Reproductive Biology and Endocrinology

Research

Effects of p-nonylphenol and resveratrol on body and organ weight and in vivo fertility of outbred CD-1 mice

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Abstract

The aim of this study was to analyse the multigenerational effects of para-nonylphenol (NP) and resveratrol (RES) on the body weight, organ weight and reproductive fitness of outbred CD-1 mice. The data indicate that in male mice, NP had an effect on the weight of selected reproductive organs and the kidneys in the parental (P) generation males. Effects on selected reproductive organs, the liver and kidneys in the F1-generation males were also seen. In females, effects of NP on body weight and kidney weight were seen in the P generation, but no effects on any measured parameter were seen in the F1 generation. RES had no effect on body weight but did have some effect on selected male and female reproductive organs in the P generation. RES altered the spleen and liver weights of P-generation males and the kidney weight of F1-generation males. Acrosomal integrity (using a monoclonal antibody against intra-acrosomal sperm proteins) was assessed for both generations of NP- and RES-treated mice. A significant reduction in acrosomal integrity was seen in both generations of NP-treated, but not in RES-treated, mice. Fewer offspring were observed in the second litter of the F2 generation of mice treated with NP; no similar effect was seen in RES-treated mice. The litter sex ratio was not different from controls. Unlike RES, NP had a negative effect on spermatogenesis and sperm quality with a resultant impact on *in vivo* fertility.

Background

In this study we selected p-nonylphenol (NP) as a representative endocrine disruptor (ED). EDs are those heterogeneous substances entering the body from the external environment that can interfere with the action of the endocrine system through diverse mechanisms, for example, receptor-mediated enzyme inhibition [1]. These substances can influence endocrine balance during the early phases of an animal's life [2].

para-Nonylphenol (4-nonylphenol) is used in the preparation of lubricating oil additives, plasticizers and surface active agents. It has also been found in polyvinyl chloride (PVC) used in the food processing and packaging industries. NP ranks among the alkyl phenols that are relatively persistent and accumulate in the lipids of living organisms [3].

p-Nonylphenol has been examined in a number of animal, usually rat, studies, with different doses and experimental protocols and also with different results. Lee [4] reported that neonatal exposure of rats to NP (8 mg/kg/day) by daily intraperitoneal injection had an effect on the weight of the reproductive organs and delayed testes descent. De Jager et al. [5] exposed adult male rats to 100 mg/kg of NP and found an effect, particularly on spermatogenesis. In a study of the fertility potential of male rats after gestation and early postnatal life, NP toxicity (100 mg/kg, 250 mg/kg, 400 mg/kg) to both testis and epididymis was found [6,7]. However, Odum and Ashby [8] did not confirm an effect of NP (8 mg/kg/day) on the reproductive tract.

As the findings of earlier papers were inconsistent, and in some cases contradictory, we decided to use oubred mice as another biomodel for analyzing low-dose NP effect. Doses of 50 and 500 μg/l in drinking water were selected and used in a multigenerational study.

Resveratrol (3,5,4'-trihydroxystilbene) – (RES) is a phytoalexin found in more than 300 edible plants and is a component of the human diet. For example, it is present in substantial amounts in red wine (4–20 mg/L) [9–12]. Resveratrol has a wide spectrum of biological activities, one of them being oestrogenicity. The reported data are based on the results of *in vitro* studies in the MCF-7 (estrogen-positive) cell line [13]. Ashby et al. [14] did not show the oestrogenic activity of RES in a uterotrophic assay. Few data are available on the *in vivo* effect of RES. A dose of 3 mg/l in drinking water was selected based on the data reported in a previous paper [11].

The objective of this study was to compare the effect of two different substances (NP, RES) on body and organ weights, the histological picture of the testes and ovaries, the acrosomal integrity of the spermatozoa and the litter size.

Materials and methods

Animals and treatments

CD1 (ICR) outbred mice (An Lab Ltd., Prague, Czech Republic) with heterozygosity and average number of pups 12–13 per litter were used for the experiments. The mice (six control pairs without treatment and six pairs in each experimental group) were kept under standardized conditions at the Institute of Molecular Genetics, Prague, with constant temperature and moisture and with a 12-h light regime without stress factors. Food (ST1, Velas a.s., Lysa nad Labem, Czech Republic) and water were available ad libitum.

p-Nonylphenol (4-Nonylphenol; empirical formula, $C_{15}H_{24}O$; mw 220.36; Sigma, Prague, Czech Republic; Fig. 1A) was selected as a xenoestrogen and used at two concentrations: 50 μg/l in drinking water and 500 μg/l in drinking water. Resveratrol (3,5,4'-trihydroxystilbene; empirical formula, $C_{14}H_{12}O_3$; mw 228.2; Sigma, Prague, Czech Republic; Fig. 1B) was selected as a phytoestrogen. One concentration of RES was employed, 3 mg/l in drinking water. Both drugs were administered in drinking water for easier determination of the precise amount of the ingested drug. Adult mice two months old (parental or P-generation) were exposed to the tested drugs for four weeks, and then the animals were mated. The F1 generation was exposed to NP and RES during gestation, lactation, the prepubertal period and the pubertal period, up to adulthood.

