Annex 1

FSANZ Response to Studies Cited as Evidence that BPA may cause Adverse Effects in Humans

STUDY	KEY FINDINGS/CLAIMS	FSANZ RESPONSE
STUDIES IN EXPERIMENTAL ANIMALS		
'Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens BPA and octylphenol' Nagel *et al* (1997) *Environmental Health Perspectives* **105**: 70-76	▪ Mice given low oral doses of BPA during pregnancy (2 or 20 µg/kg bodyweight/day) gave offspring with larger prostates at both dose levels.	▪ The study used an 'in-house' mouse strain which was subsequently destroyed thus preventing replication of the findings by other researchers. ▪ The use of small animal numbers per group raises questions regarding statistical validity. ▪ Reproductive organs in male mice can vary in size depending on social status (e.g. dominant males usually have larger prostates). The reported increase in average prostate weight (approx 30%) may be an artefact of the study design in which one male per litter was randomly selected for analysis. ▪ Microscopic analyses of prostates were not conducted. ▪ No effects on the prostate have been observed in several subsequent studies in mice and rats employing multiple dose levels and larger group sizes.
'A physiologically based approach to the study of BPA and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior' Vom Saal *et al* (1998) *Toxicology and Industrial Health* **14**: 239-260	▪ Mice given low oral doses of BPA during pregnancy (2 or 20 µg/kg bodyweight/day) gave offspring with reduced sperm production at the higher dose, higher preputial weights and lower seminal vesicle weights at the lower dose, and lower epididymal weights at both dose levels.	▪ This study shares weaknesses with the above study (Nagel *et al* 1997) such as the use of: (i) small animal numbers per group, (ii) an obsolete mouse strain, and (iii) data from only one randomly selected male per litter. ▪ An additional weakness is the unusual/unexplained findings of low dose only effect on weights. ▪ The US National Toxicology Program stated that it was not able to confirm any of the statistically significant findings in this paper and concluded that the data were inadequate for hazard assessment. ▪ No effects on these parameters have been observed in more robust studies employing multiple dose levels and larger group sizes.
'Exposure to BPA advances puberty' Howdeshell *et al* (1999) *Nature* **401**: 763-764	▪ A low oral dose of BPA (2.4 µg/kg bodyweight/day) administered to pregnant mice advanced puberty in the female offspring.	▪ A non-standard endpoint was used for assessing puberty in mice and is of questionable biological significance. ▪ Use of only a single dose level prevents dose-response considerations which are integral to toxicological hazard assessment. ▪ Statistical analysis was not adequately documented.

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'Exposure to a low dose of BPA during fetal life or in adulthood alters maternal behavior in mice' Palanza *et al* (2002) *Environmental Health Perspectives* **110**: 415-422	▪ Female mice exposed to BPA (10 µg/kg bodyweight/day) either as foetuses or in adulthood spent less time nursing their offspring and more time out of the nest compared with the control group.	▪ Statistically significant effects were reported for prenatal exposure or postnatal exposure. However, no effects were observed following combined pre- and postnatal exposure and there was no explanation for this apparent anomaly. ▪ No adverse effects were reported for the offspring. ▪ Use of only a single dose level is a deficiency (see above). ▪ The small reported differences in maternal behaviour are not considered to be adverse effects.
'Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra' Timms *et al* (2005) *Proceedings of the National Academy of Sciences USA* **102**(19):7014-7019	▪ Pregnant mice receiving oral BPA (10 µg/kg bodyweight/day) produced male offspring with increased number and size of prostate ducts. ▪ Urethral constriction was also reported and it was stated that this could contribute to urine flow disorders.	▪ The study used a single dose level and small animal numbers. ▪ Prostate weights were not reported. ▪ No effects on the prostate have been observed in more robust studies with multiple dose levels and larger group sizes. ▪ No disorders which could be attributed to altered urine flow (e.g. adverse bladder or kidney effects, altered clinical chemistry parameters) have been observed in well designed animal studies on BPA.
'No effect of route of exposure on plasma BPA throughout 24h after administration in neonatal female mice' Taylor *et al* (2008) *Reproductive Toxicology* **25**(2):169-176	▪ In neonatal mice, the route of administration (oral *vs* subcutaneous injection) gave no significant difference in plasma levels of BPA. ▪ Studies that use non-oral administration of BPA during the neonatal period should not be dismissed as unsuitable for hazard assessment.	▪ BPA concentrations were determined only in ether extracts of blood samples and only a small fraction of the administered dose was recovered (<5%). In well designed studies close to 100% of the administered BPA dose is accounted for. Therefore, no reliable conclusions can be made regarding the systemic bioavailability of BPA in this study. ▪ Well designed human studies show that BPA is rapidly and extensively detoxified following oral exposure. ▪ BPA studies using non-oral routes of administration, for which detoxification pathways are largely bypassed, should be given little weight in the hazard assessment of BPA.

