UPDATED RISK ASSESSMENT
OF
4,4'-ISOPROPYLIDENEDIPHENOL
(Bisphenol-A)

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(to be read in conjunction with published EU RAR of BPA, 2003)

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gland, its validity is hampered by serious methodological limitations. It is also noted that these findings are inconsistent with the absence of preneoplastic lesions of the mammary gland in the offspring of several standard multi-generation studies in rats and mice.

Regarding the other new studies, it can be concluded that prenatal and/or neonatal exposure to BPA does not exert promoting activity on the carcinogenesis induced by established carcinogens/initiators in specific organs.

Overall, therefore, the new information on the potential carcinogenic and/or promoting effects of BPA in prenatal and neonatal rat models supports the original conclusion from the published report that BPA does not possess any significant carcinogenic potential.

### 4.1.2.9 Reproductive toxicity

#### 4.1.2.9.1 Summary of original risk assessment report

No human data are available. BPA has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with BPA glucuronide *in vitro*. The available data also indicate that there is a marked strain difference in the response to BPA in rats. However, there are no data to indicate the underlying reasons for such differences.

It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories. Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of BPA on fertility and reproductive performance have been investigated in three good quality studies: two generation and multigeneration studies in the rat, and a continuous breeding study in the mouse. Although no effect on fertility was seen in the rat two-generation study, low dose levels were employed (0.2-200 µg/kg/day). In the multigeneration study, an effect on fertility (reduction in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of BPA. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse, it is prudent to assume that BPA may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg.

The continuous breeding study in the mouse provides some evidence that BPA can cause adverse effects on fertility. In the F₀ generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter
tested at each dose level from the F₁ generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F₁ generation. However, the significance of this finding is uncertain given that there was no effect on fertility in this generation, and where an adverse effect on fertility was seen (in the F₀ generation) there was no effect on epididymal weight. In spite of the uncertainty, the epididymis is associated with sperm transport and storage, and any reduction in the weight of this organ would be of concern. Although no effects were seen in the 2-generation rat study, it is not considered suitable for use in the risk characterisation due to the low dose levels employed (0.2-200 μg/kg/day). However, these data combined with that for the multigeneration study does provide a comprehensive dose-response range for evaluating effects on fertility in the rat. In addition, comparing the rat and mouse data it can be seen that similar toxicological profiles were observed for effects on fertility; effects were seen in both species at approximately the same dose level (i.e. reductions in litter size at 500 mg/kg/day in the rat and at 600 mg/kg/day in the mouse). Consequently, it is considered that the NOAEL of 50 mg/kg/day identified in the rat multigeneration study is also likely to produce no adverse effects in mice for which there is only a LOAEL of 300 mg/kg/day (for a small but statistically significant decrease in epididymal weight in F₁ males only). Therefore, the NOAEL of 50 mg/kg/day identified from the multigeneration study will be used for risk characterisation purposes, in relation to effects on fertility.

No evidence that BPA is a developmental toxicant was observed in standard development studies in rats and mice. In rats, a maternal LOAEL and foetal NOAEL of 160 and 640 mg/kg/day respectively, were identified. In mice, maternal and foetal NOAELs were 250 and 1000 mg/kg/day, respectively. In a rat multigeneration study, a statistically significant decrease in mean pup body weight gain, with concomitant delays in the acquisition of developmental landmarks (vaginal patency and preputial separation) was observed at 500 mg/kg on post-natal days 7-21 in males and females of all generations (F₁-F₃). These decreases in pup body weight gain and delays in development were seen in the presence of maternal toxicity. No maternal toxicity and no treatment-related effects were reported in the offspring of animals exposed to 50 mg/kg.

However, additionally, some studies have investigated the potential of BPA to affect male reproductive tract development in rats and mice. Conflicting results have been reported in these studies, in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at dose levels in the range 2 – 50 μg/kg. However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects. It is noted that in contrast to the studies showing effects on the male reproductive tract, the studies that did not find an effect of BPA also did not show any effects of DES. Furthermore, no functional changes in reproductive parameters or reproductive organ development were observed in a recent rat two-generation study using similar dose levels. The reasons for the differences in these results are unclear. Recent evidence from one study suggests that there are differences in the sensitivity of different mouse strains to the effects of oestrogens, which may be related to the selection of strains for large litter size. This difference in sensitivity may in part explain some of the differences in the current database, although the relevance of these rodent strain differences in relation to human health remains unclear.

Overall, in standard developmental studies in rodents, there is no convincing evidence that BPA is a developmental toxicant. However, the available and apparently conflicting data from studies conducted using low doses (in the μg/kg range) do raise uncertainties. Overall,
the majority of EU member states felt that the studies reporting effects at low doses could not be dismissed. However, the member states disagreed on how these studies should be used, if at all, in the risk characterisation for this endpoint. The disagreements were based on differing views about the uncertainties surrounding the reproducibility of the findings and their biological significance, if any, to human health.

This issue was referred to the Competent Authorities in June 2001. It was agreed unanimously by the Competent Authorities that further work was required to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on development at low doses. In addition, it was agreed that a provisional NOAEL of 50 mg/kg/day for developmental effects, derived from the rat multi-generation study, should be used in the risk characterisation in the interim, whilst awaiting the outcome of further testing, with the aim of identifying those scenarios which are clearly of concern irrespective of the outcome of the further testing.

4.1.2.9.2 Updated information

Member States required that a 2-generation study in the mouse involving exposure to low (μg/kg bw/day range) and high (mg/kg bw/day range) doses of BPA be conducted. This study has now become available (Tyl et al. 2007) and is summarised below.

In addition to this comprehensive 2-generation study, a large number of studies investigating the reproductive toxicity of BPA have become available since the finalisation of the RAR. Among these studies, several (approximately 40-50) have investigated the same standard reproductive and developmental endpoints as those examined by Tyl et al. (2007). These studies have been performed on a range of animal species and strains, at different life stages, over a wide array of doses, using a variety of exposure routes, for varying exposure durations, and have investigated a large assortment of endpoints (for a detailed review see Gray et al., 2004; Goodman et al., 2006; EC SCF, 2002; and EFSA, 2006). The majority of these studies have reported only small changes (unrelated to dose) in organ weight, tissue architecture, receptor expression or hormone levels of unknown pathophysiological consequences. Some have found no effect, but, overall, no consistent, reproducible, adverse effects have been observed. Furthermore the results from these studies have been in contrast to the results of investigations conducted according to internationally recognised guidelines and in compliance with GLP, including the recent 2-generation study in the mouse by Tyl et al. (2007). As we consider this investigation by Tyl et al. (2007) as the gold-standard, definitive study of the reproductive toxicity of BPA (for the endpoints examined), all the other recent publications investigating the same standard reproductive and developmental endpoints have not been evaluated in detail in this report.

However, there are also numerous recent studies which have investigated the potential developmental neurotoxicity of BPA. As these endpoints were not examined by Tyl et al. (2007), these publications have been considered in detail in this evaluation.

Additionally, one study investigated the effects of neonatal exposure to BPA on the morphology of the reproductive tract at 18 months of age in female mice (Newbold et al. 2007). Because the potential for the expression of developmental effects in old-age has not been assessed in the standard reproductive toxicity studies, an appraisal of this study is included below.
One relevant human study has been published since the finalisation of the RAR and is included in this update (Sugiura-Ogasawara et al. 2005). This is an investigation of the possible association between BPA exposure and recurrent miscarriage.