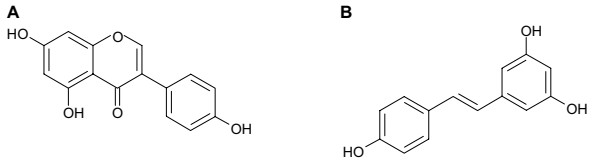

Figure 1
A: The structure of p-nonylphenol (NP). B: The structure of resveratrol (RES).

Body and organ weights

The control and experimental groups of the same sex were killed at the same time. The males were killed at 100 days of age and females at 130 days, and the reproductive organs (testes, epididymides, prostate and seminal vesicles in males and ovaries in females) and other organs (kidneys, liver and spleen) were dissected out and weighed individually. Relative organ weight was calculated as the ratio between organ weight and body weight.

Preparation of tissues and histological examination

The right testis or right ovary were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin-eosin. Leica DM XRA photomicroscope and Leica DC 100 camera were used for microphotography of histological sections. Image analysis software (Leica) was used for histology evaluation (18 semiferous tubule parameters from all groups). Adobe Photoshop 6.0 was used for final processing.

Preparation of cells

The spermatozoa of the cauda epididymis were obtained from mice killed by cervical dislocation. Spermatozoa were washed twice with phosphate-buffered saline (PBS),

Table 1: Effect of p-nonylphenol (NP; 50 μg and 500 μg) and resveratrol (RES; 3 mg) on different body parameters in male outbred CD1 mice. The control and experimental groups of the same sex were killed at identical times. The body weights and organ weights were taken individually. The ratio between the organ weight and body weight represents the relative organ weight. (* P < 0.05, ** P < 0.01).

	P				F1			
Variable	Control	NP 50	NP 500	RES	Control	NP 50	NP 500	RES
Body weight (g)	39.42 ± 1.60	40.26 ± 0.52	42.83 ± 1.80	36.05 ± 2.52	36.88 ± 0.32	36.73 ± 0.95	42.32 ± 1.17**	36.65 ± 1.67
Testis weight (mg)	121.4 ± 6.1	99.3 ± 6.2**	126.5 ± 3.3	118.7 ± 5.1	112.5 ± 2.2	102.5 ± 2.6**	107.4 ± 8.3	114.5 ± 6.8
Testis weight/Body weight	3.09 ± 0.09	2.46 ± 0.12**	2.97 ± 0.10	3.36 ± 0.21	3.05 ± 0.07	2.87 ± 0.11	2.53 ± 0.16*	3.11 ± 0.07
Epididymis weight (mg)	56.1 ± 1.0	58.3 ± 2.1	69.2 ± 2.0**	53.8 ± 3.0	53.4 ± 3.1	49.0 ± 0.9	58.2 ± 1.5	56.9 ± 7.2
Epididymis weight/Body weight	1.44 ± 0.09	1.45 ± 0.03	1.63 ± 0.07*	1.51 ± 0.06	1.45 ± 0.09	1.34 ± 0.04	1.33 ± 0.05	1.55 ± 0.04
Prostate weight (mg)	140.5 ± 15.5	126.7 ± 6.2	142.98 ± 16.3	125.6 ± 26.4	149.2 ± 7.8	130.5 ± 5.6**	150.4 ± 3.5	149.2 ± 13.5
Prostate weight/Body weight	3.58 ± 0.41	3.15 ± 0.15	3.38 ± 0.46	3.60 ± 0.66	4.05 ± 0.21	3.55 ± 0.11*	3.57 ± 0.08	4.07 ± 0.28
Seminal vesicle weight (mg)	193.1 ± 9.5	184.1 ± 6.8	205.6 ± 16.6	156.2 ± 14.3**	154.5 ± 0.3	142.3 ± 7.9	226.6 ± 15.3**	126.9 ± 12.3*
Sem.ves.weight/Body weight	4.93 ± 0.28	4.57 ± 0.18	4.67 ± 0.26	4.34 ± 0.16*	4.19 ± 0.03	3.86 ± 0.19	4.38 ± 0.44	3.47 ± 0.24
Liver weight (mg)	2580 ± 152	2816 ± 75	2805 ± 103	2048 ± 162*	2749 ± 30	2397 ± 35**	2656 ± 123	2500 ± 191
Liver weight/Body weight	65.45 ± 3.86	69.95 ± 1.86	65.49 ± 2.40	56.81 ± 4.49	74.54 ± 0.76	65.26 ± 0.95**	62.76 ± 2.91**	68.21 ± 5.21
Kidney weight (mg)	404.3 ± 15.0	354.9 ± 7.8*	415.7 ± 10.0	355.7 ± 39.3	355.8 ± 2.5	396.9 ± 13.1**	406.3 ± 5.4**	321.6 ± 59.3*
Kidney weight/Body weight	10.27 ± 0.33	8.83 ± 0.32**	9.52 ± 0.46	9.78 ± 0.52	9.65 ± 0.15	10.83 ± 0.48	9.65 ± 0.43	8.73 ± 0.21
Spleen weight (mg)	99.4 ± 3.6	101.6 ± 5.7	108.8 ± 6.7	82.2 ± 7.1*	105.3 ± 13.2	93.68 ± 5.7	110.6 ± 12.4	111.7 ± 12.7
Spleen weight/Body weight	2.53 ± 0.11	2.52 ± 0.13	2.54 ± 0.08	2.31 ± 0.21	2.86 ± 0.42	2.54 ± 0.11	2.60 ± 0.19	3.01 ± 0.28

