restricted, which adds to this concern. Overall, because of doubts regarding the extent of exposure of the target tissue, only limited significance can be given to this negative result.

4.1.2.7.3 Summary of mutagenicity

No human data are available. Nonylphenol tested negative in two bacterial assays and an *in vitro* mammalian cell gene mutation assay. An *in vivo* micronucleus test, conducted using the intraperitoneal route, was negative. A second in vivo micronucleus test, which used the oral route, was also negative, although there were methodological weaknesses in this study. These results show that nonylphenol is not mutagenic.

4.1.2.8 Carcinogenicity

Carcinogenicity has not been studied directly in humans or animals. However, some information on the carcinogenic potential can be derived from other data. On the basis of the information currently available it is considered unlikely that that nonylphenol is mutagenic, so concerns for cancer caused by a genotoxic mechanism are low. Considering the potential for carcinogenicity by a non-genotoxic mechanism, no evidence of sustained cell proliferation or hyperplasia was seen in the standard repeated exposure toxicity studies. Nonylphenol has been reported to induce cell proliferation in the mammary gland of the Nobel rat following subcutaneous exposure at levels down to 0.05 mg/kg/day, but this finding could not be reproduced in a duplicated study; furthermore there are doubts about the relevance of this model to humans because of the route of exposure and sensitivity of the strain selected. Overall, there are low concerns for carcinogenicity by a non-genotoxic mechanism.

4.1.2.9 Toxicity to reproduction

Only data from animals or *in vitro* test systems are available.

4.1.2.9.1 Studies investigating oestrogenic activity

The oestrogenic activity of nonylphenol has been investigated in a number of studies using either recombinant yeast, oestrogen sensitive MCF-7 cells or a rodent uterotrophic assay response. None of these assays have been validated as an internationally accepted toxicity test method, although the MCF-7 and uterotrophic assays have been established for a number of years as standard assays for oestrogenic activity. It should be noted that the significance to human health of oestrogenic activity detected in these assays has yet to be established.

<u>In vitro systems</u>

4-Nonylphenol was one of a number of alkyl phenols tested in a yeast assay in a study which looked at the structural features important for oestrogenic activity in this chemical group (Routledge and Sumpter, 1997). The assay uses a recombinant strain of yeast (*Saccharomyces cerevisiae*) which contains an oestrogen-inducible expression system. In the presence of oestrogens a reporter gene (Lac-Z) encoding for the enzyme ß-galactosidase is expressed, which can be monitored by measuring a colour change reaction in the culture medium. The oestrogenic activity of the test substances was expressed as a potency relative to 17ß-oestradiol by comparing the molar concentrations required to produce the same response. 17ß-oestradiol was found to be about 30 000 times more potent than nonylphenol. Tamoxifen, an oestrogen antagonist known to act via the oestrogen receptor, was shown to inhibit the activity of the alkyl

phenols, demonstrating that the assay response was due to interaction with the oestrogen receptor.

The oestrogenic activity of nonylphenol has also been assessed in an *in vitro* assay involving oestrogen sensitive human breast tumour MCF-7 cells (Soto et al., 1991). The cells are cultured in the presence of charcoal-stripped (to remove endogenous oestrogens) human serum which inhibits cell proliferation. Substances with oestrogenic activity can then overcome this inhibition. The MCF-7 cells were cultured 17ß-oestradiol and nonylphenol at several concentrations were each cultured in triplicate in multiwell plates and cell proliferation was assessed after a six-day exposure period by counting nuclei from lysed cells. Nonylphenol at a concentration of 10 µM elicited a similar proliferative response to oestradiol at a concentration of 30 pM; thus, on a molar basis the oestrogenic potency of oestradiol, as measured in this assay, is 3 000 000 times greater than that of nonylphenol. At concentrations of 1 and 0.1 µM the proliferative response produced by nonylphenol was similar to that observed in negative control cultures.

In another similar *in vitro* assay, MCF-7 and ZR-75 human breast cancer cell lines were used (White et al., 1994). Cells were cultured in quadruplicate in the presence of nonylphenol at concentrations ranging from 0.1 nM to 10 µm or 17ß-oestradiol at 10 nM. No oestrogenic activity was detected at nonylphenol concentration of 100 nM and less. At 1 and 10 µM nonylphenol elicited a proliferative response which at the higher concentration was similar to that produced by oestradiol. Thus, 17ß-oestradiol was 1000 times more potent than nonylphenol in this assay. In a further investigation, the ability of nonylphenol to stimulate transcriptional activity was determined in MCF-7 and chicken cell fibroblasts (CEFs) transfected with reporter gene pEREBLCAT and a mouse oestrogen receptor. Nonylphenol stimulated transcription at culture concentrations of 1 and 10 µM.