2-generation study in mice

The effects of BPA on fertility and reproductive performance in mice have been investigated in a two-generation study, conducted in compliance with GLP (Tyl et al., 2007). The study design and interpretation of the results were supervised by a Steering Group, that was chaired by a representative of the European Chemicals Bureau and included experts from several EU Member States. The overall design of this study was based on OECD Test Guideline 416, enhanced by incorporation of a second vehicle control group, a positive control group, a total of 6 exposure levels of BPA, the retention of additional F1 male offspring for organ weight and other assessments, and extending histopathological examinations to all treatment groups. This study was conducted in response to an ESR risk assessment conclusion that further research is needed to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on development of the male reproductive tract at low doses (0.002-0.05 mg/kg/day) in mice.

Eight groups of 28 male and 28 female CD-1 mice (F0 generation) were exposed to BPA in the diet (Purina Certified Ground Rodent Diet®, No. 5002) at concentrations of 0 (2 vehicle control groups), 0.018, 0.18, 1.8, 30, 300 or 3500 ppm, which resulted in a BPA intake close to the target doses of 0, 0.003, 0.03, 0.3, 3, 5, 50 and 600 mg/kg/day, respectively. CD-1 strain mice were used as there have been claims that this strain is specifically sensitive to low doses of BPA. Two vehicle control groups were used to better characterise the natural variability in mice of the parameters evaluated in the study. The exposure levels were selected as a range that would make possible a comprehensive assessment of the dose-response relationship for reproductive toxicity. The lowest BPA dietary concentration was selected to provide a BPA intake of about 0.003 mg/kg/day, close to that at which effects on the development of the male reproductive system have been reported by Nagel et al. 1997 and vom Saal et al. 1998. The next two higher concentrations of 0.18 and 1.8 ppm were selected as 10-fold incremental increases. Concentrations of 30 and 300 ppm were selected to produce intakes that matched the NOAEL and LOAEL, respectively, for general parental toxicity in a 3-generation dietary study in the rat (Tyl et al. 2002). The highest concentration of 3500 ppm was selected as an exposure level that would cause mild general parental toxicity, based on the results of a 13-week rangefinding study (Tyl et al. 2005). The phytoestrogen content of the batches of diet used were: genistein 177-213 ppm, daidzein 173-181 ppm, glycine 39-55 ppm and total isoflavones 390-449 ppm.

The positive control group of 28 male and 28 female mice was exposed to 17β-oestradiol (E2) in the diet at a concentration of 0.5 ppm (resulting in an E2 intake of about 0.08 mg/kg/day), to confirm the sensitivity of the mouse model to a potent endogenous oestrogen. This exposure level was selected as one that would result in effects on oestrogen sensitive reproductive parameters, based on the findings of a E2 rangefinding (Tyl et al. 2004a) and 2-generation study (Tyl et al. 2004b, 2006 - this study is briefly summarised below).

Exposure of the F0 generation commenced at 6 weeks of age and continued throughout an 8 week pre-breed exposure period, a 2 week mating period (each male was paired with a female from the same exposure group) and gestation. Exposure of F0 females to BPA continued throughout lactation until weaning on post-natal day (pnd) 21. At weaning, 28 male and 28
female F1 animals were selected from each exposure group for retention and were similarly exposed for a pre-breed, mating gestation and lactation period. An additional one F1 male from each litter was retained (termed ‘F1 retained males’) with continued exposure for 3 months until sacrifice concurrently with the parental F1 males. The remaining F1 animals were sacrificed at weaning. Parental males were sacrificed at the end of their respective delivery period and parental females were sacrificed at weaning of their litters. The study was terminated with the sacrifice at weaning of the F2 generation.

For the parental F0 and F1 generation and retained F1 males, clinical signs of toxicity, body weights and food consumption were recorded. Oestrous cycles were monitored in the last 3 weeks of the pre-breed exposure period and during the mating period for both the F0 and F1 parental animals. A necropsy was conducted on all adult animals in which reproductive (including the prostate) and other selected organs were removed and weighed. Histology of selected tissues was conducted on all vehicle control animals and on 10 parental males and females from each of the F0 and F1 BPA and E2 groups. Histology was also conducted on all vehicle control and E2 F1 retained males and on 10 randomly selected F1 retained males from each BPA group. For all parental and retained males, epididymal sperm number, motility and morphology were assessed, testicular homogenisation-resistant spermatid head count was recorded, and daily sperm production and efficiency of daily sperm production was calculated. For all parental females the number of ovarian primordial follicles was counted. Parameters assessed in the young offspring included litter size, body weight, survival, gross appearance, anogenital distance (on PND 0 and 21), vaginal patency and preputial separation. For offspring killed at weaning a gross necropsy was conducted on all; selected organs were removed and weighed from two randomly selected pups/sex/litter and histopathology was conducted on the reproductive organs of one pup/sex/litter and on all selected organs from the other selected pup/litter.

All statistical comparisons to each BPA group and the E2 positive control group values were made against the pooled values for the two vehicle control groups.

For the BPA exposed F0 and F1 parental/retained animals there were no treatment-related mortalities or clinical signs of toxicity. Evidence of general toxicity was observed in 300 ppm and 3500 ppm groups. At 300 ppm, this evidence was limited to an increased incidence of centrilobular hepatocyte hypertrophy of minimal to mild severity in F0 males (40% vs 11% in controls) and females (10% vs 2%) and F1 parental/retained males (30% vs 10%). There were no increases in liver weight at this dose level. At 3500 ppm, bodyweight gain was reduced among the F1 parental/retained males; at termination mean bodyweights of the parental and retained males were 4% and 10%, respectively, less than the vehicle controls. Kidney weights were increased in F0 males and in F1 parental/retained males. Histological examination of the kidney revealed an increased incidence of minimal to mild nephropathy in the F0 males and F1 parental/retained animals at 3500 ppm. Absolute liver weights were significantly increased in F0 males (by 18%) and females (by 20%) and in F1 parental males (by 17%) at 3500 ppm. Histological examination of the liver revealed an increased incidence of minimal to mild centrilobular hypertrophy and minimal to mild nephropathy in the F0 males (100% vs 11% in controls) and females (60% vs 2%) and F1 parental/retained males (65% vs 10% in controls) and parental females (70% vs 4%) at 3500 ppm. The increased incidence of centrilobular hypertrophy at 300 ppm (50 mg/kg/day) was not accompanied by an increase in the group mean liver weight, suggesting that the liver changes seen at this dose level were minor and without toxicological significance. Therefore, the study NOAEL for general toxicity can be set at 50 mg/kg/day on the basis of the observation of toxicologically significant effects on bodyweight, kidney and liver at the next highest dose level of 600 mg/kg/day (3500 ppm).
Concerning the reproduction system, there were no effects on F₀ or F₁ adult reproductive organ weights (F₁ prostate weights are further discussed below), sperm parameters, ovarian primordial follicle count, oestrous cyclicity or histopathology. There were no effects on F₀ or F₁ mating performance or fertility. However, gestational length was statistically significantly increased for both the F₀ and F₁ generations at 3500 ppm (19.3 days vs. 19.0 days for the vehicle controls, both generations), although the health implications of this marginal difference are questionable.

There were no statistically significant differences and no treatment related changes in prostate weight in either the F₁ parental or retained males. Prostate weights appeared slightly increased at 0.018 ppm in the F₁ parental males but because this difference was not also seen in the F₁ retained males, and statistical significance was not achieved, this was considered not to be treatment related.

