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NEUROBEHAVIORAL TOXICITY OF PERINATALLY ADMINISTERED POLYBROMINATED BIPHENYLS

By

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To my husband, Patrick Henck, who lived every minute of this dream and helped to make it come true.

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INTRODUCTION

In recent years agents have been identified in the environment which are potentially toxic. In many cases the toxic effects which they generate are readily identified in humans and/or research animals as target organ lesions. However, some substances exert toxic effects in a more insidious manner. At doses below those required to produce overt toxicity, some produce functional changes manifested as behavioral deficits. Of particular concern are lipophilic agents which can cross the placenta, are transmitted via mother's milk, and cross the blood-brain barrier. Such agents, when administered to the dam, are capable of acting directly on the developing organism to produce changes in the central nervous system manifested as functional impairment. An environmental contaminant which meets these criteria is a mixture of polybrominated biphenyls (PBB) known as fireMaster, and research efforts are essential to characterize possible deficits caused by this agent.

In 1973, a series of unfortunate events resulted in contamination of farm animals in Michigan with the flame retardant fireMaster; the entire human population living in the state at that time was subsequently contaminated. As with all environmental accidents, this received a great deal of media attention for both public health and political reasons. PBB contamination is not unique to Michigan, however. PBB have been found in catfish in the Ohio River and in plants,

soil, water, and human hair in industrialized areas of New York and New Jersey (Culliton, 1977). PBB have been used in large amounts in these areas, as well as in areas of Illinois, California, Mississippi, Tennessee and Pennsylvania. Although brominated biphenyls have not been manufactured since 1977, they persist in the environment. Therefore, the risk of contamination still exists. In addition, disposal of plastics treated with fireMaster over time has resulted in the presence of PBB in landfills due to burial and in the air as a result of burning (DiCarlo et al., 1978).

Adverse human health effects have been reported following exposure to PBB via ingestion, inhalation, or percutaneous absorption (Wolff et al., 1979). An initial characterization of the Michigan farming population exposed to PBB revealed health complaints of four major types: gastrointestinal, dermal, musculoskeletal and neurological (Anderson et al., 1978a). Other signs of potential toxicity reported in exposed individuals included slightly enhanced activity of the liver enzymes glutamic pyruvate transaminase and glutamic oxaloacetic transaminase, an increase in carcinoembryonic antigen, a slight decrease in thyroxine levels, and a decreased number of lymphocytes (Anderson et al., 1978b,c,d; Bekesi et al., 1978, 1979; Kreiss et al., 1982).

Epidemiologic studies from which these results were derived are highly inconclusive, however. Many are characterized by lack of unexposed controls and inappropriate statistical analysis for the study design utilized. Additionally, in many cases, testing was conducted on highly exposed individuals cognizant of their exposure. Not only were these results highly subjective, therefore, but also could have been

affected by endogenous depression inherent to loss of income and potential loss of health (Stross, 1981). This supposition is supported by the fact that in the majority of cases, adverse health effects were not correlated with serum or fat PBB levels. To date, dermal effects appear to be the most clear-cut; they are correlated with PBB levels and are persistent in the exposed population (Chanda et al., 1982).

Possible adverse effects on future generations is an area of great concern to the general public. Thus, a series of epidemiologic studies which examined the effects of prenatal or perinatal PBB exposure were of great interest. Children examined in these studies were reported to have deficits in cognitive and fine motor development which seemed to diminish with age (Schwartz and Rae, 1983; Seagull, 1983). These studies are as difficult to interpret as the epidemiologic studies cited previously due to small sample sizes, lack of control over the testing environment, and problems with the experimental design. However, they suggest that prenatal or perinatal exposure to PBB may produce functional deficits; the possibility requires that these potential effects must be clarified.

Because PBB are highly lipophilic, they have the ability to cross the placenta, be transferred via mother's milk and cross the blood-brain barrier. Thus, PBB have direct access to the nervous system of the developing organism and could conceivably produce functional deficits when administered prenatally or perinatally. Transfer to the developing organism via the placenta or milk has indeed been demonstrated in humans and research animals (Dent et al., 1978; Moore et al., 1978; Rickert et al., 1978; Willett and Durst, 1978; Fisher, 1980; Ecobichon, 1983; Eyster et al., 1983; Jacobson et al., 1984).

The biological effects of PBB have been well-characterized in research animals with regard to pharmacokinetics, target organ toxicity, and carcinogenesis. However, the possibility of functional toxicity due to disruption of the central nervous system has not been adequately addressed. Because the interpretation of further epidemiologic studies of the 18 children initially tested will be difficult, it is imperative to pursue this problem using research animals to provide an index of potential neurobehavioral teratogenicity and toxicity in humans exposed to PBB perinatally. This is the primary purpose of the present investigation.

The following sections provide background on the chemical, physical, and biological properties of PBB. A section is also presented on behavioral teratology: the branch of toxicology which will be used to assess functional deficits which occur due to PBB exposure during development.

A. The Michigan PBB Incident: An Historical Perspective

In the summer of 1973 workers at the Michigan Chemical Company in St. Louis, Michigan, shipped bags to Michigan's Farm Bureau Services which contained a substance known as fireMaster, then marketed as a fire retardant, under the assumption that they contained nutriMaster: a magnesium oxide supplement added to cattle feed to augment milk production (Kay, 1977). At that time Michigan Chemical was the sole manufacturer of this mixture of brominated biphenyls; Dow Chemical and duPont had rejected manufacture of brominated biphenyl compounds because of chronic toxicity hazards (Reich, 1983).

It is estimated that 500 to 1000 pounds of fireMaster-contaminated feed were sold and distributed to Michigan farmers by Farm Bureau Services: Michigan's largest feed distributor (Carter, 1976). The maximum PBB concentration ingested by cattle during this primary contamination was estimated as 4000 to 13,500 ppm (Kay, 1977). Secondary contamination was also widespread: a common practice among farmers known as feed swapping served to spread the contamination. In addition, machinery that mixed the contaminated feed passed PBB to other animal feeds. The Farm Bureau was also involved in the distribution of PBB-contaminated aureomycin to farmers (DiCarlo et al., 1978). Those animals not fit for human consumption because of PBB contamination were often slaughtered, processed, and added to farm feed; this resulted in a low level of contamination throughout the population of Michigan farm animals and, ultimately, throughout the Michigan food chain (Kay, 1977). Subsequent analysis of tissue from contaminated animals revealed levels as high as the following: cattle tissue - 2700 ppm, milk - 595 ppm, poultry tissue - 4600 ppm, and eggs - 59.7 ppm (Kay, 1977).

A farmer named Frederic Halbert began to notice abnormalities in his dairy herd in September of 1973. Symptomatology included decreased appetite, decreased milk production, increased urination, increased lacrimation, lameness, the appearance of hematomas and abscesses, abnormal hoof growth, hyperkeratosis, alopecia, persistent mastitis, severe reproductive abnormalities, wasting, and death (Jackson and Halbert, 1974). Although the supplemented feed was withdrawn after 16 days, 24 of 400 cows died within 6 months. Halbert suspected that the feed had been contaminated and fed it to 12 calves to confirm his

suspicions; within 6 weeks 5 calves were dead. Identification of the contaminant eluded investigators until March, 1974, when researchers testing contaminated feed samples accidentally left their gas chromatograph turned on during lunch and produced the late-emerging peaks characteristic of PBB (Carter, 1976). Although Michigan Chemical was implicated in the contamination because they manufactured both fireMaster and nutriMaster, they denied involvement. Their role in the incident became clear, however, when a half-used bag of fireMaster was discovered at the Farm Bureau Services mill.

The contamination was initially thought to be the problem of one farmer: Frederic Halbert. He had received the largest amount of contaminated feed and later estimated that some of his cows had ingested one-half pound of fireMaster. However, it was soon recognized that the incident might have far-reaching consequences. In May of 1974 the Michigan Department of Agriculture placed several contaminated farms under quarantine; by the end of 1975 500 farms were quarantined. In May of 1974, the Food and Drug Administration (FDA) established tolerance levels of 1 ppm PBB for meat and dairy products, 0.3 ppm for feed and 0.1 ppm for eggs. Within 6 months these levels were lowered to 0.3 ppm for meat and dairy products and to 0.05 ppm for feed and eggs because animals manifested signs of toxicity at levels below those originally established (Carter, 1976). Ultimately, 29,800 cattle, 5,920 hogs, 1,470 sheep, and 1.5 million chickens were destroyed because of unacceptable PBB levels (DiCarlo et al., 1978). In addition, 865 tons of animal feed, 17,790 pounds of cheese, 2,630 pounds of butter, 34,000 pounds of dry milk products, and nearly 5 million eggs had to be removed from the market.

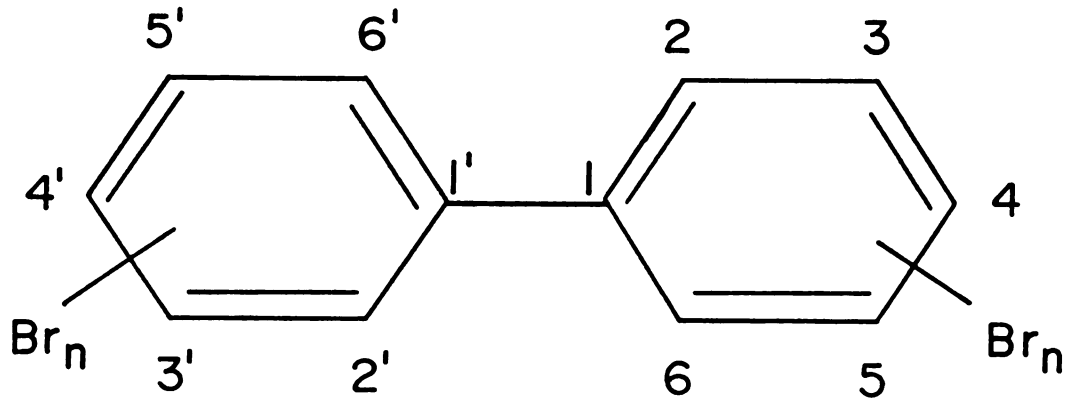
Over 2 years intervened between the initial contamination incident and the identification of the last of the farms to be quarantined. This resulted in widespread contamination of not only Michigan farm animals, but also of the human population of Michigan. Thus, the PBB incident not only had tremendous economic impact on the farming community of Michigan but also had the potential to produce deleterious effects on human health and the environment.

B. Chemical and Physical Properties of FireMaster

FireMaster is the trade name associated with a fire retardant marketed by the Michigan Chemical Company in the 1970's. FireMaster FF-1 (the material implicated in the Michigan feed contamination incident) was produced when 2% calcium silicate (an anticaking agent) was added to fireMaster BP-6 (DiCarlo et al., 1978). Both fireMaster formulations were mixtures of at least 30 different polybrominated biphenyl congeners (Figure 1), with an average bromine content of 6 atoms per molecule (Kay, 1977). Table 1 lists the brominated biphenyl congeners which have been structurally characterized for each mixture and the degree of their contribution to the mixtures. These data indicate that the composition of the two formulations differs slightly, and it has been shown that fireMaster BP-6 contains a higher proportion of congeners demonstrated to be toxic (Dannan et al., 1982b). This provides a rationale for the fact that most toxicity studies have been conducted with fireMaster BP-6. The discussion which follows will in general deal with fireMaster BP-6.

Figure 1

POLYBROMINATED BIPHENYL



Structure and ring numbering system of polybrominated biphenyls.

Table 1

PBB Congeners	Congener Designation[A]	From Standard Curves of GC-ECD Response to Pure PBB Congeners		From Electronic Integrator (Direct Measurement)		
		% in FF-1	% in BP-6	% in FF-1	% in BP-6	% in Reconstituted Mixture
2,4,5,2',5'-	(1)	3.7	4.5	2.0	3.2	4.8
2,4,5,3',4'-	(2)	0.7	4.2	0.9	5.3	3.7
2,3,6,2',4',5'-	(3)	1.3	1.4	1.2	1.5	0
2,4,5,2',4',5'-	(4)	47.1	47.8	56.5	56.1	58.9
2,3,4,2',4',5'-	(5)	8.6	12.0	7.5	11.6	11.6
2,4,5,3',4',5'-	(6)	3.3	5.5	3.2	5.2	4.2
2,3,4,5,3',4'-	(7)	4.5	5.0	3.6	2.9	4.0
2,3,4,5,2',4',5'-	(8)	24.7	15.1	22.7	12.6	12.3
2,3,4,5,2',3',4'-	(9)	1.5	1.1	0.8	0.8	0.45
Br_4		1	1	0.4	0.2	0
Br_5		1	1	0.6	0.1	0
2,3,4,5,2',3',4',5'-	(12)	2.4	0.9	1.3	0.3	0.5

[A]Congener designations were made on the basis of the elution sequence from the GC.

Relative abundance of PBB congeners in fireMaster (FF-1 and BP-6) and in a reconstituted mixture made from purified congeners.

From: Dannan et al., 1982d.

FireMaster is contaminated by approximately 200 ppm bromonaphthalenes. The majority of these are hexabromonaphthalenes; however, trace amounts of tetrabromonaphthalenes and pentabromonaphthalenes have also been detected (O'Keefe, 1976; Hass et al., 1978; Birnbaum et al., 1983). The hexabromonaphthalene component consists of two isomers: 1,2,3,4,6,7-hexabromonaphthalene and 2,3,4,5,6,7-hexabromonaphthalene in a ratio of 3 to 2 (Birnbaum et al., 1983). No contamination with bromodibenzofurans or bromodibenzo-p-dioxin (compounds with great potential toxicity) has been detected (Hass et al., 1978).

FireMaster melts at approximately 72°C and decomposes in the range of 300 to 400°C. It has a low vapor pressure, is insoluble in water, and is highly soluble in organic solvents (a property which indicates a high degree of fat solubility; Kay, 1977). It is persistent within living organisms and the environment and is highly resistant to metabolism or degradation. Environmental degradation in the form of dehalogenation can occur via ultraviolet radiation (Ruzo and Zabik, 1975). DeKok and coworkers (1977) demonstrated that irradiation at 300 nm of fireMaster BP-6 dissolved in n-hexane resulted in the appearance of 2,3',4,4',5-pentabromobiphenyl. Degradation of this congener in the presence of n-hexane and ultraviolet light resulted in the highly toxic chemical 3,3',4,4'-tetrabromobiphenyl (Hill et al., 1982). Analysis of field soil and manure from cattle contaminated with fireMaster one year after the accident revealed no degradation (Jacobs et al., 1976). However, analysis of soil surrounding the site of the Michigan Chemical Company several years after the manufacture of fireMaster revealed the presence of degradation products, including 2,3',4,4',5-pentabromobiphenyl; 2,2',4,4',5-pentabromobiphenyl and two unidentified

tetrabromobiphenyls (Hill et al., 1982). Because PBB are resistant to microbial degradation in soil (Jacobs et al., 1976), these products probably resulted from photodegradation. It may therefore be concluded that while degradation by sunlight is not a major decontamination pathway under normal circumstances, it can occur with high PBB concentration in the soil. This degradation can lead to the production of toxic compounds. Application of the major congener 2,2',4,4',5,5'-hexabromobiphenyl to the rabbit ear did not produce hyperkeratosis, while application of this congener following exposure to ultraviolet light produced marked hyperkeratosis, indicative of chloracne (Patterson et al., 1981).

PBB resist degradation not only within the environment but also within the mammalian system. The fireMaster BP-6 mixture is poorly metabolized. However, an hydroxylated pentabromobiphenyl metabolite has been identified in the pig (Kohli and Safe, 1976). The authors speculated that this metabolite could have been formed by direct hydroxylation of minor pentabromobiphenyl isomers contained in the mixture or by debromination and hydroxylation of 2,2',4,4',5,5'-hexabromobiphenyl. Oral administration of fireMaster BP-6 to dogs resulted in the appearance of 6-hydroxy-2,2',4,4',5,5'-hexabromobiphenyl in the feces (Gardner et al., 1979). Because 6-hydroxylation of a highly brominated biphenyl is rare in animals, the authors speculated that the metabolite was formed by microbial metabolism of PBB in the gut. Limited mammalian metabolic data indicate that congeners with a higher bromine content are metabolized at a much slower rate than those with a lower bromine content. Indeed, the majority of congeners comprising fireMaster persist in the body fat of animals (Willett and Durst, 1978; Rickert et al.,

1978; Domino et al., 1980). The congener 4,4'-dibromobiphenyl is metabolized by the rabbit, calf and pig to 4,4'-dibromo-3-biphenylol; 3,4'-dibromo-4-biphenylol; and 4'-bromo-4-biphenylol (Kohli and Safe, 1976; Safe et al., 1978). In addition, 3,3',4,4'-tetrabromobiphenyl; 2,2',4,5,5'-pentabromobiphenyl; and 2,2',3,4',5',6-hexabromobiphenyl have been shown to be metabolized in the rat by microsomal monooxygenase systems induced by phenobarbital (Dannan et al., 1978; Millis et al., 1984). The serum concentration of 2,2',4,5,5'-pentabromobiphenyl was decreased in highly exposed farmers and Michigan Chemical Company workers (Wolff and Aubrey, 1978), indicating that it may have been metabolized. Analytical techniques in this study did not allow for identification of the other metabolizable congeners. The fireMaster contaminant 1,2,3,4,6,7-hexabromonaphthalene is metabolized by the rat and excreted, while 2,3,4,5,6,7-hexabromonaphthalene persists. Results of these studies indicate that organisms near the top of the food chain which did not consume fireMaster directly may have received a mixture of somewhat different composition than that of the original fireMaster. This is substantiated by results of an experiment in which mink were fed fireMaster FF-1 or tissues from chickens and a cow previously fed fireMaster. Data from this study suggested that the derived PBB were more toxic than the original mixture (Aulerich and Ringer, 1979).

C. Human Health Effects Related to FireMaster Exposure

In 1976 researchers began to examine individuals exposed to fireMaster for untoward effects. Two groups of individuals were tested: families from quarantined farms and workers employed by the Michigan

Chemical Company during the time of fireMaster manufacture. As the contamination became more widespread throughout the state, subsequent research involved the general populace of Michigan.