pH 7.4, centrifuged for 30 min at 200 g and then used in experiments.

Acrosomal status tested by monoclonal antibody

Aliquots of a sperm suspension were smeared onto glass slides. The smears were dried and fixed with acetone at room temperature for 10 min, then rinsed with PBS and incubated for 45 min at 37°C with a monoclonal antibody against intra-acrosomal proteins, Hs-14 (prepared in our laboratory) [15], and used for determining the acrosomal integrity of the spermatozoa. The antibody was diluted in PBS to an immunoglobulin concentration of 20 μg/ml. After thorough washing with PBS, the smears were incubated with FITC-conjugated swine anti-mouse immunoglobulins (SEVAC, Prague, Czech Republic), diluted 1:20 in PBS, for 45 min at 37°C, washed with PBS and water, and mounted in Vectashield H-1200 + DAPI (Vector Laboratories, Inc., Burlingame, CA). For negative controls, smears were incubated with a non-specific monoclonal antibody, with the supernatant from cultured myeloma cells, and with the FITC-conjugated secondary antibody only. Samples (200 sperm cells) from each male were evaluated and viewed with a Nikon Labophot-2 fluorescent microscope equipped with 40 × Nikon Plan 40/0.65 lenses. Selected fields were chosen for photography with a COHU4 CCD camera (Inc. Electronics Division, San Diego, USA) with the aid of LUCIA imaging software (Laboratory Imaging, Ltd., Prague, Czech Republic).

Statistical analysis

The statistical differences among compared groups were analysed by one-way ANOVA and the Student-Newman-Keuls test. P value < 0.01, (<0.05 respectively) was considered significant.

Results

Effect of p-nonylphenol on body weight, organ weight and in vivo fertility

NP had no effect on body weight in the males of the P generation, but there was a significant difference at the higher dose (500 μg NP) in the F1 generation, compared to controls (Table 1). NP (50 μg) had an effect on some reproductive male organs; it decreased the absolute and relative (ratio of tested organ weight to body weight) testes weight in the P generation and decreased the absolute testes weight in the F1 generation. Fifty μg NP also decreased the absolute and relative prostate weight as compared to controls in the F1 generation. The higher NP dose (500 μg) increased the seminal vesicle weight in the F1 generation (Table 1). NP also had effect on other body organs. The lower dose statistically decreased the absolute kidney weight in the P generation and the relative liver weight in the F1 generation. Both doses statistically increased the absolute kidney weight in the F1 generation. The higher dose decreased the relative liver weight in the F1 generation (Table 1).

In females, the lower dose decreased body weight as compared to controls in the P generation but had no effect on body weight in the F1 generation. Both doses decreased the relative kidney weight compared to controls in the P generation (Table 2).

NP was tested for its potential reproductive toxicity in a two-generational model in CD1 mice. The average litter size (number of pups born) in two successive generations

Table 2: Effect of p-nonylphenol (NP; 50 μg and 500 μg) and resveratrol (RES; 3 mg) on different body parameters in female outbred CD1 mice. The control and experimental groups of the same sex were killed at identical times. The body weights and organ weights were taken individually. The ratio between the organ weight and body weight represents the relative organ weight. (* P < 0.05, ** P < 0.01).