F₁ and F₂ litter size, pup survival at birth and during lactation were not affected by BPA exposure. There were no treatment-related malformations or clinical signs during lactation. However, several effects on the offspring were apparent at 3500 ppm only. F₁ pup bodyweights were significantly less than vehicle controls on pnd 7, 14 and 21, but F₂ pup bodyweights were not affected. Absolute and bodyweight- or brain-related testes and spleen weights were reduced in the F₁ and F₂ males sacrificed at weaning. The effects on testes weight correlated with an increased incidence of hypoplasia of the seminiferous tubules at 3500 ppm (F₁: 12% vs. 1% in vehicle control; F₂: 35% vs. 4% in vehicle control). Similar effects, however, were not seen in the F₁ parental and retained males. Also, acquisition of preputial separation was slightly delayed in the F₁ parental (by 2 days, compared with negative control, adjusted for bodyweight at time of acquisition) and F₁ retained (by 1.8 days) males at 3500 ppm. Additionally, at 3500 ppm there was a slightly increased incidence of undescended testes observed at weaning sacrifice (a condition with a high and variable background rate in CD-1 mice) of the F₁ and F₂ generations, but there were no indications in adult males of a permanent effect on testes descent. Anogenital distance (AGD) was significantly reduced in F₁ pups on pnd 21 at 300 and 3500 ppm, although this was considered unlikely to be treatment-related because the effect was not also seen on pnd 0 in the F₁ generation, on pnd 0 or 21 in the F₂ offspring; in addition the fact that AGD was not consistently affected in E₂ two-generation studies (Tyl et al. 2006, Biegel et al., 1998) shows that AGD is not an oestrogen regulated endpoint.

In the E₂ positive control group no general toxicity was observed in the F₀ and F₁ parental animals. Reproductive toxicity was expressed as changes in a number of parameters, demonstrating the sensitivity of the mouse model to an oestrogenic substance. F₀ and F₁ gestational length was increased. There was a reduced number of live F₁ litters born, reduced F₁ litter sizes and F₁ fertility was reduced. Onset of puberty (vaginal patency) was accelerated in the F₁ females, preputial separation was delayed in F₁ males, female reproductive organ weights in adults and offspring of all generations were increased. Testes and epididymal weights were decreased and the incidence of seminiferous tubule hypoplasia of the testes was increased among F₁ and F₂ weanlings. Anogenital distance was reduced in male offspring of the F₁ and F₂ generations, although this is considered not to be an oestrogen regulated endpoint. Finally, there was an increased incidence of vaginal epithelial keratinization and bilateral luminal dilatation of the uterus in F₁ and F₂ weanlings. Overall, with the exception of reduced anogenital distance in male offspring, the findings with the E₂ positive control group were consistent with those from the E₂ 2-generation study (Tyl et al., 2004b, 2006, briefly summarised below).
To conclude, BPA caused effects on pregnancy and the offspring (observed as a slightly increased duration of gestation, reduced pup body weight during lactation, a slight increase in the incidence of undescended testes at weaning, seminiferous tubule hypoplasia in offspring at weaning, and delayed acquisition of preputial separation), occurring only at the highest dietary concentration 3500 ppm (intake approximately 600 mg/kg/day), an exposure level that also caused mild parental toxicity. Fertility was not affected by BPA exposure. There was no evidence of an adverse effect on the development of the male reproductive tract at low doses of BPA. Overall, the study NOAEL for both general and reproductive toxicity is 50 mg/kg/day.

The design of the E2 2-generation study (Tyl et al. 2004b, 2006) was based on OECD Test Guideline 416. Six groups of 25 male and 25 female CD-1 mice (F0 generation) were exposed to E2 in the diet (Purina Certified Ground Rodent Diet®, No. 5002) at concentrations of 0, 0.001, 0.005, 0.05, 0.15 or 0.5 ppm, which resulted in E2 intakes of about 0.2, 1.0, 10, 30 or 100 μg/kg/day, respectively. The exposure concentrations were selected as a range likely to include both effect and no-effect levels for E2 reproductive toxicity, based on the results of a rangefinding study (Tyl et al. 2004a). The F0 and F1 exposure periods, mating and sacrifice schedules and experimental observations were essentially the same as for the BPA 2-generation study (Tyl et al. 2007). The study was terminated with the sacrifice at weaning of the F2 generation.

There were no treatment-related mortalities or clinical signs of toxicity among F0 and F1 parental/retained animals. Evidence of general toxicity in the parental/retained animals was limited to significantly reduced body weight gain during gestation and food consumption during lactation for F0 females at 0.5 ppm. Reproductive effects were seen at the three highest exposure levels. At 0.5 and 0.15 ppm, F0, F1 and F2 uterus weights were increased, F1 and F2 weanling testes and epididymides weights were deceased, F1 and F2 litter size was reduced, the timing of vaginal patency was accelerated in F1 females and the timing of preputial separation was delayed in F1 males. At 0.05 ppm, uterus weights were increased in F1 and F2 generations. No general or reproductive toxicity was observed at 0.005 or 0.001 ppm. Overall, the study NOAEL for reproductive toxicity in this E2 study is 0.005 ppm (about 1 μg/kg/day) and the study LOAEL is 0.05 ppm (approx. 10 μg/kg/day).

Developmental neurotoxicity endpoints

The effect of prenatal and perinatal exposure to BPA on neurological development has been investigated in a large number of recent studies. Many developmental neurotoxicity endpoints were evaluated: locomotory and exploratory activity; grooming, cognitive, emotional, social, sexual and maternal behaviour; behavioural response to pharmacological challenge; brain morphology, immunohistochemistry, and receptor/gene expression. Thirty-one studies conducted using the oral route and three studies using the subcutaneous route of exposure are summarised below. The impact of these recent studies on the hazard assessment for BPA is assessed using a weight of evidence approach that focuses on the reliability and consistency of the evidence. This overall assessment, which is presented after the individual study summaries, draws attention to a low level of confidence in the reliability of the studies and a lack of consistency in the results, such that no firm conclusions can be drawn.

The literature search also located several developmental neurotoxicity studies in which BPA was administered by intracisternal injection. These studies have been omitted because this route is clearly of no relevance to the risk assessment for human health.
To ensure transparency with respect to any connections between investigations, the study summaries have been grouped according to the investigating team.

**Studies by collaborating researchers, mainly from Universities of Florence, Siena, Rome, Calabria and Parma, Italy**

The effects of prenatal and post natal exposure to BPA at maternal dose levels of 0.01 - 0.4 mg/kg/day on a range of behaviours and on receptor expression in the brain were investigated in a series of 12 studies conducted by the Italian collaborating researchers.

The effect of prenatal and neonatal exposure to BPA on behaviour was investigated in male and female Sprague-Dawley rats (Farabollini *et al.* 1999). Dosing was by the oral route, using a micropipette. A group of 11 Sprague-Dawley females was fed BPA dissolved in arachis oil at 0.04 mg/kg/day from 10 days prior to mating to the day of weaning (pnd 21) of their offspring. A second group of 11 females received the vehicle from 10 days prior to mating to gd 13, then 0.4 mg/kg/day BPA from gd 14 to pnd 6 and then the vehicle only until pnd 21. A control group received the vehicle from 10 days prior to mating to pnd 21. Twelve-15 pups/gender/group were selected for behavioural testing, conducted between pnd 85 and 87. The order of testing with respect to treatment group was counterbalanced to avoid confounding due to circadian rhythm and variations in testing conditions. Activity during a 5 min session in a holeboard box was recorded, immediately followed by a 5 min session in an elevated-plus maze. These tests are thought to provide measures of ‘anxiety’ and locomotion. The statistical analysis was probably conducted using the individual pup as the experimental unit. This is a weakness in the study design, which is present in many of the other BPA developmental neurotoxicity studies.