To summarise the *in vitro* oestrogenic data, there is evidence that nonylphenol has oestrogenic activity, of 3-6 orders of magnitude less potent than oestradiol.

In vivo systems

The oestrogenic activity of nonylphenol has been assessed in several studies using an assay based upon the uterotrophic response in the rat.

In the first study, five groups of immature (aged 20 - 22 days) female rats (six in each group) of a Wistar derived strain received single oral gavage doses of nonylphenol in corn oil on each of three consecutive days (ICI, 1996). The dose levels ranged from 9.5 to 285 mg/kg/day. Vehicle and positive (oestradiol benzoate 8 µg/kg, by subcutaneous route) groups were included. One day after the final dose the females were killed and the uterus was removed from each animal and weighed. Absolute uterus weight and bodyweight related uterus weight were statistically significantly increased, in a dose-dependent manner, at levels of 47.5 mg/kg/day and above. The NOAEL was 9.5 mg/kg/day. The uterine response seen in the positive control group was much greater than that of the nonylphenol groups, although a direct comparison of potency is not possible given the differing exposure routes. Similar data from the same laboratory have also been presented in peer-review literature (Odum et al., 1997). This latter report also included oral positive control groups (17ß-oestradiol, 10-400 µg/kg), which indicated that oestradiol was about 1000 times more potent in this assay than nonylphenol.

In a similar assay, groups of ten ovariectomised female Sprague-Dawley rats were dosed once daily for three consecutive days by the oral route with ethanol/oil suspensions of nonylphenol at

levels of 0 (vehicle control), 30, 100 and 300 mg/kg/day (Chemical Manufacturers Association 1997b). Positive control groups received ethynyloestradiol in ethanol at levels of 10, 30 and 80 µg/kg/day according to the same dosing regimen. One day after the final dose the females were killed and the uterus was removed from each animal and weighed. Uterus weights at 300 mg/kg/day were significantly increased (1.5-fold) in comparison with the vehicle control group. A slightly greater response (a 2-fold increase) was seen in the 30 and 80 µg/kg/day positive control groups.

In another uterotrophic assay, groups of three immature (aged 20 - 21 days) Sprague-Dawley rats each received a single intraperitoneal injection of nonylphenol at dose levels of 0, 1, 2 or 4 mg/animal (approximately 25, 50 or 100 mg/kg) (Lee and Lee, 1996). Oestradiol, administered by the same route, served as a positive control. The animals were killed 24 hours later and each uterus was removed, weighed and analysed for protein and DNA content and peroxidase (thought to be a uterotrophic marker enzyme) activity. There was a dose-dependent and statistically significantly increase in uterine weight at all levels, with associated increases in uterine protein and DNA content and uterine peroxidase activity. In further experiments, the uterotrophic activity of nonylphenol was found to be blocked by the co-administration ICI 182,780, an oestrogen antagonist, providing evidence that the effect of nonylphenol is mediated through the oestrogen receptor. Also, the potency was compared with oestradiol; in this assay oestradiol was found to be about 1000 - 2000 times more potent than nonylphenol.

Overall, these *in vitro* and *in vivo* studies show that nonylphenol has oestrogenic activity of a potency that is between 3 to 6 orders of magnitude less than that of oestradiol.

4.1.2.9.2 Effects on fertility

The effects of nonylphenol on fertility and reproductive performance have been investigated in a multigeneration study, and additionally, the testicular toxicity of nonylphenol has been studied in a repeated exposure study.

The multigeneration study was comprehensive, of good quality, and was conducted in compliance with GLP (NTP 1997). The overall study design was based on the OECD two-generation reproduction toxicity study guideline, with an extension to include the production of an F_3 generation. Groups of thirty male and thirty female Sprague-Dawley rats were exposed to nonylphenol via incorporation in the diet at concentrations of 0 (control) 200, 650 or 2000 ppm over three generations. Calculated nonylphenol intakes were, respectively, about 0, 15, 50 and 160 mg/kg/day during non-reproductive phases and rising to around 0, 30, 100 and 300 mg/kg/day during lactation.

