Archives of Environmental & Occupational Health, Vol. 60, No. 5, 2005
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Silicone Rubber Nipples: Effects of Sesame-Oil Extract on Reproduction in Mice

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ABSTRACT. The authors investigated a type of silicone rubber (SR) nipple for toxicity, caused by chemical migrants, on reproduction and pregnancy outcomes. They followed an extraction method (set forth in the 20th revised edition of the *United States Pharmacopeia*) in which sesame oil was a vehicle. They prepared the extract daily and administered it orally (50 ml/kg of body weight) into pregnant Swiss albino mice from gestation Day 0 until delivery. They gave a control group of mice the pure vehicle that was subjected to the same conditions. The authors recorded pregnancy weight gain, gestation period, litter size, stillbirths, and offspring sex ratio. They performed an enzyme-linked immunosorbent assay for pregnancy hormones (progesterone, estradiol, and prolactin) for each trimester and monitored birth weight, growth rate, and sex hormone levels (follicle-stimulating hormone, luteinizing hormone, and estradiol in females; testosterone in males) in offspring. The authors detected SR-extractable chemicals by means of gas chromatography and mass spectrometry. The decrease in weight gain from Day 6 of gestation until delivery and the shortness in the gestation period were significant in dams ($p \leq .05$). Newly born pups demonstrated a significantly ($p \leq .05$) lower body weight that continued with age, and this became highly significant ($p \leq .01$) from Day 6. Blood hormone levels in dams and offspring indicated no significance. In conclusion, the studied SR nipples indicated leachability, which could affect reproduction, without a manifest endocrine modulation.

KEY WORDS: mice, nipples, oil extract, pregnancy, silicone rubber

Silicone rubber (SR) is a synthetic elastomer that is applied extensively in the fabrication of teats, teethers, pacifiers, and feeding bottle nipples for human babies. Various chemicals, including accelerating agents, antioxidants, and plasticizers, are normally blended with SR during the industrial process to attain an elastomer with good durability and elasticity.[1]

Although some regard SR as the answer to the toxicity problems of other plastics, such as flexible polyvinyl chloride,[2] tested samples of SR products that are used in contact with food have proved to be leachable for plasticizing agents, such as di-(2-ethylhexyl) adipate and di-(2-ethylhexyl) phthalate (DEHP).[3,4]

Lipophilic agents in SR nipples, like most organics, have a higher tendency toward extraction with fatty media.[5] Therefore, in the present study, we examined a brand of SR nipples, marketed locally at a large scale, for potential adverse effects on reproduction; we used SR-extractable chemicals in a vegetable oil medium, which we administered into pregnant mice.

METHODS AND MATERIALS

Animals

We used virgin female and adult male Swiss Webster albino mice that weighed between 25 and 35 g. We dealt

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with the animals according to the Animal Care and Use Committee[6] guidelines for animal care. We housed them in macrolone cages (27 cm × 21 cm × 14 cm) with sawdust bedding, and they had free access to commercial laboratory chow and tap water. The colony room was maintained at 23 ± 1°C. It had a relative humidity level of 45% to 55% and a 12-hour light–dark cycle. We quarantined animals for a period of 6 days to check their suitability for the study, on the basis of good physical condition and freedom from clinical signs of disease or injury during this period. We subsequently mated the animals for breeding. For mating, we housed the mice overnight, using 1 male plus 2 females (triad breeding) per cage. We removed the males the next morning and checked the females for vaginal copulatory plug (7:00–8:00 AM); we defined the detection of a plug as gestational Day 0 (GD 0). We then placed each mated female in an individual cage under the same conditions until delivery, unless otherwise stated. On Day 4 postpartum, we culled litters randomly to 8 pups and left them with dams up to weaning (at the age of 21 days), after which we separated them according to sex.

Preparation of SR Extract

Our extraction of the SR sample followed the method described in the *United States Pharmacopeia*.[7] In accordance with the method, we cut the sample into small chips (0.5 cm in the larger dimension). We carried out the extraction of the sample on the basis of 4 g of material per 20 ml, using sesame oil as the extraction medium (vehicle). Extraction was carried out into glass-stoppered flasks in the oven, with incubation at 50°C for 72 hr. We prepared the extract daily and cooled it to room temperature before administering it into pregnant mice according to the protocol for the treatment of animals.

