011) and in several reviews (Arnich *et al.*, 2011; Hengstler *et al.*, 2011). The EFSA opinions issued to date are focused on the oral route of exposure, because it was found

to be the most relevant for risk assessment of food/feed. In the 1980s, a series of sub-chronic and chronic studies were performed by the USA-NTP and U.S. EPA (US Environmental Protection Agency), whereby the majority of the studies used the oral route. Doses ranged from 250 to 4000 ppm (= 250 to 4000 mg/kg feed) corresponding to maximum exposure dose of approximately 400 mg/kg b.w./day[3] in rats and from 5000 to 25,000 ppm (= 5000 to 25,000 mg/kg feed) corresponding to maximum exposure dose of approximately 5600 mg/kg b.w./day in mice (US-NTP, 1982). Doses higher than 1000 ppm (= 1000 mg/kg feed, which corresponds to approximately 100 mg/kg b.w./day) led to decreased body weight in both sexes of rats. Doses higher than 9000 ppm (= 9000 mg/kg feed), the total dose depending on the food intake, led to an increase in mean liver weight in dogs (EC 2003).

3.5.2. Controversial issues

It is recognized that regarding the risk assessment of BPA after oral uptake, several controversies still need to be addressed.

In monotonic responses, the effect either increases or decreases over the full dose range tested. The controversy is on-going whether BPA exhibits non-monotonic dose-response, and whether the effect seen with low doses but not with high doses is really present (Goodmann *et al.*, 2009; Vandenberg *et al.*, 2012; Rhomberg and Goodman, 2012). Furthermore, there are diverging views whether BPA causes adverse effects in humans related to its estrogenic activity at exposure levels present in the population (Borrell, 2010; Aschberger *et al.*, 2010; Taylor *et al.*, 2011; Yang *et al.*, 2009). Increases in prostate weights of 30-35 % compared to controls were observed in F_1 adult mouse offspring which were exposed *in utero* by orally dosing dams with 2 and 20 μg/kg/day BPA from gestational days 11 through 17 (Nagel *et al.*, 1997). Other studies did not reproduce the result (Ashby *et al.*, 1999; Cagen *et al.*, 1999). In addition, it is not yet clear whether developmental exposure to BPA can affect adiposity, glucose or insulin regulation, lipid profiles or other endpoints related to diabetes or metabolic syndrome (Miyawaki *et al.*, 2007; Somm *et al.*, 2009; Alonso-Magdalena *et al.*, 2010; Alonso-Magdalena, *et al.*, 2011; Nadal 2013; Ryan *et al.*, 2010; Wei *et al.*, 2011; MacKay *et al.*, 2013; Angle *et al.*, 2013). Other studies dealing with these endpoints had results which were contradictory to the findings of the above cited authors (e.g. Anderson *et al.*, 2013).

Furthermore, effects on mammary tissue (e.g. proliferative changes in mammary gland, Ayyanan *et al.*, 2011; Kass *et al.*, 2012; Tharp *et al.*, 2012), were demonstrated and are difficult to interpret in the context of human health.

Lastly, neurological, neurodevelopmental and neuroendocrine effects are additional areas of uncertain results because it is not clear whether the effects shown by these animal studies -even when scientifically sound results have been obtained– can be translated to the human population (see below).

Another issue is related to findings from observational epidemiological studies, suggesting associations between BPA exposure (mainly measured as spot urine concentrations) and chronic health effects such as coronary heart disease, reproductive disorders and others. However, the design of observational studies does not allow the establishment of causal relationships.

A minority view to the EFSA BPA assessment (EFSA 2010) expressed concerns resulting from studies published after 2006 in which animals were exposed during prenatal and postnatal development. The studies appear to indicate that adverse effects, in particular brain receptor programming, immune modulation and susceptibility to breast tumours, might occur at doses below the current No Observed Adverse Effect Level (NOAEL).

[3] The indicated dose levels in mg/kg b.w./day were calculated using the general conversion factor of 0.1 for rats and 0.2 for mice (EFSA 2012a)

Some of the controversial issues were recently discussed by Shelnutt et al. (2013) including a summary on the current regulatory status of BPA, a review of recent pharmacokinetic studies and studies on neurobehavioral effects, and how this new information addresses the National Toxicology Program's NTP's 2008 finding of "some concern" (Shelnutt *et al.*, 2013). The species differences in pharmacokinetics of BPA were recognized as was the lack of certain neurobehavioral effects of BPA. In addition, ongoing and planned research in cooperative studies on BPA between NTP/FDA and National Institute of Environmental Health Sciences (NIEHS) in the USA are described (Shelnutt et al., 2013).

3.5.3. Conclusion

The exposure to and toxicity of BPA have been investigated in depth and a multitude of studies have been published. In the risk assessments carried out to date, less interest has been directed towards studies in which the BPA dose was given via non-oral routes (e.g. subcutaneous, dermal).

In the existing evaluations, the following conclusions have been drawn for oral route of exposure to BPA:

- NOAEL of 5 mg/kg b.w./day in rats
- Tolerable Daily Intake (TDI) of 50 µg/kg b.w.
- developmental toxic effects only observed at doses with severe maternal toxicity in rats and mice
- an overall NOAEL for reproductive toxicity of 50 mg/kg b.w./day in rats and mice
- in terms of toxicokinetics, there is a difference between rats and humans (the latter presenting a shorter half-life) as well as between the oral and the parenteral route of exposure
- due to the first pass effect, after oral uptake, the systemic exposure to free BPA is a small fraction of the external dose in all species
- there remain unresolved issues in the risk assessment of BPA after oral and subcutaneous uptake

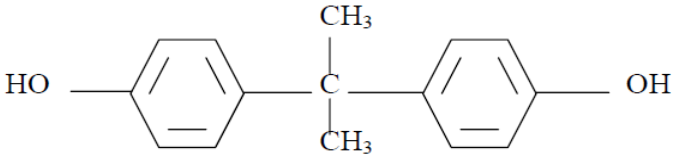
More recently, EFSA established a temporary (t)-TDI of 5 µg/kg b.w./day for oral exposure to BPA (EFSA 2014). A bench-mark dose (BMD) evaluation was used with the $BMDL_{10}$ of 3.76 mg/kg b.w./day for kidney alterations as a critical effect. The recently calculated $BMDL_{10}$ of 3.76 mg/kg b.w./day and the NOAEL of 5 mg/kg b.w./day, both based on the Tyl et al.(2002, 2008) multigeneration reproductive toxicity studies using oral exposure, are very similar, although conceptually different from a toxicokinetic point of view. Indeed, the internal exposure of the organs is different: hepatic exposure is presystemic, whereas renal exposure is systemic. The doses at the site of action (i.e. liver and kidney) differ after the oral route of exposure because of the biotransformation occurring in the liver which results in a lower dose of free BPA for kidney exposure. The SCHENIR supports the use of the newly developed t-TDI for the risk assessment of medical devices.

3.6. Identification of the relevant medical devices

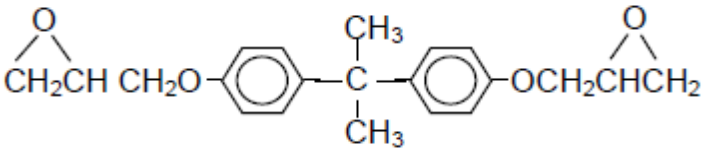
3.6.1. Medical devices

Medical devices based on polycarbonate and polysulfone, due to their chemistry, may contain BPA residues, whereas others like PVC may or may not contain BPA residues depending on their production method. In addition, some other BPA-derivatives (such as

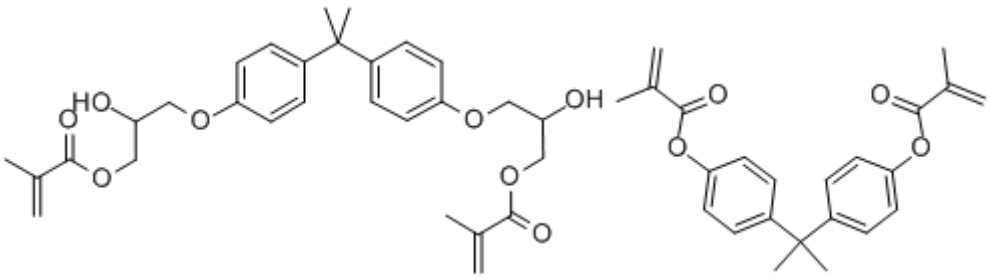
epoxy resins) are used specifically in dental materials (Fig. 1). Annex I lists medical devices which may contain BPA.



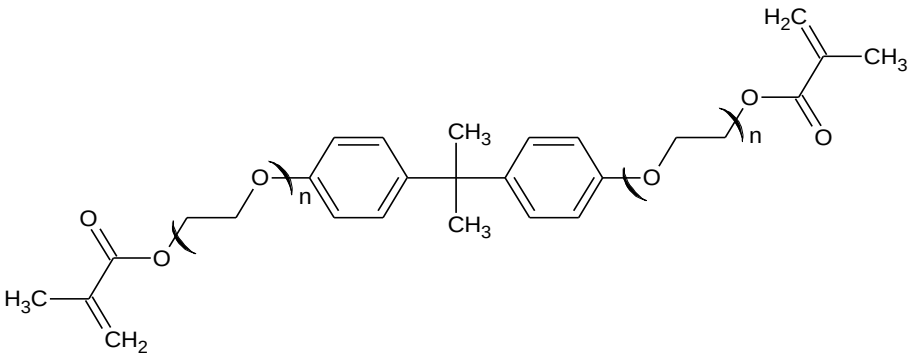
BPA



BADGE



BISGMA BISDMA



Bis-EMA

Figure 1: BADGE: Bisphenol A diglycidyl ether; Bis-GMA: Bisphenol A glycidyl methacrylate; Bis-DMA: Bisphenol A dimethylacrylate; Bis-EMA:ethoxylated bisphenol A dimethacrylate

3.6.2. Presence in and release of BPA from medical devices

Potential exposure to BPA from polycarbonate articles can derive from the incomplete polymerization of the monomer during the manufacturing process as well as from the

breakdown or hydrolysis of the polymer under certain conditions (ECB 2003, EC 2010a, Mercea 2009).

It is known that polymerization of monomers is rarely complete, and un-reacted monomers are almost always released from polymer resins (Begley *et al.*, 1990, 2005; De Meulenaer and Huyghebaert 2004). The major factor influencing residual amounts of BPA is the employment of incorrect operating conditions during the processing step. The presence of water in the polycarbonate before processing, the use of excessively high processing temperatures and the use of additives that promote degradation are the major causes of polycarbonate degradation during processing. However, the residual BPA in polycarbonate is likely to be low when proper processing and handling conditions are used. For instance, in polycarbonate articles used for food contact, the residual content is usually less than 10 µg/g of polycarbonate (ECB 2003). The presence of residual BPA in polycarbonate is supported by available results concerning migration of BPA mainly into food simulants (water, 3% acetic acid, 10% aqueous ethanol, olive oil) but also into foods, showing levels of BPA of up to 50 µg/L of infant formula from baby bottles (EC 2010b).

Moreover, breakdown or hydrolysis of the polycarbonate polymer after manufacturing can occur, thus giving rise to the free monomer from the polymer available for exposure. Factors affecting BPA release from polycarbonate (PC) used in food contact materials have been studied and recently reviewed (Aschberger *et al.*, 2010; EC 2010b; Beronius and Hanberg, 2011; Geens *et al.*, 2012). Length of contact time, high temperatures and high pHs (hydroxide aqueous solutions) increase the release, whereas the role for other factors, which have been suggested as possible releasing facilitators (i.e the composition of mineral water or the repeated use (ageing) of the articles) has not been clearly demonstrated. Residual alkaline detergent remaining on the surface of the polymer after dishwashing may increase the release of BPA. Reactions with amines and ethanol of polycarbonate were observed (Maia *et al.*, 2010; Sajiki & Yonekubo, 2004; Biles *et al.*, 1997; Jie *et al.*, 2006)

Importantly, studies on BPA content and its leaching from polycarbonate medical devices in their actual use are limited.

Polysulfones are polymers obtained by reaction between a diphenol and bis(4-chlorophenylsulfone) producing an ether-oxide. The diphenol could be either BPA or 1,4-dihydroxybenzene. Thus, in case of use of BPA during this reaction, leaching of BPA is then possible. They are mostly used in medical devices as membranes, especially in hemodialysers.

<u>PC pellets used for the manufacture of medical devices</u>

Haishima *et al.* (2001) found a total BPA content of 4.0 and 7.2 mg/kg in two types of PC pellets, using tetrahydrofuran for the dissolution of the polymer. Additionally, one type of polysulfone (PSU) was analysed resulting in a significantly higher value: 34.5 mg/kg. Furthermore, 2 PC casings lacking hollow fibres were extracted with water and methanol (10 mL each, 16h) at room temperature, while shaking. The extraction power of methanol was evident from the results: BPA released was 11.7 and 13.7 ng/casing by water extraction, and 296 and 345 ng/casing by methanol extraction.

Some data on BPA content in medical devices and/or PC pellets used for the manufacturing of medical devices were submitted in the Call for Information[4]. Low amounts of BPA were observed in PC pellets. The amount of total BPA extracted (24h soxhlet extraction in isopropanol) from PC pellets (3 replicates) used to manufacture

[4] For the data submitted in response to the Call for Information the information was available as far as provided by the applicant and complete study reports were not available for evaluation by SCENIHR.

medical devices, as well as from a finished trocar tubing was 0.2-0.3 mg/kg. No significant difference was found in the amount found in the pellets and in a finished device trocar sleeve tested. However, BPA could not be detected when using a 0.9% sodium chloride solution at 37ºC for different extraction time intervals from 1h up to 168h (Limit of Detection, LOD, approximately 2 mg/kg) using conditions selected to mimic human physiological conditions. Notably, the LOD for saline analysis is 10 times higher than the amount observed using soxhlet extraction in isopropanol. The assumption that 100% of BPA is released in one day is assumed to represent the worst-case scenario.

BPA could be extracted (4-5.8 mg/kg) only from PC-drinking cups,. In PC pellets and a PC-containing trocar, no BPA was detected in a follow-up study of medical grade PC when extracted with isopropyl alcohol and ethanol to account for potential accumulation in blood lipids (LOD approximately 0.5 mg/kg material). After sterilization by gamma radiation, similar results were obtained using the same PC-containing materials (submitted through the Call for Information).

Medical Devices used for air and/or gas circulation

In hoods of neonatal incubators and neonatal intensive care units (NICU), BPA could not be detected (LOD approximately 50 µg/m^3, ISO 16000-6 method) in the analyzed gases after a prolonged period of contact time of the material with the breathing gas[5].

Hemodialysers

Several studies have reported the leaching of BPA from hemodialysers, though the number of devices in each study was limited.

Four hemodialysers, composed of a combination of PC casing and cellulose acetate hollow-fibres (1 device), PC casings and PSU (polysulfone) fibres (2 devices), and polystyrene and PSU (1 device), were tested for BPA released (Haishima *et al.*, 2001). Water and bovine serum (250 mL each), the latter used as a simulant for human blood circulating into hollow-fibres during hemodialysis, were circulated at 10 mL/min for 16 h at room temperature in the four devices tested. BPA recovered ranged from 3.78 to 141.8 ng/module using water circulation and from 140.7 to 2090 ng/module when bovine serum was used. The highest values of BPA released corresponded to the 2 hemodialysers tested consisting of PC casings and PSU fibres were 1 and 2 µg/module. Moreover, a 17.2% (v/v) ethanol solution was found to extract comparable BPA amounts as with bovine serum, reaching the maximum release after 2 to 4h circulation.

BPA concentrations of 83.3 ng/10 mg and 122.5 ng/10 mg in PSU and PEPA (polyester-polymeralloy) hollow fibres, respectively, were reported (Murakami *et al.*, 2007). The hollow fibres, taken from individual dialyzers, were crushed and dissolved in hexane.

Fink (2008) considered the leaching of substances (including BPA) from five different types of dialyzers and PVC blood tubing. All the dialyzers had in their composition either PC or PSU: PC housing and PSU-PVP blend membranes (n=2), PC housing and polyamide-PSU blend membrane (n=1), and PP housing, PSU-PVP blend membrane (n=2). The surface area range was 1.3 - 1.8 m^2. The blood and dialysate compartment of each dialyzer was connected by 110 cm standard PVC tubes. Dialysis was simulated using two different eluents (volumes ranging 230-410 mL), reverse osmotic water and 17.2% ethanol, the latter as a substitute of bovine serum (hence, simulating human serum). Temperature, dialysis period and flow rate were adjusted to represent realistic dialysis modalities: 37 °C, 230 mL eluate/min for 4 h and also for 24 h as the worst case scenario. Three independent eluates with new dialysers each time were obtained at each elution condition. BPA was quantitated in all eluates by LC-MS/MS.

In agreement with the study of Haishima *et al.* (2001), higher levels were measured when 17.2% ethanol was used (to simulate blood), ranging from 54.8 to 4299

ng/dialyser, whereas BPA levels were 6.4 – 71.3 ng/dialyser using water as eluent (Fink 2008). Other factors influencing the amount of leaching BPA were the type of dialyzer, different batch of the same type, the size of the membrane surface and time of dialysis. In general, the longer the dialysis time the greater the leaching. Additionally, an increase in the surface area, when comparing dialyzers with the same material for housing and membranes resulted in a higher amount of BPA extracted. The maximum of leaching BPA was estimated to be 4.3 µg/dialyzer when circulating 17.2% ethanol for 24h (3.4 µg/dialyzer for 4h circulating time). Contribution of the PVC tubing to total BPA content in the eluates was negligible, and the levels found were below the limit of quantification (3.42 ng/mL). The results obtained with water and also with 17.2% ethanol were in the range reported in the previous study by Hashima (2001), except for a single batch or a type of dialyzer, which released almost twice the higher level reported before. However, this discrepancy could be attributed to the different conditions used in both studies, regarding temperature, duration and flow.

Krieter *et al.* (2013) have also reported release of BPA from 3 different dialyzers, one with a 1.7 m^2 high-flux polyethersulfone membrane, and two with 1.3 m^2 polysulfone membranes, high and low flux, respectively. All dialyzers had PC housing. BPA-free sterile water (400 mL) was circulated through the blood and dialysate compartments for 3 h, at 250 mL/min, 37 ºC and BPA was measured by ELISA. As found in other studies, eluted BPA concentrations differed significantly between dialyzers, averaged (n=6) levels found being 140.8 ± 38.7, 48.1 ± 7.7 and 6.2 ± 2.5 ng/dialyzer. These results are in the range with those reported in other studies when using water as eluent (Hashima, 2001; Fink, 2008). The highest BPA levels were eluted from the low-flux dialyzer with PS membrane, and the lowest from the dialyzer with polyethersulfone membrane.

Leaching of BPA from PSU hollow fiber membranes used in hemodialyzers and hemoconcentrators has also being recently reported (Cho *et al.*, 2012). The authors studied the kinetic elution profile in ten multiple consecutive extractions with 1 L of 17.2% ethanol at a flow rate of 200mL/min, for 1 hour. Every hour the ethanolic solution was refreshed with a new one. The experimental results were fitted to derive an equation to predict the total leachable BPA. For a PSU membrane with a total surface area of 0.5 m^2 and weight of 7.9 g, the total leachable amount of BPA was estimated as 20.7 µg, and 95% of this was released after 10 consecutive extractions. Single prolonged (6 hours) extraction tests (same conditions) were also performed on PSU membranes with surface areas of 0.4 m^2 and 0.7 m^2, respectively. BPA concentrations were measured (LC/MS) at different time points from 0.33 up to 6 hours. In agreement with the Haishima (2001) study, the BPA released reached a plateau in approximately 2 hours (read values from the figure in the publication are 1.3-1.4 ng/mL and 0.65-0.7 ng/mL for the 0.7m^2 and 0.4 m^2 PSU membranes, respectively). If the flow rate was decreased to half, the time to reach the equilibrium increased to 4 hours, but the final concentration was not dependent on the flow rate of the extraction solution.

Cardiopulmonary bypass

A study by Sakurai H. (2002, only abstract available in English) indicates the presence of BPA in cardiopulmonary bypass circuits as BPA leaching was observed during open heart surgery (see exposure section).

Effect of sterilization on medical devices:

Sterilization of medical devices is usually performed either by steam, irradiation, gas (ethylene oxide) or "gas-plasma" (hydrogen peroxide). Each method may interact with the content of the medical device, creating some by-products, as sterilization may modify/deteriorate the polymer component of the medical device which as result may modify the BPA release properties. In the specific case of plastics and polymers, effects are described since long time and extensive reviews are available in the literature

(Mendes *et al.*, 2007; McKeen, 2012). These effects include alteration of resistance, surface modification, release and/or modification of some components (Baker *et al.*, 2000; Brown *et al.*, 2002).

Shintani (2001) reported migration of BPA from four dialyzers into a saline extract for different sterilization methods and for different devices. The procedures used for sterilization were by autoclaving (121ºC, usually 15-20 min) and gamma radiation at 25 KGy. BPA levels found were 0.1-0.2 ppb (µg/L) in the saline extract.

In another study (Shintani *et al.*, 2003), BPA content after extraction in 4 mL was determined by LC-MS-UV in one PC (119 mg/kg) and three PSU membranes (43, 207 and 247 mg/kg) sterilized with ozone gas (gas concentration 300 ppm, RH 80%, T 35ºC) by extraction with ethanol. BPA was not detected after extraction of unsterilized and steam-sterilized (121ºC, 15 min) PC and PSU membranes (less than 4 – 11 mg/kg, based on the weights of the tested membranes which were approximately 20 mg and 7 mg for PSU and PC, respectively). Sterilization by means of ethylene oxide was not used in this study.

Dental materials

BPA exposure from dental materials is a concern, especially from dental sealants (Olea *et al.*, 1996), but also from composites and other polymer-based restorative materials (Van Landuyt *et al.*, 2011, 2013). BPA, as such, is not a component in dental materials[5], but may be present as a contaminant or degradation product. Some dental materials are produced using monomers synthesized from BPA, such as Bis-GMA, Bis-EMA and incidentally Bis-DMA (all methacrylates), and BADGE (epoxy), see Figure 1. Only Bis-DMA, which has an ester linkage, can be hydrolysed to release BPA. The ether linkage in Bis-GMA is stable (Schmalz *et al.*, 1999). The reports on BPA leaching from dental materials vary extensively, but it seems that only a few specific products/brands were detected as a source of BPA (Arenholt-Bindslev, 1999). One study analyzing 28 different materials found BPA in only one of them (Lewis, 1999).

In 2011, Van Landuyt and co-workers published a systematic review of 71 publications dealing with the release of substances from resin-based materials of which 11 studies investigated the release of BPA. They considered exclusively *in vitro* incubation in aqueous and organic solvents, for at least 24 hours, without a pre-incubation process. The review reported a few studies with BPA-release in water-based solutions, the highest individual value being 67 nmol/mm^2 surface area of the resin bonding material as published by Mazzaoui *et al.* (2002). Notably, this amount was measured on the resin bonding material, which is an adhesive normally not exposed to saliva.

One study (Takahashi *et al.*, 2004) found no release of BPA into water even at elevated temperature (65 ^{0}C, 24h) from PC crowns, but release of 0.28 µg (± 0.02) from 100 mg material into ethanol (65 ^{0}C, 24h). The amount of BPA released into other organic solvents was 4.72 µg (acetic acid) and 8.80 µg (acetonitrile), both at 65^{o}C, 24h. No LOD/LOQ was declared in the study.

Adhesives in orthodontic applications

Orthodontic treatment involves using fixed or removable appliances (dental braces) to correct the positions of teeth. The success of a fixed orthodontic appliance depends on the attachments (brackets and bands) being attached securely to the teeth so that they

[5] At least one manufacturer has actively informed during the call for information that their products have no additions of BPA:

http://multimedia.3m.com/mws/mediawebserver?mwsId=66666UF6EVsSyXTtNXTEnxTEEVtQEVs6EVs6EVs6E666666--&fn=bpa_letter.pdf

do not become loose during treatment. A number of epoxy-resin based adhesives are available to attach bands to teeth. Eliades and co-workers quantitatively characterized *in vitro* BPA released from orthodontic adhesives after artificial accelerated aging (Eliades *et al*., 2007). No trace of BPA was identified for either adhesive across all time intervals, implying that, if present, the amount of BPA did not exceed the detection limit of the analytical technique (0.1 mg/kg of adhesive).

Eliades and co-workers also quantified BPA released from a light-cured orthodontic adhesive used to bond lingual fixed retainers (Eliades *et al.*, 2011). Eighteen recently extracted premolars, divided into 3 groups of 6 teeth each, were embedded in plaster in an arch shape. A light-cured adhesive was bonded to a 3-strand, heat-treated twist flex wire adjusted to the lingual surface of the teeth, and the arches were immersed in double-distilled water for 10, 20, and 30 days. The concentration of BPA in the 3 extracts was investigated with gas chromatography-mass spectroscopy; all assays were performed in triplicate, and the results were averaged. Measurable amounts of BPA were identified for all groups, with the highest value (2.9 μg/L) found in the immersion media of the 30-day groups (six teeth embedded in plaster and arranged in an arch mimicking the shape of of the six mandibular anterior teeth), whereas the control (tooth storage solution) had 0.16 μg/L. The total release could not be determined as the incubation volume was not presented in the paper. The level in the control samples indicates background levels of BPA in the lab equipment used. In conclusion, BPA released from a light-cured adhesive used to bond lingual fixed retainers might be assigned to the application mode of the material that differs from conventional use.

As orthodontic bonding resins are exposed to oral fluids and are in contact with tissues throughout treatment, leaching from resin can occur at 2 times: during the setting period of the resin and later when the resin is degraded. Leaching during the first process is related to the degree of conversion. Sunitha *et al.* (2011) used high-performance liquid chromatography to assess BPA released from an orthodontic adhesive with various light-curing tip distances and to correlate the release to the degree of conversion. BPA release was greater in specimens cured with a greater light-curing tip distance. The degree of conversion decreased with increased light-curing tip distances. A negative correlation was found between BPA release and degree of conversion.

Watanabe (2004) analyzed the leaching of BPA in water at 37^{o}C for 12, 16, 25, and 34 months from brackets, denture base and temporary crown PC materials. The total BPA released was found to be 37.4 (3.56), 2.2 (0.03), and 2.8 (0.32) μg/g, respectively. The BPA content in the materials was shown to increase after 34 months (max 472 μg/g for brackets), and the MW of the PC to decrease. The BPA content in retrieved PC brackets was in the range 38 μg/g (18 months) to 697 μg/g (40 months).

Watanabe *et al.* (2001) analyzed (HPLC/UV-DAD or fluorescence) five retrieved PC brackets from five patients for their content of BPA. At retrieval points ranging from 5-15 months, the BPA content in the brackets were found in the range 56-102 μg/g bracket. (One bracket weighs approximately 22 mg). In addition, they measured the *in vitro* leaching of BPA from the brackets in water at 37 ^{o}C and 60^{o}C for 3, 6, 9, 12 months and 1, 2, 4, 6, 10, 14 weeks, respectively. The release after 12 months reached 3.8 μg/g. The release after 14 weeks reached 35 μg/g. The BPA content in PC brackets immersed in water increased to a maximum of 434 μg/g (14 wks, 60^{o}C). Calculations of exposure (3.8 μg/g, 5-15 months, 28 brackets, 50 kg b.w.): daily BPA intake of 0.1-0.3 ng/kg/day, as given by the authors.

Recently, Kloukos *et al.* (2013) reviewed all the publications on BPA leaching from orthodontic adhesive resins and polycarbonate brackets. The objective of this systematic review was to assess the short- and long-term release of components of orthodontic adhesives and polycarbonate brackets in the oral environment. Eleven studies met the inclusion criteria and all were observational studies conducted *in vivo* or *in vitro*. The BPA released from orthodontic bonding resins was found to be between 0.85 and 20.88

ng/mL *in vivo* and from traces to 65.67 ppm (mg/L) *in vitro*. Polycarbonate brackets released amounts of 22.24 µg per gram in ethanol solution and 697 µg per gram after 40 months in water. The available evidence on this topic derived from observational *in vivo* and *in vitro* studies that represent a moderate level of evidence. The variety of setups and the different units allied to the diversity of reporting among studies did not allow calculation of pooled estimates (Table 1).

Table 1 BPA released in experimental media (*in vitro*)

Reference	Orthodontic material	Time at sampling	BPA concentration
Sunitha *et al.*, 2011	Light-cured adhesive resin	Day 7	65.67 ppm (mg/L)
Eliades *et al.*, 2011	Light-cured adhesive	30-days exposure	2.9 µg/L (control group: 0.16 µg/L)
Watanabe 2004	PC brackets	34 months (immersion in water, 37 ºC)	37.4 µg/g
Watanabe *et al.*, 2001	PC brackets	12 mo. 37 ºC Immersion in water 14 weeks 60 ºC Immersion in water	3.8 µg/g 35.0 µg/g
Suzuki *et al.*, 2000	PC brackets (n=4)	Crushed brackets in ethanol solution	22.24 µg/g

Notably, with a mean weight of approximately 40 mg and a maximum number of 24 applications, the total weight of brackets potentially applied is approximately 1 g material.

Bone cements

Bi-functional methacrylates based on bisphenol-A-glycidyldimethacrylate (BisGMA) or triethylene glycol dimethacrylate (TEGDMA) may also be applied in bone cements (Vallo and Schroeder, 2005). There are commercially available cements that comprise three main resins, BisGMA and ethoxylated BPA dimethacrylate (BisEMA) and TEGDMA as a viscosity modifier and reinforcing particles. As BPA may be present as potential impurity/residue, and tens to hundreds of grams of these bone cements are used per treatment, this may result in a considerable BPA release. However, to date no data exist on BPA release from these new bone cement materials.

3.6.3. Conclusions

Bisphenol A can be present in medical devices as residue from an (incomplete) polymerization process or result from the hydrolysis of the polymer.

Bisphenol A can be extracted from medical devices consisting of polycarbonate and/or polysulfones, the latter mostly being used in the form of membranes. BPA extraction can be performed *in vitro* with water, methanol or organic solvents resulting in dissolution of the product. Extraction in methanol results in higher release of BPA compared to water extraction. For PC casings BPA release in water was between 11 and 14 ng/casing, while in methanol the release was between 296 and 345 ng/casing. Results for PC pellets used for the production of medical devices were 4 to 7 mg/kg after dissolution of the pellets. In the Call for Information, for PC drinking cups values of 4-6 mg/kg were submitted. So, PC used for the production of medical devices seems to have BPA levels similar to those of PC commonly used as food contact materials.

In hemodialyzers, water and bovine serum circulation resulted in a BPA recovery of 4 to 142 ng/module for water and 141 to 2090 ng/module for bovine serum, again indicating that water is not the best medium for BPA extraction. This was confirmed by other data showing BPA release of 6 to 71 ng/dialyzer in water and 55 to 4300 ng/dialyzer in 17.2% ethanol. Low water extraction was observed for three different dialyzers being 141, 48 and 6 ng/dialyzer, respectively. In hollow fibres isolated from individual dialyzers and dissolved in hexane, BPA content was 8.3 to 12.2 µg/g (mg/kg) material. The highest values of BPA released corresponded to the 2 hemodialyzers tested that consisted of PC casings and PSU fibres were 1 and 2 µg/module. After sterilization procedures, some BPA may have already been released from the dialyzers.

The highest amount of BPA for dental materials measured was 67 nmol/mm^2 which amounts to 15 µg/mm^2 for a resin bonding material which is commonly not exposed to saliva. For PC orthodontic brackets, the BPA release varied between 22 µg/g (crushed brackets) to 697 µg/g retrieved after 40 months of use by patients. In general, there was very limited information provided to assess the reliability of available data.

3.7. Exposure scenarios

3.7.1. Knowledge on BPA exposure

Most people are exposed to BPA through the diet. Bisphenol A in food and beverages accounts for the majority of daily human exposure (Wilson *et al.*, 2007; Chapin *et al.*, 2007; Vandenberg *et al.*, 2007; EFSA 2013). BPA may migrate into food from food and beverage containers with internal epoxy resin coatings and from consumer products made of polycarbonate plastic such as tableware, food containers, and water bottles. PC was used in the production of baby bottles, but it was discontinued subsequent to European regulation. BPA exposure results from either the release of un-polymerized monomers or the slow decay of polymer bonds in polycarbonate leading to monomer release into proximal foods and liquids. Air, dust, and skin contact with thermal paper, are other possible sources of BPA exposure. Measured concentrations of BPA in human blood, urine and other tissues have indicated that the majority of the population (91–99%) has detectable levels of BPA-conjugates in their urine, confirming that exposure is widespread in the human population (Vandenberg *et al.*, 2007; Calafat *et al.*, 2008). Some studies indicate the presence of free BPA in blood; however, their reliability and the toxicological relevance is a subject of intense academic and public debate (Calafat *et al.*, 2005; Kang *et al.*, 2006; Vandenberg *et al.*, 2007; Dekant and Völkel, 2008; Calafat *et al.*, 2008; Becker *et al.*, 2009; Bushnik *et al.*, 2010).

To date, there are limited data available on potential exposure to BPA from the use of medical devices (Beronius and Hanberg, 2011).

3.7.1.1. Methods for measurement of internal exposure in humans

Biomonitoring directly measures human daily or cumulative exposures to xenobiotics from all sources by the determination of biological fluids (blood, urine, breast milk, saliva) or tissue concentrations of the chemical or its metabolites.

Regarding BPA, the analytical method used to detect both the parent compound and its metabolites is crucial, especially at the low levels expected in biological samples, and may represent a relevant source of differences among available studies. For biomonitoring studies, independently of the detection method applied, the use of a stable isotope-labeled BPA as an internal standard was suggested (WHO 2010) as the best means to determine the effect of complex matrices (i.e. biological samples) and the stability of BPA during analysis. The use of stable isotope-labeled BPA prevents discrepancies due to contamination from external sources during sample collection and processing (see below).

The features of different methods have been summarized by FDA (US FDA, 2010b): it appeared that 1) among the methods for BPA analysis, the Mass Spectrometry (MS)-based methods are considered the best and 2) data obtained by using immunochemical methods (ELISA, RIA) are the least reliable because of low sensitivity, poor selectivity due to cross reactivity with other phenols, and strong matrix effects (especially for urine samples). Even when cross-reactivity with endogenous steroids and most analogous BPA phenolic structures were reduced (Kaddar *et al.*, 2009), immunoassays tend to overestimate serum BPA concentrations when compared to LC-MS/MS data.

Recently, the development of a new specific radioimmunoassay was described for the direct measurement of BPA-glucuronide in urine without hydrolysis that requires only the elimination of unconjugated BPA by one solvent extraction step (Harthé *et al.*, 2012). A polyclonal anti-BPA antibody showing a 95% cross-reactivity with BPA-glucuronide and insignificant cross-reactivity with most phenolic structures analogous to BPA was used. The simple sample preparation phase can significantly reduce external contamination. The method is reported to be valid, precise and accurate in the range of 0.05 mg/L to 5 mg/L, with a LOD comparable to GC and LC methods. It was reported that BPA-conjugate concentrations measured with the radioimmunoassay method correlated with total BPA concentrations measured by GC/MS in 32 urine samples (r=0.86) (Harthé *et al.*, 2012).

GC/MS methods for BPA analysis in blood and urine have been widely used (US FDA, 2010b), although they require derivatization or esterification of BPA (which is a non volatile chemical). There is a time-consuming sample preparation phase with different extraction and pre-concentration procedures that may increase the possibility of external contamination of the sample itself (e.g. by plastic disposable lab devices or solvents). Recently, classic GC–MS method based on solvent and solid phase extraction followed by derivatization was adapted and validated for determination of BPA also in tissue samples, such as human placental and fetal liver tissue (Zhang *et al.*, 2011) and in the human maternal and umbilical cord blood serum (Kosarac *et al.*, 2012).

In contrast, HPLC methods can be used to analyse BPA without any pre- derivatization step (Inoue *et al.*, 2001; Inoue *et al.*, 2003).

An LC/MS-MS method was developed to quantify both free and conjugated BPA in blood and urine samples against an internal standard (d^{14}-bisphenol A-glucuronide) (Völkel *et al.*, 2005) and further modified to analyse different matrices (i.e. breast milk) (Ye *et al.*, 2006). D^{16}-BPA or $^{13}C_{12}$-BPA can be used as an internal standard. The LOD of LC-based method is in the range of 0.1-0.4 ppb (ng/g) in blood or urine samples.

With both GC and LC-based methods, an enzymatic hydrolysis step with beta-glucuronidase is usually carried out, as BPA-glucuronide is not generally available as an analytical standard. The requirement for two separate assays may lead to critical pitfalls in terms of accuracy and external contamination (see below). To overcome these problems, BPA-glucuronide may be isolated from urine samples, purified by flash chromatography and characterized by mass spectrometry and NMR. The isolated BPA-glucuronide was found to be suitable as analytical standard for the simultaneous

quantification of BPA and BPA-glucuronide in biological matrices by UPLC/MS/MS (Lacroix *et al.*, 2011).

3.7.1.2. Internal exposure to BPA in humans from all routes

A large number of biomonitoring studies to measure BPA in urine are available from North America, Europe and South-east Asia (for overviews see Dekant and Völkel, 2008; Vandenberg *et al.*, 2007, 2010a, 2010b; US FDA, 2010b; Geens *et al.*, 2012). Importantly, in urine BPA is present in its conjugated form. Urinary biomonitoring data provide information on the internal dose, which is the result of total BPA exposure, independently from the sources: therefore, biomonitoring data in urine accounts not only for dietary exposure, but also for non-food sources (e.g. medical devices, dust, thermal and other kind of papers).

Methodological issues

The appropriateness of the study design (i.e. sampling time, most adequate fluid/tissue, appropriate sampling and storage procedures) requires a detailed knowledge of the biotransformation and toxicokinetics of any xenobiotic and/or its metabolites, as well as a suitable analytical method. This is particularly relevant for BPA, with its rapid conjugation and an elimination half-life time of only a few hours in blood. BPA concentrations in blood decrease quickly after exposure (WHO, 2010).

In addition, blood concentrations of total BPA (free plus conjugates) determined at one time point are not representative of an average exposure, because it is strongly dependent on the time of blood sampling with respect to the exposure time. Since BPA urinary excretion is almost complete within 24 hour after exposure and due to less invasive sampling, urine is the matrix of choice for assessing daily exposure to BPA in humans. Similar to blood, single spot urine samples serve as a measure of very recent BPA exposures. Urine BPA levels depend on frequency of food intake, time of sampling after food consumption, the last urination and urine production rate.

A 24 hour pooled urine collection is the most appropriate sample to determine average daily exposure to BPA. However, in large cohorts, the high number of spot urine samples collected will average out variations in urinary concentrations of total BPA among individuals arising from temporal factors within a day. It has been reported that mean values from studies reporting BPA concentrations in spot urine samples with a large number of participants correlate well with those using cumulative excretion over 24 hours (Völkel et al, 2008; WHO, 2010). Recently, Christensen *et al*. (2012) specifically investigated whether spot sample concentrations of BPA are comparable to daily average concentrations. Overall, spot urinary concentrations of BPA have variability roughly similar with corresponding 24 hour average concentrations obtained from a comparable population, suggesting that spot samples can also be used to characterize population distributions of intakes, although caution should be applied in interpreting the high end of spot sample data sets.

In order to estimate the daily BPA intake, the urinary concentrations of total BPA (free and conjugated form) should be multiplied with the 24 hour urinary output (mL) to get the daily excretion of BPA in ng/day, considering that excretion is almost complete in 24 hours (Völkel *et al.*, 2002, 2005). In addition to the urinary output, BPA concentration can also be adjusted for daily creatinine excretion or for body weight. The correction for urinary output is generally preferred over creatinine excretion (Lakind and Naiman, 2008), due to its high interindividual variation (over 1000 %) (Geens *et al.*, 2012). The correction is quite relevant when comparing data obtained in different studies, although the corrections are not always used or reported. However, the urine volume is also

related to several other factors such as liquid intake, physical exercise, and individual health and lifestyle factors (WHO, 2010).

No study has investigated whether urinary excretion of BPA and other environmental phenols differed by renal function. However, You *et al*. (2011) estimated the association between renal function and urinary excretion of BPA and they suggested that urinary excretion of BPA decreased with decreasing renal function. Because the associations might differ by age or sex, further studies are necessary to replicate these results and understand the mechanism.

For risk assessment purposes, both free BPA and its metabolites should be determined, especially because most BPA in human is present as conjugates. Koch *et al.* (2012) investigated the extent of BPA body burden in the German population from 1995 to 2009 based on 600 24 hour-urine samples and corresponding plasma samples from the Environmental Specimen Bank. They concluded that the total BPA in urine is the most appropriate and robust marker for BPA exposure assessment (if controlled for BPA contamination). Data on unconjugated BPA in urine and unconjugated or total BPA in plasma, where contamination or breakdown of the glucuronide cannot be ruled out, are of no value for human exposure assessment.

Another potentially confounding effect in determining free BPA is the deconjugation of BPA due to spontaneous hydrolysis and/or to bacterial contamination of the urine sample which may cause hydrolysis (Schöringhumer and Cichna-Markl, 2007; Ye *et al.*, 2007; Helander and Dahl, 2005).

As far as levels in urine sample are concerned, the reporting of data is a crucial issue for comparing data, which are either reported with or without creatinine-adjustment to correct for urine dilution, or adjusted by using 24 hour urine volume which is not specified. However, a common result is that BPA conjugates (mainly glucuronides) are by far the major BPA form present in human urine.

Possible artifacts in measurements of population exposure to BPA

The leaching of BPA from PC containers for sampling and storage, as well as plastic disposable lab devices, sample workups and analysis (including HPLC capillary systems and solvents) has been identified as a source of free BPA in biological samples. This kind of contamination, possibly contributing to the concentration of free BPA, has been reported in some studies (Markham *et al.*, 2010; Twaddle *et al.*, 2010; Ye *et al.*, 2011).

A recent survey conducted in France on pregnant women after delivery supported the importance of urine sample collection methodology, but also relevant for this opinion, the potential source of exposure and/or contamination due to the use of medical devices (Vandentorren *et al.*, 2011). Women who had caesarean sections had much higher levels of free and total urinary BPA than women giving birth naturally, the 95th percentile being 273.9 µg/L vs. 4.2 µg/L, with data adjusted for creatinine levels (Vandentorren *et al.*, 2011). Since the analytical methodology was correct and urine samples were stored in polypropylene tubes at -4°C first and then at -80°C, prior to be analysed, contamination with BPA from exogenous sources during storage can be excluded. However, the paper does not clearly describe the way urine was collected, but the hypothesis was given about the possibility that urinary collection devices were used in women having caesarean section, suggesting that the source of higher BPA levels could be hospital-based. Indeed, supplemental studies on the release of BPA from catheterization probes of urine at room temperature for 12 and 24 hours clearly indicate the time dependence of free BPA extraction from the probes up to approximately 300 µg/L. In this case, since the major source of BPA is external to the body of patients, it can be concluded that measured free BPA was due to contamination rather than to real exposure of patients via medical devices.

The presence of analytical artefacts cannot be excluded in many of the available studies that did not carry out the appropriate internal controls (Twaddle *et al.*, 2010).

Background contamination has been quantified by Doerge *et al.* (2010a) and found to be as high as 2 ng/mL in buffer blanks, which may be responsible for the differences in free BPA levels reported in many studies. This highlights the importance of sample preparation and clean-up in the determination of traces of BPA in complex matrices such as biological samples, to remove interfering matrix compounds and to increase selectivity by using solvent extraction or SPE clean up or a combination of sol-gel immunoaffinity columns containing anti-BPA antibodies (Cichna-Markl, 2012). Results obtained with the administration to animals or volunteers of ^{14}C-labelled or deuterated BPA are not affected by background contamination and are the most reliable methods to perform the BPA toxicokinetics studies and as input for the risk assessment. For measurements not using isotope labelled BPA, a quality control on possible BPA contamination due to sampling equipment and consumables used within the assay is necessary.

In addition, the variability of results obtained in biomonitoring studies to population exposure of BPA depends also on human inter-individual differences, which include age, diet, presence of pathologies (e.g. renal failure, hepatic dysfunction), genetic factors (e.g. polymorphisms of UDPGT enzymes) as well as the pattern of exposure. Especially during medical treatment, additional exposure to BPA may occur when using medical devices consisting of polycarbonate components.

Human data on BPA exposure

Much of the concern for BPA exposure has come from studies reporting relatively high levels of free BPA in human body fluids/tissues (Schönfelder *et al.*, 2002; Fernandez *et al.*, 2007; Lee *et al.*, 2008; Jimenez-Diaz *et al.*, 2010; Zhang *et al.*, 2011; Geens *et al.*, 2012).

The presence of high levels of free BPA in human specimens in those studies were questioned on the basis of 1) the kinetics of BPA in humans (supported by data in animals, including non human primates) showing <1% of total free BPA in serum at peak levels and an almost complete and rapid excretion in human volunteers and 2) the low aggregate intake estimates (mean<1 microg/kg b.w./day) made from urinary conjugated BPA measurements (Lakind and Naiman, 2010). This raises questions in data generation and evaluation that should be addressed, underlining the needs for a careful quality control to be included in the study design to avoid/minimize any possible source of artefact or external contamination, and confounding factors (Calafat and Needham, 2009; Dekant and Völkel, 2008; Markham *et al.*, 2010; Ye *et al.*, 2007). The major ones are related to i) stability of BPA and BPA conjugates in the sample matrix and during sample processing, ii) BPA background levels due to sample handling and work up, iii) use of appropriate internal standards and analytical methods, including adequate reporting of data.

Most biomonitoring data obtained in different biological samples available up to 2010 have been tabled in a FDA report (US FDA, 2010b) and recently reviewed (Geens et al., 2012). Comparison among results from different studies is not always simple, due to reporting difference. As an example, for statistical analysis non-detectable samples are assigned a value which is generally, but not always the LOD value, differently determined by the various authors. For this reason all data below LOD and LOQ, as well as some average (median) values should be interpreted with caution.

From blood data, which were measured in a limited number of enrolled individuals, it appeared that free and conjugated BPA levels are typically ≤ 1 µg/L (US FDA 2010b), which is consistent with the known rapid plasma clearance of BPA and its metabolites and kinetic studies conducted in humans. Only two studies (Schönfelder *et al.*, 2002; Padmanabhan *et al.*, 2008) reported much higher free BPA levels in maternal and fetal plasma as well as placenta tissue. Because they did not measure conjugates, the reported levels have a limited value and should be interpreted with caution. In a recently performed controlled human study at environmentally relevant BPA doses, where potential technical factors as sources for BPA variability in biological tissues were adequately reduced, serum concentrations of parent BPA were consistently below LOD

(1.3 nM) (Teeguarden *et al.*, 2011). The residual observed variability in free BPA levels, approximately by a factor of 4, could be attributed to interindividual variability in BPA metabolic disposition.

Liao and Kannan (2012) determined free and conjugated BPA (glucuronide and disulfate) forms of BPA in human urine and serum samples of 32 healthy volunteers, using solid-phase extraction (SPE) and liquid chromatography−tandem mass spectrometry (LC−MS/MS) techniques. They found 32% and 19% of free BPA with respect to the total BPA measured in urine and serum, respectively. Glucuronated BPA, the dominant metabolite, was present in this form in 57% and 43% of the total BPA in urine and serum, respectively.

The influence of functionally relevant polymorphic UGT2B15, the major UGT isoform responsible for BPA metabolism, on the blood concentration time profile has been recently investigated by using a validated physiologically based kinetic human model (Partosch et al, 2013). Maximum concentrations (Cmax) and AUCs in blood varied for a factor of 4.7 and 4.6 in high and low metabolisers (dose: 1 µg/kg/day) in accordance with biomonitoring data reported by Teeguarden et al. (2011). The highest Cmax-value calculated in the subject with the lowest metabolic clearance is roughly 40 pg/mL, far lower than the reported high blood concentrations which cannot be explained by a genetically impaired UGT2B15 activity (Partosch et al, 2013).

The largest-scale studies with a consistently high number of enrolled participants (n= 2517 and 5476 individuals) spread over a broad range of age, were carried out in the USA and Canada, respectively (Calafat *et al.*, 2008; Bushnik *et al.*, 2010).

In both studies, after adjusting BPA levels for creatinine, the youngest age category (6–11 years) showed the highest urinary concentrations (3.6 ng/mL in the US and 1.30 ng/mL in Canada), when compared to the average values of the general population (2.6 ng/mL and 1.16 ng/mL, respectively). Recent biomonitoring studies in Asian countries gave similar results (Zhang *et al.*, 2011).

According to WHO (WHO, 2010), the available urinary data allow to estimate the median exposures for adults and for children in the range of 0.01–0.05 µg/kg body weight (b.w.) per day and 0.02–0.12 µg/kg b.w. per day, respectively. Similarly, US FDA derived mean daily intake of BPA is <0.03 - 0.13 µg/kg b.w./day for a 60-kg adult, and 0.07 to 0.12 µg/kg b.w./day for a 36.1-kg child (age 3-14), by using FDAs standard default assumptions (US FDA, 2010a). The recent evaluation of BPA exposure by EFSA (EFSA, 2013) indicated diet to be the main source of exposure to BPA in all population groups. In the current exposure of up to 857 ng/kg b.w./day 22 for toddlers and up to 495 ng/kg b.w./day for infants of 1-5 days were estimated, while for adults (including woman of childbearing age) the exposure was up to 132 ng/kg b.w./day.

Mose et al. (2012) studied in human placentas *ex vivo* the PBA transplacental transfer rate. Results lead the authors to conclude that free BPA can cross the placenta by passive diffusion with a transplacental transfer rate of 1 (i.e. the concentration in the fetal blood was equal to the concentration in the blood of the mother), similarly to a previous study (Balakrishnan et al., 2010).

Two recent studies reported BPA concentrations (both free and conjugated forms) in amniotic fluid and fetal liver samples (Edlow et al., 2012; Nahar et al., 2012). However, the lack of procedures used to avoid contamination and deconjugation of BPA during sample handling, do not allow any conclusions to be drawn from the study results.

The occurrence of BPA in breast milk was analysed in some small-scaled studies (Cariot *et al.*, 2012; Ye *et al.*, 2006; Ye *et al.*, 2008; Otaka *et al.*, 2003; Sun *et al.*, 2004; Kuruto-Niwa *et al.*, 2007; Yi *et al.*, 2010). This information can be relevant to estimate exposure of breast-fed infants, as a consequence of exposure of the lactating mother. The lactational transfer from the maternal plasma compartment to the maternal milk compartment, both as free and conjugated BPA, has been demonstrated in rat studies (Snyder *et al.*, 2000; Doerge *et al.*, 2010c), where dams were administered a daily oral

dose of 100 µg/kg b.w. of ^{14}C- or deuterium-labelled BPA, respectively. The older study indicated that total exposure of the lactating pups was estimated to be approximately 0.3% of the dose (mg/dam)/kg pup weight, with about 80% of the total BPA concentration consisting of BPA-glucuronide (EFSA, 2010; Doerge et al., 2010c). The more recent study reported that on day 7 postpartum at 1 h after dosing, when BPA serum levels are maximal (Doerge *et al.*, 2010a), mean concentrations of 0.2 ng/mL and 1.7 ng/mL for free an total BPA, respectively, with a proportion of only 13% free BPA, were observed. Consequently, the concentrations in pup serum are estimated below 0.2 nM (45.6 pg/ml). Therefore, pup exposure via lactation is extremely low (1/300 of the maternal dose).

BPA has also been reported in human milk. In the most recent study (Cariot *et al.*, 2012), in which appropriate measures to avoid contamination by environmental BPA were taken, including milk collection (drawn manually without breast milk pumps or gloves), free BPA was absent in solvent blanks. BPA was detected only at low concentrations (≤0.12 ng/mL; lower than the LOQ) in some of the pooled, mature breast milk samples taken from donors breast-feeding for over 1 month, used as quality-control materials. In the test samples collected from additional donors (n=3) within a few days after delivery, distinctly higher levels of free BPA (0.80, 3.07, and 3.29 ng/mL) were detected compared to the donors breast feeding for over 1 month. The very small number of samples limits the possibility of generalising the obtained results and the difference in the protein/fat composition between colostrum and mature breast milk limit the possibility of comparing the two groups. In addition, since no information is available on possible treatment with medical devices during the hospitalization period of the three mothers, a parenteral exposure to BPA cannot be excluded. This information could be interesting also for the understanding of the influence of medical devices on the exposure of possible groups at risk, like the infants.

In two studies ELISA (Kuruto-Niwa, 2007) or HPLC-FLD (Sun *et al.*, 2004) methods were used to detect total BPA in breast milk: 1.4–7.1 ng/mL; median =3.0 ng/mL in samples from 101 healthy mothers in the former and 0.28–0.97 ng/mL in 23 samples in the latter. However, the scarce reliability of the methods used limit the relevance of these data. In two studies in the USA, free and total BPA in breast-milk samples were quantified by isotope-dilution HPLC-MS/MS (Ye et al, 2006; 2008). Free BPA was detected in 60% of the 20 analysed samples with a median of 0.4 ng/mL (below the LOQ) and a maximum of 6.3 ng/mL (Ye et al, 2006), with free BPA being around 36% of total BPA. In their later study, Ye *et al.* (2008) additionally analysed 4 milk samples. The free and total BPA concentrations were 0.41–1.54 ng/mL and 0.73–1.62 ng/mL, respectively, with free BPA in the individual samples accounting for 50–99% of the total. The presence of β-glucuronidase in human milk (Gaffney *et al.*, 1986, Grazioso and Buescher, 1996) could be the cause for such a high free BPA proportion. However, importantly, the glucuronide could be deconjugated in the infants' gut by intestinal β-glucuronidases of bacterial origin, although not in neonates due to the scant presence of intestinal flora, which usually develop starting from the 8th postnatal month.