There were no signs of maternal toxicity or foetal malformations (data not presented). In the holeboard test, the frequency and duration of head dipping for females was significantly reduced (2-4-fold, in comparison with controls) in both BPA groups, with the effects being more marked at 0.4 mg/kg/day. Among males, the only head dipping parameter affected was the frequency, which was reduced at 0.4 mg/kg/day. Reduced head dipping frequency is thought to indicate an increased state of anxiety. No other holeboard parameters were affected by BPA treatment. Among females, the proportion of time spent in the centre of the maze was significantly reduced at both 0.04 and 0.4 mg/kg/day and the frequency of self grooming was increased at 0.04 mg/kg/day. For males in both BPA exposed groups, the percentage of entries that were to open arms and the frequency of stretched posture (stretching the body forward without moving the paws) was significantly increased. A relatively higher proportion of entries to open arms is thought to indicate a decreased state of anxiety, so the results of maze test for males appear in conflict with the holeboard findings.

The effect of prenatal or neonatal exposure to BPA on social and sexual behaviour was investigated in the Sprague-Dawley rat (Farabollini *et al.* 2002). Groups of 7 females were administered 0.04 mg/kg/day BPA via the oral route, by feeding from a micropipette, either during gestation or the lactation period. A group of 13 control females received the arachis oil vehicle throughout gestation and lactation. On pnd 2 the offspring were culled to a standard litter size of 4/sex and cross-fostered to establish the following groups of 12 pups/sex:

1. Prenatal exposure group: born to BPA treated mothers and nursed by vehicle control mothers
2. Postnatal exposure group: born to control mothers but fostered to BPA treated dams
Behavioural testing commenced at about 14 weeks of age when the response of each rat to the introduction of an unfamiliar (‘intruder’) rat of the same sex and bodyweight was assessed over a 15 min period. The results for females not in dioestrus were excluded from the analysis. At 15 weeks, each rat was placed between two cages, one containing a sexually mature male and the other a sexually responsive female, and the time spent (‘sexual preference’) in the area adjacent to each cage was recorded. In a second phase of this test, each male was given access to a sexually responsive female and its sexual performance was scored. At 16 weeks, the sexual behaviour of each female, when paired with a mature male, was examined. On the day of the test, the stage of the oestrous cycle was determined. Behaviour was video recorded for later scoring by an observer blinded to treatment group. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably based on the individual pup, and did not take account of the litter of origin.

In the intruder test, the proportion of prenatally exposed males displaying defensive behaviour was greater (9 vs. 4, out of 12) and displaying ambivalent behaviour was less (3 vs. 8) in comparison with the control males. Also, the ratio of defensive/antagonistic was increased in this BPA group (by 280%). For the BPA postnatal males, and both BPA female groups, intruder test behaviour was similar to the control animals. There were no differences in sexual preference behaviour between the control and BPA exposed groups of either sex. In the sexual performance testing of males, there were statistically significant differences for several parameters in the BPA groups in comparison with the controls; in the postnatal group the mean number of intromissions required for ejaculation was increased (15 vs. 11 in the controls) and in the prenatal group the mean latency to first intromission (120 vs. 40 sec) and duration of female genital sniffing (40 vs. 15 sec) were increased. However, the numbers of mounts, latency to ejaculation and time between ejaculation and the next mount were similar for all groups. For the females, the results of the prenatal and postnatal BPA groups were combined; for BPA females in pro-oestrus the time taken to enter the arena occupied by the male was reduced (20 vs. 120 sec) and the mean number of displays of lordosis was increased (12 vs. 4).

The effect of prenatal and neonatal exposure to BPA on the behavioural response to pain of offspring was investigated (Aloisi et al. 2002). Groups of 7 Sprague Dawley female rats were administered 0.04 mg/kg/day BPA via the oral route, by feeding from a micropipette, during either gestation or the lactation period. As a control group 13 females received the peanut oil vehicle throughout gestation and lactation. Within 48 h of birth the offspring were cross-fostered to form the following groups:

1. Prenatal exposure group: 11 males and 9 females born to BPA exposed mothers and nursed by vehicle control mothers
2. Postnatal exposure group: born to vehicle control mothers but fostered to BPA exposed mothers
3. Control group: 16 males and 11 females, born to and nursed by mothers exposed to only the vehicle

At 22 weeks of age, each pup received a subcutaneous injection of either formalin (half the animals in each group) or saline in a hind paw. Each animal was then observed in an open field for 60 mins. Behaviours such as licking, flexing and jerking of the paw were recorded.
described by Dessi-Fulgheri et al. (2002). The results for 18 females from each exposure group were analysed. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably conducted using the individual pup as the experimental unit, and litter of origin was not taken into account.

Six types of behaviour were identified by principal component analysis, and statistically significant differences between the control and the BPA group were reported for three of these. The score for social and non-social exploration in the BPA group was significantly increased (by 34%) at 35 days and (by ~25%) at 45 days, in comparison with controls. Play with males was significantly decreased (by a factor of 2) at 45 days. Duration of grooming behaviour was reduced (by a factor of 2) on day 45.

The effect of BPA exposure after weaning on the behaviour of juvenile males was investigated in the Sprague-Dawley rat (Della Seta et al. 2006). Groups of 26 males were dosed via the oral route, by feeding from a micropipette, with BPA at 0 (peanut oil vehicle control) or 0.04 mg/kg/day from pnd 23 to 30. Another group of 26 males were similarly exposed to 0.0004 mg/kg/day ethinyl oestradiol. The behaviour of 12 males from each group was examined on pnd 45 and at pnd ~90. The report did not state whether the order of testing was counterbalanced with respect to treatment group.

At the younger age, the response to the introduction of a PVC tube into the home cage of males housed in groups of 4 was videorecorded and later scored by an observer blind to treatment status. As young adults, the sexual behaviour and performance of each male in the presence of a receptive adult female rat was similarly recorded and scored. Plasma 17β-oestradiol and testosterone level were measured in 5-8 males/group on pnd 37 and 105. It is not clear from the report if littermates were present in each treatment and age group and, if so, whether the statistical analysis took account of this.

There was no treatment-related effect on bodyweight. Three types of juvenile behaviour were identified by principal component analysis, and statistically significant differences between the control and the BPA group were reported for one. Biting/sniffing/climbing behaviours directed at the PVC tube were significantly lower in the BPA group. In the sexual behaviour assessment, 10/12 controls and 9/12 BPA treated males were active, and the data analysis was restricted to these animals. Only one element of sexual performance was significantly affected; latency to first intromission was reduced. Plasma testosterone levels were significantly lower than controls on pnd 37 (by 33%) and 105 (by 61%). Plasma 17β-oestradiol was not affected. In the ethinyl oestradiol group exploring, behaviours directed at the PVC tube and sexual performance were reduced, but there were no changes in hormone levels.

The effect of prenatal exposure to BPA on maternal nursing behaviour was investigated in CD-1 mice (Palanza et al. 2002). Groups of 10-12 mated females were exposed to BPA via the oral route, by feeding from a micropipette, to levels of 0 (corn oil vehicle control) or 0.01 mg/kg/day from gd 14-18. Pups were weaned on pnd 20. At 2-2.5 months of age female offspring (F1 generation) were mated, and dosed with either the vehicle or 0.01 mg/kg BPA from gd 14-18, creating four treatment groups as follows: 20 control F1 females receiving the vehicle only; 15 control F1 females receiving BPA; 15 BPA F1 females receiving the vehicle only; 15 BPA F1 females receiving BPA. Maternal behaviour was monitored over a 2 hour period on each of pnd 2-15. The report did not state whether the order of testing was counterbalanced with respect to treatment group. Additionally, F2 litter size, pup
bodyweights, cliff drop aversion and righting reflex were recorded. The statistical analysis provided an adjustment for litter effects.

