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4-Nonylphenol, verzweigt
(CAS-NR.: 84852-15-3)

Nonylphenol
(CAS-NR.: 25154-52-3)

The following data rely mostly on the EC Risk Assessment Report (EC-RAR) dating from August 1999. All references already cited there are not listed in this document. Only references not or not yet cited in the EC-RAR are listed here.

1 General Informations

“Nonylphenol“ is a common name for several isomeric substances of the general chemical formula $C_6H_4(OH)C_9H_{19}$. Technical Nonylphenol consists mainly of a phenol which is substituted in *para*-position with side chains of different degrees of branching. Moreover 4-NP contains 5 % (w/w) of each 2-NP and 2,4-Dinonylphenol. Due to the production process (synthesis of the side chain by polymerisation of propylene) unbranched nonylphenol is formed in small amounts only or even not at all.

Technical 4-NP is a clear or faint yellow viscous liquid with faint phenolic smell. Its physico-chemical properties give no basis for safety concerns:

- low vapour pressure (ca. 0.3 Pa at 25°C)
- neither easily flammable nor explosive
- pKa value a little bit higher than for phenol (pKa / phenol 9.9; pKa / NP ca. 10.28).

Technical NP is mainly used for the synthesis of Alkylphenolpolyethoxylates (NPnEO). Unchanged NP is used in small amounts for some special purposes.

2 Classification and Labelling

In the EC-RAR the following classification of NP is proposed:

C; R 34 Xn; R 22

3 Mutagenicity

in vitro

Two Ames-tests with NP in Salmonella typhimurium TA 1537, TA 1538, TA 98, TA 100 and E.coli with and without addition of rat-liver S9-mix (Hüls 1984; Shimizu et al, 1985) as well as a HPRT-assay in V79 cells (Hüls 1990) yielded negative results.

in vivo

There are two micronucleus assays with different routes of application available in mice, both with negative results:

- A rather new one (performed according to OECD Guideline) with *i.p.* application of different doses (50, 100 or 300 mg/kg bw; Hüls 1999);
- Another with *p.o.* application of a rather high dose (500 mg/kg bw; Hüls 1988).

4 Carcinogenicity

There are no carcinogenicity studies available with NP. Due to the negative outcome of genotoxicity tests in vitro and in vivo and due to the fact that NP does not induce constant cellular proliferation there is no experimental data basis indicating a possible carcinogenic activity of NP.

5 Reproductive Toxicity

There is no classification of this toxicological endpoint proposed in the EC-RAR. Nevertheless the rapporteur has added the following remark: *„However, it is recognised that this is a borderline decision as there is a degree of coherence across the data which raises concerns for reproductive toxicity, possibly by a mechanism involving endocrine disruption.“* Therefore the available data on reproductive toxicity of NP ist presented:

Estrogenic activity

NP showed an estrogen-like action in a several in vitro- and in vivo-assays. The relative estrogenic potency varied in the different test systems and was by a factor of 10^{-3} – 10^{-6} lower than for estradiol.(e.g. in vitro: Routledge & Sumpter 1997; Soto et al 1991; White et al 1994 or in vivo: ICI 1996; Odum et al 1997; CMA 1997b; Lee & Lee 1996).

Fertility

There is a high-quality 3-generation feeding study available on rats with application of 4-NP in the feed in concentrations of 0, 200, 650 or 2000 ppm (NTP 1997). The mean 4-NP intake was calculated as 0, 15, 50 and 160 mg/kg/d respectively during the non-reproductive phase; the 4-NP uptake for lactating animals was higher. This study shows that exposure to ≥ 650 ppm 4-NP during several generations can lead to subtle disturbances in the reproductive system of the progenies. At this dosage there are already histopathological effects at the kidneys of the F0 animals (tubular dilatation or degeneration and cysts) together with an elevated kidney weight.