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'Oral exposure to BPA increases dimethylbenzanthracene-induced mammary cancer in rats' Jenkins *et al* (2009) *Environmental Health Perspectives* **117**:910-915.	▪ In rats, combination treatment with a carcinogenic chemical and lactational exposure to BPA resulted in increased numbers of mammary tumours per animal and reduced time to first tumour.	▪ Studies have shown that a minimal fraction of BPA administered to dams is transferred to breast milk. BPA has shown no carcinogenic potential in chronic studies in mice and rats at relatively high dose levels. ▪ Histopathologic evaluation revealed no changes in the carcinoma score or tumour burden (expressed as percentage of bodyweight). ▪ With regard to tumour latency, uncertainty in the measurement method (palpation) is likely to be large and was not addressed in the publication. ▪ The shortcomings in this study and a lack of concordance with other studies indicate that this study is not useful for hazard assessment.
'Similarity of BPA pharmacokinetics in rhesus monkeys and mice: relevance for human exposure' Taylor *et al* (2011) *Environmental Health Perspectives* **119**(4):422-430	▪ BPA administered as a single oral dose exhibited similar concentration *vs* time profiles of free BPA measured in blood serum of mice and monkeys. ▪ This finding adds weight to the potential relevance to humans of low dose findings reported in some mouse studies.	▪ Close scrutiny of the data shows important differences in the BPA serum profiles in mice and monkeys. In mice, the serum concentration of free BPA 24 hours after dosing was approximately 12% of the maximum serum concentration observed at 1 hour after dosing. In monkeys, the corresponding value was only 2.5%. ▪ This difference results in a 3-fold greater systemic exposure to free BPA in mice compared to monkeys and could result in accumulation of free BPA in mice following repeated dosing. This result did not receive any comment in the paper. ▪ The conclusion that serum profiles of free BPA are similar in monkeys and mice is therefore not considered to be generally valid. ▪ Other studies have shown that mice and rats are less efficient than humans with regard to BPA detoxification. This diminishes the human relevance of low dose findings reported in some rodent studies.
'Disruption of adult expression of sexually selected traits by developmental exposure to bisphenol A.' Jašarevic et al (2011) *Proceedings of the National Academy of Sciences USA*, published online ahead of print, 27 June 2011.	▪ Male deer mice exposed to BPA through maternal diet exhibited compromised learning abilities and exploratory behaviours compared to control males. ▪ Female mice spent more time in nose-to-nose contact with control males than with males exposed to BPA. The authors' concluded that female deer mice have a reduced sexual preference for males exposed to BPA.	▪ The level of BPA in feed was 50 mg per kg of feed (i.e. 50 ppm), however feed consumption and estimated doses of BPA received by the deer mice were not reported. ▪ Male deer mice exposed to BPA showed no changes in appearance, body weight, sensory development, or adult circulating concentrations of testosterone and corticosterone. A mechanistic explanation for the reported findings is lacking. ▪ Parameters relevant to mating were not investigated. Reduced time in nose-to-nose contact may not be indicative of reduced sexual preference. ▪ This is the first study of BPA in deer mice. It is unknown whether the findings in deer mice may be applicable to other rodents let alone humans.

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EPIDEMIOLOGY STUDIES		
'Association of urinary BPA concentration with medical disorders and laboratory abnormalities in adults' Lang *et al.*, (2008). *Journal of the American Medical Association* **300**:1303-1310	▪ In a study on 1455 US adults, higher urinary BPA concentrations were associated with cardiovascular diagnoses, diabetes, and clinically abnormal concentrations of two liver enzymes.	▪ The cause of higher urinary BPA levels could plausibly be linked to higher consumption of packaged food resulting in increased fat, sugar and salt consumption which may in turn increase the risk of diabetes and heart disease. That is, a higher BPA level may not have any causal effect but is simply a marker of a poor diet. Attributing any cause and effect relationship from this study would be unsound. ▪ Urinary BPA levels reported in this study represent a snapshot of short term exposure whereas longer term factors are more likely to be relevant to the development of diabetes and cardiovascular disease. ▪ In a subsequent issue of the journal, authors of three letters to the editor criticised several aspects of the study. These criticisms included: (i) diabetes was self-reported and there was no distinction between type I and type II diabetes (ii) the severity of the self-reported diabetes was not reported; and (iii) based on statistical considerations, the potential for false positives was considered to be substantial.
'Prenatal BPA exposure and early childhood behavior' Braun *et al* (2009) *Environmental Health Perspectives* **117**(12):1945-1952	▪ Prenatal BPA exposure, as measured by maternal urinary BPA levels during pregnancy, may be associated with hyperactivity and aggression in two-year old children, especially among female children.	▪ Behaviour of the children was assessed at only one time point and this may not adequately reflect the overall behaviour pattern during early childhood. ▪ The reported statistical associations could potentially be affected by confounding factors that were not taken into account such as maternal behaviour toward the child, parental psychopathology, and alcohol/drug consumption. ▪ The authors themselves identified statistical limitations of the study and also stated that the results could be biased due to uncharacterised confounding factors and to inaccurate characterisation of BPA exposure.