Otaka et al. (2003) reported free BPA content of 0.65 and 0.70 ng/mL in 2 out of 3 human milk samples by using a GC-MS method, whereas in a more recent study (Yi et al, 2010) free and total BPA in milk samples from 100 mothers (within two weeks from delivery) were detected by LC-MS/MS at values higher than 10 ng/mL.

On the basis of available biomonitoring and exposure data, it was recently concluded that the exposure to BPA from non-food sources that by some authors was hypothesised as potentially relevant sources (Calafat *et al,*, 2009; Stahlhut *et al.*, 2009; Taylor *et al.*, 2011) is generally lower than that from exposure from food by at least one order of magnitude for most studied subgroups (Geens *et al,* 2012). Dietary exposure was indeed estimated to contribute for more than 90% to the overall BPA-exposure for non-occupationally exposed individuals (Geens *et al.*, 2012) and exposure through dust ingestion, dental surgery and dermal absorption from thermal paper accounted for less than 5%. As a consequence total BPA blood concentrations should be directly related to

contaminated food consumption, and individuals with long fasting times before urine collection should have substantially lower BPA urine levels than those with shorter fasting times. The study design and sample collection are again highlighted as a crucial issue. A specific case remains exposure to BPA through release from medical devices, especially in some subgroups as dialysis patients and infants in Intensive Care Units.

However, considering the toxicokinetics profile of BPA, any significant degree of bioaccumulation is not expected. If, following repeated exposure, BPA accumulation occurs in slowly releasing tissues, such as fat, then BPA concentrations in urine should increase with age. This is not the case in humans: individuals in the range 60-85 years old show lower levels than younger people enrolled in the study of Stahlhut *et al.* (2009).

Recently, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, Paris, France) published their report on the evaluation of the risk of BPA for human health (ANSES, 2013). The report provides information on the aggregated exposure of pregnant women and unborn children due to air, dust and food in France. The median internal exposure was 1.68 ng/kg per day, whereas the maximum internal exposure of all three exposure routes (95^{th} percentile) was estimated at 4.18 ng/kg per day of which 84% was due to food, 4% due to dust and 12% due to air exposure (ANSES, 2013). The conversion factor used from external to internal exposure was 0.03 (3%). In this evaluation, ANSES excluded possible exposure to medical devices including dental materials, and cosmetics. Similar to EFSA, ANSES has also concluded that the diet is the main source of BPA exposure for the general population.

EFSA (2013) estimated the BPA exposure due to dietary uptake of BPA. The highest exposure for children older than 6 months and up to 10 years of age was 857 ng/kg b.w./day and for infants days 1-5 after birth 495 ng/kg b.w/day. For adults, the highest exposure of 388 ng/kg b.w./day was estimated.

3.7.1.3. Non-oral exposure routes

Most studies and evaluations have focused on the potential for BPA exposure from dietary sources. BPA exposure from other routes, namely through dermal and inhalation routes, have received far less attention. Very few studies have estimated total BPA exposure from multiple sources. Data from available studies on BPA exposure from dermal and inhalation exposure routes are described below.

<u>BPA in Cosmetics and other consumer products</u>

Zhu *et al.* (2010) employed molecularly imprinted layer-coated silica nanoparticles for extraction of BPA from 3 cosmetic samples followed by determination by HPLC-fluorescence detection: Shampoo 1.71 nmol/g (398 ng/g); bath lotion 2.69 nmol/g (614 ng/g); cosmetic cream 1.46 nmol/g (333 ng/g). Cacho *et al.* (2013) determined BPA in 30 personal care products by extraction using ethylene glycol-silicone coated stir bars followed by thermal desorption–gas chromatography–mass spectrometry. BPA was found in six of the thirty analyzed samples (shower gel, hair gel, face cream, make-up remover and mouthwash) at concentrations ranging from 30.9 ng/g to 88.3 ng/g. Employing GC-MS in full scan mode, Dodson *et al.* (2012) determined up to 55 substances, including BPA, in 298 consumer products. BPA at concentrations 1-100 µg/g was found in 15 samples, including a vinyl shower curtain and pillow protector, dish and laundry detergent, tub and tile cleaner, soaps, lotions, shampoo, conditioner, shaving cream, nail polish, and sunscreen. However, uncertainty in BPA determination in consumer products performed by GC-MS in full scan mode may be very high. A large variation in BPA levels found in cosmetic products in the three studies may be associated with the uncertainty of measurements by three different methods. It may not be relevant to evaluate BPA exposure from cosmetics at present due to a few and non-comparable data. However, in

exaggerated exposure estimation, EFSA (2013) concluded that the contribution for BPA exposure due to cosmetics only contributes less than 3% of the total BPA exposure.

Dermal exposure

A common source of BPA exposure to consumers may be due to its presence in various types of papers. The amount of BPA used in thermal paper in the EU is 1,700 tons (EC, 2008). Of 13 thermal printing papers analysed, 11 were found to contain 8–17 g BPA/kg paper (Biedermann *et al.*, 2010). Vinggaard *et al.* (2000) reported BPA concentration 0.6 – 24 mg/ kg (mean 6.21 mg/ kg) in 9 kitchen paper towels/kitchen rolls made of 80 – 100% recycled paper. In toilet papers, BPA was found to be 46.1 mg/kg (Gehring, 2004). Lopez-Espinosa (2007) found BPA (geometric mean 2.38 ng/g of material) in 47.50% of samples of paper and cardboard investigated independent of the percentage of recycled material and the composition of the paper.

Biedermann *et al.* (2010) found that when taking hold of a receipt consisting of thermal printing paper for 5 s, roughly 1 µg BPA (0.2–6 µg) was transferred to the forefinger and the middle finger if the skin was rather dry and about ten times more if these fingers were wet or very greasy. Seventy-three percent of the BPA transferred to dry skin by holding thermal printer paper was extractable after 2 hours. The authors speculated that 27% of the BPA transferred from the thermal printing paper to the finger was penetrated into the skin. From these data, exposure of a person repeatedly touching thermal printer paper for 8 hours/day, such as at a cash register, could reach approximately 57 µg/day. Sun *et al.* (2001) reported that BPA content in commercially available samples of PVC wrap film, PVC gloves and PVC hose to be 68±3.5 µg/g, 60.5±2.8 µg/g and 290.1 µg/g, respectively. No data is available on BPA exposure from PVC gloves, but it cannot be ruled out that small amounts of BPA may be transferred to the skin when these gloves are used. BPA was reported in five types of plasticised PVC food wrapping films at 0, 43, 96, 98 and 483 mg/kg (Lopez-Cervantes and Paseiro-Losada, 2003). It is not known if these samples, purchased in Spain, were made in the EU or were imported or if they were produced before or after the December 2001 cessation date given by The European Council of Vinyl Manufacturers (KEMI, 2011). No more recent survey data on testing PVC for BPA could be found in the literature.

In the EU risk assessment report on BPA (ECB 2003), some worst-case scenarios for dermal exposure of BPA were described. For epoxy-based surface coatings and adhesives, the main route of exposure is dermal. Use of BPA in printing inks is considered to result in negligible exposure.

Exposure from brush application of antifouling paint (without protective clothing), based on a paint containing 40% epoxy-resin and a residual level of 10 ppm BPA in the resin, will result in a dermal exposure to 29 µg BPA. The use of wood fillers without gloves will result in 9 µg dermal exposure of BPA per event. Application of wood varnish without use of gloves will result in 3.6 µg dermal exposure of BPA. Based on residual level of 1 ppm BPA in an adhesive, dermal exposure to BPA arising from the use of adhesives was calculated to be 0.014 mg per event.

Air and Dust

Air and dust levels of BPA serve as another potential source for human BPA exposure. Staples *et al.* (1998) estimated 1000 t/year release of BPA from industrial production. Fu and Kawamura (2010) found that the concentrations of BPA (1-17,400 pg/m^3) ranged over 4 orders of magnitude in the world with a declining trend from the continent (except for the Antarctica) to remote sites. Matsumoto *et al.* (2005) found that BPA concentrations in urban ambient outdoor air in Osaka during six months ranged from 0.02 to 1.92 ng/m^3, with increasing levels from autumn to winter and decreasing levels from winter to spring.

Wilson *et al.* (2007) found that potential sources of BPA exposure in preschool children included outdoor and indoor air and house dust, as well as soil from homes and daycare centers. Concentrations in indoor air from homes and daycare centers ranged from <LOD to 193 and 8.99 ng/m^3, respectively. Concentrations in outdoor air ranged from <LOD to 44.6 and 51.5 ng/m^3 in homes and daycare centers, respectively. Concentrations in dust ranged from <LOD to 707 and 156 ng/g in homes and daycare centers, respectively. Rudel *et al.* (2003) found BPA present in 86% of house dust samples at concentrations ranging from 0.2 to 17.6 µg/g. Völkel *et al.* (2008) reported median BPA concentration in dust from 12 private houses in Germany to be 553 ng/g (range 117 to 1486 ng/kg).

Geens *et al.* (2009) found that BPA concentration in indoor dust samples from 18 Belgian homes was 535-9730ng/g; and dust samples from 2 offices contained 4685 and 8380 ng BPA/g dust. Geens *et al.* (2009) calculated 29 ng /d (or 0.4 ng /kg b.w./day) BPA intake through dust by an adult with an average dust intake of 20 mg /d and a median BPA content of 1460 ng/g. Exposure assessments, based on the different sources of BPA exposure from the National Toxicology Program, gave a range of 0.043–14.7µg/kg b.w./day BPA for 1.5 and 6 years-old children and between 0.008 and 1.5µg/kg b.w./day for adults (US National Toxicology Program, 2008). Loganathan and Kannan (2011) measured BPA in dust from 50 homes and 6 laboratories in the Eastern USA. Mean and median concentrations of BPA in dust were 843 and 422 ng/g, respectively. The authors calculated that the median daily intake of BPA via dust for adults and toddlers were 0.35 and 5.63 ng/kg b.w./day, respectively. The contribution of dust to total human intake of BPA was estimated to be <1%. Thus, despite the high concentrations of BPA measured in dust, this route of exposure seems to be a minor contributor to the total exposure.

According to EU risk assessment, BPA exposure as a result of brush application of antifouling paints (containing 40% epoxy-resin and a residual level of 10 ppm BPA in the resin), in a worst case scenario, resulted in inhalation exposure of $3x10^{-4}$ µg (ECB 2003). For wood varnish, a value of 0.02 µg for inhalation exposure to BPA per event was calculated, in a worst case scenario (ECB 2003).

Recently ANSES estimated that dust contributed for 4% to the total BPA exposure for pregnant women (ANSES, 2013).

3.7.2. Exposure to BPA from medical devices

The major source of BPA exposure by medical devices is due to medical devices made from polycarbonate (PC) and polysulfone (PSU). With regard to the possible presence of BPA in medical devices made of PVC, notwithstanding the statement that the use of BPA for polymerization and as a stabiliser for storage of vinyl chloride monomer was discontinued in Europe from December 2001 (KEMI 2011). However, PVC as a possible source of BPA exposure cannot be completely excluded because BPA-containing PVC may still be used in the EU due to medical devices coming from outside of the EU because of the global market for medical devices. For example BPA was reported in several consumer products consisting of PVC (See above).

Only limited information on BPA exposure from medical devices was found in the scientific literature. Elevated blood levels of BPA in dialysis patients have been reported (Murakami *et al.*, 2007; Krieter *et al.*, 2009; Shintani and Hayashi, 2011). Calafat *et al.* (2009) found that *total* BPA (including BPA metabolites) geometric mean urinary concentration (30.3 µg/L) among prematurely born infants undergoing intensive therapeutic medical interventions was about ten times higher than that among the general population.

Dialyzers

Shintani (2001) reported average levels of BPA (n=4) of 0.2 and 0.7 ppb (ng/mL) in the blood of patients exposed for 4 hours to dialyzers (PSU fibres, PC housing) sterilized by

autoclaving. The average concentrations in the blood of patients treated with two other dialyzers (both with PSU fibre, one with PC housing and other with polystyrene-butadiene copolymer housing) sterilized with gamma radiation, were below limit of detection (0.02 ppb or ng/mL). HPLC-MS was used for BPA detection. Samples (ca 10 mL) were collected before dialysis and after 4h of dialysis treatment. Tests were also performed using saline solutions (according to ISO 10993-7) to compare with migration into blood, using the same dialyzer sterilized by the same procedure. BPA levels found were 0.1-0.2 ppb (ng/mL) in the saline solution and 0.2 to 0.7 ppb (ng/mL) for blood. Pre-treatment BPA blood levels were below the detection limit (0.02 ppb in plasma). It was not stated in the publication if total or free BPA was measured and whether the blood was drawn from the patient or directly from the equipment.

Sajiki *et al.* (2008) reported levels of BPA in plasma of 53 hemodialysis patients, before and after hemodialysis, and in plasma of 5 healthy volunteers. The BPA measurements were performed by 3 different methods: LC/ECD, LC/MS and ELISA. The plasma BPA levels in 5 healthy persons were 0.033±0.075 ng/ml (LC/ECD) or 0.284±0.146 ng/mL (ELISA). Plasma BPA levels in patients before and after hemodialysis were 0.284 ±0.748 ng/mL and 0.642±1.443 ng/mL respectively when measured by LC/ECD, or 0.505 ±2.125 ng/mL and 0.197±0.248 ng/mL respectively when measured by ELISA, or 0.310±0.840 and 0.179±0.263 ng/mL respectively when measured by LC/MS. Individual values or range of BPA in plasma are not reported. The results of the study cannot be considered reliable because the recovery of 100 ng/ml BPA in plasma or water was only 61-72% by LC/MS and ELISA methods and the selectivity of LC/ECD method was very poor. In addition, the hemodialysis set-up used for any of the patients was not described.

Murakami *et al.* (2007) determined the amount of BPA leaching from dialyzers with PSU membranes in the blood of 15 patients. An indication for an increase in BPA was found from 4.83 ± 1.94 ng/mL blood prior to dialysis to 6.62 ± 3.09 ng/mL thereafter (increase of 1.79 ng/mL); however, a later second test with the same patients and the same dialyzers resulted in a much lower increase in BPA serum levels, from 3.78 ± 2.57 ng/ml to 4.27± 2.98 (0.49 ng/ml increase). Both the quoted differences are not statistically significant; in addition, BPA concentration was measured by ELISA, which may be prone to cross reactivity with other phenols, leading to overestimation of BPA content.

Krieter *et al.* (2013) found that despite differences in BPA elution into water from 3 different types of dialyzers with high and low-flux polysulfone and polyethersulfone membranes, the plasma levels of 18 patients with chronic kidney disease treated with any of the dialyzers did not significantly change after a 4-week treatment period. On the other hand, BPA pre-dialysis plasma concentrations in patients were significantly higher (range 9.1 ± 4.5 – 12.0 ± 6.0 ng/mL) than those found in the healthy control group (n= 24, ≤ 0.2 ±0.1 ng/mL). Fractions of protein-bound and free plasma BPA were found to have similar values in dialysis patients (74 ± 5%) and in the control group (70 ± 3%). ELISA method was used for BPA measurements.

Dental materials

Recent reports from different authorities addressing the risk assessment of BPA from various sources, especially food contact materials, have to some extent also addressed dental materials. The general conclusion is that the contribution from dental materials to the total exposure is low (Beronius and Hanberg, 2011; FAO/WHO, 2011; Environment Canada/Health Canada 2008; EC 2010b; US NTP-CERHR, 2008; EFSA, 2013). A report recently published by the Swedish National Board of Health and Welfare (Socialstyrelsen 2012) addressed "Bisphenol A in dental materials". The report summarizes research on *in vitro* and in vivo studies related to BPA from dental materials, and concludes that there is a possibility of low-dose exposure to BPA from dental materials, either as a contaminant (very low amounts) or from degradation of Bis-DMA. In the report, calculations based on the maximum values of BPA found in fissure sealants and in composite materials, in combinations with the actual amount of material used in clinical practice and a median 4-year life-time of a composite restoration, suggest a maximum exposure of 0.06 µg

BPA/day from fissure sealants, and a maximum exposure of 0.36 µg BPA/day from composite restorations.

Kang *et al.* (2011) assessed the changes in bisphenol A (BPA) levels in saliva and urine after placing lingual bonded retainers. Liquid chromatography/mass spectrometry was used to examine the BPA levels in the saliva and urine samples collected from 22 volunteers who received a lingual bonded retainer on their mandibular dentition. Samples were collected immediately before placement and 30 minutes, 1 day, 1 week, and 1 month after placement. The salivary BPA level detected in the samples collected just after placement with a mean concentration of 5 ng/mL and a maximum of 21 ng/mL.

Olea and co-workers (1996) recruited 18 healthy men and women who were treated with one molar sealant, and found no composite components before treatment (except in one subject who was excluded), whereas after treatment all saliva samples contained variable amounts of BPA ranging from 90 to 931 µg in the total saliva produced by the volunteers, who spit and collected their saliva in glass vials during the whole 60-minute period.

Arenholt-Bindslev and colleagues (1999) enrolled 8 adult male volunteers who were treated with 4 molar sealants of 2 different brands. BPA assessment was done before, immediately after, 1 hour after and 24 hours after sealant placement. Before treatment, there was no detectable BPA, but after sealant placement BPA levels ranged between 0.3- 2.8 ppm (µg/mL). For materials containing BisDMA, the BPA was found to be released immediately into the saliva after application, while at 24 hours after the placement BPA levels in saliva were back to the pretreatment levels (Schmalz *et al.*, 1999; Arenholt-Bindslev *et al.*, 1999).

Fung et al., (2000) recruited 40 adults and found BPA in some saliva specimens collected at 1 hour and 3 hours after treatment, ranging from 5.8 to 105.6 ppb (ng/mL). In addition, Joskow and co-workers (2006), using two brands of sealants, assessed saliva samples in 14 patients who received a mean number of 6 sealants. Saliva samples were collected pretreatment, immediately after, and 1 hour post treatment. Mean BPA before treatment that was 0.30 ng/mL reached 42.8 ng/mL, immediately after placement.

Zimmerman-Downs (2010) enrolled 30 adults who were treated with one or four occlusal sealants. BPA assessment was done 1 hour prior and 1, 3, and 24 hours post sealant placement. BPA was elevated significantly from baseline measurements before sealant (range 0.07 to 6.00 ng/mL) at all post-treatment time points for both groups, with main peak amounts of 3.98 and 9.08 ng/mL for one-occlusal sealant group and four occlusal-sealants group, respectively.

More recently, Han *et al.* (2012) made a survey including 62 children serving as control, without restorations in the oral cavities, and 62 children treated with more than 4 sealants. A possible relationship was found: BPA level of control was 0.40 µg/L, whereas BPA levels in saliva of treated children was 0.92 µg/L after controlling for confounders.

Kingman and co-workers (2012) collected saliva before and after (<1, 1-8 and 9-30 hours) from 172 participants receiving composite restorations. BPA concentrations in saliva significantly increased within an hour after treatment from 0.43 to 0.64 ng/mL (Geometric Mean), together with other restorations materials. At time periods (1-8h) or (9-30h) after restoration, no differences in BPA-concentrations were found compared to before treatment.

In summary, the release of BPA into the saliva occurs in patients receiving dental restorations. Placement of resin-based composite restorations polymerised *in situ* was associated with detectable increases in saliva of BPA. The release mainly occurs during the few hours directly after application and contributes to the oral exposure to BPA. An overview of the BPA releases, as discussed above for medical devices used in dentistry, are presented in the table 2 below.

Recently, Kloukas *et al.* (2013) made a systematic review of *in vivo* BPA release from dental pit and fissure sealants. Six interventional and two observational studies, examining in vivo BPA release in human salivary, blood and urinary samples, were

included. BPA levels identified in saliva ranged from traces below the method's detection limit to 30 µg/mL. In urine, BPA quantities spanned from 0.17mg/g to 45.4mg/g. The quantitative analysis showed evidence of BPA release one hour after sealant placement compared to the amount traced before restoration (Stouffer's z trend: <0.001).

From the qualititative and quantitative synthesis of available studies, it is reasonable to conclude that some BPA is released after placement of dental pit and fissure sealants in the oral cavity. The largest quantities are detected in saliva immediately after or one hour after their placement.

Table 2 BPA in saliva after application of a dental sealant

Reference	Number/ Individuals	Time	BPA concentration	Maximum BPA reported
Kingman *et al.*, 2012	150/adult	At <1 h	0.64 ng/mL	
Han *et al.*, 2012	124/Children		0.92 ng/mL	8.305 µg/L
Zimmerman-Dawns *et al.*, 2010	30/adults	At 1 h	9.08 ng/mL	
Joskow *et al.*, 2006	14/adults	At 1 h	42.8 ng/mL	96.2 ng/ml
Sasaki *et al.*, 2005	21/adults			100 ng/ml
Fung *et al.*, 2000	40 adults	At 1 h		105.6 ppb (ng/mL)
Arenholt-Bindslev *et al.*, 1999	8 male volunteers	Immediately after		2.8 ppm (µg/mL)
Olea *et al.*, 1996	28/adults	All the saliva produced in 1 h		931 µg

Note to the table: The studies are listed in the chronological order (most recent first).

Only one study was identified in the category of orthodontic adhesives releasing BPA in saliva. Kang and coworkers (2011) evaluated the changes of BPA levels in saliva before and after placing a lingual bonded retainer on the mandibular dentitions of 2 volunteers. Samples were obtained immediately before placement of the retainer and 30 min, 1 week and 1 month after placement. Mean salivary levels of BPA were 5.04 ng/mL (range 0.85-20.88 ng/mL) in the immediately collected samples.

<u>Medical procedures</u>

A study by Sakurai (2002, only abstract available in English) indicates migration of BPA from cardiopulmonary bypass (CPB) circuits during open heart surgery and when using a saline priming solution. Blood samples were obtained from 6 patients who underwent open heart surgery after the CPB process was initiated and at the termination. For the

priming solution study, eight circuits were used and as control 3 samples were collected directly from the saline in a polyethylene container. BPA levels measured in the blood were 0.3 ± 0.2 µg/L, after the commencement of CBP and 0.4 ± 0.3 µg/L when it was finished. In the priming solution from the circuits, higher levels were found: 0.9 ± 1.1 µg/L. No BPA was detected in the control samples. BPA was considered to be leached from the circuit because parts of the reservoir and of the oxygenator were made of polycarbonate containing BPA.

Calafat *et al.* (2009) found that among prematurely born infants undergoing intensive therapeutic medical interventions, the *total* BPA (including BPA metabolites) geometric mean urinary concentration was 30.3 µg/L with 946 µg/L as the highest value measured that was about ten times higher than that among children 6-11 years old (Calafat *et al.*, 2008). More than 90% of the BPA detected in the urine of the prematurely born infants was in its conjugated (e.g. glucuronide, sulfate) form. The authors attributed the high BPA levels to the recent treatment given to the infants after birth rather than to the *in utero* exposure. Notably, BPA total concentrations among infants in one health care unit were about 17 times higher than those among the infants hospitalized in the second one. The authors suggested that this difference may be due to parenteral exposure via medical devices used, which is supported for the same neonates by a strong association between di(2-ethylhexyl) phthalate (DEHP) and BPA total concentrations.

Based on the iso-propanol extraction of BPA from PC pellets (0.2-0.3 mg/kg) and assuming that 100% of this BPA would also leach from the material during clinical use within a single day, the patient exposure could be estimated as high as 0.0386 µg/kg b.w./day, considering a body weight of 58 kg. This estimation was presented in the data submitted through the Call for Information.

Women who had caesarean sections showed much higher levels of free and total urinary BPA than women giving birth naturally, the 95th percentile being 273.9 µg/L vs. 4.2 µg/L, with data adjusted for creatinine levels (Vandentorren *et al.*, 2011). However, as contamination with BPA from exogenous sources during sample storage can be excluded, the high level of BPA was attributed to the release of BPA from the urinary catheter used for collection. Indeed, supplemental studies on the release of BPA from catheterization probes into urine at room temperature for 12 and 24 hours clearly indicate the time dependence of free BPA extraction from the probes up to approximately 300 µg/L.

Conclusion

BPA release was demonstrated from dialyzers, dental materials, circulation equipment, neonatal care medical devices, and urinary catheters. Some clinical studies involving hemodialysers indicated that there were no significant BPA plasma level changes after dialysis treatment. However, one study reported a difference in BPA plasma levels between dialysis patients and controls. The release of BPA from other medical device is generally very poorly characterised in human studies.

Dental materials release BPA especially shortly (0-1 h) after placement, the levels of BPA detected in saliva ranged from 0.64 ng/mL to 30 µg/mL. This wide range in BPA measurements reflects a continuous reduced leaching of BPA from dental materials, probably due to the reduced use of bis-DMA.

3.7.3. Exposure to BPA from medical devices under different scenarios

Scenarios in hospitals

Taking into account the many possible sources of exposure of patients during hospital care and the scarcity of information related to release of BPA from medical devices, six scenarios were considered to be representative situations.

(i) External contact with a medical device containing BPA;

(ii) Contact with oral/dental material and / or orthodontic equipment;

(iii) Contact with implants such as valve, pacemaker, insulin dispenser made in polycarbonate;

(iv) Hemodialysis;

(v) Prolonged surgical procedures such as bypass operations and transplantations;

(vi) Prolonged exposure to different sources of BPA in intensive care units.

The specific case of health care workers using PVC gloves which may contain traces of BPA (around 0.05%) was not considered even through a few cases were reported describing contact dermatitis against BPA (Aalto-Korte *et al.*, 2003). The use of BPA in PVC products has been discarded by European PVC manufacturers (KEMI, 2011).

(i) External contact with a medical device-containing BPA.

This situation occurs very frequently since many medical devices such as catheters, trocars, laparoscopic and endoscopic instruments, breast pumps, prescription spectacles and lenses, etc., may contain residues of BPA (see also Annex I). In this scenario exposure may be repeated but, taking into account the small surface area of the medical device in contact with skin or mucosa, the amount of released BPA is very low. Taking the example of a disposable laryngoscope blade made of polycarbonate, the surface area in contact depends on the size and the shape. Sizes range from 00 (premature) up to 3 (large, adult). The surface area is estimated to be in the range ca. 5 to 25 cm^2. For a 1 kg premature infant and a 60 kg adult this is 5/1 = 5 and 25/60 = 0.4 cm^2/kg b.w. The application is single use. The contact medium is mucosa, the temperature is 37°C and the contact duration is a few minutes at most.

Calculation: For the release of BPA from polycarbonate, EFSA (2013) considered tableware (plates, cups etc.) made of polycarbonate and using from the literature the migration data into water, 3% acetic acid and 50% ethanol under testing conditions of 2 hours at 70ºC. These data were combined with data from the EFSA call for data obtained under the same testing conditions. An upper-bound value of 0.0013 mg/L was derived from the 2h contact times. Rounding this value up to 0.002 mg/kg and assuming a conservative surface area: volume ratio of 1000 cm^2/L, migration of BPA from PC articles equates to 2 ng/cm^2. Assuming (conservatively) that each contact use of a laryngoscope releases the same amount (on an area-related basis) as PC for 2 hours of contact at 70ºC, exposure from the laryngoscope would be 1 ng/kg b.w./use for premature infants and 0.08 ng/kg b.w./use for adults.

(ii) Contact with dental material and orthodontic equipment

Dental materials may be divided in two scenarios

- Short-term exposure in conjunction with dental treatment: one full crown restoration of a molar may release after 24 h on average 57.38 nmol (13µg)(Van Landuyt, 2011)
- Long-term exposure from the use of dental materials: After 24 h, no elevation in BPA-level (saliva, urine) is found (Kingman *et al.*, 2012; Kang *et al.*, 2011)

Additionally, there may be a possible exposure from the procedure of removing a restoration. This situation is not well covered in the scientific literature and may need further investigation.

Calculation: Given the considerable uncertainty with respect to the chemical composition of dental materials used, both now and in the past, the value of 13 µg released in the one day following treatment (Van Landuyt, 2011) is considered for short-term (acute) exposure and this would be 200 ng/kg b.w./day for a 60 kg b.w. adult. The value of 0.36 µg/day (6 ng/kg/b.w./day) from the Swedish review is considered for the long-term exposure scenario for adults undergoing composite restorations and 0.06 µg/day (2 ng/kw b.w./day) for a 30 kg child treated with fissure sealants.

For orthodontic equipment, the case of PC brackets secured using adhesives is considered. Release of BPA from adhesives and PC brackets under experimental conditions is presented in table 1 above.

Calculation 1: For short-term exposure, there was BPA release of 66 µg/L from a light-cured adhesive resin analysed at day-7 (Sunitha *et al.*, 2011). With a typical saliva production of 15 mL/kg b.w./day the short-term exposure would be 140 ng/kg b.w./day.

Calculation 2: For medium-term exposure, there was a BPA release of 2.9 µg/L from a light-cured adhesive analysed after 30 days immersion in simulant (Eliades *et al.*, 2011). With a typical saliva production of 15 mL/kg b.w./day, medium-term exposure would be 1.5 ng/kg b.w./day. Adding this to the daily exposure possible from the PC brackets (see below) gives exposure of 13.5 and 7.5 ng/kg b.w./day for a 30 kg b.w. child and a 60 kg b.w. adult, respectively.

Calculation 3: For long-term exposure, Watanabe *et al.* (2001) found that PC brackets released BPA when immersed in water. The BPA increase in the water was 3.8-fold after 12 months immersion in water at 37°C, and 14-fold after 14 weeks immersion in water at 60°C, when compared to virgin water values. BPA release started slow at 0.4 µg/g of PC until 6 months and increased to 3.8 µg/g of PC at 12 months immersion in water at 37°C. With a typical weight of approximately 40 mg and a maximum number of 24 applications, the total weight of brackets would be ca. 1 g of PC. The release would be 10 ng/day or an exposure of 0.33 and 0.17 ng/kg b.w./day for a 30 kg b.w. child and a 60 kg b.w. adult, respectively. In this long-term scenario, it is assumed that any release of BPA from the adhesive used to secure the brackets has declined to a not detectable level.

(iii) Contact with an implant

According to the implanted device such as a valve, pacemaker or a dispenser made in polycarbonate, the surface in contact with tissues and body fluids may be estimated (worst case scenario) to be 50 cm^2. EFSA (2013) considered the migration from PC baby bottle into food simulants and they derived an average migration of 0.89 µg/L and a high migration of 4.56 µg//L. The most usual test conditions employed with food simulants was 10 days contact at 40°C. Rounding-up, taking the high migration of 0.5 µg/L per day, and the conservative area volume ratio (see above) of 1000 cm^2/L, then a release value of 0.5 ng/cm^2/day can be derived.

Calculation 1: 50 cm^2 releasing 0.5 ng/cm^2/day would give exposure of 25 ng per day, this being 0.4 ng/kg b.w./day for a 60 kg b.w. adult or 0.8 ng/kg b.w./day for a 30 kg b.w. child. This release rate includes the possibility of hydrolysis of the PC surface rather than only classical diffusion-migration.

Calculation 2: Mass of 10 g PC in the medical device and with up to 10 mg/kg residual BPA gives a residual content of 100 µg. If 10% were released in the first 30 days of use of the implant (highly unlikely) this would give 333 ng/day or 6 and 11 ng/kg b.w./day

for a 60 kg adult or 30 kg child respectively. This calculation assumes that the PC does not hydrolyse *in situ*.

(iv) Haemodialysis

Patients experiencing renal failure need, before eventual kidney transplantation, regular blood separation performed by exchange between blood and osmolar dialysis fluid through a dialyser comprising a membrane and a support unit. The carter is often made by polycarbonate and hold PSU membranes (often hollow filters) which may release BPA as explained in the paragraphs 3.6.2 and 3.7.2.

The highest values of release of BPA by such dialysers, as found in the literature, are comprised between 2 µg/module (Haishima *et al.*, 2001) and 4 µg/session (Fink, 2008). According to their medical problems, weight, age and activity, the patients undergo 3 to 6 dialysis sessions each week (mean duration 3-4 hours) for the most active patients (>70kg + sport practice), down to 2 sessions a week for the aged inactive and < 50kg patients.

Calculation: In the worst care scenario, 4 µg of BPA per session, 6 times a week, for a 60 kg b.w. adult the exposure would be 57 ng/kg b.w./day.

(v) Prolonged surgical procedures

In this case of extracorporeal circulation (by-pass) or transplantation / implantation of an artificial organ such as heart, according to the size and the nature of catheters and artificial organ, the release of BPA may be estimated in the same range as in the care of hemodialysis. For infants and children, although the use of medical devices may be scaled-down, it is assumed that there is the same release of BPA but with a smaller body weight. Consequently the short-term exposure would be 685, 114, and 57 ng/kg/b.w. for an infant, child and adult of 5, 30 and 60 kg body weight respectively.

(vi) Prolonged exposure to different sources of BPA in intensive care units

a) in adults intensive care units

Adult patients hospitalized in intensive care units (ICU) are treated with the use of:

- Venous or arterial catheters (inserted by peripheral way, PICC line), with a classical duration comprised between 2-3 weeks to some months onco-hematology (Kabsy *et al.*, 2010)
- Respiratory assistance using tubing (some hundred grams)

b) in neonatal intensive care units

The neonates' BPA exposure can be via the respiratory tract (incubator walls and respiratory tubing), the blood (catheters) and via the oral route (tubing, mother's milk collected via breast pump in polycarbonate).

Neonates hospitalized in intensive care units (NICU) are treated with the same medical devices as adults, often for several months, like:

- Venous or arterial catheters
- Respiratory assistance
- Incubator (the walls are made in polycarbonate)

For an incubator hood of dimensions 80 x 60 x 70 cm (W x D x H), the surface area of polycarbonate is 24400 cm^2. In fact these dimensions are not used in the calculations, just the vapour pressure.

Calculation 1: "In hoods of neonatal incubators and neonatal intensive care units, BPA could not be detected (LOD approximately 50 µg/m^3)" (see paragraph 3.6.2, data submitted via the call for information).

Breathing volume of a prematurely born infant ca. 100 mL/kg/min gives 0.144 m^3/kg b.w./day. If the air contained BPA at the detection limit and assuming complete uptake out of the air, the exposure would be $\leq$7 µg BPA/kg b.w./day. Due to the high detection limit, this calculation is likely to be unrealistic; therefore, a second calculation was performed.

Calculation 2: The vapour pressure of BPA is 5.3 x 10^{-6} Pa at 25°C. Assuming gas ideality, since 1 mole of a gas occupies 25 L at STP at this vapour pressure, then 25L of air above pure BPA solid would contain 5.3 x 10^{-11} moles or 1.2 x 10^{-8} g of BPA. Correspondingly, one m^3 would contain 4.8 x 10^{-7} g. Residual BPA in PC is up to 10 mg/kg or a 10^{-5} mass fraction. Assuming for simplicity a linear concentration-vapour pressure relationship, BPA in air at equilibrium with PC would be ca. 4.8 x 10^{-12} g/m^3. Exposure = 7x10^{-13} g/kg b.w./day (but higher at 37°C). Although this simple calculation uses many assumptions and is subject to considerable uncertainty, it illustrates that the result of calculation 1 is likely to be an over-estimate by several orders of magnitude.

NOTE. For the vapour pressure of BPA various numbers were reported 1.6 x 10^{-9} hPa at 20°C, 4.12x10^{-9} hPa at 25°C, and 3.91x10^{-7} mm Hg at 25°C. ANSES and EFSA indicate a value of 5.3x10^{-6} Pa at 25°C (ANSES, 2013; EFSA, 2013).

Calculation 3: Calafat *et al.* (2009) found that *total* BPA (including BPA metabolites) geometric mean urinary concentration (30.3 µg/L) among prematurely born infants undergoing intensive therapeutic medical interventions was about ten times higher than that among the general population. The medical care given is not itemised and may or may not include PC incubators along with other medical devices. Fluid intake for prematurely born infants is unlikely to exceed ca. 150 mL/kg b.w./day. Insensible water loss in a heated incubator can be 0.6 - 0.7 mL/kg b.w./hr for a larger prematurely born infant up to 2-3 mL/kg b.w./hr for a very immature infant with immature skin and a large exposed skin area (Kliegman et al, 2011). Taking urine output as fluid input (150) minus insensible loss (2 x 24) gives ca. 100 mL urine/kg b.w./day. So a concentration of 30.3 µg/L urine would equate to approximately 3 µg/kg b.w./day.

(vii) Breast pump and collection vessel made of PC

Calculation: Analogy is made here with migration levels from PC baby bottles. EFSA (2013) considered the migration from PC baby bottle into food simulants and they derived an average migration of 0.89 µg/L and a high migration of 4.56 µg//L. Taking the average migration value along with a high level of consumption of milk by infants of 150 g/kg b.w./day (EFSA, 2011) would give exposure of 134 ng/kg b.w./day.

3.7.4. BPA exposure from uses of BPA containing PVC

Since no information on BPA release from PVC-made medical devices is available to evaluate the possible exposure to BPA originating from PVC, a parallel was drawn and information was used from the evaluation of exposure to DEHP from medical devices made of PVC, performed by SCENIHR (SCENIHR, 2008).

Plasticised PVC is used in certain parts of medical devices for procedures such as blood transfusion, haemodialysis, parenteral nutrition or endotracheal tubing. Di(2-ethylhexyl) phthalate (DEHP) is the main plasticiser used in PVC-based medical devices. The typical concentration of DEHP in plasticized PVC is 30% (ECB, 2004).

The five DEHP-plasticised PVC food wrapping films tested by Lopez-Cervantes and Paseiro-Losada (2003), contained BPA at 0, 43, 96, 98 and 483 mg/kg. The samples of plasticised PVC wrapping film, gloves and hose analysed by Sun *et al.* (2001) contained BPA at 68, 61 and 290 mg/kg respectively. From these 8 results, a crude average of approximately 150 mg BPA /kg PVC was derived.

Comparing the two concentrations, with DEHP at approximately 300,000 mg/kg (i.e. 30% by weight) and BPA at ca. 150 mg/kg, the concentration ratio is 2000:1. The estimation of exposure is based on the assumption that DEHP and BPA leach from plasticised PVC used in medical device, in this same ratio of 2000:1.

For adults, the SCENIHR 2008 opinion used data based on measurements of DEHP blood levels in patients before and after specific medical procedures and concluded that blood transfusions to trauma patients or during ECMO (extracorporeal membrane oxygenation) may be the short-term procedures that result in the highest acute DEHP exposure in adults, up to 10 mg/kg/day. Long-term haemodialysis was the continuously repeated procedure which may result in the highest cumulative dose of DEHP and this could be up to 2.2 mg/kg/day.

For neonates, the SCENIHR concluded that the highest short-term exposure may occur due to double volume exchange transfusion (up to 23 mg/kg/day) while ECMO is the medical treatment which may give the highest daily exposure over a prolonged period of time, at up to 14 mg/kg/day. Similarly, SCENIHR noted that the US-FDA (2002) had estimated an upper-bound daily DEHP dose to be around 3 mg/kg/day for a newborn in the neonate intensive care unit setting, considering exposure from multiple devices. Such exposures may occur for a period of weeks or even months (SCENIHR, 2008).

The estimates of possible exposure to BPA from plasticised PVC medical devices that are calculated by a 2000-fold down-scaling of these SCENIHR estimates for DEHP are shown in Table 3.

Table 3. Derivation of estimates of possible exposure to BPA from medical devices made of plasticised PVC.

	Adults short-term	**Adults longer-term**	**Neonates short-term**	**Neonates longer term**
DEHP estimate, µg/kg b.w./day [a]	10,000	2,200	23,000	14,000
BPA estimate, µg/kg b.w./day [b]	5	1	12	7

a) SCENIHR (2008).

b) Taking the estimates for DEHP and reducing by a factor of 2000 to account for the ratio of the concentrations of DEHP and BPA possible in plasticised PVC (300,000 and 150 mg/kg respectively, see text)

As stated, these estimates in Table 3 are based on the assumption that all the PVC used in the medical devices contain BPA and that the leaching propensity of BPA and DEHP from plasticised PVC are about the same. Considering the two molecules, BPA has a lower molecular weight than DEHP (228 versus 390 Daltons), but perhaps more significantly, BPA is only moderately lipophillic whereas DEHP is strongly lipophillic (log octanol-water partition coefficients of 3.3-3.5 versus 7.5). The exposure scenarios are for leaching into blood which, although largely aqueous, does have an important lipid

fraction too. Therefore, in the absence of experimental data, this assumption on relative leaching rates is reasonable.

Considering the case of non-plasticised PVC, if BPA were to be present in the plastic, it is assumed that rigid PVC has only minimal uses in medical devices and also that leaching rates from the plastic would be much lower than from plasticised PVC. Thus, this potential source of exposure is not being considered further.

3.7.5. Conclusions

Table 4 summarises the outcome of the different exposure scenario calculations. There is considerable uncertainty with respect to the chemical composition of medical devices and their release properties with respect to BPA. For each scenario considered, there are relatively few studies reported and in most cases the studies do not have much information on how the material under study is representative of the European situation. For these reasons the estimates of exposure have made conservative assumptions and extrapolations. Nevertheless, these figures should be viewed and used with some caution since they could underestimate or overestimate the true exposure of some patients.

Some of the estimated BPA exposures due to medical devices are in the same range as exposure via the food (EFSA 2013). EFSA estimated the exposure to be highest for infants and toddlers among the population older than 6 months, with the highest estimated average of 375 ng/kg b.w./day and a high dietary exposure of 857 ng/kg b.w./day. The modeled dietary exposure for teenagers, adults and elderly ranged from 116 to 159 ng/kg b.w./day for average exposure, with a high exposure ranging from 341 to 388 ng/kg b.w./day.

Table 4. BPA exposure from medical devices as estimated for various use scenarios.

	BPA exposure estimation in ng/kg b.w./day			
Exposure scenario	Prematurely born infant	infant	child	adult
External contact with a medical device containing BPA (short-term)	1			0.08
Contact with dental material				
(short-term)	na	na		200
(long-term)	na	na	2	6
Contact with orthodontic equipment				
(short-term)			140	140
(medium-term)			13.5	7.5
(long-term)			12	6
Contact with an implant				
(medium-term)			11	6
(long-term)			0.8	0.4
Hemodialysis (long-term)				57
Prolonged surgical procedures				

(short-term)		685	114	57
Prolonged exposure to different sources of BPA in intensive care units (medium-term)	3000			
Breast pump and collection vessel made of PC (medium-term)		134		
Uses of PVC (short-term) (long-term)	 12000[a] 7000[a]			 5000[a] 1000[a]

a) In the absence of data on BPA, leaching properties of DEHP have been considered for the estimation.

3.8. Toxicokinetics of bisphenol A

3.8.1. BPA biotransformation

The major BPA metabolite in human is BPA-glucuronide, which is quantified in plasma and rapidly excreted in the urine; BPA-sulphate has also been detected after oral exposure as a minor urinary metabolite (Hanioka *et al.*, 2008; Kim *et al.*, 2003; Ye *et al.*, 2005). In both monkeys and rats, BPA is biotransformed by the same reactions (EFSA, 2010): the predominant pathway is glucuronidation, with the sulfation reaction representing <20% for monkeys and <5% for rat.

The efficiency of the first pass is demonstrated by the very small amounts of unchanged parent BPA, up to 9.5% of the oral administered dose is recovered in human urine (Dekant and Völkel, 2008; Völkel *et al.*, 2008; Ye *et al.*, 2005), whereas a high % of the total amount was detected as PBA-conjugates. This is especially relevant for risk assessment, since the conjugates do not retain the biological activity of the parent BPA which is the toxicologically relevant compound (Snyder *et al.*, 2000; Shimizu *et al.*, 2002, Willhite *et al.*, 2008).

From *in vitro* data, Kurebayashi *et al.* (2010) calculated that 91% of the hepatic clearance is due to glucuronidation and 9% due to sulfation in human hepatocytes; a similar percentage was observed also for clearance in monkey and rat hepatocytes. *In vivo* a variable ratio between the two reactions was determined: indeed, glucuronides account for 80-100% and sulfates for 0-15% of the oral BPA dose measured in urine of human volunteers and in individuals unintentionally exposed to BPA (Völkel *et al.*, 2002; Ye *et al.*, 2005). According to a study in a Korean population, the ratio glucuronidation/sulfation is higher in men than in women (4 vs 1.5, respectively) (Kim *et al.*, 2003). The glucuronide/sulphate ratio was not age-dependent in either species (Doerge *et al.*, 2010b).

Sulfation of BPA is mediated by sulfotransferases (SULT); SULT1A1, which is involved in the conjugation reaction of other phenols (Campbell *et al.*, 1987), has been identified as the major isoform mediating BPA sulfation in the human liver, although recombinant SULT2A1 and 1E1 showed also some activity (Nishiyama *et al.*, 2002). Among some recombinant human (UDP)-glucuronosyltransferase isoforms, UGT2B15 showed the highest activity over the range of BPA concentrations (1-20 μM) tested (Hanioka *et al.*, 2008). Other authors, by using a complementary panel of recombinant enzymes, identified a relevant role for UGT1A9 (which is expressed both in the liver and in the gastrointestinal tract) (Doerge *et al.*, 2010b) and for UGT2B7 (Mazur *et al.*, 2010).

It has been suggested that hepatic metabolism plays a more relevant role in humans than the intestinal one, as confirmed by recent studies carried out with human microsomes pooled from different donors and from different organs (intestine, kidney, liver, and lung), reporting that the tissue intrinsic clearances for the kidney and intestine were less than 1% of liver intrinsic clearance, whereas human lung microsomes did not show glucuronidation activity towards BPA (Mazur *et al,* 2010; Trdan Lušin *et al,* 2012). In order to evaluate the possible impact of the polymorphic allelic variants genetic polymorphism in BPA conjugation, UGT2B15 variants and the *28 polymorphism in the UGT1A1 gene were studied *in vitro*_(Hanioka *et al*, 2011; Trdan Lušin *et al*, 2012). Among the six recombinant UGT2B15 allelic variants no significant difference in the Km value between wild-type and any variant UGT2B15 variant was evidenced; on the contrary UGT2B15 variants having D85Y substitution showed markedly reduced Vmax and intrinsic clearance (around 10% of the wild type enzyme) (Hanioka *et al*, 2011). When human liver microsomes genotyped for UGT1AS1*28 polymorphisms were tested, wild-type homozygotes and heterozygotes did not significantly differ, while polymorphic homozygotes significantly differed from both, showing 25% residual activity. The lower catalytic efficiency for glucuronidation is due to decreases in Vmax with negligible changes in Km, consistently with the lower expression of UGT1A1 in microsomes with a *28 promoter polymorphism.

However, due to the redundancy in UGTs for conjugation, the possible compensatory activity of SULT enzymes and the overlapping substrate specificity, it is expected that a single polymorphism would not significantly affect the total BPA glucuronidation capacity of individuals (EFSA, 2010). To support this conclusion, a recent study using a human based PBPK modelling estimated a factor of 4.7 for the maximum blood concentration (Cmax) and of 4.6 for the Area Under the Curve (AUC) between human hepatic cells from 15 different donors showing low and high intrinsic clearance for BPA (Partosch *et al,* 2013).

In addition, starting from data on UGT variants by Hanioka *et al.* (2011) for an oral dose of 1 µg/kg/day, the Cmax for the UGT2B15.2 and the UGT2B15.5 variant was 3.9-fold and 4.9-fold higher than that of the wild-type, respectively with AUC values 4.9 and 5.5-fold higher with respect to the wild type (Partosch *et al.*, 2013).

The glucuronidation and the sulfate pathways are negatively correlated: by using a PBPK model it has been evidenced that in subjects with low glucuronidation capacity the fraction of dose which is metabolized to the sulfate conjugate is higher than in subjects with high glucuronidation capacity (Partosch *et al.*, 2013).

This limited level of variability is further confirmed by the results of a recently performed biomonitoring study showing variability in BPA levels in human tissue approximately by a factor of 4, attributed to inter-individual variability in BPA metabolic disposition (Teeguarden *et al.*, 2011).

Therefore, it can be considered that the default value of 10 used to account for kinetic interindividual variability within the general population (IPCS, 2005) can cover differences due to polymorphically expressed enzyme activity involved in BPA metabolism.

More recently additional metabolic pathways have been reported in *in vitro* studies, but their relevance in vivo has not been demonstrated to date.

Rat liver microsomes and recombinant human CYPs have been shown to biotransform BPA into hydroquinone via an ipso-substitution reaction and isopropenylphenol and hydroxycumyl alcohol (HCA) were also produced as further metabolites (due to a C-C bond scission). Formation of novel metabolites via ipso-substitution pathway was about 20% of o-OH-BPA formation, via traditional oxidative pathway of P450 (Nakamura *et al*, 2011). Among the panel of 12 recombinant CYP tested, only CYP3A4 and 3A5 catalysed the ipso-substitution of BPA, whereas the hydroxylation of aromatic or hydrocarbon of BPA is catalysed by CYPS of the 2C family (CYP2C18> CYP2C19> CYP2C9) (Niwa *et al.*,

2001). Interestingly, HCA had higher ER-binding activity than BPA; for this reason, this metabolic pathway has been claimed to play a role in the estrogenic activity of BPA.

The formation of another active BPA metabolite, namely 4-methyl-2,4-bis(4-hydroxyl-phenyl) pent-1-ene (MBP), was demonstrated in incubation with rat liver S9 fraction in the presence of a NADPH-generating system (Yoshihara *et al.*, 2001, 2004). MBP showed estrogenic activity several fold higher than BPA in the yeast estrogen screening (YES) and in rat in vivo uterotrophic assay (Okuda *et al.*, 2010). MBP is formed by recombination of the radical fragment of BPA, which is the one-electron oxidation product of carbon-phenyl bond cleavage, and its formation required both microsomal and cytosolic fractions (Okuda *et al.*, 2011). Anti-CYP3A2 and anti-CYP2C11 antibodies strongly inhibited the formation of MBP, suggesting the involvement of these two isoforms in generating dimer-type metabolite, whereas the exact function of cytosol is still unclear. Similar metabolic activation was demonstrated also for various other BPA-related compounds, including BPB and BPF (Okuda *et al.*, 2011).

However, these *in vitro* studies have been carried out with recombinant enzymes or subcellular fractions in experimental conditions avoiding any competition with the predominant and more efficient metabolic pathways, involving direct conjugation of the parent compound. The relative importance of these reactions in actual *in vivo* conditions, when the other pathways are simultaneously active, is not known, although it is expected to be relevant under circumstances where glucuronidation is unable to work efficiently as a detoxification pathway of BPA.

3.8.2. Toxicokinetics after oral uptake

In humans, BPA is readily and almost completely absorbed by the oral route, as demonstrated by the high recovery in the urine of human volunteers (97% and 84% of the ingested dose in males and females, respectively) (Völkel *et al.*, 2002, 2005). Since human volunteers were dosed with deuterated BPA, it was possible to differentiate between administered BPA and BPA coming from other sources. Results were consistent with studies carried out dosing nonhuman primates (Doerge *et al.*, 2010a and b) and with some modeled data (Mielke and Gundert-Remy, 2009). Comments on these data (Vandenberg *et al.*, 2010a, 2010b) were evaluated concluding that these comments were not scientifically justified (Hengstler *et al.*, 2011).

In humans, both low and high single oral doses of BPA are well absorbed (>90%). This is a conservative estimate for neonates, since the immature pancreatic and biliary secretion and the scant presence of intestinal flora up to the 8th month can limit the absorption of BPA in infants (Ginsberg *et al.*, 2002). Because of high first-pass metabolism in the liver, the systemic availability of free BPA is low and varies between species. As a consequence, the half-life is very short, ranging from 1 to 3.5 hours (Völkel *et al.*, 2002, 2008; Tsukioka *et al.*, 2004; Shin *et al.*, 2004), as estimated by considering the excretion data and assuming that the rate-limiting step is BPA detoxication.

Oral doses of labelled BPA (methyl-d6-BPA, 100 µg /kg b.w.) given to adult non human primates are nearly completely absorbed, with very low (<1 nM) serum levels of free BPA (Doerge *et al.*, 2010b), indicating a very limited systemic availability of the parent compound and a concentration–time profile for total BPA similar to that of human volunteers administered a dose of 50–90 µg/kg b.w. BPA (Völkel *et al.*, 2002). The estimated half-life of 3.5 hours for total BPA in rhesus monkey (Doerge *et al.*, 2010b) was similar to those reported in cynomologous monkeys (4.2h; Tominaga *et al.*, 2006), and humans (3.4h, Völkel *et al.*, 2002). Similar agreement was found for other pharmacokinetic parameters obtained in non human primates (Kurebayashi *et al.*, 2002; Tominaga *et al.*, 2006; Taylor *et al.*, 2011; Tharp *et al*., 2012; Patterson *et al*., 2013). The comparison of results obtained after oral dosing and i.v. injection, expressed as the ratio $AUC_{oral}/AUC_{i.v.}$ indicated also the relevance of the first-pass effect, supporting the conclusion that presystemic conjugation mainly occurring in the liver after oral

administration is a crucial factor in determining the internal dose of free BPA after oral administration.