Treatment of Animals

Starting from GD 0, we gave the extract orally (per os) to a group of 40 pregnant dams. The daily dose was equivalent to 50 ml/kg of body weight; we used nontoxic, nonpyrogenic feeding needles to administer it (Popper & Sons, Inc., New Hyde Park, NY, USA).[7,8] During the treatment course, to collect the blood samples used to assay the pregnancy hormones, we used cardiac puncture. We killed 10 animals for each trimester, with a total of 30 animals for the 3 trimesters (6, 12, and 18 days from GD 0). The remaining 10 animals continued the daily treatment until delivery. We used 40 animals as a control group, into which we administered the pure vehicle under the same conditions and in a similar fashion as the other group.

We collected blood samples in vials containing the anticoagulant ethylenediamine tetra-acetic acid (known as EDTA), and then we subjected them to centrifugation (at room temperature) by using a microcentrifuge (Model 235B, Fisher Scientific Company, Pittsburgh, PA) at 3900 rpm for 15 minutes. We stored the harvested plasma specimens in stoppered and labeled Erlenmeyer vials at −20°C until the time of our analysis for sex hormones.

We subjected the offspring produced by the surviving treated and control subgroups to a battery of tests (parameters) to monitor prenatal and perinatal effects of SR extract on offspring.

Parameters

In accordance with the method of Dixon[9] and Hood,[10] we monitored the following pregnancy outcomes for both dam groups: (a) weight gain in the pregnant dams (the animals were weighed twice a week); (b) blood sex hormones during pregnancy, including estradiol, progesterone, and prolactin (prolactin, like human chorionic gonadotropin in humans, performs the luteotropin function of supporting the corpus luteum until the placenta produces amounts of progesterone sufficient to support pregnancy)[11,12]; (c) gestation period (GP); (d) litter size (number of pups per litter); (e) stillbirths; and (f) sex ratio (number of males/number of females).

We monitored the offspring of both groups for (a) growth rate, weighing the offspring twice a week, from delivery up to weaning, and (b) blood sex hormones in the adult offspring, assaying follicle stimulating hormone (FSH), leuteinizing hormone (LH), and estradiol in females and testosterone in males.

Assay Method of Sex Hormones

We determined the blood levels of sex hormones by means of the enzyme-linked immunosorbent assay method.[13] We obtained assay kits for these hormones from Diagnostic Systems Laboratories (in Webster, Texas). We also used the Bioelisa Reader, Model EL_x800, and the automatic Bioelisa Washer, Model ELx50 (Bio-Tek Instruments, Winooski, VT). We calibrated the Reader to plot the mean absorbance readings for the standards supplied for each hormone, yielding a calibration curve from which it reads the concentration values of unknowns. We conducted assays according to protocols supplied by the manufacturer for each individual hormone.

Data Analysis

To determine statistical significance, we used Student's *t* test (unpaired-samples test) to analyze data obtained for all parameters.[14]

Gas Chromatography and Mass Spectrometry Analysis of the SR Extract

We subjected our SR sample extract to a gas chromatography and a mass spectrometry (GCMS) qualitative analysis to identify chemical migrants expected to leach from the SR samples. According to the method used by Simoneau and Hannaert,[15] we extracted the SR extract (prepared under the experimental conditions of this study) with acetonitrile (high-performance liquid chromatography grade, Fisher

Scientific, Fair Lawn, NJ). We used a GCMS-QP2010 Series instrument (Shimadzu Corporation, Kyoto, Japan) for the GCMS analysis. We performed the chromatographic separations under the following conditions.

The capillary column was the Rtx-5MS model (Restek, Bellefonte, PA), 30 m × 250 μm inside diameter, with a film thickness of 0.25 μm. The injection was 1.0 μl. (We used the pulsed split mode, with a split ratio of 20.) The injector temperature was 225°C, the interface temperature was 250°C, and the mass spectrometer ion source temperature was 280°C. The ion source was electron ionization, with a positive ion mode. The carrier gas was helium (under a pressure of 66 kPa and a purge flow of 3.0 ml/min). The temperature program ranged from 40°C to 280°C at 48°C/min, with an initial isotherm of 2 minutes and a final isotherm of 6 minutes. The total run time was 13 minutes. The electron energy was 70 eV, and the full-scan mass range was 50–350 amu.

