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Study of the effects of ß-myrcene on rat fertility and general reproductive performance

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Abstract

ß-Myrcene (MYR) is a monoterpene found in the oils of a variety of aromatic plants including lemongrass, verbena, hop, bay, and others. MYR and essential oils containing this terpenoid compound are used in cosmetics, household products, and as flavoring food additives. This study was undertaken to investigate the effects of MYR on fertility and general reproductive performance in the rat. MYR (0, 100, 300 and 500 mg/kg) in peanut oil was given by gavage to male Wistar rats (15 per dose group) for 91 days prior to mating and during the mating period, as well as to females (45 per dose group) continuously for 21 days before mating, during mating and pregnancy, and throughout the period of lactation up to postnatal day 21. On day 21 of pregnancy one-third of the females of each group were submitted to cesarean section. Resorption, implantation, as well as dead and live fetuses were counted. All fetuses were examined for external malformations, weighed, and cleared and stained with Alizarin Red S for skeleton evaluation. The remaining dams were allowed to give birth to their offspring. The progeny was examined at birth and subsequently up to postnatal day 21. Mortality, weight gain and physical signs of postnatal development were evaluated. Except for an increase in liver and kidney weights, no other sign of toxicity was noted in male and female rats exposed to MYR. MYR did not affect the mating index (proportion of females impregnated by males) or the pregnancy index (ratio of pregnant to sperm-positive females). No sign of maternal toxicity and no increase in externally visible malformations were observed at any dose level. Only at the highest dose tested (500 mg/kg) did MYR induce an increase in the resorption rate and a higher frequency of fetal skeleton anomalies. No adverse effect of MYR on postnatal weight gain was noted but days of appearance of primary coat, incisor eruption and eye opening were slightly delayed in the exposed offspring. On the basis of the data presented in this paper the no-observed-adverse-effect level (NOAEL) for toxic effects on fertility and general reproductive performance can be set at 300 mg of ß-myrcene/kg body weight by the oral route.

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- Postnatal development

Introduction

ß-Myrcene (7-methyl-3-methylene-1,6-octadiene) (MYR) is an acyclic monoterpene found in a large variety of plants including lemongrass, verbena, hop, bay, and others (1,2). ß-Myrcene and essential oils containing this terpenoid compound are widely used as a fragrance in cosmetics, as a scent in household products, and as a flavoring additive in food and alcoholic beverages (3). Furthermore, it was reported that ß-myrcene is an analgesic substance and the active principle of lemongrass (*Cymbopogon citratus* Stapf) 'abafado', an infusion made with the pan covered in order to prevent the loss of volatile constituents (4). Lemongrass 'abafado' is widely used in Brazilian folk medicine as a sedative and as a remedy for gastrointestinal disorders (5).

The importance of human exposure to ß-myrcene, the widespread use of plants as well as essential oils containing large amounts of this monoterpene (e.g. lemongrass oil), and the relative paucity of data about its health risks prompted us to perform a rather comprehensive study of its reproductive toxicity.

The metabolism of ß-myrcene was studied in the rabbit (6) and in the rat (7), and has been shown to induce liver monooxygenases (8,9). The acute toxicity of ß-myrcene was reported to be low (10) and this monoterpene was shown to have no genotoxic activity *in vitro* (11) or *in vivo* (12). No evidence that ß-myrcene is a teratogenic substance was found (13) and the no-observed-adverse-effect level (NOAEL) for peri- and postnatal developmental toxicity in the rat was set at 0.25 g ß-myrcene/kg body weight (14).

The aim of the present study was to investigate the effects of ß-myrcene on rat fertility and general reproductive performance. This is segment I study, part of a more comprehensive evaluation of the reproductive toxicity of ß-myrcene designed in three segments as recommended by the guidelines of the Food and Drug Administration, and of the Organization for Economic Cooperation and Development (OECD).

Material and Methods

Animals

Male and female Wistar rats (Bor: spf, TNO; Fa. Winkelmann, Borchen, Germany) were kept under *spf* conditions at a constant 12-h light-dark cycle (lights on from 9:00 to 21:00 h), at a room temperature of 21 ± 1°C and 50 ± 5% relative humidity. The animals received a standard pelleted diet (Altromin 1324, Lage, Germany) and tap water *ad libitum* during the experiment. All rats were adapted to the conditions of our animal quarters for three weeks before starting the experiment.