F1 females exposed to BPA either only prenatally or only as an adult spent significantly less time nursing and in the nest and more time nest building, resting alone, grooming and out of the nest. The only significant effect observed in F1 females exposed to BPA both in utero and as an adult was increased time resting. There were no significant differences for the F2 generation parameters. The lack of consistency between the effects seen the groups exposed either prenatally or as an adult and the group exposed during both periods suggests that these intergroup differences were unlikely to have been caused by BPA exposure.

The effect of prenatal exposure to BPA on d-amphetamine reinforcing effects was investigated in CD-1 mice (Laviola et al. 2005). Groups of 10-12 mated females were exposed to BPA via the oral route, by feeding from a syringe, to levels of 0 (corn oil vehicle control) or 0.01 mg/kg/day from gd 11-18. At pnd 60, 3 males and 3 females from each litter (1/sex/dose level of d-amphetamine) were subjected to conditioned place preference testing. The order of testing with respect to treatment group was counterbalanced. On the first day of the test procedure, animals were familiarised to the apparatus. On days 2 and 4 each animal received an intraperitoneal injection of 0, 1, or 2 mg/kg d-amphetamine and were confined to one compartment of the test apparatus for 20 minutes. On days 3 and 5 each animal was injected with saline and confined in another compartment for 20 minutes. On the final day of testing, each animal was given free access to the entire apparatus for 10 minutes without d-amphetamine or saline treatment and the time spent in each compartment and total locomotion activity was recorded.

Conditioned place preference occurred in control females following injection with either d-amphetamine dose, but was not observed in the BPA exposed females. In males, a preference displayed for the d-amphetamine-associated compartment was similar for the BPA exposed and control animals. There were no significant differences in locomotor activity between the BPA and control groups.

Ceccarelli et al. (2007) investigated the effect of juvenile BPA exposure on brain development of Sprague-Dawley rats. Groups of 14 juveniles/group (sex distribution not stated) were dosed by the oral route, using a micropipette, with BPA at 0 (peanut oil vehicle control) or 0.04 mg/kg/day BPA from pnd 23 to 30. Another group of 14 juveniles were similarly exposed to 0.0004 mg/kg/day ethinyl oestradiol. Half the animals were killed on pnd 37 and half on pnd 90. Females killed on pnd 90 were killed in oestrus. Blood samples were taken and brains were processed for immunohistochemistry. ERα levels were analysed in three sexually dimorphic regions of the hypothalamus: arcuate nucleus, ventromedial nucleus and medial preoptic area. Serum testosterone and 17β-estradiol were determined.

The ERα analysis revealed just one statistically significant observation for comparisons between the control and same sex BPA groups; levels were higher (~2-fold) in the ventromedial nucleus among BPA females killed on pnd 37. On day 37, serum testosterone levels in the BPA males were significantly lower (by ~30%). 17β-estradiol levels were not affected by BPA treatment. In the ethinyl oestradiol group there were occasional differences in ERα levels, which were not consistent with the change in the BPA group. Also, testosterone levels were increased in males on pnd 37 and 17β-estradiol levels were increased in females on pnd 90 in the ethinyl oestradiol group. Overall, some evidence of differences in ERα in one of three sexually dimorphic regions of the hypothalamus was seen following BPA exposure to juvenile females.
shown by the BPA 0.06 and 0.6 mg/kg/day groups. In the locomotion test, activity after the morphine injection was increased (about 10-fold) at 0.006 and 400 mg/kg/day. In the binding assay, dopamine-induced binding was increased at 0.006, 0.6 or 400 mg/kg/day (by about 32, 18, and 56%, respectively), compared with controls. According to the authors, these findings suggest that prenatal and neonatal exposures to low BPA doses may potentiate central dopamine receptor dependent neurotransmission in the mouse. However, in the absence of a conventional dose-response relationship it is plausible that the observed inter-group differences were due to background variation.

Narita et al. (2007) provided a further investigation into the effect of prenatal and neonatal exposure to BPA on the dopaminergic system of male ddY strain mice. Mated females (group size not reported) received BPA via the diet at estimated dose levels of 0 or 400 mg/kg/day from either gd 0-7, gd 7-14, gd 14-20 or pnd 0-20. A similar series of investigations to those described by Narita et al. (2006) were conducted in 6-16 male offspring/group, at 7-9 weeks of age. In the place preference test, a clear preference for the compartment associated with morphine was seen in the males exposed for the periods gd 7-14 and pnd 0-20, but not for gd 0-7 or gd 14-20. In the locomotion test, activity after the morphine injection was markedly increased in the 7-14 and pnd 0-20 groups, but again the gd 0-7 or gd 14-20 groups were not affected. Similarly, dopamine-induced binding was potentiated in the gd 7-14 and pnd 0-20 groups, but not in the gd 0-7 or gd 14-20 groups.

Studies from Chemical Industry Institute for Toxicology (CIIT), USA

Three studies, focussing on brain structure, have been conducted at CIIT.

Kwon et al. (2000) examined the effect of prenatal and neonatal exposure to BPA on SDN-POA (Sexually Dimorphic Nucleus of the PreOptic Area) volume in female Sprague-Dawley rats (other reproductive parameters were also assessed). Groups of 8 mated females were dosed orally by gavage with 0 (vehicle control) 3.2, 32 or 320 mg/kg/day BPA from gd 13 to pnd 21. A positive control group received diethylstilbestrol at 1.5 mg/kg/day. On pnd 10, 1-3 female pups per litter were killed for measurement of SDN-POA volume. Other parameters measured in offspring included reproductive organ weights and histopathology in males and oestrous cyclicity, vaginal opening and lordosis behaviour in females. The statistical analysis was conducted using the litter as the experimental unit.

There was no evidence of maternal toxicity, based on bodyweight and organ weight analysis. BPA treatment had no effect on SDN-POA volume, or any of the other parameters investigated. In the positive control group there was an increase in maternal liver weight, increased SDN-POA volume and disrupted oestrous cycling. Thus, this study provided no evidence of an effect on SDN-POA volume of prenatal and neonatal BPA exposure maternal dose levels of up to 320 mg/kg/day.

The effect of short-term neonatal exposure to BPA on the development of the anteroventral periventricular nucleus of the hypothalamus (AVPV) was investigated in the Sprague Dawley rat (Patisaul et al. 2006). Group size was 5-8 pups/sex. BPA was administered by subcutaneous injection on pnd 1 and 2 at a dose level of approximately 100 mg/kg/day. A control group received the sesame oil vehicle. On pnd 19 the pups were killed and the brains were removed for immunohistochemical processing. The numbers of cells in the AVPV immunoreactive for ERα and/or tyrosine hydroxylase (TH) were counted.
Studies by researchers associated with Kushi academic institutes, Fukuoka, Japan

The Kushi group has conducted five studies, investigating the effects of prenatal and postnatal BPA exposure at maternal exposure levels of 0.002 - 1.5 mg/kg/day on behaviour or brain structure.