	P				F1			
Varible	Control	NP 50	NP 500	RES	Control	NP 50	NP 500	RES
Body weight (g)	37.34 ± 0.70	33.54 ± 0.47**	35.68 ± 0.62	35.05 ± 1.02	35.90 ± 1.84	33.94 ± 1.66	38.29 ± 0.90	35.72 ± 1.42
Ovary weight (mg)	13.60 ± 0.89	13.50 ± 0.60	12.91 ± 0.73	10.12 ± 0.57*	14.37 ± 0.68	13.84 ± 0.94	15.49 ± 0.85	13.89 ± 1.58
Ovary weight/Body weight	0.37 ± 0.03	0.40 ± 0.02	0.36 ± 0.02	0.29 ± 0.03*	0.40 ± 0.01	0.42 ± 0.03	0.40 ± 0.02	0.39 ± 0.05
Liver weight (mg)	2613 ± 752	2532 ± 691	2554 ± 35	2642 ± 83	2857 ± 143	2799 ± 162	2945 ± 907	2361 ± 127
Liver weight/Body weight	69.98 ± 20.14	75.49 ± 20.60	71.58 ± 0.98	77.59 ± 2.44	79.58 ± 3.98	82.47 ± 4.77	76.91 ± 23.53	66.10 ± 3.56
Kidney weight (mg)	292.7 ± 7.3	249.6 ± 4.5	250.4 ± 5.4	306.9 ± 10.3	291.8 ± 9.0	283.4 ± 9.3	287.1 ± 9.6	283.0 ± 21.0
Kidney weight/Body weight	7.80 ± 0.20	7.46 ± 0.20**	7.01 ± 0.06*	8.40 ± 0.54	8.17 ± 0.24	8.59 ± 0.24	7.44 ± 0.23	7.90 ± 0.36
Spleen weight (mg)	159.9 ± 14.2	139.2 ± 3.0	156.6 ± 9.1	134.4 ± 10.8	174.1 ± 18.6	163.0 ± 21.8	170.9 ± 4.2	149.1 ± 22.0
Spleen weight/Body weight	4.36 ± 0.33	4.10 ± 0.22	4.39 ± 0.25	3.76 ± 0.38	4.79 ± 0.29	4.85 ± 0.49	4.46 ± 0.09	4.20 ± 0.64

was compared to controls using two experimental groups (exposed to 50 μg and 500μg) in each generation. In the experimental groups, neither NP concentration had any effect on the number of pups per litter in the first litter of the F2 generation. In the second litter of the F2 generation, NP significantly decreased the number of pups per litter to 9 and 8.9 compared to 14.8 in the control group (P<0.01) see Fig. 2. The sex ratio (female/male) remained unchanged (Fig. 3).

Effect of p-nonylphenol on acrosomal integrity

The state of the acrosomal integrity in the control and experimental groups over two generations was tested by using the monoclonal antibody Hs-14. In the control groups, about 90% of mouse sperm acrosomes were positively labelled (Figs. 4, 5a). Acrosomal damage was found in the group treated with p-nonylphenol. In the P generation of animals treated with 50 μg or 500 μg NP, the acrosomal staining decreased to 77% and 80%, respectively (Fig. 4). Acrosomal damage was also found in the F1 generation of both experimental groups (50, 500 μg NP), where 65% and 64% of acrosomes were labelled, respectively (Fig. 5b).

Effect of resveratrol on body weight, organ weight and in vivo fertility

RES had no effect on body weight in either males or females in the P or F1 generation (Table 1, Table 2). RES decreased the absolute and relative seminal vesicle weight as compared to controls in the P generation and the absolute vesicle and kidney weight in the F1 generation (Table 1). In females treated with RES, the absolute and relative ovary weight was decreased compared to controls in the P generation (Table 2).

Unlike p-nonylphenol, resveratrol at a dose of 3 mg had no significant effect on litter size. (Fig. 2). The sex ratio (female/male) was also unchanged (Fig. 3).

Effect of resveratrol on acrosomal integrity assessed by the Hs-14 antibody

The state of the acrosomal integrity in the control and experimental groups over two generations was tested using the monoclonal antibody Hs-14. RES had no effect on sperm quality. The acrosomal staining was comparable in the P and F1 generations (Fig. 4).

Effect of p-nonylphenol and resveratrol on the number of sperm from the cauda epididymis

The number of spermatozoa from the cauda epididymis was comparable between the control and experimental groups. The control group had 21 and 20 × 10^6 spermatozoa/ml in the P and in the F1 generations, respectively. The NP-treated group (50 μg) had 20 × 10^6 spermatozoa/ml in the P generation and 16 × 10^6 spermatozoa/ml in the F1 generation; the higher dose (500 μg) also did not significantly affect the number of sperm in the P generation (17 × 10^6 sperm/ml) or in the F1 generation (19 × 10^6sperm/ml). After RES treatment, 19 × 10^6 spermatozoa/ml were found in both generations.