Mating procedure

Males were housed individually in a Macrolon type 3 cage with wood shavings as bedding. Three virgin females were placed inside the cage of one male for 2 h each day (7:00 to 9:00 h) and vaginal smears were evaluated for sperm. The first 24-h period following the mating procedure was called day 0 of pregnancy if sperm were detected in the smear. The mating procedure was repeated every working day until all three females became "sperm-positive" or, alternatively, for fifteen mating sessions extending over three weeks.

Treatment

Commercially available ß-myrcene (Sigma Chemical Co., St. Louis, MO) was purified up to 95% (methanol extraction and HPLC purification) at our laboratory and administered to rats once a day during the following periods: a) male rats for 91 days prior to mating and during the mating period; b) female rats for 21 days prior to mating,

during the mating period, and during pregnancy and lactation until day 21 after parturition.

Three experimental groups (15 males and 45 females per group) were treated by gavage with ß-myrcene (100, 300 and 500 mg/kg body weight) dissolved in peanut oil (pharmaceutical grade). The control group received a similar treatment but with vehicle only (peanut oil, 2.5 ml/kg body weight).

Evaluation of the animals

All F_o-males and -females were evaluated for weight development, mortality, and signs of toxicity. Pregnant females were also observed for weight gain, signs of abortion, dystocia and prolonged duration of pregnancy. All males were sacrificed by decapitation and autopsied at the end of the mating period. All major organs were inspected macroscopically and weighed. Livers and one of the two testes were fixed in a 10% neutral buffered formalin solution for routine histological processing and light microscopic evaluation of sections stained with hematoxylin-eosin. The number of spermatozoa in the remaining testis and cauda epididymis was counted as described elsewhere (15). The following indices were used: mating index = [No. of sperm-positive females ÷ No. of mated females] x 100; pregnancy index = [No. of pregnant females ÷ No. of sperm-positive females] x 100.

Cesarean section

On day 21 of pregnancy one-third of the females in each group were anesthetized by ethyl ether inhalation and killed by decapitation. The gravid uterus was weighed with its contents. Resorption as well as living and dead fetuses were counted. The number of implantation sites was determined by the method of Salewski (16). All living fetuses were immediately weighed, numbered with a marker pen, examined for externally visible malformations and fixed in a 5% formalin solution. All fetuses were examined for skeletal anomalies after clearing with potassium hydroxide and staining with Alizarin Red S (17).

Postnatal development of the offspring

All the remaining pregnant females were allowed to give birth to their offspring. From pregnancy day 20 on the dam's cages were inspected for births and the day of birth was designated as postnatal day 1. As soon as possible after birth the numbers of viable and dead newborns were recorded, and the pups were sexed and weighed. Any newborn death on postnatal day 1 was considered to be a stillbirth. The weight gain of the pups was recorded on postnatal days 6, 11, 16 and 21. Each pup was examined for signs of physical development and the days on which developmental landmarks appeared were recorded as follows: a) incisor eruption: the first sign of eruption through the gums of both lower incisors; b) fur development: the first detection of downy hair; c) eye opening: total separation of the upper and lower eyelids and complete opening of both eyes.

At weaning (postnatal day 21) all mothers were anesthetized with ethyl ether, killed by decapitation and subjected to postmortem examination.

Statistical analysis

Data were analyzed by one-way analysis of variance or, alternatively, by the Kruskal-Wallis test whenever the data did not fit a normal distribution. Differences between groups were tested by the two-tailed Student *t*-test or Mann-Whitney U-test. Proportions were analyzed by the chi-square test or, alternatively, by the Fischer exact test. Statistical evaluation was performed using a MINITAB program (MTB, University of Pennsylvania, 1984), and a difference was considered statistically significant at $P<0.05$.

Results

Body weight changes and toxicity to the parental generation

No deaths were induced and no other signs of toxicity were apparent in male rats treated orally with ß-myrcene (100, 300 and 500 mg/kg body weight) for 91 days prior to mating and during the mating period. There were no statistically significant differences in body weight gain between the control and the MYR-treated male rats (Table 1). Except for a slight increase in both absolute and relative weights of liver and kidneys of males exposed to the highest dose tested (Table 1), no other treatment-related abnormality was noted in MYR-treated rats at autopsy. Light microscopy evaluation of sections stained with hematoxylin and eosin revealed no morphological alterations in the liver or testicular tissue of male rats exposed to ß-myrcene. Moreover, no effect of MYR treatment was found either on the number of spermatids in the testis or on the number of spermatozoa in the cauda epididymis (data not shown).

