Exposure to a Low Dose of Bisphenol A During Fetal Life or in Adulthood Alters Maternal Behavior in Mice
Paola Palanza,1 Kenza L. Howesheff,2 Stefano Parmigiani,1 and Frederick S. vom Saal2

1Department of Evolutionary and Functional Biology, Farmar University, Parma, Italy; 2Division of Biological Sciences, University of Missouri, Columbia, Missouri, U.S.A.

Maternal behavior in mammals is the result of a complex interaction between the lactating dam and her developing offspring. Significant variation of any of the components of the mother-infant interaction may result in alterations of the behavior of the mother and/or of the offspring. We studied the effects of exposure of female CD-1 mice to the endocrine-disruptive chemical bisphenol A (BPA) during fetal life and/or adulthood in the last part of pregnancy on subsequent maternal behavior. Pregnant females were fed daily doses of corn oil (controls) or 10 μg/kg body weight BPA during gestation days 14–18. In addition, the currently most cited mode of exposure was simulated and females fed daily corn oil alone or the same doses of BPA on gestation days 14–18, resulting in four treatment groups: control, propyl-phenol exposure, low-BPA exposure, and both propyl and adult BPA exposure. Maternal behavior was then observed on gestation days 2–15 and offspring response were measured in the offspring exposed to BPA. Dams exposed to BPA as fetuses or in adulthood spent less time nursing their pups and more time out of the nest compared with the exposed group. Females exposed to BPA both as fetuses and in adulthood did not significantly differ from controls. Injections in propyl (fetal) or adult exposure were observed in the offspring of the exposed dams. The changes seen in maternal behavior may be the result of a direct effect of BPA on the neuroendocrine substrates underlying the initiation of maternal behavior. Key words: development, endurent disruption, house mice, maternal behavior, neuroendocrine transmission. Environ Health Perpect 111:931-932 (2003); http://dx.doi.org/10.1289/ehp.6145-421; E-mail: alvord@emich.edu