Effect of p-nonylphenol and resveratrol on spermatogenesis, evaluated by histological methods

Parental generation males treated with the lower dose of NP had normal spermatogenesis when compared with controls (Fig. 6A). No apparent histopathological changes of the seminiferous tubules (STs) were found in animals treated with the higher dose of NP either. On the other hand, the testes of F1 males showed alterations in the STs. There were two main changes linked with NP oestrogenicity: the sloughing of germ cells and the appearance of round cells in the lumen of the STs (Fig. 6B). The observed

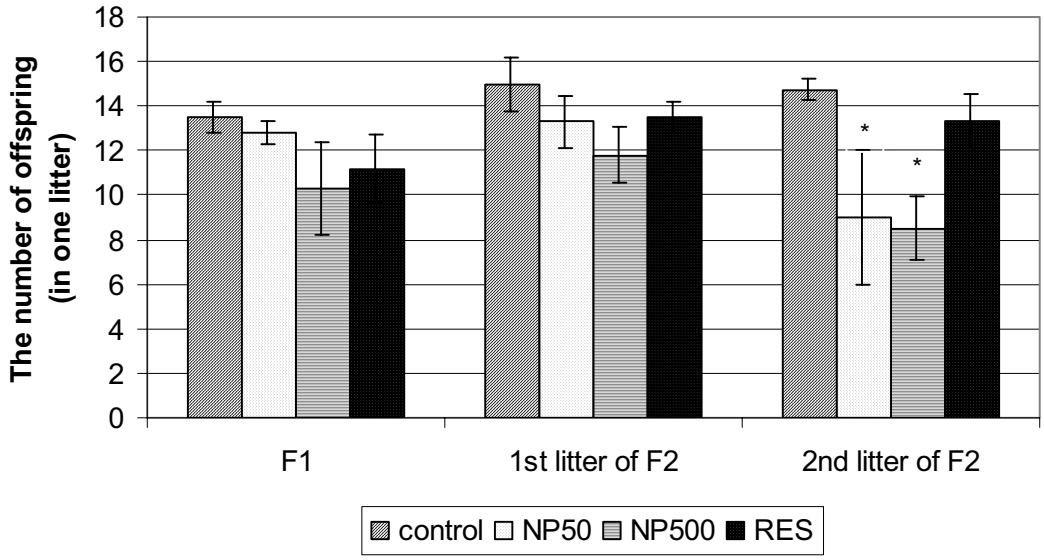

Figure 2
The effect of NP and RES on litter size. The figure shows the number of offspring (in one litter) in the F1 generation and the first and second litters of the F2 generation. A statistically significant difference was found between the control group and the groups treated by 50 or 500 μg NP in the second litter of the F2 generation (* $P < 0.01$).

premature release of germ cells into the lumen is a sign of Sertoli cell damage caused by the NP treatment. Such changes were seen in both treatment groups, in five out of six evaluated animals. There was no dose-response dependence; marked pathology was seen following the lower dose as well as the higher.

According to the Student-Newman-Keuls test the difference in mean seminiferous tubule diameter was not statistically significant between the different groups in P generation. The lower mean diameter was statistically significant (NP (50 μg) $p < 0,05$; NP (500 μg) $p < 0.01$) in both exposed groups F1 generation compared to the control group. The difference between control and the higher NP dose groups was statistically significant in both generations in seminiferous epithelium thickness. There was no statistically significant difference in lumen diameter between control and exposed groups (Tab.3). The general decrease in seminiferous tubule size is obvious from Fig. 6C.

Resveratrol treatment did not induce any alteration of testes morphology in any evaluated animal.

Effect of p-nonylphenol and resveratrol on ovaries, evaluated by histological methods

The histology of the ovaries of control and NP-treated mice appeared similar; the only difference was in the average number of atretic follicles. The Student-Newman-Keuls Test showed a statistically significant overall difference between control and the higher NP dose groups ($p < 0.01$), (data not showed). Resveratrol treatment did not affect ovarian morphology in either generation.

Discussion

We tested the effect of p-nonylphenol, an environmental toxicant with oestrogenic properties, on the body weight, reproductive organ weight and histology, and *in vivo* fertility of the CD1 outbred mouse strain. This strain of mice simulates better than inbred strains the genetic heterozygosity in the human population. NP had an effect on selected reproductive organs in the P generation. The