Females: At 650 ppm the body weight gain of F1 animals (7%) and of F3 animals (10%) is lower in comparison to the controls; at 2000 ppm this occurs in animals of all generations (9 -12%). In the F1 and F2 animals of the 2000 ppm group there were small increases in estrous lengh (14 and 18 %, resp.) and in all three generations the time to the vaginal opening was shorter (by 1.5-7.3 days for 650 ppm and by 2.9-6.0 days for 2000 ppm). The earlier onset of puberty (vaginal opening) is thought to be due to the estrogenic activity of NP but it must not necessarily be interpreted as an adverse effect per se. At 2000 ppm the ovary weights were reduced in all 3 generations (by ca. 12 %); at 650 ppm this occurred in F2 animals only. The ovaries didn't show any histological alterations. Fertility and mating performance were not changed in any of the dosage groups. Males: At 650 ppm the body weight gain of F2 animals was lower (8%) as compared to the controls, at 2000 ppm this was true for all generations (7–9 %). A reduction of sperm density (8-13 %) was detected in the epididymis at 650 and at 2000 ppm. At 2000 ppm the testicular spermatid counts were also decreased (12-13%). Since these effects appeared in the F2-generation only and were not found consistently the relevance of this finding should be interpreted with caution. There were no effects on fertility and mating performance in any dosage group. There are no definite evidences showing that the reported effects on the reproductive system represent secondary effects of the nephrotoxicity of NP. Due to the estroge-nic action of NP a specific action of NP on the male reproductive system seems plausible.

There is a new well documented 2-generation study available (Nagao et al. 2001) with pre- and postnatal gavage application of NP in doses of 2; 10; or 50 mg/kg/d resp.In F0 and F1-animals of the 50 mg/kg/d-group NP treatment led to toxic effects in liver of males and females (centrilobular hypertrophy of hepatocytes and a decrease in number of eosinophilic bodies) and kidney of males (medullary tubular dilatation, medullary cysts, focal mineralization, granular casts, and hydronephrosis). F1- and F2-pups of this dose group showed a reduced viability on pnd 0 to 4; the body weight gain of these animals remained unaffected. In males of the 50 mg/kg/d-group NP had no effect on the timing of the preputial separation. In females of the same dose group NP led to an accelarated vaginal opening. In any of the treatment groups there were adverse changes in behaviour or learning of the offspring. There were no treatment related changes seen in any reproductive parameter including

estrous cycle, mating, fertility, delivery, and lactation except for significant decreases in the numbers of implantation sites in F1 dams and in the numbers of F2 pups born alive and a significant decrease in absolute and relative ovary weight in adult F1 females (alwalys animals from the 50 mg/kg/d group only). No treatment related changes were observed in the sperm characteristics.

Another study with gavage application of NP over 10 weeks (de Jager et al. 1999a) of 0, 100, 250 or 400 mg/kg/d resp. yielded some evidences for testicular toxicity at doses which led also to general toxicity and high mortality. The survival was reduced dose-dependently (85 %/100 mg/kg; 25 %/250 mg/kg; 10 %/400 mg/kg).

Subcutaneous application of high doses of NP (500 mg/kg/d) to newborn rats (PND 1-5) led to macroscopic and/or microscopic changes at the gonades of the postpubertic animals (Nagao et al. 2000). Disturbances in estrous cycle and abnormal mating performance of females are compatible with the estrogenic action of NP at high doses. NP treatment of neonatally treated males had no influence on locomotoric activity, sperm motility or serumtestosteron levels. The study is of low relevance due to its uncommon design.

Reproductive Toxicity/Developmental Effects:

There is a valid guideline study available on rats (Initiative Umweltrelevante Altstoffe, 1992) with gavage application of NP (GD 6 to 15; maternal doses 0, 75, 150 und 300 mg/kg/d). A group of animals receiving 600 mg/kg/d had to be terminated earlier due to marked lethal effects. At 300 mg/kg/d there were still clear indications for maternal toxicity (increased mortality, reduced body weight gain and food intake, macroscopic changes in kidney and spleen), whereas at 75 mg/kg/d there were no indivations for maternal toxicity. This study gave no indications for developmental effects even at maternally toxic doses.