Similarly, no adverse effects on body weight gain and no other signs of toxicity were observed in MYR-treated female rats during the premating (21 days) and mating periods.

Outcome of fertility tests

ß-Myrcene did not present any adverse effect on fertility indices at the dose range tested. As can be seen in Table 2, the proportion of females impregnated by male rats (mating index), and the ratio of pregnant to sperm-positive females (pregnancy index) did not differ between control and MYR-treated groups. Thus, no indication was found that MYR administered orally at doses as high as 500 mg/kg could impair male or female fertility.

Table 1 - Body weight gain and organ weight changes in male rats treated orally with ß-myrcene (0, 100, 300 and 500 mg/kg body weight) for 91 days prior to mating.

Values are reported as means ± SD. Data were analyzed by ANOVA and the Student *t*-test. * P<0.05 compared to controls.

	ß-Myrcene (mg/kg body weight)			
	0	100	300	500
Body weight (g)	434 ± 26	451 ± 32	428 ± 24	413 ± 28
Body weight gain (g)				
Δ (day 1-91)	23 ± 11	27 ± 15	20 ± 12	16 ± 19
Organ weight (g)				
Liver	13.3 ± 1.7	14.3 ± 2.0	14.1 ± 1.6	15.0 ± 1.3*
Kidneys				
right	1.25 ± 0.11	1.28 ± 0.10	1.28 ± 0.12	1.38 ± 0.11*
left	1.25 ± 0.12	1.27 ± 0.07	1.30 ± 0.11	1.38 ± 0.10*
Spleen	0.66 ± 0.08	0.66 ± 0.07	0.63 ± 0.08	0.63 ± 0.10
Heart	1.27 ± 0.13	1.34 ± 0.06	1.22 ± 0.12	1.18 ± 0.10
Thymus	0.21 ± 0.05	0.19 ± 0.06	0.17 ± 0.04	0.19 ± 0.05
Brain	1.94 ± 0.10	1.96 ± 0.12	1.85 ± 0.34	1.70 ± 0.21
Testes				
right	1.77 ± 0.41	1.75 ± 0.21	1.85 ± 0.34	1.66 ± 0.22
left	1.80 ± 0.39	1.85 ± 0.18	1.83 ± 0.16	1.68 ± 0.12

Evaluation of embryo-fetotoxic effects

No adverse effect of MYR on pregnancy weight gain was noted at any dose level (Table 3). Except for a slight increase in the weights of liver and kidneys in the MYR-treated females, no other sign of toxicity to the maternal organism was observed. The body weight of MYR-treated fetuses did not differ from that of the control group at any dose level (Table 3). However, at the highest dose tested (500 mg/kg), MYR produced a slight increase in the resorption rate and a parallel decrease in the ratio of live fetuses per implantation site (Table 3).

The effects of prenatal exposure to MYR on the occurrence of fetal skeleton abnormalities are shown in Table 4. No differences between control and treated groups were observed at doses up to 300 mg of MYR per kg body weight, but the frequency of skeletal malformations was increased at 500 mg/kg. Nonetheless, the higher incidence of skeletal abnormalities observed at this dose level seems to have been due, to a large extent, to an increase in the occurrence of anomalies such as fused os zygomatic, dislocated sternum (non-aligned sternebrae) and lumbar extra ribs, the spontaneous frequencies of which are high in our rat strain. Anyhow, the higher incidence of skeletal abnormalities as well as the embryolethal effect clearly indicated that a dose as high as 500 mg MYR/kg is embryotoxic to rats.

Table 2 - Outcome of fertility tests in rats continuously exposed to ß-myrcene (0, 100, 300 and 500 mg/kg) during the premating period.

Mating index = [(No. of sperm-positive females) ÷ (No. of mated females)] x 100; Pregnancy index = [(No. of pregnant females) ÷ (No. of sperm-positive females)] x 100. There were no statistical differences between treated and untreated rats (chi-square test).

