The psoralens adversely affect reproductive function in male wistar rats

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Abstract

The psoralens occur naturally in produce and are widely used in skin therapy. Studies show that 5-methoxypsoralen and 8-methoxypsoralen reduced birth rates in rats. We determined the effect of psoralens on reproductive function in male rats. Male Wistar rats were dosed daily with 5-methoxypsoralen or 8-methoxypsoralen (75 or 150 mg/kg, p.o.), or vehicle control. Treated males had significantly smaller pituitary glands, fewer sperm per ejaculate, and fewer sperm in the vasa deferentia and epididymides than controls. Dosing significantly elevated levels of testosterone and increased relative testis weight, but did not directly affect testicular weight. Females bred to dosed males required more time to become pregnant, and these males required more breeding attempts. The findings demonstrate the importance of determining the potential risk for infertility and/or birth defects in humans who are exposed to therapeutic, dietary, or occupational psoralens. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: 5-Methoxypsoralen; 8-Methoxypsoralen; Birth rate; Testosterone; Sperm production; Birth defects; Reproductive behavior

1. Introduction

The psoralens occur naturally as secondary metabolites in various plant species including many fruits and vegetables [1–4]. The concentrations of psoralens found in produce have occasionally been reported to be hazardous to human and animal health [3–5]. Plant-related health risks associated with psoralens are due to handling and/or ingestion of psoralen-containing plant tissue [6–11]. These hazards are generally associated with photodermatitis, a skin reaction characterized by bullous eruption, pigmentation, erythema, and vesicle formation [2,12].

The psoralens are known to be toxic to a wide range of organisms [3,4] and have been proven to be carcinogenic and mutagenic [13–17]. Yet, oral administration of synthetic forms of 5-methoxypsoralen (bergapten) and 8-methoxypsoralen (xanthotoxin or methoxsalen) in combination with UVA radiation, a procedure referred to as PUVA, remains the treatment of choice for skin disorders such as psoriasis and vitiligo [18–23]. The psoralens are also used in the treatment of other skin conditions such as skin depigmentation (leprosy and leucoderma), mycosis fungoides, polymorphous dermatitis, and eczema. The United States National Toxicology Program tested 8-methoxypsoralen (xanthotoxin or methoxsalen) for subchronic toxicity in male rats using oral administration [24]. The investigators found that the high doses of 8-methoxypsoralen (200 to 400 mg/kg body weight) altered the histology of the liver, testes, and adrenals, reduced animal weight gain, increased relative liver weight, and even caused death. The authors suggested that further investigations were needed to explain the male genital organ atrophy observed during the study.

A number of recent reports documented other potential risks associated with PUVA. Complications of burns received in a tanning salon by a patient under PUVA resulted in death (2, and references therein). PUVA has been impli-
cated in the increased incidence of malignant melanoma in patients, resulting in the death of some [19]. Studies by Diawara et al. [25,26] showed that, in addition to the effects reported by scientists at the National Toxicology Program [24], oral administration of 8-methoxypsoralen and/or 5-methoxypsoralen to male and female rats also significantly reduced birth rate. Subsequent studies demonstrated that the two compounds administered to females only (mated to undosed males) reduced levels of circulating blood estrogen, and the numbers of implantation sites, pups, and corpora lutea compared with the control group [27].

In our previous studies, the reduction in the number of pups when only female rats were dosed with psoralens was less compared with the reduction when both sexes were dosed [26,27]. This finding suggests that the psoralens may have effects on both males and females. Thus, the present study was designed to determine whether exposure to the psoralens has a direct effect on reproductive function in males.

2. Materials and Methods

Experiment 1: Effect of psoralen dosing on sperm output in male rats

1.1. Animals

Male Wistar rats (21 days old) were obtained from Charles River Laboratory (Charles River; WI/BR; Wilmington, MA), and were cared for at The University of Southern Colorado according to the guidelines of the Institutional Animal Care and Use Committee.