Nonylphenol exposure commenced for the F_0 generation at about 7 weeks of age and continued until study termination when the F_3 generation were about 8 weeks old. F_0 animals were mated (one male with one female) within each dose group to produce the F_1 generation, selected F_1 animals were similarly mated to produce the F_2 generation and selected F_2 animals were mated to produce the F_3 generation. For the F_0 generation and retained F_1, F_2 and F_3 animals, clinical signs of toxicity, bodyweights and food consumption were reported. Oestrous cycles were monitored prior to mating. At the necropsy of adult animals, sperm samples were taken (but not from the F_3 generation) for analysis of density, motility (using a computer assisted sperm motion analysis system, only conducted on control and high dose group males) and morphology, a number of organs were weighed and selected organs were sampled for histopathology. Additionally, testicular spermatid counts were made. Parameters assessed in the young offspring included litter

size, bodyweights, survival, gross appearance, ano-genital distance, sexual development and, for animals killed at weaning, gross appearance of organs at necropsy and reproductive organ weights.

There was evidence of general toxicity in adults of all generations, seen as a reduction in bodyweight gain at 50 and 160 mg/kg/day and histopathological changes in the kidneys at all dose levels. These aspects are described in greater detail in section 4.1.2.6.1.

Considering the reproduction-related parameters, there were no adverse effects on fertility or mating performance. However, several other parameters were affected. Oestrous cycle length was increased by about 15% in the F_1 and F_2 females at 160 mg/kg/day, in comparison with controls. The timing of vaginal opening was accelerated by 1.5-7 days at 50 mg/kg/day and by 3-6 days at 160 mg/kg/day in females of the F_1, F_2 and F_3 generations. Also, absolute ovarian weights were decreased at 50 mg/kg/day in the F_2 generation and at 160 mg/kg/day in the F_1, F_2 and F_3 generations; however, no effect on ovarian weight was apparent in the F_1 and F_3 generations when analysed as an organ-to-bodyweight ratio. In males, changes in sperm endpoints were seen only in the F_2 generation; epididymal sperm density was decreased by about 10% at 50 and 160 mg/kg/day and spermatid count was decreased by a similar amount at 160 mg/kg/day. However, there may have been methodological problems with the epididymal sperm density measurements, because the density in all F_2 generation groups, including controls, was considerably greater (by about 25-40%) than reported for the F_0 and F_1 generation males; the age of each generation was similar at necropsy, so major differences in the sperm density would not be expected.

To summarise the reproductive aspects of this study, fertility and mating performance were not adversely affected by nonylphenol treatment. However, there were changes, albeit relatively slight, in the oestrous cycle length, timing of vaginal opening, ovarian weight and sperm/spermatid count. The effects on the oestrous cycle were seen in both the F_1 and F_2 generations (not assessed in F_3 females) and the timing of vaginal opening was influenced in all three generations; this consistency provides firm evidence of a relationship with treatment. These effects were possibly related to the oestrogenicity of nonylphenol. There is some uncertainty about the relationship to nonylphenol treatment with respect to the ovarian weight reduction because this effect was apparent after adjusting for bodyweight in only one generation and did not correlate with any histopathological changes; nevertheless, it is compatible with the anticipated direct effects of exogenous oestrogenic activity. Also, there is uncertainty regarding the cause of the apparent reduced sperm/spermatid numbers in the F_2 generation. It has been hypothesised that such changes could result from foetal or neonatal exposure to exogenous oestrogenic activity (Sharpe and Skakkebaek, 1993), but if the hypothesised mechanism were operating, semen/testicular changes should also have occurred in the F_1 generation. Furthermore, the possibility of methodological problems adds to the difficulty in interpreting the sperm/spermatid count data. However, the observation of impaired male reproductive tract development in an intraperitoneal study summarised in section 4.1.2.9.3 provides supporting evidence in favour of the sperm/spermatid count changes being causally related to nonylphenol treatment. Furthermore, the intraperitoneal study indicates that a critical window of exposure for this effect is likely to be the neonatal period. Overall, this study provided evidence that nonylphenol exposure over several generations can cause minor perturbations in the reproductive system of offspring, which are compatible with the predictable or hypothesised effects of exogenous oestrogenic activity, although these perturbations do not cause functional changes in reproduction of the rat at the dose levels tested. A clear NOAEL for these changes of 15 mg/kg/day was identified.