A marked species difference exists in BPA disposition when data obtained in human and monkeys are compared to rodents, where BPA undergoes enterohepatic recirculation (Kurebayashi *et al.*, 2003; Upmeier *et al.*, 2000; Pottenger *et al.*, 2000). After an oral dose BPA is readily and completely absorbed also in rats, metabolized in the liver with a high degree of conjugation similar in the three species (>99%): the comparison with data on free BPA in serum obtained after oral dosing and i.v. injection led to the conclusion that a high first-pass effect take place also in rats, and indeed, the total hepatic clearance for BPA conjugation as measured in vitro with cryopreserved hepatocytes is higher in rats then in monkey and human cells (Kurebayashi *et al.*, 2010). Then, at variance with human and monkeys, BPA metabolites in rats are excreted from the liver via the bile into the gastrointestinal tract, and not in the urine. The BPA conjugates are cleaved back to BPA and the free BPA is reabsorbed (enterohepatic recirculation). As a result, BPA clearance takes longer in rats than in humans, the half-life in rodents being 19-78 hours (EFSA, 2006). The occurrence of a high metabolic turnover and the occurrence of the enterohepatic recirculation was also demonstrated by a recent study using stable isotope–labeled BPA, showing a second peak in the concentration of total BPA in the plasma concentration–time profiles (Doerge *et al.*, 2010a): this second peak was totally absent in primates kinetics.

As in rats, the plasma concentration–time profiles after an oral dose of BPA to female CD-1 mice exhibited a second peak in the concentration of total BPA, indicating the presence of the enterohepatic recirculation (Taylor *et al.*, 2011), although the half-life (~4 hours) was lower than the one exhibited by the rats. The efficiency of the pre-systemic conjugation reaction was very high, with only ~1% of the administered dose was found as free BPA 30 minutes after dosing.

The availability of studies on mice, rats and monkeys with a common study design (same dose and vehicle, analytical methodology, model-independent pharmacokinetic analysis, age ranges), allowed reliable interspecies comparisons. Internal exposures to free BPA following oral administration are similarly low (Cmax <10 pM per g/kg b.w.) for adults of all three species, again supporting the dominant role of presystemic Phase II metabolism. Although there are major differences in BPA metabolism and disposition between rodents (enterohepatic recirculation and extensive fecal excretion of unconjugated BPA) and primates (extensive urinary excretion of conjugated BPA) that directly affects BPA half-life, which, again, is longer in rats than in primates. Based on the analysis of oral versus intravenous toxicokinetic data (Doerge et al., 2010a, 2010b; 2011; 2012), the oral systemic bioavailability of unconjugated BPA is 2.8%, 0.2% and 0.9 % in rats, mice and monkeys, respectively. Gayrard et al. (2013) also reported that the absolute bioavailability for unconjugated BPA in blood was below 1% after orogastric dosing in dogs. The systemic availability of unconjugated BPA in humans has not been evaluated experimentally, however, modelled data and controlled biomonitoring studies indicated that internal exposure in humans to unconjugated BPA is very low (1-10%) (Mielke and Gundert-Remy, 2012; ANSES, 2013).

The comparison of BPA kinetics in adult vs. neonatal animals in the three species was also performed, highlighting another major interspecies difference related to neonatal development in the Phase II metabolism of BPA.

Oral administration of the same BPA dose (100 µg/kg b.w.) to PND3 (Post Natal Day 3) in rat pups produced higher Cmax in serum of total and free BPA (6- and 74-fold, respectively) when compared to adults. The fraction present as conjugates increased with age time (93.4, 96.9 and 98.9% at PND 3, 10 and 21, respectively), indicating a progressive development of metabolic and excretory functions toward the adult situation (when 99.5% BPA is in conjugated form). Similar results supporting the presence of first-pass metabolism, albeit at levels markedly lower than in the adult rat, were reported in neonatal rats following oral delivery of 10 µg BPA/kg b.w. (Prins *et al.*, 2011): free BPA constituted 29%, 21% and 31% of total BPA levels at 0.5, 1 and 2 h, respectively. The

relative deficiency in Phase II metabolism in newborn versus adults is even more pronounced in the mouse (Doerge *et al,* 2011b). The similarity in the toxicokinetics in newborn mice following subcutaneous (s.c.) or oral administration (AUC ratio = 1.0) appeared to be mice-specific and was explained by the metabolic immaturity, rapid oral absorption, and rapid distribution of unconjugated BPA (Doerge *et al*., 2011b). With advancing postnatal age, due to the increasing maturation of metabolic and elimination processes, the typical differences in toxicokinetics between the parenteral and the oral routes were evidenced similarly to the rat. The Cmax and AUC 0–∞ values for unconjugated BPA in PND 3 pups were 189- and 260-fold greater than in adults, respectively (Doerge et al, 2011b). However, at PND3, the internal exposure metrics (Cmax and AUC) following oral administration are similar for rats and mice, within a factor of 2.

The pharmacokinetics of BPA in neonatal non-human primates are clearly different from neonatal rodents mainly because the degree of conjugation was not affected by developmental age and consequently there was no significant age related change in internal exposure metrics for free BPA in non-human primates.

The glucuronide/sulfate ratio was not significantly affected by age from early perinatal period to adulthood in the three species.

The comparison of age-related BPA kinetics evidenced that newborn rodents have approximately 10 times higher plasma levels of free BPA than PND5 monkeys, when treated with the same oral BPA dose (Doerge, 2010a and b; Doerge et al, 2011a). These data provide evidence for a different developmental profile of hepatic and intestinal conjugation of BPA in rodents and monkeys, consistent with literature data describing a higher degree of immaturity of rats at birth as compared to primates, in relation to UGT activity (Coughtrie *et al*, 1988; Matsumoto *et al,* 2002).

Many of the human UGT1A and 2B isoforms catalyzing BPA glucuronidation are homologous with those in monkeys and share the same tissue distribution and substrate specificity towards steroid hormones (Doerge *et al.*, 2010b). For SULT enzymes, no age-dependency has been described (Pacifici *et al.*, 1993: Duanmu *et al.*, 2006) and consequently, in humans the sulfation activity is comparable at birth and in the adult. These considerations are supported by the results from the study of Calafat (Calafat *et al.*, 2009), showing that >90% of the BPA excreted in the urine by prematurely born infants was in its conjugated (e.g. glucuronide, sulfate) form, clearly indicating that prematurely born infants are able to metabolize BPA. In addition, the concentrations of free and total BPA were linear over the range of detected BPA levels, suggesting that the enzyme(s) responsible for the conjugation of BPA were not saturated in the tested prematurely born infants. More recently, Nachman *et al*. (2013) measured the content of unconjugated and BPA-glucuronide in the urine of 11 healthy neonates plus 1 young infant, all but one receiving infant formula. The average concentration of BPA glucuronide, as measured in all of the duplicate urine samples, was 0.87± 0.51 ng/mL (median: 0.66 ng/ml). Unconjugated BPA was not found in any of the urine samples, further demonstrating that neonates and infants are capable of conjugating BPA to the BPA-glucuronide.

The large inter-species differences in internal free BPA dosimetry emphasize the importance of using physiologically based pharmacokinetic (PBPK) modelling to estimate internal exposure in adults, children and infants following different exposure scenarios. The input of enzyme kinetics parameters obtained *in vitro* with human samples in the PBPK models could be an important improvement in BPA risk assessments.

Although dietary BPA exposure would be a more appropriate and convenient route than bolus exposure, most of the studies have been carried out to date with oral administration of single or repeated bolus dose. Even when animals were fed a diet containing BPA (Dolinoy *et al.*, 2007; Cox *et al.*, 2010) serum, concentrations of BPA were not measured and it remains controversial whether the quantities of BPA supplied to mice are representative of actual exposure conditions. Only one study (Sieli *et al.*,

2011) measured serum concentrations of isotopically tagged dimethyl-d6-BPA and its conjugates resulting from dietary exposure in female mice and compared concentrations with those in mice exposed through single oral bolus exposure, as in previous studies (Doerge *et al.*, 2010a, 2010b; Taylor *et al.*, 2011).

For mice receiving the oral bolus (20 mg/kg b.w.), maximum concentration (Cmax) of unconjugated BPA-d6 (21.0 ± 3.9 ng/mL, mean ± SE) occurred within 1 hour after treatment and declined slowly thereafter, reaching barely detectable concentrations after 24 hours. The estimated dietary exposure dose was 13 mg/kg b.w. over the first 24 hours, and peak BPA-d6 concentration (18.8 ± 4.4 ng/mL) was observed at 6 hours after the initiation of the BPA-d6–supplemented diet then declining significantly by 11 hours. A similar trend was evidenced for total serum BPA, where concentrations of the conjugated form were up to 70–100 times higher than those of unconjugated BPA (Sieli *et al.*, 2011). To compare the oral bolus and diet groups at the same external dose, the dose of the diet-exposed group was scaled to 20 mg/kg b.w., and the AUC(0–24 hour) was not significantly different in the dietary exposed group (227.4 ± 41.1 and 201.0 ± 20.6 ng-h/mL in the diet and oral bolus group, respectively). A lower total BPA absorption (81%) was estimated for the diet group: the slightly higher bioavailability associated with diet exposure (113%) was tentatively explained by a "food-effect" (Sieli et al., 2011).

These findings and the hypothesis on the effect of food on BPA bioavailability might account for the considerable inter- and intrameal variability in BPA urinary excretion, consistent with an estimated range of exposure from 3.29 to 73.29 µg found in biomonitoring studies (Teeguarden et al., 2011). An additional finding, although not explained yet, is related to concentrations of unconjugated or active BPA-d6 in the diet group which were found to be higher after 7 days of dietary exposure than at 24 hours after exposure, an effect which has not been observed when BPA is administered as a single bolus (Doerge et al., 2010a; Taylor et al., 2011).

Different PBPK models have been developed: some are extrapolated from animal models (rat: Teeguarden *et al.*, 2005 and Shin *et al.*, 2010; monkey: Fisher *et al.*, 2011) and others which are human based models (Edginton and Ritter, 2009; Mielke and Gundert-Remy, 2009). Yang *et al.* (2013) developed a PBPK model for neonatal and adult rats with implications for the extrapolation of toxicity studies from neonatal rats to neonatal monkeys or infant humans. In view of the kinetic differences among rodents and primates, those based on primates are considered more appropriate for the extrapolation of data to the human situation, unless the rat based model is refined to account for the entrohepatic re-circulation and features typical of rodent kinetics (Shin *et al.*, 2004) used to describe and predict the blood and tissue concentration time profiles after oral and iv doses in rats and in humans. This PBPK model was employed as an example to estimate the oral dose required to achieve the actual total BPA concentrations in human blood as reported in Korean pregnant women (Shin *et al.* 2010). Additionally, the Teeguarden et al. model (2005) was later extended to humans.

Conclusion

Major differences exist in BPA metabolism and disposition between rodents (enterohepatic recirculation and extensive fecal excretion of unconjugated BPA) and primates (extensive urinary excretion of conjugated BPA), that directly affect BPA half-life, which is longer in rats than in primates. Moreover, at the neonatal stage there is a major difference between rodents (rats and mice) and non-human primates. For neonatal rodents, the conjugation of BPA develops with increasing age being very low (especially in mice) at birth. In neonatal non-human primates (NHP), the degree of conjugation was not affected by developmental age. Even in prematurely born infants, >90% of the BPA excreted in the urine was conjugated indicating that prematurely born infants are able to conjugate BPA. Pharmacokinetics in rats and mice with an enterohepatic recirculation may result in the exposure to free BPA, especially in neonatal rats and mice, being higher than in NHP and humans. Therefore, studies in postnatal rats or mice may over-predict adverse outcomes for humans (Shelnutt *et al.*, 2013).

The large inter-species differences in internal free BPA dosimetry emphasize the importance of using physiologically based pharmacokinetic (PBPK) modeling to estimate internal exposure in adults, children and infants following different exposure scenarios. The input of enzyme kinetics parameters obtained *in vitro* with human samples in the PBPK models could be an important improvement in BPA risk assessments.

Based on recent new toxicokinetic data on different animal species and BPA PBPK models that have become available, EFSA has recently derived a so called human equivalent dose (HED) for oral BPA uptake for extrapolating animal internal BPA exposure data into human internal BPA exposure data (EFSA 2014). The HED is an accepted method for linking a critical effect from the dose-response relationship in animals to predict a level without harmful effects in humans. For the HED approach, EFSA decided to use the above mentioned PBPK model of Fisher *et al*. (2011) to derive internal dosimetrics for oral BPA, as done by Yang *et al.* (2013). These models make it possible to predict the internal BPA exposures in laboratory animals and humans in a route specific manner.

3.8.3. Toxicokinetics after uptake by other routes

Studies on toxicokinetics of BPA available to date have demonstrated a significantly lower internal exposure to free BPA after oral intake as compared to parenteral exposure, essentially due to the highly efficient pre-systemic conjugation to glucuronides and sulfate occurring in the liver and partially in the gut after oral administration independently on the species.

Many toxicological studies showing adverse effects used s.c. injections of BPA or alternatively BPA was injected into discrete regions or delivered by osmotic pumps to ensure reproducible dosing. There are differences due to pre-systemic clearance occurring following oral exposure and the slow release of BPA from the oil suspensions during injection/infusion, making a direct comparison inappropriate. For route to route extrapolation, s.c. studies may only be useful if tissue and/or plasma values are used. However, the results of s.c. studies are appropriate for hazard identification or for risk assessment purposes for specific exposure scenarios, when BPA exposure occurs via transcutaneous or parenteral route, as it could be with some medical devices.

3.8.3.1. Toxicokinetics after dermal and transcutaneous uptake

Dermal absorption

Toxicokinetic study in humans involving dermal exposure are not available, indicating the extent of BPA dermal absorption and the internal dose metrics for free and conjugated BPA. However, in vivo study in rats as well as in vitro studies on cutaneous penetration using pig skin and human skin samples have been carried out.

The European Union in the RAR (ECB, 2003) estimated that the bioavailability of BPA applied on skin was around 10%, but considering the physico-chemical properties of BPA, skin penetration could be expected to be higher: indeed, BPA has a moderate water solubility (K_{ow} of 3.2), log $P_{o/w}$ of BPA is 2.2 and a relatively low molecular weight.

The fate of BPA after topical application or skin contact was examined in only a few studies. The oldest one was carried out using deuterated-BPA on isolated perfused bovine udders (Kietzmann *et al.*, 1999), a model that is poorly representative of human skin and not recommended by current guidelines for dermal absorption studies. A study was carried out with ^{14}C-BPA, using full thickness human skin *in vitro* (Mørck *et al.*, 2010), following OECD guideline 428 on skin penetration studies However, in addition to poor data reporting, results were apparently obtained with an extremely high dose of BPA (17.5 mM corresponding to 3.99 g/L, inconsistent with BPA solubility of 120-300 mg/L water at 25°C (EFSA 2010)): the exaggerated BPA application does not allow any conclusion to be drawn from this study.

The percutaneous absorption of ^{14}C-BPA was measured with porcine skin after 10 hours of exposure (Kaddar *et al.*, 2008). The histological and biochemical properties of porcine skin have been repeatedly shown to be close to that of human skin (Jacobi *et al.*, 2007) as well as the thickness of both the stratum corneum and the epidermis. The proportion of radioactivity found in the receptor fluid varied from 0 to 5% of the applied dose over time (0–24 hour), with around 15% recovered in the treated skin (dermis +epidermis) at 10 hours, to give an amount of absorbed and potentially 'absorbable' material of 15-20%. Data do not allow an absorption flux for BPA to be calculated. The apparent sequestration by the fatty compartment of the dermis could be explained with the log $P_{o/w}$> 2; on the other hand the possible biotransformation to more water-soluble metabolites through conjugation is expected to progressively increase the transfer in the receptor fluid at longer time. Unfortunately, the study did not report any measurement of possible metabolite formation and did not report on exposure times longer than 10 hours (Kaddar *et al.*, 2008).

BPA metabolites were measured in a recent dermal absorption study with ^{14}C-BPA (50–800 nmol) for 72 hours, using both human skin explants and short-term cultures of pig ear skin (Zalko *et al.*, 2011). In short-term cultures (72 hours), the proportion of radioactivity diffused into culture media (trans-dermal passage in the receptor fluid) was ≈50–60%, most of which was associated with BPA–conjugates (ca. 90% of radioactivity in the receptor fluid). However, skin viability (related to metabolic competence) did not significantly modify the absorption rate of BPA thus, questioning the results obtained on metabolites. In human skin explants, the percutaneous absorption measured at 72 hours was 46-58% (of which ca. 10% associated to metabolites); 20–30% of the radioactivity applied on explants was retained at the application site (Zalko *et al.*, 2011). The different results with respect to the Kaddar study could be attributed to longer exposure times (72 hours).

Marquet *et al.* (2011) performed an *in vivo* and *ex vivo* adsorption study through rat and human skin. Rats were treated with different doses of [^{14}C]-BPA dissolved in acetone (4 mg BPA/mL, 50 µl/cm^2). BPA penetrated rapidly into the skin: indeed 1h post exposure the maximal penetration flux was obtained and more than 10% of the applied dose was recovered in the treated skin (with a maximum value of 19% measured after a 4 hour exposure). Moreover, a mean value of 31 ± 10 µg BPA/cm^2 for the skin content did not change significantly at any time during exposure, indicating that there was no accumulation of BPA in the skin. The percentage of dermal absorption was obtained by summing up the excreted radioactivity and the one recovered in the carcass and in the skin at the administration site. Recovery was ≥94% in all the different experimental conditions. After an 8 hour exposure, the dermal absorption was approximately 26-29% (at both 8 and 72 hours post exposure time). The amount of radioactivity in the skin decreased progressively during post exposure time (paralleled by increase in the excreted radioactivity), indicating that BPA penetrated in the skin is only partially associated to the stratum corneum (no tape stripping was applied to the treated skin, so that the stratum corneum content could not be quantitated). Dermal absorption increased linearly, with exposure time around 46-51% measured after 24 and 30 hour exposure time.

The half-life of BPA after dermal absorption was estimated to be 28 hours (compared with the half-life of 10 hours measured after i.v. administration in the same study) (Marquet et al, 2011). When dermal absorption was measured *ex vivo* in human and rat skin at the studied dose, BPA was not cytotoxic for the skin and did not affect the skin's integrity.

Ex vivo and *in vivo* percutaneous absorption fluxes of BPA after 24h exposure in the rat were in the same range (1.48 and 2.2 µg/cm^2/h) (Marquet *et al.*, 2011). They found approximately 12-fold lower flux (0.12 ± 0.09 µg/cm^2/h) in human skin samples treated *in vitro* in the same conditions (^{14}C-BPA in acetone: 4 mg BPA/mL; 50 and 200 µl/cm^2 for human), however, inter- and intra-individual variability of up to tenfold was observed. The extent of BPA metabolism was estimated by measuring BPA metabolites in the

receptor fluid after a 24 hour exposure to BPA on fresh dermatomed rat and human skin samples. For both human and rat skin, unmodified BPA accounted for more than 97% of the radioactivity detected in the receptor fluid, in contrast to results obtained by Zalko *et al.* (2011). However, the study's design shows weaknesses by using acetone for BPA dissolution which induces skin damage thus, promoting BPA penetration even though the viability of the skin was demonstrated.

Recently, the dermal penetration rate of ^{14}C-BPA was determined in human skin in an *in vitro* test method performed according to the OECD Test Guideline 428 and in compliance with good laboratory practices (GLP) (Demierre *et al.*, 2012). Results indicated a recovery in the receptor fluid of 8.6% of the applied dose (1.82 µg/cm^2) and a total amount of bio-available BPA of 9.3% after 24 h incubation, thus, summing up the amount detected in the treated skin after tape stripping. However, the authors excluded the radioactivity recovered in the 15-tape stripping (34.9% of the applied dose), considering it entirely associated to the stratum corneum. According to the EFSA Guidance Document on dermal absorption (EFSA 2012b), only the first two tapes can be strictly considered equivalent to the stratum corneum; therefore, radioactivity recovered in tapes 3-15 (approximately 15-20%) should be added as potentially bioavailable. On this basis, SCENIHR estimated the dermal absorption in the range of 25-30%. At variance EFSA recently assumed that the amount present in the stratum corneum will remain deposited on the skin surface, considering a dermal absorptivalue of 10% (EFSA, 2014).

The differences of the Demierre *et al*. (2012) results with respect to previous studies have been related to the experimental model and the more realistic exposure conditions in terms of time (24 hours) and BPA concentrations (1.82 BPA µg/cm^2). Indeed, the applied dose was slightly higher than the one estimated to be transferred from thermal paper to a single finger (1.13 BPA µg/cm^2) and not expected to increase even in case of prolonged or repeated contacts (Biedermann *et al.*, 2010). In addition, in order to be closer to sweat composition, ^{14}C-BPA was dissolved in pure water and not in organic solvents (alcohol or acetone) as in the previous studies (Mørck *et al.*, 2010; Zalko *et al.*, 2011; Marquet *et al.*, 2011). The application of very high BPA doses can greatly influence BPA dermal penetration. Indeed, skin penetration results from passive transfer, and therefore, it is strictly dependent on the gradient between the applied concentration and the concentration in the receptor fluid. The 100-fold higher dose used in the study of Marquet *et al.* (2011) can thus, explain the higher flux in human skin (0.12 and 0.022 µg/cm^2/h, respectively).

By using their previously published and validated PBPK-model (Mielke and Gundert-Remy, 2009), modified to include absorption through skin, Mielke et al (2011) simulated concentrations in blood, liver and kidney after dermal intake of a daily dose of 71 µg/day (0.97 µg/kg/day) as estimated by Biedermann et al. (2010) as the worst case exposure scenario for the dermal route with varying extent of absorption (10%, 13%, 46% and 60%) to account for the various data available in the literature. They compared concentrations which would result from the intake of BPA on the dermal route with those which would result from the oral administration at the TDI. Results indicated that after dermal exposur,e concentrations in blood, kidney were higher and in the liver were lower as compared to the oral route due to the high first pass in the liver. Whatever the extent of dermal absorption used, the dermal dose of 0.97 µg/kg/day gives rise to an AUC and Cmax lower (up tp 10- and even 70-fold for the highest absorption value used) than 50 µg/kg/day (corresponding to the t-TDI) by the oral route.

Summarizing, the available data indicate that at the estimated exposures, BPA penetrated rapidly into the skin by passive transfer at a percentage around 25-30% of the applied dose. The possible skin metabolism is controversial, since contrasting results were reported. However, not considering metabolism in the skin prior to systemic distribution will represent a conservative approach. Therefore, as a worst-case, a

systemic bioavailability equal to 30 % of the applied dermal dose can be used for risk assessment purposes.

Subcutaneous injection

Toxicokinetics studies were performed on rats and mice after oral and s.c. administration of 100 µg/kg b.w. BPA using the same experimental design (Doerge et al, 2010a, 2011a). Levels of free and conjugated deuterated BPA were measured in neonatal, immature and adult animals (post-natal day, PND 3, 10 and 21). Animals were given a single dose, which was demonstrated to be within the linear range of pharmacokinetics, so that extrapolation to lower doses is feasible.

Administration of 100 µg/kg b.w. BPA by s.c. injection to PND 3 rats produced 34-fold higher Cmax and 17-fold higher AUCs for free BPA compared to oral administration. The age-related changes in serum levels of free BPA observed after oral administration were not evidenced after s.c. injection. This indicates that first pass effect is not relevant after s.c. treatment and confirmed that on the contrary the first pass effect is relevant after the oral exposure even in early postnatal pups, although characterised by a lower conjugation activity.

In mice, administration of the same dose of BPA by either gavage or s.c. injection, indicated that, unlike adult mice, serum levels of free BPA were consistently detected in pups of all ages at early post-dosing time points (Doerge *et al*, 2011a). These results are in line with previously reported data by Taylor *et al.* (2008), although the differences in the experimental design between the two studies make quantitative comparison quite difficult. However, this information may be relevant for the interpretation of some toxicity studies carried out by administering BPA by s.c. injection. This feature can be explained by considering that the particularly pronounced metabolic immaturity of PND 3 mice abrogates the route of administration effect observed for injection of BPA into neonatal rats (Doerge *et al.*, 2010a) and monkeys (Doerge *et al.*, 2010b); this difference is no longer present at PND 10 and 21 pups which have developed their conjugation activities, so that s.c. injection results as expected in higher levels of free BPA, having by-passed the presystemic BPA clearance in the GI tract and liver, typical of the oral route.

Similar results were obtained in another study (Prins *et al.*, 2011), comparing BPA pharmacokinetics in neonatal rats following s.c. injection or oral delivery of 10 µg BPA/kg b.w.. Free and total BPA at max were 1.77 and 2.0 ng/mL, respectively following s.c. injection with an initial peak of free BPA in blood that was not seen after oral exposure (after which max values for free and total BPA were 0.26 and 1.02 ng/mL, respectively). After s.c. injection, 88%, 52% and 31% of BPA was in the free form at 30 min, 1 hour and 2 hours, respectively. After 2 hours, the differences in serum free BPA levels were no longer statistically different between the oral or s.c.-treated groups (Prins *et al.*, 2011).

3.8.3.2. Toxicokinetics after intravenous administration

In rhesus monkey administered with stable isotope–labeled $^{13}C_{12}$-BPA (100 µg/kg b.w.) to avoid background contamination, the total BPA levels were higher following i.v administration (29±19% of the administered dose at 5 min post-injection) than after oral administration of the same dose (0.21±0.14% of total BPA at 30 min post-gavage), confirming that the systemic availability of free BPA in monkeys is much lower after oral exposure than after parenteral exposure (Doerge *et al.*, 2010b).

Overall, these findings are in line with those obtained in the rat with a similar experimental design (Doerge *et al.*, 2010a). Free BPA was rapidly eliminated from the circulation (> 50% of circulating BPA was conjugated 5 min after i.v. injection), with a half-life of 0.66 h following i.v. administration; however, the fraction present as conjugated BPA was substantially lower following i.v. administration compared to oral (55% vs. 99.5%) due to the absence of first-pass conjugation. After i.v. exposure the

percentage of free BPA was higher in adult rats than in monkeys (52 ± 10% vs. 29 ± 19% at 5 min post-injection) (Doerge *et al.*, 2010a and 2010b).

When [^{14}C]-BPA (10, 50, 100, 500, and 1,000 µg/kg b.w.) was administered i.v. to rats (n = 3–6) regardless of the dose administered, the percentage of [^{14}C] excreted in faeces and urine were not significantly different, confirming no saturation of the excretion pathway (Marquet et al, 2011). The total recovery was in the range of 90–101% of the administered dose. Radioactivity was predominantly excreted in faeces (63–75% of the excretion over the 72 hours), whereas within this period, urinary excretion accounted for about 12–22% of the total excreted [^{14}C] and occurred essentially in the first 24 hours after administration (Marquet et al, 2011).

The i.v. administration to female CD-1 mice of stable isotope–labeled $^{13}C_{12}$-BPA (100 µg/kg b.w.) indicated a rapid distribution of free BPA into the tissues ($t_{1/2}$ = 0.2 h) with a nearly as rapid terminal elimination phase ($t_{1/2}$ = 0.8 h). Clearance of the parent compound was mainly due to the rapid formation of Phase II conjugates: unconjugated d6-BPA was undetectable in serum by 8 h (<0.2 nM). However, the total BPA (mostly accounting for conjugated forms) was eliminated more slowly from the circulation ($t_{1/2}$ = 6.6 h), due to enterohepatic recirculation, suggested by the presence of an apparent "re-entry peak" at 2 hours for total but not for free BPA (Doerge at al, 2012). Therefore, enterohepatic re-circulation does not appear to significantly affect the serum/tissue levels of free BPA, but prolongs the elimination of BPA conjugates, as after "re-entry", a first-pass effect occurs again.

When the possible sequestration by the adipose tissue was investigated following i.v. administration of deuterated BPA (100 ug/kg b.w.), limiting interference by background BPA levels, it showed that free BPA was rapidly taken up into adipose tissues ($t_{1/2}$= 0.07 h), with maximal levels observed at 0.25 h, followed by a slower terminal elimination phase similar to that for conjugated BPA ($t_{1/2}$ = 7.0 h) (Doerge at al., 2012). The maximal level of free BPA in adipose tissue did not exceed the initial measured serum level. These data support a rapid equilibrium of BPA in the blood with tissues, including fat, such that the redistribution of BPA from adipose tissue follows in parallel.

About 2.5–4 ng deuterated BPA/g for 100 mg tissue were detected (Doerge *et al.*, 2012), These values are within the same concentration range previously reported for free BPA in human adipose tissue even though different methodologies were used (Fernandez *et al.*, 2007; Geens et al., 2012).

Despite a high adipose tissue/serum partition ratio (6.9) and minimal competence in conjugation (% unconjugated BPA = 90%), BPA was eliminated from adipose tissue at a rate similar to that for BPA conjugates in the whole organism. These observations confirm the non-persistent nature of BPA in vivo, mainly due to its extremely efficient conjugation and elimination in urine (primates and partially rodents) and bile (rodents only) and are in line with its aqueous solubility (100–300 mg/l), moderate lipid partitioning (log Ko/w = 3.3), and the fat/serum distribution ratio in female rats and mice (5 and 7, respectively) (Doerge et al., 2011b; 2012). No significant bioaccumulation was reported when 11 adult female rhesus macaques were fed 400 µg/kg deuterated BPA (dBPA) daily for 7 days (Taylor et al, 2011).

The possible accumulation of BPA in adipose and other tissues, due to chronic exposure to low levels hypothesised by some authors (Stahlhut *et al.*, 2009; Hugo *et al.*, 2008; Nunez *et al.*, 2001), is, therefore, not supported by experimental data.

3.8.3.3. Toxicokinetics after inhalation

No data are available on kinetics following inhalation exposure, which on the other hand seems not to be a relevant route of exposure for the general population (Wilson *et al.*, 2007; Geens *et al.*, 2009; von Goetz *et al.*, 2010). However, this route of exposure may be relevant for medical devices in view of tracheal intubations. The only information

available is the lack of BPA-glucuronidation shown by human lung microsomes (Trdan Lušin et al, 2012), suggesting the absence of relevant pre-systemic inactivation after absorption through the lung epilthelium.

3.8.4. Special considerations on susceptible populations

Different susceptibilities for BPA have been postulated for some specific subgroups, including foetuses, infants, and older people. The age dependence of the toxicokinetics of BPA and its conjugated metabolites was studied by applying PBPK modelling to estimate levels of BPA in the blood in young children after oral exposure (Edginton and Ritter, 2009; Mielke and Gundert-Remy, 2009). The oral absorption can be considered complete, that is around 90% (although this % is a conservative estimate for neonates, since the immature pancreatic and biliary secretion and the scant presence of intestinal flora up to the 8th month can limit the absorption of BPA in infants (Ginsberg *et al.*, 2002).

Edginton and Ritter (2009) built their PBPK model using information from toxicokinetic studies in adults, and scaled to children <2 years of age, by replacing the age-dependent physiologic parameters relevant for kinetics in newborn. The average free BPA modelled plasma concentrations at steady state in newborns and 3 months-old infants were 11 and 2 times greater than that in adults (after a dose of 1 µg/kg b.w./day).

The model by Mielke and Gundert-Remy (2009) included not only the age-dependent UDPGT-mediated BPA conjugation but also took into account the sulfation pathway, assuming SULT activity toward BPA to be about 15% of that of glucuronidation, independently of the age (being already expressed at high levels starting from intrauterine life). They reported a children/adult ratio in free BPA in blood of about 3 (0.44 µg/L versus 0.13 µg/L): the difference between the two studies may be explained with both the pattern of exposure and the consideration of sulfation in BPA metabolism. The simulation by Mielke and Gundert-Remy underlines the importance of taking both pathways (i.e. glucuronidation and sulfation) into account and suggests that the well-expressed sulfation activity in the newborn can compensate at least partly for the lower glucuronidation activity in neonates.

In order to perform a comparison of BPA dosimetry across species including humans, Yang *et al.* (2013) applied the monkey-based PBPK model of Fisher *et al*. (2011) for the prediction of internal dosimetrics in human newborns and adults. After a simulated daily oral administration repeated up to 14 days, the Cmax was 0.23 nM and 0.51 nM and the AUC was 1.53 and 1.80 nM per hour for human newborns and adults, respectively, with a ratio lower than the ones previously predicted by Edginton and Ritter (2009) and Mielke and Gundert-Remy (2009).

Pregnant women show slightly elevated glucuronidation activity when compared to non-pregnant women, and therefore, are characterized by a higher efficiency in detoxifying BPA. This is relevant also for *in utero* exposure for the embryo/foetus, the exposure of which depends on maternal blood concentrations. The issue of *in utero* exposure has been extensively discussed in the EFSA opinion (EFSA, 2010).

There is no indication that the elderly are at risk, since their metabolic capacity associated to phase II enzymes is not affected. However, chronic diseases such as those characterised by impaired hepatic or renal functionality can be of relevance, especially if associated to the prolonged use of medical devices as for dialysis patients.

3.8.5. Conclusions

The available data on BPA in animals and humans indicate that there is a marked difference between the possible routes of exposures: the internal exposure after oral

intake being much lower as compared to dermal or parenteral exposure. In addition, after oral administration a species-specific difference exists in BPA kinetic, indicating that BPA is eliminated faster in humans than in rats, resulting in a lower internal exposure to free BPA in humans. The direct consequence of these differences are: i) the limited representativeness of rat toxicity data in risk assessment when not associated to specific route of exposure result in in a relative higher exposure after oral intake ii) the indication that the default assumption that human are more susceptible than rodents is not completely correct at least for the kinetic component.

The oral absorption can be considered complete, that is around 90% (although this % is a conservative estimate for neonates, since the immature pancreatic and biliary secretion and the scant presence of intestinal flora up to the 8th month can limit the absorption of BPA in infants (Ginsberg *et al.*, 2002). However, the systemic bioavailability of free BPA is dramatically reduced by the first pass effect to 2.8%, 0.2%, 0.9% and less than 1% in rats, mice, monkeys, and dog, respectively. The systemic availability of unconjugated BPA in humans has not been evaluated experimentally, however, modelled data indicated that internal exposure in humans to unconjugated BPA is very low (1-10%). This estimate was confirmed by results from controlled biomonitoring studies in humans showing that unconjugated BPA in serum is below the LOD of 0.3 ng/ml (= 1.3 nM), confirming that internal exposure to unconjugated BPA is extremely low.

After dermal exposure, the absorption fraction can be considered around 25-30% of the applied dose, which is directly systemically bioavailable.

For all the parenteral routes of exposure (including i.v., i.p., trandermal or subcutaneous), the chemical is 100% systemically bioavailable: however, the clearance of free BPA from the circulation appeared to be quite fast, as indicated by controlled studies in non human primates showing a half-life of 0.66h with >50% of circulating BPA already conjugated 5 min after i.v. injection.

The available modelled data, obtained considering after oral exposure, also point out that newborns and babies up to 6 months constitute a potentially susceptible subpopulation due to immature BPA metabolism. However, the default uncertainty factor which is used to account for the toxicokinetic variability in the general population seems to be large enough to cover the variability in the newborn population exposed via the oral route. Analogously, inter-individual differences in the expression of the isoenzyme mainly responsible for BPA glucuronidation are within a factor of 4, again covered by the usual uncertainty default factor, at the estimated dietary exposures.

Based on animal data and PBPK modelling, it is possible to provide internal dose metrics for neonatal-to-adult stages and for different routes of exposure. More recently EFSA derived a human equivalent dose (HED), a concept that can be used to derive internal human exposure data for BPA, to be applied to Points of Departure derived from animal studies (EFSA 2014).

3.9. Toxicity

3.9.1. General toxicity studies

3.9.1.1. Acute toxicity

Oral LD50 values above 2,000 mg/kg b.w./day were reported in the rat and mouse, and dermal LD50 values above 2,000 mg/kg b.w./day have been reported in the rabbit. For inhalation, a 6h exposure to 170 mg/m^3 (the highest attainable concentration) produced no deaths in rats; slight and transient slight nasal tract epithelial damage was observed.

These data indicate that BPA is of low acute toxicity by all routes of exposure relevant to human health (EC, 2003, 2008, 2010a,b).

The effects of single oral exposure to BPA in humans are not well documented. In a kinetic study in healthy volunteers, a dose of 5 mg BPA (range 54.3 to 87.7 µg/kg) was well tolerated (Völkel *et al.*, 2002).

3.9.1.2. Chronic toxicity (repeated-dose) studies

Mice and rats

Oral

Several repeated dose toxicity studies have been performed in rodents which were extensively revised in the previous evaluations and are, therefore, not all recapitulated here.

Dietary studies in mice indicated that the liver is a target organ in this species, with changes being observed in the size and nucleation state of hepatocytes in 2-year and 90-day studies (US NTP, 1982). It was not possible to identify a no effect level for males in the 90 day study as the effect was observed at all dose levels used in males, the lowest doses being 120 mg/kg b.w./day. In females, in the 2-year study a no-adverse-effect level of 650 mg/kg b.w./day was established based on reduction of body weight gain. Thus, the LOAEL in males is 120 mg/kg b.w./day and the NOAEL 650 mg/kg b.w./day in females. A NOAEL of 74 mg/kg b.w./day has been established for rats from a 2-year study based on reduced bodyweight gain at the next dose level of 148 mg/kg b.w./day.

Tyl *et al.* (2002, 2008) conducted a dose-range finding study and two large multigenerational studies in rats and mice using dietary administration of BPA with doses ranging from 1 or 3 µg/kg b.w./day up to 500 or 600 mg/kg b.w./day. These studies demonstrated effects on the liver, kidney and body weight at doses of 50 mg/kg b.w./day and higher. Chronic inflammation of the liver was seen from 50 mg/kg b.w./day in the 3-generation study, but with no convincing dose-response relationship. These liver effects in rats were thus considered to be background variation and not treatment-related. Renal tubule degeneration of the kidney was also seen in this 3-generation study in females at 500 mg/kg b.w./day but not at 50 mg/kg b.w./day. Hence, the NOAEL for kidney effects is 50 mg/kg b.w./day. In mice, the NOAEL based on effects on liver was 5 mg/kg b.w./day. Stump *et al.* (2010), used a wide dose range in rats, performing a study on neurotoxicity according to OECD 426 and based on reduced body weight or body weight gain respectively identified a lowest no-observed-adverse-effect level (NOAEL) of 5.85 mg/kg b.w./day.

Inhalation

In an inhalation study in rats, slight inflammation and hyperplasia of the olfactory epithelium were observed at an exposure level of 50 mg/m^3 (6 hours/day, 5 days/week for 13 weeks) the NOAEL being 10 mg/m^3 (EC, 2003, 2008).

Dogs

In a 90-day dietary study in dogs, a no effect level of approximately 80 mg/kg b.w./day was identified, with increases in relative liver weight being the only finding observed at approximately 270 mg/kg b.w./day. In the absence of histopathology this finding is of uncertain toxicological significance. (EC 2003, 2008).

In conclusion, BPA is of low acute toxicity, and the lowest NOAEL for subchronic exposure currently available is approximately 5 mg/kg b.w./day, based on effects on the liver as target organ, as identified in several studies. The next lowest NOAEL is 50 mg/kg b.w./day, based on effects on the kidney.

3.9.2. Genotoxicity

In vitro assays

Studies of the potential of BPA to induce mutations, chromosomal aberrations, sister chromatid exchange and transformation in a variety of *in vitro* test systems are largely negative, including studies with Salmonella typhimurium, Chinese hamster V79 cells, Syrian hamster embryo cells and mouse lymphoma cells (NTP, 2008). However, deoxyribonucleic acid (DNA) damage was induced by BPA in MCF-7 and MDA-MB-231 cells (Iso *et al.*, 2006). DNA adduct formation in Syrian hamster ovary cells (Tsutsui *et al.*, 1998, 2000) and a number of positive findings have been reported for the potential for BPA to inhibit purified microtubule polymerization, affect the spindle apparatus and produce aneuploidy in *in vitro* studies with Chinese hamster V79 cells or oocytes from BALB/c or MF1 mice (US NTP, 2008).

BPA appears to have demonstrated aneugenic potential *in vitro*, positive results being observed without metabolic activation in a micronucleus test in Chinese hamster V79 cells and in a non-conventional aneuploidy assay in cultured Syrian hamster embryo cells. Additionally, in cell-free and cellular systems there is information that shows BPA disrupts microtubule formation. BPA as been shown to produce adduct spots in a post-labelling assay with isolated DNA and a peroxidase activation system, but it does not appear to produce either gene mutations or structural chromosome aberrations in bacteria, fungi or mammalian cells *in vitro*. (EFSA 2010)

In the study by Tiwari *et al.* (2012), negative results were obtained at concentrations up to 200 µg/plate in an Ames assay using tester strains of *S. typhimurium* TA 98, TA 100 and TA 102 in the presence and absence of S9 metabolic activation.

In the study by Audebert *et al.* (2011), BPA was shown to be negative for induction of phosphorylated histone γ-H2AX, a marker for induction of DNA double strand breaks, in HepG2 and LS174T (human epithelial colorectal adenocarcinoma cells).

Concerning the studies available before 2010, EFSA (2010) noted that the conduct of these studies had some deficiencies and the negative results cannot be taken as conclusive. BPA does not appear to be aneugenic *in vivo*, because a recently conducted, standard mouse bone marrow micronucleus test has given a negative result (EFSA 2010). Any aneugenic potential of BPA seems to be limited to *in vitro* test systems and is not of concern when follow up *in vivo* studies show negative results. The relevance of the finding that BPA can produce rat hepatic DNA adduct spots in a postlabelling assay is not entirely clear. However, given the absence of positive results for gene mutation and clastogenicity in cultured mammalian cell tests, it seems unlikely that these are of concern for human health. The newer studies are not indicative of an *in vitro* genotoxicity of BPA.

In vivo studies

Hunt *et al.* (2003) investigated the effects of short-term, low-dose exposure to BPA on the meiotic processes of female mice during the final stages of oocyte growth. Although BPA can affect chromosomal structure during replication in *in vitro* studies, the outcomes of similar assessments when the chemical is administered to laboratory mice are inconsistent and inconclusive. The striking findings of meiotic aneuploidy in oocytes of mice (Hunt *et al.*, 2003; Susiarjo *et al.*, 2007) have not been independently replicated, and the failure to observe clear effects on fertility or cancer associated with BPA exposures during development suggests that the findings are of limited biological significance.

In the study by Masuda *et al.* (2005) intended to simulate stomach environment and its influence on genotoxicity by studying the reaction of BPA and nitrite under acidic conditions, BPA did not induce micronuclei in peripheral blood reticulocytes when administered at 228 mg/kg b.w. by oral gavage to male ICR mice.

Following several treatment modalities (single oral gavage treatment at 0.2 and 20 mg/kg b.w., seven daily administrations of 0.04 mg/kg b.w. by oral gavage or seven weeks drinking water at 0.5 mg/l) Pacchierotti *et al.* (2008), evaluated potential aneugenic effects of BPA on mouse female germ cells. Following six daily administrations of BPA of 0.002, 0.02 and 0.2 mg/kg b.w. by oral gavage, effects on male germ and induction of micronuclei in bone-marrow cells were investigated (Pacchierotti *et al.*, 2008). No significant induction of hyperploidy or polyploidy in oocytes and zygotes was observed at any dose-level and treatment condition employed. Similarly, no induction of hyperploidy or polyploidy in epydidimal sperms were observed in male mice. Negative results on induction of micronuclei in bone marrow cells of male mice were also obtained.

Izzotti *et al.* (2009) studied BPA induction of DNA adducts, detected by ^{32}P-postlabelling in liver and mammary cells of female CD-1 mice (BPA in drinking water, dose equivalent to 200 mg/kg b.w./day for 8 days). Treatment related bulky DNA adducts (two major DNA adducts) were observed in liver and in mammary cells. The authors attributed the formation of adducts to the reactive metabolite BPA-3,4-quinone (BPAQ). However, as the chemical characterization of DNA adducts was not performed, unspecific covalent binding to DNA cannot be excluded.

The results of Naik *et al.* (2009) indicate that no significant increases of chromosomal aberrations or micronuclei were induced at 10, 50 and 100 mg/kg b.w. or five daily administrations at 10 mg/kg b.w. by oral gavage. It can be concluded that BPA under the reported experimental conditions was not clastogenic and did not elicit micronuclei induction, which would be indicative of a clastogenic and/or aneugenic potential at dose-levels employed.

De Flora *et al.* (2011) did not find induction of micronuclei in bone marrow cells and positive comet assay in peripheral blood cells after a treatment with 200 mg/kg b.w. BPA for 10 consecutive days.

Ulutaş *et al.* (2011) studied the effect of BPA (oral administration of 125 and 250 mg/kg b.w./day for four weeks) in the alkaline comet assay. No effect was observed at the lower dose-level (125 mg/kg b.w./day) whereas at 250 mg/kg b.w., the positive results might be explained by cytotoxicity which was not clearly ruled out.

Dobrzyńska and Radzikowska (2013) showed that BPA induced statistically significant increases of DNA breaks (DNA tail moment in the alkaline comet assay) in male germ cells at 24 hours and 5 weeks from last administration of test compound and in bone marrow, spleen, kidney and lung cells at 24h from last administration of 5, 10, 20 or 40 mg/kg b.w. per day in drinking water for 2 weeks. The increases observed were not dose-related and were obtained following collection of organs/tissues 24h or 5 weeks after last administration. Significant increases observed indiscriminately at 24h and at 5 weeks from last administration raise questions about the reliability of the results. In addition, the authors did not evaluate cytotoxicity. Hence, no conclusion could be drawn from this study.

Tiwari et al. (2012) investigated oral exposure of BPA investigated for induction of micronuclei and structural chromosome aberrations in bone marrow cells and primary DNA damage in blood lymphocytes using single cell gel electrophoresis ("Comet assay"). Furthermore, plasma concentrations of 8-hydroxydeoxyguanosine (8-OHdG), lipid peroxidation and glutathione activity were also evaluated to assess potential induction of oxidative DNA damage in rats following the oral administration of test compound once a day for 6 consecutive days at dose-levels of 2.4 µg, 10 µg, 5 mg and 50 mg/kg b.w./day. The observed increases achieved statistical significance at a dose-level of 10 µg/kg b.w./day and higher. Similarly, the analysis of primary DNA damage evaluated by comet assay, in isolated peripheral blood lymphocytes showed marked and dose-related increases. However, the study of Tiwari *et al.* (2012) has several shortcomings which include the staining procedure used to detect micronuclei in the bone marrow cells, the micronuclei data reporting, the observation of chromosomal aberration figures which are not generally induced by known chemical clastogens, and the absence of evaluation of

cytotoxicity in the comet assay. Hence, the reported dose-related increases of both micronuclei and structural chromosome aberrations in bone marrow cells in a dose range between 10 µg/kg b.w. up to 50 mg/kg b.w. for six days are difficult to interpret and cannot be considered reliable in view of the methodology used. In a preliminary study with two doses (10 µg/kg b.w./day and 5 mg/kg b.w./day) in male rats, oral BPA by gavage for 6 days induced for both doses a decrease in sperm production (Tiwari and Vanage 2013). In addition, the highest dose of 5 mg/kg b.w./day induced sperm DNA damage as demonstrated in a comet assay.

Overall Conclusions on genotoxicity of BPA

The genotoxicity of BPA has been reviewed elsewhere (Haighton *et al.*, 2002; ECB, 2003; EFSA, 2006; US NTP-CERHR, 2008; EFSA, 2010; WHO, 2010). Additional and new publications were reported and evaluated in this part. BPA did not induce *in vitro* gene mutation in bacteria (Masuda *et al.*, 2005; Tiwari *et al.*, 2012) and *in vivo* micronuclei in rodent bone marrow assays (Masuda *et al.*, 2005; Pacchierotti *et al.*, 2008; Naik *et al.*, 2009; De Flora *et al.*, 2011). BPA is aneugenic in an *in vitro* study in mammalian cells by Johnson and Parry (2008) due to a spindle disrupting effects of BPA. This effect has also been demonstrated by induction of colchicine-like metaphases (C-metaphases) in mammalian cells *in vitro* (Tayama *et al.*,2008) and *in vivo* by induction of prematurely separated chromatids in metaphase II of mouse oocytes (Pacchierotti *et al.*, 2008) and c-metaphases in mouse bone marrow cells *in vivo* (Naik *et al.*, 2009).Obviously, BPA does not interact with DNA directly but it acts on the mitotic spindle apparatus, an effect which is thought to be thresholded (COM Guidance on a Strategy for Testing of Chemicals for Mutagenicity, Department of Health, UK, 2000).

The large margin between the dose-levels found negative *in vivo* for induction of aneuploidy in rodent germ cells (Pacchierotti *et al.*, 2008) and for induction of micronuclei in somatic bone marrow cells (Masuda *et al.*, 2005; Pacchierotti *et al.*, 2008; Naik *et al.*, 2009; De Flora *et al.*, 2011) provides adequate reassurance on the lack of aneugenic effects of BPA *in vivo*.

In conclusion, BPA is not likely to pose a genotoxic hazard to humans.

3.9.3. Carcinogenicity

BPA studies

In the traditional rodent cancer bioassay (US NTP, 1982), BPA was tested in F 344 rats at 2 dose levels of approximately 74 and 140 mg/kg b.w./day, and in B6C3F1 mice at dose levels of 130 and 835 mg/kg b.w./day in male mice and 835 and 1670 mg/kg b.w./day in female mice. The number of animals was 50 per group. Gross morphological and histopathological investigations were performed, whereas no clinical chemistry or haematological investigations were performed, which was the standard procedure at that time. There was a marginally increased rate of leukemias in male rats, which disappeared after Bonferroni adjustment; leukemias were also seen in female rats and male mice; the increased incidence not being statistically significant. Statistically significant increase interstitial cell carcinomas of the testes were attributed to the ageing of the rats. It should be mentioned that the part of the study done in rats is somewhat compromised by the fact that among male rats in the control group, only about 50% of the rats survived, whereas in females the survival rate was about 70%. In mice, survival was about 80%.

The authors of the study concluded that under the condition of the bioassay (US NTP, 1982) there was no convincing evidence that the substance was carcinogenic to F 344 rats or B6C3F1 mice of both sexes. Other assessments (e.g. FAO/WHO, 2011) followed this interpretation of the study results.

No inhalation or dermal carcinogenicity studies are available, although in repeat exposure inhalation toxicity studies, BPA did not exhibit properties that raise concern for potential

carcinogenicity. Only minimal inflammation was seen in the upper respiratory tract at 50 mg/m^3 in a 13-week study and the severity did not increase up to concentrations close to the maximum attainable concentration in the experimental system used, 150 mg/m^3 (EC 2003).

The United States National Toxicology Program (NTP) bioassay did not include exposures during the peri-natal period. Later studies addressed this point.

Studies that included perinatal (gestational and/or lactational) exposures to BPA (oral doses to the dam from ~10 to 250 µg/kg b.w./day) have reported, among other lesions, proliferation of mammary ductal epithelium and squamous metaplasia of prostatic epithelium in offspring, conditions suggested to predispose to neoplasia (Timms *et al.*, 2005; Moral *et al.*, 2008). Additional treatments with initiating or promoting agents have led to earlier onset of mammary tumours (Jenkins *et al.*, 2009) or prostatic intraepithelial neoplasia (Prins *et al.*, 2011). Further studies used transgenic animals. An overview of carcinogenic studies including those using a co-treatment with a known carcinogen are presented in table 5.