Endocrine-disrupting chemicals (EDCs) are synthetic chemicals (i.e., pesticides or Naturally occurring substances (i.e., phytosterols, etc.) are released into the environment and can interfere with the endocrine system of vertebrates (1). Certain EDCs can mimic, or antagonize the endogenous sex hormones (estrogens and androgens) and alter the normal hormone balance during development, which is crucial in regulating sexual differentiation of the neuroendocrine system of vertebrates. In traditional toxicology studies, male and female mice are used for test compounds to assess the capacity to induce genotoxic abnormalities or lethality after administration of a dose typically much higher than what would be expected in the environment. Concerns include the main effects of EDCs, not on the features that will be observed in the test organisms themselves, but to oppose to lose, that nonrepeatability of the test is necessary during critical periods in the development of vertebrates. Functional changes, such as changes in behavioral responses or sex hormone levels, have not typi- cally been examined in neurotoxicology, and this has been a concern. A study by van der Toorn and van der Toorn (2,3), "Functional changes in the challenge to the environment with many stressors in the case of neuroendocrinological damage." A central nervous system (CNS) conflict may become evident only upon a specific kind of behavioral challenge, and the consequences of exposure to endocrine disrupters can be subtle. Examination of both learned and unlearned behaviors may reveal subtle deficits in CNS function, which may not be accom- plished by demonstrable morphological pathology. In many species have shown that exposure to low doses of EDCs during early development alters behavior in animal models of rats and mice and induced neurobehavioral alterations in humans, including a decrease in intelligence quotient (3,4). The neuroendocrine-molecular systems regulate the development and organization and adult expression of behaviors critical to maturation survival and reproduction, such as reproductive aggression, exploration, and sexual and paternal behaviors (5). The expression of these behaviors demonstrates the fit of an individual, and changes in neuroendocrine alterations induced by EDCs may impact the survival and fitness of an individ- ual in the environment. Ethology, the evolutionary study of behavior, may provide a framework for integrating a functional perspec- tive (i.e., evolutionary significance) to studies on prenatal mechanisms that can affect for behavioral alterations induced by developmental exposure to the endocrine-disrupting chemicals. Animal models aid in elucidating the impact of endocrine disrupters on brain development and behavior by taking into consideration the behavioral alterations and animal. In the present study, we hypothesized that ethological alterations of maternal behavior may be a sensitive index of pertur- bations due to exposure to very low doses of endocrine EDCs, which may act directly on the neuroendocrine system of the dam and/or on the development of the offspring. Maternal behavior in mammals is regulated both by maternal hormones and substrates by the maternal offspring in terms of the initial endocrinology. In mice, the endocrine disruption is due to alteration of the behavior of the offspring arising from this study. Therefore, it is well known that stress-related effects in maternal behavior occurs in the neuroendocrine system (5) and/or the offspring during develop- ment (6). For instance, it is well known that stress-related effects in maternal behavior occurs in the neuroendocrine system (6) and/or the offspring during development (7). Significant alterations of any of the components of the maternal behavior may result in alteration in the behavior of the mother and/or of the offspring during development (7). Thus, it is well known that stress-related effects in maternal behavior occurs in the neuroendocrine system (8) and/or the offspring during development (9). This study is part of the neurodevelopmental impact of Endocrine Disrupters on Brain Development and Behavior. (1,2) The present study was supported by grants from the National Research Council of Canada (NSERC) and from the Italian Ministry of University and Scientific Research (60% FIN-EXCVPRC2000) and the University of Parma (Università di Parma). Reference 1 received 3 January 2001; accepted 9 April 2001.
spouse significantly alters the development and formation of the reproductive organs of the female. We conducted a detailed histological and behavioral examination of the ovaries in the presence of a-posteriori analysis. Ovaries were observed in the female brain during the light phase of the cycle when mice are most active. As a result, we observed that females were fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additional 10% of the body weight. In addition, the female was fed Purina 5001 (50% by weight) and Purina chow (25% by weight) with an additiona...
Development of maternal recognition and uterine body height; the higher the uterine, the closer the pup was to the uterine body. The experiment was performed on the 14th day of pregnancy. The animals were divided into two groups: control (C) and BPA (BPA-OIL). The BPA-OIL group was injected with BPA (5 mg/kg) on the 14th day of pregnancy, while the control group was injected with OIL (vehicle) only. The experiment was performed on pregnant rats. The results were analyzed using one-way ANOVA, and the differences were considered significant at p < 0.05. The data are presented as mean ± standard error. Statistical analysis was performed using GraphPad Prism 8 software. The data are presented as mean ± standard error. The difference between the groups was considered significant at p < 0.05. The final analysis was performed using the Student t-test. doi: 10.1101/743571

Results
Maternal Body Weight during Gestation
For the F1 generation, the average body weight during gestation was similar across the treatment groups (mean ± SD: control = 293 ± 17 g; BPA = 271 ± 17 g; BPA-OIL = 285 ± 17 g). The body weight of the dams exposed to BPA-OIL was significantly higher than that of the control group (p < 0.05).

Maternal Behavior
Figure 1 shows the number of maternal behaviors observed in the control and experimental groups. The number of maternal behaviors observed in the control group was significantly higher than that in the experimental group (p < 0.05). The BPA-OIL group showed significantly lower maternal behavior compared to the control group. The mean score was calculated for the following variables: (a) licking and grooming; (b) nursing; (c) aggression; (d) vocalization; (e) play behavior. The differences were considered significant at p < 0.05. The data are presented as mean ± standard error. The final analysis was performed using the Student t-test. doi: 10.1101/743571

Figure 2 shows the average percentage of time spent in maternal behavior across different days. The percentage of time spent in maternal behavior was significantly lower in the BPA-OIL group compared to the control group (p < 0.05). The differences were considered significant at p < 0.05. The data are presented as mean ± standard error. The final analysis was performed using the Student t-test. doi: 10.1101/743571

Figure 3 shows the average percentage of time spent in maternal behavior across different days. The percentage of time spent in maternal behavior was significantly lower in the BPA-OIL group compared to the control group (p < 0.05). The differences were considered significant at p < 0.05. The data are presented as mean ± standard error. The final analysis was performed using the Student t-test. doi: 10.1101/743571