The testicular toxicity of nonylphenol was investigated in Sprague Dawley rats in a briefly reported repeated dose study (de Jager et al., 1999a). Groups of 20 male rats were dosed once daily by the oral (gavage) route at doses levels of 0 (vehicle control, cotton seed oil), 100, 250 or 400 mg/kg/day for a period of 10 weeks, from the age of 12 weeks. The animals were killed at the end of the dosing period and a detailed evaluation of the reproductive organs was conducted. Testes and epididymal weight were recorded. The total cauda epididymal sperm numbers were determined. The testes were stored in Bouin's fixative and processed for histological examination, which included the identification of the stages of spermatogenesis present and the measurement of the seminiferous tubule diameter, lumen diameter and epithelial thickness.

Three, 15 and 18 animals from the 100, 250 and 400 mg/kg/day groups, respectively, died during the dosing period; no further information on these deaths was presented. Clinical signs of toxicity were not reported. The bodyweight gain of surviving animals was not affected by treatment, although bodyweight gain was reduced among the decedents. In comparison with the control group, lower testicular and epididymal weight, tubule and lumen diameter and seminiferous epithelial diameter were seen in surviving animals at 250 and 400 mg/kg/day and the sperm count was reduced at 400 mg/kg/day, but because of the very small groups sizes due to mortality, little toxicological significance can be accorded to these findings. At 100 mg/kg/day, testes and epididymal weight were not affected, but tubule and lumen diameter and seminiferous epithelial diameter were statistically significantly lower than found in the control group; the mean tubule diameter was reduced by 10%, but data for the other two parameters were not presented. Testicular abnormalities were identified by histopathology at both 250 and 400 mg/kg/day. In one animal at 250 mg/kg/day vacuolization and cell necrosis with sloughing of the epithelium was seen in about 40% of tubules. Both surviving animals at 400 mg/kg/day had tubular vacuolization, cell necrosis and derangement, with very few secondary spermatocytes and sperm being present.

This study provides evidence of nonylphenol-related testicular toxicity at exposure levels which also cause mortality. A LOAEL for testicular toxicity of 100 mg/kg/day can be designated. The observation of mortality at 100, 250 and 400 mg/kg/day in this gavage study contrasts with the findings of studies involving dietary administration summarised in the Repeated Dose Toxicity section (Hüls, 1989; Chemical Manufacturers Association, 1997a; NTP, 1997). This difference can probably be accounted for by the method of administration; gavage dosing is likely to produce higher peak concentrations of nonylphenol in the blood than dietary administration.

4.1.2.9.3 Developmental toxicity

A good quality standard oral rat developmental toxicity study and two studies, one using the intraperitoneal route and one using the oral route, looking specifically at the potential effects on the developing male reproductive tract are available.

The standard rat developmental toxicity study is well-reported, conducted according to OECD guideline 414 and in compliance with GLP (Initiative Umweltrelevante Altstoffe, 1992). Groups of timed-mated females of the Wistar strain were administered by oral gavage corn oil solutions of nonylphenol from days 6 to 15 of pregnancy at dose levels of 0, 75, 150 and 300 mg/kg/day. A further group receiving 600 mg/kg/day was terminated prematurely because many females died during the first few days of treatment. Sufficient females were allocated to the study to produce at least 21 pregnant females in each group. Surviving females were killed on day 20 of pregnancy and the foetuses were subjected to routine external, visceral and skeletal examinations.

There was clear evidence of maternal toxicity at 300 mg/kg/day, manifested as a reduction in bodyweight gain and food consumption, mortality of two females and the macroscopic organ changes in the kidney (pale or irregular shape in seven mothers) or spleen (reduced size in two mothers). Similar macroscopic changes were seen occasionally at 150 mg/kg/day and at a high incidence in females from the prematurely terminated 600 mg/kg/day group. No maternal toxicity was seen at 75 mg/kg/day. Post-implantation loss, litter size, foetal weights and incidence of both major and minor foetal abnormalities were not affected by treatment. To conclude, this study provides no evidence of developmental toxicity in the rat at exposure levels which are toxic to the mother; thus the maternal NOAEL was 75 mg/kg/day and the foetal NOAEL was 300 mg/kg/day.