Table 5. Pre- and perinatal exposure in carcinogenic studies with and without inducing agents

Author	species	dose (mg/kg b.w./day)	treatment duration	co-treatment	outcome
All organs					
Takashima *et al.*, 2001	rat	0, 400-600 (one dose level) in drinking water	10 week before mating until end of lactation	N-nitrosobis-(2-hydroxypropyl)-amine	BPA during development does not exert promoting effects on BHP-induced thyroid, lung, liver, thymus and esophagus carcinogenesis
Ichihara al., 2003	rat	0, 0.05, 7.5, 30, and 120 by gavage	pregnancy and lactation	3,2-dimethyl-4-aminobiphenyl	Without DMAB treatment, incidences of prostatic intraepithelial neoplasia (PIN), carcinoma, and atypical hyperplasia were not increased
Uterus					
Yoshida *et al.*, 2004	rat	0, 0.006 and 6 oral	GD 2 to PND 21	N-ethyl-N´-nitro-nitrosoguanidine	incidence of uterine preneoplastic or neoplastic lesions induced by ENNG was not increased by BPA exposure
Leydig cell division					
Nanjappa *et al.*, 2012	rat	0.0025, 0.025 gavage	GD 12 to PND 21		prepubertal period and increased Leydig cell numbers were shown in the testes of adult male rats at 90 days
Prostate					
Ho *et al.*, 2006	rat	0.01 sub-cutaneous	PND 1, 3 and 5	17β-estradiol (E) and testosterone (T) by sub-cutaneous implantation for 16 weeks	BPA/E+T 10/10 E+T 4/10 prostatic intraepithelial neoplasia (PIN)
Prins *et al.*, 2011	rat	0.01 sub-cutaneous 0.01 oral	PND 1, 3 and 5	17β-estradiol (E) and testosterone (T) by sub-cutaneous implantation for 16 weeks	PIN BPA/E+T >E+T; no difference between oral and sc
	rat	0 or 0.025 subcutaneous	GD 8–23	N-nitroso- N-methylurea	BrdU/apoptosis ratio was significantly increased and apoptosis was significantly decreased in mammary parenchyma and stroma

Tang *et al.*, 2012	rat	0.01 sub-cutaneous admin-istration in three doses	PND 1, 3, and 5	E+T	in prostate hypomethylation of the promoter of nucleosom binding protein-1 persisting until day 100; hypermethylation of hippocalcin-like1 which shows changes throughout life; persistent overexpression of four of eight genes functioning in methylation/demethylaton of DNA
Mammary gland					
Durando *et al.*, 2007	rat	25 µg/kg b.w. subcutaneous	GD8 to GD23	additional groups: NMU (25 mg/kg)	1. No NMU tretamen: increased proliferation/apoptosis ratio 2-NMU treatment: increased percentage of hyperplastic ducts and induced the development of neoplastic lesions
Murray *et al.*, 2007	rat	0, 0.0025, 0.025, 0.250, or 1 subcutaneous	GD 9 through PND 1		3–4-fold increase mammary hyperplastic ducts in all dose groups; increased cribriform structures in the 0.250 and 1 mg/kg b.w.
Vandenberg *et al.*, 2008	mice	0.25, 2.5, 25 µg/kg b.w SC	GD 8 through PND 16		intraductal hyperplasias, alveolar buds
Moral *et al.*, 2008	rat	25, 250 µg/kg b.w. orally	GD 10 to delivery		increase in mammary hyperplastic ducts
Jenkins *et al.*, 2009	rat	0, 25, and 250 µg BPA/kg b.w. orally dosing to dams	during lactation (PND1-PND20)	additional groups received 30 mg DMBA/kg body weight 0n PND 50	1.no DMBA treatment: increased cell proliferation and decreased apoptosis at 50 but not 21 days postpartum 2. DMBA treatment: dose-dependent increase in mammary tumour multiplicity and reduced tumour latency compared to controls
Betancourt *et al.*, 2010	rat	25, 250 µg/kg b.w. orally	GD 10 to GD21	additional groups received DMBA; 30 mg/kg b.w.. on PND 50, or PND100.	1.no DMBA treatment: increased cell proliferation 2. DMBA treatment only on day 100 but not on day 50: increased tumor incidence while decreasing tumor latency
Jones *et al.*, 2010	Brca1 knockout	0.000250 continously sc	4 weeks		increased mammary epithelial cell proliferation and hyperplasia

	mouse				
Jenkins *et al.*, 2011	transgenic MMTV-erbB2 mammary tumour mouse	0.0005, 0.005, 0.050, 0.5 orally	PND 56-252		decreased tumour latency and increased tumour multiplicity
Weber Lozada and Keri (2011)	mouse	0.025, 0.250 by gavage	GD8-21	DMBA	reduction of tumour latency of mammary tumours
Ayyanan *et al.*, 2011	mouse	0.6 µg, 1.2 mg/kg-b.w./day	unclear		adjusted number of buds increased
Kass *et al.*, 2012	rat	0.7, 64 µg/kg b.w./day	GD9 until weaning in F0		In F1 on GD21 but not on day 18 delayed histological MG differentiation
Tharp *et al.*, 2012	rhesus monkeys	0.400 orally	GD 100-165		statistically significant difference in the number of mammary buds/ductal units
Vandenberg *et al.*, 2013	mice	0.25, 2.5, 25 or 250 µg/b.w./day via osmotic mini-pumps	GD8-PND16		advanced gland development at 0.25 and 2.5 µg/kg b.w. but not at higher doses

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General tumorigenicity

In a study, carcinogenicity (including thyroid and lungs) was induced by N-nitrosobis-(2-hydroxypropyl)-amine (BHP) (Takashima *et al.*, 2001) The effect of BPA exposure during development in this model was investigated giving oral doses of 0 or 10,000 ppm (mg/kg in feed) BPA for 10 weeks prior to mating, and through mating, gestation and lactation. Intakes of BPA were reported to be about 400-600 mg/kg b.w./day. Beginning at 5 weeks of age and continuing for 12 weeks, offspring in each group received either tap water or tap water containing 2000 ppm (mg/L) BHP. Dam body weight was lower in the BPA group compared to the control group during the gestation period and at weaning. Otherwise, BPA had no effect on food intake and maternal serum levels of triiodothyronine, thyroxine, and thyroid-stimulating hormone, weights or histopathological alterations of maternal organs, including uterus and ovary, on mating, fertility, duration of gestation, live-born pups, implantation loss, or offspring viability through PND 21. In pups from dams exposed to BPA compared to pups from control dams, body weights were higher (by 11%) in females at 3 days of age and lower in males and females at 10 days and 2 weeks of age (16–22% decreases in males and 12–19% decreases in females). Prenatal and postnatal BPA exposure was not associated with significant differences in the development of BHP-induced neoplasms in the offspring (Takashima *et al.*, 2001). The results of this study indicate that oral exposure to 400-600 mg/kg b.w./day BPA during development does not exert promoting effects on BHP-induced thyroid, lung, liver, thymus and esophagus carcinogenesis in rats.

Doses of 0, 0.05, 7.5, 30, and 120 mg BPA/kg b.w./day were administered by gavage to female F344 rats during pregnancy and lactation (Ichihara al., 2003). At 5 weeks of age, 21 male rats/group were injected subcutaneously with 50 mg/kg b.w./day 3,2-dimethyl-4-aminobiphenyl (DMAB) 10 times at 2-week intervals. DMAB is an experimental aromatic amine that serves as an experimental model for arylamine and heterocyclic amine mutagens (Ravoori *et al.*, 2008). There were no consistent or dose-related effects on multiple endpoints. Without DMBA treatment, incidences of prostatic intraepithelial neoplasia (PIN), carcinoma, and atypical hyperplasia were not increased by exposure to BPA, and there were no increases in tumours of the non-reproductive organs. No effect was observed on serum testosterone levels. This screening study showed that exposure of rat dams up to 120 mg/kg b.w./day BPA during the gestation and lactation periods did not predispose their offspring to prostate cancer development later on in life (65 weeks of age).

Effects of maternal exposure to BPA on uterine carcinogenesis were studied in offspring of Donryu rats (a strain of rats with a high rate of spontaneous and ENNG-induced uterine tumours) administered BPA (0, 0.006 and 6 mg/kg b.w./day, n = 12, 15 and 19/group respectively) daily by gavage from GD 2 to PND 21 (Yoshida *et al.*, 2004). At 11 weeks of age, 35-36 animals/group were injected in the uterine horn with N-ethyl-N´-nitro-nitrosoguanidine (ENNG) to initiate uterine carcinogenesis. About 24 weeks later, the uteri of the 24–30 surviving animals/group were examined histologically to detect tumours and other lesions. In dams exposed to BPA, there were no clinical signs of toxicity or effects on body weight, implantation sites, or gestation length and on litter size. BPA exposure had no effect on the pups. The incidence of uterine preneoplastic or neoplastic lesions induced by ENNG was not increased by BPA exposure.

Prostate

The effect of short-term neonatal exposure to BPA on susceptibility of Sprague Dawley rats to prostate cancer was investigated after s.c. injection of BPA on PND 1, 3 and 5. The dose administered was 10 µg/kg b.w. s.c. corresponding to 41.8 µg/kg b.w. by the oral route that was based on AUCs after oral and s.c. administration allowing the calculation of an oral systemic availability of 23.9% (Ho *et al.*, 2006). From PND 90, in 50% of the rats of every group 17β-estradiol (E) and testosterone (T) were administered by s.c. implantation for 16 weeks in a dose which is reported to induce prostatic intraepithelial neoplasia (PIN) in 33% of Sprague Dawley rats. The second half of rats served as control. At 28 weeks, BPA exposure alone showed no effects on dorsal prostate

weight, histopathology alterations, proliferation index, or apoptotic index. In animals that were given E and T simultaneously for 16 weeks from PND 90, the group of rats with BPA exposure showed a statistically significant increased incidence and severity of PIN (100 [10/10] vs. 40% [4/10] incidence in controls). In the BPA/E+T group compared to the E+T group, the proliferation index was increased and the apoptosis index was decreased in regions where PIN was observed. Furthermore, the authors found hypomethylation of the PDE4 gene and increased expression of that gene at 90 and 200 days of age, with or without E+T exposure in adulthood.

A study by the same group (Prins *et al.*, 2011) used the identical protocol with the modification that a group with oral dosing was included whereby s.c. and oral dosing was at the same level, namely 10 µg/kg b.w.. Kinetic studies revealed an oral systemic availability of 23.9 % of the dose. The study confirmed the findings of the earlier study. Notably, identical effects were elicited by s.c. and oral dosing, although the internal dose after oral dosing was about 25% of the s.c. dose. Thus, no dose-response relationship could be demonstrated.

In a mechanistic study, the group of Prins (Tang *et al.*, 2012) evaluated methylation and expression of several genes throughout life in the rat prostate gland by BPA given by s.c. administration in three doses of 10 µg/kg b.w. (corresponding to 36 µg/kg each) each on PND 1, 3, and 5 with a low (0.1 µg/kg b.w.) and a high (2500 µg/kg b.w.) dose of 17ß-estradiol-3-benzoate (EB) as controls. Furthermore, a group of the treated rats received additional treatment, with estradiol (E) plus testosterone (T) released via a s.c. implanted capsule to produce increased intraepithelial neoplasias in the prostate, a model which has been used by the group since 1981 (Lee *et al.*, 1981). Further results were obtained in prostate cell lines. A whole array of changes were observed *in vivo*: hypomethylation of the promoter of nucleosom binding protein-1 persisting until day 100; hypermethylation of hippocalcin-like1 which shows changes throughout life; persistent overexpression of four of eight genes functioning in methylation/demethylaton of DNA not related to DNA methylation at their promotors. The results are somewhat inconclusive because a) no dose dependency was observed for the treatment with EB the dose differing by a factor of 25, 000 b) no clear difference existed in the parameters for the group undergoing additional treatment with E+T and c) more than 50 statistical tests were performed when testing the *in vivo* results. Although the authors claim that Bonferroni *post hoc* test was performed to correct for multiple testing, it is uncertain to which data it was applied.

Mammary gland effects

The effect of BPA was investigated in the N-nitroso- N-methylurea (NMU) model for inducing mammary tumors in Wistar rats (Durando *et al.*, 2007). On gestational day (GD) 8–23, *s.c.* (via miniature osmotic pumps) doses of 0 or 0.025 mg/kg b.w./day (corresponding to 0.9 mg/kg b.w. day on the oral route) BPA were given. Offspring were killed before puberty (PND 30), after puberty (PND 50), or in adulthood (PND 110 and 180). In mammary gland stroma and epithelium proliferation, apoptotic cells were determined and morphometric analyses were performed using adequate methods. Part of the offspring was examined for responsiveness towards the established carcinogen N-nitroso-N-methylurea (NMU). On PND 50, NMU was administered *intraperitoneally* to 10–16 offspring from the vehicle control group at 25 or 50 mg/kg b.w. and to 21 offspring from the BPA group at 25 mg/kg b.w./day. Based on findings from a pilot study, 25 mg/kg b.w. NMU was considered a sub-carcinogenic dose and 50 mg/kg b.w. NMU was considered a positive control.

Anogenital distance on PND 1 or 5 and postnatal body weights were unaffected in pups exposed to BPA. Vaginal opening was 5 days earlier in pups exposed in intra-uterine life to subcutanous BPA (mean PND 34 to PND 39 in controls). On PND 50, the BrdU/apoptosis ratio was significantly increased and apoptosis was significantly decreased in mammary parenchyma and stroma of BPA-exposed animals; the effects

were not observed on PND 30 or PND 110. Significantly increased percentages of hyperplastic ducts, density of stromal nuclei, and numbers of mast cells were observed in the BPA group on PND 110 and PND 180. In rats exposed to 25 mg/kg b.w./day NMU on PND 50, incidence of hyperplastic lesions on PND 180 was significantly higher in the group with prenatal BPA exposure compared to controls (mean incidence of 35.5% compared to 15.7% in controls). Other results were not statistically significant. As no dose-response relationship was investigated, the results on the mammary gland cell proliferation should be considered as supporting evidence and indicator for a possible concern.

Murray *et al.* (2007) studied whether prenatal BPA exposure induced mammary tumours in rats. Wistar-Furth rat dams were exposed via subcutaneously implanted osmotic pumps to BPA 0, 0.0025, 0.025, 0.250, or 1 mg/kg b.w./day from GD 9 through PND 1. Vehicle control exposure was 50% DMSO.

The number of hyperplastic ducts was increased in all dose groups on PND 50; the study authors noted that the effect on PND 50 was quantitatively similar in all dose groups (i.e. 3–4-fold increase) lacking a dose-response. Some of the hyperplasias were classified as carcinoma *in situ* with cribriform structures. These were observed in the 0.25 (corresponding to 9 mg/kg/d oral dosing) and 1 (corresponding to 35.7 mg/kg/d oral dosing) mg/kg b.w./day groups. The incidence was 25% (1 out of 4) at PND50 and 33% (2 out of 6) for the two highest dosed groups (0.25 and 1 mg/kg). The study authors concluded that fetal BPA exposure at dose levels of 0.250 and 1 mg/kg b.w./day via subcutaneously implanted minipumps (corresponding to oral doses of 9 mg/kg b.w./day and 36 mg/kg b.w./day) is able to induce development of preneoplastic and neoplastic mammary lesions in rats. There were some limitations to this study. Background diet, drinking water, bedding, and cage provided "negligible" estrogenicity. The forming of ductal end buds usually appears at puberty (Lucas *et al.*, 2007). Bud formation and ductal hyperplasia might be seen as an adverse outcome or as part of the normal ductal development depending on the status of the animals in terms of mammary gland develoment. A positive control like estradiol, lacking is this study, is then needed for comparison and identification of the adverse outcome. It is unclear whether these findings are biologically related to carcinogenic hazard. However, the results show a difference clearly induced by BPA and confirm earlier observations of this research group for the possibility for an effect on the developing mammary gland (Durando *et al.*, 2007).

The same research group used the same exposure protocol (s.c. osmotic pumps, dosing 0.25, 2.5, 25 µg/kg b.w) for long-term evaluation of mammary gland alterations in mice (Vandenberg *et al.*, 2008). Mice were exposed from gestation day 8 until day 16 of lactation. At 3, 9 and 12 -15 months of age female offspring were killed and mammary tissue samples collected and evaluated. An increase in the volume fraction of alveolar buds in the mammary tissue was observed for the 0.25µg/kg b.w.. BPA group only, at month 3 and 9. A dose-response relationship was not present. At 9 months, an increase in the incidence of beaded ducts was also noted for all doses investigated, although a dose-response relationship was not present. At month 12-15, the incidence of beaded ducts was increased only for the lowest dose. Proliferation of the cells was indicated by the Ki-67 antigen staining. Histological analysis of mammary glands at 9 months was unremarkable as to periductal stroma width, periductal collagen density, proliferative index (Ki-67 staining) and %ER and %AR positive cells.

BPA exposure during pregnancy and lactation affected the development of the mammary glands in the exposed female offspring. It is unclear whether these findings are biologically related to a carcinogenic hazard. No dose-response was observed, and the authors interpreted the results as indicating non-monotonicity.

In a study by Moral *et al.* (2008) pregnant rats were given 25 µg BPA/kg b.w. or 250 µg BPA/kg b.w. from GD 10 to GD21. Female litters were euthanized at 21, 35, 50, and 100 days. Analysis of mammary gland morphology was performed from whole-mounted mammary tissue. Proliferative index was determined by quantifying bromodeoxyuridine incorporation in the epithelial cells. BPA exposure induced changes in the mammary

gland that were time and dose specific. High-dose exposure resulted in increased number of undifferentiated epithelial structures of the breast tissue. Proliferative index did not show an effect of BPA. The study results are well described. However, it remains unclear what the findings indicate.

Neonatal/prepubertal rats were exposed to BPA via lactation from nursing dams treated orally with 0, 25, and 250 µg BPA/kg body weight/day from PND 1 to PND 21 (Jenkins *et al.*, 2009). In addition, female offspring were exposed to 30 mg DMBA/kg body weight at 50 days of age. Lactational BPA exposure resulted in increased cell proliferation and decreased apoptosis at 50 but not 21 days PND. This means that no effect was seen at the end of exposure. When additional DMBA treatment has been performed, lactational exposure to BPA demonstrated a dose-dependent increase in mammary tumor multiplicity and reduced tumor latency. The effect of DMBA is similar to that seen in other studies. It is, however, not clear whether the findings indicate a carcinogenic hazard.

A similar protocol was used in the study of Betancourt *et al.*, 2010. Oral treatment with 0, 25 or 250 µg BPA/kg b.w. was given to pregnant rats from GD 10 to GD21. For tumorigenesis experiments, prenatally exposed female offspring received a single dose of 30 mg/kg b.w. DMBA by gavage on PND 50, or PND 100. Prenatal exposure of the dam to 250 µg BPA/kg b.w. increased cell proliferation. Prenatal exposure of the dam to 250 µg BPA/kg b.w. combined with a single exposure of female offspring to DMBA had an effect only when dosed on PND 100, but not when dosed on PND 50. In those animals, tumor incidence increased significantly and tumor latency was decreased in comparison to the control group. The effect of DMBA is similar as seen in other studies. It is, however, not clear whether the findings indicate a carcinogenic hazard.

Three studies originating from the research group of Soto *et al.* demonstrated an effect of prenatal BPA exposure on mammary gland development, i.e. ductal hyperplasia and in one study carcinoma in situ development. These studies were performed with very low background estrogen levels in the feed which may have had an effect on the normal development in the controls. A limitation is the lacking of a positive control like estradiol for comparing the estrogenic effects induced by BPA. However, the differences between the non-treated and prenatal/postnatal BPA exposed animals are clear, so it cannot be excluded that BPA affects early development of mammary tissue. Therefore, these studies should be considered as an indicator for a possible concern.

Recently, Ayyanan *et al.* (2011) also showed that perinatal exposure of mice to BPA at doses ranging from 1.2 µg to 1.2 mg BPA/kg b.w./day and DES at 0.12 and 1.2 µg/kg b.w./day via drinking water. Exposure to low doses of oral BPA had no significant effect on litter size, sex ratio, or body weight at weaning. The number of terminal end buds, estrogen-induced proliferative structures, was altered in a dose-dependent fashion, but for only one dose (3 µg/kg b.w./day), an increase was suggested. In addition, adult F1 females showed an increase in mammary epithelial cell numbers at three months of age for both BPA (low 6-12 µg/kg and high 600-1200 µg/kg intake) and DES exposed F1 females. However, the study reported effects on increasing adjusted number of buds at a dose of 0.6 µg/kg b.w./day. As the paper does not clearly describe the procedure how the number of buds was adjusted and because the dose is really unclear (differences between method section and results section differ up to a factor of 1000), the study cannot be considered valid.

More recently, Tharp *et al.* (2012) of the group of Soto, investigated the histopathology of mammary gland in the offspring of rhesus monkeys given orally 400 µg of BPA per kg of body weight daily from gestational day 100 to term. This regimen resulted in 0.68 ± 0.312 ng/mL of free BPA and 39.09 ± 15.71 ng/mL of conjugated BPA in serum measured after dosing with deuterated BPA. The serum concentration is about 250 times higher than the predicted concentration in humans at a high realistic dose of 1 µg/kg b.w./day by the oral route. Morphometric analysis of the mammary glands removed from female offspring at birth showed that only the density of mammary buds was significantly increased in BPA exposed monkeys. Other parameters like total area, ductal area,

number of ducts, and terminal ends showed no difference between BPA exposed and control animals. In general the development of their mammary gland seemed more advanced when compared to unexposed monkeys.

BPA induced proliferative changes in the mammary gland of male CD-1 mice when BPA was given to pregnant and lactating mice at doses of 0.25, 2.5, 25 or 250 µg/b.w./day via osmotic mini-pumps (Vandenberg *et al.*, 2013). In this study, mammary glands were examined at 3-4, 7-9 and 12-16 months in the adult male offspring. The reported changes were seen in the ductal area and at branching points. Animals exposed to 0.25 or 2.5 µg/kg b.w./day showed more advanced gland development than the controls, whereas animals having dosed with 25 or 250 µg/kg b.w./day had no statistically different results compared with controls. These results point at a non- monotonic dose-response to BPA. At later time periods, effects were similar. However, the dose-response relationship had a different pattern.

Jones *et al.* (2010) used the Brca1 knockout mouse model of breast cancer susceptibility (gene 1 (BRCA1) related mammary cancer). Continuous exposure to 250 ng/kg b.w. BPA through an osmotic pump for 4 weeks (corresponding to 125 µg/kg b.w./day by the oral route) increased mammary epithelial cell proliferation and hyperplasia in adult Brca1 knockout mouse mammary glands compared with wild type mice. The authors also presented *in vitro* mechanistic investigations in MCF-7 cells supporting the hypothesis that loss of BRCA1 function in mammary cells would enhance BPA-induced cell proliferation via interference with the ER-alpha signalling pathway.

Jenkins *et al.* (2011) investigated in a transgenic MMTV-erbB2 mammary tumour mouse model, whether BPA increased the susceptibility of females to mammary cancer after chronic oral exposure to BPA at levels of 0, 2.5, 25, 250, 2500 µg BPA/L in drinking water for the whole adult life (PND 56-252). In this model, BPA decreased tumour latency and increased tumour multiplicity, enhanced tumour volume and higher incidence of lung metastasis in a way that the authors describe a non-monotonic dose-response as the effects were observed at one of the two lower doses (0.5 and/or 5 µg BPA/kg b.w./day), but not at 50 or 500 µg BPA/kg b.w./day. In contrast, the cell proliferation index of mammary epithelial cells (evaluated on PND 112) and the apoptotic index increased in a dose-dependent manner, with statistical significant results at the highest dose (500 µg BPA/kg b.w./day).

Similar to the study of Ichihara al., 2003, Weber Lozada and Keri (2011) used the DMBA mammary tumour mouse model to assess the effects of foetal exposure to BPA on mammary tumour development in adults. When mice were exposed *in utero* to 25 µg/kg b.w. and 250 µg/kg b.w. by oral gavage of the pregnant dams, the offspring showed an increased susceptibility to DMBA mammary gland induction when treated with DMBA postnatally. A dose-response in the reduction of tumour latency of mammary tumours was observed in mice treated with BPA before birth. The foetal exposure to BPA led to early vaginal opening in FVB/N female mice. In these studies, the mouse strain FVB/N was used because of its intrinsic propensity to develop mammary tumors with various genetic manipulations. The administration of BPA only had no effect on mammary gland development. BPA was administered from postcoital day 8 until birth, while DMBA was administered twice one dose each at week 5 and 6 after birth. Additionally, tumour growth promotion was observed for both BPA and 17β-oestradiol treated mice after injection of estrogen dependent MCF-7 human breast cancer cells in ovariectomized nude mice. However, the effect of BPA was reduced when compared to 17β-oestradiol.

Kass *et al.* (2012) found in F1 bred female offspring of BPA and DES treated dams a delay in histological mammary gland differentiation and altered milk yield pattern during lactation. BPA with theoretical doses of 0.5 µg BPA/kg b.w./day and 50 µg BPA/kg b.w./day was administered via the drinking water from GD9 until PND21. Direct exposure to BPA or DES in the drinking water did not produce signs of embryotoxicity (i.e. all pregnant dams successfully delivered their pups, and the number of live-born pups per litter was similar among groups), abnormal maternal or nursing behavior, or changes in

body weight gain in the F0 dams. The reproductive parameters were not significantly changed in the F1 females, with the exception of the number of resorption sites in BPA 50 µg/kg b.w. and DES treated dams. The number of pups born of the F1 females was decreased but did not reach significance.

Leydig cell division

In a study in which pregnant and lactating Long-Evans rats were given BPA via gavage (2.5 and 25 µg/kg b.w./day) from gestational day 12 to postpartum day 21, Leydig cell division was stimulated in the prepubertal period and increased Leydig cell numbers were shown in the testes of adult male rats at 90 days (Nanjappa *et al.*, 2012).

Conclusion on BPA carcinogenicity

From all the study results taken together, it can be concluded that in standard carcinogenic testing protocols according to OECD, BPA has no carcinogenic activity. In additional in multigeneration studies, (Tyl *et al.*, 2002; Tyl *et al.*, 2008) no indication of increased cancerogenicity was observed; in particular, preneoplatic lesion of the mammary gland were absent in all offspring. In contrast, several studies in rats, using s.c. exposure via osmotic pumps, demonstrated an effect of prenatal BPA exposure on mammary gland development, i.e. ductal hyperplasia, and in one study carcinoma development in situ. These studies were performed with very low background estrogen levels in the feed which may have had an effect on the normal development in the controls. A limitation is the lack of a positive control like estradiol for comparing the estrogenic effects induced by BPA. Similar effects were indicated in studies in mice and rhesus monkeys, supporting the observations in rats. The differences observed between the non-treated and prenatal/postnatal BPA exposed animals is clear, so this effect cannot be excluded. These studies should be considered as an indicator for a possible concern, although the relevance for humans is not clear.

Studies using s.c. administration of BPA indicated that BPA may have the ability to increase the effects of well-known carcinogens even at very low BPA levels, acting as promotor. The studies had limitations which render them unsuitable to assess whether BPA has such an effect following prenatal or peri-natal exposure. The main limitation is that in the studies with positive outcome additional treatment with a strong initiating or additional promoting agent(s) has been performed. Furthermore, in most of the studies multiple statistical testing has been performed without proper adjustment to avoid positive results by chance. An additional problem in the statistical analysis is the lack of considering litter effects.

Further studies were performed in transgenic animals, the results of which cannot be extrapolated directly to the human situation.

In conclusion, the studies indicating effects on mammary gland development raise some concern for a possible effect after prenatal exposure to BPA. However, the existing studies performed according to OECD guidelines do not show a carcinogenic effect of BPA.

3.9.4. Neurotoxicity and behavioural toxicity

Neurological studies in laboratory animals (rat, mouse, sheep and/or non-human primate) assayed pathology, neurochemistry, neuroendocrine system, sensory systems, locomotor and spontaneous activity, social and sexual behaviours, anxiety, and learning and memory at various stages of development. Exposure was primarily during the periods of gestation and lactation.

The experimental evidence does not support brain developmental neuropathological changes (e.g. cortical thickness, cerebellum height, height of hippocampal layers) at rat

maternal dietary exposures below 164 mg/kg b.w./day (Stump *et al.*, 2010). Brain biochemical changes (e.g. monoaminergic, cholinergic, glutamatergic, nuclear receptor expression and signalling) were reported in rodents at dietary exposures below 5 mg/kg b.w./day. However, no additional functional testing was performed. Thus, the *in vivo* consequences of the changes are unclear.

Only a few studies have specifically focused on the impact of BPA on morphometric and cellular brain sex differences. Depending on the hypothesized mode of action, not all studies included both sexes. In some cases, only one sex was impacted, whereas in others, the overall differences between the sexes were reduced or eliminated. The controversial results limit their interpretation (reviewed in Wolstenholme *et al*. 2011,).

BPA does not appear to affect sensory systems, spontaneous activity or female sexual behaviour in rodents. For neonatal reflexes, sensory response, spontaneous motor activity and other open field behaviours, a minimum NOAEL of 164 mg/kg b.w./day for rat maternal dietary exposure can be identified. Minimum NOAELs (corresponding to the highest dose tested in individual studies) of 200 µg/kg b.w. per day (Ryan *et al.*, 2010) and 320 mg/kg b.w./day (Kwon *et al.*, 2000) for rodent maternal dietary exposure could be identified for lordosis; for other components of sexual/sociosexual behaviours, NOAELs could not be identified. For learning and memory in rodents, conflicting data exist, although the weight of evidence does not suggest these to be a concerning hazard identification endpoint (Kwon *et al*., 2000).

Neuroendocrine data in rodents and sheep suggest effects on female hypothalamic-pituary-gonadal (HPG) axis organization (≥50 µg/kg b.w. per day, non-oral route) and function (≥5 mg/kg b.w./day, non-oral route), that is at doses higher than the Point of Departure used for TDI derivation (Katoh *et* al., 2004). The specific mechanisms by which this occurs remain to be identified, but some data suggest that the pattern of luteinizing hormone release may be altered by exposure, resulting in blunted secretion and resistance to feedback.

Recent studies (Ishido *et al.*, 2011; Kim *et al.*, 2011; Wolstenholme *et al.*, 2011; Eilam-Stock *et al.*, 2012) have examined the effects of BPA at doses lower than 50 µg/kg b.w.. The endpoints were neuroanatomical/genomic and behavioural.

The study by Wolstenholme *et al.*, 2011 investigated the effect of dietary exposure towards BPA (5 µg/kg b.w./day) on social interactions in the pups and also some gene expressions. The female offspring showed increased social interactions in a free 30-min social interaction test. However, BPA did not affect social preference for the stimulus animal in a social preference test. In the Plus Maze task, anxiety, time spent in the open arms, closed arms and the number of crosses between arms were similar in the two groups. Gene expression analysis revealed that mRNA for the glutamate transporter Slc1a1 was enhanced by exposure to BPA in female brains and that expression of two of the three DNA methyltransferase genes, Dnmt1 and Dnmt3a, was modulated by BPA. Whereas expression of estrogen receptors' genes was not affected by BPA, oxytocin receptor gene was to some extent reduced in males. Although the association of the behavioural results at weaning age with the small changes in gene expression found at the fetal stage is weak, and do not fully support novel mechanistic hypotheses, these findings confirm previous data on the sex-dimorphic effects of BPA on social behaviour.

The effects of a single subcutaneous BPA administration (40 µg/kg b.w.) on memory and synaptic plasticity in adult male rats was examined by Eilam-Stock *et al.* (2012) in adult animals. Single subcutaneous administration of BPA interferes with memory consolidation possibly impairing the formation of dendritic spines by reducing a marker of neural plasticity and synaptic remodeling as PSD95 in the hippocampus. This study is remarkable as a single dose is sufficient to produce marked effects. However, notably, in rats 40 and 80 µg/kg b.w. given s.c. corresponds to roughly 1 and 2 mg/kg b.w. oral dosing, considering kinetic differences.

The study of Jones and Watson (2012) investigated the behaviour in the Morris Water Maze (MWM), Elevated Plus Maze (EPM) and Forced Swimming Test (FST) after oral

administration of BPA in doses between 5 µg/kg b.w./day and 5000 µg/kg b.w./day during gestation until PND 14 day to investigate their effects after delivery. No effect of BPA was observed in the MWM, but on both the EPM and FST, low and high doses (5 µg/kg and 5000 µg/kg b.w./day) of BPA eliminated sex differences found between controls. However, interpretation of the result is difficult and might be due to statistical noise.

Three doses of 50 µg/kg b.w. and 50 mg/kg b.w. BPA each by subcutaneous injection (corresponding to an oral dose higher than 1 mg/kg b.w.) from PND0 to PND2 showed significant and sex-specific alterations of gene expression of estrogen receptor alpha (ERα), ER beta (ERβ) and kisspeptin (Kiss1) in the anterior and mediobasal hypothalamus on PND 4 and 10 of rats but not in other regions of the brain (Cao *et al.*, 2012). The authors suggest that effects observed with BPA are very different from those of the positive control (oestradiol) and hence mechanismswere involved which are different from estrogenic action.

BPA induced locomotor hyperactivity when administered *intracisternally* at PND 5 in the brain of male rats (Ishido *et al.*, 2004). A difference noted was that control animals received the vehicle olive oil only, while BPA treated rats received BPA dissolved in at least 50% ethanol complemented with olive oil. There was a dose dependent effect on the motor activity with a NOAEL of 0.02 µg/pup and a LOAEL of 0.2 µg/pup, both administered as a single dose. The same group reported similar results for BPA in another study (Masuo *et al.*, 2004). In an additional study only one dose was investigated (20 µg/pup) and BPA was compared with two of its derivatives (3-hydroxybisphenol A, bisphenol A 3,4-quinone). With the same treatment modality (ethanol and olive oil), the derivatives did not induce a motor hyper activity whereas BPA did. So, the effects could not be attributed to the ethanol present in the administered solution (Ishido *et al.*, 2011). The same protocol was applied in a study by Kiguchi *et al.*, (2008) and the results the authors report are similar to the previous studies with the exception that the motor hyperactivity was observed in the light phase in contrast to the studies of Ishido *et al.*, 2004, and Masuo *et al.*, 2004, in which the hyperactivity was observed in the dark phase of the day. When comparing doses in the study of Kiguchi a higher dose was needed to induce the effect (40 µg versus 0.2 µg and 20 µg/pup), the 20 µg dose being ineffective (Kiguchi *et al.*, 2008). The effect seems to be limited to a period directly after the administration, as at week 8-10 after the treatment no effect of the BPA administration on motor hyperactivity was observed (Kiguchi *et al.*, 2007).

There are several studies published in the last three years investigating the effect of BPA in animal models for anxiety, for learning and memory, and for social behaviour.

Anxiety

Concerning anxiety, there exist a number of studies in rodents (e.g. Cox *et al.*, 2010; Tian *et al.*, 2010; Zhang *et al.*, 2009; Patisaul and Bateman, 2008; Ryan and Vanderbergh, 2006; Gioiosa *et al.*, 2007; Fujimoto *et al.*, 2006). However, the results of these studies were controversial with either significant or not significant sex differences, which might be partly explained by the testing conditions used which were different in the studies and may have not exposed the animals in the window of susceptibility. The most recent studies (Matsuda *et al.*, 2012; Jones and Watson, 2012; Patisaul *et al.*, 2012; Jasarevic *et al.*, 2012; Xu *et al.*, 2012; Gioiosa *et al.*, 2013; Kundakovic *et al.*, 2013) assessed the effects of BPA on anxiety-like behaviour and brain biochemistry. The studies explored different exposure schedules and different doses.

Matsuda *et al.*, 2012 showed that only in male offspring and not in females of C57BL/6J mice dosed from GD10 to PND20 to BPA (0.25 µg/kg b.w./day subcutaneously) assessed at 4 weeks and at 8 weeks exhibited increased anxiety and dopamine concentrations and turnover in different brain areas were altered. In the rat study of Jones and Watson (2012) anxiety responses (Elevated Plus Maze (EPM); Forced Swimming Test (FST)) were measured after repeated oral exposure between GD 1 and PND 21 to BPA doses 5, 50, 500, or 5000 µg/kg b.w./day. Males showed greater anxiety-like behaviour than females

in the EPM and the lowest and the highest dose of BPA eliminated significant sex differences. In the FST test only for the lowest BPA dose eliminated sex differences. They also assessed spatial learning capacities in the Morris Water Maze and reported no effects of BPA or an interaction of BPA with sex. This study is reported in a way which excludes evaluation of the extent of the overall BPA effect. In contrast, Wolstenhome *et al.* (2011) did not find effects on anxiety in the Elevated Plus maze following oral administration of BPA.

In outbred deer mice (Peromyscus maniculatus bairdii) (Jasarevic *et al.* 2012), BPA at doses of 50 mg, and 5 mg kg feed weight showed increased anxiety in the Elevated Plus Maze and reduced exploratory behaviours in male offspring whose dams were fed with a diet supplemented with either ethinyl estradiol or BPA (50 mg, 5 mg, or 50 µg/kg feed weight) starting from 2 weeks before mating up to the end of the lactation period. The drawback of the study is that food consumption was not measured, and, therefore, the BPA doses were calculated by a formula were imprecise.

Patisaul *et al.* (2012) exposed Wistar rats via drinking water (1 mg/L) in the intrauterine and postnatal period (GD 6 through PND 40) to BPA (estimated dose of BPA between 100 and 1000 µg/kg b.w./day). BPA-exposed animals showed increased anxiety as juveniles, and as adults displayed a disappearance of the normal sexual dimorphism in exploratory behavior. Down-regulation of expression of ERβ in the amygdale was reported, a key area in modulation of affective responses. Administration of a soy-enriched diet appeared to mitigate the BPA effects.

Xu *et al.* (2012) explored in mice the effects of different exposure windows (GD 7-20 or PND 1-14) with BPA (0.4 or 4 mg/kg b.w./day) by oral route. Both exposure periods and both doses increased anxiety- and depression-like behaviours in mice of both sexes measured by EPM, Open Field, dark light transition task and mirrored maze. The gestational exposure exhibited a stronger effect on anxiety-like state only in females. AMPA GluR1 receptor subunit was inhibited in hippocampus and amygdala in both sexes.

Gioiosa *et al.* (2013) exposed mouse dams from GD 11 to PND 8 with an oral low-dose of BPA (10 µg/kg b.w./day). After birth, offspring of exposed mothers were cross-fostered by mothers not exposed, whereas offspring from not-exposed mothers were nursed by exposed mothers. The exposed F1 animals were tested in EPM, open-field and novelty test to measure anxiety and emotional response to novelty. There were sex differences in the control group: control females were less anxious, more active and more prone to explore a novel environment than control males. Females exposed to BPA pre- and postnatally showed evidence of increased anxiety and were less prone to explore a novel environment relative to the control females, showing a behavioural profile more similar to control males than females. In this study, the direction of the behavioural changes was consistent and affected similarly by the pre- and postnatal exposures, although with a greater effect associated with postnatal exposure only in females. The study has limitations due to incomplete statistical considerations, as no adjustment of the p-value ($p<0.05$) was made for multiple testing, although 15 endpoints were evaluated with six comparisons per endpoint. In addition, the authors investigated only one dose level of BPA. Thus, the results cannot be used for risk assessment.

In the study of Kundakovic *et al.* (2013), BALB/c mice were exposed from the day of mating to the end of pregnancy to BPA (2, 20 or 200 µg/kg b.w./day). Whole arrays of endpoints were evaluated. Six behavioural endpoints, including anxiety-like behaviour, were assessed and expression of five genes at two locations in the brain as well as DNA methylation at eleven loci and two brain regions were measured. The results showed that maternal exposure during pregnancy induces sex-specific, dose-dependent (linear and curvilinear), and brain region- specific changes in expression of genes encoding estrogen receptors (ERs; ERα, ERβ Er γ) and altered mRNA levels of epigenetic regulators DNA methyltransferase (DNMT) 1 and DNMT3A in the juvenile cortex and hypothalamus, paralleling changes in estrogen-related receptors. At the behavioural level BPA exposure induced persistent, largely sex-specific effects on social and anxiety-like behaviour, leading to disruption of sexually dimorphic behaviours in adult mice. The results may

only be seen as generating a hypothesis, as the statistical model is a not-prespecified exploratory model.

Most of the studies, with the exception of the study of Kundakovic *et al.* (2013) that found decreased anxiety in males, reported increased anxiety, mainly in males but occasionally also in females following *in utero* exposure and exposure during lactation. The doses of BPA were lower than 5 mg/kg b.w./day.

Effects on learning and memory

In previous studies (Xu *et al.*, 2010; Tian *et al.*, 2010; Carr *et al.*, 2003), developmental exposure to BPA seemed to interfere with learning and memory capacities in different learning tasks in rodents. However, the studies were not considered valid for risk assessment due to methodological shortcomings. In addition, in the study by Stump *et al.* (2010, see description of tests applied and results above), the authors did not report any effects on learning and memory.

In the study by Xu *et al.* (2013), adult mice were exposed to oral doses of BPA (0.4, 4, or 40 mg/kg b.w./day) for 12 weeks. Mice were assessed at the end of treatment in two learning tasks, the Morris Water Maze and the Passive Avoidance test. BPA (0.4 or 40 mg/kg/day) extended the average escape path length to the hidden platform in Morris Water Maze task and shortened the step-down latency 24 h after footshock of the males, but no changes were found in females. BPA reduced numeric synaptic density and an enlarged synaptic cleft and reduced length of active zone and PSD thickness, in the hippocampus of male mice. Western blot analyses further indicated that BPA down-regulated expressions of synaptic proteins (synapsin I and PSD-95) and synaptic NMDA receptor subunit NR1 and AMPA receptor subunit GluR1 in the hippocampus of the males.

Eilam-Stock *et al.*, 2012 investigated the effects of a single subcutaneous BPA injection (40 µg/kg b.w.) on memory and synaptic plasticity in adult male rats. Memory tests applied included the Object Recognition (OR) and the Object Placement (OP) tasks. The authors reported that BPA significantly impaired both OR and OP and decreased spine density in the hippocampus and medial prefrontal cortex. Additionally, BPA significantly decreased PSD-95, a measure of neural plasticity in the hippocampus and increased pCREB, a transcription factor, in the prefrontal cortex. Together, these findings show that BPA may block the formation of new memories by interfering with neural plasticity processes in the adult brain. The Panel noted that the study was performed in adult animals, limiting its value in assessment of developmental neurotoxicity.

The study by Inagaki and coworkers (2012), performed mainly in adult ovariectomised (OVX) female 0 rats (to abolish any estrogenic modulation of behaviour) administered with BPA at levels from 0.4 µg/kg b.w. to 400 µg/kg b.w. (6 does levels) by subcutaneous route, found that BPA did not impair memory response *per se* in either OR and OP memory task, but it significantly antagonized the effects of 17β estradiol as enhancer of learning and memory performances and dendritic spine formation (lowest dose of BPA effective in blocking the facilitatory effect of estradiol in OP test equal to 4 µg/kg b.w.; in OR test equal to 40 µg/kg b.w.). A group of normally cycling rats were also used and exposed to a single dose level of 40 µg/kg b.w.: while BPA did not affect OP performance at any phase of the estrous cycle, OR memory was inhibited by BPA only on proestrous when endogenous E2 levels are at the highest.

These two studies, though exploring the effects of a single administration of BPA in adult animals, added some mechanistic information to explain the effects reported for developmental BPA on learning and memory processes and spinogenesis, which could implicate the interference of BPA with steroid-modulated synaptogenesis occurring during brain ontogenesis in physiological conditions (see the review by Hajszan and Leranth, 2010).

Studies with exposure during pregnancy

Jones and Watson (2012) failed to evidence any effects of oral gavage of BPA (doses 5, 50, 500, and 5000 µg/kg b.w./day) during gestation and lactation on spatial learning in the Morris Water Maze.

On the contrary, Jasarevic *et al.* (2012) reported that male deer mice orally exposed during gestation and lactation to 0.05, 5 or 50 mg/kg feed weight BPA equivalent to 0.25, 25 or 250 µg/kg b.w./day had impaired learning performance in the Barnes Maze, while females outperformed males.

Finally, Ferguson *et al.* (2012) in their robust study using two very low doses of BPA (2.5 or 25 µg/kg b.w./day) given by oral gavage on gestational days 6-21 and then to offspring from birth to weaning did not show significant effects on spatial learning.

There are several papers presenting evidence indicating effects of BPA exposure during development on social responses, including social/affiliative interactions in female rodents, sexual behaviour and aggression in males (Xu *et al.*, 2011; Jones *et al.*, 2011 ; Cox *et al.*, 2010; Tian *et al.*, 2010; Palanza *et al.*, 2008; Patisaul and Bateman, 2008; Giosa *et al.*, 2007). Overall, the direction of the effects ranged from pro-social effects to reduction of social motivation. Although most of these studies have been reviewed by EFSA in 2010, the social behaviour endpoint was not addressed separately in the EFSA 2010 opinion.

In the 2011 study by Wolstenholme *et al.,* the female offspring born to females fed with a BPA-supplemented diet during pregnancy (about 1.25 mg BPA/kg diet estimated to be equivalent to approximately 120 µg/kg b.w./day) showed slightly increased social interactions in a free 30-min social interaction test. The effect on males was in the same direction, but less significant. However, BPA did not affect social preference for a stimulus animal when compared to an inanimate object. Gene expression analysis performed in whole-brain embryos revealed mRNA for the glutamate transporter Slc1a1 was enhanced by exposure to BPA in female brains and that expression of two of the three DNA methyltransferase genes, Dnmt1 and Dnmt3a, was modulated by BPA. Notably, expression of estrogen receptor genes was not affected by BPA, but oxytocin receptor gene (highly responsive to estrogen modulation and involved in social behaviour) was reduced in males.

In a second study assessing transgenerational effects of BPA in mice through four generations, Wolstenholme *et al.* (2012) exposed the F0 generation only to BPA (about 5 mg/kg diet, equivalent to approximately 1.0 mg/kg b.w./day21) through pregnancy and lactation. Subsequent generations were not exposed to BPA. Brains from embryos from mothers exposed to BPA had lower gene transcript levels for several estrogen receptors, oxytocin, and vasopressin as compared with controls in the F1 generation; decreased vasopressin mRNA persisted into the F4 generation, at which time oxytocin was also reduced but only in males. Changes in gene expression were paralleled by alterations in social behaviour in F1 and in F2 and F4. The effects were in the direction of slightly reduced social interaction in F1 and increased social interaction in either F2 and F4.

The CEF panel noted that these two studies have some methodological limitations (only one dose level used, litter effect not properly controlled). However, the extent of the effects on F1 in the two studies is comparable although possibly in two opposite directions (considering that different BPA dosages were used). The different direction of effects in F2 and F4 was an unexpected result and might suggest inconsistency, but transgenerational effects might result from modulation of several genes implicated in the control of complex functional endpoints.

Effects on sensory-motor functions

In previous studies, no convincing evidence of a consistent BPA-related effect on motor activity was demonstrated at low oral doses (Stump, 2010).

Newer studies on changes in sensory-motor function following pre- and post-natal exposure were published by Ferguson *et al.* (2012) and Viberg *et al.* (2011).

Ferguson *et al.*, 2012 studied in rats the effect of exposure towards 2.5 and 25 µg/kg b.w. daily from GD 6 to 21 and PND 1 to 21. Whereas in positive controls (5 and 10 µg/kg b.w./day EE), clear effects were seen in open field assessments and the Barnes maze escape box, few consistent or dose-related effects resulted from developmental treatment with BPA at the doses tested.

Viberg *et al.* (2011) showed that there were significant alterations in behavior and cognitive functions in mice after two months and after five months, for the locomotion, rearing, and total activity variables after a single oral administration of 3.3 or 4.8 mg/kg b.w. BPA but not of 0.23 mg/kg b.w. on PND 10.

<u>Studies with endpoints in brain biochemistry, neurogenesis, neuroanatomy and gene expression (*ex vivo* studies)</u>

The study by Cao *et al.*, 2012 shows that 50µg/kg b.w. BPA by subcutaneous injection daily from postnatal day 0 (PND 0) to PND 2 had regional and sex-specific alterations of gene expression of estrogen receptor alpha (ERα), ER beta (ERβ) and kisspeptin (Kiss1) that are all decreased in the anterior and mediobasal hypothalamus on PND 4 and 10. Notably, the effects of BPA are very different from those of estradiol (positive control), supporting the view that the interference of BPA with early hypothalamic organization involves mechanisms different from its estrogenic action. A more recent study by Cao *et al.* (2013) found that offspring of rats receiving BPA orally from gestational day 6 to PND 21 (2.5 or 25 µg/kg b.w.) show significant changes in estrogen receptors ESR1 and ESR2 in hypothalamus and amygdale at birth. Specifically, both doses of BPA increased expression of ESRs in both sexes comparably to 5 or 10 µg/kg b.w. ethinyl estradiol.

The study by He, Paule and Ferguson (2012) indicates that BPA can have sex-specific effects on hypothalamic medial preoptic area volume and that these effects manifest as larger volumes in males, with oral exposure during pregnancy and by gavage to the pups during the period of lactation at doses of 2.5 or 25 µg/kg b.w.. These alterations in rats have been linked to changes in sexual dimorphic behaviour. The consequences of these morphological changes if replicated in humans are not known, but the medial preoptic area has a pivotal role in the regulation of sexual and parental behaviour in mammals including primates.

Two studies addressed the controversial issue of BPA effects on hippocampal neurogenesis (considered as a clear adverse effect by the 2013 ANSES report). Kim *et al.* (2011) found increased neurogenesis after treating mice by oral gavage for 2 weeks in late adolescence with 1 mg/kg b.w./day oral BPA, whereas Komada *et al.* (2012) described a similar effect in the fetus after *in utero* exposure to 200 µg/kg b.w./day.

In the study by Xu *et al.* (2013), mouse dams were orally exposed to BPA (4, 0.4 or 0.04 mg/kg b.w./day) from GD 7 through PND 21. Results showed that BPA (lower and higher doses) significantly reduced the numeric synaptic density of pyramidal cells in hippocampus CA1 region on PND 14, 21 and 54 in male offspring ($p < 0.001$). The reduced density was paralleled by significant modification of structural parameters indicative of synaptic functionality (enlargement of synaptic cleft by 0.4 and 4 mg/kg BPA and reduction of the active synapse zone as indicated by decreased Post Synaptic Density at 0.4 and 4 mg/kg BPA on PND 14 and PND 21, respectively. BPA also reduced the expression of synapsyn 1 and PSD95 in a dose-dependent fashion at all the time endpoints analysed. In addition, exposure to BPA markedly reduced the expression of both glutamate NMDA and AMPA receptors in the hippocampus on PND 14, 21 and 56 at the doses of 0.04 and 4 mg/kg 3118 b.w./day.

Finally, two *in vitro* studies showed that BPA concentrations in the nanomolar range inhibits NGF- induced neurite extension in PC12 cells (Seki *et al.*, 2011), while BPA significantly enhanced spinogenesis when added to isolated hippocampal slices obtained from untreated adult male Wistar rats (BPA concentrations ranging from 1 nM to 10 µM), with mechanisms likely independent from estrogen receptors (Tanabe *et al.*, 2012).

Conclusion

There is uncertainty with regard to the interpretation of the data regarding neurological effects of BPA. In several studies an increased anxiety was observed. However, studies on anxiety (rodent and non-human primate) have a behavioural endpoint which is sensitive for a number of factors such as study design, testing apparatus, inclusion of only one sex, and age at examination. New data confirm previous data that BPA has an effect on sex-dimorphic social behaviour. However, it is disputed whether elimination of sexual dimorphism could be considered as an adverse effect in extrapolating to humans. Gene expression in the brain was also altered either after prenatal BPA exposure and BPA exposure in adult mice. Other effects of BPA on hypothalamic organisation involve mechanisms different from its estrogenic properties because the effects of oestradiol were different. The variety of read-out parameters and the effects observed warrant further investigation of the possible neurological and behavioural effects of BPA.

3.9.5. Immunotoxicity

In previous reviews on BPA, it was concluded that BPA is capable of inducing skin sensitization responses in humans with low prevalence being a weak sensitiser (EFSA 2010, FAO/WHO 2011, EC 2010a, ANSES 2011). Some individual cases were also reported describing contact dermatitis against BPA (Aalto-Korte *et al.*, 2003). The results of rodent studies suggest that BPA may modulate immune homeostasis especially regarding the induction of T-cells and cytokine production directing the immune response into an allergy prone profile. (EFSA 2010, FAO/WHO 2011, EC 2010a, ANSES 2011). However, the results were insufficient to conclude on immunotoxic activity of BPA, and the immune system was considered to be an area of interest for further research.

The study of Lee *et al.* (2012) evaluated possible mechanisms of sensitization induced by BPA through investigating the cytokine profiles after BPA exposure. Mice were injected intraperitoneally with BPA (5 mg/kg b.w.). Total non-specific IgE antibodies and β-hexosaminidase and histamine (both inducing degranulation of mast cells) in serum were increased. The results are indicative for an effect of BPA on the immune system at doses higher than the PoD considering the route of exposure, however, no specific immune responses were measured.

Kendziorsky *et al.* (2012) investigated the response of BPA in a specific mouse strain prone to develop pyometra. BPA was observed to induce pyometra in one of two mice strains investigated similar to 17α-ethinyl estradiol. For both compounds only one dose induced pyometra, and a dose-response relationship was not established. The authors concluded that there was a strain specific estrogen sensitivity resulting in pyometra in C57Bl/6 versus CD1 mice. However, only in one out of five animals treated with BPA pyometra was observed. No effects of BPA were observed on fertility in both C57Bl/6 and CD1 mouse strains. The results of the study are of no value for the risk assessment.

Nakajima *et al.* (2012) exposed mice to 10 µg/ml BPA in their drinking water from 1 week before pregnancy until PND 22. The treatment with BPA, followed by postnatal allergic sensitization with ovalbumin and a challenge at PND 22, promoted the development of ovalbumin-induced allergic asthmatic responses (airway hyperreactivity, increase in eosinophilic granulocytes).

In three studies, data of the US NHANES were used for evaluation of possible BPA effects on allergies (Clayton *et a*l., 2011, Savage *et a*l., 2012, Vaydia *et al*., 2012). All three studies used spot urinary BPA levels for their evaluations. Clayton *et al*., (2011) evaluated urinary BPA levels and the presence of viral antibodies and allergy in children older than 6 years of age. BPA showed no association with allergy diagnosis, whereas the evaluations of the CMV antibodies showed contradicting results in diferent age groups. Both low and high BPA urinary levels were associated with a higher antibody levels. Savage *et a*l., (2012) evaluated urinary BPA levels and the sensitization against aeroallergens and food allergens by measurement of antigen specific IgE levels in sera.

For BPA, no associations with IgE levels were observed, whereas for some other chemicals investigated an association was observed. However, Vaydia et al., (2012) did find an association between urinary BPA levels and allergic asthma based on total IgE determinations and allergen specific IgE levels. BPA was associated with a higher likelihood of allergic asthma in females but not in males.

In two studies, BPA exposure of mothers and presence of wheeze in their children was investigated. Spanier et al. (2012) measured spot urinary levels in pregnant women at week 16 and 26 of gestation and at birth. In general, BPA levels were not associated with the occurrence of wheeze in the children. However, mean prenatal BPA above versus below the median was positively associated with wheeze at 6 months of age but not at 3 years. Another mother and child cohort was reported by Donohue et al., (2013), in which urinary BPA levels were evaluated in relation to occurrence of wheeze. Higher prenatal BPA levels were associated with a lower ocurrence of wheeze at five years of age. In contrast, post-natal BPA levels in the children indicated that higher BPA levels were associated with an increase in wheeze and asthma.