The effect of prenatal and neonatal exposure to BPA on behaviour and brain development was investigated in the Wistar rat (Kubo et al. 2001). Groups of 5 mated females were administered BPA via the drinking water at 0 (vehicle control) or approximately 1.5 mg/kg/day during gestation and the lactation period. Open field behaviour was investigated in offspring (11-14/group, sex and litter distribution not reported) at 6 weeks of age. The report did not state whether the order of testing was counterbalanced with respect to treatment group. A passive avoidance test was conducted at 7 weeks (11-14/group). At 20 weeks of age the volumes of the sexually dimorphic nucleus of the preoptic area (SDN-POA) and locus coeruleus were measured (6-7/sex/group). It was not clear if the statistical analysis was conducted using the litter or individual pup as the experimental unit.

In the open field, the distance moved, rearing frequency and time spent in centre of the field were all significantly greater for control females than control males. In the passive avoidance test the latency to enter the dark chamber following a shock was significantly longer in control males compared with control females of the same group. For the BPA group, these gender-related differences were not present. This was due to differences in the behaviour of both sexes; the three open field parameters were higher and the passive avoidance test latency period shorter for BPA males in comparison with control males, and vice versa for the BPA females. However, there were no statistically significant differences when the results for BPA males and females were compared with their gender controls. The volume of the SDN-POA in the control group was significantly greater in males than females, and a similar difference was also present in the BPA group. In contrast, a gender difference observed in the control group for the volume of the locus coeruleus (~14% greater for females) was reversed in the BPA group (volume was greater for males). However, it should be noted that the difference between control males and BPA males was not statistically significant, and neither was the difference between control females and BPA females.

Kubo et al. (2003) investigated the effect of prenatal and neonatal exposure to BPA on behaviour and brain development in a second study. Groups of 5-6 mated female Wistar rats were administered BPA via the drinking water at 0 (vehicle control), 0.03 or 0.3 mg/kg/day (estimated intakes). As positive controls, groups of 5 mated females received either diethylstilbestrol (~0.0065 mg/kg) or resveratrol (~1.5 mg/kg/day) via the drinking water. The animals were probably exposed throughout gestation and the lactation period, although the report is not clear on this. Open field testing was conducted at 6 weeks of age on 24 animals/group; the sex and litter distribution of the tested pups was not reported. Sexual behaviour of males (in the presence of a receptive untreated female) and females (in the presence of a sexually vigorous untreated male) was assessed at 11-12 weeks of age in 7-13 animals/sex/group. The report did not state whether the order of testing was counterbalanced with respect to treatment group. At 14 weeks of age the volume of the SDN-POA and locus coeruleus was measured (7-8/sex/group). Also, the number of neurones in the locus coeruleus was estimated. The study was conducted as 3 replicated blocks. The data were analysed using the individual pup as the experimental unit.

As with the previous Kubo et al. (2001) study, in the open field the distance moved, rearing frequency and time spent in centre of the field for control females were all significantly
greater than control males. With the exception of the distance moved in the BPA 0.03 mg/kg/day group where the normal significant sex difference was present, these sex differences were less marked and did not achieve statistical significance in the BPA group. The masking of the sex difference was due primarily to a greater distance moved and rearing frequency among BPA males and a reduced amount of time spent in the centre for the BPA females, in comparison with their gender controls. With regard to the assessment of sexual behaviour there was no evidence of a treatment-related effect in either males or females. In the diethylstilbestrol group, the open field distance moved, rearing frequency and time spent in centre of the field were significantly increased for both sexes. In the resveratrol group there were no effects on open field behaviour. Consistent with the Kubo et al. (2001) study, the volume of the SDN-POA was similar in both the control and BPA exposed groups and the gender difference observed in the control group for the volume of the locus coeruleus (greater for females) was reversed in the BPA group. However, a ‘conventional’ dose response relationship was not apparent for the locus coeruleus differences as the extent of the reversal was greater in the 0.03 mg/kg/day group. Differences in the locus coeruleus neurone count matched those observed for volume of this brain. In both the diethylstilbestrol and resveratrol groups the normal locus coeruleus sex difference was reversed but SDN-POA was not affected.

Fujimoto et al. (2006) conducted a range of behavioural testing in the offspring of mothers exposed to BPA during the last week of gestation. Groups of 6 Wistar rats received BPA via the drinking water from gd 13 to pnd 0 at 0 (vehicle control) or approximately 0.015 mg/kg/day. The following behavioural tests were conducted in the offspring (20-24/sex/group in each test): open field at 6 weeks of age, elevated plus maze at 7 weeks, passive avoidance at 8 weeks and forced swimming test at 9 weeks. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably conducted using the individual pup as the experimental unit.

In the open field test, treatment related differences were observed for rearing behaviour. The duration of rearing was significantly greater for BPA males (by ~50%) in comparison with the control males. Among the control offspring, the rearing frequency and duration was significantly greater for the females as compared with males, but this gender difference was not present in BPA group, due to both a comparative increase in rearing activity in males and a slight decrease in females of the BPA group. BPA treatment had no affect on the other parameters measured in the open field test. Behaviour in the elevated plus maze and passive avoidance test was similar for controls and the BPA group. In the forced swimming test, the duration of immobility was significantly greater (by ~75%) and the duration of limb movements was significantly less (by ~8%) for BPA males compared with control males. For females, the duration of diving was significantly greater (by ~28%) in the BPA group. Also, the significant gender difference in duration of struggling seen in the controls (longer for females) was not seen in the BPA group, due to both a comparative increase in duration for males and a slight decrease for females of the BPA group.

Kawai et al. (2003) investigated the effect of prenatal exposure on ‘aggressive’ behaviour in male CD-1 mice. Groups of 7-9 females were dosed by the oral route, using a micropipette, with BPA at 0 (corn oil vehicle control), 0.002 and 0.02 mg/kg/day from gd 11-18. ‘Aggression’ testing was conducted 8, 12 and 16 weeks of age in groups initially comprising of 26-32 male offspring randomly selected from each treatment group. Each male was placed in a cage with an ‘opponent’ mouse (a male specially selected from the control group, used for testing once/day) and their behaviour was observed for 7 mins. The report did not state whether the order of testing was counterbalanced with respect to treatment group. About 10
The consistency assessment shows that there is no discernable and reproducible pattern to the behavioural testing results. Most of the studies investigating effects at the receptor/neurotransmitter level and brain morphology have not been replicated by independent laboratories; so consistency cannot be assessed.

Overall, taking together the low confidence in the reliability of the developmental neurotoxicity studies and the lack of consistency in the results of behavioural testing, no conclusions can be drawn from these studies. This opinion is very similar to that of EFSA (2006), who reviewed nine of the developmental neurotoxicity studies.

Developmental effects on female reproductive tract expressed in old-age.