In the intraperitoneal study, which was briefly reported, the effects of nonylphenol on male reproductive tract development were investigated in neonatal Sprague-Dawley rats (Lee, 1998; additional information was obtained by personal communication with the author). Age-matched male pups were randomly allocated to either the control or treated groups. Daily doses of nonylphenol were administered by the intraperitoneal route at a dose volume of 5-10 µg/injection, for varying schedules between the day of birth (day 0) and 30 days of age. Control animals received the vehicle (dimethylsulfoxide) only, by the same route. The pups were killed at 31 days of age; terminal observations included external appearance of genital area, ano-genital distance, the presence of undescended testes, and reproductive organ weights (which were reported as bodyweight-related values).

In the initial experiment, groups of at least three pups were dosed at 0, 0.08, 0.8 and 8 mg/kg/day, from birth to 15 days of age. At 0.8 and 8 mg/kg/day there was a statistically significant, dose-dependent, reduction in testes, epididymis, seminal vesicle and prostate weight; typically weights were about 15 to 25% less than found in the control group. Additionally, ano-genital distance was reduced at 8 mg/kg/day, only. Reproductive organ weights were not affected at 0.08 mg/kg/day. Next, groups of three or four pups received nonylphenol at 0 or 8 mg/kg/day, either from days 1 to 18 of age, days 6 to 24 or days 13 to 30, to see if there is a vulnerable phase of development. Reproductive organ weights were significantly reduced in the groups for which dosing commenced on day 1 or 6, but not in the group dosed from day 13. In a third experiment, the influence of the oestrogen receptor antagonist ICI 182,780 on nonylphenol-impaired reproductive organ weight development was investigated in groups of six or seven pups dosed with nonylphenol at 8 mg/kg/day from days 1 to 5 of age. The antagonist was administered by the intraperitoneal route at a dose of 0.5 mg/kg and dose volume of 5-10 µg/injection, 10 minutes after the nonylphenol dose. It was found that ICI 182,780 blocked the effects of nonylphenol on organ weights. Administration of ICI 182,780 alone had no effect on reproductive organ weight. The incidence of undescended testes was reported in groups of between 6 and 34 pups dosed with nonylphenol at 8 mg/kg/day, days 1 to 5, days 1 to 10 or days 1 to 18; this was 33%, 55% and 62%, respectively. Undescended testes were not observed in vehicle control pups, in pups receiving a single dose of nonylphenol on day 1, or when ICI 182,780 was administered concurrently with nonylphenol.

In a final experiment, eight male pups, selected from two litters, were dosed by the intraperitoneal route from days 1 to 15 of age with nonylphenol at 8 mg/kg/day and then reared to sexual maturity. Their fertility was assessed by serial pairing with either six or twenty untreated female rats and recording the number of females which became pregnant. Vehicle control male pups, selected from the same two litters, were used for comparison. Among the controls, pregnancies resulted from almost all pairings. In contrast, in the nonylphenol treated group, two males were completely infertile, failing to impregnate any females; three were

initially fertile, but failed to impregnate females in later pairings; two showed comparable fertility to the controls; the remaining male died near the start of the fertility trial. Necropsy findings were reported for five of the nonylphenol-treated males; all were observed with undescended testes and/or either slight or marked testicular atrophy.