In a special study on rats with focus on testicular toxicity the animals were exposed to NP in doses of 100; 250 and 400 mg/kg/d first *in utero,* then via the milk and finally by gavage application until sexual maturaty (de Jager et al. 1999b). Females were treated with NP starting from gd 7 and during lactation, F1-males were treated for further 10 weeks after weaning with doses of 100 or 250 mg/kg/d. There are no informations concerning effects of NP treatment on body weight gain of F0 females available. The dams of the group with the highest dose (400 mg/kg/d) had no progenies. There is no information given whether this was due to the maternal mortality or due to fetal resorption. There were no anomalies or stillbirths in the F1-generation. The body weight gain of the F1 animals was significantly reduced in the groups treated further with 100 and 250 mg/kg/d, respectively (by 11 and 20 % resp.). In both treatment groups the absolute weights of testes and epididymes were reduced in comparison to the controls, but not the relative organ weights. Treatment with 250 mg/kg/d led to increased mortality and to reduced sperm counts. Whether this was due to developmental toxicity or due to the direct exposure after weaning is unclear. It should be mentioned that in the other rat study with NP exposure of adults by gavage over 10 weeks there was a rather high mortality in the 250 mg/kg/d group (de Jager et al. 1999a).

In another study (Lee 1998) neonatal rats were treated with NP by *i.p.* application (0.08, 0.8 oder 8 mg/kg bw/d) for different intervals ranging from pnd 1 to 30. There were indications for NP-mediated effects on the male reproductive system. Due to serious shortcomings concerning the study design and in light of the fact that these results proved to be not reproducable in a similar repetitive study by Odum & Ashby (2000) this study is jugded as being invalid for evaluation of NP.

Lee et al. (1999) also treated neonatal rats with i.p. applications of 8 mg/kg bw/d NP from pnd 1 to 15. Also the relevance of this study is flawed by serious shortcomings concerning the study design as discussed in detail by Odum & Ashby (2000).

4-NP was administered to pregnant rats via the feed in concentrations of 0; 25; 500 or 2000 ppm, resp.) starting from gd 7 and continued with treatment also of the progenies after weaning (Ferguson et al. 2000). NP had no effects on gestation time, birth weights, litter sizes and sex ratios of pups. Neurobehavioural tests on progenies (open-field, running wheel activity, behaviour, sweet preference) showed no differences compared to the controls. At 2000 ppm NP body weight gain and food consumption were reduced indicating a toxic action of NP.

Summary:

Mutagenicity:

NP shows in standard assays in vitro and in vivo no mutagenic activity. Therefore classification of NP is not warranted (M: -).

Carcinogenicity:

There are no carcinogenicity studies available with NP. Furthermore NP shows no mutagenic or cell proliferating activity. Therefore classification of NP is not warranted (C: -).

Reproductive Toxicity/Fertility:

NP has a weak estrogenic activity and leads after repeated gavage application of high doses to very slight disturbances of the reproductive system of the male rat and to slight effects on the rat testes. In summary the results show that NP has a low potential of reproductive toxicity presumably due to the interaction of NP with the estrogen receptor. Arguments for an only weak effect are:

- The effects in the multi generation study were only marginal and there were no functional disturbances of the reproduction,
- All effects in vivo were found at doses which were already in the range or even above the LOAEL for systemically toxic effects (LOAEL 15 mg/kg/d for kidney effects),

- The testicular toxicity was reported only in 2 special studies after administration of rather high doses which also led to increased mortality,
- No effects on reproductive organs observed in a 90 day feeding study.

The estrogenic activity reported in screening tests is for itself not a sufficient basis for classification. Taking the whole data base into consideration there is no indication for effects of NP on the male fertility. But the effects of NP on the female reproductive system reported in two multigeneration studies at doses of about 50 mg/kg bw/d (accelerated vaginal opening and reduced ovary weights) leed to some suspicion that NP might have an adverse effect on female fertility. Therefore classification of NP in category 3 (R_F: 3) is proposed.

Reproductive Toxicity/Development:

It was shown that the onset of puberty of young rats treated with nonylphenol was around 7 days earlier in comparison to the control. This effect is considered adverse. As a consequence, according to EC criteria classification in category 3 (R_D,R_E: 3) is warranted.

References (not cited in RAR):

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[4] Nagao T, Saito Y, Usumi K, Nakagomi M, Yoshimura S, Ono H (2000) Disruption of the reproductive system and reproductive performance by administration of nonylphenol to newborn rats. Hum Exp Toxicol 19: 284-296

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[7] Odum J, Pyrah IT, Foster JR, Van Miller JP, Joiner RL, Ashby J (1999b) Comparative activities of p-nonylphenol and diethylstilbestrol in noble rat mammary gland and uterotrophic assays. Regulatory Toxicol Pharmacol 29: 184-195.

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