We interpreted the mass spectra by comparison with the mass spectral library software of the National Institute of Standards and Technology.

RESULTS

Pregnancy Outcomes

Table 1 presents the GP weight gain in dams. Compared with the control group, the SR-extract-treated group exhibited a significant decrease ($p \leq .05$) in weight gain, from Day 6 of gestation (G6) until delivery.

The GP in the treated dams was significantly shorter ($p \leq .05$) than the GP in the control group dams, but we observed no significant changes in the litter size, number of stillbirths, and sex ratio (Table 2).

Compared with the control group, the newly born pups demonstrated a significantly ($p \leq .05$) lower body weight, which continued with age; we recorded a highly significant difference ($p \leq .01$), starting from day 6 after birth (Table 3).

Blood Sex Hormone Levels

Our statistical analysis of hormonal data did not show significant changes in the concentrations of the blood sex hormones assayed in dams or their adult offspring (Table 4). We could not detect FSH and LH in the adult female offspring using the assay method applied. Because pituitary gonadotropin secretion controls ovarian steroidogenesis, we could assess FSH and LH here by indirect assays that reflect their influence on the target ovarian tissues.[16] That is, the insignificant result indicated by estradiol in the adult female offspring might be considered an indirect measurement for these 2 gonadotropins, indicating that they did not undergo significant changes.

Migrants Detected in the SR Extract

By means of the GCMS analysis, we identified 6 chemicals in the SR extract (Figure 1): 2-decenal, 2-heptenal,

Table 1.—Effect of Sesame Oil Extract of Silicone Rubber on Maternal Gestational Period Weight Gain

Treatment	Gestation period (d)						
	0	3	6	9	12	15	18
Control	29.09 ± 0.85	30.01 ± 0.74	31.83 ± 0.76	35.38 ± 0.77	39.20 ± 1.07	43.38 ± 1.16	47.20 ± 0.89
SR	28.50 ± 0.49	28.73 ± 0.62	29.67 ± 0.62*	32.31 ± 0.84*	35.60 ± 1.22*	40.50 ± 1.13*	43.93 ± 1.43*
LSD	1.77	1.90	1.99	2.55	3.40	2.28	3.27

Note. The authors administered sesame oil extract of silicone rubber orally at 50 ml/kg daily during the gestation period. Weight gain is shown in grams. Values represent the mean ± SEM; n = 7/group. Control = dams given pure sesame oil (vehicle); SR = dams given sesame oil extract of silicone rubber; LSD = least significant difference; 0 = Day 0 of the gestation period.
*Significantly different from the control ($p \leq .05$).

Table 2.—Effect of Sesame Oil Extract of Silicone Rubber on Pregnancy Outcomes

Treatment	Gestation period (days)	Litter size	No. of stillbirths	Sex ratio
Control	21.63 ± 0.18	7.00 ± 1.05	0.00	1.04 ± 0.13
SR	20.50 ± 0.34*	9.00 ± 0.86	0.67 ± 0.33	1.17 ± 0.51
LSD	0.86	2.72	1.03	1.01

Note. The authors administered sesame oil extract of silicone rubber orally at 50 ml/kg daily during the gestation period. The sex ratio is males to females. Values represent the mean ± SEM; n = 7/group. Control = dams given pure sesame oil (vehicle); SR = dams given sesame oil extract of silicone rubber; LSD = least significant difference.
*Significantly different from the control ($p \leq .02$).