1.2. Dietary treatments

Bergapten (5-methoxypsoralen) and xanthotoxin (8-methoxypsoralen) were obtained from Sigma Chemical Company (Milwaukee, Wisconsin). Dietary treatments were prepared by incorporating the chemical into AIN-93G (ICN Pharmaceuticals Inc., Costa Mesa, California) powdered diet as previously described [27]. The mixture was then blended into a warm agar/water solution for 5 min and immediately dispensed into 30-mL plastic cups. The dietary treatments were allowed to solidify and kept in the refrigerator until needed. The following treatments were used for bergapten and xanthotoxin: 0 (vehicle control), 1250, and 2500 μg chemical/g (ppm) diet. Each rat was housed in a separate cage (1 rat per cage; 10 rats per treatment group) and had access to the diet in non-spill stainless steel containers and to deionized water. Based on the amount of agar/water solution added to the powdered diet mixture, the average daily food intake, and daily weight of individual rats, the average daily dose of psoralens administered during the study were determined to correspond to the following doses/d: 0 mg/kg body wt for the vehicle control, 75 mg/kg body wt for bergapten or xanthotoxin at 1250 ppm (range of 64 to 116 mg/kg), and 150 mg/kg body wt for bergapten or xanthotoxin at 2500 ppm (range of 125 to 235 mg/kg). These concentrations were based on previous research [24–27,29].

As a clear indicator of the intrinsic toxic effect of the psoralens [24,30], we observed during independent studies that xanthotoxin exposure significantly reduced reproductive potential of male and female rats compared with control groups in the absence of UVA irradiation. Therefore, the test animals were fed the experimental diet for eight weeks on a 12:12 (L:D) cycle without UVA exposure during this experiment.

1.3. Sperm output

Sperm counts were obtained by electroejaculation of the males [31] 37 and 57 d after the dietary treatments, i.e., when the test animals were 58 and 78 d old, respectively (the rats were 21 d old when the experiment was started). The male rat was secured on his back to a wooden board. A small probe (2 mm diameter, 1.5 cm long) lubricated with vaseline was inserted into the rectum to stimulate the prostate and seminal vesicles. The probe was operated on a 110 v, 60-cycle alternating current, and the oscillator was run at 30 Hz with a maximum voltage of 3 v. Voltage was brought up slowly from 0 to 3 v and then brought down to 0 during a ten second-period. This treatment was repeated until ejaculation occurred, which was observed for most test animals after approximately one minute of stimulation. As the animal ejaculated, the glans was gently squeezed to remove all the semen. The ejaculate was collected in a microtube and enough eosin was added to bring the total volume to 1 mL. The sample was vortexed for 5 min and sperm were counted under light microscopy using a hemacytometer. On day 79, final weight was taken and animals were sacrificed with an overdose of CO2. Relative liver weight was recorded.

Experiment 2: Effect of the psoralens on reproductive organs

The effect of psoralen dosing on the production of circulating testosterone, diameter of testicular tubules, and weight of testes, seminal vesicles, prostate, epididymis, and pituitary glands was determined.

1.4. Animals and treatments

Animals and experimental conditions were the same as for Experiment 1. However, test animals were kept on the experimental diet for four weeks and three dietary groups with ten males per group were used: 0 mg/kg body wt (vehicle control), bergapten at 150 mg/kg body wt, and xanthotoxin at 150 mg/kg body wt.
1.5. Tissue weights

Animals were sacrificed by an overdose of CO2 30 d after the start of the experiment to observe psoralen-induced subacute toxicity. The following observations were recorded: male body initial and final weight, weight of testes, seminal vesicles, prostate, epididymides, and pituitary glands. Circulating blood testosterone levels were also measured in each animal at the time of tissue collection.

1.6. Histologic examination

At the time of tissue collection, testes were immediately fixed in 10% buffered formalin solution. Formalin-induced shrinkage of tissue was assumed to be consistent for all treatment groups. For each testis, cross-sections (1 to 2 mm thick) were made and immersed in glycol methacrylate [32]. Sections (2.5 to 3 μm) obtained from these cross-sections were stained with periodic acid Schiff’s reagent and hematoxylin (PAS/H), and morphometrically examined by light microscopy. Tubule diameters of 100 nearly round seminiferous tubules were measured for each testis using an ocular micrometer at 250×. Diameter measurements were averaged for each rat for statistical analysis.

1.7. Testosterone concentrations

Testosterone concentrations were measured in serum and testes according to the previously reported radioimmunoassay for 17β-estradiol [27] with the following differences. Testosterone antiserum (No. T4267) and testosterone were obtained from Sigma Chemical Company, St Louis MO. 3H-Testosterone was purchased from Amersham (80 Ci/mmol). Testicular homogenates and sera were extracted with diethyl ether, and recovery was monitored in each sample. The sensitivity was 23.9 pg/mL, the intraassay coefficient of variation was 5.34%, and interassay coefficient of variation was 8.10%.