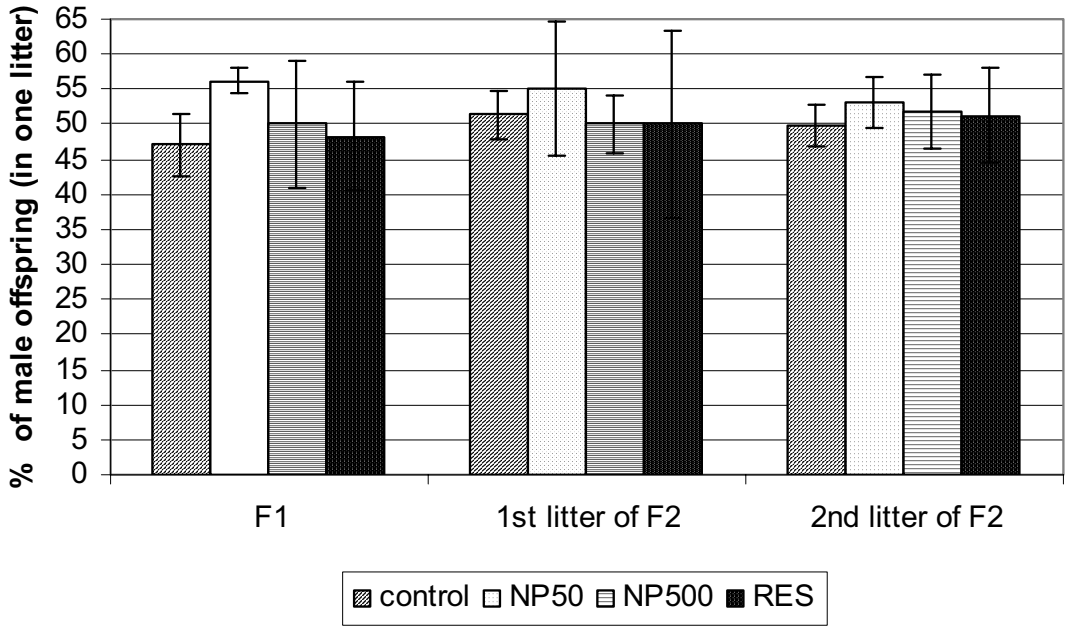

Figure 3
Males born in one litter (%) in the control group and in the experimental groups treated by NP and RES in the F1 generation and the first and second litters of the F2 generation. The sex ratios (female/male) did not differ.

damage to the reproductive organs increased in the F1 generation, when NP influenced the animals during gestation, lactation and the pubertal period. In the group treated by the lower dose of NP, the prostate weight was decreased, and in the group treated by the higher dose, a lower body weight was found. Parental generation males treated with the lower dose of NP had normal spermatogenesis when compared with controls. No marked histopathological changes of the seminiferous tubules were found. Interestingly, we detected damage to the acrosome in spermatozoa already in the P generation. The acrosomal status (% of acrosome-intact cells) of spermatozoa decreased (compared to control) in the P generation by about 14% (50 μg NP) or 10% (500 μg NP). A marked decrease was observed in the F1 generation: 26% (50 μg NP) and 26% (500 μg NP). Both NP doses had a similar effect on acrosomal damage in the P and F1 generations. The data indicate that we did not find any dose-dependence effect. Our previous study confirmed that monoclonal antibodies against intra-acrosomal sperm proteins are valuable and sensitive markers for the evaluation of sperm quality [15–20]. A correlation between sperm quality and fertility has been demonstrated using animal and human pathological spermatozoa [18–22] and also by our own results (prepared for publication). We can conclude that although spermatogenesis was established in the P generation, NP had an effect on sperm quality. In contrast to the quality, the number of spermatozoa was similar to the control group (in the P and F1 generations). Depending on duration of exposure, there were progressive degenerative changes in the reproductive organs. The damage to male reproductive organs and gametes is evidently not crucial in the P generation, as there was no effect on the number of pups born in the F1 generation. Finally, a statistically significant decrease in the number of pups appeared in the second litter of the F2 generation. However, the sex ratio of live pups and the number of live pups per litter did not differ among treatment and control groups in the tested generation.

Unlike NP, RES had no damaging effect on spermatogenesis, sperm number or sperm quality. We did not find any