It has been estimated that between the onset of fireMaster contamination in 1973 and the spring of 1974, when farms began to be quarantined, more than 10,000 Michigan residents were exposed (Cordle et al., 1978). This estimate is not representative of the total number of people exposed, as the last farms were not quarantined until 1975 (Carter, 1976).

In an initial study by the Michigan Department of Public Health, serum and fat PBB levels of 110 people from quarantined farms were compared to those of 104 people from surrounding farms thought not to be contaminated. Serum levels of the majority of exposed farmers tested were in the range of .002-.019 ppm (however, a maximum value of 2.26 ppm was achieved), while those of the majority of their children were in the range of .02-.09 ppm (Cordle et al., 1978). Paired samples of adipose tissue and blood revealed an average fat:blood ratio of 175:1. This observation is consistent with data from research animals which indicate that PBB preferentially distribute to and persist in fatty tissues (Matthews et al., 1977; Fries et al., 1978; Willett and Durst, 1978; Tuey and Matthews, 1980; Miceli and Marks, 1981; Domino et al., 1982). Trace amounts of PBB were found in the serum of farmers from supposedly uncontaminated farms, which negated the use of these individuals as controls.

Subsequent epidemiologic studies used a group of Wisconsin farmers as controls. In one such study in which individuals from Michigan

cognizant of their high degree of exposure to PBB were asked to list current health problems, those from quarantined farms were found to have far more health complaints than did those from Wisconsin farms. The nature of these complaints was categorized as follows: gastrointestinal, dermal, musculoskeletal and neurological (Anderson et al., 1978a). Gastrointestinal complaints included loss of appetite and weight, nausea, vomiting, abdominal pain, and diarrhea. Dermal symptoms included appearance of a rash, acne, increased sensitivity to the sun, darkened or thickened skin, and discoloration or deformity of the fingernails or toenails. Arthritis and tendonitis were among the musculoskeletal signs noted. Neurological symptoms were among the earliest manifestations of human health effects related to the PBB incident (Valciukas et al., 1978) and included marked tiredness and fatigue, a large decrement in the capacity for physical and intellectual work, an increase in the number of hours slept per day, muscle weakness, poor memory, perception changes, unusual patterns of reactivity (e.g., slowness in answering questions), paresthesias, loss of balance, and reduced energy of expression and movement.

Farmers were exposed by the oral route to a mixture which was in all probability altered by the metabolism of farm animals, whereas Michigan Chemical Company workers were exposed directly to fireMaster primarily via the dermal route, with additional exposure by inhalation and direct ingestion (Wolff et al., 1979a). The prevalence of symptoms in chemical workers was far less than in farmers with the exception of skin abnormalities. Chemical workers also had gastrointestinal, musculoskeletal and neurological complaints (Anderson et al., 1978c), and in addition exhibited "chest" symptoms. It was suspected that the "chest"

symptoms might be due to inhalation of respiratory irritants such as bromine and chlorine, rather than solely to fireMaster. A high incidence of skin abnormalities was felt to be due to the dermal route of exposure.

Serum and fat levels of PBB were found to be higher among exposed chemical workers than among exposed farmers. PBB levels in males were as follows: Michigan Chemical Company non-production workers 0.017 ppm (serum) and 3.88 ppm (fat); quarantined farm residents 0.032 ppm (serum) and 4.92 ppm (fat); and Michigan Chemical Company production workers 0.604 ppm (serum) and 196.5 ppm (fat) (Wolff et al., 1979a). The fat:serum ratio for production workers was 287:1, considerably higher than that of exposed farmers. Wolff and coworkers (1979a) postulated that the tissue level and distribution discrepancies between the two groups may have been due to a different intensity of exposure, the difference in exposure route, and the composition of the PBB contaminant (unchanged fireMaster vs. an animal-mediated mixture).

Data from exposed farmers and chemical workers revealed a lack of correlation between serum and fat PBB levels and prevalence of symptoms. This lack of correlation was demonstrated in several epidemiologic studies (Bekesi et al., 1979; Brown et al., 1981; Stross et al., 1981) and, coupled with a high incidence of depression among individuals from quarantined farms, led Stross and coworkers (1981) to postulate that many of the subjective complaints observed might be a result of reactive depression occurring due to economic losses and worry over potential health effects. However, certain changes in nonsubjective indices were also reported and are discussed subsequently.

Activity of serum glutamic pyruvate transaminase and serum glutamic oxaloacetic transaminase was elevated in exposed Michigan dairy farmers when compared to Wisconsin dairy farmers (Anderson et al., 1978b). A trend toward elevation of activity of these enzymes, generally considered to indicate signs of liver toxicity, was also observed in Michigan Chemical Company production workers (Anderson et al., 1978c). Both groups also showed an increase in carcinoembryonic antigen (CEA) (Anderson et al., 1978c,d). This antigen represents nonspecific material resulting from pathologic cell change; there is a lack of organ specificity. Environmental factors such as cigarette smoking, excess alcohol consumption and/or occupational exposure to vinyl chloride can affect CEA titer. CEA titer is positively correlated with serum PBB concentration, although PBB effects may be additive to those of other factors known to result in enhanced CEA titer.

Hormonal effects have also been reported in individuals exposed to PBB. A high prevalence of primary hypothyroidism was observed in workers from a plant manufacturing decabromobiphenyl and decabromobiphenyl oxide (Bahn et al., 1980). Thyroxine levels were reduced in members of Michigan's cohort of highly exposed individuals (Kreiss et al., 1982). This reduction was not significantly correlated with PBB levels, indicating that this may be an adverse effect associated with congeners of higher bromine content.

The immune system may also be affected by PBB exposure. Lymphocyte numbers have been shown to be depleted in farmers and in Michigan Chemical production workers. Comparison of 45 farmers from quarantined farms to Wisconsin farmers and New York residents revealed a decrease in the number and percent of peripheral blood lymphocytes, an increased

number of "null" cells, and an altered response to tests designed to evaluate the functional integrity of immune system cells (Bekesi et al., 1978). The authors stated that these changes are not found in healthy, normal subjects. Similar effects were observed in Michigan Chemical workers when compared to Wisconsin farmers (Bekesi et al., 1979). However, these individuals had a normal response to tests evaluating functional integrity of the cells, and no correlation was observed between PBB effects on the immune system and plasma PBB levels. The possibility of causes other than PBB exposure were not investigated. Women from highly exposed farms have indicated that their children have an increased incidence of respiratory illnesses (Weil et al., 1978). However, this observation is highly subjective and the effects of PBB exposure on the immune system remain unclear.

PBB tissue levels and health effects associated with PBB exposure have been found to persist in the population. Serum PBB levels did not decrease significantly in farmers or Michigan Chemical workers when assessed two years after the most intense presumed exposure (Wolff et al., 1979a). However, examination of half the 3683-member cohort of Michigan residents exposed to PBB revealed a small but significant decrease in serum PBB over 1-year and 2-year periods (1 μg/l in 1 year) assessed 1977 through 1979 (Kreiss et al., 1982). Serum PBB levels of Michigan Chemical Company workers were also assessed during this time period. Levels did not change significantly, indicating that exposure may still have been occurring for these individuals (Wolff et al., 1979b).

Three years after the accident, exposed individuals were re-examined for cutaneous effects. Highly exposed farmers exhibited halogen acne, hair loss, skin redness, skin peeling and scaling, itching, increased sweating, and increased growth of fingernails and toenails (Chanda et al., 1982). Only halogen acne was observed in Michigan Chemical Company workers, but at a higher incidence than in farmers. This is probably a reflection of the initial dermal exposure route.

PBB have been found to be extremely persistent in human breast milk. In the original Department of Public Health study, five women were studied who were pregnant at the time of the fireMaster accident, one of whom had a breast milk PBB concentration of 92.66 ppm, with a corresponding plasma level of 1.068 ppm (Cordle et al., 1978). Of the five women examined, the average ratio of breast milk to plasma was 102:1. This ratio was supported by the data of Eyster and coworkers (1983), who determined the ratio to be 107:1 to 119:1 using a sample size of 46. These researchers also established a positive correlation between serum, adipose, and breast milk PBB levels. The ratio of adipose to serum PBB levels was only 100:1 in lactating women, as opposed to 140:1 in non-lactating women, indicating that the fat mobilization which occurs during lactation alters PBB partitioning (Brilliant et al., 1978; Eyster et al., 1983). Brilliant and coworkers (1978) conducted a random sample survey from Michigan mothers three years after the fireMaster accident, with the following results: 96% of 53 women in Michigan's lower peninsula and 43% of 42 women in the upper peninsula had detectable levels of PBB in their breast milk. Because of the positive correlation between adipose tissue and breast milk PBB levels, these data indicate that at this point in time 9 of 10 lower peninsula

Michigan residents had detectable serum levels of PBB. Miller and coworkers (1984) studied PBB levels in breast milk over a 32-month period from May 1976 to December 1978 and determined that the proportion of specimens with detectable PBB levels decreased slightly with time. They estimated by linear extrapolation that slightly less than half of the breast milk samples tested would still have detectable levels (0.05 ppm or greater) by the year 2000.

The significance of the presence and persistence of PBB in breast milk lies in the fact that PBB may be transferred to the nursing infant via the milk. Indeed, this is probably the major route of exposure for the developing infant. Human milk PBB concentration was determined to be 107 to 119 times that of maternal serum, while the concentration in umbilical cord blood was one-tenth that of maternal serum, and in the placenta was one-sixth to one-tenth that of maternal serum (Eyster et al., 1983). These data indicate that partial passage of PBB through the human placenta does occur. This was confirmed by Jacobson and coworkers (1984) in a study of 313 women living in the most highly contaminated area of Michigan. These authors concluded that because umbilical cord serum PBB concentration was less than that of maternal serum, that the placenta can act as a barrier to PBB, but does not totally exclude it.

These data indicate that the developing human infant may be at risk, depending on the maternal body burden of PBB. However, no adverse effects have been reported regarding decreased fertility or an increased incidence of birth defects in highly exposed individuals. Assessment of fertility in highly exposed males revealed no difference in sperm counts, motility, or morphology (Rosenman et al., 1979). Although

visible physical defects have not been observed, the possibility of functional deficits exists.

In individuals from quarantined farms some of the earliest symptoms reported following the PBB incident were neurological in origin (Valciukas et al., 1978). Neurological complaints appeared to increase in number from 1973 to 1976, at which time a plateau was reached. Behavioral testing of 170 randomly selected members of the Michigan cohort and subsequent comparison of results from 93 Wisconsin farm residents revealed that PBB-exposed male farmers 50 years of age and older performed significantly less well on tests designed to assess spatial relations, association, and visual perception (Valciukas et al., 1978). A group of 21 PBB-exposed farm residents with persistent medical complaints had more difficulty than an urban control group on tests of memory for prose, short-term retention of words and cognitive flexibility (Brown and Nixon, 1979). Because these deficits were positively correlated with measures of depression on the Minnesota Multiphasic Personality Inventory but not with adipose tissue PBB concentrations (average = 3.94 ppm), these researchers stated that the observed memory dysfunction was related to psychological dysfunction rather than to PBB body burden. A group of 25 Michigan Chemical Company workers with a mean adipose tissue PBB concentration of 9.33 ppm did not exhibit cognitive deficits, leading these authors to affirm that the memory deficits observed in farmers were not due to PBB body burden (Brown et al., 1981). Many of the neurological complaints reported by Michigan farmers were subjective in nature. This fact, coupled with results of the Brown and Nixon (1979) and Brown (1980) studies, indicate that the

neurological consequences of exposure to PBB in adults are unclear and warrant further clarification.

The functional effects of prenatal or perinatal exposure to fire-Master appeared to be initially quite clear. Weil and coworkers (1978) examined 33 children from quarantined farms born between September 1, 1973 and December 31, 1975 in September of 1977. No effects were observed on physical growth or upon physical or neurological assessment. Developmental milestones were achieved at the same age as those of controls. However, parents stated that these children had more illnesses, especially of the respiratory tract. Nineteen of these children were administered five tests from the McCarthy Scales of Children's Abilities. An inverse relationship was observed between adipose tissue PBB levels and performance on 4 of the 5 tests designed to assess early cognitive and fine motor development (Seagull, 1983). Subsequent evaluation of 18 of these children at 4 to 6 years of age revealed a normal range of performance on the McCarthy and Wechsler Preschool and Primary Scale of Intelligence (Schwartz and Rae, 1983). When children with PBB fat levels greater than 0.1 ppm (arbitrarily chosen as representing a high level of exposure) were assessed separately, an inverse relationship was found between PBB fat levels and performance on some perceptual and perceptual-motor tasks. Nevertheless, because these children were subjected to many different tests with no adjustment of the acceptable significance level and because of the arbitrary nature of their assignment to the testing group, it is difficult to determine if the effects observed were "real" or merely due to enhanced statistical error. These data might indicate that the behavioral deficits observed

initially in these children diminished with time. However, it is difficult to compare these studies. One of the greatest problems involves the small sample size, particularly because few children with higher PBB levels were examined. With regard to the Seagull study, children were tested at the same time and in the same place where they had been subjected to extensive medical testing (Nebert et al., 1983). In addition, they faced the added trauma of a subsequent fat biopsy. Stress alone could have affected their performance, or stress could have unmasked subtle functional deficits caused by PBB exposure but for which compensation had taken place. Schwartz and Rae subjected these children to a more extensive battery of tests, but testing was conducted in the childrens' schools (Nebert et al., 1983), an environment to which they were habituated.

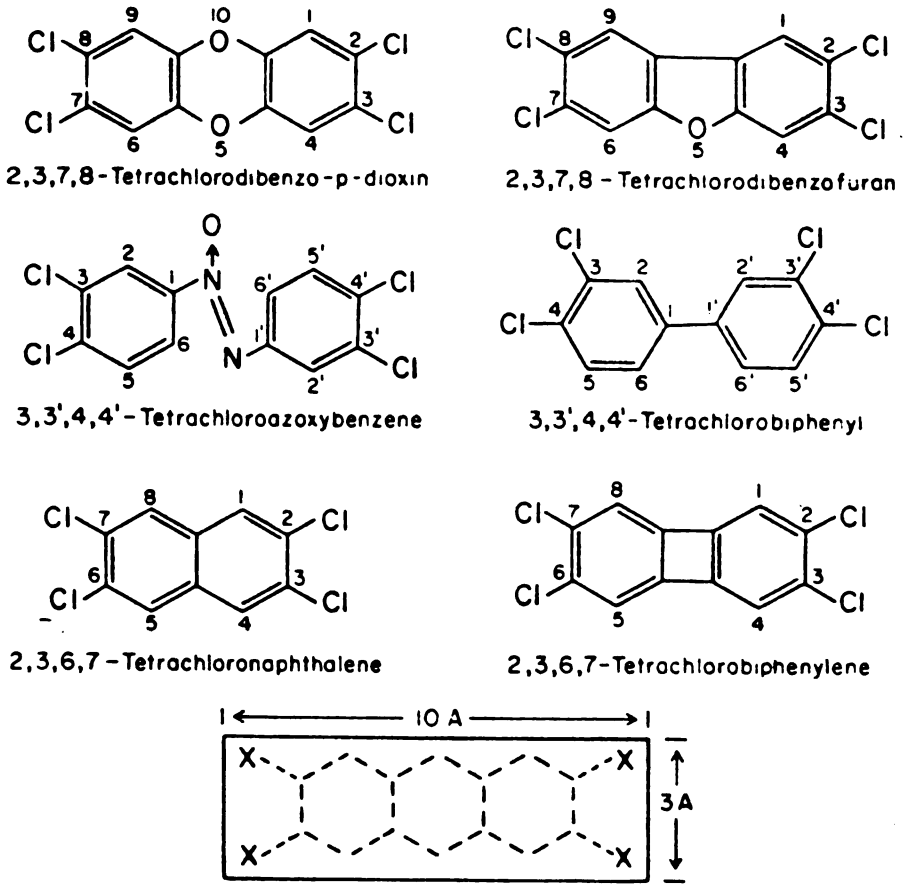
The evidence suggesting that exposure to fireMaster produced functional deficits, particularly in children, is not firm. However, the existing evidence, coupled with the fact that PBB can be transferred to the developing infant in utero or via lactation, indicates that further clarification of these possible effects is needed.

D. Toxicity of Polybrominated Biphenyls

1. The Polyhalogenated Aromatic Hydrocarbon Toxic Syndrome

The polybrominated biphenyl congeners which comprise fireMaster belong to a class of compounds of similar structure known as the polyhalogenated aromatic hydrocarbons (PHAH). This class includes the chlorinated dibenzo-p-dioxins, dibenzofurans, azoxybenzenes, naphthalenes, chlorinated biphenyls and brominated biphenyls. Figure 2

Figure 2



The isosteric congeners and ring numbering systems of various halogenated aromatic hydrocarbons. The diagram at the bottom depicts the postulated recognition site on the cytosolic receptor to which these isosteric compounds bind.

From: Poland and Knutson, 1982.

indicates the structures of several of these compounds. The PHAH are considered together because: a) their chemical structures are similar (they are approximate isostereomers), b) they produce a similar and characteristic pattern of toxicity (although they vary greatly in potency), c) they are believed to act by a common mechanism, d) all are widespread in the environment, and e) their chemical stability, lipophilicity and resistance to degradation result in persistence in the environment and concentration in the food chain (Poland and Knutson, 1982).

The toxic syndrome produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in many species of experimental animals is most representative of this class of compounds. This syndrome includes wasting (decrease in body weight gain with a decrease in adipose tissue), lymphoid involution (with effects on thymus, spleen and lymph nodes), hepatotoxicity (including hepatomegaly due to hyperplasia and hypertrophy of parenchymal cells, with proliferation of the smooth endoplasmic reticulum, increased activity of hepatic mixed function oxygenases, increased DNA synthesis, and increased DNA content), halogen acne, gastric lesions, urinary tract hyperplasia, subcutaneous edema and hydropericardium in chicks, decreased fertility, teratology (with the most prominent anomalies in the kidney and palate), and carcinogenesis (including hepatocellular carcinoma, stratified squamous cell carcinoma of the hard palate and nasal turbinates, and keratinizing squamous cell carcinoma of the lung; see Poland and Knutson, 1982).