Outcome	ß-Myrcene (mg/kg body weight)			
	0	100	300	500
Mated females (No.)	45	45	45	45
Mated males (No.)	15	15	15	15
Sperm-positive females (No.)	44	39	37	41
Pregnant females (No.)	33	29	29	32
Mating index (%)	98	87	82	91
Pregnancy index (%)	75	74	78	78

Table 3 - Effects of ß-myrcene (0, 100, 300 and 500 mg/kg) administered by gavage during the premating, mating and gestation periods on parameters evaluated at the time of cesarean section performed on pregnancy day 21 for fetal body weight.

Proportions were analyzed by the chi-square test. Live fetuses per litter, uterus weight, maternal weight gain and fetal body weight are reported as means ± SD and were analyzed by one-way analysis of variance. Mean litter weight was taken as the unit of analysis for fetal body weight.

	ß-Myrcene (mg/kg body weight)			
	0	100	300	500
Gravid uterus weight (g)	67.0 ± 10.2	61.6 ± 9.8	71.0 ± 16.8	63.4 ± 12.7
Maternal weight gain (g)				
Δ (day 0-21)	79.0 ± 11.8	75.6 ± 11.4	84.3 ± 11.8	74.5 ± 19.4
Δ (day 0-21) - (uterine weight)	12.0 ± 14.6	13.9 ± 9.8	13.3 ± 12.1	11.1 ± 15.8
Litters (No.)	12	12	10	10
Implantation sites (No.)	131	121	120	114
Resorptions (No.)	4	5	4	12
Resorptions/implantations (%)	3.0	4.1	3.3	10.5*
Live fetuses (No.)	127	116	116	102
Live fetuses/implantations (%)	97.0	95.9	96.7	89.5*
Live fetuses per litter	10.6 ± 2.1	9.7 ± 1.8	11.6 ± 3.0	10.2 ± 2.4
Fetal body weight (g)	4.42 ± 0.43	4.51 ± 0.71	4.36 ± 0.46	4.25 ± 0.49

Perinatal toxicity and postnatal development of the exposed offspring

As shown in Table 5, duration of pregnancy was not affected by treatment with MYR at any dose level. No adverse effect of MYR on labor was noted in this experiment, and pup mortality in the treated groups was not above that observed in the vehicle-control group on the first day of life (stillbirths) or throughout lactation, i.e. from postnatal day 2 through day 21 (Table 5). Furthermore, no differences between control and MYR-treated groups were found with regard to maternal or offspring weight changes during the lactation period (Tables 5 and 6). In spite of the absence of MYR-induced effects on offspring body weight development, exposure to this monoterpene seemed to have caused a slight retardation in the appearance

Table 4 - Occurrence of skeletal abnormalities in fetuses from dams treated orally with ß-myrcene (0, 100, 300 and 500 mg/kg) during the premating, mating and pregnancy periods.

* P<0.05 compared to controls (chi-square test). Abbreviations: o.c. not ossified = ossification centre not ossified; incpl. ossif. = incomplete ossification; irreg. ossif. = irregular ossification; dumbbell = dumbbell shaped; bicent. = bicentric; part. = partial; sym. = symmetrical.