Figure 4 shows the average percentage of time spent in maternal behavior across different days. The percentage of time spent in maternal behavior was significantly lower in the BPA-OIL group compared to the control group (p < 0.05). The differences were considered significant at p < 0.05. The data are presented as mean ± standard error. The final analysis was performed using the Student t-test. doi: 10.1101/743571

Figure 5 shows the average percentage of time spent in maternal behavior across different days. The percentage of time spent in maternal behavior was significantly lower in the BPA-OIL group compared to the control group (p < 0.05). The differences were considered significant at p < 0.05. The data are presented as mean ± standard error. The final analysis was performed using the Student t-test. doi: 10.1101/743571

Environmental Health Perspectives • Volume 110 | Supplement 3 | June 2002

417
early lactation (from PND 2 to PND 5) than control mothers (data not shown). The OIL-BPA dams also showed a similar trend with increased nest-building activity compared with control dams early in lactation (data not shown). The BPA-BPA dams were not significantly different from controls in non-building behavior.

Bathing. There was a significant interaction between life stage at exposure and treatment for bathing behavior (p < 0.06). Dams exposed to BPA (including BPA-OIL, BPA-BPA, and OIL-OIL groups) spent significantly more time bathing away from the nest than OIL-OIL dams (p < 0.02; Figure 1C), regardless of the decrease in bathing behavior reported above. There was also a significant interaction between PND and treatment (p < 0.05), reflecting the fact that the difference between controls and the BPA-exposed females was greater during the middle period of lactation on PNDs 9-14 (Figure 2D-F).

Grooming. For grooming behavior, dams were a significant interaction between life stage at exposure and treatment (p < 0.01). Both BPA-OIL and OIL-BPA dams spent significantly more (p < 0.01) more time self- grooming relative to controls (OIL-OIL) dams (Figure 1D). However, the rate of self- grooming was similar between BPA-BPA and control dams. The interaction between PND and treatment was not significant (Figure 3A-C).

Active. There was a significant interaction between life stage at exposure and treatment for the variable active behavior (p < 0.05). OIL-BPA dams were significantly more active than OIL-OIL dams across the observation period (p < 0.05), whereas BPA-BPA and BPA-OIL dams were similar to controls (Figure 3E). There was a significant interaction between PND and treatment for active behavior (Figure 3F).

Eating/drinkng. There was a significant interaction between life stage at exposure and treatment for eating and drinking (p < 0.01). Although the post hoc comparisons did not find significant differences between the treatment groups, the dams previously exposed to BPA (BPA-OIL) and dams gav- ernationally exposed to OIL-BPA-OIL tended to spend more time in eating and drinking behavior than controls (Figure 1E). There was no significant interaction between PND and treatment for eating and drinking behavior.

Non-related behavior. The variable of non-related behavior was calculated by combining the observations for active, eating/drinking, grooming, and resting behavior that occurred away from the pups. There was a significant interaction between life stage at exposure and treatment for non- related behavior (p = 0.01). BPA-OIL and OIL-BPA dams, but not BPA-BPA dams, spent significantly more time out of the nest, thus away from their pups, than controls (p < 0.05; Figure 1F). The interac- tion between PND and treatment was not significant (Figure 2D-F).

Eating/drinkng behavior. BPA exposure did not significantly influence either in-nest, licking, or focused nest-building behavior.

Offspring Postnatal Development Linear parameters of birth and growth were not significantly different across the number of pups per liter alive on the day of birth. In fact, the mean (standard deviation) number of pups per liter, body weight at birth, based on treatment. Regardless of treatment, males weighted significantly more than females on the day of birth, as well as during PND 3-15 (p < 0.001). Treatment did not influ- ence the body weight of offspring on PNDs 3-15.

Cliff drop avoidance reflex. PND was a significant factor in the analysis of cliff avoidance reflexes (p < 0.01); and the off- spring completed the avoidance response more quickly as they aged (Figure 5A-C). The interaction of PND and treatment was not significant, but BPA-BPA dams did not differ significantly from controls.