Accidental introduction of many of the PHAH into the environment has resulted in adverse health effects for wild and domestic

animals, as well as humans. An early survey of workers employed in the manufacture of the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T - compounds determined at that time to be contaminated with TCDD) revealed such signs of toxicity as halogen acne, hyperpigmentation, eye irritation, hepatotoxicity, neuromuscular deficits, psychological alterations (manifested as a high score on the manic scale of the Minnesota Multiphasic Personality Inventory), and porphyria cutanea tarda (Poland et al., 1971). TCDD has also been identified as a contaminant in pentachlorophenol. Workers employed in the manufacture of pentachlorophenol displayed symptoms similar to those of workers exposed to 2,4-D or 2,4,5-T. Halogen acne has been identified in humans following exposure to chloronaphthalenes, chlorobiphenyls, chlorodiphenyloxides, certain petroleum products, and solid chlorophenols (Kimbrough, 1972).

Severe human health effects were observed in members of the population from a province in Japan who ingested rice oil contaminated with polychlorinated biphenyls (PCB) as Kanechlor 400. Victims of the Yusho ("oil") incident exhibited many of the same subjective symptoms as those experienced by individuals contaminated with TCDD and with fire-Master. These included halogen acne, increased eye discharge, lethargy, gastrointestinal disturbances, numbness and other neurological signs, and pigmentation of the skin (Kuratsune et al., 1972). Babies born to affected women had unusually grayish, dark-brown stained skin and similar pigmentation of the gingiva and nails. One fetus which was stillborn had marked halogen acne. The health effects of the Yusho incident have persisted. Because of the structural similarity between PCB and PBB and the persistent human health effects observed upon

exposure to various PHAH, it is clear that members of the PBB cohort must be monitored on a chronic basis to quickly identify health effects as they occur.

2. Absorption, Distribution and Excretion

The fireMaster mixture appears to be rapidly absorbed from the gastrointestinal tract. Willett and Durst (1978) detected the major brominated biphenyls of fireMaster in the plasma of cattle within 2 to 4 hours of oral administration. Single-dose studies indicated that the concentration of these biphenyls in blood plasma of cattle was maximal 24 hours after exposure. A single oral dose of the major congener 2,2',4,4',5,5'-hexabromobiphenyl administered to rats resulted in 93% absorption, while four doses administered daily resulted in 90% absorption (Matthews et al., 1977). With regard to the congeners comprising fireMaster, there appears to be less absorption with increasing halogenation (Matthews et al., 1978). This is supported by the observation that five times more hexabromobiphenyls than heptabromobiphenyls or octabromobiphenyls were transferred to the milk of lactating cows fed 10 mg fireMaster per day for 60 days (Fries and Marrow, 1975).

Much of the early research on distribution and clearance of fireMaster and its components was conducted in cattle in an effort to identify the actual composition of the contaminant to which humans were exposed via milk and beef. Subchronic feeding of fireMaster to cattle resulted in steady-state levels in plasma within 15 days and steady-state levels in milk fat within 30 days (Fries et al., 1978; Willett and Durst, 1978). The biological half-life in cows was 60 days, and the rate of elimination was dependent on stage of lactation. Residues were

distributed in proportion to fat concentration in tissues; however, unexpectedly, residues in the brain (a lipid-rich tissue) were lower than in all other tissues. Robl and coworkers (1978) administered fireMaster FF-1 to calves and cows and confirmed that the highest residue levels (measured as 2,2',4,4',5,5'-hexabromobiphenyl) were found in fatty tissues. Residues in the fat of calves and cows and in the milk of cows increased with increasing dose and duration of administration.

Free PBB were not detectable in the urine of cows following multiple oral administration of fireMaster (Willett and Durst, 1978). At fireMaster doses which did not produce toxicoses, approximately 50% of the total dose was excreted in the feces, which indicates a major elimination route for PBB in non-lactating cows. After fireMaster withdrawal, fecal concentration declined to 1 to 2% of that during dosing, but still represented the major excretory route. In contrast, lactating cows removed 3 times the quantity of PBB as that removed in feces. This would indicate that a substantial degree of human contamination could have occurred via cows' milk. Indeed, Fries and coworkers (1978) estimated that people who consumed milk from contaminated cows could have ingested as much as 10 g of the animal-mediated fireMaster.

Domino and coworkers (1982) determined that 2,2',4,4',5,5'-hexabromobiphenyl (HBB) was distributed to the tissues of a rat following a single oral dose of fireMaster FF-1 according to a 3-compartment linear model. Compartment 1 consisted of whole blood, spleen, kidney, and heart; compartment 2 was made up of cerebral grey and white matter

and cerebellum; and compartment 3 constituted the subcutaneous fat. Peak tissue levels occurred at the following times after administration: whole blood, spleen, and lung within 4 hours; heart, liver, and testes within 8 hours; grey matter, white matter, cerebellum, kidney, and jejunum within 12 hours; and inguinal subcutaneous fat within 7 to 10 days (Domino et al., 1980). A multi-exponential decay of HBB residues was observed in most tissues to a similar degree; however, residues continued to accumulate in fat. Following a single intraperitoneal dose of fireMaster BP-6 to rats, the fat:serum ratio rose from 6 to 36 weeks post-administration from 222:1 to 722:1, which indicates more rapid clearance of PBB from serum than from fat (Miceli and Marks, 1981). Half-lives following a single intraperitoneal dose of fireMaster BP-6 to rats were determined to be the following: serum, 23.1 weeks; spleen, 9.0 weeks; lung, 11.2 weeks; liver, 11.5 weeks; adrenal gland, 43.3 weeks; brain, 63 weeks, and fat, 69.3 weeks (Miceli and Marks, 1981). A growing adipose tissue compartment was needed to describe the pharmacokinetics of single intravenous or multiple oral doses of HBB in growing rats (Tuey and Matthews, 1980). Predictions from the 3-compartment linear model indicated that the biological half-life in emaciated rats would be 60.5 days following a single oral dose, while that for obese rats would be 311 days (Domino et al., 1982), thus indicating the importance of the size of the adipose tissue compartment. The actual measured half-life for "normal" rats was 145 days.

Multiple oral doses of HBB to rats resulted in an initial high rate of excretion, probably due to elimination of unabsorbed compound (Tuey and Matthews, 1980). Excretion was slow, with only 3% of the dose having been eliminated after 42 days, and the majority of that during

the first 3 to 4 days. A slight amount of biliary excretion was observed following single intravenous or multiple oral doses of HBB (Matthews et al., 1977; Tuey and Matthews, 1980). About 35% of HBB excreted in the bile during the first week following administration was reabsorbed. Data using several different routes of administration indicate that substantial residues of fireMaster or HBB would remain in the body of the rat at the end of its life because of the persistence of PBB in lipid-rich tissues. This is not the case for humans. When a blood-flow limited compartmental model constructed for the rat was extrapolated to man, the analysis indicated that the body burden half-life of PBB in man would be 6.5 years (Tuey and Matthews, 1980).

A single oral dose of fireMaster FF-1 to rats resulted in a differential distribution of congeners. More pentabrominated biphenyls were observed in the brain than in the blood, while the opposite was true for heptabrominated biphenyls (Domino et al., 1980). Thus, differential absorption, distribution and metabolism of the congeners which comprise fireMaster might indicate their differential involvement in the characteristic toxic syndrome and provide a reason for the appearance of various symptoms over an extended time period.

Pregnancy and lactation change the distribution and excretion pattern of PBB in the rat, as discussed previously for humans and cattle, although lactation does not appear to be as significant a route of excretion in rats as in humans and cattle (Rickert et al., 1978). However, placental passage to developing offspring and passage through the milk both serve to reduce the body burden of the dam.

Pharmacokinetic data indicate that PBB are very persistent within the body, depending on adipose tissue stores. This persistence provides adequate time for PBB to be released from the fat and thus exert toxic effects. The consequences are described in the following sections.

3. PBB-Mediated Toxicity

The fireMaster mixture and associated congeners are not acutely toxic. The single-dose oral LD_{50} for fireMaster BP-6 in rats was determined as 21,500 mg/kg (Hilltop, 1970). Studies with octabromobiphenyl revealed an oral LD_{50} of 2000 mg/kg in rats and of greater than 12,500 mg/kg in Japanese quail (Aftosmis et al., 1972a). Toxicity due to skin absorption of octabromobiphenyl in the rabbit was also minimal, with a lethal dose greater than 10,000 mg/kg (Aftosmis et al., 1972b). The acute percutaneous absorption potential of hexabromobiphenyl was low, with a lethal dose greater than 5000 mg/kg. Administration of 1000 mg/kg octabromobiphenyl to occluded skin 5 hours/day for 10 days produced enlargement of the liver. No primary irritation was observed on the skin of rabbits due to octabromobiphenyl, nor did it serve as a skin sensitizer in guinea pigs. Mild irritation was observed upon instillation to the rabbit eye. Application of 60 mg fireMaster FF-1 to the ear of rabbits resulted in hyperkeratosis identical to that produced by TCDD (Kimbrough et al., 1977).

Mortality occurs upon subchronic administration of fireMaster; the degree depends on species. The 313-day LD_{50} for fireMaster FF-1 fed to mink was 3.95 ppm (an approximate cumulative dose of 106 mg); 90% mortality occurred with a cumulative dose of 197 mg (Aulerich and

Ringer, 1979). Mink fed meat from a fireMaster-contaminated chicken (cumulative dose of derived fireMaster = 22 mg) for 10 months had a 10% mortality rate, while those fed meat from a fireMaster-contaminated cow (cumulative dose of derived fireMaster = 98 mg) were all dead in 3 months. Only 2 of 6 guinea pigs survived oral administration of 100 ppm fireMaster BP-6 for 30 days (cumulative dose ≈100 mg, based on a mean body weight of 600 g and a mean daily food intake of 25 g), and all died within 15 days following administration of 500 ppm (cumulative dose ≈400 mg). Oral administration of fireMaster FF-1 to Fischer 344 rats for 90 days resulted in an LD_{50} of 65 mg/kg/day for males (cumulative dose ≈1800 mg, based on a mean body weight of 300 g) and an LD_{50} of 149 mg/kg/day for females (cumulative dose ≈2700 mg, based on a mean body weight of 200 g; Gupta et al., 1981). Survival time was significantly decreased in male rats fed 0.3-10 mg/kg/day and in male mice fed 10 mg/kg/day for 125 days with an observation time of 23 to 24 months (Gupta et al., 1983b). Thus, the rank order of species for mortality, in order of decreasing susceptibility, is guinea pig > mink > rat > mouse.

It is difficult to determine the mortality rate due to the original contamination in farm animals, as the exact amount of fireMaster received by the animals is unclear. Although mortality occurred in Halbert's herd, subsequent studies have not demonstrated mortality at fireMaster levels greater than those at which his herd was contaminated and have provoked questions as to whether the mortality rate may have been a reflection of farm management procedures, nutritional deficiencies or indigenous microbial and parasitic infections (Moorhead et al., 1977).

Signs of toxicity observed upon subchronic administration of fireMaster or its associated congeners are similar to those outlined previously for PHAH. One of the earliest manifestations of toxicity in the Halbert cattle was anorexia (Jackson and Halbert, 1974). As the toxic syndrome progressed, the affected cattle began to lose weight, although appetites remained normal (suggesting poor feed efficiency). Subsequent examination of these animals revealed chronic inflammatory changes in all layers of the intestinal tract, indicating compromised absorption and thus providing a possible reason for poor feed efficiency (Cook et al., 1978). Cattle fed 25 g fireMaster BP-6/day for cumulative doses of 825 to 1650 g exhibited anorexia, emaciation, diarrhea, and dehydration; necropsy revealed edema of the abomasal folds, mucoid enteritis, hemorrhage, and edema of the rectal mucosa (Moorhead et al., 1977).

Hyperplastic gastritis, correlated with body weight loss, was observed in female monkeys fed 1.5 ppm fireMaster FF-1 for 36 weeks (cumulative dose = 70 mg). Body weight loss of a greater magnitude was observed in female monkeys fed 0.3 ppm fireMaster FF-1 for 15 months (cumulative dose = 22 mg; Lambrecht et al., 1978). Mink exhibited food rejection and weight loss after ingestion of 2.5 ppm fireMaster FF-1 for 4 months (cumulative dose of approximately 20 mg; Aulerich and Ringer, 1979). Decreased food consumption and body weight gain were observed in male and female rats administered 100 mg fireMaster FF-1/kg/day for 90 days (approximate cumulative doses of 2700 and 1800 mg, respectively; Gupta et al., 1981). Decreased body weight gain with no appreciable change in food consumption was observed in male and female rats at approximate cumulative doses of fireMaster FF-1 as low as 113 and 75 mg,

respectively (3 mg/kg/day for 125 doses over 6 months), and in male, but not female, mice at a cumulative dose of 38 mg (10 mg/kg/day for 125 doses over 6 months; cumulative dose based on a mean body weight of 30 g; Gupta et al., 1983a). Examination of the male rats at 24 months of age revealed hyperplastic gastropathy and gastric ulcers.

Deficiencies in the immune system have been reported for humans exposed to fireMaster (Bekesi et al., 1978, 1979; Weil et al., 1978), as well as for domestic and research animals. Initially contaminated cattle exhibited enhanced vulnerability to common environmental pathogens and, upon necropsy, showed lymphocyte infiltration into liver, kidney, small intestine, lung, spleen, and lymph nodes (Cook et al., 1978).

A decreased thymus to body weight ratio was observed in male mice administered 30 mg/kg fireMaster FF-1 by 30 days of administration (approximate cumulative dose = 27 mg) and in male and female rats administered FF-1 by 15 days (approximate cumulative doses of 135 and 90 mg, respectively; Gupta et al., 1981). Administration of 125 doses of fireMaster FF-1 to rats resulted in a decreased thymus to body weight ratio following doses of 0.3 (females) or 1 (male) mg/kg/day (approximate cumulative doses of 7.5 and 37.5 mg, respectively; Gupta et al., 1983a). In addition, males fed 1 mg/kg/day exhibited a decreased spleen to body weight ratio.

Thymic involution was observed in dogs fed .0625, .25, 1 or 4 mg/kg fireMaster BP-6 for 61 days and was accompanied by degenerating lymphocytes at all doses administered, depletion of lymphocytes, particularly in the T-cell zone of the lymph nodes (1 and 4 mg/kg), and a

decrease in plasma cells (4 mg/kg; Farber et al., 1978). Changes in the spleen included extramedullary hematopoiesis and a decreased number of lymphocytes in the white pulp (1 and 4 mg/kg). Hematopoiesis was decreased in bone marrow.

Examination of immune function was conducted in the Balb/c mouse: a strain which is immunosensitive to PBB. At a dose of 1000 ppm fireMaster BP-6 surviving mice were athymic and incapable of mounting an antibody-mediated response, while mice administered 1 to 100 ppm exhibited preferential wasting of the thymic cortex (Fraker, 1980). Although mice administered the lower doses exhibited a normal T-cell-dependent delayed type sensitization reaction to dinitrofluorobenzene, the IgM and IgG primary response was decreased in a dose-dependent manner. This latter effect involved both B and helper T cells. These data demonstrated that fireMaster was immunotoxic to mice at doses which produced no overt clinical signs of illness.

Halogen acne is a characteristic sign of PHAH toxicity and has been observed in humans contaminated with fireMaster (Chanda et al., 1982). Skin abnormalities were observed in contaminated cattle and included abnormal hoof growth, matted hair, alopecia, and thickening of skin on the neck, thorax and shoulder (Jackson and Halbert, 1974). Calves administered fireMaster had hyperkeratosis over the entire body, while rhesus monkeys exhibited alopecia (Lambrecht et al., 1978). As mentioned previously, the rabbit ear test for halogen acne was positive for fireMaster FF-1 and for sunlight degradation products of 2,2',4,4', 5,5'-hexabromobiphenyl (Kimbrough et al., 1977; Patterson et al., 1981).

Decreased body weight gain, feed efficiency, reproductive performance (to be discussed subsequently) and skin lesions may in part be related to altered thyroid function. Morphologic changes in the thyroid gland characterized by hypertrophy and hyperplasia of the follicular cells, as well as by the presence of large membrane-limited colloid droplets and electron-dense lysosomal bodies within the cytoplasm, were observed in rats fed 5, 50 or 500 ppm fireMaster BP-6 for 5 weeks (a cumulative dose as low as approximately 263 mg). Microscopic examination of these rats revealed a decrease in size of the Golgi apparatus, with a resultant decrease in secretory vesicles (Kasza et al., 1978b). These authors speculated that the lesions observed would interfere with the synthesis and secretion of thyroxine. This was confirmed by the work of Gupta and coworkers (1983a), who determined that 0.3 mg fireMaster/kg/day for 125 days administered to rats (cumulative dose = approximately 11 mg) resulted in decreased serum thyroxine levels; serum triidothyronine (T_3) was also decreased, but at levels of 3 mg/kg/day and greater. The thyroid hyperplasia observed by Kasza et al. (1978b) and Sleight et al. (1978) with fireMaster and by Norris et al. (1975) with octabromobiphenyl was felt to result from long-term stimulation by thyroid stimulating hormone because of an absence of positive feedback. These results support the finding of decreased serum thyroxine levels in Michigan's cohort of highly exposed individuals (Kreiss et al., 1982). A possible explanation for the lowered serum thyroxine levels is that some of the congeners which comprise fireMaster induce UDP-glucuronyl transferase activity. Glucuronidation of thyroxine in the liver may be enhanced and thus biliary excretion would be enhanced (Akoso et al., 1982a).

Effects on the urinary tract have been observed in some species, although not to the degree that some fireMaster-related effects have been seen. Nephrosis and nephritis were observed in the cattle originally exposed to fireMaster (Jackson and Halbert, 1974). Administration of 25 g fireMaster BP-6/day to heifers produced enlargement and a pale gray to tan coloration of the kidneys; necropsy revealed changes in the collecting ducts and convoluted tubules (Moorhead et al., 1977). Gupta and coworkers (1983b) observed a significantly higher incidence of chronic progressive nephropathy in male rats administered 1, 3 or 10 mg fireMaster FF-1/kg/day for 6 months and observed to 24 months of age.