	ß-Myrcene (mg/kg body weight)			
	0	100	300	500
Fetuses examined (No.)	127	116	116	102
Fetuses with skeleton abnormalities (%)	35.4	27.5	35.3	64.7*
Fetuses with anomalies in (%)				
Forelimbs	2.36	8.62	2.58	1.96
finger (o.c. not ossif.)	2.36	7.75	1.72	0.98
humerus (bent)	0	0	0	0.98
Skull	9.44	11.2	12.0	15.6
os interparietalis (incpl. ossif.)	3.93	1.72	5.17	4.90
os parietalis (incpl. ossif.)	2.36	1.72	0.86	1.96
os supraoccipitalis (irreg. ossif.)	0.78	0	0	0
(incpl. ossif.)	0	0	1.72	0
os zygomatic (fused)	5.51	1.72	6.03	8.82
p.j. os maxill. (incpl. ossif.)	0.78	0	0	0
(bone hole)	0	0	0	1.96
Sternum	10.2	6.89	6.03	16.6
(bent)	2.36	0	0	2.94
(dislocated)	3.14	5.17	3.44	9.80
ossif. centre 1/2 (fused)	0.78	0	0.86	0.98
ossif. centre 2 (irreg. shaped)	0	0	0.86	0.98
ossif. centre 2 (narrow)	0	0	0	0.98
ossif. centre 5 (additional)	3.14	0	0	0.98
ossif. centre 5 (incpl. ossif.)	0.78	0	0	0
ossif. centre 5 (narrow)	0	0	0	1.96
ossif. centre 5 /6 (additional)	0	1.72	0.86	0
Thorax	15.7	6.03	13.7	21.5
Ribs (extra lumbar)	12.5	4.31	12.9	19.6
(wavy)	3.14	1.72	0.86	3.92
Vertebral column	8.66	5.17	10.3	27.4
(bent)	0	3.44	0.86	13.7
Lumbar vertebra (additional)	0	0.86	0	0.98
Thoracic vertebra				
(o.c. bicent., part.)	3.93	4.31	7.75	9.80
(o.c. bicent., sym.)	0.78	0	1.72	0.98
(o.c. dumbbell)	1.57	0.86	0	2.94

Table 5 - Duration of pregnancy, number of stillbirths, postnatal mortality and weight gain of offspring of rats treated orally with ß-myrcene (0, 100, 300 and 500 mg/kg body weight) during pregnancy and lactation.

Data were analyzed by the chi-square test (proportions), Kruskal-Wallis (duration of pregnancy) and ANOVA (body weight and litter size). % of stillbirths = [No. of stillbirths/total of pups born] x 100; % of pups dead = [No. of pups dead/No. of viable pups on day 1] x 100. * P<0.05 compared to control group.

	ß-Myrcene (mg/kg body weight)			
	0	100	300	500
Duration of pregnancy (days)	23.0 ± 0.5	23.1 ± 0.5	23.0 ± 0.4	23.0 ± 0.4
Total of pups born	173	146	176	174
Viable pups on day 1	161	133	174	159
Stillbirths (%)	12 (6.9)	13 (8.9)	2 (1.1)*	15 (8.6)
Viable pups on day 21	143	122	166	132
Pups dead between postnatal days 2 and 21 (%)	18 (11.1)	11 (8.3)	8 (4.6)*	27 (17.0)
Postnatal weight gain (g)				
day 1				
body weight	6.0 ± 0.9	5.5 ± 0.8	5.3 ± 0.6	5.4 ± 0.7
litter size	8.1 ± 2.4	8.3 ± 4.3	9.2 ± 3.0	8.0 ± 2.9
day 7				
body weight	11.9 ± 2.0	10.6 ± 2.3	10.7 ± 1.5	10.3 ± 1.9
litter size	7.5 ± 2.7	8.1 ± 4.3	8.8 ± 3.0	7.4 ± 2.8
day 14				
body weight	22.0 ± 3.9	20.0 ± 4.4	20.0 ± 4.4	20.0 ± 4.0
litter size	7.5 ± 2.7	8.1 ± 3.6	8.7 ± 3.0	7.4 ± 2.8
day 21				
body weight	34.0 ± 7.2	31.0 ± 11.0	31.0 ± 6.6	32.0 ± 11.0
litter size	7.5 ± 2.7	8.1 ± 3.6	8.7 ± 3.0	7.4 ± 2.8

Table 6 - Weight development of female rats treated orally with ß-myrcene (0, 100, 300 and 500 mg/kg body weight) during pregnancy and lactation.

Values are reported as means ± SD. Data were analyzed by ANOVA and the Student *t*-test and no significant differences were detected. Dams submitted to cesarean section on pregnancy day 21 were not included. Days of pregnancy and days of lactation are indicated by subscripts P and L, respectively.