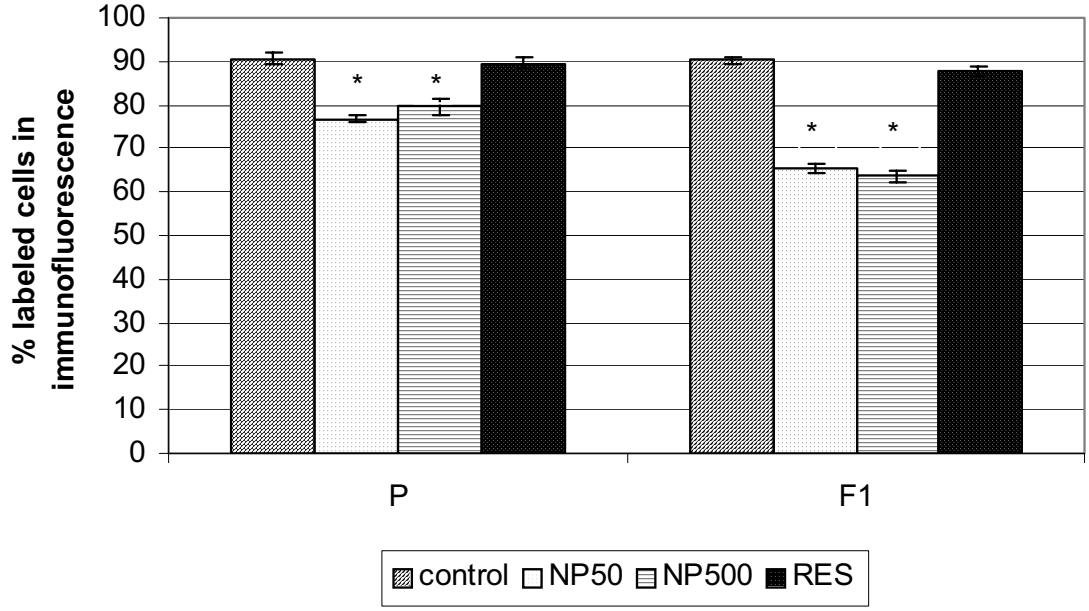

Figure 4
The effect of NP and RES on acrosomal integrity. Acrosomal integrity was evaluated as the number of spermatozoa with a labelled acrosome using a monoclonal antibody against intra-acrosomal proteins (Hs-14). Two hundred cells were evaluated in each group. A statistically significant difference was found between the control group and the experimental groups treated with 50 or 500 μg NP in the first and second generations (* $P < 0.01$).

differences in the morphology of the ovaries. We conclude that phytoestrogen RES had no effect on *in vivo* fertility in our experimental system.

We have compared the results presented here with those of our previous paper [15], in which we tested two rather low doses of bisphenol-A. The lower dose of BPA had a stronger effect on all tested parameters than the higher dose of BPA. Bisphenol-A had an effect on spermatogenesis in the males of the experimental groups, where infertile males without spermatozoa were found. The lower dose of BPA also significantly decreased the number of pups in the F1 and F2 generations in comparison with the control group. Unlike BPA, NP treatment in our study had a similar effect at both concentration used. Neither NP dose had any effect on the number of spermatozoa in the cauda epididdymis. Contrary to our results with BPA, NP had a comparable toxic effect on the quality of spermatozoa at both doses in the P and F1 generations, but the effect on *in vivo* fertility manifested itself later, in the second litter of the F2 generation, when the number of pups was significantly decreased.

On the other hand, resveratrol treatment showed no adverse effect both on sperm quality and fertility. Histology of reproductive organs of both sexes did not indicate any sign of damage.

A deleterious effect of NP treatment appeared following long-term treatment with low NP doses. This observation is alarming, considering the persistence of harmful NP products in the environment.

The data presented here add to the biological information available for the study of testicular dysgenesis syndrome, with underlying entities such as poor semen quality, testicular cancer, undescended testes and hypospadias, which may be increasing because of adverse environmental influences [23].