The liver is the primary target organ of PBB. Calves administered the original fireMaster contaminant developed massive liver abscesses; histopathology of the liver revealed hemorrhagic necrotic hepatitis, fatty metamorphosis, and replacement of liver cells by large fat vacuoles (Jackson and Halbert, 1974).

Lipid accumulation in the liver and an increased liver to body weight ratio were observed 2 months following a single oral dose of 1 g/kg fireMaster FF-1 (approximately 300 mg) to rats (Kimbrough et al., 1977, 1978). In addition, livers of these rats exhibited enlarged hepatocytes with foamy or vacuolated cytoplasm, general cellular pleomorphism, brown pigment in macrophages and Kuppfer cells, and interstitial fibrosis and necrosis of single cells.

Similar hepatic changes as well as enlarged livers, hepatocellular hypertrophy, accentuated hepatic lobular markings, and/or elevated activities of the liver enzymes gamma-glutamyl transpeptidase, glutamic pyruvic transaminase and alkaline phosphatase were observed in rats, mice, guinea pigs, mink and rhesus monkeys (Hilltop, 1970;

Aftosmis, 1972a,b; Kasza et al., 1978a; Lambrecht, 1978; Aulerich and Ringer, 1979; Gupta and Moore, 1979; Gupta et al., 1981, 1983). Histopathologic changes in the liver included proliferation of smooth endoplasmic reticulum and Golgi apparatus; swelling, disorganization, and single cell necrosis of hepatocytes; fatty infiltration; and bile duct proliferation (Kasza et al., 1978a; Gupta et al., 1981, 1983a). Hepatic porphyria was also observed upon acute or subchronic administration of fireMaster to rats and mice (Kimbrough et al., 1978; Gupta et al., 1981, 1983b).

PBB induce hepatic microsomal enzymes (to be discussed subsequently); this induction leads to decreased uroporphyrinogen decarboxylase activity and the resultant enhanced urinary porphyrin excretion and may also be related to carcinogenesis (Jones and Sweeney, 1977). This is in keeping with the fact that fireMaster is an hepatocarcinogen. Neoplastic nodules, considered to be precursors of hepatocarcinogenesis, were observed in rats within 1 year of single oral dose of 200 or 1000 mg fireMaster (cumulative dose of FF-1/kg approximately 60 mg) (Kimbrough et al., 1978, 1981) or following 1 mg/kg/day for 125 doses (cumulative dose of approximately 38 mg; Gupta et al., 1983b). Hepatocellular carcinoma was observed within 2 years of oral dosage of rats and mice with fireMaster FF-1 using the following exposure regimens: a single dose of 1000 mg/kg to rats (41.4% incidence); 12 doses of 100 mg/kg/day to rats (67.8% incidence); 125 doses of 3 (male) or 10 (female) mg/kg/day to rats (21% incidence males, 35% incidence females); and 125 doses of 10 mg/kg/day to mice (95% incidence males, 88% incidence females) (Kimbrough et al., 1981; Gupta et al., 1983b).

Using the assay of Pitot and the principle of a 2-stage process of carcinogenesis, Jensen and coworkers (1982) determined that both fireMaster BP-6 and the major congener 2,2',4,4',5,5'-hexabromobiphenyl were promoters, rather than initiators, of hepatocarcinogenesis in rats. The fireMaster mixture had greater promoting ability than did the hexabromobiphenyl.

4. Toxicity Due to Mixed-Function Oxygenase Induction

The mixed function oxygenase (MFO) system, which utilizes one of many cytochromes P-450 as the terminal oxidase, is located in the endoplasmic reticulum of many mammalian cells and is responsible for the metabolism of many xenobiotics and endogenous compounds (Dent, 1978). These are known as Phase I reactions. These enzymes can be induced by two major classes of inducing agents, represented by phenobarbital (which induces the cytochrome P-450 form) and 3-methylcholanthrene (which induces the cytochrome P-448, or P_1-450 form).

Most PHAH, including PBB, are potent inducers of MFO. For induction to occur, halogens must occupy at least 3 or, for maximum potency, 4 sites (Poland and Knutson, 1982). The relative toxicity of PCB, halogenated dibenzo-p-dioxins and dibenzofurans has been correlated with the capacity to induce, in particular, the cytochrome P-448-dependent aryl hydrocarbon hydroxylases (AHH) such as benzo(a)pyrene hydroxylase (Goldstein et al., 1979). Extrapolation from studies of PCB indicates that biphenyls with halogens in 3 or more positions meta and para to the carbon bridge, but with no halogens in ortho positions, are 3-methylcholanthrene (3-MC) type inducers and are thus more toxic.

Although 3-MC is considered to be the classical cytochrome P-448-type inducer, TCDD is 30,000 times more potent (Goldstein et al., 1979). It has been proposed that the toxicity and AHH induction produced by TCDD and other PHAH is mediated through a cytosolic receptor of molecular weight 136,000 to which these compounds are reversibly and competitively bound with high affinity (Poland and Knutson, 1982). Poland and Glover (1977) have postulated that the TCDD receptor is a planar rectangle which requires halogens in 3 of 4 corners for identification. Chlorinated biphenyls which are halogenated similarly but unsubstituted in a position ortho to the carbon bridge interact with the TCDD receptor but are 10^3 to 10^4 times less potent than TCDD. This is presumably because of the energy barrier to rotation which must be overcome for biphenyl rings to assume a coplanar configuration. This barrier increases with increasing ortho substitution.

The fireMaster mixture produces a mixed type of microsomal enzyme induction, with inducing properties of both phenobarbital and 3-MC (Dent et al., 1976a). It has been estimated that approximately 76% of fireMaster is comprised of Pb-type inducers, while 24% is comprised of 3-MC-type inducers (Render et al., 1982). Those congeners in the mixture with 2 or more bromines ortho to the carbon bridge (2,2',4,5,5'-pentabromobiphenyl; 2,2'4,4',5,5'-hexabromobiphenyl; 2,3',4,4',5,5'-hexabromobiphenyl; 2,2',3,4,4',5'-hexabromobiphenyl; 2,2',3,3',4,4',5-heptabromobiphenyl; and 2,2',3,4,4',5,5'-heptabromobiphenyl) induce microsomal enzymes usually induced by phenobarbital, cause hepatomegaly and proliferation of the endoplasmic reticulum of rat liver, but in general do not produce the toxic syndrome characteristic of TCDD (Moore et al., 1978b; Moore et al., 1979; Aust et al., 1981; Dannan et al.,

1982a). There are no pure 3-MC-type inducing congeners in the fireMaster mixture (Aust et al., 1981). However, congeners with 0 or 1 ortho bromines (3,3',4,4'-tetrabromobiphenyl; 2,3',4,4',5-pentabromobiphenyl; 2,3',4,4',5,5'-hexabromobiphenyl; 2,3,3',4,4',5-hexabromobiphenyl; and the minor contaminant 2,3,6,7-tetrabromonapththalene) are mixed-type inducers which may preferentially behave more like 3-MC-type and may also induce UDP-glucuronyl transferase in rat liver (Goldstein et al., 1979; Dannan et al., 1982a; Akoso et al., 1982a; Robertson et al., 1981, 1983, 1984). These congeners produce toxicity similar to that produced by TCDD including, in the liver, swollen hepatocytes, lipid vacuoles and numerous membrane arrays and vesicles, and, in the thymus, disappearance of the cortex followed by atrophy and subsequent immunotoxicity (Aust et al., 1981).

Toxicity studies have concluded that the primary toxicity of fireMaster is due to congeners which are 3-MC-type inducing agents (Aust et al., 1981; Akoso et al., 1982b; Dannan et al., 1982a). Dannan and coworkers (1982a) administered a single intraperitoneal dose of 90 mg/kg of fireMaster FF-1, fireMaster BP-6 or a reconstituted mixture formulated to resemble fireMaster BP-6 to rats (see Table 1). FireMaster BP-6 was found to be more toxic, a more effective 3-MC-type inducer, and to be comprised of 25% toxic congeners (2,3',4,4',5-pentabromobiphenyl; 2,2',3,4,4',5'-hexabromobiphenyl; 2,3',4,4',5,5'-hexabromobiphenyl; and 2,3,3',4,4',5-hexabromobiphenyl), while fireMaster FF-1 was comprised of 15% toxic congeners. Results from studies of the reconstituted mixture indicated that the toxicity of fireMaster is due to the effects of the congeners studied and that no other component in these mixtures (i.e., trace contaminants) contributes to these effects.

Enhanced microsomal enzyme activity can have significant impact on drugs and chemicals administered concurrently with fireMaster. FireMaster BP-6 has been shown to enhance the disappearance of drugs from plasma which are metabolized by MFO (Cagen et al., 1977). Thus, PBB can decrease the effectiveness of therapeutic agents. An additional detriment is that PBB may enhance the toxicity of agents which require metabolic activation. Mice fed fireMaster were more susceptible to the lethality and renal and hepatic damage induced by chloroform and carbon tetrachloride: agents which require metabolic activation (Kluwe et al., 1978). PBB may also enhance the metabolism of critical endogenous substances, with resulting adverse effects. Examples of this will be discussed in the following section on reproduction.

5. Effects of FireMaster on Reproduction and Development

The effect of fireMaster BP-6 administration on steroids in rats was evaluated in offspring from dams administered 10 or 100 mg/kg from day 8 of gestation through weaning of pups onto rat chow containing the same concentrations of fireMaster. Estradiol activity was assessed by measurement of the uterus to body weight ratio in treated rats; no PBB-related effect was observed. However, exogenous estradiol failed to increase the uterus to body weight ratio in fireMaster-exposed offspring to as great an extent as in controls (McCormack et al., 1979). In addition, the length of the estrous cycle was significantly increased (Johnston et al., 1980). The hepatic microsomal metabolism of estradiol, estrone, and ethinylestradiol was increased by at least 2 times (Bonhaus et al., 1981). This enhanced metabolism resulted in a decreased concentration of exogenous estrogen or its metabolites following

administration of [^{3}H]estradiol. In addition, fewer cytosolic estrogen receptors were observed.

Administration of 10 or 100 mg/kg fireMaster BP-6 to pregnant and lactating rats resulted in decreased duration of progesterone-induced anesthesia in their offspring (McCormack et al., 1979). A decrease in serum progesterone was correlated with increased menstrual cycle length in rhesus monkeys administered 0.3 mg/kg fireMaster FF-1 for 6 months (Lambrecht et al., 1978). The hepatic metabolism of progesterone to 16α- and 6β-hydroxyprogesterone was stimulated in male and female offspring exposed to 100 mg/kg fireMaster (Arneric et al., 1980). Administration of 3-MC had little effect on progesterone metabolism; therefore, phenobarbital-type inducing congeners would be more important in steroid metabolism. This is important in light of the widely-held view that fireMaster toxicity results from the 3-MC-type inducing congeners. It may be that steroid-dependent reproductive and behavioral toxicity (to be discussed subsequently) results more from the phenobarbital-type inducing congeners. Estrogen-positive feedback facilitated by progesterone is critical for the initiation of puberty in female rats (Arneric et al., 1980). Therefore, enhanced metabolism of either steroid could prevent this positive feedback and thus delay the onset of puberty. This has indeed been demonstrated: Harris et al. (1978) and McCormack et al. (1981) observed a delay in vaginal opening (a parameter indicative of the onset of puberty) in female offspring exposed to PBB perinatally.

No change was observed in the seminal vesicle to body weight ratio of male offspring from dams administered 10 or 100 mg/kg fireMaster BP-6. However, exogenously administered testosterone failed to

increase this ratio to as great an extent in fireMaster-exposed offspring as in controls (McCormack et al., 1979). The prostate to body weight ratio was decreased, indicating a deficit in anabolic steroids such as testosterone (Johnston et al., 1980). The in vitro metabolism of testosterone by 7α-hydroxylase, 6β-hydroxylase, 16α-hydroxylase and 17-oxidoreductase was stimulated in fireMaster-exposed offspring in an age- and sex-related manner (Newton et al., 1982). In contrast, a dramatic inhibition of Δ^4-3-oxosteroid 5α-reductase was observed in these animals. However, this enzyme catalyzes the 5α-reduction of testosterone to dihydrotestosterone and dihydroandrosterone: substances which may mediate specific androgenic functions. Therefore, the overall result is to decrease androgenic activity.

The enhanced metabolism of sex steroids may result in decreased fertility. This has indeed been demonstrated following fireMaster exposure. Cows contaminated during the first trimester had gestation periods which were prolonged by 2 to 4 weeks (Jackson and Halbert, 1974). Many calves were delivered dead or died shortly after birth. In addition, the udders of cows which had recently freshened shrank shortly after exposure, while the udders of cows which delivered calves did not develop. Decreased fertility was observed in cows administered 250 mg fireMaster for 60 or 202 days; a high rate of calf mortality was observed, primarily because of dystocea due to prolonged gestation and high birth weight (Willett et al., 1982). The relationship of these reproductive effects to PBB exposure in cows is unknown, as doses of fireMaster which resulted in overt toxicity did not affect serum concentrations of estrogen or progesterone, nor did they affect the periodicity of estrous cycles (Willett et al., 1983). Rhesus

monkeys which had demonstrated decreased serum progesterone and lengthened menstrual cycles due to fireMaster exposure exhibited excessive postconceptual bleeding and an increased number of abortions (Allen et al., 1976; Lambrecht et al., 1978). All live offspring had low birth weights. Although mink fed fireMaster for 313 days did not exhibit reproductive problems, the average litter size, kit weight at birth, and kit survival were decreased at a level as low as 1 ppm (Aulerich and Ringer, 1979). Thus far, no adverse effects on fertility have been observed in human females exposed to fireMaster.

Examination of testes from originally exposed cattle revealed decreased numbers or absence of spermatozoa (Cook et al., 1978). These animals, therefore, had subfertile or infertile testes. Male rats administered 100 mg/kg fireMaster FF-1 for 90 days exhibited degenerative and hyperplastic changes in the ductus deferens; 4 of 9 rats also developed prostatitis (Gupta and Moore, 1979). Although some men who were members of Michigan's PBB cohort complained of decreased sexual desire, analysis revealed no differences in the distribution of sperm counts, motility, or morphology (Rosenman et al., 1979). Therefore the paternal contribution to fireMaster-induced toxicity in the developing mammal is unclear.

It has been demonstrated that PBB cross the placenta in human and other animal species (Rickert et al., 1978; Fisher, 1980; Ecobichon, 1983; Eyster et al., 1983; Jacobson et al., 1984). Therefore, the possibility exists that doses of PBB which are not embryolethal may produce a toxic response in the developing animal. The nature of the response may differ somewhat from that of adults, as Dent (1978) has demonstrated that the pattern of enzyme induction is different in the

developing rat from that of adults. This may result in exposure to different congeners. In these animals enzymes stimulated by 3-MC are induced prior to those stimulated by phenobarbital, while the opposite pattern is observed in adults.

Fisher and coworkers (1980) administered 800 mg/kg fireMaster BP-6 by gavage to pregnant rats on day 10 of gestation 4 or 24 hours before dissection. A significant number of PBB-exposed fetuses failed to rotate to the ventroflexed position, retained an open anterior neuropore, and did not develop anterior limb buds, a visceral yolk sac circulation or the control complement of somites. These problems were still apparent 42 hours after culturing the embryos. A significant decrease in protein and DNA content was observed in the cultured embryos approximately 24 hours after removal from the dam, but not at 4 or 42 hours. The authors concluded that removal of the embryo from the affected environment would aid in recovery from injury and that development would proceed at a normal but asynchronous level. No evidence of chromosomal aberrations was observed in offspring from dams administered 100 mg/kg fireMaster at 2-day intervals from days 6 through 19 of gestation (Ficsor and Wertz, 1976).

Beaudoin (1977) administered a single dose of 40, 200, 400 or 800 mg/kg fireMaster BP-6 to rats on one day of pregnancy, from day 6 through 14. Resorptions followed treatment at all days, but were most prevalent in rats administered 800 mg/kg fireMaster on days 6 through 11. No skeletal malformations were observed; however, soft tissue anomalies included cleft palate and diaphragmatic hernia (800 mg/kg fireMaster administered on gestation days 11, 12, or 13 -- approximate

cumulative doses for a 300 g dam of 2.6, 2.9, or 3.1 g, respectively). These data indicate that a critical developmental period exists during which fireMaster may be either embryolethal or teratogenic in the rat. An approximate cumulative maternal dose of 80 mg (10 mg fireMaster BP-6 per day, days 7 through 15 of gestation) resulted in no maternal or developmental toxicity (Harris et al., 1978), while an approximate cumulative dose of 195 mg (1000 ppm in the diet, days 7 through 20 of gestation) resulted in fetal weight loss and late fetal mortality (Corbett et al., 1975). However, when the same approximate cumulative dose was administered in the diet every 2 days (100 mg/kg every 2 days, days 6 through 19 of gestation), no adverse effects were observed in dams or offspring (Ficsor and Wertz, 1978). The reason for this apparent cumulative effect is unclear, as relatively minor amounts of fireMaster would be eliminated over the course of 1 day. The induction of aryl hydrocarbon hydroxylase (2.9 times control) and epoxide hydratase (3.4 times control) has been observed at a cumulative dose as low as 10 mg (50 ppm fireMaster BP-6, days 8-21 of gestation; Dent et al., 1978).

Mice appear to be more susceptible to the teratology produced by PBB and are particularly vulnerable during certain periods of development. A dose of 200 ppm fireMaster in the diet caused an increase in number of pups born dead and in resorptions when administered on days 4 through 19 of gestation, while the same dose administered on days 8 through 19 of gestation only produced a decrease in fetal body weight (Preache et al., 1977). Exencephaly has been observed at a cumulative maternal dose of approximately 4 mg (100 ppm, days 7 through 18 of gestation), while cleft palate and hydronephrosis have been observed at

cumulative maternal doses of at least 8 mg of 2,2',4,4',5,5'-hexabromobiphenyl or fireMaster (Corbett et al., 1975; Welsch and Stedman, 1984). Cleft palate was found to coincide with cystic malformation of the midbrain and adjacent structures (Welsch and Stedman, 1984), indicating that the developing central nervous system may be susceptible to PBB-induced toxicity.