Conclusion

BPA is able to elicit skin sensitization in humans, probably as a result of it being a weak sensitiser. Studies on a possible relationship between prenatal and/or postnatal BPA exposure and allergic responses are not consistent. Other studies on immunotoxic responses are insufficient to draw final conclusions: although effects on the immune system are suggested, there is uncertainty on the immunotoxicity of BPA. In view of the suggested effects of BPA on the immune system, further investigation into potential immunotoxicity of BPA are warranted.

3.9.6. Cardiovascular effects

None of the large-scale experimental animal studies (90 days, 2 years carcinogenicity study) suggest effects on cardiovascular function (see Risk Assessment Report EC ECB 2003, 2008). In conclusion, the toxicological data do not indicate a clear effect of BPA on cardiovascular function.

3.9.7. Metabolic disorders

Summary of previous opinions

In the EU-RAR of 2003, updated in 2010 (ECB 2003, EC 2010a,b), metabolic effects of BPA were not mentioned. Whereas EFSA did not give reports addressing the effects of BPA on the metabolism of experimental animals in the 2006 Opinion (EFSA 2006), in the 2010 EFSA Opinion (EFSA 2010) publications were cited with effects of BPA on insulin secretion in mice (Ropero et al., 2008) and increased adipogenesis in the female offspring of rats exposed prenatally to BPA (mean oral dose 70 µg/kg b.w./day) (Somm et al., 2009) and aggravated insulin resistance in mice during pregnancy at s.c. doses of 10 or 100 µg/kg/d (Alonso-Magdalena et al., 2010). The study of Ryan et al. (2010) was cited showing no indications of increased susceptibility to induced obesity by high fat diet and of glucose intolerance in adult mice exposed prenatally to BPA (0.25 µg/kg b.w./day orally). The NTP-CEHR monograph (US NTP 2008) reviewed the study of Alonso-Magdalena et al. (2006) and the study of Miyakawa et al. (2007). Because of the limited data, the NTP-CEHR monograph did not make a firm conclusion concerning metabolic effects of BPA. In the FAO-WHO opinion (2011) the experts expressed their view that the data of Miyakawa et al. (2007), Somm et al. (2009), Alonso-Magdalena et al. (2010) and Ryan et al. (2010) warranted further assessment of the potential effects of BPA on adiposity, glucose or insulin regulation, lipids and other endpoints related to diabetes or metabolic syndrome.

The 2011 ANSES report reviewed the studies also considered by FAO-WHO and as well as a study by Rubin *et al.* (2001). This study showed obesity in the offspring of Sprague-

Dawley female exposed via drinking water, at approximately 0.1 mg or 1.2 mg from GD6 throughout the period of lactation. According to the ANSES report, effects of BPA on lipogenesis in experimental animals were proven (including adipocyte hypertrophy, predisposition to obesity, elevated cholesterol levels and triglyceride levels and over-expression of lipogenic proteins) following pre-and perinatal exposure in adults. The ANSES risk assessment report (2013) confirmed this view and stated that the increase in body weight in experimental animal studies, together with increases in plasma lipids (such as cholesterol and triglycerides) and lipogenesis, are critical effects. According to ANSES (2013), the Miyakawa *et al.* (2007) study in ICR mice is considered the pivotal study for risk assessment, and a LOAEL of 0.26 mg/kg b.w./day was derived based on an increase in body weight and an increase in cholesterolemia in females.

Since the EFSA opinion of 2010, the WHO Expert meeting of 2010 and the ANSES report of 2011, several additional experimental studies have reported metabolic effects of BPA (including effects on body weight/obesity, lipogenesis or adipogenesis) and/or effects related to glucose or insulin regulation. Studies published over the last 5 years include Miyawaki *et al.*, 2007; Somm *et al.*, 2009; Alonso-Magdalena *et al.*, 2010; Ryan *et al.*, 2010 and Wei *et al.*, 2011. Findings from these studies include reports of glucose intolerance and hyperinsulinaemia in the 6-month-old male offspring of OF-1 mice treated from GD9 to GD16 with BPA at 10 or 100 µg/kg b.w. per day. In the study by Alonso-Magdalena *et al.* (2010), the administration was by subcutaneous injection of 10 µg/kg b.w./day or 100 µg/kg b.w./day. Importantly, the s.c. administration has a systemic availability of 100%, whereas the systemic availability of an oral dose is 2% (Doerge *et al.*, 2012).

Thus, 10 µg/kg b.w./ day and 100 µg/kg b.w./day s.c. corresponds to 500 µg/kg b.w./day and 5000 µg/kg b.w./day by the oral route. The endpoints measured were manifold and the dose-response relationship was not monotonic for all of them. Somm *et al.* (2009) observed adipocyte hypertrophy and increased mass of parametrial white adipose and brown adipose tissue on postnatal day (PND) 21 in female offspring of Sprague-Dawley rats who were orally treated with BPA approximately 70 µg/kg b.w. per day in drinking-water from GD 6 to PND 21. Furthermore, increased cholesterol on PND 31 was observed in female offspring of ICR mice orally treated with BPA (in drinking-water) at approximately 260 or 2600 µg/kg b.w. per day. Exposure was from GD 10 to weaning via the dam and then after weaning with the same drinking water treatment as the dam (Miyawaki *et al.*, 2007). In the most recent study of Wei at al. (2011), doses of 50µg/kg, 250µg/kg and 1250 µg/kg were given by oral gavage throughout gestation and lactation.

Effects were increased body weight, elevated serum insulin and impaired glucose tolerance in adult offspring. It was striking that effects were only observed at the 50 µg/kg/d dose and only in male offspring. Effects in males were accelerated and more severe when offspring were fed a high-fat-diet. In this group, severe metabolic syndrome, dyslipidaemia, hyperleptinaemia, hyperglycaemia, hyerinsulinaemia and glucose intolerance were observed. In contrast, Ryan *et al.* (2010) observed changes in body weight and size in mice which are not longer apparent when the animals reach adulthood. The study results taken together are inconsistent: the effects were seen only in females (Somm *et al.*, 2009), but also in males only (Wei *et al.*, 2011). Also the doses where effects have been observed are at variance in the studies and contradictory results have been observed.

The animal studies have shown an increase (Miyawaki *et al.*, 2007; Rubin *et al.*, 2001; Somm *et al.*, 2009, Wei et al., 2011, Mackay et al., 2013), a decrease (Honma *et al.*, 2002; Nagel *et al.*, 1997), or no effect on body weight (Ryan *et al.*, 2010, Marmugi et al., 2012, Mackay et al., 2013, Anderson et al., 2013) after early life exposure to BPA and the effect can occur in both or only one sex (Somm *et al.*, 2009; Alonso-Magdalena *et al.*, 2010, Mackay et al., 2013). The discrepancy among these animal studies may arise

from variety of experimental conditions, such as dosing regimens, animal species and strains, and timing of evaluation of effects.

In vivo studies studies involving prenatal exposure

Xu *et al.* (2011) suggested that an increased preference of adult rats for a sweet taste, potentially resulting in obesity, could be linked to prenatal exposure to BPA. Female Sprague Dawley rats were exposed to BPA in drinking water at doses of 0.01, 0.1 and 1.0 mg/L from G11 to lactation day 21. A significant sex difference in preference for a sweet taste was evident in both BPA-treated and non-BPA-treated offspring, with all females including controls showing a preference for saccharin-containing drinking water compared with plain water. There was no evidence of a treatment-related effect. However, male offspring showed an increased preference for 0.25% (but not for 0.5%) saccharin, and for 15% sucrose, compared with male controls. The preference for 15% sucrose was reversed in BPA-treated females compared with controls, implying the feminization of males and masculinization of females. Male offspring from dams receiving 0.1 mg/L BPA who were administered 15% sucrose in their drinking water postnatally also showed increased body weight gain, a higher percentage of body fat and higher tail blood pressure compared to the control group. The inconsistency in the response to saccharin (preference for 0.25% but not for 0.5% saccharin) is noted, interpretation of the saccharin preference results was difficult. In addition, there is no explanation why only the middle dose of BPA pups for the sucrose preference test was chosen. These drawbacks limit the conclusions that can be drawn from the study.

Wei *et al.* (2011) administered doses of 0, 50, 250 or 1250 μg BPA/kg b.w. per day orally by gavage in corn oil to pregnant Wistar rats from GD0 to PND2. The offspring (n=16 per group, 2 from each of 8 litters) were maintained on either a normal or a high fat diet for 16 weeks, with monitoring of body weight and blood parameters (triglycerides, cholesterol, low- and high-density lipoprotein) and periodic glucose tolerance and insulin tolerance tests throughout the experimental period: morphology and function of the pancreas was assessed at termination at week 27. The authors present only results for the 50 μg/kg b.w./day dose in their paper, which limits the interpretation of the data. Offspring exposed prenatally to 50 μg BPA/kg b.w. per day and maintained on a normal diet showed increased weight gain from week 17 (females) or week 19 (males), and serum insulin levels were higher at week 15 for males and at week 26 for females. Effects were more evident in animals fed a high fat diet. No effects of BPA were observed at the two higher doses (250 or 1250 μg BPA/kg b.w. per day). Serum leptin was elevated in 50 ug BPA/kg b.w. BPA-treated animals compared with controls at week 26; the animals also had a higher body fat percentage and showed hypertrophy of adipocytes. Mitochondrial structure and insulin granule characteristics in pancreatic β-cells were altered by BPA at 50 ug/kg b.w. per day and mRNA expression of islet-associated transcription factors were reduced compared to controls. This investigation was carried out in the 50 μg/kg b.w./day group only.

In the study of MacKay *et al.* (2013), CD mice were exposed from GD 1 until PND21 to diets containing 0, 1 or 20 μg BPA/kg, estimated to be equivalent to an average of 0.19 and 3.49 μg/kg b.w. per day prenatally and 0.36 and 7.2 μg/kg b.w. per day of BPA postnatally. Offspring were weaned initially onto a normal diet, then as adults exposed to either a normal or high-fat diet (HFD). Female offspring at the higher BPA dose level and fed a high fat diet showed increased body weight gain as adults compared with controls and the DES positive control, and also ate more. They had increased adiposity and leptin concentrations with reduced propio-melanocortin mRNA expression in the arcuate nucleus and estrogen receptor α expression patterns similar to those seen in males, which the authors considered to be suggestive of a masculinising effect of BPA. Male offspring showed no similar BPA-linked effect on body weight gain; however, males at both levels of BPA showed a dose-related increase in weight in the retroperitoneal and

intrascapular brown adipose fat pads compared with control and DES-exposed mice, and similar effects were seen in female offspring at the higher dose but not at the lower level of BPA. The extent of the effects was small. Males exposed to the high dose of BPA showed impaired glucose tolerance on both diets.

Anderson *et al.* (2013) exposed mice starting at two weeks before mating until the end of lactation (PND 21) to 0, 50 ng, 50 µg or 50 mg of BPA/kg of diet corresponding to 0, 10.75 ng, 10.75 µg, and 10.75 mg/kg b.w./day. A subset of animals, 1 male and 1 female/litter, was followed until 10 months of age on standard diet or diets containing BPA at the same levels as administered to the dams. The authors found increased energy expenditure as evidenced by increased oxygen consumption and carbon dioxide production in all BPA-treated animals. Notably, however, that the dose-response relationship was inconsistent. Spontaneous activity was increased, but only in females. Food consumption in females was reduced to a statistically significant extent but without a clear dose-response, whereas in males the reduction of food intake was not statistically significant. Body weight and body fat was not statistically different from control in either sex and glucose tolerance and insulin release were also unchanged.

A further study was published by Angle *et al.* (2013). Pregnant CD-1 mice BPA were given BPA at oral doses ranging from 5 - 50,000 µg/kg/day from GD 8 until GD 18. In the male offspring from the animals, a large number of endpoints were measured (body weight; gonadal and renal fat pad weight; adipocyte number and adipocyte volume; food (metabolic energy) consumption; glucose and insulin tolerance tests; serum hormones (such as insulin, leptin, adiponectin) at different time points and statistical assessment explored several models. Most of the endpoints showed non-monotonic changes which are difficult to assess. No pathophysiolocal model can be derived from the data and no biological explanation can be given at present.

In vivo studies in adult mice and rats

D'Cruz *et al.* (2012) dosed male rats (n = 6 per group) with BPA (0.005, 0.5, 50 and 500 µg/kg b.w. per day orally) for 45 days. 17-β-estradiol (50 µg/kg b.w./day) was used as a positive control. A whole array of endpoints was measured: Plasma glucose, plasma insulin and enzymes involved in glucose metabolism were investigated. In addition, testicular levels of insulin, insulin signalling molecules, glucose transporter-2, antioxidant enzymes and steroidogenesis were also evaluated. Levels of plasma glucose and insulin were significantly increased even at the lowest level of BPA exposure of 5 ng/kg b.w. per day, whereas the testicular glucose level significantly decreased, again at all dose levels. Levels of insulin and various insulin signalling molecules were significantly decreased in testis by BPA in a dose-related manner even at the lowest dose of 5 ng/kg b.w./day. A significant decrease in testicular superoxide dismutase and catalase activities was measured following BPA exposure at all doses, and lipid peroxidation was increased, together with decreases in testicular marker proteins and key enzymes of steroidogenesis. There was evidence of testicular damage as evidenced by loss of germ cells and decrease in the spermatids in rats treated with 500 µg BPA, as well as in the positive control. The authors concluded that low doses of BPA affect insulin signalling and glucose, possibly leading to impairment of testicular function.

Batista *et al.* (2012) administered subcutaneously a dose of 100 µg BPA/kg b.w./day divided into two subcutaneous injections to 3-month old Swiss albino OF1 mice for 8 days. Whole body energy homeostasis and responses of insulin sensitive peripheral tissues were assessed. Higher plasma insulin concentrations in the fed state and increased glucose-stimulated insulin secretion in isolated pancreatic islet of Langerhans were shown for BPA treated animals, in addition to changes in insulin signaling. Glucose tolerance testing showed that BPA-treated mice were insulin resistant. Whole-body energy homeostasis, as assessed by food intake, locomotor behaviour and nocturnal energy expenditure was reduced. In contrast, the respiratory exchange ratio was unchanged. Hence, the findings of this study were contradictory in themselves.

Male CD1 mice were dosed with 0, 0.05, 0.5, 5 or 50 mg/kg BPA in the diet, estimated by the authors to be equivalent to 0, 5, 50, 500 and 5000 µg/kg b.w./day for 28 days (Marmugi *et al.*, 2012) After the treatment period, measurements were taken for body weight gain, liver weight and weight of perigonadic white adipose tissue (pWAT), hepatic lipid content and fatty acid composition, plasma levels of insulin, triglycerides, glucose, total cholesterol, and low- or high-density lipoprotein (LDL, HDL) cholesterol. In addition, the effects of BPA on gene expression in the liver were assessed using microarrays. No effect was seen on body weight gain and relative liver weight, but pWAT weight was significantly increased in mice receiving 50 µg/kg b.w./day (but not at higher dose levels). Plasma insulin levels were significantly increased following exposure to 5, 50, and 500 µg BPA/kg b.w./day: the lowest dose produced the greatest effect. Plasma glucose and tota, LDL- or HDL-cholesterol were not different from control. Mice exposed to 500 µg BPA/kg b.w./day showed a significant increase in plasma triglyceride levels. The effects were confirmed in a further study in C57BL/6J mice given the same BPA doses, although the changes were generally of a smaller magnitude. The results of the microarray assays showed a stimulatory effect of BPA on expression of key enzymes involved in lipogenesis, cholesterol biosynthesis and, to a lesser extent, enzymes involved in glucose metabolism. A stronger response was seen in the liver of mice dosed with 50 µg/kg b.w./ day than those dosed with 5000 µg/kg b.w./day. Hence, a non-monotonic dose-response seems to exist.

Bodin *et al.* (2013) investigated possible effects of BPA on the development of type 1 diabetes (T1DM). They gave 0, 1 and 100 mg/L BPA in the drinking water (corresponding to intakes of 0, 150 or 15000 µg/kg b.w./day) to non-obese pre-diabetic (NOD) mice. The prevalence and extent of insulitis did not differ between groups at week 7. At week 12, markedly increased effects were seen compared with controls only in female mice and only exposed to 1 mg/l BPA in drinking water, being less severe in the dosing group of 100 mg/l. Prevalence and extent of insulitis was decreased in male mice exposed to BPA compared with controls. Serum glucose levels were increased in the 1 mg/ml BPA group, indicating an accelerated onset of T1DM, but this was not seen in the animals exposed to 100 mg/l BPA. Insulin levels did not differ significantly between the groups. Serum levels of T4, cytokines and autoantibodies did not differ between the groups.

In the studies of the Jayashree group (Jayashree *et al.*, 2013; Indumathi *et al.*, 2013), BPA (0, 20 or 200 mg/kg b.w./day orally) was administered for 30 days to adult male Wistar rats. The effects of BPA were investigated on insulin signal transduction and glucose oxidation in skeletal muscle and liver. After 30 days of treatment serum insulin was significantly increased by BPA in a dose-related manner whereas glucose oxidation was reduced at both dose levels in liver and in skeletal muscle, and glycogen content of the liver was also reduced. In skeletal muscle, treatment with BPA at both 20 and 200 mg/kg b.w., significantly decreased the levels of insulin receptor, of protein kinase B and of glucose transporter-4 levels (both plasma membrane and cytosolic fraction). However, the mRNA levels for these proteins were unchanged. In the liver, both mRNA and protein levels were significantly decreased at the highest BPA-exposed group.

Female F-344 rats were dosed with 0.025, 0.25 or 2.5 mg BPA/L in drinking water from five to 15 weeks of age (Rönn *et al.*, 2013). The drinking water contained 5% fructose (n= 12 per group) and BPA intake, calculated by the authors, was between 4.6 (week 9) and 5.6 (week 2) µg/kg b.w./day at the lowest dose, between 46.3 (week 6) and 61.6 (week 3) µg/kg b.w./day at the mid dose and 400.3 (week 9) and 595.3 (week 2) µg/kg b.w./day at the highest dose. The authors measured adipose tissue volume and liver fat content by magnetic resonance imaging (MRI). Further endpoints were cholesterol, triglycerides and apolipoprotein A-1a, changes in body weight and weight of the perirenal fat pad. There were no significant effects of BPA exposure on body weight or weight of the perirenal fat pad, and no differences were seen in total or visceral adipose tissue volumes between the groups. However, liver fat content was significantly higher in rats receiving the two higher doses of BPA compared with controls ($p = 0.04$). BPA exposure also increased the apolipoprotein A-I levels in plasma ($p < 0.0001$), a favourable

modification in the lipid profile because apolipoprotein A-I is the main component of the high density lipoprotein (HDL).

Conclusion on metabolic effects of BPA in animals

Studies in rats and mice which were prenatally and postnatally exposed to BPA indicate that an effect on metabolic parameters was elicited, measured by effects on glucose or insulin or lipogenesis. The animal studies have shown an increase (Miyawaki *et al.*, 2007; Rubin *et al.*, 2001; Somm *et al.*, 2009, Wei et al., 2011, Mackay et al., 2013), a decrease (Honma *et al.*, 2002; Nagel *et al.*, 1997), or no effect on body weight (Ryan *et al.*, 2010, Marmugi et al., 2012, Mackay et al., 2013, Anderson et al., 2013) after early life exposure to BPA and the effect can occur in both or only one sex (Somm *et al.*, 2009; Alonso-Magdalena *et al.*, 2010, Mackay et al., 2013). The discrepancy among these animal studies probably arise from variation in experimental conditions, such as dosing regimes, animal species and strains, and timing of evaluation of effects. There is no consistent evidence that BPA is obesogenic in adulthood after intrauterine exposure or in longer-term studies. In some of the studies, the authors claim that the findings indicate a non-monotonic dose-response as effects seen at a lower dose were not observed in higher doses. However, effects were seen only at one dose level and not in at least two dose levels with a decline at higher dose levels, thus corroborating the existence of non-monotonicity.

Epidemiological studies

Some epidemiological studies also reported an association in adults of actual BPA levels in urine with cardiometabolic disorders (Lang *et al.*, 2008, Melzer *et al.*, 2010), or with obesity in children and adolescents (Trasande *et al.*, 2012), but the validity of these results from the cross-sectional NHANES data were afterwards disputed (LaKind *et al.*, 2012). Other epidemiological studies indicate inconsistent findings for an association between prenatal BPA exposure and a low birth weight, a predictor of obesity later in life (Lee *et al.*, 2008, Miao *et al.*, 2011, Padmanabham *et al.*, 2008, Wolff *et al.*, 2008). Hence, no firm conclusion can be drawn at present.

In vitro studies

Several *in vitro* studies were published after 2010 reporting the effects of BPA on insulin secretion, mitochondrial morphology and function and gene expression in different cell types.

In the presence of low glucose concentrations, 3 mM BPA had no effect (Soriano *et al.*, 2012) Insulin secretion stimulated by high glucose levels (8-17.7 M) was further increased by treatment with BPA concentrations (10-10 M, 10-9 M and 2x10-9 M) in mouse and human islets, in primary rat islet cells and in a rat insulinoma cell line (Soriano *et al.*, 2012; Song *et al.*, 2012; Lin *et al.*, 2013). These concentrations correspond to 22,8 ng/L, 228 ng/L and 465 ng/L, far above concentrations calculated in humans after oral exposure to BPA which are in the 10-12 M range (Teeguarden *et al.*, 2013). The results from Soriano *et al.* (2012) suggest that BPA´s effects on insulin secretion, KATP channel activity and glucose-induced [Ca2+] oscillations in pancreatic □-cells are linked to the presence of ERβ.

BPA-induced toxicity and apoptosis was associated with changes in the morphology and the membrane potential of mitochondria of pancreatic cells induced by BPA concentrations (10-12 M - 10-8 M) in the human hepatic cell line HepG2 (Huc *et al.*, 2012).

In isolated human adipose tissue taken from children, BPA at 10-8 M increased the expression of 11β-hydroxysteroid-dehydrogenase, PPARα and lipoprotein lipase and, in addition, induced lipid droplet accumulation in adipocytes at terminal differentiation (Wang *et al.*, 2013). Using transfection gene reporter assays with monkey kidney cells, Sheng and co-workers (2012) observed a BPA (10-9 M to 10-7 M)-induced suppression

of thyroid hormone receptor transcription through a non-genomic pathway. However, the relevance of the model for the *in vivo* situation is unclear.

Conclusions on metabolic activity

Several studies in laboratory animals published in the last 5 years and more recently directly address the issue of whether developmental exposure to BPA can affect adiposity, glucose or insulin regulation, lipid profiles or other endpoints related to diabetes or metabolic syndrome. Effects were increased body weight, elevated serum insulin, and impaired glucose tolerance in adult offspring. There is inconsistency in the results, as in some studies, effects were observed only in male offspring while in other studies, effects were only observed in female offspring. In addition, effects were not consistently induced by similar dosages and studies showing a lack of increase or even a decrease in body weight were reported. BPA exposure also affected various metabolic endpoints in adult rodents exposed to BPA. In some of the studies, the findings have been taken as evidence for a non-monotonic dose-response because effects seen at a lower dose were not observed at higher doses. However, effects were sometimes seen only at one dose level. There are no studies which demonstrate effects of different effect size at two consecutive dose levels and/or no or a reduced effect at a higher dose, thus corroborating the existence of non-monotonicity. In addition, epidemiological studies do not show unequivocal convincing evidence for metabolic effects of BPA. In view of the inconsistent results, no firm conclusions can be drawn at present, but the data warrant further assessment of the potential effects of BPA on adiposity, glucose or insulin regulation, lipids and other endpoints related to diabetes or metabolic syndrome.

In vitro BPA was found to increase cellular insulin secretion only at high glucose levels, whereas at low glucose levels, a high BPA exposure was needed. In addition, lipid droplet accumulation was induced by BPA in human adipose tissues at relative high concentrations that could also induce cytotoxicity and apoptosis of the cells.

3.9.8. Reproductive and developmental toxicity

A host of studies is available on the effects of BPA on reproduction and prenatal development some of which were performed according to internationally agreed guidelines and in compliance with GLP. A wealth of *in vitro* results and studies on non-intact animals (such as ovariectomized rodents) is available, but their value for risk assessment is questionable. There are also uncertainties as to reproducibility of several individual studies. The text below focuses on GLP-compliant guideline-based generation and developmental studies. These studies were conducted in rats and mice. Chapin *et al.* (2008) performed a comprehensive review of all available animal studies. A selection of studies critical for the determination of reproductive and developmental toxicity and the derivation of NOAELs is revisited below.

Morrissey *et al.* (1987) studied the developmental toxicity of BPA in CD rats (0, 160, 320, or 640 mg/kg b.w./day) and CD-1 mice (0, 500, 750, 1000, or 1250 mg/kg b.w./day) dosed daily by gastric intubation on Gestational Days 6 through 15. In rats, maternal weight gains during gestation, weight gain corrected for gravid uterine weight and weight gain during treatment were significantly reduced at all BPA doses. Gravid uterine weight and average foetal body weight per litter were not affected by BPA. No increase in percentage resorptions per litter or percentage foetuses malformed per litter was detected. In mice, maternal mortality occurred at all BPA doses, reaching 18% at the high dose, which also produced a significant decrease in maternal body weight gain during gestation and treatment. Weight gain corrected for gravid uterine weight was not affected by BPA. Reductions in gravid uterine weight and average foetal body weight were observed with the 1250 mg/kg dose of BPA. Relative maternal liver weight was increased at all doses of BPA. There was a significant increase in the percentage of resorptions per litter with 1250 mg BPA/kg b.w./day. Malformation incidence was not altered by BPA. Thus, BPA treatment at maternally toxic dose levels during

organogenesis produced foetal toxicity in mice but not in rats and did not alter foetal morphologic development in either species.

Ema *et al.* (2001) conducted a two-generation study in Crj:CD(SD) IGS rats using doses of 0.2, 2, 20 and 200 µg/kg b.w./day by oral gavage, starting exposure 10 and 2 weeks before mating in males and females, respectively. No compound-related clinical signs or effects on body weight or food consumption were observed in any generation. There were no compound-related changes in surface righting reflex, negative geotaxis reflex, mid-air righting reflex, pinna detachment, incisor eruption, eye opening, testes descent, preputial separation, or vaginal opening in F1 and F2 generations, or behavior in the open field or water filled multiple T-maze tests in the F1 generation. No test compound-related changes in estrous cyclicity, copulation index, fertility index, number of implantations, gestation length, litter size, pup weight, pup sex ratio, pup viability, or other functional reproductive measures were noted in any generation. A few significant changes in the anogenital distance (AGD) per cube root of body weight ratio were found at 0.2 and 20 µg/kg b.w. in F1 males, at 2, 20, and 200 µg/kg b.w. in F1 females and at 20 and 200 µg/kg b.w. in F2 females. However, the changes in the AGD were consistently small (within 5% of control values), and no continuous changes in the AGD or AGD/cube root of body weight ratio were detected. There were no compound-related changes in epididymal sperm counts or motility in F0 and F1 males. No compound-related necropsy findings or effects on organ weight, including the reproductive organs, were found in any generation. Histopathologic examinations revealed no evidence of compound-related changes in any organs including the reproductive organs of both sexes. The data indicate that oral doses of BPA of between 0.2 and 200 µg/kg b.w. over two generations did not cause significant compound-related changes in reproductive or developmental parameters in rats.

Tyl *et al.* (2002) performed a three-generation study in Sprague Dawley rats using a wide range of dietary doses of 0.001 to 500 mg/kg b.w./day. Adult systemic toxicity occurred at 50 mg/kg b.w./day including reduced body and organ weight gain, with hepatic pathology at 500 mg/kg/day. Ovarian weights as well as total pups and live pups/litter on postnatal day (PND) 0 were decreased at 500 mg/kg b.w./day, which exceeded the adult maximum tolerated dose (MTD). Mating, fertility, gestational indices; ovarian primordial follicle counts; estrous cyclicity; precoital interval; gestational length; offspring sex ratios; postnatal survival; nipple/areolae retention in preweanling males; epididymal sperm number, motility, morphology; daily sperm production (DSP), and efficiency of DSP were all unaffected. Adult systemic toxicity no observed adverse effect level (NOAEL) was 5 mg/kg b.w./day; reproductive and postnatal NOAEL was 50 mg/kg/day. There were no treatment-related effects in the low-dose region (0.001-5 mg/kg b.w./day) on any parameters and no evidence of nonmonotonic dose-response curves across generations for either sex. The authors conclude that BPA should not be considered a selective reproductive toxicant, based on the results of this study.

Kobayashi *et al.* (2002) exposed Crj:CD(SD) IGS rat dams to 0, 4 or 40 mg/kg/day from GD6 through PND20. There were no significant changes in body weight, liver weight, kidneys weight, testes weight, AGD, the ratio of AGD to body weight, or the ratio of AGD to the cube root of body weight in BPA exposed pups compared to the vehicle-exposed control. The authors conclude that prenatal and postnatal exposure (indirect exposure) to BPA (4–40 mg/kg b.w./day, GD 6–PND 20) does not affect somatic growth or AGD of F1 generation of male and female rats.

Tyl *et al.* (2008) conducted a two-generation study of BPA in CD-1 mice. F0 and F1 mice (28 sex/group/generation) were fed diets containing BPA (99.70–99.76% purity) at 0.018, 0.18, 1.8, 30, 300, or 3500 ppm. Target intakes were 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg b.w./day, respectively. A concurrent positive control group of dietary 17beta-estradiol (0.5 ppm; 28 per sex) confirmed the sensitivity of CD-1 mice to an endogenous estrogen. There were no clinical signs of toxicity or treatment-related deaths in F0 or F1 males or females. Increases in weight, both absolute and relative to body or brain, of the kidney and liver were consistently observed in F0 and F1 adults (males and females) at

the highest dose level. At 50 mg/kg b.w./day hepatocyte hypertrophy was noted and at 600 mg/kg b.w./day body and organ weight effects were also observed. Incidence of minimal to mild hepatocyte centrilobular hypertrophy was increased in both generations at 300 and/or 3500ppm. Renal nephropathy incidence was increased in F0 males and in F1 males and females of the 3500 ppm group.

There were no BPA-related effects on adult mating, fertility or gestational indices, ovarian primordial follicle counts, estrous cyclicity, precoital interval, offspring sex ratios or postnatal survival, sperm parameters or reproductive organ weights or histopathology (including the testes and prostate). BPA exposure had no effect on numbers of implantation sites or resorptions or on mating, fertility, or gestational indices in F0 or F1 mice. Gestational length was increased in F0 and F1 females from the 3500 ppm group; the study authors stated the effect was of unknown biological significance. Epididymal sperm concentration was decreased in F0 males of the 3500 ppm group, but no effect was observed in F1 parental or retained males. There was no effect on daily sperm production, efficiency of daily sperm production, or sperm motility or morphology in either generation. The study authors did not consider the decrease in sperm concentration in F0 animals to be treatment-related, based on lack of consistency between generations, no detectable effect on any other andrological endpoint, and no detectable effect on fertility. Estrous cyclicity and numbers of ovarian primordial follicle counts were not affected by BPA exposure in F0 or F1 females. The only gross observation in reproductive organs was a slightly increased incidence of gross ovarian cysts in F0 females from the 3500 ppm group. The incidence of paraovarian cysts was increased in F0 and F1 females from the 3500 ppm group. In F1 pups from the 3500 ppm group, body weights were reduced during PND 7, 14, and 21 in F1 females and both sexes combined and on PND 7 and 21 in F1 males. Preputial separation (absolute age and adjusted for body weight on day of acquisition) was delayed in F1 males of the 3500 ppm group. The study authors reported no gross findings in F1 or F2 weanlings. The incidence of undescended bilateral testes was increased in F1 and F2 weanling males of the 3500 ppm group. The incidence of hepatic cytoplasm alteration (clear hepatocellular cytoplasm, slightly more basophilic cytoplasm, and/or minute vacuoles) was apparently increased in F1 males from the 300 and 3500ppm groups and F1 females and F2 males from the 3500ppm group. The incidence of seminiferous tubule hypoplasia was increased in F1 and F2 weanlings from the 3500ppm group. The study authors identified bisphenol A NOELs of 30ppm (5 mg/kg b.w./day) for systemic effects, 300ppm (50 mg/kg b.w./day) for developmental toxicity, and 300ppm (50 mg/kg b.w./day) for reproductive toxicity. Therefore, BPA was not considered a selective reproductive or developmental toxicant in mice.

Kobayashi *et al.* (2010) exposed C57BL/6J mice to dietary levels of 0.33,3.3 or 33 ppm BPA. from GD6 through PND22, and the weanlings (F(1) and F(2)) from each F(0) and F(1) dam group, respectively, dosing was continued until sacrifice. There were no treatment-related changes in body weight, body weight gain, food consumption, gestation length, or the number of live births on postnatal day 1 in F(0) dams between the control group and BPA groups. Sex ratio and viability were similar in all F(1) pups. No treatment-related changes were observed in body weight, food consumption, developmental parameters, anogenital distance, or weight of any of the organs (liver, kidney, heart, spleen, thymus, testis, ovary, or uterus) in F(1) and F(2) adults in either sex. The epididymis weight was slightly higher with 0.33 and 3.3 ppm in F(1) males, but this slight increase was neither dose dependent nor seen across generations. There were no treatment-related effects of BPA on cauda epididymal sperm count or sperm motility in F(1) or F(2) males. These findings indicate that dietary exposure to bisphenol A between 0.33 and 33 ppm does not adversely affect reproduction or development as assessed in two generations of mice.

Stump *et al.* (2010) conducted a developmental neurotoxicity study in Crl:CD(SD) rats with dietary concentrations of 0.15, 1.5, 75, 750, and 2250 ppm daily from gestation day 0 through lactation day 21. F(1) offspring were evaluated using the following tests: detailed clinical observations (PNDs 4, 11, 21, 35, 45, and 60), auditory startle (PNDs 20

and 60), motor activity (PNDs 13, 17, 21, and 61), learning and memory using the Biel water maze (PNDs 22 and 62), and brain and nervous system neuropathology and brain morphometry (PNDs 21 and 72). For F(1) offspring, there were no treatment-related neurobehavioral effects, nor was there evidence of neuropathology or effects on brain morphometry. Based on maternal and offspring body weight reductions, the no-observed-adverse-effect level (NOAEL) for systemic toxicity was 75 ppm (5.85 and 13.1 mg/kg/day during gestation and lactation, respectively), with no treatment-related effects at lower doses or nonmonotonic dose-responses observed for any parameter. There was no evidence that BPA is a developmental neurotoxicant in rats, and the NOAEL for developmental neurotoxicity was 2250 ppm, the highest dose tested (164 and 410 mg/kg/day during gestation and lactation, respectively).

In a preliminary study with two doses (10 µg/kg b.w./day and 5 mg/kg b.w./day) in male rats, oral BPA by gavage for 6 days induced for both doses a decrease in sperm production (Tiwari and Vanage 2013).

Conclusions

Overall, female reproductive toxicity occurred with an overall NOAEL of 50mg/kg b.w./day and a LOAEL of 500 mg/kg b.w./day, derived from the Tyl *et al.* (2002) multigeneration study. However, at the LOAEL for female reproductive effects, significant body weight reduction and hepatic toxicity occurred. As to developmental toxicity, BPA does not cause malformations or birth defects in rats or mice at levels up to the highest doses evaluated: 640 mg/kg b.w./day (rats) and 1250 mg/kg b.w./day (mice) (Morrissey *et al.*, 1987). BPA does not alter male or female fertility after gestational exposure up to doses of 500 mg/kg b.w./day in the rat (Tyl *et al.*, 2002) and 600 mg/kg b.w./day in the mouse (Tyl *et al.*, 2008), being highest dose levels evaluated. BPA does not permanently affect prostate weight at doses up to 475 mg/kg b.w./day in adult rats or 600 mg/kg b.w./day in mice. BPA does change the age of puberty in male or female rats at high doses (ca. 500 mg/kg b.w./day). Neurodevelopmental toxicity was not observed at the highest dose tested (164/410 mg/kg b.w./day, Stump *et al.*, 2010).

On the basis of the above studies, it can be concluded that BPA is essentially not a specific reproductive or developmental toxicant. General toxicity effects such as body and organ weights and liver histopathology and nephropathy often occur simultaneously with reproductive or developmental effects, which are only observed at high dosages.

3.9.9. Conclusions on toxicity

General toxicity

The key studies for setting a NOAEL were considered to be the multi-generation studies in rats and mice performed by Tyl *et al.* investigating the general and reproductive toxicity of BPA over a wide range of oral doses (Tyl *et al.,* 2002, 2008). Based on these studies, a NOAEL of 5 mg/kg b.w./day was derived based on liver toxicity, whereas the NOAEL for reproductive toxicity was set at 50 mg/kg b.w./day. In these multi-generation studies in rats and mice, alterations in kidney weight were also observed at the low administered doses, but renal weight increased in mice and decreased in rats, although the relative kidney weight also increased in rats. For the higher doses investigated in both mice and rats, histopathological alterations were noted in the kidneys (Tyl *et al*., 2002, 2008). Using the results of Tyl *et al.* (2002, 2008), EFSA (EFSA 2014) has recently applied a BMD approach (EFSA 2009, 2011) for further refining the risk assessment with kidney weight as the critical endpoint.

With the BMD approach a confidence interval ($BMDL_{10}$ – $BMDU_{10}$) of the BMD_{10}, being a dose resulting in a 10% deviation from vehicle treated control animals, was obtained.

$BMDL_{10}$ indicates the lower part (5%) of the 90% confidence interval and $BMDU_{10}$ indicates the upper part (95%) of the confidence interval. A $BMDL_{10}$ of 3633 (right kidney) and 3887 (left kidney) µg/kg b.w./day, and a $BMDU_{10}$ of 99220 (right kidney) µg/kg b.w./day and 120100 (left kidney) µg/kg b.w./day was calculated for changes in male mouse kidney weight based on Tyl et al. (2008). For the risk assessment, EFSA used the mean value of the $BMDL_{10}$ results obtained for the left and right kidney resulting in an established $BMDL_{10}$ of 3760 µg/kg b.w./day with kidney weight as the critical endpoint. The $BMDL_{10}$ of 3.76 mg/kg b.w./day derived by EFSA is still derived for a general toxicity endpoint and the result is very close to the previously used NOAEL of 5 mg/kg b.w./day.

For BPA, the two derived values (NOAEL and $BMDL_{10}$) are conceptually different from a toxicokinetic point of view. Indeed, the internal exposure of the organs is different: the hepatic exposure is presystemic, whereas the renal one is systemic. The doses at the site of action (i.e. liver and kidney) differ after the oral route of exposure, because of the biotransformation occurring in the liver resulting in a lower dose of free BPA for kidney exposure. The SCENIHR supports the use of the kidney effects as the PoD and the newly developed t-TDI for the risk assessment of medical devices.

Genotoxicity

BPA was found not to interact with DNA directly as it does not induce gene mutation in bacteria (Masuda *et al*., 2005; Tiwari *et al*., 2012) and micronuclei *in vivo* in rodent erythropoietic cells (Masuda *et al*., 2005; Pacchierotti *et al*., 2008; De Flora *et al*., 2011, Naik *et al*., 2009). There was also no induction of chromosomal aberrations observed in bone marrow cells of mice treated *in vivo* with BPA (Naik *et al*., 2009), however, BPA acts on the mitotic spindle apparatus. The large margin between the dose-levels found negative *in vivo* for induction of aneuploidy in rodent germ cells (Pacchierotti *et al.*, 2008) and for induction of micronuclei in somatic bone marrow cells (Masuda *et al.*, 2005; Pacchierotti *et al.*, 2008; Naik *et al.*, 2009; De Flora *et al.*, 2011) provides adequate reassurance on the lack of aneugenic effects of BPA *in vivo*. So, BPA is not likely to pose a genotoxic hazard to humans.

Carcinogenicity

From all the study results taken together, it can be concluded that in standard carcinogenic testing protocols according to OECD BPA has no carcinogenic activity. In additional multigeneration studies (Tyl *et a*l., 2002; Tyl *et al.*, 2008), no indication of increased cancerogenicity was observed; in particular, preneoplatic lesions of the mammary gland were absent in all offspring. In contrast, three studies from the same group in rats have demonstrated an effect of prenatal BPA exposure on mammary gland development, i.e. ductal hyperplasia, and one study showed some carcinoma development. These studies were performed with very low background estrogen levels in the feed which may have had an effect on the normal development in the controls. A limitation is the lack of a positive control like estradiol for comparing the estrogenic effects induced by BPA. Similar effects were indicated in studies in mice and rhesus monkeys, supporting the observations in rats. Therefore, it cannot be excluded that BPA affects early development of mammary tissue, although their relevance for adversity in humans is not clear. These studies should, therefore, be considered as an indicator for a possible concern.

Studies using s.c. administration of BPA indicated that BPA may have the ability to increase the effects of well-known carcinogens even at very low BPA levels. The studies had limitations which render them unsuitable to assess whether BPA has a carcinogenic potential by prenatal or peri-natal exposure. The main limitation is that in the studies with positive outcome additional treatment with a strong initiating or additional promoting agent(s) has been performed. Furthermore, in most of the studies the statistical analysis does not consider litter effects, and in addition, multiple statistical testing has been performed without proper adjustment to avoid positive results by

chance. Further studies were performed in transgenic animals, the results of which can not be extrapolated directly to the human situation.

The current situation shows no carcinogenic effects in OECD guideline studies. However, studies indicating effects on mammary gland development raise some concern for a possible effect late in life after prenatal exposure.

Reproductive toxicity

Overall, female reproductive toxicity occurred with an overall NOAEL of 50mg/kg b.w./day and a LOAEL of 500 mg/kg b.w./day, derived from the Tyl *et al.* (2002) multigeneration study. However, at the LOAEL for female reproductive effects significant body weight reduction and hepatic toxicity occurred. As to developmental toxicity, Bisphenol A does not cause malformations or birth defects in rats or mice at levels up to the highest doses evaluated: 640 mg/kg b.w./day (rats) and 1250 mg/kg b.w./day (mice) (Morrissey *et al.*, 1987). BPA does not alter male or female fertility after gestational exposure up to doses of 500 mg/kg b.w./day in the rat (Tyl *et al.*, 2002) and 600 mg/kg b.w./day in the mouse (Tyl *et al.*, 2008, highest dose levels evaluated). BPA does not permanently affect prostate weight at doses up to 475 mg/kg b.w./day in adult rats or 600 mg/kg b.w./day in mice. BPA does change the age of puberty in male or female rats at high doses (ca. 500 mg/kg b.w./day). Neurodevelopmental toxicity was not observed at the highest dose tested (164/410 mg/kg b.w./day, Stump *et al.*, 2010).

On the basis of the above studies, it can be concluded that BPA is not a specific reproductive or developmental toxicant. General toxicity effects such as body and organ weights and liver histopathology and nephropathy often occur simultaneously with reproductive or developmental effects, which are only observed at high dosages.

Immunotoxicity

BPA is able to elicit skin sensitization in humans probably because it is a weak sensitiser. Studies on a possible relationship between prenatal and/or postnatal BPA exposure and allergic responses are not consistent. Other studies on immunotoxic responses are insufficient to draw final conclusions.

Metabolic effects

Several studies in laboratory animals published in the last 5 years and more recently directly addressed whether developmental exposure to BPA can affect adiposity, glucose or insulin regulation, lipid profiles or other endpoints related to diabetes or metabolic syndrome. Effects were increased body weight, elevated serum insulin and impaired glucose tolerance in adult offspring. There is inconsistency in the results as effects were observed in some studies only in male offspring while in other studies effects were only observed in female offspring. In addition, effects were not consistently induced by similar dosages. BPA exposure also affected various metabolic endpoint in adult rodents exposed to BPA. In some of the studies, the findings could be taken as evidencing a non-monotonic dose-response as effects were seen at a lower dose which was not observed in higher doses. However, effects were seen only at one dose level. There are no studies which demonstrate effects of different effect size at two dose levels and no or a reduced effect at a higher dose, thus corroborating the existence of non-monotonicity. Additionally, epidemiological studies do not show unequivocally conclusive evidence for metabolic effects of BPA. Although no firm conclusion can be drawn at present, the data warrant further assessment of the potential effects of BPA on adiposity, glucose or insulin regulation, lipids and other endpoints related to diabetes or metabolic syndrome.

In vitro BPA was found to increase cellular insulin secretion only at high glucose levels, whereas at low glucose levels a high BPA exposure was needed. In addition, lipid droplet accumulation was induced by BPA in human adipose tissues at relative high concentrations. These high concentrations could also induce cytotoxicity and apoptosis of the cells.

BPA is a chemical that has been investigated in studies performed according to OECD protocols and compliant to GLP principles. The array of studies performed is such as requested for a high production volume chemical according to the REACH legislation plus one neurotoxicity study as requested for pesticides. In these studies, the lowest NOAEL (5 mg/kg b.w./day orally) was found in a developmental study in rats (Tyl *et al.*, 2002) as an effect on the liver. Centrilobular hepatocyte hypertrophy was also seen in the 3-generation study in mice (Tyl *et al.*, 2008) at an oral dose of 50 mg/kg b.w./day. Other effects in the same study were increased kidney weight and nephropathy. No developmental and no fertility endpoint other than reduced sperm concentration at the highest dose was changed in this study which investigated oral doses of 0.003, 0.03, 0.3, 5, 50, or 600 mg BPA/kg b.w./day. No carcinogenicity was observed in a classical NTP study. The results of gentoxicity testing were grossly negative. Testing for immuntoxicity revealed some effects; however, without indicating that BPA is clearly an immuntoxic substance. New studies with "untypical" endpoints have raised concerns. The endpoints encompass cell proliferation in the male and femal breast after intrauterine and postnatal exposure, anxiety and behavioural endpoints also after intrauterine and postnatal exposure and changes in metabolic endpoints also after intrauterine and postnatal exposure. The doses which elicit those effects are much lower than 5 mg/kg b.w./day and in some studies given only once. At present, it is under discussion whether the observations are real effects and what the meaning of the observations is with regard to the adversity in humans. However, there is the possibility that BPA may have NOAEL in "untypical" endpoints which may be as low as several µg/kg b.w./day orally and even below 1 µg/kg b.w. when given by the subcutaneous route.

Overall conclusions

In conclusion, there are several indications that BPA has biological effects below the current NOAEL of 5 mg/kg b.w./day and the recently established $BMDL_{10}$ of 3.76 mg/kg b.w./day (oral repeated exposure). However, the evidence has been mainly obtained in dedicated studies focussing on specific outcome parameters like adiposity and hormone levels, and not in general toxicity studies. Some of those parameters resulted in contradicting results in various studies (e.g. decrease, no effect or increase in body weight). In addition, dose-response relationships could not be established. There is a possible concern for prenatal BPA exposure and an effect on mammary gland development. In addition, the effects on the metabolism and adiposity needs further investigation in large scale studies with a wide dose range of BPA.

3.10. Epidemiological studies

Studies

There are a limited but increasing number of epidemiological studies that investigated an association between BPA exposure and health outcomes. Most of them use cross-sectional designs which limit their interpretability, especially for outcomes that have long latency periods (e.g. cardiovascular disease, diabetes). Frequently, a single measure of urinary BPA is used to categorize exposure, which given the short half-life of BPA, is another limitation. Fortunately, many of those epidemiological studies utilized the same laboratory at the United States Centers for Disease Control and Prevention for quantification of urinary BPA concentrations (Wolff *et al.*, 2007, 2008b, 2010; Lang *et al.*, 2008; Braun *et al.*, 2009; Mok-Lin *et al.*, 2009; Meeker et al., 2010a; Meeker *et al.*, 2010b; Melzer *et al.*, 2010, 2011, 2012; Mendiola *et al.*, 2010; Clayton *et al.*, 2011; Carwile and Michels 2011; Silver *et al.*, 2011; and Shankar *et al.*, 2012a,b,c). This eliminates one potential source of variability in comparisons across these studies.

We have identified 34 peer-reviewed epidemiological studies that examined associations between BPA exposure and human health outcomes.

Occupational and non-occupational exposure to BPA was investigated in relation with some reproductive outcomes, including serum sex steroid hormone concentrations,

semen quality, oocyte count, recurrent miscarriage, endometriosis, endometrial hyperplasia and cancer, and polycystic ovarian syndrome (Hanaoka *et al.*, 2002; Hiroi *et al.*, 2004; Takeuchi *et al.*, 2004; Sugiura-Ogasawara *et al.*, 2005; Itoh *et al.*, 2007; Cobellis *et al.*, 2009; Mok-Lin *et al.*, 2009; Galloway *et al.*, 2010; Li *et al.*, 2010a,b,c; Meeker et al., 2010a; Meeker *et al.*, 2010b; Mendiola *et al.*, 2010; Bloom *et al.*, 2011 and Hao *et al.*, 2011).

Interestingly, three epidemiological studies investigated the association of urinary BPA concentrations with semen quality. Men who were partners of pregnant women in the USA (Mendiola *et al.*, 2010), male partners in infertile couples who were patients in an infertility clinic (Meeker *et al.*, 2010) and workers with occupational exposure to BPA in China (Li *et al.*, 2010c). Although all three studies, with a relatively modest sample size (ranging from 190 to 302 men), reported associations of increased urinary BPA concentration and one or more measures of reduced semen quality, this was statistically significant in only one of them. Limitations include their cross-sectional designs and incomplete assessment of occupational co-exposure in one of the three studies.

The evidence for an association of BPA with altered age of pubertal onset in girls was investigated in two epidemiological studies, a cross-sectional study in New York City, New York, USA (Wolff *et al.*, 2008a) and in a prospective cohort study of 1151 female children from Cincinnati, Ohio, San Francisco, California, and New York City, New York, USA (Wolff *et al.*, 2008a, 2010). Results are limited and inconsistent. Research needs to include large prospective studies on the association of BPA with pubertal development. A research gap is the lack of studies on male pubertal development in relation to BPA exposure.

Only one study examined the association between BPA exposure and cancer (Yang *et al.*, 2009). In a breast cancer case–control study of 152 women, serum samples were used to quantify BPA exposure. Although cases had higher median serum BPA concentrations than controls, differences were not statistically different. Because BPA has a short half-life, current serum BPA concentrations may not be relevant to the etiological window of development for breast cancer, which is years to decades before clinical recognition.

It is difficult to draw any conclusions from two published epidemiological studies that have examined the association of BPA with perinatal outcomes because contradictory results. Six published epidemiological studies have examined the association of BPA with perinatal outcomes, body mass index and neurodevelopment. Two studies examining perinatal outcomes relied on a single serum measure of BPA at birth (Padmanabhan *et al.*, 2008; Chou *et al.*, 2011); others relied on a single urinary BPA concentration during pregnancy (Wolff *et al.*, 2008b; Philippat *et al.*, 2012), another estimated BPA exposure by personal air sampling measurements and exposure history. Wolff and colleagues (Wolff *et al.*, 2008b) found that urinary BPA concentrations in pregnant women in the third trimester were associated with modest elevations (although not statistically significant) in birth weight. Philipat *et al.* (2012) found that head circumference increased in association with BPA increased concentrations. There is only one cross-sectional pilot study examining the association of urinary BPA concentration with body mass index (Wolff *et al.*, 2007). Chou *et al.* (2011) who measured BPA in maternal blood and umbilical cord blood found elevated prenatal BPA exposure increased the risk of low birth weight, smaller size for gestational age and adverse actions of adipokines in neonates, especially in male infants. Although these results could suggest evidence that maternal exposure may be correlated with adverse birth outcomes, most of these studies are cross-sectional, relied on a single measure of exposure or did not adequately adjust for important potential confounders.

Only one prospective cohort study had examined the relationship of serial BPA urinary concentrations in pregnant women with neurobehavioural outcomes (Braun *et al.*, 2009; 2011). This study found a positive association between urinary BPA concentrations measured during pregnancy and externalizing behaviours (i.e. aggression and hyperactivity). In the follow-up of 3-year-old children, the investigators found gestational

BPA exposure to affect behavioural and emotional regulation domains at 3 years of age, especially among girls.

Six cross-sectional analyses of data from the United States National Health and Nutrition Examination Survey (NHANES) reported associations of BPA exposure with self-reported diagnosis of pre-existing cardiovascular disease, hypertension, obesity and diabetes (Lang *et al.*, 2008; Melzer *et al.*, 2010, 2011; Carwile *et al.*, 2011: Silver *et al.*, 2011; Shankar *et al.*, 2012a,b,c). Two other studies in US (Metlzer *et al.*, 2012) and China (Wang *et al.*, 2012) reported an association between BPA exposure and coronary disease at the time of diagnosis and obesity and insuline resistance, respectively. In addition, a study found associations between urine BPA and immune function and allergy (Clayton *et al.*, 2011). These cross-sectional analyses have several important weaknesses that limit their interpretation. A major limitation is the use of a single spot urine sample that reflects recent BPA exposure only (past several hours) and may not adequately measure BPA exposure during the relevant etiological window for cardiovascular disease and diabetes, which might be years or decades earlier.

Strong conclusions based on cross-sectional analyses are not possible. Prospective studies with serial exposures to BPA assessed during etiologically relevant windows, years before development of disease, are needed. Additional studies, especially of a longitudinal design with repeated BPA measurements, are needed to further elucidate these associations.