There are a number of design weaknesses to this study: the group sizes were generally very small; the pups were apparently not weight-matched at the start of treatment; and the intraperitoneal route of administration, which could result in unrealistically high exposure of the reproductive organs, is of questionable relevance to the human risk assessment involving the inhalation, dermal and oral routes. Nevertheless, the consistent observation throughout the series of experiments of reduced reproductive organ weight or undescended testes, supported by observations of reduced ano-genital distance and, in animals reared to sexual maturity, reduced fertility, provide evidence that nonylphenol exposure during the neonatal period impairs male reproductive tract development in the rat. The period of maximum vulnerability to this effect appears to be prior to the age of 13 days. The blocking influence of the oestrogen receptor antagonist ICI 182,780 suggests that the effect of nonylphenol on the male reproductive tract may be mediated through action on the oestrogen receptor. However, in view of corrosive properties of nonylphenol and use of the intraperitoneal route of administration, it is possible that non-specific irritation of the undescended testes may have contributed to the observed effects. The author has stated that about 50% of the nonylphenol treated pups had peritoneal cavity adhesions, while none were seen in control animals, which supports this hypothesis. Although adhesions were seen, there were no treatment-related clinical signs of toxicity or increased mortality. The blocking influence of ICI 182,780 may possibly have resulted from dilution of the injected nonylphenol (this alternative explanation was not tested as the study did not include a control group receiving nonylphenol followed by a vehicle only injection). It should be noted that precise information on clinical signs, mortality and general macroscopic necropsy findings were not available from the author. No effects were seen in pups dosed at 0.08 mg/kg/day but, because of the very small numbers of animals receiving doses other than 8 mg/kg/day, information on the NOAEL and dose-response relationship can be gained from this study. Overall, because of the design weaknesses and the possibility that non-specific irritation may have contributed to the observed effects on the male reproductive tract, it is not possible to draw any firm conclusions from this study with respect to specific reproductive toxicity of relevance to humans. Consequently, this study carries little weight in the overall assessment of the available reproductive toxicity data base.

In the third study, which was briefly reported, the effects of nonylphenol exposure from the *in utero* period to sexual maturity of nonylphenol exposure were investigated in an oral (gavage) study (de Jager et al., 1999b). Groups of 10 mated females were dosed once daily with nonylphenol at levels of 0 (vehicle control, cotton seed oil), 100, 250 and 400 mg/kg/day from day 7 of pregnancy to weaning of their litters. Twenty F_1 generation males were randomly selected from each group for dosing as for the mother until 10 weeks of age. The selected F_1 males were then killed. Testes and epididymal weight were recorded. The total cauda epididymal sperm numbers were determined. The testes were stored in Bouin's fixative and processed for histological examination, which included the identification of the stages of spermatogenesis present and the measurement of the seminiferous tubule diameter, lumen diameter and epithelial thickness.

Concerning maternal toxicity, no information was presented on maternal bodyweights, but it was stated that no females showed any physical or behavioural abnormalities. No offspring were born from the mothers receiving 400 mg/kg/day; it is not clear from the report if this was because of maternal deaths or embryonic/foetal resorption.

There were no malformations or still births among the F_1 offspring. No physical or behavioural abnormalities were seen among the selected F_1 males, although possibly two animals at 250 mg/kg/day died since the group size at termination of the study was reduced to 18; this contrasts with the de Jager (1999a) study conducted in adult males in which 15 out of 20 animals died at 250 mg/kg/day (see section on Effects on Fertility). F_1 bodyweight gain over the course of the study was significantly reduced at both 100 and 250 mg/kg/day (by 11 and 20%, respectively), relative to the control group. F_1 absolute testicular and epididymal weights were less than the controls at both 100 and 250 mg/kg/day, but this effect was not evident when organ weights were expressed relative to bodyweight; the differences in absolute organ weight are thought likely to be related to the intergroup bodyweight differences. Total cauda epididymal sperm count was reduced at 250 mg/kg/day (by 36%, relative to controls), but at 100 mg/kg/day sperm counts were similar to those of the control group. Seminiferous tubule diameter was slightly lower in both nonylphenol treated groups (by about 10%); surprisingly, these slight differences were declared to be highly statistically significantly different from the control group. The authors also stated that the tubule lumen diameter and seminiferous epithelium thickness were highly statistically significantly less than the control group in both nonylphenol groups, but the data were not presented. Although these quantitative tubular changes were consistent with those of the de Jager (1999a) study, in the present study these may be related to the fact that testicular weight was lower in these groups. Histopathology revealed pathological changes in the testes of one F_1 male from the 100 mg/kg/day group; in the tubules, cell necrosis, vacuolation and sloughing of the germinal epithelium were described. However, no such histopathological abnormalities were seen at 250 mg/kg/day, so the changes outlined above cannot be attributed to nonylphenol treatment.

This study provides evidence of a reduction in sperm count at 250 mg/kg/day, a dose level which may have caused mortality, although it is not possible to state whether this is a developmental effect or as a result of direct exposure to the males after weaning. It is not clear if the changes in the tubular measurements represent specific reproductive toxicity or non-specific secondary consequences of the reduction in bodyweight gain.