Experiment 3: Effect of male dosing on reproductive function

This experiment used dosed males and undosed females to investigate effects on mating behavior and success, including the number of breeding attempts; numbers of implantation sites; fetuses, empty spaces, and reabsorbed fetuses; and placental, ovarian, and uterine (full and empty) weight. The effect on individual fetuses resulting from mating undosed females with dosed males was also determined. Another goal of Experiment 3 was to compare the level of circulating and testicular testosterone to determine the potential impact of the psoralens on steroidogenesis. The psoralens induced mRNAs of cytochrome (CYP) 1A1 in female rats during our previous studies [27]. Another objective of Experiment 3 was a further exploration of xanthotoxin-induced modulation of the CYP1A family, an observation that is consistent with psoralen-induced reduction in circulatating estrogen levels. By contrast with Experiment 1, in which sperm output was evaluated by electroejaculating males, Experiment 3 evaluated sperm counts in the vas deferens and the epididymis at necropsy.

1.8. Animals and treatments

Animals were purchased and maintained as described for Experiment 1. Two dietary groups with five males per group were used: 0 mg/kg body wt (vehicle control) and xanthotoxin (Sigma Chemical Company, Milwaukee, WI) 150 mg/kg body wt. Females were obtained from Charles River Laboratory (Charles River; WI/BR; Wilmington MA) after they had reached sexual maturity (45 d). Animals were maintained on regular chow diet and deionized water in individual cages during the experiment. Females were acclimated for a week before the start of breeding. Animals were all appropriately labeled so that each of the five males in each dietary group was bred to three females; i.e., a total of 15 females per group were tested. Vaginal lavage was checked daily (9:00 AM) in each female and used for microscopic determination of estrous cycle stage [33]. On the morning of proestrus, each female was transferred to the appropriate male cage at around 4:00 PM for overnight breeding. The feeder was removed from the male cage during the time the female was present.

Females were removed from male cages early the next morning and the vaginal lavage was immediately examined for the presence of sperm, which was used as evidence of mating and subsequent pregnancy. A female was bred again if sperm were not found. The date of breeding and number of breeding attempts (i.e., the number of days on which males were caged with females) were recorded. Dams and fetuses were sacrificed two days before expected parturition (Day 19 of pregnancy). All males were sacrificed 79 d after the start of experiment after pregnancy had been established for each of the females in the study.

To simulate conditions of the experiment in which both males and females had been dosed [26] and the experiment in which only females were dosed [27], all males (control and treated) received UVA irradiation using fluorescent lamps (40-W Philips F Black Light, burn in 20,000 h, Light Bulb Supply, Denver, Colorado) adjusted at approximately 40 cm above the rat cages to produce 300 μW/cm2 long-wave UVA radiation. The exposure to UVA lasted for 45 min/d during the daytime portion of the 12:12 (L:D) light cycle for the duration of the experiment. Females were on the same 12:12 (L:D) cycle but were not exposed to UVA.

1.9. Northern blot analysis

RNA was prepared from frozen male liver samples as previously described [27]. Briefly, RNA from each sample was subjected to electrophoresis. Blots were washed at high stringency and exposed to autoradiographic film. Autorad band intensity was determined by scanning densitometry
The cDNA probes utilized were 1511 to 12202 for rat CYP1A1 [34], 228 to 2810 for rat UGT1A6 [35], and 18 to 1728 for rat UGT2B1 [36]. Plasmids containing cDNA probes were kindly provided to MRF by Dr. J. Ritter, Medical College of Virginia, Virginia Commonwealth University, Richmond.

1.10. Evaluation of females

The body weight of each female was determined upon arrival, three weeks later (before any pregnancy-related weight gain), and on the day of sacrifice. At the time of sacrifice by CO2 overdose, the weights of full and empty uteri, and individual fetuses were recorded. The number of reabsorbed fetuses, implantation sites, corpora lutea, developed fetuses, and empty spaces, and the size (length and girth) of individual fetuses, fetal deformities, and discoloration were also recorded. The number of breeding attempts (number of days on which males were caged with females) before successful pregnancy for each female, the frequency of presence and absence of sperm in the vaginal lavage after an overnight breeding attempt, the number of pseudopregnancies, and the number of days to pregnancy were determined for each female.