It should be noted that terata were only observed at maternally toxic doses in these studies, indicating that the hazard to embryonal development is not exclusive. This was not the case with the toxic polychlorinated biphenyl (PCB) congener 3,3',4,4',5,5'-hexachlorobiphenyl, which caused cleft palate and hydronephrosis in mice at doses which had no apparent adverse effects on dams (Marks et al., 1981). Because of the close structural similarity between PBB and PCB, it may be postulated that the degree of teratogenesis depends upon the predominant congeners. Further research is required to identify the most teratogenic brominated biphenyl congeners.

A great deal of evidence exists that PBB are extensively transferred to offspring via mothers' milk (Brilliant et al., 1978; Dent et al., 1978; Moore et al., 1978; Rickert et al., 1978; Ecobichon, 1983; Eyster et al., 1983; Jacobson et al., 1984). Vodicnik and Lech (1980) determined that the body burden of structurally related PCB was almost entirely eliminated by lactating rats by day 20 postpartum. Administration of fireMaster BP-6 in the diet of lactating rats on days 1 through 14 postpartum resulted in a higher PBB concentration in the livers of offspring than in livers of dams (Rickert et al., 1978). This provides direct evidence that PBB actually bioconcentrate in the neonate

when exposure occurs via the milk. Data from the same study indicated that PBB levels were less in offspring exposed *in utero* than in those exposed via the milk, indicating that transfer of PBB through milk is much more important to the appearance of PBB in the newborn than is placental transfer.

A mixed type of enzyme induction was observed in lactating dams and their offspring following fireMaster exposure, indicating that the inducing components were transferred in the milk (Moore *et al.*, 1978a). Postnatal exposure of offspring from dams administered 50 ppm fireMaster BP-6 in the diet (approximate cumulative dose of 11 mg) enhanced aryl hydrocarbon hydroxylase (AHH) activity 4.5 times and epoxide hydratase (EH) activity 2.8 times (Dent, 1978). This is compared with an enhancement of 2.9 times for AHH and 3.4 times for EH in offspring exposed during gestation only. These data indicate that cytochrome P-448 (represented by AHH) may increase in predominance with age in the rat, while cytochrome P-450 (represented by EH) does not change. This increase in enzyme activity was correlated with a greater increase in liver to body weight ratio than that observed in offspring exposed to fireMaster during gestation only (Cagen *et al.*, 1979). Enzyme induction in offspring but not dams was found to occur at a dose as low as 1 ppm in the diet (approximate cumulative dose of 270 μg) administered on days 0 through 18 of lactation (Moore *et al.*, 1978a). This effect persisted in the next generation, even though exposure of the original offspring ended on day 18 of lactation.

Because PBB cross the placenta and are transferred via mothers' milk, it would be expected that exposure during both gestation

and lactation would be more detrimental than exposure during either gestation or lactation. Dent and coworkers (1978) demonstrated that administration of 50 ppm fireMaster BP-6 during gestation and lactation increased AHH activity 5.8 times and EH activity 7 times. This increase was greater than that observed following administration of the same dose during gestation or lactation. McCormack and coworkers (1980, 1981) administered 10 or 100 ppm fireMaster BP-6 to gravid female rats from day 8 of gestation through day 28 of lactation (approximate cumulative doses of 6.2 and 62 mg, respectively). Offspring from these dams (100 ppm) exhibited delays in fur development, and opening of eyes, external auditory ducts, and vaginas, as well as hepatic histopathology, and induction of AHH and EH to at least 328 days of age. Offspring exposed initially (F_1-100 and F_1-10) were bred with littermates to produce an F_2 generation (F_2-100 and F_2-10), and these in turn were bred with littermates to produce an F_3 generation (F_3-100 only). An increase in liver to body weight ratio and hepatic histopathology were observed in the F_1-100, F_1-10, and F_2-100 offspring. In addition, induction of AHH and EH occurred in F_1-100, F_1-10, F_2-100 and F_2-10 offspring. Although no functional alterations were observed in offspring of the F_3 generation, these animals still had measurable tissue PBB levels.

One of the most interesting observations of these studies was that the concentration of congeners comprising fireMaster was altered from mother to offspring and from generation to generation (McCormack et al., 1981). The congener 2,2',3,4',5',6-hexabromobiphenyl was seen to disappear from liver, which is in keeping with the fact that this congener is metabolized (Dannan et al., 1978). In addition, the liver

concentration of 2,3',4,4',5,5'-hexabromobiphenyl (one of the most toxic congeners) decreased through the generations, as did the toxic response. Thus, although PBB may be transferred through generations, the toxicity may decline with time.

6. Behavioral Effects of PBB Exposure

PBB not only cross the placenta and are transferred via mothers' milk, but also cross the blood-brain barrier and concentrate in lipid-rich brain tissue (Domino et al., 1980). When these factors are considered together, the possibility arises that PBB may exert detrimental effects on the central nervous system during development, ultimately resulting in functional deficits. Epidemiologic data have hinted that this is true (Weil et al., 1978; Schwartz and Rae, 1983; Seagull, 1983). However, no confirmation is probably forthcoming, as these children are now over 10 years of age, and their participation in testing appears to have diminished with time. Therefore, functional testing (i.e., behavioral testing) has been conducted in research animals in an attempt to identify the nature of deficits produced by PBB.

Effects of PBB on behavior have not been as well characterized as other aspects of PBB toxicity. Initial behavioral studies were conducted with adult animals. Fischer 344 rats and B6C3F1 mice were administered 0.03 through 30 mg/kg fireMaster FF-1 (FF-1) or 0.168 through 16.8 mg/kg 2,2',4,4',5,5'-hexabromobiphenyl (HBB) by gavage for 22 doses. Administration of 30 mg/kg FF-1 and 16.8 mg/kg HBB to rats resulted in a deficit in reflexes, decreased grip strength (FF-1 only), and decreased open field activity (Tilson et al., 1978). Rectal

temperature was decreased in mice, but few behavioral effects were observed. Administration of either 3 or 10 mg/kg FF-1 to rats for 6 months also resulted in decreased open field activity in female, but not male rats, as well as decreased forelimb grip strength (both sexes), hindlimb extensor response, or startle response (Tilson and Cabe, 1979). These authors determined that HBB was less toxic with regard to neuromuscular and behavioral parameters than was FF-1. It is noteworthy, however, that a congener of fireMaster considered to be nontoxic exerted neurobehavioral effects.

Learning deficits were assessed by Geller and coworkers (1979) by training male rats administered 1, 3 or 6 mg/kg FF-1 for 20 days to an operant discrimination task using an auditory discriminative stimulus (DS). No adverse effects were observed on acquisition or performance of the task. However, animals administered 1, but not 3 or 6 mg/kg, FF-1 were slower to respond to the DS than were controls. Extra responses which occurred in the absence of the DS exhibited a biphasic dose-response; rats administered 1 mg/kg FF-1 showed an increase in extra responses (hyperactivity), those administered 3 mg/kg were comparable to controls, and those administered 6 mg/kg showed a decrease in extra responses (CNS depression). This study demonstrated that in addition to causing nonspecific neurobehavioral effects, PBB can produce deficits in central nervous system function.

Administration of HBB to mice during gestation resulted in cystic malformation of the midbrain and adjacent structures in offspring (Welsch and Stedman, 1984). Functional deficits have actually been produced in male offspring of female rats administered 0.5 or 5 mg/kg FF-1 by gavage for 20 days prior to breeding. Those offspring from dams

administered 5 mg/kg FF-1 displayed a significant decrease in pain threshold with time compared to the increased threshold of controls and offspring from dams administered 0.5 mg/kg (Gause et al., 1984). FF-1-exposed animals were more active than controls but demonstrated no deficits in acquisition or performance of a 2-lever discrimination task. However, pharmacologic challenge with d-amphetamine or phenobarbital resulted in disruption of this task to a lesser degree in FF-1 exposed rats than in controls.

A decrease in motor activity was observed in offspring of mice administered 100 ppm fireMaster during lactation but was not observed at the same dose administered during gestation (Preache et al., 1977). This decreased activity was correlated with an increase in mortality.

Pre-conception and perinatal administration of PHAH appears to produce a characteristic behavioral response in rhesus monkeys. Hyperactivity lasting 6 months or more has been observed in the absence of overt toxicity in offspring of female monkeys fed fireMaster FF-1, Aroclor 1248 (a PCB mixture) or TCDD (Bowman et al., 1981; Schantz and Bowman, 1983a,b). Offspring exposed to Aroclor 1248 at a dose which produced halogen acne also exhibited an increased number of learning errors in a discrimination task which persisted over a period of 16 months (Bowman et al., 1981). Due to the structural similarity of PBB to PCB and to the identical hyperactivity response, it might be expected that PBB would produce similar learning errors.

These data indicate that perinatal administration of PBB may cause behavioral deficits. However, the exact nature of these deficits has not been characterized. The fact that learning dysfunction in children may have resulted from perinatal PBB exposure underscores the

need for a full characterization of these deficits. This characterization requires testing of research animals using the skills of a relatively new science: behavioral teratology.

E. Behavioral Teratology

Behavioral teratology is a discipline which has developed rapidly during the first 20 years of its existence. It is directly related to behavioral toxicology: a field of endeavor which relies on the methodology of psychopharmacology. Norton (1982) has stated that two major differences separate the fields of behavioral pharmacology and behavioral toxicology: a) the behavioral effect of a drug is often predictable, while that of a toxic agent is generally not, and b) drug effects are often reversible, while the effects of toxic agents may be irreversible due to structural damage. Despite these differences, it is still possible to obtain highly useful information about the behavioral effects of a toxic agent utilizing tests developed to study drugs. Animals may be subjected to some of these tests at a very young age, thus facilitating study of the effects of a toxic agent on functional development.

Behavioral teratology (also known as psychoteratology or functional teratology) has been defined as the study of abnormal behavioral development which results from damage to the embryo or fetus (Vorhees, 1983a). Insult may occur not only gestationally, but also postnatally, pregestationally, and spermatogenically. Deviations in the functional capacity of organisms exposed to environmental chemicals may result from subtle morphological or biochemical alterations within the central nervous system or within systems which modulate central nervous system

function. What is particularly insidious about behavioral teratology is that it may occur at exposure levels not toxic to the dam and at levels which do not cause malformations in the offspring (Spyker, 1975).

The central nervous system exhibits extended development in most mammalian species. The nervous system begins as the neural plate on gestation day 19 in man, day 9.5 in the rat, and day 7 in the mouse (Hoar and Monie, 1981). Development of the nervous system proceeds initially with formation of central nervous system structures, followed by development of neurons. Neuronal development occurs in man primarily during the last 2.5 months of gestation, in rats from gestation days 13 through 20, and in mice from gestation days 10 through 20. Many neurons of the central nervous systems of rats and mice do not make their final synaptic connections until several weeks after birth. Myelination of nerves appears to be the slowest process. Full myelination does not generally occur in the human prior to adolescence and in the rat prior to 3 months of age.

The extended nature of central nervous system development renders it extremely susceptible to toxic insult. As with teratology, the type and pattern of behavioral deficit produced by a toxic chemical is a function of the developmental stage at which the agent is administered (Vorhees and Butcher, 1982). As an example, Vorhees and Butcher (1982) stated that administration of vitamin A early in organogenesis resulted in activity effects, while administration during late organogenesis resulted in learning deficits in homing behavior. Swimming deficits could be demonstrated when exposure occurred either early or late in organogenesis.

During late prenatal and early postnatal development neurons make their final synaptic connections. The time at which this occurs depends on the particular neurotransmitter involved. The functional consequences of this differential rate of neuronal maturation have been well characterized using various measures of spontaneous locomotor activity. Open field activity in the rat exhibits a characteristic pattern of development. Activity increases from day 0 postpartum to peak at approximately day 16. This peak is followed by a decline and subsequent increase in activity to plateau at adult levels on approximately day 24 (Campbell et al., 1969; Mabry and Campbell, 1974). Using neurotoxins designed to specifically eliminate neuronal populations utilizing the major neurotransmitters, it has been demonstrated that the original peak is due to the functional maturation of noradrenergic systems, while the decline is due to maturation of cholinergic, serotonergic, and possibly dopaminergic, systems (Campbell et al., 1969; Mabry and Campbell, 1974; Melberg et al., 1976; Lucot et al., 1981; Lucot and Seiden, 1982). Administration of toxic agents at a particular time during the development of this behavior will result in a shift in the pattern. Thus, not only is developmental stage of the organ system important during gestation, but also during the final stages of functional maturation which occur postnatally.

It is obvious from the preceding discussion that different types of behavior are acquired at different times during development. These may be differentially affected by toxic agents. In order to fully characterize the behavioral teratogenic potential of a toxic agent, deficits in these different behaviors must be assessed. There is no one behavioral test which can identify deficits in all aspects of behavior.

Therefore, the most common approach to testing has been an apical one. Test measures utilizing this approach are designed to measure effective integration of sensory, motor, motivational, learning, and/or memory processes (Adams and Buelke-Sam, 1981). They are intended to overlap in order to identify an agent as a behavioral teratogen and to suggest which neurological systems are targets for the agent.

Spyker (1975) has suggested that the discriminative power of behavioral assessment is a function of the range of behavioral endpoints examined. She has outlined the following categories to be included in a test battery: 1) morphological and physical characteristics, 2) maturational landmarks, 3) growth, 4) specific reflexes, responses, and sensory-motor capacities, 5) activity levels, 6) neuromuscular ability, 7) sensory functions, 8) learning ability, 9) emotionality, and 10) sexual parameters. These categories are relatively exhaustive, and obviously all cannot be thoroughly examined for each test chemical. Therefore, an appropriate test battery should be chosen based on knowledge of the pharmacology of the test chemical, as well as on results of preliminary testing. Muller et al. (1984) have stated that conducting many tests can lead to inflation of the type I error rate (probability of a false positive) for the entire study. Therefore, a rational approach to assessment of behavioral teratology would be to conduct exploratory studies to identify which behavioral test methods are most appropriate for the particular toxic agents. Once identified, a smaller number of appropriate tests could be employed in a confirmatory analysis, as suggested by Muller et al. (1984).

One important aspect of behavioral teratology involves the well-developed compensatory mechanisms of the central nervous system. Although a toxic agent may cause cellular or biochemical changes within the nervous system, functional deficits may not be manifested because of structural redundancy. Deficits may be unmasked by utilizing psychoactive agents to stress the system and thus remove functional reserves (Rech et al., 1968; Hughes and Sparber, 1978; Rech et al., 1983). However, some deficits may only be unmasked by the stress of aging. Spyker (1975) has therefore emphasized the necessity of long-term behavioral evaluation of animals exposed to suspected behavioral teratogens.

Table 2 provides a list of toxic agents reported to be experimental behavioral teratogens in at least one mammalian species. It has been suggested that doses of a toxic agent used for assessment of behavioral teratology should be below those required to produce gross malformations and/or maternal toxicity (Vorhees, 1983a). It is unknown how many of the agents listed follow these criteria. However, the list is extensive and will undoubtedly grow as more testing is conducted. Vorhees and Butcher (1982) have stated that almost any agent can be a behavioral teratogen if administered at large enough doses during vulnerable periods of development. In addition, all agents that have been found to be morphologic teratogens in the central nervous system are behavioral teratogens at non-malforming doses, and psychoactive agents are also in general psychoteratogenic. Vorhees (1983a) has suggested that any agent which exhibits CNS toxicity, teratogenicity, crosses the placenta, and/or alters oogenesis, spermatogenesis, or uterine competence should be tested for behavioral teratogenicity. This obviously includes a multitude of agents.

TABLE 2

Compounds Reported to be Experimental Behavioral Teratogens in at Least One Mammalian Species[a]

Class	Agent
Alcohol	Ethanol
Amino Acids	Phenylalanine
	p-Chlorophenylalanine
Anticonvulsants	Phenobarbital
	Diphenylhydantoin
Anesthetics	Halothane
	Phencyclidine
Antidepressants	MAO inhibitors
	Tricyclics
	Lithium
Antimitotics	Hydroxyurea
	5-Azocytidine
	Methylazoxymethanol
	X-irradiation
	Co^{60}
Anxiolytics	Benzodiazepines
	Meprobamate
Antipsychotics	Phenothiazines
	Reserpine
	Butyrophenones
Cannabinoids	Marijuana
Cholinergics	Physostigmine
	Diisopropylfluorophosphate
	Scopolamine
	Nicotine
Food Additives	Red No. 40
	Antioxidants
	Aspartame
	Brominated vegetable oil
	Calcium carragenan
	Red No. 3 (erythrosine)
	Monosodium glutamate
	Yellow No. 5 (tartrazine)
Hormones	ACTH
	Adrenalectomy
	Gonadotrophins
Metals	Lead
	Methylmercury

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TABLE 2 (continued)

Class	Agent	Class	Agent
Miscellaneous	Anabolic steroids	Salicylates	Acetyl, methyl, sodium
	Atmospheric pressure		
	Barbital	Stimulants	d-Amphetamine
	Carbon monoxide		Caffeine
	α-Methyl-p-tyrosine		Fenfluramine
	Polychlorinated biphenyls		
	Reticular stimulation	Stress	Restraint
	Actinomycin D		Cold
	Microwaves		Shock
			Handling
Narcotics	Heroin		Drug-induced
	Methadone		Various
	Morphine		
	Naloxone	Vitamins	Hypervitaminosis A
	Propoxyphene		Thiamin deficiency
Nutrition	Undernutrition	Others	Benzene[b]
	Essential fatty acid deficiency		Chlordecone[c]
			Chlorpromazine[d]
	Zinc deficiency		Cyclophosphamide[e]
	Excess iron		Methylparathion[f]
			Polybrominated biphenyls[g]
Pesticides	Carbofuran		Prochlorperazine[h]
	Diazinon		TCDD[i]

a) From: Vorhees and Butcher, 1982, with the following exceptions:

b) Tilson et al., 1980
c) Squibb and Tilson, 1982
d) Robertson et al., 1980
e) Preache and Gibson, 1976
f) Gupta et al., 1985
g) Schantz and Bowman, 1983b
h) Rech et al., 1983
i) Schantz and Bowman, 1983a

Japan and Great Britain have mandated that neurobehavioral evaluations be conducted on all new drugs during premarket reproductive testing; United States regulatory agencies are considering this possibility (Vorhees, 1983). This underscores the importance of research endeavors in behavioral teratology to identify appropriate testing batteries and to identify those agents potentially capable of producing subtle behavioral deficits upon neonatal or prenatal exposure.