Table 3.—Effect of Sesame Oil Extract of Silicone Rubber on Offspring Growth Rate

Treatment	Gestation period (d)									
	0	3	6	9	12	15	18	21	24	27
Control	1.59 ± 0.08	3.09 ± 0.14	5.74 ± 0.17	7.08 ± 0.32	8.50 ± 0.36	9.97 ± 0.36	12.26 ± 0.48	14.86 ± 0.70	18.18 ± 0.89	20.97 ± 0.96
SR	1.18 ± 0.14*	2.21 ± 0.25*	3.87 ± 0.31**	5.67 ± 0.29**	7.17 ± 0.16**	8.21 ± 0.08**	9.97 ± 0.33**	12.06 ± 0.38**	14.59 ± 0.49**	17.71 ± 0.49**
LSD	0.33	0.73	1.60	1.39	0.88	1.71	1.77	2.03	3.36	2.63

Note. The authors administered sesame oil extract of silicone rubber orally at 50 ml/kg daily during the gestation period. Growth rate is shown in grams. Values represent the mean ± SEM; n = 7/group. Control = dams given pure sesame oil (vehicle); SR = dams given sesame oil extract of silicone rubber; LSD = least significant difference; 0 = body weight taken at birth.
*Significantly different from the control ($p \leq .05$).
**Highly significant difference from the control ($p \leq .01$).

Table 4.—Effect of Sesame Oil Extract of Silicone Rubber on the Level of Blood Sex Hormones in Dams and Their Adult Offspring

	Dams									Offspring	
	Progesterone (ng/ml)			Estradiol (pg/ml)			Prolactin (ng/ml)			Females	Males
Treatment	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	Estradiol (pg/ml)	Testosterone (ng/ml)
Control	41.56 ± 4.32	88.61 ± 5.53	128.62 ± 4.89	35.84 ± 3.15	46.94 ± 6.59	52.88 ± 5.83	1.64 ± 0.07	1.72 ± 0.04	1.90 ± 0.05	28.25 ± 2.27	7.59 ± 0.99
SR	52.79 ± 7.45	99.14 ± 5.86	117.29 ± 7.51	35.33 ± 2.36	43.35 ± 3.73	56.34 ± 5.56	1.72 ± 0.09	1.65 ± 0.04	1.85 ± 0.06	31.86 ± 3.63	8.43 ± 0.62
LSD	17.92	46.66	27.24	9.12	15.05	14.31	0.21	0.37	0.19	10.48	2.21

Note. The authors administered sesame oil extract of silicone rubber orally at 50 ml/kg daily to pregnant mice. Level of blood sex hormones in dams is shown during the first, second, and third trimesters (at 6, 12, and 18 days of pregnancy, respectively). Values represent the mean ± SEM (dams, n = 7/group; offspring, n = 8/group). Control = dams given pure sesame oil (vehicle); SR = dams given sesame oil extract of silicone rubber; LSD = least significant difference.