1.11. Evaluation of males

For males, the initial and final animal weight and weights of pituitary glands, testes, epididymides, vasa deferentia, and livers were recorded. Previous researchers reported that sperm production in Fisher 344 rats could not be reliably determined based on epididymal sperm count alone [37]. Thus, sperm were collected from the vas deferens and cauda epididymis separately, and caput plus corpus epididymis combined. For the vas deferens, sperm were collected by flushing the organ with 1 mL PBS in a small centrifuge tube, which was vortexed for 1 min. A 1:10 dilution was then made in eosin solution and sperm counts were obtained using a hemacytometer. For the epididymis (cauda or corpus plus caput combined), organs were sliced in 1 mL PBS using scissors and the solution was vortexed for 1 min at 6 g. As with the vas deferens, a hemacytometer was used to take counts from a 1:10 sperm/PBS:eosin dilution. Previous workers have collected sperm from the vas deferens or the epididymis [38,39].

Circulating follicle stimulating hormone (FSH) levels were measured in serum by radioimmunoassay using a kit (RPA 550; Amersham, Piscataway NJ). All samples were assayed at once. The intrassay coefficient of variation was 6.6%, with a sensitivity of 180 pg/tube.

Statistical methods

For each of the three experiments, data were analyzed using General Linear Model of ANOVA [28]. For each variable, means statistically different were separated at the 5% level using Fisher’s Protected LSD test.

3. Results

Experiment 1: Effect of psoralens bergapten (B) and xanthotoxin (X) on sperm output in Wistar rats. *Statistically different from the control group at the 5% level for each variable according to Duncan’s multiple range test. Bars are standard errors. (Control n = 5, xanthotoxin n = 5).
Effects of dietary 5-methoxypsoralen (bergapten) and 8-methoxypsoralen (xanthotoxin) on liver somatic index and weight gain in male Wistar rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative liver weight* (mean ± sem)</th>
<th>Body weight change** (g, mean ± sem)</th>
<th>Body weight change (% of control weight change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.67 ± 0.44*a</td>
<td>290.5 ± 5.5bc</td>
<td>Reference</td>
</tr>
<tr>
<td>Bergapten 75 mg/kg body wt</td>
<td>5.63 ± 0.14abc</td>
<td>307.5 ± 12.5c</td>
<td>5.9</td>
</tr>
<tr>
<td>Bergapten 150 mg/kg body wt</td>
<td>6.80 ± 0.08a</td>
<td>259.4 ± 8.3a</td>
<td>−10.7d</td>
</tr>
<tr>
<td>Xanthotoxin 75 mg/kg body wt</td>
<td>5.41 ± 0.33ab</td>
<td>280.5 ± 15.0abc</td>
<td>−3.4</td>
</tr>
<tr>
<td>Xanthotoxin 150 mg/kg body wt</td>
<td>6.20 ± 0.54bcd</td>
<td>271.8 ± 18.5abc</td>
<td>−6.4</td>
</tr>
<tr>
<td>Bergapten + xanthotoxin (75 mg/kg body wt each)</td>
<td>7.07 ± 0.33d</td>
<td>249.1 ± 3.8a</td>
<td>−14.2d</td>
</tr>
</tbody>
</table>

n = 10/treatment group.
* (Liver wt/body wt) × 100.
** Body weight change = final wt – initial wt.
a,b,c Means within a column followed by the same letter are not statistically different at the 5% level according to Fisher’s Protected Least Significant Difference test.

Experiment 2: Effect of the psoralens on reproductive organs

In this experiment, oral administration of bergapten and xanthotoxin significantly reduced weights of seminal vesicles, prostates, epididymides, and pituitary glands compared with untreated animals (Table 2). Although the psoralens did not significantly affect total testicular weight, relative testicular weights were increased significantly by xanthotoxin treatment. Testicular tubule diameter showed no significant psoralen-induced adverse effect (Table 2).

Experiment 3: Effect of male dosing on reproductive function

As compared with control, treated males required a significantly greater number of breeding attempts to establish pregnancy, associated with a greater frequency of an absence of sperm in the vaginal smear following an overnight breeding attempt (Table 3).

The girth of litters from females bred to xanthotoxin-treated males was significantly smaller compared with controls, but there were no differences in the other fetal parameters as a result of treatment (Table 3). The number of placentas (13.6 control vs. 14.4 xanthotoxin), implantation sites (14.3 control vs. 14.7 xanthotoxin), fetuses (13.4 control vs. 13.6 xanthotoxin), and reabsorbed fetuses (0.09 control vs. 0.46 xanthotoxin) were not different. Full uterine weight (56.5 g control vs. 55.3 g xanthotoxin), empty uterine weight (4.7 g control vs. 4.9 g xanthotoxin), and ovarian weight (0.22 g control vs. 0.20 g xanthotoxin) were also not affected.