F. Statement of Purpose

The purpose of this investigation was to determine if perinatal exposure of rats to fireMaster BP-6 produces behavioral deficits. Pursuit of this question in rats was deemed necessary to provide an index of potential neurobehavioral teratogenicity and toxicity in humans exposed to PBB perinatally.

This research was initiated based on several lines of evidence. Results of epidemiologic studies indicated that PBB exposure may result in neurobehavioral deficits. Of particular importance are studies in which children exposed to PBB either prenatally or perinatally were reported to exhibit deficits in cognitive and fine motor development which seemed to diminish with age. Interpretation of these epidemiologic studies is difficult due to small sample sizes, lack of control over the testing environment, and the probable existence of Type I error inherent in the experimental design. The value of further epidemiologic study of these 18 children is questionable because their participation has diminished with time. Therefore, this problem must be pursued using research animals.

PBB are lipophilic and persist in the environment and within mammals. They have been shown to cross the placenta and to bioaccumulate in mother's milk of humans and research animals, indicating direct access of PBB to the developing organism. PBB also cross the blood-brain barrier and persist within the brain, providing a prolonged period of time over which damage to the central nervous system can potentially occur.

Effects on the central nervous system have been identified as functional deficits in rats and mice exposed to PBB as adults. However, a thorough investigation has not been conducted on functional deficits which may occur from perinatal exposure. Therefore, the present study was conducted using the following objectives to clarify the response in offspring exposed to PBB perinatally.

Objectives

1. To determine which exposure regimen produces the most severe behavioral deficits in rat offspring: administration of BP-6 to dams during gestation alone, lactation alone, or gestation and lactation.
2. To establish a dose of BP-6 which produces behavioral deficits in perinatally exposed rats in the absence of gross malformations, overt toxicity, and/or maternal toxicity.
3. To determine if behavioral deficits observed at an early age in offspring from PBB-treated dams persist through adulthood.
4. To characterize the nature of behavioral deficits produced in rats exposed to BP-6 perinatally with regard to changes in locomotor activity, sensory processing, punished responding, short-term memory, and/or cognitive functions.

5. To identify the presence of sexual dimorphism in behavioral responses and the effect of BP-6 on those responses.

6. To determine through behavioral testing which neurotransmitter systems are affected by perinatal BP-6 exposure.

CHAPTER 1

COMPARATIVE STUDIES ON THE BEHAVIORAL TERATOLOGY OF PBB ADMINISTERED DURING GESTATION, GESTATION AND LACTATION, OR LACTATION

A. Introduction

Several lines of evidence exist which indicate that PBB may disrupt the developing nervous system; this evidence has been elaborated upon in the General Introduction. To characterize the neurobehavioral toxicity of perinatally administered PBB, studies were conducted in rats using the "leapfrog" experimental design suggested by Muller and coworkers (1984). This approach combines pilot studies (designed to evaluate methodology), exploratory studies (designed to evaluate data analysis; hypothesis generating) and confirmatory studies (designed to confirm an hypothesis) to extract the maximum amount of information from the animals tested.

Initial probe studies were hypothesis-generating in nature. They were conducted with the following goals in mind: 1) to identify the exposure regimen which produces behavioral changes of the greatest magnitude in offspring from dams administered PBB; 2) to establish dose levels for a confirmatory study based on the most detrimental exposure regimen; 3) to establish a sensitive battery of behavioral tests to be used in a confirmatory study; 4) to provide sufficient data for power analysis to determine an appropriate sample size for a confirmatory

study; and 5) to identify potential neurotransmitter effects using appropriate psychoactive agents.

Results of these studies are presented in this chapter.

B. Materials and Methods

1. Experimental Subjects and Dosing Procedure

Virgin female Sprague-Dawley rats, 6-8 weeks of age, were obtained from Harlan Sprague-Dawley, Inc., Indianapolis, IN. They were housed in temperature- and humidity-controlled animal care facilities with a 12-hour light-dark cycle (lights on 1300-0100 hours daily). Food (Wayne Lab Blox, Chicago, IL) and water were supplied ad libitum.

Following at least one week of acclimation to the laboratory environment, vaginal smears were obtained daily from each female. The presence of cornified cells or cornified and nucleated cells in the vaginal lavage indicated that the female was in estrus and therefore receptive. Each receptive female was placed overnight in the cage of two non-dosed male Sprague-Dawley breeders from the same source of supply. Day 0 of gestation was considered to be the day on which sperm were detected in the vaginal lavage.

Dams were administered PBB as fireMaster BP-6 (lot #6244-A, manufactured by the Michigan Chemical Company, St. Louis, MI). Although fireMaster FF-1 (fireMaster BP-6 plus 2% calcium silicate) was the formulation responsible for the Michigan environmental incident, a great deal of animal research has been conducted using the more common fireMaster BP-6 formulation. In addition, it has been demonstrated that BP-6 contains a greater amount of known toxic congeners than does FF-1 (Dannan et al., 1982d). Thus, toxic potential would be maximized with

the use of this formulation. The following studies were, therefore, conducted using fireMaster BP-6 (hereafter referred to as BP-6).

Two to four dams per dose group were randomly assigned to one of the following exposure regimens:

	EXPOSURE REGIMEN	BP-6 DOSES ADMINISTERED
1.	Days 6-18 of gestation	0, .6, 6, 12 or 60 mg/kg/day
2.	Day 6 of gestation - day 24 lactation	0, .12, .6, 2 or 6 mg/kg/day
3.	Days 0-24 of lactation	0, .6, 6 or 60 mg/kg/day

BP-6 was administered daily via the oral route in a peanut butter "treat". The effectiveness of administration of test chemicals in a peanut butter vehicle to pregnant rats has been demonstrated (Gupta et al., 1985). The administered dose is ingested completely each day, the containment problems of contaminated ground rat chow are eliminated, and the stress and potential behavioral modification of repeated daily gavage are avoided (Hutchings et al., 1979).

The appropriate amount of BP-6 was dissolved in a small amount of heated peanut oil. This solution was made to the desired concentration in peanut butter (the ratio of peanut oil:peanut butter was 1:9). Control females received a mixture of 1 part peanut oil (unheated) to 9 parts peanut butter.

Body weights and general appearance of all dams were monitored throughout gestation and lactation. In addition, gestation length, number of pups born alive, sex ratio of the pups, and mortality from birth to weaning were assessed. All pups remained with their biological dams following parturition. This would indicate that offspring from dams administered BP-6 during gestation only were actually exposed to

some level of BP-6 during both gestation and lactation, as PBB are readily transferred to pups via the milk in lactating rats (Dent et al., 1978; Moore et al., 1978; Rickert et al., 1978). Cross-fostering would have served no advantage in the gestation plus lactation or lactation alone exposure regimen. At 3 days of age all litters were culled to 4 pups per sex where possible. At this time indelible ink was injected subcutaneously into the paws of the pups in a specific pattern for the purpose of identification. Pups remained with their dams until weaning on postpartum day 24, and were housed 2 or 3 per cage thereafter according to BP-6 treatment and sex. All offspring were weighed on postpartum days 0, 3, 7, 14, 21, 28 and 60. Those offspring maintained until one year of age for behavioral testing were also weighed at 6, 9 and 12 months of age.

2. Assessment of Physical and Behavioral Developmental Endpoints

Beginning on day 1 postpartum, all surviving offspring were assessed for acquisition of a variety of physical and behavioral developmental endpoints to assess developmental delays. The test battery utilized was one demonstrated by Vorhees and coworkers (1979) to be sensitive in uncovering the wide variety of developmental delays elicited by such behavioral teratogens as monosodium glutamate and hydroxyurea. A description of these tests follows. For all tests each litter from each treatment regimen was represented by one value; this value was the day on which the final pup in the litter first exhibited acquisition of a behavior or reached a physical endpoint, unless otherwise indicated. Offspring were tested from 1300-1500 hours in a well-lighted room separated from that in which dams were housed.

a. Pinna detachment. Detachment of both pinna from the sides of the head was assessed from postpartum day 1.

b. Incisor eruption. Eruption of upper and lower incisors was monitored from postpartum day 1.

c. Forward locomotion. Pups were assessed for acquisition of various forms of forward locomotion, beginning on day 1 postpartum. Locomotion was monitored from the most primitive form, pivoting, to forward locomotion with the head low and body low, to forward locomotion with the body raised and head low, to the adult form of locomotion with the head raised and body raised.

d. Surface righting. Beginning on day 2 postpartum, each pup was placed on its back on a slate tabletop and timed until righting occurred and all four feet touched the tabletop. Acquisition of the righting reflex was considered to occur when all test pups in a litter righted in $\leq$2 seconds in at least 1 of 2 trials.

e. Cliff avoidance. All pups were placed on the edge of a slate tabletop, with forepaws and noses protruding over the edge. Observed daily from day 2 postpartum, acquisition was considered to be when all pups within a litter showed retraction and/or sideward movement beginning within 10 seconds and requiring no longer than 30 seconds to complete.

f. Negative geotaxis. Beginning on day 1 postpartum all pups were placed nose down on a wire-mesh screen inclined first at 15°, and then at 25°, for a period of 3 minutes at each angle. Acquisition was considered to be when all pups of a litter turned 180° to face nose up at both angles.

g. Swimming. Acquisition of various aspects of swimming behavior was assessed in pups from dams administered PBB during gestation alone or gestation and lactation beginning at day 6 postpartum. As with locomotion, several endpoints were assessed, and were as follows:

a) direction

0 = sank
1 = floated
2 = swam in a circle or arc
3 = swam in a straight line

b) angle

0 = submerged (nose and head)
1 = nose at surface
2 = nose and top of head at surface
3 = nose, top of head and ears at surface

c) use of limbs

0 = no paddling
1 = paddling with 4 limbs
2 = paddling with hind limbs only, forelimbs stationary

Swimming was conducted in a tank heated to approximate the temperature of the dam. Following swimming, pups were thoroughly dried and placed under a warming lamp. Because this test yielded little additional information and was considered to be highly stressful to the pups, it was decided to eliminate it from the neonatal test battery. Therefore, swimming evaluation was included in the behavioral analysis of pups from dams administered PBB during gestation, or during gestation and lactation, but not during lactation.

h. Auditory startle. At 8 days of age each pup was placed in a plexiglas mouse cage filled with bedding and located in a sound-attenuating, dimly-lighted chamber. Following a 1-minute acclimation period, a bicycle bell approximately 20 cm from the pup was rung, and

the flinch reaction of the pup was assessed visually. The bell was again rung 15 seconds after the first ring. Litter success was considered to be the day on which all pups flinched following both auditory stimuli.

i. Eye opening. Opening of both eyes was considered to indicate success.

j. Visual placing. Beginning on day 16 postpartum, pups were assessed for functioning of the visual system as the investigator held each by the tail and approached a small diameter metal bar mounted 21 cm above a table. Visual placing was considered to be when the pup reached for the bar prior to touching it with its vibrissae or forepaws.

3. Open Field Activity

Open field activity of offspring from dams administered BP-6 during gestation alone was assessed on days 18, 19 and 20 postpartum, as outlined in the method of Vorhees et al. (1979). However, review of the literature revealed that rat pups exhibit a characteristic pattern in the development of open field activity which occurs from days 0-24+ days postpartum (Campbell et al., 1969; Mabry and Campbell, 1974). Toxic chemicals have been shown to disrupt this developmental pattern (Campbell et al., 1969; Mabry and Campbell, 1974; Melberg et al., 1976; Lucot et al., 1981; Lucot and Seiden, 1982; Rech et al., 1983); therefore, in order to determine if perinatal BP-6 exposure resulted in disruption of this pattern, pups from dams administered BP-6 during gestation and lactation or lactation alone were assessed for open field activity every other day from postpartum days 12 through 24. In all cases the open field apparatus consisted of a 50 (L) x 50 (W) x 16 (H) cm plexiglas enclosure marked off into 10x10 cm squares. Each test session was 3

minutes in duration; the number of squares traversed during this period, as well as latency to move from the center square and number of rearings, were recorded. Testing was conducted between 1300 and 1500 hours in a well-lighted room separated from that in which dams were housed.

4. Drug-induced Stimulation of Accommodated Locomotor Activity

Beginning at approximately 6 months of age, all offspring from dams administered BP-6 during gestation and lactation or lactation alone were assessed for drug-induced stimulation of accommodated locomotor activity. Offspring exposed to BP-6 during gestation alone were not tested because few effects had been observed in this group with studies at earlier time points.

All drugs were dissolved in distilled water and administered intraperitoneally as a 1 mg/ml solution. Each rat was placed in a 30 (L) x 35 (W) x 16 (H) cm plastic cage containing a cylinder 15 cm in diameter, around which activity was linearly directed. The motor activity cage contained bedding of the same type as that found in the home cage (Bed O'Cobs, The Andersons, Maumee, OH). Activity was monitored by a Stoelting activity meter, model 31400, which records the number of times the animal disrupts the electromagnetic field generated over the surface of the apparatus. The entire motor activity apparatus was located in a sound-attenuating box to eliminate environmental disruptions. All experiments were controlled by electromechanical programming circuits. The number of activity counts per minute was recorded for each animal. It is well known that female rats tend to be more active than males (Norton, 1982) and that nocturnal activity exceeds diurnal activity. In order to equalize activity as much as

possible, males were tested from 0800 to 1300 hours (at the end of the nocturnal phase), while females were tested from 1200 to 1700 hours (during the diurnal phase).

Motor activity was measured for each animal at 6 months of age for 1 hour to allow the animal to acclimate to its surroundings. At the end of 1 hour the rat was quickly removed from the motor activity cage and injected intraperitoneally with distilled water on day 1 and 2 mg/kg d-amphetamine (Sigma Chemical Company, St. Louis, MO) on day 2. Activity was then monitored for an additional 3 hours. Because the response of female rats to d-amphetamine seemed somewhat variable, and because activity in female rats has been correlated with stage of the estrous cycle (Norton, 1982), vaginal cytology of all females was assessed immediately prior to placement in the motor activity cage. To provide an adequate number of data points, each female was retested with d-amphetamine at a different stage of the estrous cycle than that at which she was tested initially. At least 2 weeks intervened between the two determinations.

At 9 months of age all offspring were assessed for scopolamine-induced stimulation of locomotor activity. The initial 1-hour accommodation period was followed by injection of 0.5 (males) or 1 mg/kg (females) scopolamine hydrochloride (Sigma Chemical Company, St. Louis, MO) and subsequent observation for 3 hours. Stage of the estrous cycle was determined as described previously.

Motor activity of all offspring was monitored following injection of fenfluramine at 12 months of age. d,l-Fenfluramine hydrochloride (A.H. Robins, Richmond, VA) was injected following the initial 1-hour acclimation period; activity was subsequently monitored for 3

hours. Performance at various stages of the estrous cycle was not assessed because many of the females were anestrous by this age.

5. Data Analysis

The probability of an effect occurring by chance (α) was 0.05 for all analyses. While this does not provide a rigorous treatment of the data, these studies were exploratory in nature and conducted for the purpose of identification of all possible effects which would need confirmation in the final study. Data of individuals from all litters within each treatment group were pooled for statistical comparison. Treatment effects on individual body weights and physical and developmental behavioral endpoints were assessed for statistical significance by a one-way analysis of variance (ANOVA, Steel and Torrie, 1980). The effects of treatment, sex, and observation day on open field activity were assessed via multifactorial ANOVA, as were the effects of treatment and observation time on spontaneous locomotor activity, and the effects of treatment, estrous cycle stage, and observation time on the locomotor activity of female rats. Individual pairwise comparisons between control and treatment means were made by the least significant difference test (Miller, 1972). A complete analysis of the contribution of litter to overall effects was not assessed since this was an exploratory study. The effects of litter were addressed in the confirmatory final study.

C. Results

1. Administration During Gestation

a. Developmental parameters. Body weights of dams administered 12 or 60 mg/kg/day BP-6 from days 6 through 18 of gestation (Table 3) were less than those of controls from days 9 through 15 of

TABLE 3

Mean (± S.E.M.) Body Weights of Female Rats Administered PBB During Gestation

Day of Gestation	Body Weight (grams)				
	Control	0.6 mg/kg/day	6 mg/kg/day	12 mg/kg/day	60 mg/kg/day
0	250.3±12.7[a]	274.0± 4.2[b]	253.7± 7.2[a]	224.7± 0.3[a]	269.5± 4.5[b]
3	257.7± 7.3	280.7± 7.2*	264.0± 0.4	239.3± 4.8	285.0± 5.0*
6	265.7± 9.7	287.7± 6.5	270.3± 3.3	250.0± 3.8	282.0± 6.0
9	273.7± 6.9	295.3± 8.5*	273.0± 3.2	251.7± 1.5*	249.0± 3.0*
12	290.7±10.5	300.3± 5.0	280.3±12.1	257.7± 2.9*	254.0± 4.0*
15	302.7± 6.9	318.7± 1.5	291.0± 9.5	275.3± 3.0*	246.5± 5.5*
18	328.7± 5.8	351.0±10.2	341.0± 5.5	310.0± 7.7	266.5± 3.5
21	367.0±11.9	381.7±12.9	378.3± 8.3	326.0±11.7[c]	291.5±12.5*[c]

Day of Lactation	Body Weight (grams)				
	Control	0.6 mg/kg/day	6 mg/kg/day	12 mg/kg/day	60 mg/kg/day
0	273.3± 4.3	283.5± 4.5	290.3± 5.6	---	---
3	277.3± 5.9	287.5± 0.5	299.0± 6.4	---	---
6	285.3± 7.4	296.5± 7.5	309.3± 6.4	---	---
9	294.7± 9.7	317.5± 1.5	313.0± 6.5	---	---
12	305.3± 5.7	316.5± 2.0	323.0± 1.0	---	---
15	311.0±14.5	320.0± 5.0	318.0± 7.6	---	---
18	307.3± 7.5	320.5±10.5	318.3± 3.8	---	---
21	318.7± 6.9	325.0± 7.0	324.7±12.8	---	---
24	304.0±10.2	328.0± 6.0	316.0± 7.6	---	---

[a] n=3 [b] n=2 [c] No pups born alive.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

gestation (both doses) and also on day 21 of gestation (60 mg/kg/day dose group only). Body weights of these dams were not assessed during lactation because all pups were born dead. No prepartum or postpartum body weight deficits were observed in dams administered 0.6 or 6 mg/kg/day BP-6 during gestation. The general appearance of all PBB-treated dams was comparable to that of controls throughout the study.