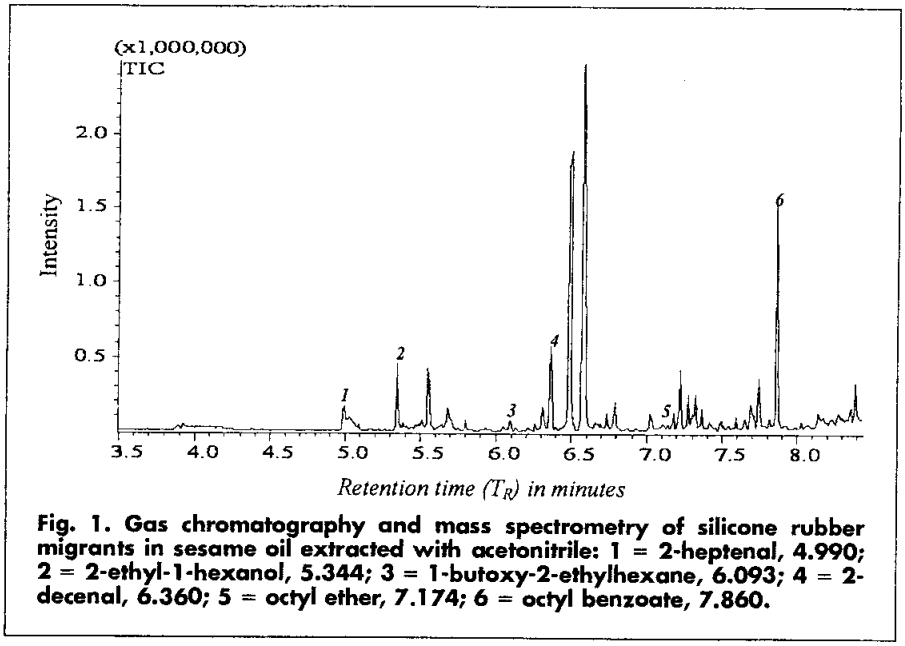

Fig. 1. Gas chromatography and mass spectrometry of silicone rubber migrants in sesame oil extracted with acetonitrile: 1 = 2-heptenal, 4.990; 2 = 2-ethyl-1-hexanol, 5.344; 3 = 1-butoxy-2-ethylhexane, 6.093; 4 = 2-decenal, 6.360; 5 = octyl ether, 7.174; 6 = octyl benzoate, 7.860.

1-butoxy-2-ethylhexane, octyl benzoate, octyl ether, and 2-ethyl-1-hexanol.

COMMENT

The chemical migrants that we detected in the SR extracts in this study are in agreement with those in the published data. According to Lund and Petersen[17] and Wypych,[18] these SR migrants represent additives and reaction products. Reaction products are generated primarily during the industrial process of this elastomer.

With regard to the noxious effects on reproduction caused by exposure to toxic chemicals, researchers have found that migrants from plastics may cause alterations in reproductive behavior. They may contribute to subfecundity, infertility, pregnancy loss, growth retardation, intrauterine fetal demise, birth defects, and ovarian failure by means of a complex mechanism that may involve hormonal or immune disruption, DNA adduct formation, altered cellular proliferation, or inappropriate cellular death.[19]

According to Klaassen,[20] lipophilic toxicants traverse the placenta more easily and attain the maternal–fetal equilibrium more rapidly. Oil-SR extractable agents are expected to behave alike.

Hood[10] noticed that maternal exposure to various chemical and physical stressors led to intrauterine growth retardation of the conceptus and had an adverse effect on the postnatal growth and development of rat pups, which might be attributed in part to maternal effect during pregnancy, as well as to the effect of being reared by a stressed mother. The SR extract in the present study caused analogous effects. This parallels the prenatal effects on mice offspring we observed in our previous study on the aqueous extract of high-density polyethylene plastic sample.[21] The effects induced here by the SR sample are attributed to its extract as a mixture of various chemical migrants, a method applied to test the toxicity of polymeric materials.[8] Whether one migrant or more—in the extract—was behind these effects is a matter that requires further investigation.

In the seeming absence of a manifest alteration in the levels of pregnancy sex hormones, we expect immunogenic responses to SR extract to be behind the effects observed in the pregnancy outcomes. With regard to the short GP in this study, the work of other scientists indicated that exposure to various environmental contaminants is associated with preterm delivery.[22,23] Many of these compounds are believed to induce intrauterine immune responses that are implicated in the etiology of preterm labor. Various migrants from plastics, like many other xenobiotics, are expected to induce their adverse effects through immunogenic pathways.[18] A disruption of the delicate balance of cytokines upon exposure to toxicants increases the production of proinflammatory cytokines at the maternal–fetal interface. These cytokines activate the preterm parturition mechanism through a cascade of reactions.[24]

Latini et al[25] found that prenatal exposure to DEHP is significantly associated with shorter pregnancy duration. In a survey they performed, they found detectable concentrations of DEHP or its metabolite mono-(2-ethylhexyl) phthalate in the cord blood of newborns with lower gestational age. These scientists reported that inflammatory immune responses toward these toxicants, in the uteri of exposed mothers, were the cause of preterm delivery. Our hypothesis that intrauterine inflammatory immune responses toward the SR extract were the cause of preterm delivery in the current

study appears to be in agreement with the finding of these researchers.

In conclusion, the investigated SR nipples demonstrated leachability, and their extract could induce adverse effects on reproduction in mice under the experimental conditions applied in this study. However, it appears that endocrine modulation is not particularly involved in that matter.

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The King Fahd Medical Research Center, Faculty of Medicine, King A. Aziz University (KAAU), Jeddah, Saudi Arabia, kindly provided the mice used in this study.

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