The numbers of sperm collected from the vasa deferentia and the epididymides in treated males were significantly reduced compared with controls (Table 4). Dosing reduced the size of the pituitary gland and increased relative testis weight, but did not affect the weights of seminal vesicle (0.72 g control vs. 0.66 g xanthotoxin), prostate (0.28 g control vs. 0.22 g xanthotoxin), whole epididymis (1.12 g control vs. 1.11 g xanthotoxin), or vas deferens (0.22 g control vs. 0.20 g xanthotoxin). The levels of serum and testicular testosterone were significantly higher in treated males (Table 4). There was no effect of treatment on epididymal testosterone, serum LH, or serum FSH (Table 4). All dosed animals had a significant induction of mRNA’s for CYPIA1, UGT1A6, and UGT2B1 in livers compared with the control animals (Fig. 2).

Psoralen-induced teratogenic effects were observed in

Table 2
Effect of the dietary 5-methoxypsoralen (bergapten, 150 mg/kg) and 8-methoxypsoralen (xanthotoxin, 150 mg/kg) on reproductive organs in male Wistar rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pituitary wt (mg)</th>
<th>Seminal vesicle wt (g)</th>
<th>Prostate wt (g)</th>
<th>Testis wt (g)</th>
<th>Relative testis wt*</th>
<th>Epidydimal wt (g)</th>
<th>Testicular tubule diameter (μm)</th>
<th>Serum testosterone (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.0 ± 1.0a</td>
<td>0.70 ± 0.06a</td>
<td>0.23 ± 0.03a</td>
<td>1.3 ± 0.05a</td>
<td>0.5 ± 0.01a</td>
<td>0.39 ± 0.02a</td>
<td>272.3 ± 3.2a</td>
<td>1.268 ± 0.11a</td>
</tr>
<tr>
<td>Bergapten</td>
<td>8.0 ± 0.9b</td>
<td>0.37 ± 0.07b</td>
<td>0.13 ± 0.02b</td>
<td>1.3 ± 0.07b</td>
<td>0.6 ± 0.03b</td>
<td>0.34 ± 0.02b</td>
<td>264.3 ± 5.6b</td>
<td>1.600 ± 0.09b</td>
</tr>
<tr>
<td>Xanthotoxin</td>
<td>6.0 ± 1.0b</td>
<td>0.27 ± 0.04b</td>
<td>0.10 ± 0.03b</td>
<td>1.2 ± 0.06b</td>
<td>0.7 ± 0.04b</td>
<td>0.23 ± 0.01b</td>
<td>259.5 ± 6.1b</td>
<td>1.237 ± 0.14b</td>
</tr>
</tbody>
</table>

Data expressed as mean ± sem, n = 10/treatment group.
a,b Means within a column followed by the same letter are not statistically significant at the 5% level according to Fisher’s Protected Least Significance test.

* (Testis wt/BODY wt) × 100.
two of 177 fetuses from treated males. These fetuses were totally deformed or had hind limbs missing. In addition, 11 out of the 177 fetuses from dosed males had marked discolored patches on the abdomen or were pale (almost white), suggesting a potential cardiovascular effect. No deformities or discolorations were observed in 174 fetuses from controls. The litter incidence of external malformations or discoloration in the psoralens group was not significantly different from the control (Table 3).

4. Discussion

Gunnarskog et al. [40] reported a “marked increase in low-birth weight infants” born to women exposed to PUVA (oral administration of psoralen drugs in the presence of UVA to treat psoriasis) prior to pregnancy. The authors suggested that this effect was not due to maternal smoking. Because it was unclear whether the disease itself (psoriasis) was implicated in the observed low birth weight, the authors concluded that PUVA did not have significant mutagenic or teratogenic effects. Because females were not subjected to PUVA in the present study, our findings of comparable fetal weight/litter from PUVA-exposed and control sires does not specifically address the Gunnarskog observation. However, the reduced fetal girth observed during the current study suggests that the psoralens might have been implicated in results observed by Gunnarskog and colleagues [40].