Developmental parameters are presented in Table 4. Gestation length was increased for females administered 60 mg/kg/day BP-6 (not shown) when compared to controls (23.5±0.3 vs. 22.0±0 days). Gestation length for all other PBB-treated females was comparable to that of controls. All pups were born dead from both PBB treatment groups (12 and 60 mg/kg/day) determined by body weight data of the mother to have involved maternal toxicity. Those from dams administered 60 mg/kg/day BP-6 exhibited gross malformations such as hydrocephaly and underdeveloped limbs. These anomalies were not observed in pups exposed to 12 mg/kg/day BP-6. No differences from control were observed in litter size, sex ratio of the offspring, or pup mortality between birth and weaning for offspring from dams administered 0.6 or 6 mg/kg/day BP-6. Body weights of these offspring (Table 5) were comparable to those of controls from days 0 through 28 postpartum.

b. Physical and behavioral developmental endpoints. The postpartum day on which a particular behavior was manifested is indicated in Table 6. Only time to eye opening was altered (decreased) in pups from dams administered 6 mg/kg/day BP-6. Eye opening in Sprague-Dawley rats has been reported to occur at approximately 15 to 16 days of age (Vorhees et al., 1979; Comer and Norton, 1982; Vorhees, 1983b). In the present study eye opening for the controls occurred on day 18

TABLE 4

Mean (± S.E.M.) Developmental Parameters for Offspring from Dams Administered PBB During Gestation

Parameter	0 mg/kg/day	0.6 mg/kg/day	6 mg/kg/day
Length of gestation (days)	21.7±0.3[a]	22.0±0[b]	22.3±0.3[a]
Number of pups born			
Male	5.0±1.0	4.5±0.5	6.0±0
Female	6.0±0	5.5±1.5	5.7±0.9
Number of pups born dead	0±0	0±0	0±0
Number of pups dead by weaning	0±0	0±0	0±0

[a] n = 3 litters

[b] n = 2 litters

TABLE 5

Mean (± S.E.M.) Body Weights of Offspring from Dams Administered PBB During Gestation

Day Postpartum	Body weight (grams)		
	Control	0.6 mg/kg/day	6 mg/kg/day
0	5.8±0.2[a]	6.7±0.5[b]	6.1±0.2[a]
3	8.6±0.1	10.5±0.6	8.6±0.3
7	12.3±0.5	13.6±0.4	12.4±0.3
14	24.9±0.9	23.2±0.3	24.5±0.3
21	36.5±1.3	26.3±0.5	36.3±0.6
28	68.7±1.1	63.2±1.1	67.2±1.2

[a]Mean of individuals from 3 litters.

[b]Mean of individuals from 2 litters.

TABLE 6

Mean (± S.E.M.) Postpartum Day Acquisition for a Specific Behavior by Offspring from Dams Administered PBB During Gestation

Behavior	DOSE GROUP		
	Control	0.6 mg/kg/day	6 mg/kg/day
Pinna detachment	3.7±0.7 (3)[a]	3.0±0 (2)	2.7±0.3 (3)
Incisor eruption	9.7±0.9 (3)	8.7±0.9 (2)	9.5±0.5 (3)
Locomotion			
a) Pivoting	7.7±1.5 (3)	5.5±0.5 (2)	4.0±0.6 (3)
b) Forward locomotion, body low, head low	11.0±1.2 (3)	10.0±0 (2)	9.3±1.5 (3)
c) Forward locomotion, body raised, head low	15.7±0.7 (3)	11.5±0.5 (2)	14.0±1.2 (3)
d) Forward locomotion, body raised, head raised	18.2±0.3 (3)	20.0±1.0 (2)	17.7±1.5 (3)
Surface righting	12.7±0.7 (3)	11.0±1.0 (2)	9.3±1.3 (3)
Cliff avoidance	12.3±0.3 (3)	11.5±0.5 (2)	10.3±1.2 (3)
Negative geotaxis	10.7±0.9 (3)	10.0±0 (2)	10.0±1.0 (3)
Swimming			
a) Swam in straight line	19.3±0.7 (3)	19.0±1.0 (2)	19.3±0.7 (3)
b) Nose, top of head, ears at surface	18.0±2.0 (3)	15.0±1.0 (2)	15.7±4.3 (3)

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TABLE 6 (continued)

Behavior	DOSE GROUP		
	Control	0.6 mg/kg/day	6 mg/kg/day
Auditory startle	15.7±0.7 (3)	15.5±1.5 (2)	16.3±1.5 (3)
Eye opening	18.0±0 (3)	17.5±0 (2)	15.7±0.3 (3)*
Visual placing	22.0±1.5 (3)	19.5±0.5 (2)	19.7±0.3 (3)

[a]Number of litters tested.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

postpartum. Therefore, the significant decrease in mean time to eye opening observed in PBB-exposed pups resulted from comparison to an anomalous control mean. These problems would be rectified by testing a larger number of animals. Values for all other behavioral and developmental parameters were comparable among PBB-exposed and control offspring.

c. Open field activity. Open field behavior of the pups, assessed from days 18 to 20 postpartum, is presented in Table 7. Significant differences in squares traversed, latency to move from the center square, and rearings for PBB-exposed offspring may relate to a shift in the pattern of development of open field activity. However, the small number of animals assessed, coupled with the fact that testing began after the peak activity normally observed on day 15 to 16 postpartum (see section B.3), indicates that more testing would be required to fully characterize these effects.

2. Administration During Gestation and Lactation

a. Developmental parameters. Body weights of dams administered 0.12, 2 or 6 mg/kg/day BP-6 from day 6 of gestation through day 24 of lactation were initially greater than those of controls and remained so throughout gestation for dams administered 0.12 or 2 mg/kg/day (Table 8). Body weight deficits were observed transiently during lactation in dams administered 0.6 or 6 mg/kg/day BP-6. The transient nature of these deficits would indicate that these doses were not highly toxic to the dams. No differences from control were observed in appearance of the dams treated with BP-6.

TABLE 7

Mean (± S.E.M.) Open Field Values for Neonatal Rats Exposed to PBB *In Utero*

Day Postpartum	DOSE GROUP								
	Control			0.6 mg/kg/day			6 mg/kg/day		
	Squares Traversed	Latency (sec)	Rearings	Squares Traversed	Latency (sec)	Rearings	Squares Traversed	Latency (sec)	Rearings
18 Male	38.8±8.1	3.4±1.0	5.6±2.2	39.3±7.4	4.2±1.5	7.7±1.2	33.7± 5.8	3.5±0.9	8.4±1.0
Female	44.8±7.9	5.3±1.0	6.6±1.0	49.4±6.9	2.3±0.5	8.0±1.5	51.9±11.0	4.2±0.8	10.9±3.7
19 Male	18.6±4.2	4.2±1.2	3.2±0.8	37.2±7.5	1.9±0.8*	6.7±1.4	26.4± 6.2	3.0±0.8	9.6±2.4*
Female	28.3±5.7	4.9±1.3	3.3±0.7	31.3±4.8	1.6±0.6*	8.0±1.0*	33.2± 5.1	1.0±0.4*	4.5±1.1
20 Male	24.2±5.3	4.3±1.0	4.2±1.2	41.6±5.3	2.8±0.8	2.8±0.9	63.1± 9.6*	7.4±1.7	2.6±0.5
Female	23.5±5.5	3.3±0.8	2.3±0.6	41.2±9.3	3.4±0.9	5.3±1.6	66.2±11.4*	8.3±2.0*	3.1±0.9

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

TABLE 8

Mean (± S.E.M.) Body Weights of Female Rats Administered PBB During Gestation and Lactation

Day of Gestation	Body Weight (grams)				
	Control	0.12 mg/kg/day	0.6 mg/kg/day	2 mg/kg/day	6 mg/kg/day
0	230.8± 4.6[a]	257.5± 7.5*	222.8±4.0	261.3± 6.6*	254.5± 7.6*
3	244.0± 1.6	270.0± 8.9*	234.5±2.5	271.0± 8.0*	263.3± 4.3*
6	257.3± 4.8	276.0± 9.6	244.5±2.6	282.5± 5.6*	265.8± 7.2
9	268.8± 5.4	281.5±11.2	253.0±3.7	288.5± 5.6	269.0± 8.4
12	277.8± 7.8	301.0± 8.3*	264.5±3.1	303.0± 6.8*	280.8± 6.9
15	294.5± 5.4	321.8± 8.1*	278.3±3.0	317.3± 6.9*	300.5± 8.3
18	330.5± 8.2	364.0±13.9*	317.3±8.5	362.0± 6.9*	342.3± 9.6
21	373.5±10.6	401.0±17.6	356.8±6.7	399.8±11.5	382.5±11.4

Day of Lactation	Body Weight (grams)				
	Control	0.12 mg/kg/day	0.6 mg/kg/day	2 mg/kg/day	6 mg/kg/day
0	285.3± 7.3	296.8±12.6	273.3±5.7	312.8± 9.1*	273.8± 6.6
3	279.0± 4.3	308.3± 8.7*	277.5±1.0	311.3± 9.0*	270.3± 4.7
6	290.3± 6.8	308.5± 8.1	287.3±1.7	303.5± 9.4	286.8± 3.3
9	307.3± 4.9	322.0± 7.4	296.0±5.9	318.8± 7.9	288.0± 3.6*
12	320.3± 5.6	336.0± 6.7	297.8±3.4*	318.3± 8.7	301.8± 3.1*
15	320.3± 4.1	334.8± 9.9	300.8±5.9	321.0±11.4	304.0± 2.5
18	327.0± 7.8	336.0± 9.6	303.5±1.5	319.0±11.2	303.3± 5.5
21	325.8±11.5	324.8±13.9	301.5±3.6	323.8±11.7	308.5± 4.3
24	318.5± 3.6	322.5± 9.3	299.5±3.2	322.5± 9.3	307.0± 6.4

[a] n=4 for all determinations.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

Developmental parameters are presented in Table 9. Gestation length, litter size, initial pup mortality, and sex ratio of offspring exposed to PBB were comparable for the same indices as observed in controls. However, of 32 pups from dams administered 6 mg/kg/day BP-6, six were dead by weaning; pre-weaning mortality was negligible in all other treatment groups. These data indicate that a dose of 6 mg/kg/day BP-6 administered to dams perinatally is overtly toxic to offspring.

Body weights of offspring from dams administered 0.12 mg/kg/day BP-6 were significantly greater than those of controls from days 14 to 28 postpartum; this effect was no longer observed 60 days postpartum (Table 10a and b). This transient increase in body weight has not been observed previously in PBB-exposed rat offspring and may be related to the increase in maternal body weight observed in this group. However, body weights of offspring from dams administered 2 or 6 mg/kg/day BP-6 were actually less than those of controls, despite an initial increase in maternal body weight. Body weight deficits of offspring exposed to 0.6, 2 or 6 mg/kg/day BP-6 were still observed 60 days postpartum. Compared to controls, body weights of offspring exposed to 6 mg/kg/day BP-6 were decreased to the greatest extent and for the longest period of time.

b. Physical and behavioral developmental endpoints. The postpartum day on which a particular behavior was manifested is indicated in Table 11. Delayed development of forward locomotion and cliff avoidance were observed in offspring from dams administered 0.6 or 2, but not 0.12 or 6 mg/kg/day BP-6. This apparent biphasic effect has

TABLE 9

Mean (± S.E.M.) Developmental Parameters for Offspring from Dams Administered PBB During Gestation and Lactation

Parameter	Control	0.12 mg/kg/day	0.6 mg/kg/day	2 mg/kg/day	6 mg/kg/day
Length of gestation (days)	21.5±0.3[a]	22.0±0	21.8±0.3	21.5±0.3	21.3±0.3
Number of pups born					
Male	6.3±1.4	5.3±1.1	6.0±1.1	5.5±0.5	4.3±1.3
Female	6.3±0.5	6.8±1.9	5.0±1.1	6.8±0.6	6.0±0.7
Number of pups born dead	0±0	0.3±0.3	1.3±0.5	0.3±0.3	0.3±0.3
Number of pups dead by weaning	0±0	0.3±0.3	0.8±0.5	0.8±0.5	1.5±0.6

[a] n = 4 litters in all cases

TABLE 10a

Mean (± S.E.M.) Body weights of Offspring from Dams Administered PBB During Gestation and Lactation

Day Postpartum	Control		0.12 mg/kg/day		0.6 mg/kg/day	
	Male	Female	Male	Female	Male	Female
0	6.6±0.1[a]	6.4±0.1	6.6±0.1	6.5±0.1	6.1±0.1*	5.9±0.1*
3	8.7±0.2	8.5±0.1	9.0±0.4	9.0±0.7	7.7±0.2*	7.1±0.3*
7	14.6±0.7	14.9±0.4	16.0±1.0	16.1±0.7	14.4±0.9	11.9±0.8*
14	20.2±1.6	18.7±1.2	30.8±1.2*	29.3±0.8*	24.3±1.0*	23.9±1.2*
21	37.0±1.2	35.7±0.8	50.2±2.0*	48.5±1.7*	38.1±1.7	37.5±1.9
28	70.5±1.1	66.8±0.9	82.0±3.7*	76.8±2.5*	61.8±2.5*	58.0±2.7*
60	296.2±3.7	206.4±2.6	296.2±7.2	208.4±3.5	241.1±6.6*	175.6±6.0*

[a]Mean of individuals from 4 litters for all determinations.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

TABLE 10b

Mean (± S.E.M.) Body weights of Offspring from Dams Administered PBB During Gestation and Lactation

Day Postpartum	2 mg/kg/day		6 mg/kg/day	
	Male	Female	Male	Female
0	6.6±0.1	6.5±0.1	6.1± 0.2*	5.8±0.1*
3	8.2±0.4	8.1±0.3	6.4± 0.2*	6.7±0.3*
7	12.2±0.7*	12.7±0.7*	8.8± 0.3*	9.6±0.3*
14	21.4±1.3	21.9±1.1*	16.1± 1.0*	16.7±0.9
21	35.3±2.2	36.7±1.9	25.6± 0.8*	25.8±1.0*
28	57.5±3.1*	58.0±2.7*	35.8± 2.9*	34.5±3.2*
60	246.1±6.9*	185.2±2.8*	171.7±15.7*	143.0±7.5*

[a]Mean of individuals from 4 litters for all determinations.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

TABLE 11

Mean (± S.E.M.) Postpartum Day Acquisition for a Specific Behavior by Offspring from Dams Administered PBB During Gestation and Lactation

Behavior	DOSE GROUP				
	Control	0.12 mg/kg/day	0.6 mg/kg/day	2 mg/kg/day	6 mg/kg/day
Pinna detachment	4.0±0.5	2.3±0.3	4.0±0.8	2.3±0.6	2.6±0.7
Incisor eruption	8.0±0.4	8.5±0.7	9.0±1.1	7.3±1.3	8.0±0.4
Locomotion					
a) Pivoting	4.0±0.4	3.5±0.5	5.5±1.6	2.8±0.6	4.0±0.7
b) Forward locomotion, body low, head low	8.5±0.9	8.5±1.3	13.5±1.0*	10.0±0.4	9.0±1.2
c) Forward locomotion, body raised, head low	15.0±1.7	13.5±0.9	14.5±1.2	15.5±1.2	14.5±1.2
d) Forward locomotion, body raised, head raised	17.5±0.7	18.8±0.3	20.0±1.1	22.5±0.9*	19.3±0.6
Surface righting	12.8±1.8	11.0±1.1	12.8±1.2	11.5±1.2	15.3±1.1
Cliff avoidance	9.8±0.8	10.0±1.6	14.8±1.0*	14.3±1.0*	11.8±0.6
Negative geotaxis	8.0±0.8	8.5±1.0	10.0±2.4	9.3±0.5	9.5±0.9

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TABLE 11 (continued)

Behavior	DOSE GROUP				
	Control	0.12 mg/kg/day	0.6 mg/kg/day	2 mg/kg/day	6 mg/kg/day
Swimming					
a) Swam in straight line	17.5±0.5	a	19.5±1.7	a	19.5±0.5
b) Nose, top of head, ears at surface	17.0±0.6	a	19.5±1.7	a	19.5±1.5
c) Paddling with hind limbs, forelimbs stationary	25.0±1.0	a	24.0±0.8	a	19.8±1.1
Auditory startle	16.0±1.1	15.3±0.9	16.0±1.1	14.3±2.5	18.5±0.3
Eye opening	16.5±0.3	15.8±0.5	16.5±0.7	16.3±0.3	14.8±2.6
Visual placing	21.5±1.0	17.8±0.6*	21.3±1.4	18.3±1.0	22.0±1.3

Mean of individuals from 4 litters for all determinations.

*Significantly different from control mean by ANOVA and least significant different test, $p<0.05$.