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'Occupational exposure to BPA and the risk of self-reported male sexual dysfunction' Li *et al* (2010a) *Human Reproduction* **25**:519-527 'Relationship between urine BPA level and declining male sexual function' Li *et al* (2010b) *Journal of Andrology* **31**:500-506 'Urine BPA level in relation to semen quality' Li *et al* (2011) *Fertility and Sterility* **95**(2):625-630	▪ Findings from this study, published in 3 separate journal articles, are that urine BPA levels in male chemical factory workers are associated with a higher risk of sexual dysfunction and decreased semen quality.	▪ A major shortcoming of this study is that no data were available on occupational exposure to chemicals other than BPA. The workers examined in this study were employed in manufacturing plants that produced epoxy resins and BPA. It is likely that such workers would have been exposed to a variety of different chemicals by non-oral routes (e.g. inhalation, dermal contact). However, BPA was the only chemical assayed in urine. ▪ A separate study found no significant associations between urinary BPA concentration and any sperm parameter in 375 men from four U.S. cities (Mendiola et al (2010) *Environmental Health Perspectives* **118**,1286-1291).
'Association of urinary BPA concentration with heart disease: evidence from NHANES 2003/06' Melzer *et al* (2010) *PLoS One* **5**(1):e8673	▪ Higher urinary concentrations of BPA are associated with heart disease in the general adult population of the USA.	▪ Cholesterol and triglyceride levels were not taken into account as confounding factors. ▪ The deficiencies described above for the Lang *et al* (2008) study also apply for this study. ▪ In contrast to Lang *et al* (2008), there was no association with diabetes.

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'Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during *in vitro* fertilization' Fujimoto *et al* (2011) *Fertility and Sterility* **95**(5):1816-1819.	▪ Higher blood levels of BPA in women undergoing *in vitro* fertilisation (IVF) may negatively affect the maturation of ova and reduce the probability of fertilisation. ▪ For the nine Asian women in the study, the statistical calculations predicted that a doubling of BPA blood level would be associated with a 9% decrease in the probability of obtaining a mature ovum. ▪ A doubling in female serum BPA concentration was predicted to result in a 55% decrease in the probability of fertilisation, while a doubling in male serum BPA concentration was predicted to result in a 12% reduction in fertilisation probability for the five Asian men in the study.	▪ This publication was brief (3 pages) and lacked detail concerning the raw data used as input for the statistical calculations. For some statistical analyses, it appears that that the number of model variables was excessive with respect to the number of experimental data points available. This can lead to unreliable statistical conclusions. ▪ When all study participants were considered, there was no association between BPA and ova maturation. ▪ It was stated by the Authors that the small number of participants in the study precluded conclusive evaluation of the associations detected between BPA and ova maturation. They also stated that the results may have been biased by the possibility that some meiosis events were misclassified. ▪ Several factors relevant for the success of the IVF procedure were also ignored (e.g. the amount of human chorionic gonadotrophin (hCG) administered, oestrogen and progesterone levels at the time of hCG administration, whether fresh or frozen sperm were used for fertilisation, the number of oocytes retrieved from each women). ▪ An additional limitation of the study is that BPA was the only environmental chemical considered in the statistical analyses. ▪ The Authors concluded that the study was preliminary and that further studies are needed. ▪ It is likely that the reported associations occurred by chance and that the low levels of BPA exposure reported in the paper have no association with adverse IVF outcomes.

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'Impact of early-life BPA exposure on behavior and executive function' Braun *et al* (2011) *Pediatrics*. Published online 24 October 2011	▪ Higher prenatal BPA exposure, as measured by maternal urinary BPA levels during pregnancy, was reported to be associated with increased anxious and depressed behaviour, inhibition and poorer emotional control in 3-year old girls. ▪ In contrast, increased prenatal BPA exposure was associated with *decreased* hyperactivity, anxiety and depression in 3-year old boys. ▪ No associations were observed between BPA exposure during childhood and behavioural parameters for 3-year old boys or girls.	▪ The statistical analysis conducted in this study is not considered to be robust. It was stated that *p* values of <0.10 were considered to be indicative of an association of a behavioural effect with BPA exposure, whereas a *p* value <0.05 is conventionally used as a cut-off for statistical significance. ▪ Scatterplots which purport to show an association with BPA exposure and behavioural effects show no clear trends. ▪ Confounding factors which were not taken into account in a similar study published by the same authors (Braun *et al* 2009; described above) also apply to this study. ▪ The authors stated that their findings '… should be interpreted cautiously, given the imprecision of the observed associations among girls and the low statistical power for interactions between gender and BPA exposures' and that 'The clinical relevance of these findings is unclear at this point.'