Dosing significantly reduced weights of pituitary glands, prostate, seminal vesicles, and epididymides, and increased relative testis weight during Experiment 2 (Table 2). It is unclear why some accessory glands differed significantly in weight during Experiment 2, but not during Experiment 3. The main differences between the two studies are the duration of exposure to psoralens and the route of administration (oral vs. topical).
tion of the dosing period (8 weeks for experiment 2 versus 11 weeks for experiment 3) and the exposure to UVA radiation during Experiment 3. The increase in relative testicular weight supports the possibility that an increase in circulating LH caused a trophic effect and increased testicular tissue mass, although such an increase was shown in the current study only at a $P$ value <0.07. Additionally, the increased circulating and testicular testosterone (Table 4) would also result from increased LH effects on steroidogenesis. No differences were found in the weight of the vasa deferentia of treated and untreated animals during Experiment 3; however, sperm counts from these organs were significantly reduced in dosed males (Table 4). Because testis weight was not significantly affected, it appears that the brain and the hypothalamic-pituitary axis may be more susceptible to the effect of the psoralens than is the testis. This possibility was recently suggested by Turner et al. [41] after they found similar results while studying the effect of a nonsteroidal aromatase inhibitor, anastrozole, on pituitary and testicular function and fertility in male rats.

Our previous studies demonstrated that xanthotoxin compromises the development of ovarian follicles in rats [42], which is consistent with its observed reduction in circulating 17β-estradiol levels [27]. Sertoli cells are the male equivalent of female granulosa cells; their main function is to protect and nourish the growing sperm. Although this similarity of function does not make these cells equivocal, it is important to note that the high number of breeding attempts, the reduced fetal girth, and possibly the abnormality of the testis weight supports the possibility that an increase in circulating 17β-estradiol levels [27]. Sertoli cells are the male equivalent of female granulosa cells; their main function is to protect and nourish the growing sperm. Although this similarity of function does not make these cells equivocal, it is important to note that the high number of breeding attempts, the reduced fetal girth, and possibly the abnormality of the testis weight supports the possibility that an increase in circulating LH effects on steroidogenesis. No differences were found in the weight of the vasa deferentia of treated and untreated animals during Experiment 3; however, sperm counts from these organs were significantly reduced in dosed males (Table 4). Because testis weight was not significantly affected, it appears that the brain and the hypothalamic-pituitary axis may be more susceptible to the effect of the psoralens than is the testis. This possibility was recently suggested by Turner et al. [41] after they found similar results while studying the effect of a nonsteroidal aromatase inhibitor, anastrozole, on pituitary and testicular function and fertility in male rats.

Induction of CYP1A1 is of interest because such an induction would lower circulating levels of estrogen in women, and inducers of this isoenzyme have been proposed as chemopreventive agents for estrogen-dependent cancer [43]. Both CYP1A1 and CYP1A2 are effective in 2-hydroxylation of estrogen [44]. Gwang [30] also found that ip administration of xanthotoxin to male Sprague-Dawley rats induced liver CYP1A, CYP2B, and UGT2B1 mRNAs, proteins, and catalytic activities in a dose-dependent manner [35]. The induction of UGT1A6 mRNA with CYP1A1 in the current study, as well as our previous studies [27], is an indication that xanthotoxin may be a direct or indirect ligand for the Ah-receptor and modulate the AhR/Arnt signal transduction pathway. The induction of UGT2B1, however, suggests that xanthotoxin can also act through other signaling pathways.

The observations reported here provide evidence for psoralen-induced reproductive effects in male rats. These effects were not evaluated by the National Toxicology Program research team [24] and differ from those reported in our study of female-only dosed animals [27]. During the current study, females were not dosed during the breeding experiment (Experiment 3) and were only potentially exposed to the psoralen through seminal fluid. Consequently, it is important to note that the high number of breeding attempts, the reduced fetal girth, and possibly the abnormalities observed in offspring of females mated to treated males result from a direct effect on the male, specifically the sperm. Since copulatory behavior was not visually monitored, it is also possible that treatment could have depressed libido, resulting in increased numbers of breeding attempts. The mechanism of xanthotoxin-induced male reproductive toxicity remains unclear and requires further investigation; however, a direct toxic effect on fertilization, implantation, and/or placental development cannot be ruled out. These findings demonstrate the importance of determining the potential risk for infertility and/or birth defects in humans who are exposed to therapeutic (PUVA), dietary (produce consumption), or occupational (agricultural or industrial) psoralens.

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