Casas and co-workers (Casas *et al.*, 2013) pointed out that although there is little published data in mother-child cohorts, many measurements are ongoing (Kasper-Sonnenberg *et al.*, 2012) and they recommend that cohorts start working towards combined and comparison studies. Recommendations for further data collection on BPA include: i) a better evaluation of exposure to BPA in children; ii) repeated measurements of BPA; iii) validation and harmonisation of questionnaires, and iv) detection methods and measurement of BPA. Furthermore, evaluation of mother child cohorts is needed to gain insight in the relation between BPA exposure and effects in children.

Recently, Maserejian and coworkers (2012) investigated whether greater exposure to dental composites is associated with psychosocial problems in children. They performed an analysis of treatment-level data from the New England Children's Amalgam Trial, a 2-group randomized safety trial comparing amalgam with the treatment plan of bisphenol A-glycidyl methacrylate (bisGMA)-based composite and urethane dimethacrylate-based polyacid-modified composite (compomer), among 534 children aged 6 to 10 years at baseline. They found that children with higher cumulative exposure to bisGMA-based composite had poorer follow-up scores on 3 of 4 BASC-SR global scales: Emotional Symptoms, Clinical Maladjustment, and Personal Adjustment. Associations were stronger with posterior-occlusal (chewing) surfaces, where degradation of composite was more likely. They concluded that greater exposure to bisGMA-based dental composite restorations as potential source of BPA was associated with impaired psychosocial function in children, whereas no adverse psychosocial outcomes were observed with either urethane dimethacrylate-based compomer or amalgam treatment levels.

Conclusions

Conclusions valid for the risk assessment based on cross-sectional analyses are not possible. In addition, many of the available cross-sectional analyses have several important weaknesses that limit their interpretation. The major limitation is the use of a single spot urine sample that reflects recent BPA exposure only (past several hours) and may not adequately measure BPA exposure during the relevant etiological window for cardiovascular disease and diabetes, which might be years or decades earlier. However, also based on the outcome of animal studies, some effects like neurobehavioral outcomes, even when observed in one study, need further investigation for confirmation of negation of the observed effects.

There is a need for further clarification and interpretation of the relationship between BPA exposure and adverse health effects. Prospective studies with serial exposures to BPA

assessed during etiologically relevant windows, years before development of disease, and thus prolonged follow up periods are needed.

Specific recommendations for use of existing data include i) the development of conversion models for the different media used for measurement of BPA, ii) and inter-laboratory comparisons and calibrations.

Recommendations for further data collection on BPA include: i) a better evaluation of exposure to BPA in children; ii) repeated measurements of BPA; iii) validation and harmonisation of questionnaires; and iv) detection methods. Additionally, evaluation of mother child cohorts is needed to gain insight in the relation between BPA exposure and effects in children.

3.11. Alternatives to BPA currently use

Some chemicals, similar to BPA, are considered to be able to partially replace BPA in the industrial applications, and, therefore, used in the manufacture of resins and plastics. An example is bisphenol-S [bis(4-hydroxyphenyl)sulfone, (BPS)] whose two phenolic rings are joined together with sulfur. The material containing BPS is of interest in the preparation of high temperature resistant thermosetting thermoplastic polymers (Spitsbergen *et al.*, 1971). Epoxy resin based on BPS has the advantage of resistance to deformation by heat and thermal stability. Such improved epoxy resins have other advantages in briefer gel periods and more rapid development of mechanical properties in cured systems, better resistance to organic solvent attack, increased dimensional stability and better wetting of glass reinforcement. BPS is commonly used as a monomer in the production of epoxy resins (Rwei *et al.*, 2003), cyclic carbonates (Kim *et al.*, 2001), and sulphonated poly(ether ketone ether sulphone) (Changkhamchom and Sirivat 2010). It is also an important chemical additive in pesticides, dyestuffs, colour-fast agents, leather tanning agents, dye dispersants, and fiber improvers.

To date, BPS replaced BPA as a developer in dyes for thermal paper in Japan (Watanabe *et al.*, 2004) and China (Liu, 2005). Therefore, the production and demand for BPS increases year by year. Resultantly, BPS could be a widespread environmental pollutant in future as well as BPA. Even more so, it has been found that BPS is much less biodegradable than BPA (Ike *et al.*, 2006). In this study of eight bisphenol compounds, BPS was the most persistent. Recently, BPS was also detected in canned food (Viñas *et al.*, 2010). Although it has not been studied as much as BPA, preliminary studies show that it shares hormone-mimicking properties as well (Hashimoto *et al.*, 2001; Chen *et al.*, 2002; Kuruto-Niwa *et al.*, 2005; Kitamura *et al.*, 2005). However, studies on the endocrine disrupting properties of BPS have focused on its interaction with human estrogen receptor alpha (hERa). Recently, interactions of BPS with other nuclear receptors were also reported (Molina-Molina et al., 2013). Several alternatives for BPA were investigated including BPS, BPF and halogenated BPA derivatives tertrachlorobisphenol A (TCBPA) and tetrabromobisphenol A (TBBPA) for their *in vitro* interaction with several nuclear receptors (e.g. estrogen receptor α, estrogen receptor β, androgen receptor, and pregnane X receptor). Although some differences were noted, all nuclear receptors investigated were activated by one or more of the bisphenol alternatives. BPS, BPF and BPA effectively activated both estrogen receptors, whereas BPA, TCBPA and TBBPA were pregnane X receptor agonists. Relative to BPA. the alternatives BPS and BPF and TCBPA and TBBPA showed reduced endocrine activity in the *in vitro* assays used.

Bisphenol-F, [bis(4-hydroxyphenyl)methane, (BPF)], which has no substituent at the bridging carbon, has a broad range of industrial applications. The BPF monomer is polymerized to prepare the epoxy resins and polycarbonates used to manufacture lacquers and varnishes, coatings, adhesives plastics, and other products (Jana *et al.*, 2005). BPF was detected in environmental samples (Fromme *et al.*, 2002; Stachel *et al.*, 2003) and several reports have confirmed its estrogenic effect using various *in vivo* (Yamasaki *et al.*, 2002) and *in vitro* assays (Hashimoto and Nakamura, 2000; Hashimoto

et al., 2001; Cabaton *et al.*, 2009). Moreover, anti-androgenic activity of BPF has also been observed in several human recombinant cell lines carrying hAR (Satoh *et al.*, 2004; Cabaton *et al.*, 2009).

Like BPA, both BPS and BPF are also an emerging group of environmental contaminants (Fukazawa *et al.*, 2001; de Wit *et al.*, 2010) and interact with and disrupt thyroid hormone receptor signaling (Kitamura *et al.*, 2002). In addition, TBBPA and TCBPA are potent peroxisome proliferator-activated receptor gamma (PPARγ) agonists (Riu *et al.*, 2011).

3.12. Recommendations for research

General

For several endpoints like effects on metabolism, neurobehavioral effects, and effects on mammary gland development, the reported studies indicate that BPA exposure may affect these endpoints in animals. However, the overall evidence is equivocal and sometimes based on limited studies. Especially low dose potentially non-monotonic effects are very difficult to identify as such and need further confirmation. Therefore, using a weight of evidence approach, as formulated in the conclusions of the various sections, it was concluded that the evidence was not substantiating the suggested adverse health effects. Therefore, recommendations were formulated for further clarification of these issues.

Exposure

This is the area in which more and better information is needed, regarding the composition and the release of BPA from medical devices in the actual use conditions. For sterilization of medical devices, it is known that steam sterilization may result in release of BPA from PC medical devices. Whether ethylene oxide (EtO) sterilization induces release of BPA from PC or PSU medical devices is yet unknown. Research into the use and consequences of EtO sterilization with regard to BPA release is also recommended.

Hazard

Currently a series of studies is being performed with US public grants investigating some of the issues dealing with BPA exposure (Shelnutt *et al.*, 2013). A two year chronic study design is used conducted under GLP guidelines as the core study. Various investigators will receive at various times designated animals or animal tissues for testing and analysis. This approach is chosen to bridge between regulatory GLP studies and more dedicated experimental scientific studies. This programme addresses some of the concerns and controversies regarding BPA adverse effects, in particular non-monotonicity of dose-response and possible low dose effects. Some of the presented research recommendations will probably be overcome by this extensive US research program (Shelnutt *et al.,* 2013). Even when the data analysis will need time, the results from such a coordinated research effort would provide good evidence for the remaining data gaps.

There are several indications that BPA does have biological effects below the current NOAEL of 5 mg/kg b.w./day and the recently calculated $BMDL_{10}$ of 3.76 mg/kg b.w./day (oral repeated exposure). However, the evidence has been mainly obtained in dedicated studies focussing on specific outcome parameters like adiposity and hormone levels, and not in general toxicity studies. Some of those parameters resulted in contradicting results in various studies like a decrease, no increase or increase in weight. Additionally, dose-response relationships could not be established. There is a possible concern for prenatal BPA exposure and an effect on mammary gland development. In addition, the effect on the metabolism and adiposity needs further investigations in large scale studies with a wide dose range of BPA.

Although effects on the immune system are suggested the data are insufficient to draw final conclusions on the immunotoxicity of BPA. In view of these suggested effects of BPA

on the immune system further investigations to the potential immunotoxicity of BPA are warranted.

Some of the above mentioned effects warrant further investigation for confirmation or negation. The currently performed study by the FDA's National Center for Toxicological Research (NCTR) in the USA with animals under a strict exposure regimen and the studies to be conducted on these animals by various research groups may clarify some of these controversial issues and give indications for specific further research priorities.

There is a need for further clarification and interpretation of the relationship between BPA exposure and adverse health effects in man. Prospective epidemiological studies are needed with serial exposures to BPA assessed continually during etiologically relevant windows, years before development of disease, and thus prolonged follow up periods are needed.

Recommendations for further prospective epidemiological data collection include: i) a better evaluation of exposure to BPA in children; ii) repeated measurements of BPA; iii) validation and harmonisation of questionnaires. Additionally, evaluation of mother child cohorts is needed to gain insight in the relation between BPA exposure and effects in children. It has been suggested (Casas *et al.*, 2013) that for mother-child cohorts, many measurements are already ongoing.

4.OPINION

Background

Currently many scientific discussions are ongoing on the possible adverse effects of BPA. The exposure of the population is mainly via food as a result of the use of BPA in food packaging. More specifically safety concerns have been expressed for vulnerable groups such as infants, pregnant and breast-feeding women exposed to BPA through other products. Medical devices are a specific product category in which BPA is often found. Examples include implants, catheters, and most dental devices. Some BPA-containing medical devices may have direct and/or indirect contact with the patients (e.g. hemodialyzer apparatus, filters, bypasses, tubing, pumps, instruments, surgical equipment, blood pathway circuits and respiratory tubing circuits). These products are used on all types of patients (e.g. adults, children). Therefore, an Opinon was asked from the SCENIHR regarding the use of BPA in medical devices. This Opinion describes the risk assessment of exposure to BPA via medical devices, for which the exposure routes are not limited to oral applications.

What is BPA?

Bisphenol A [Bis(4-hydroxyphenyl)propane], BPA is a high-production volume industrial chemical. BPA is a key building block of polycarbonate plastic and a precursor for the manufacturing of monomers of epoxy resins. Polycarbonate is used in a wide variety of products including medical devices, for its balance of toughness, dimensional stability optical clarity, high heat resistance and electrical resistance. In addition to polycarbonate medical devices, various dental materials are fabricated from monomers such as bisphenol A glycidyl methacrylate (Bis-GMA) and bisphenol A dimethacrylate (Bis-DMA) derived from BPA. BPA-resins are also used in inks and adhesives. In addition to BPA itself, polymers produced using BPA like polysulfone (PSU) that are used in medical devices are also considered because they can release BPA. For example, the BPA derived polymer polysulfone (PSU) is used as membrane in hemolysis dialyzers.

Previous risk assessments

In the existing evaluations, the following conclusions have been drawn for oral route of exposure to BPA:

- No Observed Adverse Effect Level (NOAEL) of 5 mg/kg b.w./day in rats and mice
- Tolerable Daily Intake (TDI) of 50 µg/kg b.w.
- developmental toxic effects only observed at doses with severe maternal toxicity in rats and mice
- an overall NOAEL for reproductive toxicity of 50 mg/kg b.w./day in rats and mice
- in terms of toxicokinetics there is a difference between rats and humans (the latter presenting a shorter half-life) as well as between the oral and the parenteral route of exposure.
- due to the first pass effect, after oral uptake, the systemic exposure to free BPA is a small fraction of the external dose in all species.
- there are still unresolved issues in the risk assessment of BPA after oral uptake.

More recently EFSA established a temporary (t)-TDI of 5 µg/kg b.w./day for oral exposure to BPA (EFSA 2014). For the establishment of this t-TDI, a bench mark dose (BMD) evaluation was used with the $BMDL_{10}$ of 3.76 mg/kg b.w./day for kidney alterations as the critical effect.

The main focus of these evaluations was on the oral route of exposure. Especially for medical devices manufactured from polycarbonate plastics, other exposure routes such as subcutaneous and intravenous (e.g. during heamodialysis) are important.

General exposure

The human population is exposed to BPA through the diet, while air, dust, water,and skin contact primarily through thermal paper are other possible sources of exposure. Bisphenol A in food and beverages accounts for the majority of daily human exposure. BPA exposure results from either the release of non-polymerized monomers or from the slow decay of polymer bonds in polycarbonate leading to monomer release into proximal foods and liquids. A number of studies in various countries have indicated that the vast majority of the population (91–99%) does have detectable levels of BPA-conjugates in urine. The measured BPA levels reflect the recent exposure of the past several hours before the sample collection as there is a rapid conjugation and short elimination half-time of a few hours of BPA in blood.

Notably, regarding BPA determination, the analytical method used to detect both the parent compound and its metabolites is crucial especially at the low levels expected in biological samples. The sampling and analytical methods used, therefore, can represent a relevant source of differences among available studies.

In urine, BPA is present mainly in its conjugated form. Urinary biomonitoring data provide information on the internal dose, which is the result of total BPA exposure, independently from the sources. Therefore, biomonitoring data in urine account not only for dietary exposure, but also for non-food sources (e.g. medical devices, dust, thermal and other kind of papers). Since BPA urinary excretion is almost complete within 24 hours after exposure and due to the less invasively sampling, urine is the matrix of choice for assessing daily exposure to BPA in humans. Blood concentrations of total BPA (free plus conjugates) determined at one time point are not representative of an average exposure, because it is strongly dependent on the time of blood sampling with respect to the exposure time.

On the basis of available biomonitoring and exposure data, it was recently concluded that the exposure to BPA from non-food sources that some authors hypothesized as being potentially relevant sources, is generally lower than that from exposure from food by at least one order of magnitude for most studied subgroups. Dietary exposure was indeed estimated to contribute for more than 90% to the overall BPA-exposure for non-occupationally exposed individuals (Geens *et al.*, 2012; EFSA, 2013; ANSES, 2013) and

exposure through dust ingestion, dental surgery and dermal absorption from thermal paper accounted for less than 5%. However, the contribution due to medical devices has never been taken into account.

EFSA (2013) estimated the BPA exposure due to dietary uptake of BPA. The highest exposure for children older than 6 months and up to 10 years of age was 857 ng/kg b.w./day and for infants days 1-5 after birth 495 ng/kg b.w./day. For adults a highest exposure of 388 ng/kg b.w./day was estimated.

Skin absorption

The available data indicate that at the estimated exposures, BPA penetrated rapidly into the skin by passive transfer at a percentage between 10% and up to 47% of the applied dose. The possible skin metabolism is controversial, since contrasting results were reported; therefore, excluding a pre-systemic BPA clearance due to dermal biotranformation, as a worst-case a systemic bioavailability equal to 30 % of the applied dermal dose can be used for risk assessment purposes.

SC exposure/administration

Subcutaneous administration resulted in much higher free BPA levels compared to the oral administration. However, differences in circulating free BPA after subcutaneous and oral administration can disappear within two hours as demonstrated in a study in rats.

IV administration

After intravenous administration a rapid distribution to organs and rapid clearance from blood was described. Initial high serum levels of free BPA may partly distribute and sequester into fatty tissues. BPA was eliminated from adipose tissue at a rate similar to that for BPA conjugates in the whole organism indicating the non persistent nature of BPA. Free BPA was no longer detectable in serum of mice at 8h after i.v. administration

Inhalation

No information was available for inhalation exposure that might occur after intubation for inhalation support in intensive care units. However, in vitro studies showed a lack of conjugation of BPA by lung cells.

For risk assessment purposes, the bioavailability of free BPA is crucial as this is the active compound. However, data on both free and conjugated BPA are required to assess the exposure and fate of BPA.

Exposure from medical devices

Medical devices based on polycarbonate and polysulfone, due to their chemistry, can contain BPA residues whereas others like PVC may or may not contain BPA residues depending on their production method. In addition, some other BPA-derivatives (such as epoxy resins) are used specifically in dental materials. The major factor influencing the residual amount of BPA levels is the employment of incorrect operating conditions during the processing step. Moreover, breakdown or hydrolysis of the polycarbonate polymer after manufacturing can occur, thus giving rise to the free monomer from the polymer available for exposure. In polycarbonate articles used for food contact, the residual content is usually less than 10 µg/g of polycarbonate (ECB, 2003).

Exposure can be estimated by either measuring the BPA content of the medical devices or by extraction assays for potential release. Extraction of BPA was much more prominent in aqueous ethanol (17.2% v/v) and bovine serum (mimicking human serum) than in water. For PC casings of hemodialyzers and hollow fibres used in hemodialyzers extracted amounts of BPA were ranging from 0.2 – 12.2 mg/kg. Under simulated use conditions release in bovine serum was up to 2090 ng/dialyzer, and in aqueous ethanol (17.2% v/v) up to 4299 ng/dialyzer. For dental materials the leakage is limited to resins composed of Bis-DMA (Bisphenol A dimethacrylate) which has an ester linkage that can

be hydrolysed to BPA, whereas the ether linkage in Bis-GMA (Bisphenol A glycidyl methacrylate) was found to be stable.

Little information concerning BPA exposure resulting from the use of medical devices,is available. For the placing of dental composite resin restorations, measurements have shown the release of BPA mainly during the few hours directly after application. Values measured were up to 30 µg/mL saliva, and 931 µg in total saliva volume produced. Calculations based on the maximum values of BPA found in fissure sealants and in composite materials, in combinations with the actual amount of material used in clinical practice and a median 4-year life-time of a composite restoration, suggest a maximum exposure of 0.06 µg BPA/day from fissure sealants, and a maximum exposure of 0.36 µg BPA/day from composite restorations. Contact with dental materials gave an estimated short-term (<24 hours) exposure of 140 to 200 ng/kg body weight per day for children and adults, respectively. These BPA releases contribute to the oral exposure to BPA and are included in the biomonitoring studies to the total exposure to BPA as mentioned above.

Measurements in dialysis patients found BPA values up to 6.6 ng/mL blood. In prematurely born infants undergoing intensive therapeutic medical interventions for BPA geometric mean urinary concentration of 30.3 µg/L was observed with the highest value measured 946 µg/L, which was about ten times higher than that among children 6-11 years old. More than 90% of the BPA detected in the urine of the prematurely born infants was in its conjugated (e.g. glucuronide, sulfate) form.

Taking into account the many possible sources of exposure of patients during hospital care and the scarcity of information related to release of BPA from medical devices, six critical exposure scenarios were evaluated to estimate potential exposure to BPA from medical devices (Table 6). The highest exposures estimated occurred during prolonged medical procedures in infants (685 ng/kg body weight per day) and exposure of prematurely born infants in NICU (3000 ng/kg body weight per day).

The use of medical devices consisting of BPA containing PVC for treatment of adults (1000 ng/kg b.w./day), and treatment of prematurely born infants (7000 ng/kg body weight per day, 7 µg/kg body weight per day) also results in potential high exposures. Short-term exposure via medical devices consisting of BPA containing PVC might even be higher (adults up to 5000 ng/kg b.w./day, infants up to 12000 ng/kg b.w./day). However, it is worth noting that exposure to BPA via BPA-containing PVC has been estimated based on extrapolation from data on phthalate leakage from PVC and are, therefore, affected by a high degree of uncertainties.

Some of the estimated BPA exposures due to medical devices are in the same range as exposure via the food (EFSA 2013). EFSA estimated the exposure to be highest for infants and toddlers among the population older than 6 months, with the estimated average of 375 ng/kg b.w./day and a highest estimated dietary exposure of 857 ng/kg b.w./day. The modeled dietary exposure for teenagers, adults and the elderly ranged from 116 to 159 ng/kg body weight per day for average exposure, with a high exposure ranging from 341 to 388 ng/kg body weight per day.

Table 6. BPA exposure from medical devices as estimated for various use scenarios.

	BPA exposure estimation in ng/kg b.w./day			
Exposure scenario	Prematurely born infant	infant	child	adult
External contact with a MD containing BPA (short-term)	1			0.08
Contact with dental material				
(short-term)	na	na		200
(long-term)	na	na	2	6
Contact with orthodontic equipment				
(short-term)			140	140
(medium-term)			13.5	7.5
(long-term)			12	6
Contact with an implant				
(medium-term)			11	6
(long-term)			0.8	0.4
Hemodialysis (long-term)				57
Prolonged surgical procedures (short-term)		685	114	57
Prolonged exposure to different sources of BPA in intensive care units (medium-term)	3000			
Breast pump and collection vessel made of PC (medium-term)		134		
Uses of PVC				
(short-term)	12000			5000
(long-term)	7000			1000

BPA metabolism and toxicokinetics in humans

The unchanged parent BPA is the toxic species, which is readily detoxified in the body. The major BPA metabolite in human is BPA-glucuronide, which is quantified in plasma and rapidly excreted in the urine; BPA-sulphate was also detected after oral exposure as a minor urinary metabolite. After oral exposure there is a very fast first pass effect in the liver that results in very small amounts of unchanged parent BPA, up to 9.5% of the oral administered dose is recovered as non-conjugated BPA in human urine. In humans, a polymorphism exists for the conjugation of BPA. However, the polymorphism was found to results in a limited level of variability of BPA conjugation as indicated by biomonitoring studies. Therefore, it can be considered that the default value used to account for kinetic interindividual variability within the general population (IPCS, 2006), can cover

differences due to polymorphically expressed enzyme activity involved in BPA metabolism.

In humans, both low and high single oral doses of BPA are well absorbed (>90%). This is a conservative estimate for neonates. The half-life of BPA in humans is very short, ranging from 1 to 3.5 hours, and because of high first-pass metabolism in the liver the systemic availability is expected to be low. Based on the analysis of oral versus intravenous toxicokinetic data, the oral systemic bioavailability of unconjugated BPA is 2.8%, 0.2%, 0.9% and less than 1% in rats, mice, monkeys, and dogs respectively. The systemic availability of unconjugated BPA in humans has not been evaluated experimentally, however, controlled biomonitoring studies indicated that internal exposure in humans to unconjugated BPA is very low (1-10%).

Studies on toxicokinetics of BPA available to date in animals have demonstrated a significantly lower internal exposure to free BPA after oral intake as compared to parenteral exposure. This is essentially due to the highly efficient pre-systemic conjugation to glucuronides and sulfate which occurs mainly in the liver and partially in the gut after oral administration independently of the species. Thus, the internal exposure to free BPA after oral intake is lower as compared to dermal or parenteral exposure, although also for these latter routes of exposure the metablization in the liver quickly diminishes free circulationg free BPA.

After dermal exposure, the absorption fraction can be considered approximately 25-30% of the applied dose as a worst case assumption, which is directly systemically bioavailable.

For all the parenteral routes of exposure (including i.v., i.p., or subcutaneous) the chemical is 100% systemically bioavailable: however, the clearance of free BPA from the circulation appeared to be quite fast, as indicated by controlled studies in non human primates showing a half-life in blood of 0.66 h with >50% of circulating BPA already conjugated 5 min after i.v. injection.

The available modeled data, obtained after oral exposure, also point out that newborns and babies up to 6 months constitute a potentially susceptible subpopulation due to immature BPA metabolism but that the default factor which is used to account for the toxicokinetic variability in the general population seems to be large enough to cover the variability in the newborn population exposed via the oral route. Analogously, inter-individual differences in the expression of the isoenzyme mainly responsible for BPA glucuronidation are within a factor of 4, again covered by the usual the default factor, at the estimated dietary exposures.

Pharmacokinetics in animals

There are major differences in BPA metabolism and disposition between rodents (enterohepatic recirculation and extensive fecal excretion of unconjugated BPA) and primates (extensive urinary excretion of conjugated BPA), that directly affects BPA half-life. Indeed, rodents (mice and rats) show a prolonged clearance of BPA due to the existence of the so-called entero-hepatic recirculation. After uptake from the GI-tract in rats there is a high degree of conjugation of BPA in the liver. However, BPA is excreted from the liver via bile into the GI-tract where it can be cleaved again resulting in free BPA that can be recirculated or excreted via the feces. So, there may be a higher exposure to free BPA especially in neonatal mice and rats after a specific oral dose when compared to humans. In addition, there is another major interspecies difference related to neonatal development in the Phase II metabolism of BPA, which is strongly age-dependent in rodents. Thus, there may be a higher exposure to free BPA especially in neonatal mice and rats after a specific oral dose when compared to humans. Indeed in primates the degree of conjugation was not affected by developmental age, and consequently there was no significant age-related change in internal exposure metrics for free BPA in primates. It may be considered that for neonatal effects, studies in mice and rats may over-predict adverse outcomes in humans (Shelnutt *et al.*, 2013).

Toxicity of BPA

Several repeated dose toxicity studies have been performed in mainly rodents. BPA was found to be of low acute toxicity, and the lowest NOAEL for subchronic oral exposure currently available is approximately 5 mg/kg b.w./day, based on effects on the liver as target organ, as identified in several studies. The next lowest NOAEL for oral exposure is 50 mg/kg b.w./day, based on effects on the kidney.

Using the same studies from which the NOAEL of 5 µg/kg b.w./day was derived recently with the bench mark dose (BMD) approach a $BMDL_{10}$ of 3.76 mg/kg b.w./day was calculated (EFSA 2014). The $BMDL_{10}$ represents the lower level of the confidence interval of the effect resulting in a 10% deviation from vehicle treated control animals. The critical endpoint for this $BMDL_{10}$ was alteration in kidney weight. The two Points of Departure (PoD), i.e. dose for liver toxicity and dose for kidney toxicity, are quantitatively very similar, although conceptually different from a toxicokinetic point of view. Indeed, the internal exposure of the organs is different: the hepatic exposure is presystemic, whereas the renal exposure is systemic. The doses at the site of action (i.e. liver and kidney) differ after the oral route of exposure because of the biotransformation occurring in the liver resulting in a lower dose of free BPA for kidney exposure. The SCHENIR supports the use of the newly developed t-TDI for the risk assessment of medical devices.

BPA is not a mutagen in *in vitro* test systems, nor does it induce cell transformation. BPA was shown to affect chromosomal structure in dividing cells in *in vitro* studies, but evidence for this effect in *in vivo* studies is inconsistent and inconclusive. In addition, BPA was found to be genotoxic in *in vitro* micronucleus assay. These findings were not confirmed by *in vivo* studies. Therefore, BPA is not likely to pose a genotoxic hazard to humans.

In standard carcinogenic testing protocols according to OECD BPA has no carcinogenic activity. In addition, in multigeneration studies no indication of increased cancerogenicity was observed. Studies using subcutaneous administration of BPA indicated that BPA may have the ability to increase the effects of well-known carcinogens even at very low BPA levels. The studies had limitations which render them unsuitable to assess whether BPA itself has a carcinogenic potential by prenatal or peri-natal exposure.

Prenatal exposure to BPA by subcutaneous injection and oral administration at doses between 2.5-1000 µg/kg b.w. induced mammary gland alterations including cell proliferation, some described as pre-neoplastic and neoplastic lesions, in the offspring. Results observed in rhesus monkey also indicated alterations of glandular tissue in the mammary gland. However, the variability in mammary gland development in this species makes it difficult to draw clear conclusions for the risk assessment. In contrast, similar alterations were not observed in the pups of the mouse multigeneration studies with continuous oral BPA exposure.

In summary, at present there are no indications for carcinogenic effects of BPA in OECD guideline studies, but some effects in the mammary gland have been reported. The observed effects on mammary gland development do need further investigation as the biological significance of such alterations as well as the relevance for humans are at the moment not known.

Studies on anxiety (rodent and non-human primate) have a behavioural endpoint which is highly depending on study design, including testing apparatus, inclusion of only one sex, age at examination. There is uncertainty with regard to the interpretation of the data. Recent data confirm previous data on the sex-dimorphic effects of BPA on social behaviour. However, it is uncertain whether elimination of sexual dimorphism could be considered adverse for humans. Other effects described in the recent studies may indicate that the effects observed with BPA on hypothalamic organization involves

mechanisms different from its estrogenic action because they are very different from those of oestradiol which was used as positive control.

BPA is able to elicit skin sensitization in humans, because it is a weak sensitiser. Studies on a possible relationship between prenatal and/or postnatal BPA exposure and allergic responses are not consistent. Although effects on the immune system are suggested the data are insufficient to draw final conclusions on the immunotoxicity of BPA. In view of these suggested effects of BPA on the immune system further investigation to determine potential immunotoxicity of BPA is warranted.

The toxicological data do not indicate a clear effect of BPA on cardiovascular function.

Several published studies in laboratory animals have directly addressed the issue of whether developmental exposure to BPA can affect adiposity, glucose or insulin regulation, lipid profiles or other endpoints related to diabetes or metabolic syndrome. Animal studies, however, have shown contrasting results (e.g. increase, decrease and no effect on body weight). The discrepancy among the various animal studies may arise from variation in experimental conditions, such as dosing regimen, animal species and strains, and timing of evaluation of effects. A number of studies in prenatally- and postnatally exposed rats and mice suggest that BPA exposure has an effect on metabolic function. In some of the studies, the findings have been claimed as evidence of a non-monotonic dose-response as effects were seen at a lower dose which was not observed in higher doses. However, effects were only seen at one dose level. There are no studies which demonstrate effects of different effect size at two dose levels and no or a reduced effect at a higher dose thus corroborating the existence of non-monotonicity. There is, however, no convincing evidence that BPA is obesogenic later in life after intrauterine exposure or in longer-term studies. Therefore, regarding a metabolic effect of BPA. No clear conclusions can be drawn at the moment due to a lack of consistent evidence. Inconsistent results were also obtained in epidemiological studies. Therefore, this issue still warrants further investigation.

A large number of studies is available on the effects of BPA on reproduction and prenatal development some of which performed according to internationally agreed guidelines and in compliance with GLP principles. A wealth of *in vitro* results and studies on non-intact animals (such as ovariectomized rodents) is available, but their value for risk assessment is questionable. There are also uncertainties as to reproducibility of several individual studies. These studies were conducted in rats and mice. Overall, female reproductive toxicity after oral exposure occurred with an overall NOAEL of 50 mg/kg b.w./day and a LOAEL of 500 mg/kg b.w./day, derived from the Tyl *et al.* (2002) multigeneration study. However, at the LOAEL for female reproductive effects, significant body weight reduction and hepatic toxicity occurred. As to developmental toxicity, Bisphenol A does not cause malformations or birth defects in rats or mice at levels up to the highest doses evaluated: 640 mg/kg b.w./day (rats) and 1250 mg/kg b.w./day (mice). BPA does not alter male or female fertility after gestational exposure up to doses of 500 mg/kg b.w./day in the rat (Tyl *et al.*, 2002) and 600 mg/kg b.w./day in the mouse (Tyl *et al.*, 2008, highest dose levels evaluated). BPA does not permanently affect prostate weight at doses up to 475 mg/kg b.w./day in adult rats or 600 mg/kg b.w./day in mice. BPA does change the age of puberty in male or female rats at high doses (ca. 500 mg/kg b.w./day). Neurodevelopmental toxicity was not observed at the highest dose tested (164/410 mg/kg b.w./day, Stump *et al.*, 2010).

On the basis of the above studies, it can be concluded that BPA is not a specific reproductive or developmental toxicant. General toxicity effects such as body and organ weights and liver histopathology and nephropathy often occur simultaneously with reproductive or developmental effects, which are only observed at high dosages.

A number of studies in prenatally- and postnatally exposed rats and mice indicate that BPA exposure has an effect on metabolic function as evidenced by effects on glucose or insulin in regulation or lipogenesis, and may affect bodyweight gain at least in short-term studies. There is, however, no convincing evidence that BPA is obesogenic later in life

after intrauterine exposure or in longer-term studies. In some of the studies the findings have been considered as evidencing a non-monotonic dose-response as effects were seen at a lower dose which was not observed in higher doses. However, again effects were seen only at one dose level. There are no studies which demonstrate effects of different effect size at two dose levels and no or a reduced effect at a higher dose thus corroborating the existence of non-monotonicity. Additionally, epidemiological studies do not show unequivocal convincing evidence for metabolic effects of BPA.

A limited, but increasing number of epidemiological studies investigated an association between BPA exposure and health outcomes, including altered behavior after dental BPA exposure. Most of them use cross-sectional designs not suitable for identifying a cause-effect relationship, limiting their interpretability, especially for outcomes that have long latency periods (e.g. cardiovascular disease, diabetes). In addition, many of these cross-sectional analyses have several important weaknesses that limit their interpretation. The major limitation is the use of a single spot urine sample that reflects recent BPA exposure only (past several hours) and may not adequately measure BPA exposure during the relevant etiological window for cardiovascular disease and diabetes, which might be years or decades earlier. Conclusions based on cross-sectional analyses are not possible.

For further clarification and interpretation of the relationship between BPA exposure and adverse health effect additional studies are needed. These should include prospective studies with serial exposures to BPA assessed during etiologically relevant windows, years before development of disease, and thus prolonged follow up periods. Specific recommendations for use of existing data include i) the development of conversion models for the different media used for measurement, ii) inter-laboratory comparisons and calibrations. Recommendations for further data collection on BPA include: i) a better evaluation of exposure especially in children; ii) repeated measurements over time; iii) validation and harmonization of questionnaires; and iv) adequate detection methods. It has been suggested (Casas *et al.*, 2013) that although there is little published data in mother-child cohorts, many measurements are ongoing which should start working towards combined and comparison studies

Conclusions

There are several indications that BPA does have biological effects below the current NOAEL of 5 mg/kg b.w./day and the recently calculated $BMDL_{10}$ of 3.76 mg/kg b.w./day both derived from multigeneration reproductive toxicity studies after oral exposure. However, the evidence has been mainly obtained from dedicated studies focussing on specific outcome parameters like adiposity and hormone levels, and not from general toxicity studies. Some of those parameters resulted in contradicting results in various studies and dose-response relationships could not be established. Regarding possible low dose effects, the studies raise some concern for prenatal BPA exposure and an effect on mammary gland development and effects on altered behaviour/anxiety, although the studies are not sufficiently robust to be used in risk assessment. In addition, the possible effects on metabolism and adiposity need further investigations in large scale studies with a wide dose range of BPA. In addition, results of BPA effects on anxiety need further investigations. So far, epidemiological studies performed do not provide consistent outcomes to conclude on possible human health effects. The currently performed study by the FDA's National Center for Toxicological Research (NCTR) in the USA with animals under a strict exposure regimen and the research to be conducted on these animals by various research groups may clarify some of the controversial issues.

For medical devices several exposure scenarios were evaluated such as external short-term contact with a medical device, short and long-term contact with dental materials, medium- and long-term contact with an implanted medical device, long-term contact via hemodialyzers and medium-term contact in intensive care units with various medical devices.

The highest exposures estimated occurred during prolonged medical procedures in infants (685 ng/kg b.w./day), and during treatment of prematurely born infants in NICU

(3000 ng/kg b.w./day). The use of medical devices consisting of BPA containing PVC during treatment of of adults (1000 ng/kg b.w./day) and prematurely born infants (7000 ng/kg b.w./day, 7 µg/kg b.w./day) as single use or after short-term exposure (adults up to 5000 ng/kg b.w./day, infants up to 12000 ng/kg b.w./day) might even be higher. However, a serious limitation of these data is that the levels of exposure due to BPA containing PVC use was estimated by extrapolating the migration properties of DHEP, as no data on BPA leakage itself were available.

Contact with dental materials gave an estimated short- term (<24 hours) oral exposure of 140 to 200 ng/kg body weight per day for children and adults, respectively, whereas long -term exposure ranges from 2 to 12 ng/kg b.w./day. Some of the estimated BPA exposures due to medical devices are in the same range as exposure to BPA via food (EFSA 2013). Depending on the type of medical device, when the exposure route is parenteral, BPA may be 100% systemically bioavailable, while the bioavailability of free BPA after oral exposure is considered 1% of the ingested dose as the worst case. With the exception of haemodialysis practice, exposure due to medical devices generally occurs for a limited period of time.

It can be concluded that the oral long-term exposure via dental material is far below the current oral t-TDI of 5 µg/kg b.w./day derived from animal studies and pose no risk for human health. The same applies to the short-term (relatively high) exposure to BPA released from dental materials that is still below the recently established t-TDI, also considering that the peak of release is limited to few hours after application.

For the risk assessment for medical device giving rise to parenteral exposure, the exposure data of prematurely born infants in a NICU are used (3000 ng/kg b.w./day). The worst case scenario for exposure to BPA via use of medical devices consisting of BPA containing PVC has been estimated to result in a potential higher exposure (up to 7000 ng/kg b.w./day) for these prematurely born infants. However, it is worth noting that exposure to BPA via BPA-containing PVC has been estimated based on extrapolation from data on phthalate leakage from PVC and are, therefore, affected by a high degree of uncertainties. In addition, European PVC manufacturers do not use BPA in their PVC production. Hence, it is unlikely that such a high exposure will be reached due to the use of medical devices consisting of PVC.

Considering possible internal doses and bioavailability of free BPA for the maximum estimated exposure to medical devices (3 µg/kg b.w./day with 100% systemic bioavailability), the systemic exposure is about 60-fold higher when compared to the internal exposure of free BPA using the oral t-TDI (being 0.05 µg/kg b.w./day based on a TDI of 5 µg/kg b.w./day with 1% systemic bioavailability). When this systemic exposure due to medical devices is compared against the internal exposure at the $BMDL_{10}$ in rats and mice (3.76 mg/kg b.w./day), assuming 1% systemic bioavailability, the factor between the internal exposure via medical devices (3 µg/kg b.w./day) and the internal exposure at the $BMDL_{10}$ (37.6 µg/kg b.w./day) is about 12. The factor of 12 is lower than the usual safety factor of 100 for assessing a margin of safety (MOS) when extrapolating non toxic exposure doses for humans from results obtained in animal studies. For prolonged medical procedures in infants with an estimated exposure of 685 ng/kg b.w./day, the margin of safety is 55, while for the other exposure scenarios estimated the MOS is well above 100.

Based on these data it is concluded that there may be some risk for adverse effects of BPA when the BPA is directly available for systemic exposure after non-oral exposure routes, especially in neonates. It should be considered that with the exception of dialysis patients, the exposure is generally of limited duration. However, better data on exposure would be beneficial for the refinement of this risk assessment. In addition, in view of the controversial issues regarding possible low dose effects and their relevance for human health, especially after prenatal and/or perinatal exposure, raising some concern for exposure to BPA via medical devices in prematurely born infants. Further research under

well controlled exposure conditions, is warranted to confirm or negate these possible low dose effects and their relevance for human health.

It should be realised that the benefit of using these medical devices should also be considered: the survival of specifically these prematurely born infants often depends on the availability of the same medical devices which result in a relative high BPA exposure due to treatment. The possibility to replace BPA in these products should be considered against their efficiency in the treatment, as well as the toxicological profile of the alternative materials.

Specific answers to the Terms of Reference

The SCENIHR was requested to assess the following:

1. To determine whether levels of exposure to BPA from the use of the various medical devices containing BPA could give reasons for concern from the health point of view and, if possible, to provide indications on limit values for BPA release from medical devices.

It can be concluded that most of the exposure scenarios via medical devices results in an exposure that is below the recently derived t-TDI of 5 µg/kg body weight per day established by EFSA based on kidney toxicity as critical endpoint with a $BMDL_{10}$ of 3.76 mg/kg b.w./day. However, the internal systemic exposure due to certain medical treatments may be higher than the internal exposure resulting from the oral t-TDI as established for life long oral exposure. This maximal internal exposure due to medical devices is about 12-fold below the internal exposure based on the $BMDL_{10}$ observed in an oral toxicity study in rats and mice. This is lower than the usual factor (100x) used for assessing a margin of safety (MOS) when extrapolating low to no risk exposure doses for humans from results obtained in animal studies. For prolonged medical procedures in infants with an estimated exposure of 685 ng/kg b.w./day, the margin of safety is 55, while for the other exposure scenarios estimated the MOS is well above 100. Based on these data there may be some risk for adverse effects of BPA, when the BPA is directly available for systemic exposure after non-oral exposure routes.

2. To identify whether any particular medical devices containing BPA could result in human exposures which will give reasons for concern under their normal use patterns or other foreseeable circumstances (e.g. high release of BPA due to the nature of the material of the medical device or to particular contact conditions).

The identification of exposure from medical devices is the weakest part of the evaluation. More appropriate data on the content and release of BPA from medical devices in the actual conditions of use would be beneficial for answering the question in a more quantitative way. However, based on the available information, the highest exposure is estimated to occur in NICU via a multitude of medical devices used, especially in prematurely born infants. There is some reason for concern also in view of the controversial issues on possible low dose effects in prematurely born infants in NICU.

3. To identify, any patient group e.g. infants, pregnant and breastfeeding women who would be particularly at risk in light of the answer to the above questions.

Although not yet unequivocally demonstrated the possible metabolic effects, effects on mammary gland development and neurodevelopmental toxicity should be considered and raise some concern. These effects are observed in animals after prenatal exposure and exposure early in life. Although differences in kinetics between rodents and primates have been demonstrated, indicating a higher internal exposure in newborn rodents, at the same level of external exposure, it can be assumed that the unborn child and the newborn might be a population specifically at risk. However, it should be realised that the benefit of medical devices should also be considered: the survival of these premature infants often depends on the availability of the same medical devices which result in a relatively high BPA exposure due to treatment.

4. In case reasons for concern related to BPA are identified, to propose possible alternative approaches that could reduce potential risks either by identifying alternative practices or by identifying alternatives to the use of BPA in medical devices. If no clear answer can be provided on this point, the SCENIHR is asked to formulate recommendations for research that could help provide scientific evidence to that end.

Several alternatives for BPA exist and are increasingly used, notably Bisphenol S and Bisphenol F and some halogenated bisphenol A derivatives (e.g. tetrachlorobisphenol A and tetrabromobisphenol A). For some of the alternatives similar effects as for BPA were reported regarding endocrine activity in *in vitro* assays, although in general the alternatives had reduced activity/potency when compared to BPA. The toxicological profile of the alternatives to BPA is much less known, and at present it is not possible to compare the potential risk associated with alternatives to the risk due to BPA exposure.

Although internal BPA exposure via medical devices is generally below the internal exposure due to the recently derived oral t-TDI of 5 µg/kg body weight per day, for the worst case scenario an internal exposure above the internal dose of the oral t-TDI was noted. This internal exposure was below the internal dose of the oral $BMDL_{10}$ obtained in animal studies resulting in a margin of safety of 12. For prolonged medical procedures in infants the margin of safety is 55, while for the other exposure scenarios estimated the MOS is well above 100. Some concerns exist regarding controversial issues like possible low dose effects of BPA and their relevance for human health. Studies are currently beind conducted in the USA to account for these uncertainties. Once new unequivocal and reproducible evidence for adverse effects at lower exposures becomes available, the risk of BPA via medical devices needs to be reconsidered in relation to the estimated exposure scenarios used in this Opinion. Recommendations for further research especially in the area of exposure through medical devices are presented in section 3.13.

The possibility to replace BPA in medical devices should be considered against their efficiency in the treatment, as well as the toxicological profile of the alternative materials.

5.MINORITY OPINION

None

6.LIST OF ABBREVIATIONS

AGD	Anogenital distance
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (receptor)
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Agency for Food, Environment and Occupational Health and Safety, Paris, France)
AUC	Area Under the Curve
BADGE	Bisphenol A diglycidyl ether
BASC-SR	Behavior Assessment System for Children – Self Reported
BFDGE	Bisphenol F diglycide ether
BHP	N-nitrosobis-(2-hydroxypropyl)-amine
Bis-DMA	Bisphenol A dimethacrylate (2,2-di(4-Methacryloxyphenyl)propane)
Bis-EMA	Ethoxylated bisphenol A dimethacrylate (2,2-bis(4-(2-Methacryloxyethoxy)-phenylpropane)

1	Bis-GMA	Bisphenol A glycidyl methacrylate
2	BMD	Bench Mark Dose
3 4	BMDL	Bench Mark Dose Low (lower value of 90% confidence interval of BMD)
5 6	BMDU	Bench Mark Dose Upper (upper value of 90% confidence interval of BMD)
7	BPA	Bisphenol A, Bis(4-hydroxyphenyl)propane
8	BPAQ	BPA-3,4-quinone
9	BPB	Bisphenol B, 2,2-Bis(4-hydroxyphenyl)butane
10	BPF	Bisphenol F, Bis(4-hydroxydiphenyl)methane
11	BPS(U)	Bis(4-hydroxyphenyl)sulfone
12	BRCA1	Breast Cancer 1 (gene)
13	b.w.	body weight
14 15	CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (EFSA panel)
16	CERHR	Center for the Evaluation of Risks to Human Reproduction (USA)
17	Cmax	Maximum concentration
18	CMV	Cytomegalovirus
19	CPB	Cardiopulmonary bypass
20	CYP	Cytochrome P450
21	dBPA	deuterated BPA
22	DEHP	Di(2-ethylhexyl) phthalate
23	DES	Di-ethylstilbestrol
24	DMAB	3,2-dimethyl-4-aminobiphenyl
25	DMBA	7,12-Dimethylbenz(a)anthracene
26	DMSO	Dimethyl Sulfoxide
27	DNA	Deoxyribonucleic acid
28	DNMT	DNA methyltransferase
29	DSP	Daily sperm production
30	EB	17ß-estradiol-3-benzoate
31	ECB	European Chemicals Bureau
32	ECDC	European Centre for Disease prevention and Control
33	ECHA	European Chemicals Agency
34	ECMO	Extracorporeal membrane oxygenation
35	EE	Ethinyl Estradiol
36	EFSA	European Food Safety Authority
37	ELISA	Enzyme-Linked Immuno Sorbent Assay
38	EMA	European Medicines Agency
39	ENNG	N-ethyl-N´-nitro-nitrosoguanidine

1	EPM	Elevated Plus Maze
2	ER	Estrogen Receptor
3	EtO	Ethylene oxide
4	EU-RAR	EU Risk Assessment Report
5	FAO	Food and Agriculture Organization
6	FDA	Food and Drug Administration (USA)
7	FST	Forced Swimming Test
8	GC	Gas Chromatography
9	GC-MS	Gas Chromatography-Mass Spectrometry
10	GD	Gestational Day
11	GI	Gastro-intestinal
12	GLP	Good Laboratory Practices
13	HCA	Hydroxycumyl alcohol
14	HDL	High-density lipoprotein
15	HED	Human Equivalent Dose
16	HPG	hypothalamic-pituary-gonadal (axis)
17	HPLC-MS	High Pressure Liquid Chromatography-Mass Spectrometry
18 19	HPLC/UV-DAD	High Pressure Liquid Chromatography/Ultraviolet-Diode Array Detector
20	ICU	Intensive Care Unit
21	IPCS	International Programme on Chemical Safety
22	ISO	International Organization for Standardization
23	i.v. (IV)	intraveneously
24	LC	Liquid Chromatography
25	LC-ECD	Liquid Chromatography-ElectroChemical Detector
26	LC-MS	Liquid Chromatography-Mass Spectrometry
27	LD50	Lethal Dose at which 50% of the animals die
28	LDL	Low density lipoprotein
29	LOAEL	Lowest Observed Adverse Effect Level
30	LOD	Limit of Detection
31	LOQ	Limit of Quantification
32	MBP	4-methyl-2,4-bis(4-hydroxyl-phenyl) pent-1-ene
33	MOS	Margin of Safety
34	MRI	Magnetic Resonance Imaging
35	mRNA	Messenger Ribonucleic Acid
36	MS	Mass Spectrometry
37	MTD	Maximum Tolerated Dose
38	MWM	Morris Water Maze
39	NHANES	National Health and Nutrition Examination Survey (USA)

NICU	Neonatal Intensive Care Unit
NIEHS	National Institute of Environmental Health Sciences (USA)
NOAEL	No Observed Adverse Effect Level
NCTR	National Center for Toxicological Research (USA)
NHP	Non Human Primates
NMDA	N-Methyl-d-Aspartate (receptor)
NMU	N-nitroso- N-methylurea
NTP	National Toxicology Program (USA)
NTP-CERHR	National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction (USA)
OECD	Organisation for Economic Co-operation and Development
8-OHdG	8-hydroxydeoxyguanosine
OP	Object Placement
OR	Object Recognition
OVX	Ovariectomised
PBPK	Physiologically Based Pharmacokinetic (modeling)
PC	Polycarbonate
PEPA	Polyester-polymeralloy
PIN	Prostatic intraepithelial neoplasia
PND	Post Natal Day
ppm	parts per million (translates into mg/L or ng/mg)
PPAR	Peroxisome proliferator-activated receptor
PSD	Postsynaptic density
PSU	Polysulfone
PVC	Polyvinyl chloride
pWAT	Perigonadic white adipose tissue
REACH	Registration, Evaluation, and Authorisation of Chemicals (EU Regulation)
RIA	Radioimmunoassay
RNA	Ribonucleic Acid
s.c. (SC)	subcutaneous
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SE	Standard Error
SPE	Solid-phase extraction
SULT	Sulfotransferase
TBBPA	Tetrabromobisphenol-A [2,2-bis(4-hydroxy-3,5-dibromophenyl)propane]

TCBPA	Tetrachlorobisphenol-A [2,2-bis(4-hydroxy-3,5-dichlorophenyl)propane]
TDI	Tolerable Daily Intake
T1DM	Type 1 diabetes mellitus
TEGDMA	Triethylene glycol dimethacrylate
UDPGT	Uridinediphosphate- glucuronosyltransferase
UGT	UDP-glucuronyltransferase
UPLC	Ultra Performance Liquid Chromatography
WHO	World Health Organization

7.REFERENCES

Aalto-Korte K, Alanko K, Henriks-eckerman ML, Estlander T, Jolanki R (2003) Allergic contact dermatitis from bisphenol A in PVC gloves – Contact Dermatitis, 49, 202-205.

Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. (2006) Environ Health Perspect. The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resis¬tance. 114, 106 – 112.

Alonso-Magdalena P., Vieira E., Soriano S., Menes L., Burks D., Quesada I., Nadal A. (2010) Bisphenol-A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environmental Health Perspectives 118, 1243–1250.

Alonso-Magdalena P, Quesada I, Nadal A. (2011) Endocrine disruptors in the etiology of type 2 diabetes mellitus. Nat Rev Endocrinol 7, 346-353.

Anderson OS, Peterson KE, Sanchez BN, Zhang Z, Mancuso P, Dolinoy DC. (2013) Perinatal bisphenol A exposure promotes hyperactivity, lean body composition, and hormonal responses across the murine life course. FASEB J. 27, 1784-1792.

Angle BM, Do RP, Ponzi D, Stahlhut RW, Drury BE, Nagel SC, Welshons WV, Besch-Williford CL, Palanza P, Parmigiani S, Vom Saal FS, Taylor JA. (2013) Metabolic disruption in male mice due to fetal exposure to low but not high doses of bisphenol A (BPA): Evidence for effects on body weight, food intake, adipocytes, leptin, adiponectin, insulin and glucose regulation. Reprod Toxicol. 2013 Jul 17. pii: S0890-6238(13)00231-1. doi: 10.1016/j.reprotox.2013.07.017. [Epub ahead of print]).

ANSES (2011) Health effects of Bisphenol A. Request nos. 2009-SA-0331 and 2010-SA-0197. Collective Expert Report. ANSES (Agence National de Securite Sanitaire Alimentation, Environment, Travail, French Agency for Food, Environmental and Occupational Health & Safety) Paris, France, September 2011.

ANSES (2013) Evaluation des risques du bisphenol A (BPA) pour la santé humaine. Substances reprotoxiques et perturbateurs endocrines. (Agence National de Securite Sanitaire Alimentation, Environment, Travail, French Agency for Food, Environmental and Occupational Health & Safety, Paris, France), Paris, France, Mars 2013. http://www.anses.fr/fr/content/evaluation-des-risques-sanitaires-li%C3%A9s-au-bisph%C3%A9nol

Arenholt-Bindslev D, Breinholt V, Preiss A, Schmalz G.(1999) Time-related bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants. Clinical Oral Investigations 3, 120-125, 1999.

Arnich N., Canivenc-Lavier M.C., Kolf-Clauw M., Coffigny H., Cravedi J.P., Grob K., Macherey A.C., Masset D., Maximilien R., Narbonne J.F., Nesslany F., Stadler J., Tulliez J. (2011) Conclusions of the French Food Safety Agency on the toxicity of bisphenol A. Int J Hyg Environ Health 214, 271-275.

Aschberger K., Castello P., Hoeskstra E., Karakitsios S., Munn S., Pakalin S. and Sarigiannis D. (2010). Bisphenol A and baby bottles: challenges and perspectives- JRC-European Commission EUR 24389.