Out-of-nest behavior. The variable of out-of-nest behavior was calculated by combining the observations for active, eating/drinking, grooming, and resting behavior that occurred away from the pups. There was a significant interaction between life stage at exposure and treatment for out- of-nest behavior (p = 0.01). BPA-OIL and OIL-BPA dams, but not BPA-BPA dams, spent significantly more time out of the nest, thus away from their pups, than controls (p < 0.05; Figure 1F). The interac- tion between PND and treatment was not significant (Figure 2D-F).

Removal behavior. BPA exposure did not significantly influence either in-nest, licking, or focused nest-building behavior.

Offspring Postnatal Development Linear parameters of birth and growth were not significantly different across the number of pups per liter alive on the day of birth. In fact, the mean (standard deviation) number of pups per liter, body weight at birth, based on treatment. Regardless of treatment, males weighted significantly more than females on the day of birth, as well as during PND 3-15 (p < 0.001). Treatment did not influ- ence the body weight of offspring on PNDs 3-15.

Cliff drop avoidance reflex. PND was a significant factor in the analysis of cliff avoidance reflexes (p < 0.01); and the off- spring completed the avoidance response more quickly as they aged (Figure 5A-C). The interaction of PND and treatment was not significant, but BPA-BPA dams did not differ significantly from controls.

Out-of-nest behavior. The variable of out-of-nest behavior was calculated by combining the observations for active, eating/drinking, grooming, and resting behavior that occurred away from the pups. There was a significant interaction between life stage at exposure and treatment for out- of-nest behavior (p = 0.01). BPA-OIL and OIL-BPA dams, but not BPA-BPA dams, spent significantly more time out of the nest, thus away from their pups, than controls (p < 0.05; Figure 1F). The interac- tion between PND and treatment was not significant (Figure 2D-F).

Removal behavior. BPA exposure did not significantly influence either in-nest, licking, or focused nest-building behavior.
spring of BPA. (III) dams reared to take longer than offspring of control (C3H-OL) dams to complete the righting reflex, with the difference occurring on PNDs 3 and 5 (Figure 3D-F).

Discussion
This detailed analysis of maternal behavior has shown a significant alteration in maternal behavior of female mice that were exposed to a low dose of BPA. Females that were exposed to BPA only as fetuses or only as adult dams in late pregnancy exhibited reduced levels of moving behavior toward their offspring and higher amounts of behaviors outside the nest (active, resting, and self-grooming) regardless of PND. An unexpected finding here is the absence of an effect on maternal behavior in females first exposed to BPA during fetal development and then again in adulthood during late pregnancy. One hypothesis is that maternal exposure to BPA results in permanent changes in systems that maintain homestasis. This shift in hemostatic mechanisms may alter the subsequent response to chemical exposure at a later life stage relative to the response that would occur with no prior exposure to the chemical. There has been speculation that short-term exposure
As an alternate hypothesis concerning the basis of disruption of maternal behavior by BPA, the researchers tested the hypothesis that a subchronic level of maternal behavior disruption by BPA -related effects on the dams’ metabolism and milk production. The study found that exposing dams to BPA throughout their pregnancy and lactation period resulted in decreased milk production and weight gain in the offspring. The authors also noted that the offspring of BPA-exposed dams had decreased body weight and decreased body fat content. These findings suggest that BPA exposure during pregnancy and lactation can have significant effects on maternal behavior and offspring development.
influence the synthesis of the neuroendocrine substrates underlying the expression of maternal behavior. The effects of stress on the neuroendocrine system have been investigated extensively in rodents and non-human primate models (1, 2, 3). This research suggests that maternal behavior can be influenced by stressors such as social, environmental, and physiological factors. For instance, maternal behavior in rats can be altered by exposure to stressful stimuli, such as social isolation or maternal separation (1). These findings indicate that maternal behavior is influenced by a complex interplay of endocrine and neural mechanisms, and that maternal behavior can be modulated by stress. Therefore, understanding the neural and endocrine processes underlying maternal behavior is crucial for developing effective interventions to improve maternal health and well-being. 

References and Notes


Environmental Health Perspectives • Volume 113, Suppl. 3 • June 2005