4.1.2.9.4 Summary of toxicity to reproduction

No human data are available. Nonylphenol has been shown to have oestrogenic activity in a number of *in vitro* and *in vivo* assays. The potency of this oestrogenic activity in these assays ranged from 3 to 6 orders of magnitude less than that of oestradiol. The effects of nonylphenol on fertility and reproductive performance have been investigated in a good quality oral (dietary administration) multigeneration study in the rat. This study provided evidence that nonylphenol exposure over several generations can cause minor perturbations in the reproductive system of offspring, namely slight changes in the oestrous cycle length, the timing of vaginal opening and possibly also in ovarian weight and sperm/spermatid count, although functional changes in reproduction were not induced at the dose levels tested. The NOAEL for these changes was 15 mg/kg/day. The observed perturbations in offspring are compatible with the predictable or hypothesised effects of exogenous oestrogenic activity. Evidence of testicular toxicity, seen as seminiferous tubule vacuolation, cell necrosis and a reduction in tubule diameter, was reported at exposure levels which also cause mortality in a repeated dose gavage study in rats. The LOAEL for testicular toxicity was 100 mg/kg/day. The toxicity of nonylphenol appears to be enhanced by gavage administration in comparison to dietary administration, presumably because higher peak blood concentrations of nonylphenol are achieved by gavage.

No evidence that nonylphenol is a developmental toxicant was seen in a standard oral developmental toxicity study in the rat; maternal and foetal NOAELs were 75 and

300 mg/kg/day, respectively. In contrast, in a gavage study involving *in utero*, lactational and direct post-weaning exposure, there was a reduction in sperm count at 250 mg/kg/day, although it is not possible to state whether this is a developmental effect or as a result of direct exposure after weaning. In an intraperitoneal study designed to investigate the effects of nonylphenol on male reproductive tract development of neonatal rats, evidence of impaired development was observed. However, this study was difficult to interpret, such that these results carry little weight in the overall assessment of the available data.

Overall, the observations of oestrogenic activity in the *in vitro* and *in vivo* assays, minor perturbations in the reproductive system of offspring in the multigeneration study, and testicular changes in gavage studies collectively raise concerns for reproductive toxicity, possibly mediated through action on the oestrogen receptor. These concerns for reproductive toxicity are addressed in the risk characterisation, although there are uncertainties. The oestrogenic activity assays are merely screening tests. The effects on reproduction-related parameters in the multigeneration study were marginal and there was no evidence of functional changes in reproduction; furthermore any changes that were seen occurred at exposure levels in excess of the LOAEL for repeated dose toxicity (LOAEL for renal toxicity is 15 mg/kg/day, NOAEL for reproductive changes is 15 mg/kg/day). Evidence of testicular toxicity was reported in two repeated exposure studies designed specifically to investigate the effects on this organ, but only at doses which also caused mortality. No evidence of testicular toxicity was seen in standard repeated dose studies involving dietary administration. Development was not affected in a standard rat oral developmental toxicity study.

4.1.3 Risk characterisation

The risk characterisation below is divided into two parts. The first provides an overview of the toxicological assessment, pointing out the effects of nonylphenol (and the concentrations at which they occur) and making clear where there are critical gaps in the data. The second part contrasts the effects data with measured and modelled exposures. From the effects and exposure information available it is clear that not all of the possible effects will be expressed. The risk characterisation therefore concentrates on the key effects and the circumstances under which they are likely to occur.

4.1.3.1 General aspects

Few significant human data are available so this assessment of the hazardous properties of nonylphenol is based mainly on animal data.

Most of the information on the toxicokinetics of nonylphenol concerns oral exposure and is based on a small number of limited rat and human studies, supported by a read across from data relating to octylphenol, an alkyl phenol with a close structural relationship to nonylphenol. The available data, though sparse, do provide the basis for a general understanding of the main features of the toxicokinetic profile. Absorption from the gastrointestinal tract is initially rapid, and probably extensive. The major metabolic pathways are likely to involve glucuronide and sulphate conjugation, and there is evidence of extensive first pass metabolism of nonylphenol absorbed through the gastrointestinal tract. Because of first pass metabolism, the bioavailability of unconjugated nonylphenol is probably limited following oral exposure, at no more than 10-20% of the administered dose. Nonylphenol is distributed widely throughout the body, with the highest concentration in fat. The major routes of excretion are via the faeces and urine.