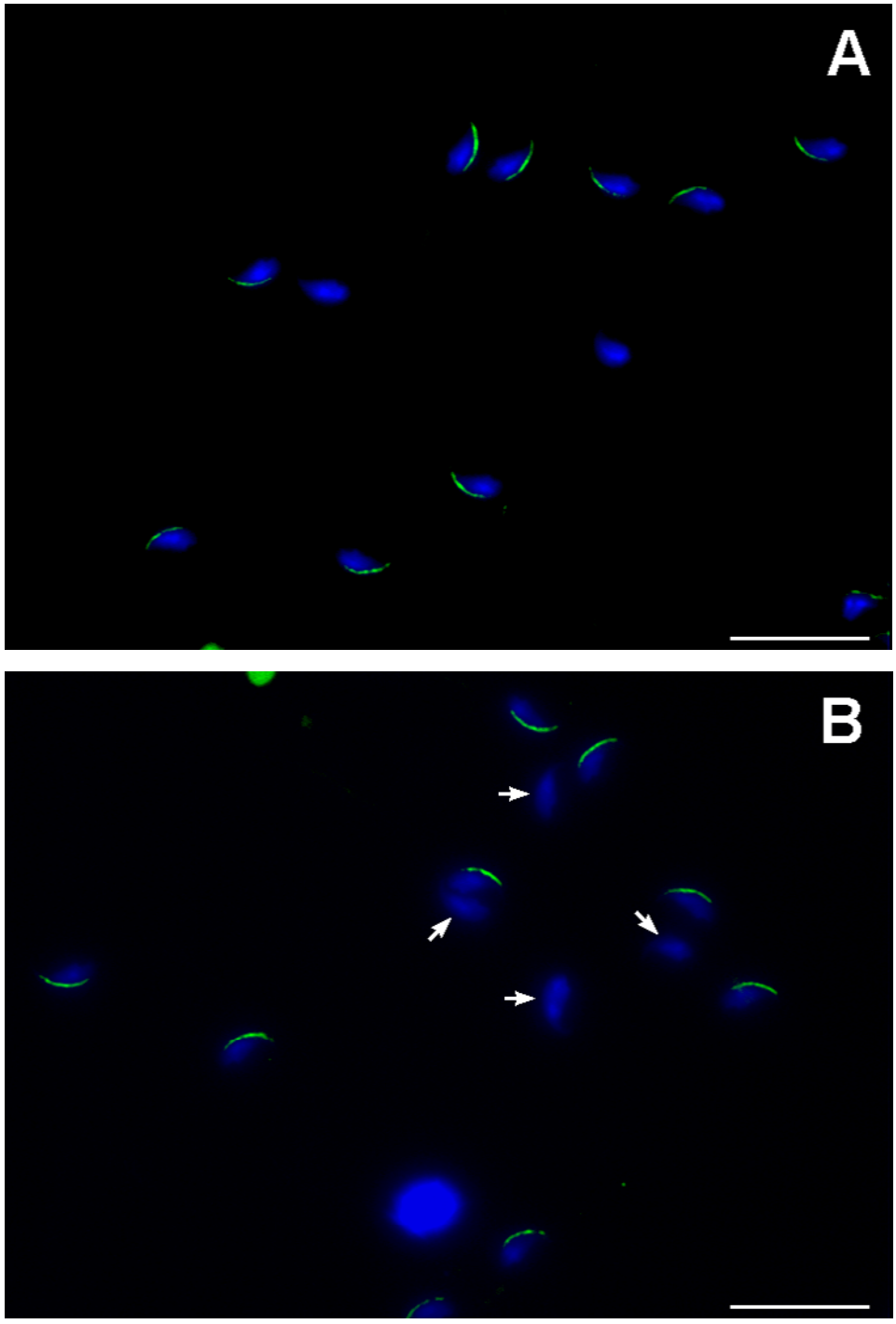

Figure 5
Immunofluorescent visualization of acrosomes (green) with a monoclonal antibody against intra-acrosomal proteins (Hs-14). A: Most spermatozoa (90%) were labelled in the control group. B: The loss of proteins from the acrosome of spermatozoa (arrow) in the F1 generation after 500 μg NP treatment. Blue – nucleus labelled by DAPI. Scale bars = 20 μm.

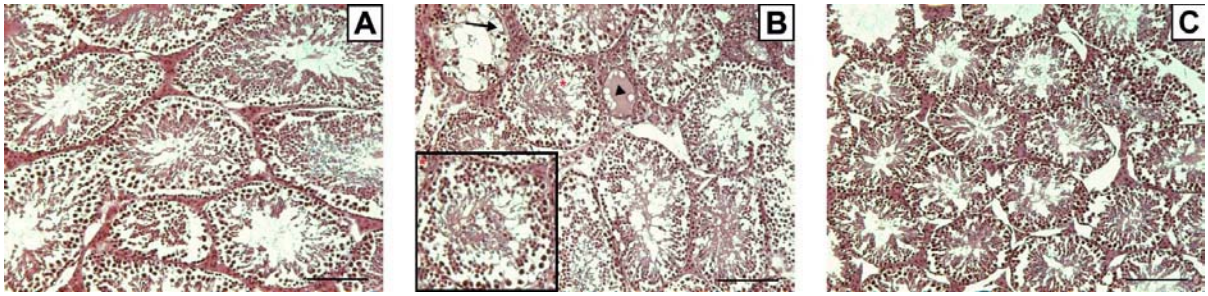

Figure 6
Light photomicrographs of testes. A: Normal spermatogenesis and a normal arrangement of late spermatids were found in the control group. B: NP 50 μg treated group, F1 generation. Sloughing of germinal cells (arrows) and the appearance of round cells in seminiferous tubules (ST) (see insert, detail of ST) were found in the testes of both NP-treated groups. Note ST tubule without germ cells (arrowhead). C: NP 500 μg treated group, F1 generation. The decrease in STs diameter is obvious. Haematoxylin-eosin stain. Scale bars = 100 μm.

Table 3: Effect of p-nonylphenol (NP; 50 μg and 500 μg) on different semiferous tubule parameters in male outbred CD1 mice. Student-Newman-Keuls Multiple RangeTest. (* P < 0.05, ** P < 0.01).

	P			F1		
Variable	Control	NP 50	NP 500	Control	NP 50	NP 500
Seminiferous tubule diameter (μm)	144.96 ± 4.5	144.02 ± 6.5	128.11 ± 4.8	176.02 ± 5.9	158.5 ± 6.5*	147.03 ± 4.8**
Epithelium thickness (μm)	50.54 ± 1.8	49.0 ± 2.1	41.62 ± 2.3**	53.11 ± 1.5	46.93 ± 2.1*	40.43 ± 2.4**
Lumen diameter (μm)	51.59 ± 3.3	63.84 ± 4.8	55.53 ± 4.0	77.27 ± 2.5	80.80 ± 6.1	74.11 ± 3.6

The results provide much-needed information for detecting other symptoms in the study of reproductive problems. Our results also confirm the usefulness of monoclonal antibodies directed against intra-acrosomal proteins as markers detecting sperm damage.

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