Ashby J, Tinwell H, Haseman J. (1999) Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. Regul Toxicol Pharmacol 30, 156-166.

Audebert M, Dolo L, Perdu E, Cravedi JP, Zalko D. (2011) Use of the γH2AX assay for assessing the genotoxicity of bisphenol A and bisphenol F in human cell lines. Arch Toxicol. 85, 1463-1473.

Ayyanan A, Laribi O, Schuepbach-Mallepell S, Schrick C, Gutierrez M, Tanos T, Lefebvre G, Rougemont J, Yalcin-Ozuysal O, Brisken C. (2011) Perinatal exposure to bisphenol a increases adult mammary gland progesterone response and cell number. Mol Endocrinol. 25, 1915-1923.

Baker DA, Hastings RS, Pruitt L (2000): Composition and tension resistance of medical grade ultra-high molecular weight polyethylene; the effect of morphology, sterilization, aging and temperature. Polymer 2000 41 (2) 795-808.

Balakrishnan B, Henare K, Thorstensen EB, Ponnampalam AP, and Mitchell MD, 2010. Transfer of bisphenol A across the human placenta. American Journal of Obstetrics and Gynecology 202, 393.e1-7.

Batista TM, Alonso-Magdalena P, Vieira E, Amaral ME, Cederroth CR, Nef S, Quesada I, Cameiro EM, Nadal A. (2012) Short-term treatment with bisphenol-A leads to metabolic abnormalities in adult male mice. PLoS One 7 (3), e33814.

Becker K, Güen T. Seiwert M et al. (2009) GerES IV: phthalate metabolites and bisphenol A in urine of German children. Internat J Hyg Environ Health. 212: 685–692.

Begley T., Castle L., Feigenbaum A., Franz R., Hinrichs K., Lickly T., Mercea P, Milana M, O'Brien A, Rebre S, Rijk R, Piringer O. et al. (2005).Evaluation of migration models in support of regulations for food-contact plastics. Food Addit Contam 22: 73:90.

Begley T.H., Dennison J.L., Hollifield H.C. (1990). Migration into food of polyethylene terephthalate (PET) cyclic oligomers from PET microwave packaging. Food Addit Contam 7: 797:803.

Beronius A and Hanberg A (2011) Sources of exposure to bisphenol A. IMM report 2/2011, Karolinska Institut, Stockholm, Sweden. http://ki.se/content/1/c6/12/58/71/IMMrapport2-2011.pdf

Betancourt AM, Eltoum IA, Desmond RA, Russo J, Lamartiniere CA. (2010) In utero exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat. Environ Health Perspect. 118, 1614-1619.

Biedermann, S., Tschudin, P., Grob, K. (2010) Transfer of bisphenol A from thermal printer paper to the skin. Analytical and Bioanalytical Chemistry 398, 571-576 (2010).

Biles JE, McNeal TP, Begley TH, Hollifield HC (1997). Determination of Bisphenol-A in Reusable Polycarbonate Food-Contact Plastics and Migration to Food-Simulating Liquids. Journal of Agricultural and Food Chemistry 45: 3541-3544. Corrected in J. of Agric. Food Chem. 46:2894.

Bloom MS, Vom Saal FS, Kim D, Taylor JA, Lamb JD, Fujimoto VY. (2011) Serum unconjugated bisphenol A concentrations in men may influence embryo quality indicators during in vitro fertilization. Environ Toxicol Pharmacol. 32, 319-323.

Bodin J, Bølling AK, Samuelsen M, Becher R, Løvik M, Nygaard UC. (2013) Long-term bisphenol A exposure accelerates insulitis development in diabetes-prone NOD mice. Immunopharmacol Immunotoxicol. 35, 349-358.

Borrell B. (2010) Toxicology: The big test for bisphenol A. Nature. 464, 1122-1124.

Braun JM, Yolton K, Dietrich KN, Homung R, Ye X, Calafat Am, Lanphear BP. (2009) Prenatal bisphenol A exposure and early childhood behavior. Environ Health Perspect 117, 1945-1952.

Braun JM, Kalkbrenner AE, Calafat AM, Yolton K, Ye X, Dietrich KN, Lanphear BP. (2011) Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics. 128, :873-882.

Brown SA, Merritt K, woods T.O, Mc Namees S.G, Hitchins VM (2002). Effect of disinfection and sterilization methods on tensile strength of material used for single use devices. Biomed Instrum. Technol. 36, 23-27.

Bushnik T, Haines D, Levallois P, Levesque J, Van Oostdam J and Viau C (2010) Lead and bisphenol A concentrations in the Canadian population. Health Reports 21: 7–18.

Cabaton N, Dumont C, Severin I, Perdu E, Zalko D, Cherkaoui-MAlki M, Chagnon MC. (2009) Genotoxic and endocrine activities of bis(hydroxyphenyl)methane (bisphenol F) and its derivatives in the HepG2 cell line. Toxicology 255, 15-24.

Cacho JI, Campilo N, Vinas P and Hernandez-Cordoba M (2013) Stir bar sorptive extraction with EG-Silicone coating for bisphenols determination in personal care products by GC-MS. J Pharm Biomed Anal 79: 255-260.

Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR.(1999) Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. Toxicol Sci. 50, 36-44, 1999.

Calafat AM, Kuklenyik Z, Reidy J A, Caudill S P, Ekong J and. Needham LL (2005) Urinary concentrations of Bisphenol A and 4-Nonylphenol in a human reference population. Environ Health Perspect 113: 391–395.

Calafat AM, Weuve J, Ye XY, Jia LT, Hu H, Ringer S, Huttner K, Hauser R, (2009). Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environmental Health Perspectives 117, 639-644.

Calafat AM, Needham LL. (2009) What additional factors beyond state-of-the-art analytical methods are needed for optimal generation and interpretation of biomonitoring data? Environ Health Perspect. 117, 1481-1485.

Calafat AM, Ye X, Wong L-Y, Reidy JA, Needham LL. (2008). Exposure of the U.S. Population to Bisphenol A and 4-tertiary-Octylphenol: 2003–2004. Environmental Health Perspectives , 116, 39-44.

Campbell NR, Van Loon JA and Weinshilboum RM, (1987). Human liver phenol sulfotransferase: Assay conditions, biochemical properties and partial purification of isozymes of the thermostable form. Biochemical Pharmacology 36:1435–1446.

Cao J, Mickens JA, McCaffrey KA, Leyrer SM, Patisaul HB. (2012) Neonatal Bisphenol A exposure alters sexually dimorphic gene expression in the postnatal rat hypothalamus. Neurotoxicology. 33, 23-36.

Cao J, Rebuli ME, Rogers J, Todd KL, Leyrer SM, Ferguson SA, Patisaul HB. (2013) Prenatal bisphenol a exposure alters sex-specific estrogen receptor expression in the neonatal rat hypothalamus and amygdala. Toxicol Sci. 133, 157-173.

Cariot, A., A. Dupuis, M. Albouy-Llaty, B. Legube, S. Rabouan, V. Migeot (2012). "Reliable quantification of bisphenol A and its chlorinated derivatives in human breast milk using UPLC–MS/MS method." Talanta 100: 175-182.

Carr R, Bertasi F, Betancourt S, Bowers S, Gandy BS, Ryan P, Willard S. (2003) Effect of neonatal rat bisphenol A exposure on performance in the Morris Water Maze. J Toxicol Environ Health A 66, 2077-2088.

Carwile J.L., Michels K.B. (2011) Urinary bisphenol A and obesity: NHANES 2003-2006. Environ Res. 111, 825-830.

Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin AE, Fernandez MF, Koch HM, Mendez MA, Sunyer J, Rubio S, Vrijheid M. (2013) Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Env Int 56, 10-18.

Changkhamchom S, Sirivat A, (2010). Synthesis and properties of sulfonated poly(ether ketone ether sulfone) (S-PEKES) via bisphenol S: Effect of sulfonation. Polymer Bulletin, 65, 265–281.

Chapin R, Adams J, Boekelheide K, Gray L, Hayward S, Lees P, McIntyre B, Portier K, Schnorr T, Selevan S, Vandenberg J, Woskie S (2007) NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A. http://cerhr.niehs.nih.gov/chemicals/bisphenol/bisphenol.html

Chapin RE, Adams J, Boekelheide K, Gray LE Jr, Hayward SW, Lees PS, McIntyre BS, Portier KM, Schnorr TM, Selevan SG, Vandenbergh JG, Woskie SR (2008). NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res B Dev Reprod Toxicol. 83, 157-395

Chen MY, Ike M, Fujita M. (2002). Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols. Environ Toxicol 17:80–86.

Cho S., Choi Y.S., Luu H. and Guo J. (2012).Determination of total leachable bisphenol A from polysulfone membranes based on multiple consecutive extractions. Talanta 101; 537–540

Chou WC, Chen JL, Lin CF, Chen YC, Shih FC, Chuang CY. (2011). Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: A birth cohort study in Taiwan. Environ Health 10:94.

Christensen KL, Lorber M, Koch HM, Kolossa-Gehring M, Morgan MK. (2012) Population variability of phthalate metabolites and bisphenol A concentrations in spot urine samples versus 24- or 48-h collections. J Expo Sci Environ Epidemiol. 22, 632-640.

Cichna-Markl M. (2012) Sample clean-up by sol–gel immunoaffinity chromatography for the determination of bisphenol A in food and urine Methods 56 (2012) 186–191

Clayton EM, Todd M, Dowd JB, Aiello AE. (2011) The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006. Environ Health Perspect 119, 390-396.

Cobellis L, Colacurci N, Trabucco E, Carpentiero C, Grumetto L. (2009) Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women. Biomed Chromatogr 23, 1186-1190.

COM (Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment) Guidance on a Strategy for Testing of Chemicals for Mutagenicity, Department of Health, UK, 2000 http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL2.pdf

Coughtrie MW, Burchell B, Leakey JE, Hume R. (1988) The inadequacy of perinatal glucuronidation: immunoblot analysis of the developmental expression of individual UDP-glucuronosyltransferase isoenzymes in rat and human liver microsomes. Mol Pharmacol 34, 729-735.

Cousins IT, Staples CA, Klečka GM, Mackay D. 2002. A multimedia assessment of the environmental fate of bisphenol A. Human and Ecological Risk Assessment (HERA). 8: 1107-1136

Cox KH, Gatewood JD, Howeth C, Rissman EF. (2010) Gestational exposure to bisphenol A and cross-fostering affect behaviors in juvenile mice. Horm Behav 58, 754–761.

D'Cruz SC, Jubendradass R, Jayakanthan M, Rani SJ, Mathur PP. (2012) Bisphenol A impairs insulin signaling and glucose homeostasis and decreases steroidogenesis in rat testis: an in vivo and in silico study. Food Chem Toxicol. 50, 1124-1133.

De Flora S, Micale RT, La Maestra S, Izzotti A, D'Agostini F, Camoirano A, Davoli SA, Troglio MG, Rizzi F, Davalli P, Bettuzzi S. (2011) Upregulation of clusterin in prostate and DNA damage in spermatozoa from bisphenol A-treated rats and formation of DNA adducts in cultured human prostatic cells. Toxicol Sci.122, 45-51.

Dekant W and Völkel W (2008) Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. Toxicol App Pharmacol 228, 114–134.

De Meulenaer B. and Huyghebaert A. (2004). Packaging and other food contact materials residues. In: Handbook of Food Analysis, Vol.2 (Nollet LML ed) 2nd ed. New York: Marcel Dekker, 1297-1330

Demierre AL, Peter R, Oberli A, Bourqui-Pittet M. (2012) Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. Toxicol Lett. 213, 305-308.

De Wit CA, Herzke D, Vorkamp K. (2010) Brominated flame retardants in the Arctic environment--trends and new candidates. Sci Total Environ 408, 2885-2918.

Dobrzyńska MM, Radzikowska J. (2013) Genotoxicity and reproductive toxicity of bisphenol A and X-ray/bisphenol A combination in male mice. Drug Chem Toxicol. 36, 19-26.

Dodson RE, Nishioka M, Standley LJ, Perovich LJ, Brody JG and Rudel RA (2012) Endocrine disruptors and asthma-associated chemicals in consumer products. Environmental Health Perspectives 120, 935-944.

Doerge DR, Twaddle NC, Vanlandingham M and Fisher JW, (2010a). Pharmacokinetics of bisphenol A in neonatal and adult Sprague-Dawley Rats. Toxicology and Applied Pharmacology 247, 158-165.

Doerge DR, Twaddle NC, Vanlandingham M and Fisher JW, (2011a) Pharmacokinetics of Bisphenol A in neonatal and adult CD-1 mice: Inter-species comparisons with Sprague-Dawley rats and rhesus monkeys Toxicology Letters 207, 298– 305.

Doerge DR, Twaddle NC, Vanlandingham M and Fisher JW, (2012) Pharmacokinetics of bisphenol A in serum and adipose tissue following intravenous administration to adult female CD-1 mice Toxicology Letters 211, 114– 119.

Doerge DR, Twaddle NC, Woodling KA and Fisher JW, (2010b). Pharmacokinetics of bisphenol a in neonatal and adult rhesus monkeys. Toxicology and Applied Pharmacology 248, 1-11

Doerge, D. R., M. Vanlandingham, N. C. Twaddle and K. B. Delclos (2010c). Lactational transfer of bisphenol A in Sprague-Dawley rats. Toxicol Lett 199, 372-376.

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

Dolinoy DC, Huang D and Jirtle RL, (2007). Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proceedings of the National Academy of Sciences of the United States of America 104, 13056-13061.

Donohue KM, Miller RL, Perzanowski MS, Just AC, Hoepner LA, Arunajadai S, Canfield S, Resnick D, Calafat AM, Perera FP, Whyatt RM (2013) Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. J Allergy Clin Immunol, 131, 736-742.

Duanmu Z, Weckle A, Koukouritaki SB, Hines RN, Falany Jl, Falany CN, Kocarek TA and Runge-Morris M. (2006). Developmental expression of aryl, estrogen and hydoxysteroid sulfotransferases in pre- and post-natal human liver. Journal of Pharmacology and Experimental Therapeutics 316, 1310-1317.

Durando M, Kass L, Piva J, Sonnenheim C, Soto AM, Luque EH, Munoz-de-Toro M. (2007) Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in wistar rats. Envir Health Perspect 115, 80-86.

ECB, European Chemicals Bureau (2003). European Union Risk Assessment Report: 4,4' ISOPROPYLIDENEDIPHENOL (Bisphenol-A). CAS No:80-05-7. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, 3rd Priority List, Luxembourg: Office for Official Publications of the European Communities
http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/1304/1/EUR%20843%20EN.pdf

ECB (2004) European Commission Scientific Committee on toxicity, ecotoxicity and the environment (CSTEE). Opinion on the results of a second Risk Assessment of: bis(2-ethylhexyl)phthalate [DEHP]: Human Health Part. CAS No.: 117-81-7; EINECS No.: 204-211-0. Adopted by the CSTEE during the 41th plenary meeting of 8 January 2004.

EC (2008) European Union Risk Assessment Report Human Helath Addendum of April 2008 (to be read in conjunction with published EU RAR of BPA 2003) 4,4'-Isopropylidenediphenol (Bisphenol-A) Part 2 Human Health. http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/15069/1/lbna24589enn.pdf

EC, European Commission (2010b) European Union Risk Assessment Report 4,4'-ISOPROPYLIDENEDIPHENOL (Bisphenol-A), Part 1 Environmental Health – Environment Addendum of April 2008 (to be read in conjunction with published EU RAR of BPA, 2003) http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/15063/1/lbna24588enn.pdf

EC, European Commission (2010a) European Union Risk Assessment Report 4,4'-ISOPROPYLIDENEDIPHENOL (Bisphenol-A), Part 2 Human Health – Human Health Addendum of April 2008 (to be read in conjunction with published EU RAR of BPA, 2003) http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/15069/1/lbna24589enn.pdf

Edginton A.N., Ritter L. (2009) Predicting plasma concentrations of bisphenol A in children younger than 2 years of age after typical feeding schedules, using a physiologically based toxicokinetic model. Environmental Health Perspectives 117, 645-652 (2009)

Edlow AG, Chen M, Smith NA, Lu C and McElrath TF. (2012) Fetal bisphenol A exposure: concentration of conjugated and unconjugated bisphenol A in amniotic fuid in the second and third trimesters. Reproductive Toxicology, 34, 1-7.

EFSA (2006). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2,2-BIS(4-HYDROXYPHENYL) PROPANE (Bisphenol A). The EFSA Journal 428, 1- 75. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620772817.htm

EFSA (2009) Guidance of the Scientific Committee on a request from EFSA on the use of the bench mark dose approach in risk assessment. The EFSA Journal 1150, 1-72. http://www.efsa.europa.eu/it/efsajournal/doc/1150.pdf

EFSA (2010). Scientific Opinion of the Panel on food contact materials, enzymes, flavourings and processing aids (CEF) on Bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of Bisphenol A. The EFSA Journal 8, 1829. http://www.efsa.europa.eu/en/efsajournal/doc/1829.pdf

EFSA (2011) Technical Report. Use of BMDS and PROAST software packages by EFGSA scientific panels and units for applying the Bench MRK Dose (BMD) approach in risk assessment. European Food Safety Authority (EFSA), Parma, Italy. http://www.efsa.europa.eu/en/supporting/doc/113e.pdf

EFSA (2012a) EFSA Scientific Committee. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 10, 2579. http://www.efsa.europa.eu/en/efsajournal/doc/2579.pdf

EFSA (2012b) EFSA panel on plant protection products and their residues (PPR). Scientific Opinionon; Guidance on dermal absorption. EFSA Journal 10, 2665.

EFSA (2013). DRAFT Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs – Part: exposure assessment. Draft Scientific

Opinion Endorsed for Public Consultation. http://www.efsa.europa.eu/en/consultations/call/130725.pdf

EFSA (2014) Draft Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. ENDORSED FOR PUBLIC CONSULTATION DRAFT SCIENTIFIC OPINION. EFSA Panel on EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) , European Food Safety Authority (EFSA), Parma, Italy. January 2014.

Eilam-Stock T, Serrano P, Frankfurt M, Luine V (2012) Bisphenol-A impairs memory and reduces dendritic spine density in adult male rats. Behavioral Neuroscience, 126, 175-185.

Eliades T, Hiskia A, Eliades G and Athanasiou AE (2007) Assessment of bisphenol-A release from orthodontic adhesives. Am J Orthod Dentofacial Orthop 131: 72-75

Eliades T, Voutsa D, Sifakakis I, Makou M and Katsaros C (2011) Release of bisphenol-A from a light-cured adhesive bonded to lingual fixed retainers. Am J Orthod Dentofacial Orthop 139: 192-195

Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A. (2001) Rat two-generation reproductive toxicity study of bisphenol A. Reprod Toxicol. 15, 505-523.

Environment Canada/Health Canada (2008) Screening Assessment for the Challenge - Phenol, 4,4' -(1-methylethylidene)bis- (Bisphenol A). Available on-line at: http://www.ec.gc.ca/ese-ees/3C756383-BEB3-45D5-B8D3-E8C800F35243/batch2_80-05-7_en.pdf

FAO/WHO (2011) Toxicological and Health Aspects of Bisphenol A. Report of Joint FAO/WHO Expert Meeting. World Health Organization. 2011. ISBN 978 92 141 56427 4. http://www.who.int/foodsafety/chem/chemicals/BPA_Summary2010.pdf

Ferguson SA, Law CD, Abshire JS. (2012) Developmental treatment with bisphenol A causes few alterations on measures of postweaning activity and learning. Neurotoxicol Teratol. 34, 598-606.

Fernandez MF, Arrebola JP, Taoufiki J, Navalon A, Ballesteros O, Pulgar R, Vilchez J L and Olea N, (2007). Bisphenol-A and chlorinated derivatives in adipose tissue of women. Reproductive Toxicology 24, 259-264.

Fink K. (2008). Toxins in Renal Disease and Dialysis Therapy: Genotoxic Potential and Mechanisms. Dissertation zur Erlangung des naturwissenschaftlichen Doktorgrades der Bayerischen Julius-Maximilians-Universität Würzburg.

Fisher JW, Twaddle NC, Vanlandingham M, Doerge DR. (2011) Pharmacokinetic modeling: prediction and evaluation of route-dependent dosimetry of bisphenol A in monkeys with extrapolation to humans. Toxicol. Appl. Pharmacol., 257 (2011) 122–136.

Fleisch AF, Sheffield PE, Chinn C, Edelstein BL and Landrigan PJ (2010) Bisphenol A and Related Compounds in Dental Materials. Pediatrics 126:760-768.

Fromme H. Küchlerb T, Ottoc T, Pilzc K, Müllerb J, Wenzel A. (2002). Occurrence of phthalates and bisphenol A and F in the environment. Water Res. 36, 1429–1438.

Fu P and Kawamura K (2010) Ubiquity of bisphenol A in the atmosphere. Environmental Pollution 158: 3138-3143.

Fujimoto T, Kubo K, Aou S.(2006) Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. Brain Res. 1068, 49-55.

Fukazawa H, Hoshino K, Shiozawa T, Matsushita H, Terao Y. (2001) Identification and quantification of chlorinated bisphenol A in wastewater from wastepaper recycling plants. Chemosphere 44, 973–979.

Fung EY, Ewoldsen NO, St Germain HA Jr, Marx DB, Miaw CL, Siew C et al. (2000), Pharmacokinetics of bisphenol A released from a dental sealant. Journal of the American Dental Association 131, 51-58.

Gaffney, P. T., R. L. Buttenshaw, M. Ward and R. D. Diplock (1986). "Breast milk beta-glucuronidase and neonatal jaundice." Lancet 1(8490): 1161-1162.

Galloway T, Cipelli R, Guralnik J, Ferrucci L, Bandinelli S, Corsi AM, Money C, McCormack P, Melzer D (2010). Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. Environmental Health Perspectives, 118, 1603–1608.

Gayrard V, Lacroix MZ, Collet SH, Viguié C, Bousquet-Melou A, Toutain PL, Picard-Hagen N. (2013) High bioavailability of bisphenol A from sublingual exposure. Environ Health Perspect. 121, 951-956.

Geens T, Aerts D, Berthot C, Bourguignon J-P., Goeyens L., Lecomte P., Maghuin-Rogister G., Pironnet A-M. Pussemier L., Scippo M-L., Van Loco J., Covaci A. (2012) A review of dietary and non-dietary exposure to bisphenol-A. Food and Chemical Toxicology 50, 3725–3740, 2012.

Geens T, Roosens L, Neels H and Adrian Covaci A (2009) Assessment of human exposure to Bisphenol-A, Triclosan and Tetrabromobisphenol-A through indoor dust intake in Belgium. Chemosphere 76: 755–760

Gehring MJ. (2004) Verhalten der endokrin wirksamen Substanz Bisphenol A bei der kommunalen Abwasserentsorgung ,Dissertation, 2004 Technische Univ. Dresden.

Genuis, SJ, Beesoon S, Birkholz and Lobo RA (2011) Human Excretion of Bisphenol A: Blood, Urine and Sweat (BUS) Study. Journal of Environmental and Public Health, 2012: Article ID 185731, 1-10

Ginsberg G, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, Smolenski S, Golbe R, (2002). Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicological Sciences 66, 185-200.

Gioiosa L, Fissore E, Ghirardelli G, Parmigiani S, Palanza P (2007) Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. Horm Behav 52, 307-316.

Gioiosa L, Parmigiani S, Vom Saal FS, Palanza P. (2013) The effects of bisphenol A on emotional behavior depend upon the timing of exposure, age and gender in mice. Horm Behav. 63, 598-605.

Goodman JE, Witorsch RJ, McConnell EE, Sipes IG, Slayton TM, Yu CJ, Franz AM, Rhomberg LR. (2009) Weight-of-evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. Crit Rev Toxicol. 39, 1-75.

Grazioso, C. F. and E. S. Buescher (1996). "Inhibition of neutrophil function by human milk." Cell Immunol 168: 125-132.

Haighton LA, Hlywka JJ, Doull J, Kroes R, Lynch BS, Munro IC (2002). An evaluation of the possible carcinogenicity of bisphenol A to humans. Regul Toxicol Pharmacol. 35 (2 Pt 1), 238-254.

Haishima Y., Hayashi Y., Yagami T. and Nakamura A. (2001). Elution of Bisphenol-A from Hemodialysers consisting of polycarbonate and polysoulfone resins. Journal of Biomedical Materials Research 58 (2):209-215.

Hajszan T, Leranth C. (2010) Bisphenol A interferes with synaptic remodeling. Front Neuroendocrinol. 31, 519-530.

Han DH, Kim MJ, Jun EJ, Kim JB. (2012) Salivary bisphenol-A levels due to dental sealant/resin: a case-control study in Korean children. Journal of Korean Medical Science 2012;27: 1098-104.

Hanaoka T, Kawamura N, Hara K, Tsugane S. (2002) Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. Occupational and Environmental Medicine, 59, 625–628.

Hanioka N, Naito T and Narimatsu S, (2008). Human UDP-glucuronosyltransferase isoforms involved in bisphenol A glucuronidation. Chemosphere 74, 33-36.

Hanioka N, Oka H, Nagaoka K, Ikushiro S, Narimatsu S (2011) Effect of UDP-glucuronosyltransferase 2B15 polymorphism on bisphenol A glucuronidation. Arch Toxicol 85: 1373-1381.

Hao J, Wang J, Zhao W, Ding L, Gao E, Yuan W. (2011). Effect of bisphenol A exposure on sex hormone level in occupational women]. Wei Sheng Yan Jiu. 40, 312-4, 319.

Harthé C., Rinaldi S., Achaintre D., Rolland de Ravel M., Mappus E., Pugeat M., Dèchaud H. (2012) Bisphenol A-glucuronide measurement in urine samples Talanta, 100, 410-413.

Hashimoto Y, Moriguchi Y, Oshima H, Kawaguchi M, Miyazaki K, Nakamura M (2001) Measurement of estrogenic activity of chemicals for the development of new dental polymers. Toxicol. In vitro 15, 421–425.

Hashimoto Y, Nakamura M. (2000) Estrogenic activity of dental materials and bisphenol A related chemicals in vitro. Dent. Mater. J. 19, 245–262.

He Z, Paule MG, Ferguson SA (2012). Low oral doses of bisphenol A increase volume of the sexually dimorphic nucleus of the preoptic area in male, but not female, rats at postnatal day 21. Neurotoxicology and Teratology, 34, 331-337.

Helander A, Dahl H. (2005) Urinary tract infection: a risk factor for false-negative urinary ethyl glucuronide but not ethyl sulfate in the detection of recent alcohol consumption. Clin Chem. 51, 1728-1730.

Hengstler JG, Foth H, Gebel T, Kramer PJ, Lilienblum W, Schweinfurth H, Völkel W, Wollin KM, Gundert-Remy U. (2011) Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A. Crit Rev Toxicol. 41, 263-291.

Hiroi H, Tsutsumi O, Takeuchi T, Momoeda M, Ikezuki Y, Okamura A, Yokota H, Taketani Y. (2004). Differences in serum bisphenol A concentrations in premenopausal normal women and women with endometrial hyperplasia. Endocrine Journal, 51, 595–600.

Ho SM, Tang WY, Belmonte J, Prins GS (2006) Developmental exposure estradiol and bisphenol A (BPA) increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant (PDE4D4) in the rat prostate Cancer Res, 66, 5624–5632.

Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T. (2002) Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reprod Toxicol, 16, 117-122.

Huc L, Lemarie A, Gueraud F, Helies-Toussaint C (2012). Low concentrations of bisphenol A induce lipid accumulation mediated by the production of reactive oxygen species in the mitochondria of HepG2 cells. Toxicology in vitro, 26, 709-717.

Hugo E.R., Brandebourg T.D., Woo J.G., Loftus J., Alexander J.W., Ben-Jonathan N.(2008). Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. Environmental Health Perspectives 116, 1642–1647.

Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ. (2003) Bisphenol a exposure causes meiotic aneuploidy in the female mouse. Curr Biol. 13, 546-553.

Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, Inaguma S, Suzuki S, Shirai T. (2003) Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. J Toxicol Sci. 28, 165-171.

Ike M, Chen MY, Danzl E, Sei K, Fujita M. (2006) Biodegradation of a variety of bisphenols under aerobic and anaerobic conditions. Water Sci. Technol. 53, 153–159.

Inagaki T, Frankfurt M and Luine V. (2012) Estrogen-induced memory enhancements are blocked by acute bisphenol A in adult female rats: role of dendritic spines. Endocrinology, 153, 3357-3367.

Indumathi D, Jayashree S, Selvaraj J, Sathish S, Mayilvanan C, Akilavalli N and Balasubramanian K. (2013). Effect of bisphenol-A on insulin signal transduction and glucose oxidation in skeletal muscle of adult male albino rat. Human & Experimental Toxicology, In Press.

Inoue K, Yamaguchi A, Wada M, Yoshimura Y, Makino T, Nakazaw H. (2001) Quantitative detection of bisphenol A and bisphenol A diglycidyl ether metabolites in human plasma by liquid chromatography-electrospray mass spectrometry. J Chromatogr B Biomed Sci Appl. 765, 121-126.

Inoue K., Wada M., HiguchiT., Osgio S., Umeda T., Yoshimura Y., Nahazawa H. (2002) Application of liquid chromatography-mass spectrometry to the quantification of bisphenaol A in humen semen. J Chromatogr B Analyt Technol Biomed Life Sci 773, 97 – 102.

Inoue K, Kawaguchi M, Funakoshi Y, Nakazawa H. (2003) Size-exclusion flow extraction of bisphenol A in human urine for liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 798, 17-23.

IPCS (2005) Chemical-specific adjustment factors for interspecies differences and human variability: Guidance document for use of data in dose/concentration-response assessment World Health Organization , Geneva, Switzerland.

Ishido M, Masuo Y, Kunimoto M, Oka S, Morita M. (2004). Bisphenol A causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. J Neurosci Res 76: 423-433.

Ishido M, Masuo Y, Terasaki M, Morita M. (2011). Rat Hyperactivity by Bisphenol A, but Not by Its Derivatives, 3-hydroxybisphenol A or Bisphenol A 3,4-quinone. Toxicology Letters 206: 300–305.

Iso T, Watanabe T, Iwamoto T, Shimamoto A, Furuichi Y.(2006) DNA damage caused by bisphenol A and estradiol through estrogenic activity. Biol Pharm Bull. 29, 206-210.

Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T, Tsugane S. (2007). Urinary bisphenol-A concentration in infertile Japanese women and its association with endometriosis: a cross-sectional study. Environmental Health and Preventive Medicine, 12, 258–264.

Izzotti A, Kanitz S, D'Agostini F, Camoirano A, De Flora S. (2009) Formation of adducts by bisphenol A, an endocrine disruptor, in DNA in vitro and in liver and mammary tissue of mice. Mutat Res. 679, 28-32.

Jacobi, U., Kaiser, M., Toll, R., Mangelsdorf, S., Audring, H., Otberg, N., Sterry, W., Lademann, J. (2007). Porcine ear skin: an in vitro model for human skin. Skin Res. Technol. 13, 19–24.

Jana SK, Okamoto T, Kugita T, Namba S. (2005) Selective synthesis of bisphenol F catalysed by microporous H-beta zeolite. Appl. Catal. A 288, 80–85.

Jasarevic E, Geary DC and Rosenfeld CS (2012). Sexually selected traits: a fundamental framework for studies on behavioral epigenetics. ILAR Journal, 53, 253-269.

Jayashree S, Indumathi D, Akilavalli N, Sathish S, Selvaraj J and Balasubramanian K, (2013). Effect of Bisphenol-A on insulin signal transduction and glucose oxidation in liver of adult male albino rat. Environmental Toxicology and Pharmacology, 35, 300-310

Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J, Lamartiniere CA (2009) Oral exposure to bisphenol a increases dimethylbenzanthracene-induced mammary cancer in rats. Environ Health Perspect. 117, 910-915.

Jenkins S, Wang J, Eltoum I, Desmond R and Lamartiniere CA, (2011). Chronic oral exposure to bisphenol A results in a nonmonotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice. Environmental Health Perspectives, 119, 1604-1609.

Jie H., Ke H., Qing Z., Lei C., Yongqiang W. and Zibin Z. (2006). Study on depolymerization of polycarbonate in supercritical ethanol. Polym. Degrad.Stabil. 91:2307–2314.

Jiménez-Díaz I, Zafra-Gómez A, Ballesteros O, Navea N, Navalón A, Fernández MF, Olea N, Vílchez JL. (2010) Determination of Bisphenol A and its chlorinated derivatives in placental tissue samples by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 878, 3363-3369.

Johnson GE, Parry EM.(2008). Mechanistic investigations of low dose exposures to the genotoxic compounds bisphenol-A and rotenone. Mutat Res. 651, 56-63.

Jones LP, Sampson A, Kang HJ, Kim HJ, Yi YW, Kwon SY, Babus JK, Wang A, Bae I. (2010) Loss of BRCA1 leads to an increased sensitivity to Bisphenol A. Toxicol Lett. 199, 261-268.

Jones BA, Shimell JJ and Watson NV (2011). Pre- and postnatal bisphenol A treatment results in persistent deficits in the sexual behavior of male rats, but not female rats, in adulthood. Hormones and Behavior, 59, 246-251.

Jones BA and Watson NV, (2012). Perinatal BPA exposure demasculinizes males in measures of affect but has no effect on water maze learning in adulthood. Hormones and Behavior, 61, 605-610.

Joskow R, Barr DB, Barr JR, Calafat AM, Needham LL, Rubin C. (2006) Exposure to bisphenol A from bis-glycidyl dimethacrylate-based dental sealants. Journal of the American Dental Association 2006; 137: 353-62.

Kabsy Y, Baudin G, Vinti H, Novellas S, Mannone L, Chevallier P, Mounier N (2010) Peripherally inserted central catheters in onco-hematology – Bull Cancer. 97, 1067-1071.

Kaddar N, Bendridi N, Harthé C, de Ravel MR, Bienvenu AL, Cuilleron CY, Mappus E, Pugeat M, Déchaud H. (2009) Development of a radioimmunoassay fort eh measurement of bisphenol A in biological samples. Anal Chim Acta. 645, 1-4, 2009.

Kaddar N, Harthe C, Déchaud H, Mappus E, Pugeat M (2008). Cutaneous penetration of bi¬sphenol A in pig skin. J Toxicol Environ Health (A) 71, 471–473.

Kang JH, Kondo F and Katayama Y (2006) Human exposure to bisphenol A. Toxicology 226, 79–89.

Kang Y.G., Kim J.Y., Kim J., Won P.J., Nam J.H. (2011) Release of bisphenol A from resin composite used to bond orthodontic lingual retainers. Am J Orthod Dentofacial Orthop. 6, 779-789.

Kasper-Sonnenberg M, Wittsiepe J, Koch HM, Fromme H and Wilhelm M, (2012) Determination of bisphenol a in urine from mother-child pairs-results from the duisburg birth cohort study, Germany. J Toxicol Environ Health A, 75, 429-437.

Kass L, Altamirano GA, Bosquiazzo VL, Luque EH and Munoz-de-Toro M. (2012) Perinatal exposure to xenoestrogens impairs mammary gland differentiation and modifies milk composition in Wistar rats. Reproductive Toxicology, 33, 390-400.

Katoh K, Matsuda A, Ishigami A, Yonekura S, Ishiwata H, Chen C, Obara Y. (2004) Suppressing effects of bisphenol A on the secretory function of ovine anterior pituitary cells. Cell Biol Int. 28, 463-469.

KEMI (2011) Bisfenol A - Rapport från ett regeringsuppdra. Rapport 2/11

Kendziorski JA, Kendig EL, Gear RB, Belcher SM (2012) Strain specific induction of pyometra and differences in immune responsiveness in mice exposed to 17α-ethinyl estradiol or the endocrine disrupting chemical bisphenol A. Reprod Toxicol. 34, 22-30

Kietzmann, M., Kranke, P., Moder, M., Schrader, S., Wahren, M., (1999). Application of deuterated compounds for investigations of percutaneous absorption of chemical substances. Isot. Environ. Health Stud. 35, 127–134.

Kiguchi M, Fujita S, Lee J, Shimizu N, Koshikawa N. (2007). Behavioral responses to methylphenidate and apomorphine in rats exposed neonatally to bisphenol-A. J Oral Sci 49: 311-318.

Kiguchi M, Fujita S, Oki H, Shimizu N, Cools AR, Koshikawa N. (2008). Behavioural characterisation of rats exposed neonatally to bisphenol-A: responses to a novel environment and to methylphenidate challenge in a putative model of attention-deficit hyperactivity disorder. J Neural Transm 115: 1079-1085.

Kim YH, Kim CS, Park S, Han SY, Pyo MY, Yang M. (2003). Gender differences in the levels of bisphenol A metabolites in urine. Biochem Biophys Res Commun 312:441–448.

Kim MR, Kim HS, Park DW, Lee JK. (2001) Synthesis of cyclic carbonates based on diglycidyl ether of bisphenol S by quaternary ammonium salts. React. Kinet. Catal. Lett. 72, 373–381.

Kim K, Park H, Yang W and Lee JH. (2011) Urinary concentrations of bisphenol A and triclosan and associations with demographic factors in the Korean population. Environmental Research, 111, 1280-1285.

Kim ME, Park HR, Gong EJ, Choi SY, Kim HS and Lee J. (2011) Exposure to bisphenol A appears to impair hippocampal neurogenesis and spatial learning and memory. Food and chemical toxicology, 49, 3383-3389.

Kim Y-J, Yun H-J and Ryu J-C. (2011) Expression profiling of estrogen responsive genes on bisphenol A, 4-nonylphenol and 17β-estradiol treatment using in house cDNA microarray. BioChip Journal, 5, 86-94.

Kingman A, Hyman J, Masten, SA, Jayaranm B, Smith C, Eicmiller F, Arnold MC, Wong PA, Schaeffer JM, Solanki S, Dunn WJ. (2012) Bisphenol A and other compounds in human saliva and urine associated with the placement of composite restorations. J Am Dent Assoc 143,1292-1302.

Kitamura S, Jinno N, Ohta S, Kuroki H, Fujimoto N. (2002) Thyroid hormonal activity of the flame retardants tetrabromobisphenol A and tetrachlorobisphenol A. Biochem. Biophys. Res. Commun. 293, 554–559.

Kitamura S, Suzuki T, Sanoh S, Kohta R, Jinno N, Sugihara K, Yoshihara S, Fujimoto N, Watanabe H, Ohta S. (2005) Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. Toxicol. Sci. 84, 249–259.

Kliegman RM, Stanton BF, St Geme III JW, Schor NF, Behrman RE (Editors), Nelson Textbook of Paediatrics. 19th Edition. 2011 Chapter 91 The high risk infant. R. M.. Elsevier Saunders, Philadelphia, PA, USA, 2011.

Kloukos D, Pandis N, Eliades T. (2013) Bisphenol-A and residual monomer leaching from orthodontic adhesive resins and polycarbonate brackets: a systematic review. Am J Orthod Dentofacial Orthop. 2013 Apr; 143(4 Suppl):S104-12.e1-2. doi: 10.1016/j.ajodo.2012.11.015

Kobayashi K, Miyagawa M, Wang RS, Sekiguchi S, Suda M, Honma T. (2002) Effects of in utero and lactational exposure to bisphenol A on somatic growth and anogenital distance in F1 rat offspring. Ind Health. 40, 375-381.

Kobayashi K, Ohtani K, Kubota H, Miyagawa M. (2010) Dietary exposure to low doses of bisphenol A: effects on reproduction and development in two generations of C57BL/6J mice. Congenit Anom (Kyoto). 50, 159-170.

Koch HM, Kolossa-Gehring M, Schroter-Kermani C, Angerer J and Bruning T. (2012). Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: A retrospective exposure evaluation. J Expo Sci Environ Epidemiol, 22, 610-616.

Komada M, Asai Y, Morii M, Matsuki M, Sato M and Nagao T. (2012). Maternal bisphenol A oral dosing relates to the acceleration of neurogenesis in the developing neocortex of mouse fetuses. Toxicology, 295, 31-38.

Kosarac I., Kubwabo C., Lalonde K. Foster W. (2012) A novel method for the quantitative determination of free and conjugated bisphenol A in human maternal and umbilical cord blood serum using a two-step solid phase extraction and gas chromatography/tandem mass spectrometry Journal of Chromatography B, 898, 90– 94, 2012.

Krieter D.H., Fischer R., Lemke H-D., Canaud B. and Wanner C. (2009). Bisphenol A (BPA) as an Uremic Toxin: Large Differences in Elution from Dialyzers and Elevated Plasma Levels in Maintenance Dialysis Patients. Poster presentation at the EDTA (European Dialysis and Transplant Association) 2009, Milan.

Krieter DH, Canaud B, Lemke HD, Rodriguez A, Morgenroth PA, von Appen K, Dragoun GP and Wanner C (2013). Bisphenol A in Chronic Kidney Disease. Artif Organs. 37, 283-290.

Kundakovic M, Gudsnuk K, Franks B, Madrid J, Miller RL, Perera FP, Champagne FA.(2013) Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. Proc Natl Acad Sci U S A. 110, 9956-9961.

Kurebayashi H, Betsui H, Ohno Y. (2003). Disposition of a low dose of 14C-bisphenol A in male rats and its main biliary excretion as BPA glucuronide. Toxicol Sci 73:17–25.

Kurebayashi H, Harada R, Stewart RK, Numata H, Ohno Y. (2002). Disposition of a low dose of bisphenol A in male and female cynomolgus monkeys. Toxicol Sci 68:32–42.

Kurebayashi H, Okudaira K, Ohno Y (2010). Species difference of metabolic clearance of bisphenol A using cryopreserved hepatocytes from rats, monkeys and humans. Toxicol Lett 198:210–215.

Kuruto-Niwa R, Nozawa R, Miyakoshi T, Shiozawa T, Terao Y. (2005) Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system. Environ. Toxicol. Pharmacol. 19, 121–130.

Kuruto-Niwa, R., Y. Tateoka, Y. Usuki and R. Nozawa (2007). Measurement of bisphenol A concentrations in human colostrum. Chemosphere 66, 1160-1164.

Kwon S, Stedman DB, Elswick BA, Cattley RC, Welsch F.(2000). Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. Toxicol Sci. 55, 399-406.

Lacroix M.Z., , S. Puel, S.H. Collet , T. Corbel, N. Picard- Hagen, PL. Toutain, C. Viguié, V. Gayrard, (2011) Simultaneous quantification of bisphenol A and its glucuronide metabolite (BPA-G) in plasma and urine: Applicability to toxicokinetic investigations Talanta 85, 2053– 2059, 2011.

LaKind, J.S., M. Goodman, and D.Q. Naiman (2012), Use of NHANES data to link chemical exposures to chronic diseases: a cautionary tale. PLoS One, 7 (12): p. e51086.

Lakind JS, Naiman DQ. (2008) Lakind JS, Naiman DQ. (2008). Bisphenol A (BPA) daily intakes in the United States: estimates from the 2003-2004 NHANES urinary BPA data. J Expo Sci Environ Epidemiol. 18, 608-615

Lakind JS, Naiman DQ. (2010). Daily intake of bisphenol A and potential sources of exposure: 2005–2006 National Health and Nutrition Examination Survey. J Expo Sci Environ Epidemiol advance online publication 17 Mar 2010; doi:10.1038/jes.2010.9

Lang I.A., Galloway T.S., Scarlett A., Henley W.E., Depledge M., Wallace R.B., Melzer D. (2008) Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 300, 1303–1310.

Lee C, Prins GS, Henneberry MO, Grayhack JT (1981) Effect of estradiol on the rat prostate in the presence and absence of testosterone and pituitary. J Andro 2:293-299)

Lee J, Lee SJ, Lim KT. (2012) CTB glycoprotein (75kDa) inhibits IgE releasing, TNF-α and IL-6 expressed by bisphenol A in vivo and in vitro. Food Chem Toxicol. 50, 2109-2117

Lee YJ, Ryu HY, Kim HK, Min CS, Lee JH, Kim E, Nam BH, Park JH, Jung JY, Jang DD, Park EY, Lee KH, Ma JY, Won HS, Im MW, Leem JH, Hong YC, Yoon HS. (2008). Maternal and fetal exposure to bisphenol A in Korea. Reprod Toxicol 25, 413–419.

Lewis JB, Rueggeberg FA, Lapp CA, Ergle JW. (1999) Identification and charakterisation of estrogen-like components in commercial resin-based dental restorative materials. Clin Oral Investig 3, 107 – 113.

Li DK, Zhou Z, Miao M, He Y, Qing D, Wu T, Wang J, Weng X, Ferber J, Herrinton LJ, Zhu Q, Gao E, Yuan W. (2010a). Relationship between urine bisphenol-A (BPA) level and declining male sexual function. Journal of Andrology, 31, 500–506.

Li DK, Zhou Z, Miao M, He Y, Wang J, Ferber J, Herrinton LJ, Gao E, Yuan W. (2010b). Urine bisphenol-A (BPA) level in relation to semen quality. Fertility and Sterility, 95, 625–630.

Li DK, Zhou Z, Qing D, He Y, Wu T, Miao M, Wang J, Weng X, Ferber JR, Herrinton LJ, Zhu Q, Gao E, Checkoway H, Yuan W. (2010c). Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. Human Reproduction (Oxford, England), 25, 519–527.

Liao C, Kannan K. (2012) Determination of free and conjugated forms of bisphenol A in human urine and serum by liquid chromatography-tandem mass spectrometry. Environ Sci Technol. 2012 May 1;46(9):5003-9. doi: 10.1021/es300115a. Epub 2012 Apr 19.

Lin Y, Sun X, Qiu L, Wei J, Huang Q, Fang C, Ye T, Kang M, Shen H and Dong S. (2013). Exposure to bisphenol A induces dysfunction of insulin secretion and apoptosis through the damage of mitochondria in rat insulinoma (INS-1) cells. Cell Death Dis, 4, e460.

Liu Z, Wolff MS, Moline J. (2005). Analysis of environmental biomarkers in urine using an electrochemical detector. J Chromatogr B Analyt Technol Biomed Life Sci. 819, 155-159.

Loganathan SN, Kannan K. (2011). Occurrence of Bisphenol A in Indoor Dust from Two Locations in the Eastern United States and Implications for Human Exposures. Arch Environ Contam Toxicol 61, 68-73.

Lopez-Cervantes J. and Paseiro-Losada P., (2003). Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. Food Additives & Contaminants, 20, 596-606.

Lopez-Espinosa MJ, Granada A, Araque P, Molina JM, Puertollano MC, Rivas A, Fernández MF, Cerrillo I, Olea-Serrano MF, López C, Olea N.(2007) Oestrogenicity of paper and cardboard extracts used as food containers. Food Additives and Contaminants, 24, 95-102.

Lucas JN, Rudmann DG, Credille KM, Irizarry AR, Peter A, Snyder PW (2007) The rat mammary gland: morphologic chamges as an indicator of systemic hormonal pertubations induced by xenobiotiocs. Toxiol Pathol 35, 199-207.

MacKay H, Patterson ZR, Khazall R, Patel S, Tsirlin D and Abizaid A. (2013) Organizational Effects of Perinatal Exposure to Bisphenol-A and Diethylstilbestrol on Arcuate Nucleus Circuitry Controlling Food Intake and Energy Expenditure in Male and Female CD-1 Mice. Endocrinology, 154, 1465-1475.

Maia J., Cruz J.M., Sendón R., Bustos J. et al. (2010). Effect of amines in the release of bisphenol A from polycarbonate baby bottles. Food Research International 43:1283-1288, 2010.

Manabe A, Kaneko S, Numazawa S, Itoh K, Inoue M, Hisamitsu H, Sasa R, Yoshida T. (2000) Detection of bisphenol-A in dental materials by gas chromatography-mass spectrometry. Dent Mater J. 19, 75-86.

Markham DA, Waechter JM Jr, Wimber M, Rao N, Connolly P, Chuang, JC, Hentges S, Shiotsuka RN, Dimond S, Chappelle AH. (2010). Development of a method for the determination of bisphenol A at trace concentrations in human blood and urine and elucidation of factors influencing method accuracy and sensitivity. J Anal Toxicol 34:293–303.

Marmugi A, Ducheix S, Lasserre F, Polizzi A, Paris A, Priymenko N, Bertrand-Michel J, Pineau T, Guillou H, Martin PG and Mselli-Lakhal L. (2012) Low doses of bisphenol A induce gene expression related to lipid synthesis and trigger triglyceride accumulation in adult mouse liver. Hepatology, 55, 395-407.

Marquet F., Payan J-P., Beydon D., Wathier L., Grandclaude M-C., Ferrari E. (2011). In vivo and ex vivo percutaneous absorption of [14C]-bisphenol A in rats: a possible extrapolation to human absorption? Arch Toxicol 85, 1035–1043.

Maserejian NN, Trachtenberg FL, Hauser R, McKinlay S, Shrader P, Tavares M, Bellinger DC. (2012a) Dental composite restorations and Psychosocial function in children. Pediatrics. 130, e328-338.

Masuda S, Terashima Y, Sano A, Kuruto R, Sugiyama Y, Shimoi K, Tanji K, Yoshioka H, Terao Y, Kinae N (2005) Changes in the mutagenic and estrogenic activities of bisphenol A upon treatment with nitrite. Mutat Res. 585, 137-146.

Masuo Y, Ishido M, Morita M, Oka S. (2004). Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats.Neural Plast 11: 59-76.

Matsuda S, Matsuzawa D, Ishii D, Tomizawa H, Sutoh C, Nakazawa K, Amano K, Sajiki J, Shimizu E. (2012). Effects of perinatal exposure to low dose of bisphenol A on anxiety like behavior and dopamine metabolites in brain. Prog Neuropsychopharmacol Biol Psychiatry. 39, 273-279.

Matsumoto J, Yakota H and Yuasa A, (2002). Developmental increases in rat hepatic microsomalUDP-glucuronosyltransferase activities toward xenoestrogens and decreases during pregnancy. Environmental Health Perspectives 110, 193-196

Matsumoto H, Adachi S and Suzuki Y (2005) BisphenolA in ambient air particulates responsible for the proliferation of MCF-7 human breast cancer cells and its concentration changes over 6 months. Arch Environ Contam Toxicol 48: 459–466.

Mazur Christopher S., John F. Kenneke, Janet K. Hess-Wilson, and John C. (2010) LiPSUPScomb Differences between Human and Rat Intestinal and Hepatic Bisphenol A Glucuronidation and the Influence of Alamethicin on In vitro Kinetic Measurements Drug Metabolism and Disposition 38:2232–2238, 2010.

Mazzaoui SA, Burrow MF, Tyas MJ, Rooney FR, Capon RJ. (2002) Long-term quantification of the release of monomers from dental resin composites and a resin-modified glass ionomer cement. J Biomed Mater Res. 63, 299-305.

McKeen LW (2012): The effect of sterilization methods on plastics abs elastomers, 3rd Edition Thechnology & Enginiiering Elsevier ed. 2012 pp 355.

Meeker JD, Calafat AM, Hauser R. (2010a) Urinary bisphenol A concentrations in relation to serum thyroid and reproductive hormone levels in men from an infertility clinic. Environ Sci Technol. 44, 1458-1463.

Meeker J.D., Ehrlich S., Toth T.L., Wright D.L., Calafat A.M., Trisini A.T., Ye X., Hauser R. (2010b) Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reproductive Toxicology 30, 532-539.

Melzer D, Gates P, Osborn NJ, Henley WE, Cipelli R, Young A, Money C, McCormack P, Schofield P, Mosedale D, Grainger D and Galloway TS. (2012) Urinary bisphenol a concentration and angiography-defined coronary artery stenosis. PLoS One, 7, e43378.

Melzer D, Harries L, Cipelli R, Henley W, Money C, McCormack P, Young A, Guralnik J, Ferrucci L, Bandinelli S, Corsi AM and Galloway T. (2011) Bisphenol A exposure is associated with in vivo estrogenic gene expression in adults. Environmental Health Perspectives, 119, 1788-1793.

Melzer D, Osborne NJ, Henley WE, Cipelli R, Young A, Money C, McCormack P, Luben R, Khaw KT, Wareham NJ and Galloway TS. (2012) Urinary bisphenol A concentration and risk of future coronary artery disease in apparently healthy men and women. Circulation, 125, 1482-1490.

Melzer D., Rice N.E., Lewis C., Henley W.E., Galloway T.S. (2010) Association of urinary bisphenol A concentration with heart disease: evidence from NHANES 2003/06. PloS One 5, e8673.

Mendes GC, Branddo TR, Silva CL (2007) Ethylene oxide sterilization of medical devices/ A review. Am J Infect Control. 35, 574-581.

Mendiola J, Jørgensen N, Andersson AM, Calafat AM, Ye X, Redmon JB, Drobnis EZ, Wang C, Sparks A, Thurston SW, Liu F, Swan SH. (2010). Are environmental levels of bisphenol A associated with reproductive function in fertile men? Environmental Health Perspectives, 118, 1286–1291.

Mercea P. (2009). Physicochemical Processes Involved in Migration of Bisphenol A from Polycarbonate. Journal of Applied Polymer Science 112 (2): 579-593.

Miao, M., Yuan W, Zhu G, He X, Li DK. (2011) In utero exposure to bisphenol-A and its effect on birth weight of offspring. Reprod Toxicol, 32, 64-68.

Mielke H, Gundert-Remy U. (2012) Physiologically based toxicokinetic modelling as a tool to support risk assessment: three case studies. J Toxicol. 2012; 2012:359471

Mielke, H. Partosch F., and Gundert-Remy U.(2011) The contribution of dermal exposure to the internal exposure of bisphenol A in man Toxicol Lett. 28, 190-198.