	ß-Myrcene (mg/kg body weight)			
	0	100	300	500
No.	21	17	19	22
Days of pregnancy and lactation period				
1_P	220 ± 10	222 ± 10	223 ± 13	224 ± 12
6_P	234 ± 10	237 ± 10	239 ± 13	234 ± 15
10_P	246 ± 22	243 ± 11	250 ± 14	246 ± 14
15_P	261 ± 15	259 ± 11	268 ± 17	257 ± 14
21_P	304 ± 15	298 ± 16	307 ± 17	288 ± 21
1_L	225 ± 20	224 ± 12	224 ± 18	219 ± 17
7_L	244 ± 17	241 ± 11	249 ± 18	239 ± 20
14_L	257 ± 17	254 ± 16	269 ± 16	253 ± 20
21_L	260 ± 14	260 ± 22	271 ± 16	260 ± 16
Weight gain (g)				
$\Delta (1_P\text{-}1_L)$	4.4 ± 19	-0.4 ± 13	-0.3 ± 10	4.0 ± 17
$\Delta (1_L\text{-}21_L)$	35 ± 15	37 ± 26	47 ± 14	38 ± 12

of incisor eruption, primary coat and eye opening (Table 7). This effect was not dose-related and the MYR-induced delay was more evident with incisor eruption (300 mg/kg) and eye opening (100 and 300 mg/kg). Except for this minor effect on physical maturation, no other indication was found that MYR at doses up to 500 mg/kg impaired the postnatal development of the treated offspring.

Discussion

Except for a slight increase in the absolute and relative weights of liver and kidneys, no other effects were noted in male rats continuously exposed to ß-myrcene for 91 days prior to mating and during the mating period. Since ß-myrcene has proved to be an inducer of hepatic monooxygenases (8), liver enlargement probably resulted from the marked hypertrophy of the endoplasmic reticulum due to the induction of microsomal enzyme synthesis in treated animals (18).

On the other hand, the mechanism underlying the ß-myrcene-induced enlargement of kidneys is still far from being entirely understood. ß-Myrcene was reported to cause a sex-specific hyaline droplet nephropathy in male rats very similar to that produced by *d*-limonene, a monocyclic monoterpene (19). *d*-Limonene-induced hyaline nephropathy was shown to be due to an epoxide metabolite (*d*-limonene-1,2-oxide) that binds to an $\alpha_{2\mu}$-globulin thereby preventing its lysosomal degradation and leading to an accumulation of this low molecular weight protein in the cytoplasm (hyaline droplets) of the proximal tubule cells (20). In the case of MYR-induced kidney damage the $\alpha_{2\mu}$-globulin ligand is still unknown, but it should be noted that microsomal oxidation of MYR olefinic bonds seems to yield epoxide metabolites structurally similar to *d*-limonene1,2-oxide (21). Anyhow, since the hyaline droplet nephropathy is sex-specific (i.e. female rats do not produce $\alpha_{2\mu}$-globulin), and the increase in kidney weight was noted in both males and females, the renal enlargement cannot be attributed only to the accumulation of hyaline droplets. One possible explanation for the kidney enlargement would be an induction of renal microsomal enzyme synthesis by MYR and *d*-limonene.

Table 7 - Physical signs of postnatal development of offspring of rats treated orally with ß-myrcene (0, 100, 300 and 500 mg/kg body weight).

* P<0.05 compared to controls (chi-square test).

Postnatal day	Primary coat (%)				Incisor eruption (%)				Eye opening (%)			
	0	100	300	500	0	100	300	500	0	100	300	500
7	78	67*	48*	59*	1.4	-	-	-	-	-	-	-
8	99	94	95	90	2.8	38	4.2	1.5	-	-	-	-
9	100	100	100	97	41	79	27*	33*	-	-	-	-
10	-	-	-	100	78	99	57*	81	-	-	-	-
11	-	-	-	-	98	100	87*	100	-	-	-	-
12	-	-	-	-	100	-	98	-	-	-	-	-
13	-	-	-	-	-	-	100	-	3.5	4.1	0.6	3
14	-	-	-	-	-	-	-	-	26	12	5.4	40
15	-	-	-	-	-	-	-	-	68	43*	37*	50*
16	-	-	-	-	-	-	-	-	93	73*	73*	87
17	-	-	-	-	-	-	-	-	100	83*	100	99
18	-	-	-	-	-	-	-	-	-	98	-	100
19	-	-	-	-	-	-	-	-	-	100	-	-