In a study designed to investigate the effects of BPA on the development of the reproductive tract, groups of 24 neonatal CD-1 mice received subcutaneous injections of BPA at dose levels of 0 (corn oil vehicle control), 0.01, 0.1 or 1 mg/kg/day from pnd 1 to 5 (Newbold et al. 2007) The neonatal mice were drawn from the pooled offspring of an unspecified number of dams. The neonates were randomly fostered to litters each comprising of 8 female pups per dam. It was not stated whether fostered litter-mates received the same experimental treatment. The female mice were weaned on pnd 21 and maintained without further experimental treatment until sacrifice at 18 months of age. The uterus and ovaries/oviducts were removed and processed for examination by light microscopy. At 18 months, 18, 23, 20 and 16 out of 24 females from the control, 0.01, 0.1 and 1 mg/kg/groups, respectively, survived. Group mean bodyweights at 18 months were similar. The incidence of histopathological changes in the ovaries and uterus is presented below:

Table 4.20 Incidence (%) of histopathological changes in reproductive tract (Newbold et al. 2007)

<table>
<thead>
<tr>
<th>Histopathological finding</th>
<th>Dose level (BPA mg/kg/day)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>Ovary/oviduct</td>
<td>Presence of corpora lutea</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Ovarian cysts</td>
<td>39</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Parovarian cysts</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Progressive proliferative lesion of oviduct</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Uterus</td>
<td>Cystic endometrial hyperplasia</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Adenomyosis</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Wolffian duct remnants in uterine wall</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Leiomyoma</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Atypical hyperplasia</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Stromal polyp</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

3 Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use: Negishi 2004, Carr 2003, Ryan and Vandenberg 2006 and Adriani 2003. The reliability of these studies is judged to be adequate because the behavioural testing has been conducted according to acceptable methods, the group sizes are quite close or equal to those recommended in the OECD TG 426, and the litter has been used as the statistical unit. The effects found in these studies indicate that there is a possible risk for developmental neurotoxicity of BPA at very low exposure levels (0.1-0.25 mg/kg/d). These effects cannot be dismissed based on the other unreliable studies in the DNT database. The above mentioned countries would therefore prefer one of two possible conclusions: 1) the available, but limited data are used for the risk assessment or 2) there is a need for further information (the countries certainly evaluate the database as sufficient to justify a concern warranting further investigation of developmental neurotoxicity), similarly to the proposed conclusion in the final expert panel report on the reproductive and developmental toxicity of BPA performed by NTP, US in November 2007.
As shown in the above table, the incidence of a number of findings was increased in the
BPA-treated groups. In particular, the incidence of cystic endometrial hyperplasia and
ovarian cysts was statistically significantly increased at 0.1 mg/kg/day, in comparison with
controls. However, these differences did not follow a dose-related pattern and could not
therefore be attributable to BPA treatment. It is also noted that these findings are inconsistent
with the results of a study by Yoshida et al (2004) (summarised in the carcinogenicity
section) in which no effects of transplacental and lactational exposure to BPA (up to 6
mg/kg/day) were seen on ovarian and uterine histopathology in 15 months-old female rats.

Human case-control study

Sugiura-Ogasawara et al. (2005) investigated the possible association between recurrent
miscarriage and serum BPA levels in a case-control study. A group of 45 women (mean age
31.6 years) with a history of 3 or more consecutive first-trimester miscarriages who attended
Nagoya City University Hospital during a 17 month period were selected for the study. The
subject selection method was not reported, although it was stated that cases with uterine
abnormalities or chromosome abnormalities in either partner were excluded from the study.
Thirty-two healthy non-pregnant women (mean age 32.0 years), with no history of pregnancy
or infertility served as controls. The occupation of most of the cases was either housewife (20
subjects) or office worker (17 subjects), whereas the control subjects were doctors, nurses
and secretaries employed by Nagoya Hospital. Immunological tests for parameters such as
antinuclear bodies (ANAs, a sensitive marker for systemic lupus erythematosus),
antiphospholipid antibodies (aPLs) and natural killer (NK) activity and blood tests for
hypothyroidism, diabetes mellitus and hyperprolactinaemia were conducted for all cases, but
not for controls. Serum BPA levels were measured using an ELISA method in single fasting
blood samples taken from all subjects 5-9 days after ovulation (Sugiura-Ogasawara, 2006).

The mean (± SD) serum BPA levels for the cases were significantly higher than controls
(2.59 ± 5.23 vs. 0.77 ± 0.38 ng/ml). However, median BPA levels in the two groups were
similar (0.71 vs. 0.705), so whether there was a genuine difference between the groups (as
claimed by the author) is questionable. The large SD for cases and similar median values for
cases and controls indicates that a small number of women with very high BPA levels were
responsible for the higher group mean levels. A comparison of BPA levels within the cases
showed that the higher levels of BPA were associated with ANA-positive status (10 cases)
but not with hyperthyroidism (8 cases), luteal phase defect (9 cases), hyperprolactinaemia (5
cases) or aPL-positive status (6 cases). Thirty-five cases achieved pregnancies subsequent to
the study, of which 17 miscarried again. The mean serum BPA levels for those who
miscarried again was higher, though not statistically significantly, than those who did not
(4.39 ± 8.08 vs. 1.22 ± 1.07 ng/ml), although the median values for the two groups were
similar (0.71 vs. 0.91 ng/ml).

There are a number of limitations to this study (as pointed out by Berkowitz, 2006). Group
sizes were relatively small, so the subjects may not be representative of the populations from
which they are drawn. The cases and controls were not comparable in terms of occupation
and may not have been comparable in terms of the presence of potential confounding factors.
The BPA levels for the cases were measured at a time-frame that was not relevant to the
induction of their miscarriages. The reliability of the reported BPA measurements is
uncertain because the ELISA method is not optimal for BPA in serum due to cross-reactivity and other problems (Fukata et al. 2006, discussed in section 4.1.1.3.2). A parametric statistical test (Welch’s test) was used to compare mean BPA levels for the cases and controls, but the distribution of results indicate that use of a non-parametric would have been appropriate. Overall, given the significant limitations in the design and conduct of this study and lack of a convincing difference in serum BPA levels between the cases and controls, no conclusions can be drawn regarding an association between BPA exposure and recurrent miscarriage.

4.1.2.9.3 Impact of new information and summary of reproductive toxicity

The new 2-generation study in mice (Tyl et al. 2007) provides a comprehensive, definitive, investigation of the effects of BPA on reproduction at exposure levels spanning the low (μg/kg bw/day) to high (mg/kg bw/day) ranges. This study has shown that BPA causes adverse effect on pregnancy and the offspring, observed as a slightly increased duration of gestation, reduced pup bodyweight during lactation, a slight increase in the incidence of undescended testes at weaning, seminiferous tubule hypoplasia in offspring at weaning, and delayed acquisition of preputial separation, at 600 mg/kg/day, an exposure level that also caused mild parental toxicity. Fertility was not affected by BPA exposure, which resolves the previous uncertainty regarding the the NOAEL for fertility in mice. A study NOAEL for reproductive toxicity of 50 mg/kg/day has been identified. As there was no evidence of an adverse effect on the development of the male reproductive tract at μg/kg bw/day doses of BPA, the study resolves the uncertainties surrounding the potential to produce adverse effects on development at low doses. Thus, a NOAEL of 50 mg/kg/day for reproductive toxicity, which was a provisional position in the original risk assessment report, should be used in the risk assessment.

No conclusions could be drawn from the new developmental toxicity studies or from a human study investigating the possible association between recurrent miscarriage and BPA exposure. Therefore, these studies do not influence the conclusions of the original risk assessment report.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Toxicokinetics: The toxicokinetics of BPA have been well studied in rats both in vivo and in vitro, and have been investigated to a lesser extent in mice and cynomolgus monkeys. Two studies have investigated the toxicokinetics and fate of an oral dose of labelled BPA in human volunteers.

In the species studied (rats, mice, monkeys, humans), the available evidence suggests that following oral administration, BPA is rapidly and extensively absorbed from the gastrointestinal tract. Analysis of plasma AUC values suggests that the extent of absorption from the GI tract is up to 86% in rats and up to 85% in monkeys. The only relevant human

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4 Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.
Considering all of the available genotoxicity data, and the absence of significant tumour findings in animal carcinogenicity studies, it does not appear that BPA has significant mutagenic potential *in vivo*. Any aneugenic potential of BPA seems to be limited to *in vitro* test systems and is not of concern. 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.