Mielke, H., Gundert-Remy, U. (2009) Bisphenol A levels in blood depend on age and exposure Tox. Lett. 190, 32–40.

Miyawaki J., Sakayama K., Kato H., Yamamoto H., Masuno H. (2007) Perinatal and postnatal exposure to bisphenol A increases adipose tissue mass and serum cholesterol level in mice. Journal of Atherosclerosis and Thrombosis, 14, 245–252.

Mok-Lin E., Ehrlich S., Williams P.L., Petrozza J., Wright D.L., Calafat A.M., Ye X., Hauser R. (2010) Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. International Journal of Andrology 33, 385–393.

Molina-Molina JM, Amaya E, Grimaldi M, Sáenz JM, Real M, Fernández MF, Balaguer P, Olea N. (2013). In vitro study on the agonistic and antagonistic activities of bisphenol-S

and other bisphenol-A congeners and derivatives via nuclear receptors. Toxicol. Appl. Pharmacol. 272, 127-136.

Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J, Russo J. (2008) Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. J Endocrinol. 196, 101-112.

Mørck, T.J., Sorda, G., Bechi, N., Rasmussen, B.S., Nielsen, J.B., Ietta, F., Rytting, E., Mathiesen, L., Paulesu, L., Knudsen, L.E., (2010). Placental transport and in vitro effects of Bisphenol A. Reprod. Toxicol. 30, 131–137.

Morrissey RE, George JD, Price CJ, Tyl RW, Marr MC, Kimmel CA. (1987). The developmental toxicity of bisphenol A in rats and mice. Fundam Appl Toxicol. 8, 571-582.

Mose T, Mathiesen L, Karttunen V, Nielsen JKS, Sieppi E, Kummu M, Mørck TA, Myöhänen K, Partanen H, Vähäkangas K, Knudsen LE and Myllynen P, (2012). Meta-analysis of data from human ex vivo placental perfusion studies on genotoxic and immunotoxic agents within the integrated European project NewGeneris. Placenta, 33, 433-439.

Murakami, K., Ohashi A., Hori H., Hibiya M. et al. (2007). Accumulation of bisphenol a in hemodialysis patients.Blood Purif 25(3): 290-294.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM.(2007). Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reprod Toxicol. 23, 383-390.

Nachman RM, Fox SD, Golden WC, Sibinga E, Veenstra TD, Groopman JD and Lees PSJ, (2013). Urinary Free Bisphenol A and Bisphenol A-Glucuronide Concentrations inNewborns. The Journal of Pediatrics, http://dx.doi.org/10.1016/j.jpeds.2012.11.083Nadal A. (2013) Obesity: Fat from plastics? Linking bisphenol A exposure and obesity. Nat Rev Endocrinol. 9, 9-10.

Nadal A. (2013) Obesity: Fat from plastics? Linking bisphenol A exposure and obesity. Nat Rev Endocrinol. 9, 9-10.

Nagel SC, Vom Saal Frederick S, Thayer Kristina AI, Dhar Minati G, Boechler Michael, Welshons Wade V,(1997) Relative Binding Affinity-Serum Modified Access (RBA-SMA) Assay Predicts the Relative In Vivo Bioactivity of the Xenoestrogens Bisphenol A and Octylphenol, Environmental Health Perspectives, 105, 70-76.

Nahar MS, Kim JH, Sartor MA, Dolinoy DC. (2013) Bisphenol A-associated alterations in the expression and epigenetic regulation of genes encoding xenobiotic metabolizing enzymes in human fetal liver. Environ Mol Mutagen. doi: 10.1002/em.21823. [Epub ahead of print]

Naik P, Vijayalaxmi KK. (2009). Cytogenetic evaluation for genotoxicity of bisphenol-A in bone marrow cells of Swiss albino mice. Mutat Res. 676, 106-112.

Nakajima Y, Goldblum RM, Midoro-Horiuti T (2012) Fetal exposure to bisphenol A as a risk factor for the development of childhood asthma: an animal model study. Environ Health. 11:8

Nakamura S, Tezuka Y, Ushiyama A, Kawashima C, Kitagawara Y, Takahashi K, Ohta S and Mashino T. (2011) Ipso substitution of bisphenol A catalyzed by microsomal cytochrome P450 and enhancement of estrogenic activity. Toxicology Letters, 203, 92-95.

Nanjappa MK, Simon L, Akingbemi BT (2012). The industrial chemical bisphenol A (BPA) interferes with proliferative activity and development of steroidogenic capacity in rat Leydig cells. Biol Reprod. 86, 135, 1-12.

Nishiyama T, Ogura K, Nakano H, Kaku T, Takahashi E, Ohkubo Y, Sekine, K, Hiratsuka A, Kadota S and Watabe T, (2002). Sulfation of Environmental Estrogens by Cytosolic Human Sulfotransferases. Drug Metabolism and Pharmacokinetics 17, 221-228.

Niwa, T., Fujimoto, M., Kishimoto, K., Yabusaki, Y., Ishibashi, F., Katagiri, M. (2001).Metabolism and interaction of bisphenol A in human hepatic cytochrome P450 and steroidogenic CYP17. Biol. Pharm. Bull. 24, 1064–1067.

Nunez AA, Kannan K, Giesy JP, Fang J, Clemens LG. (2001). Effects of bisphenol A on energy balance and accumu¬lation in brown adipose tissue in rats. Chemosphere 42(8):917–922.

Okuda, K., Takiguchi, M., Yoshihara, S. (2010). In vivo estrogenic potential of 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, an active metabolite of bisphenol A, inuterus of ovariectomized rat. Toxicol. Lett. 197, 7–11.

Okuda K., T. Fukuuchi, M. Takiguchi, S.Yoshihara (2011) Novel Pathway of Metabolic Activation of Bisphenol A-Related Compounds for Estrogenic Activity Drug Metabolism and Disposition 39:1696–1703, 2011

Olea N, Pulgar R, Pérez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A et al. (1996) Estrogenicity of resin-based composites and sealants used in dentistry. Environmental Health Perspectives 1996; 104: 298-305.

Otaka, H., A. Yasuhara and M. Morita (2003). "Determination of bisphenol A and 4-nonylphenol in human milk using alkaline digestion and cleanup by solid-phase extraction." Analytical Sciences 19, 1663-1666.

Pacchierotti F, Ranaldi R, Eichenlaub-Ritter U, Attia S, Adler ID. (2008). Evaluation of aneugenic effects of bisphenol A in somatic and germ cells of the mouse. Mutat Res. 651, 64-70.

Pacifici, G.M., Kubrich, M., Giuliani, L., de Vries, M., Rane, A. (1993). Sulphation and glucuronidation of ritodrine in human foetal and adult tissues. Eur. J. Clin. Pharmacol. 44, 259–264.

Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L, Tao L, Kannan K. (2008) Maternal bisphenol-A levels at delivery: a looming problem? J Perinatol 28, 258-263.

Palanza P, Gioiosa L, vom Saal FS, Parmigiani S. (2008). Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environ Res. 108, 150-157.

Partosch F., Mielke, H.,Gundert-Remy, U. (2013) Functional UGT-glucuronyltransferase 2B15 polymorphism and Bisphenol A concentrations in blood: results from physiologically based kinetic modelling Arch Toxicol 87, 1257-1264.

Patisaul H. (2010) Assessing risks from bisphenol-A. Evaluating human health risks from endocrine disruptors such as BPA is difficult, but animal studies suggest trouble is afoot. American Scientist 98, 30.

Patisaul HB, Bateman HL. (2008). Neonatal exposure to endocrine active compounds or an ERbeta agonist increases adult anxiety and aggression in gonadally intact male rats. Horm Behav. 53, 580-588.

Patisaul HB, Sullivan AW, Radford ME, Walker DM, Adewale HB, Winnik B, Coughlin JL, Buckley B, Gore AC. (2012) Anxiogenic effects of developmental bisphenol A exposure are associated with gene expression changes in the juvenile rat amygdala and mitigated by soy.PLoS One. 7, e43890.

Patisaul HB, Todd KL, Mickens JA, Adewale HB.(2009). Impact of neonatal exposure to the ERalpha agonist PPT, bisphenol-A or phytoestrogens on hypothalamic kisspeptin fiber density in male and female rats. Neurotoxicology. 30, 350-357.

Patterson TA, Twaddle NC , Roegge CS, Callicott RJ, Fisher JW and Doerge DR, (2013). Concurrent determination of bisphenol A pharmacokinetics in maternal and fetal rhesus monkeys. Toxicology and Applied Pharmacology, 267, 41–48.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, Silva MJ, Brambilla C, Pin I, Charles MA, Cordier S, Slama R. (2012) Exposure to phthalates and phenols during pregnancy and offspring size at birth. Environ Health Perspect. 120, 464-470. Erratum in: Environ Health Perspect. 120, 470, 2012.

Plastics Europe – Polycarbonate/BPA Group. (2007). Applications of Bisphenol-A. www.bisphenol-a-europe.org/uploads/BPA%20applications.pdf

Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, Waechter JM Jr. (2000). The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicol Sci 54, 3–18.

Prins GS, Ye SH, Birch L, Ho SM, Kannan K. (2011) Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats. Reprod Toxicol. 31, 1-9.

Ravoori S, Feng Y, Neale JR, Jeyabalan J, Srinivasan C, Hein DW, Gupta RC (2008). Dose-dependent reduction of 3,2'-dimethyl-4-aminobiphenyl-derived DNA adducts in colon and liver of rats administered celecoxib Mutat Res. 638:103-9.

Rhomberg LR, Goodman JE. (2012) Low dose effects and nonmonotonic responses of endocrine disrupting chemicals: has the case been made? Regul Toxicol Pharmacol. 64, 130-133, 2012.

Riu A, Grimaldi M, Le Maire A, Bey G, Phillips K, Boulahtouf A, Perdu E, Zalko D, Bourguet W, Balaguer P. (2011) Peroxisome proliferator-activated receptor γ is a target for halogenated analogs of bisphenol A. Environ. Health Perspect. 119, 1227–1232.

Rönn M, Kullberg J, Karlsson H, Berglund J, Malmberg F, Orberg J, Lind L, Ahlstrom H and Lind PM. (2013) Bisphenol A exposure increases liver fat in juvenile fructose-fed Fischer 344 rats. Toxicology, 303, 125-132.

Ropero A, Alonso Magdalena P, García-García E, Ripoll C, Fuentes E, Nadal A. (2008) Bisphenol A disruption of the endocrine pancreas and blood glucose homeostasis. Int. J. Androl. 31, 194–200.

Rubin et al. (2001 Rubin, B.S., et al., Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect, 2001. 109, 675-680.

Rudel RA, Camann DE, Spengler JD, Korn LR and Brody JG (2003) Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environ Sci Technol 37, 4543–4555.

Rwei SP, Kao SC, Liou GS, Cheng KC, Guo W. (2003) Curing and pyrolysis of epoxy resins containing 2-(6-oxido-6H-dibenz(c, e) (1,2) oxaphosphorin-6-yl)-1,4-naphthalenediol or bisphenol S. Colloid Polym. Sci. 281, 407–415.

Ryan K.K., Haller A.M., Sorrell J.E., Woods S.C., Jandacek R.J., Seeley R.J. (2010) Perinatal exposure to bisphenol-A and the development of metabolic syndrome in CD-1 mice. Endocrinology 151, 2603–2612.

Ryan BC, Vandenbergh JG. (2006). Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. Horm Behav. 50, 85-93.

Sajiki J, Hasegawa Y, Hashimoto H, Makabe Y, Miyamoto F, Yanagibori R, Shin J, Shimidzu Y and Morigami T (2008) Determination of bisphenol A (BPA) in plasma of hemodialysis patients using three methods: LC/ECD, LC/MS, and ELISA. Toxicology Mechanisms and Methods 18: 733-738.

Sajiki J, Yonekubo J (2004). Leaching of bisphenol A (BPA) from polycarbonate plastic to water containing amino acids and its degradation by radical oxygen species. Chemosphere, 55, 861–867.

Sakurai H., Maeda M., Miyahara K., Nakayama M. et al. (2002). Extraction of bisphenol-A from cardiopulmonary bypass circuit. Kyobu Geka 55(9):770-772 (only abstract available in english)

Sargis R.M., Johnson D.N., Choudhury R.A., Brady M.J. (2010) Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. Obesity (Silver Spring, Md.) 18, 1283–1288.

Sasaki N, Okuda K, Kato T, Kakishima H, Okuma H, Abe H, Tuchida K, Kubono K (2005) Salivary bisphenol-A levels detected by ELISA after restoration with composite resin. J Mater Sci: Mater Med 16, 297 – 300.

Satoh K, Ohyama K, Aoki N, Iida M, Nagai F. (2004). Study on anti-androgenic effects of bisphenol a diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and their derivatives using cells stably transfected with human androgen receptor, AR-EcoScreen. Food Chem Toxicol.42, 983-993.

Savage JH, Matsui EC, Wood RA, Keet CA (2012) Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. J Allergy Clin Immunol, 130, 453-460 e457

SCENIHR (2008). Opinion on the safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk. http://ec.europa.eu/health/archive/ph_risk/committees/04_scenihr/docs/scenihr_o_014.pdf

SCENIHR (2012) Memorandum on the use of the scientific literature for human health risk assessment purposes – weighing of evidence and expression of uncertainty.http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_s_001.pdf

Schönfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. (2002). Parent bisphenol A accumulation in the human maternalfetal-placental unit. Environ Health Perspect 110, A703–A707.

Schöringhumer K, Cichna-Markl M.(2007). Sample clean-up with sol-gel enzyme and immunoaffinity columns for the determination of bisphenol A in human urine. J Chromatogr B Analyt Technol Biomed Life Sci. 850, 361-369.

Schmalz G, Preiss A, Arenholtt-Bindslev D (1999) Bisphenol A content of resin monomers and related degradation products. Clin Oral Investig 3, 114-119.

Seki N, Nakajima M, Kishikawa R, Hosaka K, Foxton RM, Tagami J (2011). The influence of light intensities irradiated directly and indirectly through resin composite to self-etch adhesives on dentin bonding. Dent Mater J. 30, 315-322.

Shankar A and Teppala S. (2012a) Urinary bisphenol A and hypertension in a multiethnic sample of US adults. J Environ Public Health, 2012, 481641.

Shankar A, Teppala S and Sabanayagam C. (2012b) Bisphenol A and Peripheral Arterial Disease: Results from the NHANES. Environmental Health Perspectives, 120, 1297-1300.

Shankar A, Teppala S and Sabanayagam C. (2012c). Urinary bisphenol a levels and measures of obesity: results from the national health and nutrition examination survey 2003-2008. ISRN Endocrinol, 2012, 965243.

Shelnutt S, Kind J, Allaben W (2013). Bisphenol A: Update on newly developed data and how they address NTP's 2008 finding of "some concern". Food Chem Toxicol 57, 284-295.

Sheng ZG, Tang Y, Liu YX, Yuan Y, Zhao BQ, Chao XJ and Zhu BZ. (2012). Low concentrations of bisphenol a suppress thyroid hormone receptor transcription through a nongenomic mechanism. Toxicology and Applied Pharmacology, 259, 133-142.

Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, Ohno Y and Ozawa S, (2002). Sulphation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. Toxicol In vitro 16, 549-556.

Shin BS, Kim CH, Jun YS, Kim DH, Lee BM, Yoon CH, Park EH, Lee KC, Han SY, Park KL, Kim HS, Yoo SD. (2004). Physiologically based pharmacokinetics of bisphenol A. J. Toxicol. Environ. Health A 67:1971–1985.

Shin BS, Hwang SW, Bulitta JB, Lee JB, Yang SD, Park JS, Kwon MC, Kim do J, Yoon HS and Yoo SD. (2010) Assessment of bisphenol A exposure in Korean pregnant women by physiologically based pharmacokinetic modeling. J Toxicol Environ Health A, 73, 1586-1598.

Shintani H and Hayashi F (2011) Determination of the Endocrine Disrupter Bisphenoi-A in the Blood of Uremia Patients Treated by Dialysis. Pharm Anal Acta S11:001. doi:10.4172/2153-2435.S11-001

Shintani H. (2001).Determination of the endocrine disrupter Bisphenol-A in the bllod of uremia patients treated by dialysis. Chromatographia 53 (5/6(331-333).

Shintani H., Suzuki E. and Sakurai M. (2003). Determination of compounds inhibiting bacterial growth ins sterilized medical devices. Chromatographia 58 (3/4): 193-199.

Sieli P.T., Jašarevic E, Warzak DA, Mao J, Ellersieck MR, Liao C, Kannan K, Collet SH, Toutain PL, vom Saal FS, Rosenfeld CS. (2011) Comparison of serum bisphenol A concentrations in mice exposed to bisphenol A through the diet versus oral bolus exposure Envir Health Perspect 119, 1260-1265.

Silver MK, O'Neill MS, Sowers MR and Park SK. (2011) Urinary bisphenol A and type-2 diabetes in U.S. adults: data from NHANES 2003-2008. PLoS One, 6, e26868.

Snyder RW, Maness SC, Gaido KW, Welsch F, Sumner SCJ, Fennell TR. (2000). Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol 168:225–234.

Socialstyrelsen (2012) (Swedish National Board of Health and Welfare), Bisfenol A i dentala material. – Bisphenol A in dental materials 2012-6-48. ISBN:978-91-87169-48-9, 59pp. http://www.socialstyrelsen.se/publikationer2012/2012-6-48

Somm E., Schwitzgebel V.M., Toulotte A., Cederroth C.R., Combescure C., Nef S., Aubert M.L., Hüppi P.S.(2009) Perinatal exposure to bisphenol A alters early adipogenesis in the rat. Environmental Health Perspectives 117,1549–1555, 2009.

Song L, Xia W, Zhou Z, Li Y, Lin Y, Wei J, Wei Z, Xu B, Shen J, Li W and Xu S. (2012) Low-level phenolic estrogen pollutants impair islet morphology and beta-cell function in isolated rat islets. J Endocrinol, 215, 303-311.

Soriano S, Alonso-Magdalena P, García-Arévalo M, Novials A, Muhammed SJ, Salehi A, Gustafsson JA, Quesada I, Nadal A.(2012) Rapid insulinotropic action of low doses of bisphenol-A on mouse and human islets of Langerhans: role of estrogen receptor β. PLoS One. 7, e31109.

Spanier AJ, Kahn RS, Kunselman AR, Hornung R, Xu Y, Calafat AM, Lanphear BP (2012) Prenatal exposure to bisphenol A and child wheeze from birth to 3 years of age. Environmental Health Perspectives, 120, 916-920.

Spitsbergen JC, Loewrigkeit P, Bluestein C, Sugarman J, Lauze WL. (1971) 26th Annual Techn. Conf. Reinforced Plastics/Composites Division, The Society of the Plastics Industry, Inc., Section 19-C, 1

Stachel B, Ehrhorn U, Heemken OP, Lepom P, Reincke H, Sawal O, Theobald N. (2003) Xenoestrogens in the River Elbe and its tributaries. Environ. Pollut. 124, 497–507.

Stahlhut RW, Welshons WV, Swan SH. (2009) Bisphenol A data in NHANES suggest longer than expected half-life, substantial non-food exposure, or both. Environ Health Perspect. 2009;117:784–789.

Staples CA, Dorn PB, Klecka GM, Oblock ST and Harris LR (1998) A review of the environmental fate, effects, and exposures of bisphenolA. Chemosphere 36: 2149–2173.

Stump DG; Beck MJ; Radovsky A; Garman RH; Freshwater L; Sheets LP; Marty MS; Waechter JM; Dimond SS; Van Miller JP; Shiotsuka RN; Beyer D; Chappelle AH; Hentges SG, (2010). Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats. Toxicological Sciences 115, 167-182.

Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K. (2005). Exposure to bisphenol A is associated with recurrent miscarriage. Human Reproduction (Oxford, England), 20, 2325–2329.

Sun Y, Wada M, Kuroda, N, Hirayama K, Nakazawa H and Nakashima K (2001) Simultaneous determination of phenolic xenoestrogens by solid-phase extraction and high-performance liquid chromatography with fluorescence detection. Analytical Sciences 17: 697-702.

Sun, Y., M. Irie, N. Kishikawa, M. Wada, N. Kuroda and K. Nakashima (2004). "Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection." Biomed Chromatogr 18(8): 501-507.

Sunitha C, Kailasam V, Padmanabhan S, Chitharanjan AB (2011). Bisphenol A release from an orthodontic adhesive and its correlation with the degree of conversion on varying light-curing tip distances. Amer J Orthodont Dentofac Orthoped, 140, 239-244.

Susiarjo M, Hassold TJ, Freeman E, Hunt PA.(2007). Bisphenol A exposure in utero disrupts early oogenesis in the mouse. PLoS Genet. 3 (1):e5.

Suzuki K, Ishikawa K, Sugiyama K, Furuta H, Nishimura F. (2000). Content and release of bisphenol A from polycarbonate dental products. Dent Mater J 19, 389-395.

Takahashi A, Higashino F, Aoyagi M, Kyo S, Nakata T, Noda M, Shindo M, Kohgo T, Sano H (2004). Bisphenol A from dental polycarbonate crown upregulates the expression of hTERT. J Biomed Mat Res, 71B, 214–221.

Takashima Y et al. (2001). Lack of effects of bisphenol A in maternal rats or treatment on response of their offspring to N-nitrosobis(2-hydroxypropyl)amine. Journal of Toxicologic Pathology, 14:87–98).

Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y. (2004). Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocrine Journal, 51(2):165–169.

Tanabe N, Yoshino H, Kimoto T, Hojo Y, Ogiue-Ikeda M, Shimohigashi Y and Kawato S. (2012). Nanomolar dose of bisphenol A rapidly modulates spinogenesis in adult hippocampal neurons. Molecular and Cellular Endocrinology, 351, 317-325.

Tang WY, Morey LM, Cheung YY, Birch L, Prins GS, Ho SM (2012) Neonatal exposure to estradiol/bisphenol A alters promoter methylation and expression of Nsbp1 and Hpcal1 genes and transcriptional programs of Dnmt3a/b and Mbd2/4 in the rat prostate gland throughout life. Endocrinology. 153, 42-55.

Tayama S, Nakagawa Y, Tayama K. (2008) Genotoxic effects of environmental estrogen-like compounds in CHO-K1 cells. Mutat Res. 649, 114-125.

Taylor JA,. Welshons WV, Vom Saal FS (2008) No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24 h after administration in neonatal female mice Reproductive Toxicology 25, 169–176.

Taylor, J.A., Vom Saal, F.S., Welshons, W.V., Drury, B., Rottinghaus, G., Hunt, P.A.,Toutain, P.L., Laffont, C.M., VandeVoort, C.A., (2011). Similarity of bisphenol A

pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. Environ. Health Persp. 119, 422–430.

Teeguarden JG, Waechter JM Jr, Clewell HJ 3rd, Covington TR, Barton HA. (2005). Evaluation of oral and intravenous route pharmacokinetics, plasma protein binding, and uterine tissue dose metrics of bisphenol A: A physiologically based pharmacokinetic approach. Toxicol Sci 85, 823–838.

Teeguarden J, Hanson-Drury S, Fisher JW, Doerge DR (2013) Are typical human serum BPA concentrations measureable and sufficient to be estrogenic in the general population? Food Chem Toxicol. 62, 949-963.

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

Terasaki M, Nomachi M, Edmonds JS, Morita M. (2004) Impurities in industrial grade 4,4'-isopropylidene diphenol (bisphenol A): possible implications for estrogenic activity. Chemosphere. 2004 May; 55(6):927-31. PubMed PMID: 15041297.

Terasaki M, Shiraishi F, Nishikawa T, Edmonds JS, Morita M, Makino M. Estrogenic activity of impurities in industrial grade bisphenol A. Environ SciTechnol. 2005 May 15;39(10):3703-7.

Tharp AP, Maffini MV, Hunt PA, VandeVoort CA, Sonnenschein C and Soto AM. (2012) Bisphenol A alters the development of the rhesus monkey mammary gland. Proceedings of the National Academy of Sciences of the United States of America, 109, 8190-8195.

Tian YH, Baek JH, Lee SY, Jang CG. (2010) Prenatal and postnatal exposure to bisphenol a induces anxiolytic behaviors and cognitive deficits in mice. Synapse. 64, 432-439.

Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS. (2005) Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. Proc Natl Acad Sci U S A. 102, 7014-7019.

Tiwari D, Kamble J, Chilgunde S, Patil P, Maru G, Kawle D, Bhartiya U, Joseph L, Vanage G. (2012) Clastogenic and mutagenic effects of bisphenol A: an endocrine disruptor. Mutation Research, 743, 83-90.

Tiwari D and Vanage G. (2013). Mutagenic effect of Bisphenol A on adult rat male germ cells and their fertility. Reproductive Toxicology, 40, 60-68.

Tominaga T, Negishi T, Hirooka H, Miyachi A, Inoue A, Hayasaka I, Yoshikawa Y, (2006). Toxicokinetics of bisphenol A in rats, monkeys and chimpanzees by the LC–MS/MS method Toxicology 226, 208–217.

Trasande, L., Attina TM, Blustein J. (2012) Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA, 308, 1113-1121.

Trdan Lušin T, Roskar R and Mrhar A. (2012) Evaluation of bisphenol A glucuronidation according to UGT1A1*28 polymorphism by a new LC-MS/MS assay. Toxicology, 292, 33-41.

Tsukioka T, Terasawa J, Sato S, Hatayama Y, Makino T, Nakazawa H. (2004). Development of analytical method for determining trace amounts of BPA in urine samples and estimation of exposure to BPA. J Environ Chem 14:57–63.

Tsutsui T, Tamura Y, Suzuki A, Hirose Y, Kobayashi M, Nishimura H, Metzler M, Barrett JC. (2000) Mammalian cell transformation and aneuploidy induced by five bisphenols. Int J Cancer. 86, 151-154.

Tsutsui T, Tamura Y, Yagi E, Hasegawa K, Takahashi M, Maizumi N, Yamaguchi F, Barrett JC. (1998) Bisphenol-A induces cellular transformation, aneuploidy and DNA adduct formation in cultured Syrian hamster embryo cells. Int J Cancer.75, 290-294.

Twaddle NC, Churchwell MI, Vanlandingham M, Doerge DR. (2010). Quantification of deuterated bisphenol A in serum, tissues, and excreta from adult Sprague-Dawley rats using liquid chromatography with tandem mass spectrometry. Rapid Commun Mass Spectrom 24, 3011–3020.

Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter JM Jr. (2008) Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicol Sci. 104, 362-384.

Tyl RW, Myers CB, Marr MC, Castillo NP, Veselica MM, Joiner RL, Dimond SS, Van Miller JP, Stropp GD, Waechter JM, Hentges SG. (2008) One-generation reproductive toxicity study of dietary 17beta-estradiol (E2; CAS No. 50-28-2) in CD-1 (Swiss) mice. Reprod Toxicol 25, 144 – 160.

Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM. (2002) Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol Sci. 68, 121-146.

Ulutaş OK, Yildiz N, Durmaz E, Ahbab MA, Barlas N and Cok I. (2011). An in vivo assessment of the genotoxic potential of bisphenol A and 4-tert-octylphenol in rats. Archives of Toxicology, 85, 995-1001.

Upmeier A, Degen GH, Diel P, Michna H, Bolt HM. (2000). Toxicokinetics of bisphenol A in female DA/Han rats after single i.v. and oral administration. Arch Toxicol 74:431–436.

US FDA (Food and Drug Administration), 2010a. Update on Bisphenol A for Use in Food Contact Applications: January 2010 Available from http://www.fda.gov/NewsEvents/PublicHealthFocus/ucm197739.htm

US FDA (Food and Drug Administration), (2010b). Memorandum of 11/16/2009, Summary of Bisphenol A Biommonitoring Studies [FDA-2010-N-0100-0001] Available from http://www.regulations.gov/search/Regs/home.htmL#docketDetail?R=FDA-2010-N-0100

US FDA (US Food and Drug Administration) Safety, (2002). Assessment of di(2-ethylhexyl)phthalate (DEHP) released from medical devices. Center for Devices and Radiological Health, 2002.

US FDA, (2013). Bisphenol A (BPA): Use in food contact application. US Food and Drug Administration, Silver Spring, MD, USA. <http://www.fda.gov/NewsEvents/

US NTP (1982). Carcinogenesis bioassay of bisphenol A (CAS No. 80-05-7) in F344 rats and B6C3F1 mice (feed study). Research Triangle Park, NC, United States Department of Health and HumanServices, National Toxicology Program (TR-215; http://ntp.niehs.nih.gov/go/14366).

USA National Toxicology Program (NTP, 2008) NTP-CERHR (2008). Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. http://ntp.niehs.nih.gov/ntp/ohat/bisphenol/bisphenol.pdf

Vallo CI, Schroeder WF (2005). Properties of acrylic bone cements formulated with Bis-GMA. J Biomed Mat Res Part B: Appl Biomat. 748, 676-685.

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007). Human exposure to bisphenol A (BPA). Reprod Toxicol 24: 139-177.

Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, Sonnenschein C, Rubin BS, Soto AM.(2008) Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice. Reprod Toxicol. 26, 210-219.

Vandenberg LN, Chauhoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G. (2010a). Urinary, circulating and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect 118, 1055–1070.

Vandenberg LN, Chahoud I, Padmanabhan V, Paumgartten FJ, Schoenfelder G. (2010b). Biomonitoring studies should be used by regulatory agencies to assess human exposure levels and safety of bisphenol A. Environ Health Perspect 118, 1051–1054.

Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, Von Saal FS, Welshons WV, Zoeller RT, Myers JP. (2012) Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev 33, 378-455.

Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM. (2013) The male mammary gland: a target for the xenoestrogen bisphenol A. Reprod Toxicol. 37, 15-23.

Vandentorren S, Morin L, Sarter H, Bidondo ML, Oleko A and Leridon H (2011) Bisphenol-A and phthalates contamination of urine samples by catheters in the Elfe pilot study: Implications for large-scale biomonitoring studies. Environmental research. 06:761-64.

Van Landuyt KL, Nawrot T, Geebelen B, De Munck J, Snauwaert J, Yoshihara K, Scheers H, Godderis L, Hoet P, Van Meerbeek B (2011) How much do resin-based dental materials release? A meta-analytical approach. Dental Materials, 27, 723-747.

Van Landuyt KL, Nawrot T, Geebelen B, De Munck J, Snauwaert J, Yoshihara K, Scheers H, Godderis L, Hoet P, Van Meerbeek B. (2013) Corrigendum to "How much do resin-based dentalmaterials release? A meta-analytical approach" [Dental 27 (8) (2011) 723–747] Dental materials 29, 919.

Viberg H, Fredriksson A, Buratovic S and Eriksson P. (2011) Dose-dependent behavioral disturbances after a single neonatal Bisphenol A dose. Toxicology, 290, 187-194.

Viñas P, Campillo N, Martínez-Castillo N, Hernández-Córdoba M. (2010) Comparison of two derivatization-based methods for solid-phase microextraction-gas chromatography-mass spectrometric determination of bisphenol A, bisphenol S and biphenol migrated from food cans. Anal Bioanal Chem. 397, 115-125

Vinggaard AM, Korner W, Lund KH, Bolz U and Petersen JH (2000) Identification and quantification of estrogenic compounds in recycled and virgin paper for household use as determined by an in vitro yeast estrogen screen and chemical analysis. Chem Res Toxicol 13: 1214–1222.

Völkel W, Bittner N and Dekant W, (2005). Quantitation of Bisphenol A and Bisphenol A Glucuronide in Biological Samples by High Performance Liquid Chromatography-Tandem Mass Spectrometry. Drug metabolism and disposition: the biological fate of chemicals 33, 1748-1757, 2005.

Völkel W, Colnot T, Csanady GA, Filser JG and Dekant W, (2002). Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chem.Res.Toxicol. 15, 1281-1287,2002.

Völkel W, Kiranoglu M, and Fromme H (2008) Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. Toxicol Lett 179: 155–162, 2008.

Von Goetz N, Wormuth M, Scheringer M and Hungerbühler K (2010) Bisphenol A: How the most relevant exposure sources contribute to total consumer exposure. Risk Anal 30: 473-487.

Wada K., Sakamoto H., Nishikawa K., Sakuma S., Nakajima A., Fujimoto Y., Kamisaki Y.: Life style–related diseases of the digestive system: endocrine disruptors stimulate lipid accumulation in target cells related to metabolic syndrome. Journal of Pharmacological Sciences 105: 133–137 (2007).

Wang T, Li M, Chen B, Xu M, Xu Y, Huang Y, Lu J, Chen Y, Wang W, Li X, Liu Y, Bi Y, Lai S, Ning G. (2012) Urinary bisphenol A (BPA) concentration associates with obesity and

insulin resistance. J Clin Endocrinol Metab. 97, E223-7. doi: 10.1210/jc.2011-1989. Epub 2011 Nov 16.

Wang J, Sun B, Hou M, Pan X, Li X. (2013) The environmental obesogen bisphenol A promotes adipogenesis by increasing the amount of 11β-hydroxysteroid dehydrogenase type 1 in the adipose tissue of children. Int J Obes (Lond). 37, 999-1005.

Watanabe M, Hase T, Imai Y. (2001) Change in the bisphenol A content in a polycarbonate orthodontic bracket and its leaching characteristics in water. Dent Mater J.; 20, 353-358.

Watanabe M, Fukazawa H, Shiraishi F, Shiraishi H, Shiozawa T, Terao T. (2004) Analysis and estrogenic activity of bisphenol A and other chemicals released from waste paper by pulping. J. Environ. Chem. 14, 65–71.

Watanabe M. (2004) Degradation and formation of bisphenol A in polycarbonate used in dentistry. J Med Dent Sci. 51:1-6.

Weber Lozada K, Keri RA. (2011) Bisphenol A increases mammary cancer risk in mouse models of breast cancer. Biol Reprod. 85, 490-497.

Wei J., Lin Y., Li Y., Ying Ch., Chen J., Song L., Zhou Z., Lv Z., Xia W., Chen X., Xu S.(2011) Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high fat diet. Endocrinology 152, 3049-3061 (2011)

WHO (2010) Joint FAO/WHO Expert Meeting to Review Toxicological and Health Aspects of Bisphenol A. Summary report. Available from:

http://www.who.int/foodsafety/chem/chemicals/bisphenol_release/en/index.htmL

WHO/FAO (2010) Joint FAO/WHO Expert Meeting to Review Toxicological and Health Aspects of Bisphenol A. Summary Report including Report of Stakeholder Meeting on Bisphenol A. http://www.who.int/foodsafety/chem/chemicals/BPA_Summary2010.pdf

Willhite CC, Ball GL, McLellan CJ. (2008). Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. J Toxicol Environ Health B Crit Rev 11, 69–146.

Wilson NK, Chuang JC, Morgan MK, Lordo RA, and Sheldon, LS (2007) An observational study of potential exposures of preschool children to pentachlorophenol, Bisphenol-A, and nonylphenol at home and daycare. Environ Res 103: 9–20.

Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J, Calafat AM. (2008b). Prenatal phenol and phthalate exposures and birth outcomes. Environmental Health Perspectives, 116, 1092–1097.

Wolff MS, Teitelbaum SL, Windham G, Pinney SM, Britton JA, Chelimo C, Godbold J, Biro F, Kushi LH, Pfeiffer CM, Calafat AM. (2007). Pilot study of urinary biomarkers of phytoestrogens, phthalates, and phenols in girls. Environmental Health Perspectives, 115, 116–121.

Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S, Forman J. (2008a). Environmental exposures and puberty in inner-city girls. Environmental Research, 107, 393–400.

Wolff MS, Teitelbaum SL, Pinney SM, Windham G, Liao L, Biro F, Kushi LH, Erdmann C, Hiatt RA, Rybak ME, Calafat AM; Breast Cancer and Environment Research Centers. (2010). Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls. Environmental Health Perspectives, 118, 1039–1046.

Wolstenholme, JT, Rissman EF and Connelly JJ. (2011) The role of Bisphenol A in shaping the brain, epigenome and behavior. Hormones and Behavior,59, 296-305.

Wolstenholme JT, Edwards M, Shetty SR, Gatewood JD, Taylor JA, Rissman EF and Connelly JJ. (2012) Gestational exposure to bisphenol a produces transgenerational changes in behaviors and gene expression. Endocrinology, 153, 3828-3838.

Xu X, Ye Y, Li T, Chen L, Tian D, Luo Q and Lu M. (2010) Bisphenol-A rapidly promotes dynamic changes in hippocampal dendritic morphology through estrogen receptor-mediated pathway by concomitant phosphorylation of NMDA receptor subunit NR2B. Toxicology and Applied Pharmacology, 249, 188-196.

Xu XH, Wang YM, Zhang J, Luo QQ, Ye YP and Ruan Q, (201)0. Perinatal exposure to bisphenol-A changes N-methyl-D-aspartate receptor expression in the hippocampus of male rat offspring. Environmental toxicology and chemistry, 29, 176-181.

Xu X, Tian D, Hong X, Chen L and Xie L. (2011) Sex-specific influence of exposure to bisphenol-A between adolescence and young adulthood on mouse behaviors. Neuropharmacology, 61, 565-573.

Xu X, Hong X, Xie L, Li T, Yang Y, Zhang Q, Zhang G and Liu X. (2012) Gestational and lactational exposure to bisphenol-A affects anxiety- and depression-like behaviors in mice. Hormones and Behavior, 62, 480-490.

Xu X, Xie L, Hong X, Ruan Q, Lu H, Zhang Q, Zhang G and Liu X. (2013). Perinatal exposure to bisphenol-A inhibits synaptogenesis and affects the synaptic morphological development in offspring male mice. Chemosphere 91, 1073-1081.

Xu X, Liu X, Zhang Q, Zhang G, Lu Y, Ruan Q, Dong F, Yang Y. (2013) Sex-specific effects of bisphenol-A on memory and synaptic structural modification in hippocampus of adult mice.) Horm Behav. 2013 May;63(5):766-75. doi: 10.1016/j.yhbeh.2013.03.004. Epub 2013 Mar 19.

Yamasaki K, Takeyoshi Y, Yakabe Y, Sawaki M, Imatanaka N, Takatsuki M. (2002) Comparison of gene reporter assay and immature rat uterotrophic assay of twenty-three chemicals. Toxicology 170, 21–30.

Yamasaki H., Nagake Y., Makino H. (2001). Determination of Bisphenol A in Effluents of Hemodialyzers. Nephron 88, 376-378.

Yang M, Ryu JH, Jeon R, Kang D, Yoo KY. (2009). Effects of bisphenol A on breast cancer and its risk factors. Archives of Toxicology 83, 281–285.

Yang X, Doerge DR and Fisher JW, (2013) Prediction and evaluation of route dependent dosimetry of BPA in rats at different life stages using a physiologically based pharmacokinetic model. Toxicology and Applied Pharmacology, 270, 45-59.

Ye, X., Bishop AM, Needham LL, Calafat AM. (2008). "Automated on-line column-switching HPLC-MS/MS method with peak focusing for measuring parabens, triclosan, and other environmental phenols in human milk." Anal Chim Acta 622, 150-156.

Ye X, Bishop AM, Reidy JA, Needham LL, Calafat AM. (2007). Temporal stability of the conjugated species of bisphenol A, parabens, and other environmental phenols in human urine. J Expo Sci Environ Epidemiol 17:567–572.

Ye X, Kuklenyik Z, Needham LL, Calafat AM. (2005). Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatographytandem mass spectrometry. Anal Bioanal Chem 383:638–644.

Ye, X., Kuklenyik Z, Needham LL, Calafat AM. (2006). Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching-high performance liquid chromatography-isotope dilution tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 831, 110-115.

Ye X, Wong LY, Bishop AM and Calafat AM. (2011) Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. Environmental Health Perspectives, 119, 983-988.

Yi, B., C. Kim and M. Yang (2010). "Biological monitoring of bisphenol A with HLPC/FLD and LC/MS/MS assays." J Chromatogr B Analyt Technol Biomed Life Sci 878, 2606-2610.

Yoshida M, Shimomoto T, Katashima S, Watanabe G, Taya K, Maekawa A. (2004). Maternal exposure to low doses of bisphenol a has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. J Reprod Dev. 50, 349-360.

Yoshihara S, Makishima M, Suzuki N, Ohta S. (2001). Metabolic activation of bisphenol A by rat liver S9 fraction. Toxicol. Sci. 62, 221–227.

Yoshihara S, Mizutare T, Makishima M, Suzuki N, Fujimoto N, Igarashi K, and Ohta S (2004) Potent estrogenic metabolites of bisphenol A and bisphenol B formed by rat liver S9 fraction: their structures and estrogenic potency. Toxicol Sci 78, 50–59.

You L, Zhu X, Shrubsole MJ, Fan H, Chen J, Dong J, Hao CM, Dai Q. (2011). Renal function, bisphenol A, and alkylphenols: results from the National Health and Nutrition Examination Survey (NHANES 2003-2006). Environ Health Perspect. 119, 527-533.

Zalko D, Jacques C, Duplan H, Bruel S and Perdu E (2011) Viable skin efficiently absorbs and metabolizes bisphenol A. Chemosphere. 82, 424-430.

Zhang H, Yamada H, Tsuno H. (2008). Removal of endocrine-disrupting chemicals during ozonation of municipal sewage with brominated byproducts control. Environ Sci Technol. 42, 3375-3380.

Zhang J., Cooke G.M., Curran I.H.A., Goodyer C.G., Cao X.-L. (2011) GC–MS analysis of bisphenol A in human placental and fetal liver samples Journal of Chromatography B, 879 (2011) 209–214.

Zhu R, Zhao W, Zhai M, Wei F, Cai Z Sheng N and Hu Q (2010) Molecularly imprinted layer-coated silica nanoparticles for selective solid-phase extraction of bisphenol A from chemical cleansing and cosmetics samples. Anal Chim Acta 658: 209-216.

Zimmerman-Downs JM, Shuman D, Stull SC, Ratzlaff RE. (2010) Bisphenol A blood and saliva levels prior to and after dental sealant placement in adults. Journal of Dental Hygiene 2010;84: 145-50.

8. ANNEXES

Annex I

Eucomed comments on the use of BPA in Medical Devices (2012) Response to the SCENIHR call for information on BPA in medical devices

A brief survey of our members identified the following examples of device types with materials derived from BPA:

- Catheters for minimally invasive surgery
- Catheters for cardiac ablation
- Trocars
- Surgical and cardiovascular instruments
- Surgical meshes
- Laparoscopic instruments
- Endoscopes
- Spine cement mixing/delivery systems
- Polycarbonate packaging for products that are steam sterilized
- Photopheresis devices for cancer treatment
- Pacemakers
- Pacemaker catheter systems/catheter delivery systems
- Stents
- Stent delivery systems
- Blood oxygenator and dialysis membrane housings
- Luer fittings
- Needle hub
- Insulin Pump Infusion Sets
- Neurostimulators (implantable)
- Drug pumPS (implantable)
- Neuromodulation catheter system/catheter delivery systems
- Balloon Dilatation Catheters

Polycarbonate medical devices reported in Beronius and Hanberg 2011 (Plastic Europe 2007)

Blood oxygenators

☐ Cardiotomy reservoirs

☐ Dialysers

☐ Respirators

☐ Dentists´ operating lamPS

☐ Safety valves for respirators
☐ Breast pumPS
☐ Inhaler housings
☐ Prescription spectacles
☐ i.v. connectors
☐ Scalpel cases
☐ Laparoscope handles
Contact lens holders
☐ Syringe toPS
☐ Medical packaging film
☐ Ampoules
☐ Three - way stop cocks and stop cocks Manifolds
☐ Tweezers with integrated lighting
☐ Single - use operating instruments

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- Neurostimulators (implantable)
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(Information provided by Eucomed, 2012)

Annex II

Summary of BPA concentrations in or released from medical devices /medical grade materials.

Chromatographic methods are considered more suitable for BPA analysis in biological samples than immunochemical methods. ELISA methods are less reliable due to possible cross reactivity of BPA parent compound and the conjugated metabolites and other BPA similar phenolic structures, what can lead to an overestimation of free BPA concentrations; also their sensitivity is low and may be subjected to matrix effects (especially in urine samples).

Methods based on mass spectrometry detection (MS) are the most reliable. MS-MS detection provides high sensitivity and selectivity, allowing the confirmation of the identity of BPA based on fragment formations.

A crucial issue in BPA analysis at low levels in human samples, and a potential source of different results published in the literature, is the differentiation in the measurement of free BPA and its metabolites. Additionally, the contamination with BPA from external sources, during sample collection and processing in the laboratory has to be assessed through the analysis of blank samples to guarantee the reliability of the results. Moreover, the possible hydrolysis of the conjugated metabolites into BPA free form during sample storage needs to be considered. In that sense, those methods using an internal standard, typically a stable isotope labeled BPA with use of MS, are more reliable since they provide a means to determine the effect of complex matrices of blood and urine samples, and the stability of BPA in the biological samples during analysis.

Reference	Sample	Extraction method	Method of analysis	LOD	BPA concentration	Remarks	Reliability
Haishima *et al.* (2001)	PC pellets (2 types) PS pellets PC casings(2) (lacking hollow fibres)	Dissolution, THF Extraction (shaking, 16h, RT, 10 mL): water methanol	HPLC GC-MS LC-MS , and NMR spectroscopy	0.65 ppb (HPLC) 0.16 ppb (GC-MS)	4.0 and 7.2 ug/g 34.5 ug/g 11.7 and 13.7 ng/casing 296 and 345 ng/casing	Total content in the material. Released amount from the material under the indicated conditions	+
data submitted in response to the Call for Information (2012)	PC pellets PC trocar tubing	Extraction, 37ºC, 24h: IP Ethanol IP Ethanol 0.9 % sodium chloride, 37ºC time point up to 168 h	HPLC		0.247 - < 0.52 ug/g < 0.5 ug/g 0.28 - < 0.50 ug/g < 0.5 ug/g < 2 ug/g	Total content extracted with IP according to the authors No detailed information on method performance to assess the reliability of the data	+
Haishima *et al.* (2001)	Hemodialyzers: PCcasing/ PS fiber (2) PCcasing/ cellulose acetate fiber (1) Polystyrenecasing/PS fiber (1)	Recirculation (250mL, 10 mL/min, 16 h, RT): Water Bovine serum	HPLC GC-MS LC-MS , and NMR spectroscopy	0.65 ppb (HPLC) 0.16 ppb (GC-MS)	Water Bovine serum (ng/module) 31.0-141.8 1010-2090 34.1 196.1 3.78 140.7	Released amount from the material under the indicated conditions. Higher BPA released was found for all types in bovine serum compared with water	+

Shintani (2001)	Hemodialyzers: PC casing/ PS fiber) autoclaving sterilization (2) PCcasing/ PS fiber - gamma-ray sterilization (1) Polystyrene-butadiene coplymer casing/PS fiber-gamma-ray sterilization(1) PCcasing/ PS fiber)-autoclaving sterilization (2)	Perfusion on patients, 4 h, 3 times a week, for 3 months consecutively Saline solution - 800 mL (according to ISO 10993-7)	HPLC-ECD HPLC-MS	0.02 ng/mL plasma	0.2 - 0.7 ng/mL (mean value, n=4) < LOD (mean value, n=4) < LOD (mean value, n=4) 0.1 -0.2 ng/mL	Measured values in blood of uremia patients after dialysis. No BPA was detected in the the blood samples collected before dialysis	Not reliable as the sensitivity and specificity of the method is insufficient to detect and quantify the concentrations in serum of normal subjects. The value of 0.2-0.7 ng/ml is not reliable.
Yamasaki *et al.* (2001)	Hemodializers: Polystyrene casing/PS fiber (A,B) PCcasing/ PS fiber (C,D) PCcasing/ EVAL fiber (E)	Filled with reverse osmotic water. Recirculation of saline solution (200 mL), 200 mL/min, 4h Blood samples from hemodialysed patients (dialysate flow rate 500 mL/min)	HPLC	*Not given*	ND (A, B) 0.23 ppb (C), 1.14 ppb (D); 0.19 ppb (E) ND (A, B) 0.16 ppb (C), 0.75 ppb (D); 0.20 ppb (E) ND (C) (n=3) 0.49; 0.67: 1.15 ppb (D)	Lack of data on method performance. Released BPA amount from the material under the indicated conditions. Saline solution was recirculated after the removal of the osmotic water and washing with 800 mL of	not reliable

						saline solution Mean value 0.77 ppb in blood. Patients with almost no residual renal function	
Shintani *et al.* (2003)	Membranes, ozone gas sterilized: PS, 20 mg aprox. (3) PC , 7 mg aprox. (1) both types unsterilised and steam-sterilized (121ºC, 15 min)	Extraction, ethanol	LC-UV-MS	0.02 ppb	43 – 207 mg/kg ozone gas sterilized: 119 ppm < LOD	Released amount from the material under the indicated conditions. BPS also detected in PS membranes (355; 63; 32 mg/kg)	reliable
Murakami *et al.* (2007)	Hollow fibres: PS PEPA (Polyester-polymeralloy) Hemodialyzers with PS membranes	Extraction (10 mg) with hexane. Redisolution of residue in dimethylsulfoxide. Perfusion on 15 patients, who previously underwent hemodialysis for at least three months with a PS dyaliser	ELISA	0.3 ng/mL	83.3 ng/10 mg 122.5 ng/10 mg Mean increase, after 1 month session: from 4.83±1.94 to 6.62±3.09 ng/mL (1st test) from 4.09± 2.78 to 4,27±2.98 ng/mL (2nd test)	Released amount from the material under the indicated conditions (mean values, n=6) The quoted differences are certainly not statistically significant. 56.6 % cross-reactivity with water-soluble	not reliable, because of cross-reactivity

						form of BPA.	
Fink (2008)	Dyalizers with PS or PC (5) Surface area (1.3-1.8 m2)	Simulated dyalisis, 37 °C, 230 mL eluate/min for 4 h and 24 h as worst case scenario reverse osmotic water 17.2% ethanol	LC-MS-MS		6.4 – 71.3 ng/dialyzer 54.8 - 4299 ng/dialyzer	Range of BPA amount eluted from the dialysers in a period time from 4 to 24 h. BPA leaching increased with the membrane surface area and with dialysis time.	reliable
Krieter *et al.* (2013)	Hemodializers: 1.3 m^2 polysulfone membranes, high flux 1.3 m^2 polysulfone membranes, low flux. 1.7 m2 high-flux polyethersulfone membrane	Recirculation of sterile water (400 mL), 3 h, 250 ml/min, 37 ºC Perfusion on patients (18), 4 week treatment	ELISA		Total amount ng/dialyzer, mean values (n=6): 48.1 ± 7.7 140.8 ± 38.7 6.2 ± 2.5 No significant plasma levels changes after treatment with any of dialyzers	BPA eluted under the indicated conditions BPA plasma levels in the control group (n=24) were significant lower(≤ 0.2	not reliable because of unspecific analytical method

						±0.1 ng/mL) compared to pre-dialysis values of patients (9.1 ± 4.5 – 12.0 ± 6.0 ng/mL). Lack of data on method performance.	
Cho *et al.* (2012)	Hemodialyzer/hemoconcentrators with polysulfone membranes: 0.5 m^2 (7.9 g) 0.4 m^2 0.7 m^2	Ten consecutive extractions (1 h each) with 1L of 17.2 % ethanol at 37ºC, 200 mL/min. One single extraction, 6 h, with 1L of 17.2 % ethanol at 37ºC, 200 mL/min.	LC/MS	0.02 ng/ml (LOQ)	19.7 ug ≈1.3-1.4 ng/ml ≈0.65-0.7 ng/ml	The released amount from the material corresponded to 95 % of the predicted amount using an elution profile equation. Aproximated values read from the published figure. Limited information on method performance.	reliable
data submitted in response to the Call for Information (2012)	Neonatal incubators	Analysis of gas in contact with the hoods	ISO 16000-6	50 ug/m3	< LOD	Very limited information provided to assess the reliability of the	unknown

						data	
Sakurai H. (2002) only abstract in english	cardiopulmonary bypass circuits with PC parts (8)	Priming with a saline solution Blood samples taken from 6 patients once the CBP was initiated and at the termination	GC		0.9 ± 1.1 ug/l 0.3 ± 0.2 ug/l (after the commencement of CBP) 0.4 ± 0.3 ug/l (at the termination of CBP)	No BPA detected in 3 controls samples. Info in the abstract not enough for the evaluation of the data. The quoted differences are certainly not statistically significant. Info in the abstract not enough to assess the reliability of the data.	unklnown
Lewis *et al.* (1999)	commercially available dental composites and sealants	Dissolution in acetonitrile (removal of fillers by centrifugation)	HPLC	No quant.		BPA not verified in any material. Bis-DMA verified in 3 products in the same product line	unknown
Manabe *et al.* (2000)	commercially available dental composites and sealants	Dissolution in methanol (removal of fillers by centrifugation)	Derivatization w TMS GC/MS	1 ng/mg	6.6/15.4/18.5/20.2 ng/mg material	In 4 materials (un-polymerized). Not detected in 2 mater.	reliable
Mazzaoui *et al.* (2002)	different dental materials	Extraction into water or water/ethan	HPLC/MS	-	0 from 3 materials, 13 ± 8 or 67	BPA extracted from 1 sealant	reliable

		ol mix			± 4 mmol/m^2 (1d, water) or 122 ± 18 or 399 ± 61 mmol/m^2 (90d, 75% ethanol)	and 1 bonding material	