表 8-5　ノニルフェノールの生殖・発生毒性試験結果

動物種等	投与方法	投与期間	投与量	結　　果	文献
ラット SD 雌 9 –11匹/群	混餌 (大豆フリー)	F_0:妊娠7日- 離乳(出産後21日) F_1: 生後21日–77日	NP (Schenectady International Inc.) 0、25、500、2,000 ppm	F_0: 25 ppm以上： 摂餌量減少 いずれの群においても妊娠期間、F_1の出生時体重、性比、同腹生児数に影響なし F_1: 雄25 ppm以上及び雌2,000 ppm： 体重増加抑制 雄2,000 ppm： 摂餌量減少 雄雌2,000 ppm： 水及び食塩水の摂取量増加 母動物：LOEL=25 ppm 次世代：(雄) LOEL=25 ppm (雌) LOEL=2,000 ppm	Ferguson et al., 2000
ラット SD 雌雄	混餌	3世代	4-NP (混合物) 0、200、650、2,000 ppm (0、9 - 35、30 - 100、100 - 350 mg/kg/日 相当著者換算) (0、15、50、160 mg/kg/日 相当EU換算) (EU, 2001)	F_0： 雌雄：影響なし F_1： 650 ppm以上： 雌：子宮重量増加 2,000 ppm： 雄：体重増加抑制 雌：体重増加抑制、膣開口6日早期化 F_2： 650 ppm以上： 雄：体重増加抑制、精巣上体精子濃度低下 雌：体重増加抑制、膣開口2日早期化、卵巣相対重量減少 2,000 ppm： 雄：精巣精子細胞数減少 雌：膣開口6日早期化 F_3： 650 ppm以上： 雄：体重増加抑制 雌：体重増加抑制、膣開口2日早期化 2,000 ppm： 雌：膣開口6日早期化 次世代：(雌雄) NOAEL=200 ppm (15 mg/kg/日 相当 EU換算)	NTP, 1997; Chapin et al., 1999

動物種等	投与方法	投与期間	投与量	結　　果	文献
ラット SD 雌雄 雄：6週齢 雌：13週齢 25匹/性/群	強制経口 (コーン油)	F_0雄は交配前12週間、F_0雌は交配前2週間、交配は最大2週間 F_0雄は交配後剖検、F_0雌は妊娠、出産、哺乳期を通じて投与、F_1の離乳後剖検 F_1は離乳後投与、同じ投与群内で交配、F_1雌雄の剖検はF_0に準じる	NP（三井化学） 0、2、10、50 mg/kg/日	F_0： 50 mg/kg/日： 雄：腎臓の絶対及び相対重量の増加、胸腺の絶対及び相対重量の減少、肝臓の相対重量の増加、下垂体相対重量増加 肝細胞小葉中心性肥大、上皮管の好酸性小体の減少 TSH（甲状腺刺激ホルモン）濃度上昇、F_1生存率低下（生後0-4日のみでそれ以降の成長に影響なし） 雌：卵巣の絶対及び相対重量の減少（組織病理学的変化なし）、 F_1生存率低下（生後0-4日のみでそれ以降の成長に影響なし） F_1： 50 mg/kg/日： 雄：腎臓及び肝臓の相対重量の増加、血清中FSH（卵胞刺激ホルモン）濃度上昇、T3（トリヨードチロニン）濃度低下（生後22日）、着床数及びF_2生存児数の減少 雌：卵巣の絶対及び相対重量の減少、膣開口早期化、LH（黄体化ホルモン）及びTSH濃度低下、T3濃度上昇（生後22日）、着床数及びF_2生存児数の減少 親世代(雌雄)：NOAEL=50 mg/kg/日以上 (但し、一般毒性はNOAEL=10 mg/kg/日) 次世代(雌雄)：NOAEL=10 mg/kg/日	Nagao et al., 2001

NP: nonylphenol
太字はリスク評価に用いたデータを示す。

8.3.6　遺伝毒性

ノニルフェノールの遺伝毒性試験結果を表 8-6に示す。*in vitro* 試験では、ネズミチフス菌及び大腸菌を用いた復帰突然変異試験並びにチャイニーズ・ハムスター肺線維芽細胞株 (CHL)を用いる染色体異常試験で代謝活性化の有無に関わらず陰性と報告されている (GDCh BUA, 1988;Shimizu et al., 1985;厚生省, 1996)。

調査した範囲内では *in vivo* 試験の報告はない。