New information on the mutagenicity of BPA has shown that BPA produces an increase in congression failure, a misalignment of chromosomes during the metaphase stages of meiosis II in oocytes of female mice. However, in view of several methodological weaknesses and flaws identified in these new data along with the reporting inadequacies, and taking into account the known mutagenicity and toxicological profile of BPA, these results cannot in themselves be taken as conclusive evidence of an effect of BPA on germ cell meiosis. Furthermore, these findings have not been confirmed in more recent publications. Therefore, the original conclusion from the published assessment that BPA has no significant mutagenic potential *in vivo*, is still valid.

**Carcinogenicity:** There are no human data contributing to the assessment of whether or not BPA is carcinogenic. In animals, BPA has not shown any significant carcinogenic activity in two standard oral cancer bioassays in rats and mice. 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/m³ 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/m³.

New information on the potential carcinogenic and/or promoting effects of BPA in prenatal and neonatal rat models indicates that BPA does not possess significant carcinogenic potential and does not exert promoting activity on the carcinogenesis induced by established carcinogens/initiators in specific organs. Taking into account all of the animal data available the evidence suggests that BPA does not have carcinogenic potential.

**Reproductive toxicity:** BPA has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with BPA glucuronide *in vitro*. It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories. Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of BPA on fertility and reproductive performance have been investigated in four good quality, comprehensive, studies: a 2-generation study in the rat, a multigeneration study in the rat, a continuous breeding study in the mouse and a 2-generation study in the mouse. Although no effect on fertility was seen in the rat 2-generation study, low-dose levels were employed (0.2-200 µg/kg/day). In the multigeneration study, an effect on fertility (reduction
in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of BPA. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse continuous breeding study, it is prudent to assume that BPA may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg/day and below in the multigeneration study. In the mouse 2-generation study, using dose levels of 0.003-600 mg/kg/day, no effects on fertility, reproductive organ weights and histopathology or sperm production were observed. However, the continuous breeding study in the mouse provided some evidence of an adverse effect on fertility. In the F0 generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter tested at each dose level from the F1 generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F1 generation. However, the significance of this finding is uncertain given that there was no effect on fertility in this generation, and where an adverse effect on fertility was seen (in the F0 generation) there was no effect on epididymal weight. Furthermore, there were no effects on epididymal weight in the mouse two generation study. Overall, a NOAEL of 50 mg/kg/day can be identified for effects on fertility based on the rat multi-generation study.

No evidence that BPA is a developmental toxicant was observed in standard developmental toxicity studies in rats and mice. In rats, a maternal LOAEL and foetal NOAEL of 160 and 640 mg/kg/day, respectively, were identified. In mice, maternal and foetal NOAELs were 250 and 1,000 mg/kg/day, respectively. In a rat multigeneration study, a statistically significant decrease in mean pup body weight gain, with concomitant delays in the acquisition of developmental landmarks (vaginal patency and preputial separation) was observed at 500 mg/kg on post-natal days 7-21 in males and females of all generations (F1-F3). These decreases in pup body weight gain and delays in development were seen in the presence of maternal toxicity. No maternal toxicity and no treatment-related effects were reported in the offspring of animals exposed to 50 mg/kg. Similarly, effects on F1 (but not F2) pup bodyweight gain were observed in the mouse two generation study at 600 mg/kg/day, a dose level that also caused mild parental toxicity. Additionally, there was an increase in the incidence of undescended testes and seminiferous tubule hypoplasia in F1 and F2 offspring at weaning at 600 mg/kg/day, although similar effects were not seen in adult F1 males. No adverse effects were seen at 50 mg/kg/day and below in the mouse study.

Several additional studies have focused on the potential of BPA to affect male reproductive tract development in rats and mice. Conflicting results have been reported in these studies, in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at low dose levels, in the range 2–50 µg/kg. However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects. Also, no effects on male reproductive tract development were observed in a recent mouse two generation study, which was conducted specifically to help resolve the uncertainties surrounding the potential for BPA to affect development at low doses. Giving most weight to the negative ‘gold standard’ mouse two generation study, and taking account of the fact that there were no functional changes in reproductive parameters or reproductive organ
development at low dose levels in the rat multigeneration and two generation studies, it is concluded that BPA does not have an adverse effect on male reproductive tract development at low dose levels.

The effect of prenatal and perinatal exposure to BPA on neurological development has been investigated in a large number of recent studies. Many developmental neurotoxicity endpoints were evaluated: locomotory and exploratory activity; grooming, cognitive, emotional, social, sexual and maternal behaviour; behavioural response to pharmacological challenge; brain morphology, immunohistochemistry, and receptor/gene expression. Although a number of these studies claimed to have detected a BPA-related effect on development, no firm conclusions could be drawn about developmental neurotoxicity because of a low level of confidence in the reliability of the studies and a lack of consistency in the results.5

Also, no conclusions could be drawn from a human study investigating the possible association between recurrent miscarriage and BPA exposure.

Thus, a NOAEL of 50 mg/kg/day can be identified for developmental toxicity, taken from the rat multigeneration study and the mouse two-generation study.

Summary: Overall, the hazardous properties of BPA have been evaluated in animals to the extent that the minimum data requirements according to Article 9(2) of Regulation 793/93 have been met. The key health effects of eye irritation, respiratory tract irritation, skin sensitisation, local effects on the respiratory tract and systemic effects on body weight, liver and kidney following repeated exposure and reproductive toxicity have been identified. No dose response information is available for eye irritation. A NOAEL of 10 mg/m^3 has been identified for repeated dose toxicity to the respiratory tract. A NOAEL of 50 mg/kg has been identified for systemic effects following repeated exposure. In relation to reproductive toxicity, a NOAEL of 50 mg/kg has been established in multi- and two-generation studies for effects on fertility and development.

For the purposes of risk characterisation, absorption via the oral and inhalation routes will be assumed to be 100%; dermal absorption will be taken to be 10%.

To conduct the risk characterisation for workers and consumers, it is necessary to compare human exposure for the inhalation/dermal route with oral N(L)OAELs from repeated dose animal studies, because of the absence of significant inhalation/dermal toxicity data. A direct comparison between exposure and systemic effects (with the exception of effects on the liver) must take account of the first pass effect, which has been shown to limit systemic bioavailability by the oral route (see toxicokinetic section). To compensate for this limited oral bioavailability (shown to be around 5-10% of the administered dose in rodents – see toxicokinetic section), the oral animal N(L)OAELs have been reduced by a factor of 10 for the comparison of inhalation or dermal exposure and systemic effects. For effects on the

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5 Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use: Negishi 2004, Carr 2003, Ryan and Vandenberg 2006 and Adriani 2003. The reliability of these studies is judged to be adequate because the behavioural testing has been conducted according to acceptable methods, the group sizes are quite close or equal to those recommended in the OECD TG 426, and the litter has been used as the statistical unit. The effects found in these studies indicate that there is a possible risk for developmental neurotoxicity of BPA at very low exposure levels (0.1-0.25 mg/kg/d). These effects cannot be dismissed based on the other unreliable studies in the DNT database. The above mentioned countries would therefore prefer one of two possible conclusions: 1) the available, but limited data are used for the risk assessment or 2) there is a need for further information (the countries certainly evaluate the database as sufficient to justify a concern warranting further investigation of developmental neurotoxicity), similarly to the proposed conclusion in the final expert panel report on the reproductive and developmental toxicity of BPA performed by NTP, US in November 2007.