Data from the present study suggest that continuous exposure of male rats to ß-myrcene for 91 days prior to mating and during the mating period did not cause any histological changes in the testis and did not impair male fertility. Since ß-myrcene is a highly lipophilic substance that reaches a rather high concentration in the testes (Webb J, Chahoud I and Paumgartten FJR, unpublished results), it seems unlikely that the absence of adverse effects on male fertility is due to an insufficient exposure of male germ cells to this monoterpene. The results also suggest that female fertility was not affected by continuous exposure to ß-myrcene for 21 days prior to mating and during the mating period. The percentages of MYR-treated females that copulated (mating index) and were impregnated by males (pregnancy index) did not differ from those obtained for the vehicle-control group at any dose level. In addition, MYR had no detectable effect on the frequency of pre-implantation losses since no difference in the number of implantation sites per dam was found when treated animals were compared to the controls. Thus, apparently no adverse effect on reproductive function was caused by MYR from gametogenesis up to implantation of the blastocyst in the maternal uterus.

Notwithstanding the absence of adverse effects on female fertility in the present study, MYR, at doses as high as 1.0 and 1.5 g/kg, was shown to impair female offspring fertility in a segment III-designed study in rats (14). It should be emphasized, however, that not only the doses, but also the periods of exposure to MYR were quite different in the two studies. While in the present experiment only adult females were treated with MYR, in the segment III study female offspring were exposed while still in utero, from pregnancy day 15 on and throughout lactation. Therefore, the absence of adverse effects on female fertility in the present study might have been due either to the lower dose levels tested, or to the different period of exposure, or to both. In any case, MYR-induced impairment of female fertility in the segment III study was apparent only at very high doses when a pronounced perinatal mortality occurred as well (14).

Except for liver and kidney enlargement, no indication of MYR-induced maternal effects was found at any dose level and no embryotoxic effects were detected at doses lower than 500 mg/kg body weight. On the other hand, a slight increase in the resorption rate and a higher incidence of skeletal abnormalities indicated that, in the present study, 500 mg MYR/kg was an embryotoxic dose. Absence of signs of maternal toxicity at 500 mg MYR/kg was also found in segment II-(13) as well as in segment III-designed studies in rats (14). Nonetheless, in the segment II study MYR-induced embryotoxic effects were observed only at a higher and maternally toxic dose (13). Since in the segment II study MYR was administered during the second week (pregnancy days 6 to 15) whereas in the present study treatment continued throughout pregnancy, the highest susceptibility of the embryos in the latter may have been due to a longer exposure to this monoterpene. It should also be pointed out that, in the present study, most of the skeletal abnormalities whose incidence was increased in MYR-treated animals were anomalies which occurred at a high frequency in the historical group as well as in the vehicle-control group. Under these circumstances, the toxicological significance of the fetal skeleton findings seems to be minor.

Parturition, perinatal pup mortality as well as postnatal weight gain of the exposed offspring during the lactation period were not affected by MYR at any of the doses tested. The only effect of MYR on postnatal development detected in the present study was a substance-produced delay on the day of appearance of some milestones of somatic maturation. No dose-response relationship was found and retardation was more evident at 300 mg/kg (incisor eruption and eye open-

ing) and 300 mg/kg (eye opening) than at the highest dose tested (500 mg/kg). Since this slight effect was not related to the dose and was not accompanied by any other indication that postnatal development was impaired in the treated animals, it was not taken into account for setting the present study-derived NOAEL .

Contrasting with the absence of toxicity in the present experiment, an increased pup mortality on the first day of life and during the first week of lactation, as well as a reduced pup birth weight, were found after treatment with 500 mg MYR/kg in the segment III study (14). A possible explanation for this discrepancy is the development of tolerance to the toxic effects of ß-myrcene, since in the segment III study treatment began on pregnancy day 15, whereas in the present study administration of this monoterpene to the females started 21 days before mating. The induction of liver microsomal enzymes by MYR (8) and the observed cross-tolerance with pentobarbital effects (9) are findings that give additional support to this interpretation.

On the basis of the data presented in this paper the NOAEL for the toxic effects of ß-myrcene on fertility and general reproductive performance can be set at 300 mg/kg body weight by the oral route. This dose is about the same NOAEL as found in a segment-III-designed study and approximately half the NOAEL obtained for MYR-induced embryotoxicity (segment II) in the rat. Although no quantitative data on human exposure to ß-myrcene are available, it seems very unlikely that dose levels comparable to this experimentally derived NOAEL could be attained when humans are exposed to this olefinic monoterpene through the use of MYR-containing essential oils or folk medicine potions.

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