[a]Data not obtained.

been clarified using a greater number of animals in the final, confirmatory portion of the study.

c. Open field activity. Results of open field testing are presented in Figures 3 and 4. These data were subjected to a multifactorial ANOVA. ANOVA of data for squares traversed revealed significant effects for treatment [$F(3,559) = 3.22$, $p<0.025$], observation day [$F(6,559) = 26.59$, $p<0.001$], treatment and observation day interaction [$F(18,559) = 3.57$, $p<0.001$], and dose and sex interaction [$F(3,559) = 3.81$, $p=0.01$]. The normal pattern of development of open field activity was shifted for offspring exposed to 0.12, 0.6 or 6 mg/kg/day BP-6. In addition, offspring from dams administered 6 mg/kg/day BP-6 traversed significantly fewer squares than did controls on days 22 (females) or 24 (males and females) postpartum. ANOVA for latency to move from the center square revealed significant effects for treatment [$F(3,563) = 6.03$, $p<0.001$] and observation day [$F(6,563) = 16.35$, $p<0.001$]. Treatment-related effects on latency were transient in nature. Analysis of number of rearings revealed a significant effect for treatment [$F(3,563) = 38.78$, $p<0.001$], observation day [$F(6,563) = 23.81$, $p<0.001$], sex [$F(1,563) = 6.10$, $p<0.025$], and treatment and observation day interaction [$F(18,563) = 8.80$, $p<0.001$]. A deficit in number of rearings was observed in offspring of all BP-6 treatment groups, with females affected to a greater extent than males. In addition, the pattern of development of rearing behavior was shifted for offspring from BP-6-treated dams.

3. Administration During Lactation

a. Developmental parameters. Body weights of dams administered BP-6 from days 0 through 24 of lactation are presented in

Figure 3. Open field activity of male offspring from dams administered PBB during gestation and lactation. Dams received 0 (○), 0.12 (▽), 0.6 (△) or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of individual values of all male offspring from four litters per dose. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

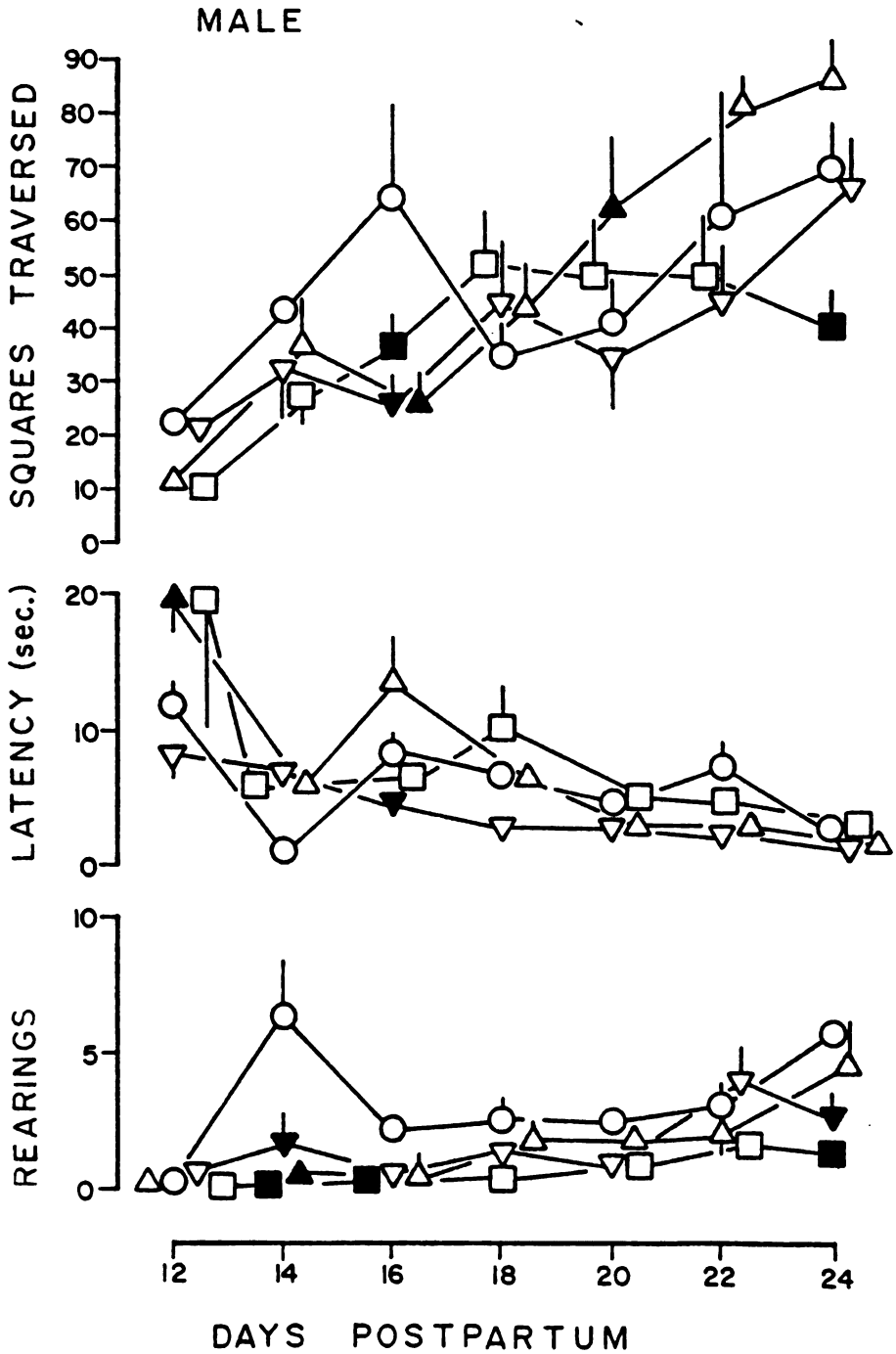

MALE
SQUARES TRAVERSED
90
80
70
60
50
40
30
20
10
0
LATENCY (sec.)
20
10
0
REARINGS
10
5
0
12
14
16
18
20
22
24
DAYS POSTPARTUM

Figure 3

Figure 4. Open field activity of female offspring from dams administered PBB during gestation and lactation. Dams received 0 (○), 0.12 (▽), 0.6 (△) or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of individual values of all female offspring from four litters per dose. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

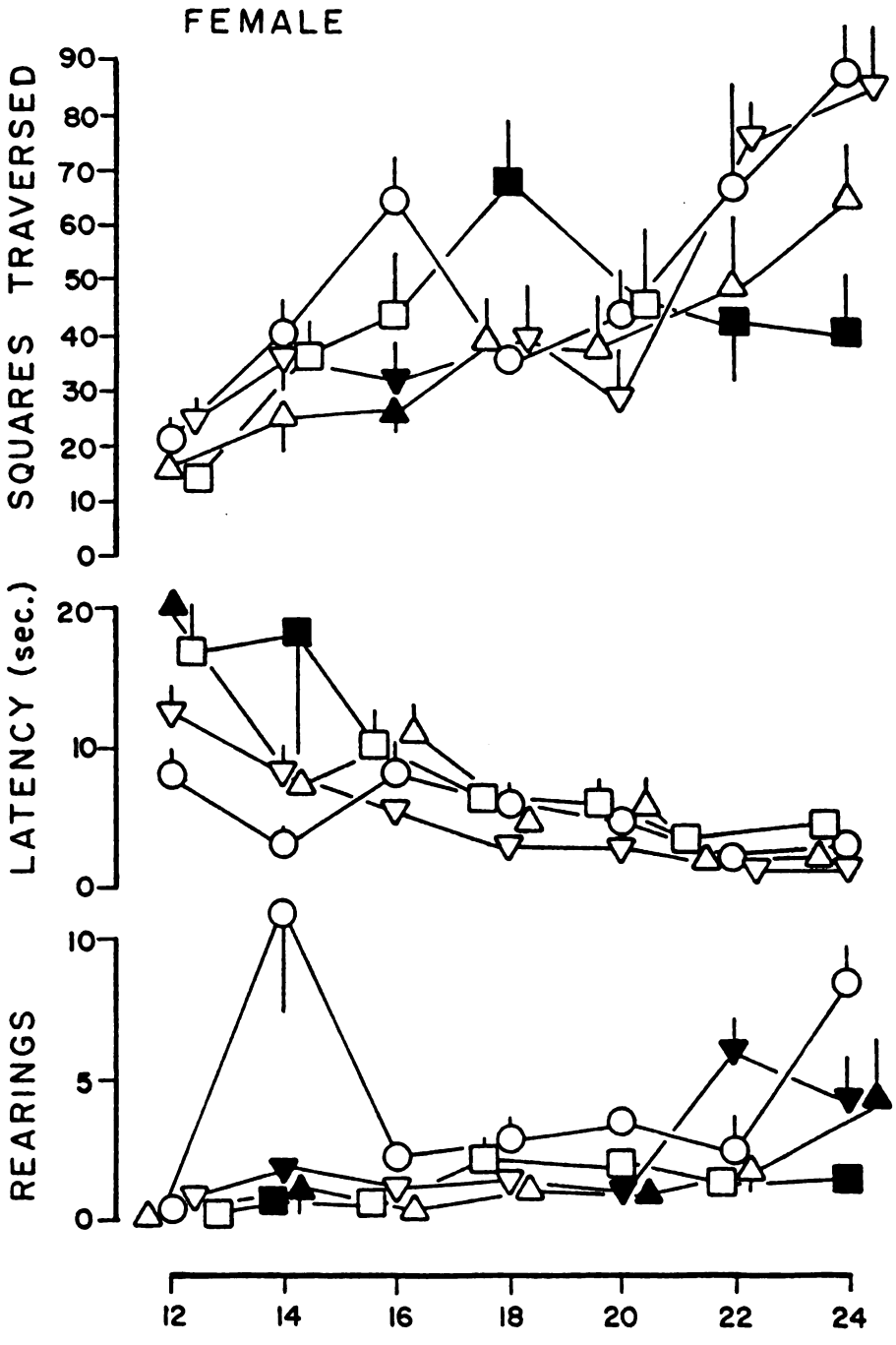

FEMALE
SQUARES TRAVERSED
90
80
70
60
50
40
30
20
10
0
LATENCY (sec.)
20
10
0
REARINGS
10
5
0
12
14
16
18
20
22
24
DAYS POSTPARTUM

Figure 4

Table 12. Body weight changes observed during gestation were transient in nature and not related to PBB treatment. Only those dams administered 60 mg/kg/day BP-6 during lactation exhibited a body weight deficit when compared to controls. Body weights of these dams were not obtained after day 12 postpartum due to excessive mortality of the offspring. Thus, surviving pups exposed to PBB during lactation were exposed to doses which did not produce maternal toxicity, as assessed by body weight. All PBB-treated dams were comparable in appearance to controls.

No PBB-related differences were observed in gestation length, litter size, sex ratio of the offspring, or pup mortality between birth and weaning for offspring from dams administered 0.6 or 6 mg/kg/day BP-6 (Table 13). However, all offspring from dams administered 60 mg/kg/day BP-6 were dead by day 15 postpartum. Body weight deficits were observed in offspring exposed to 6 mg/kg/day BP-6 (Table 14). Males were affected to a greater degree than were females, and for a greater length of time (days 7 to 60 versus days 21 to 28 postpartum).

b. Physical and behavioral developmental endpoints. The postpartum day on which a particular behavior was manifested is indicated in Table 15. Precocious development of forward locomotion with the body raised and head low was observed in offspring from dams administered 0.6 or 6 mg/kg/day BP-6. In addition, offspring exposed to 0.6 mg/kg/day BP-6 developed the auditory startle reflex at an earlier age than did controls. Based on data previously discussed for offspring exposed to PBB during gestation or gestation and lactation, and on data obtained by Vorhees and coworkers (1979), acquisition of the developmental parameters was prolonged for controls. Testing with a

TABLE 12

Mean (± S.E.M.) Body Weights of Female Rats Administered PBB During Lactation

Day of Gestation	Body Weight (grams)			
	Control	0.6 mg/kg/day	6 mg/kg/day	60 mg/kg/day
0	233.3±5.4[a]	253.5± 7.8	256.8± 7.1	234.3±12.4
3	244.3±3.9	269.0± 7.3	266.8± 5.5	247.8±10.0
6	256.5±5.5	277.0± 6.3	276.0± 5.9	257.5± 9.2
9	268.3±4.8	288.0± 7.1	284.8± 7.3	267.0±13.4
12	275.8±6.7	298.8± 7.0	303.3± 8.2*	275.5± 8.4
15	299.3±7.2	317.3± 7.2	313.8± 8.9	291.8±11.0
18	341.5±9.0	351.5± 7.8	357.0± 7.1	324.8±13.1
21	390.3±4.3	394.3± 6.2	399.5± 9.4	360.3±14.1*

Day of Lactation	Body Weight (grams)			
	Control	0.6 mg/kg/day	6 mg/kg/day	60 mg/kg/day
0	281.3±4.0	293.3± 7.1	293.5± 5.9	282.0± 8.7
3	289.0±3.7	302.0± 9.4	302.8± 4.8	254.3± 6.7*
6	297.3±5.6	300.8±12.7	308.3± 9.8	260.0±10.7*
9	301.5±3.2	315.5± 6.2	317.8± 9.8	264.0±12.6*
12	309.8±2.8	322.0± 4.0	318.3±12.5	277.0±14.9*[b]
15	307.3±3.4	326.5± 6.4	316.5±11.8	---
18	309.8±5.1	337.0± 8.4	323.8± 7.6	---
21	307.5±6.4	322.5± 4.4	320.0± 9.7	---
24	304.5±8.2	327.5± 6.2	324.0±13.0	---

[a] n=4 for all determinations.

[b] No further data due to excessive mortality of offspring.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

TABLE 13

Mean (± S.E.M.) Developmental Parameters for Offspring from Dams Administered PBB During Lactation

Parameter	Control	0.6 mg/kg/day	6 mg/kg/day
Length of gestation (days)	21.3±0.3[a]	21.8±0.3	22.0±0
Number of pups born			
Male	6.5±1.0	6.0±0.4	5.3±0.8
Female	5.8±0.9	5.3±0.5	6.8±1.0
Number of pups born dead	1.3±0.6	0.3±0.3	1.0±0.4
Number of pups dead by weaning	0.8±0.8	0±0	0±0

[a] n = 4 litters in all cases

TABLE 14

Mean (± S.E.M.) Body weights of Offspring from Dams Administered PBB During Lactation

Day Postpartum	Control		0.6 mg/kg/day		6 mg/kg/day	
	Male	Female	Male	Female	Male	Female
0	6.3±0.1[a]	6.0±0.1	6.8±0.1	6.4±0.2	6.7±0.1	6.2±0.1
3	8.8±0.2	8.2±0.2	9.3±0.3	8.4±0.3	8.1±0.2	7.7±0.1
7	15.4±0.4	14.0±0.5	15.0±0.5	13.6±0.5	11.9±0.5*	11.7±0.4
14	27.1±0.9	25.1±1.0	28.3±0.5	26.5±0.6	21.9±0.6*	21.9±0.4
21	42.1±1.3	38.4±1.1	47.9±1.1*	44.9±1.0	31.6±1.0*	32.2±0.9*
28	62.1±2.7	60.4±2.0	76.7±2.4*	69.2±1.6	41.9±2.2*	45.3±1.4*
60	269.9±2.6	187.1±4.0	293.2±5.0	194.2±3.0	205.1±7.7*	157.0±4.3

[a]Mean of individuals from 4 litters for all determinations.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

TABLE 15

Mean (± S.E.M.) Postpartum Day Acquisition for a Specific Behavior by Neonatal Rats from Dams Administered PBB During Lactation

Behavior	DOSE GROUP		
	Control	0.6 mg/kg/day	6 mg/kg/day
Pinna detachment	2.8±0.6	2.5±0.3	2.5±0.3
Incisor eruption	9.5±0.9	8.8±0.6	7.8±0.8
Locomotion			
a) Pivoting	6.0±1.1	3.3±1.0	4.5±0.3
b) Forward locomotion, body low, head low	10.8±0.5	8.5±0.9	8.8±1.0
c) Forward locomotion, body raised, head low	17.0±0.9	12.3±0.3*	12.5±0.7*
d) Forward locomotion, body raised, head raised	19.3±0.9	20.3±1.7	17.8±0.3
Surface righting	12.0±0.9	10.3±0.5	12.3±0.6
Cliff avoidance	13.0±1.2	11.8±0.5	12.5±1.7
Negative geotaxis	9.8±0.9	8.8±1.0	9.3±0.8
Auditory startle	17.3±0.9	14.0±0.7*	17.0±0
Eye opening	16.5±0.7	16.3±0.3	17.0±0
Visual placing	19.3±0.8	18.3±0.6	19.0±0.4

Mean of individuals from 4 litters for all determinations.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.05$.

greater number of animals would be required to clarify these anomalous results.

c. Open field activity. Results of open field testing are presented in Figures 5 and 6. These data were analyzed by ANOVA at each time point; interactions of sex and testing day were not assessed. Although the PBB-related effects were not as great as those observed in offspring from dams administered BP-6 during gestation and lactation, male and female pups exposed to 0.6 or 6 mg/kg/day BP-6 during lactation failed to achieve peak activity on the same day as controls (day 16 postpartum). In addition, female pups exposed to either BP-6 dose exhibited significantly fewer rearings than did controls during the latter half of the 24-day observation period. PBB treatment of dams during lactation thus appeared to shift the pattern of development of open field activity in offspring.

4. Accommodated Locomotor Activity

a. Administration of BP-6 during gestation and lactation

i. Distilled water. Spontaneous locomotor activity following distilled water injection of male and female offspring from dams administered BP-6 during gestation and lactation is presented in Figure 7. Multifactorial ANOVA revealed significant effects for PBB treatment [$F(3,800) = 68.49$, $p<0.001$], observation time [$F(15,800) = 114.75$, $p<0.001$], and the treatment and observation time interaction in males [$F(45,800) = 4.84$, $p<0.001$]. Offspring from dams administered 0.12 mg/kg/day BP-6 were more active than controls throughout the accommodation period and most of the observation time following distilled water injection. Activity of male offspring from dams administered 0.6 or 6 mg/kg/day BP-6 was essentially comparable to that of controls. The

Figure 5. Open field activity of male offspring from dams administered PBB during lactation. Dams received 0 (○), 0.6 (△), or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of individual values of all male offspring from four litters per dose. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

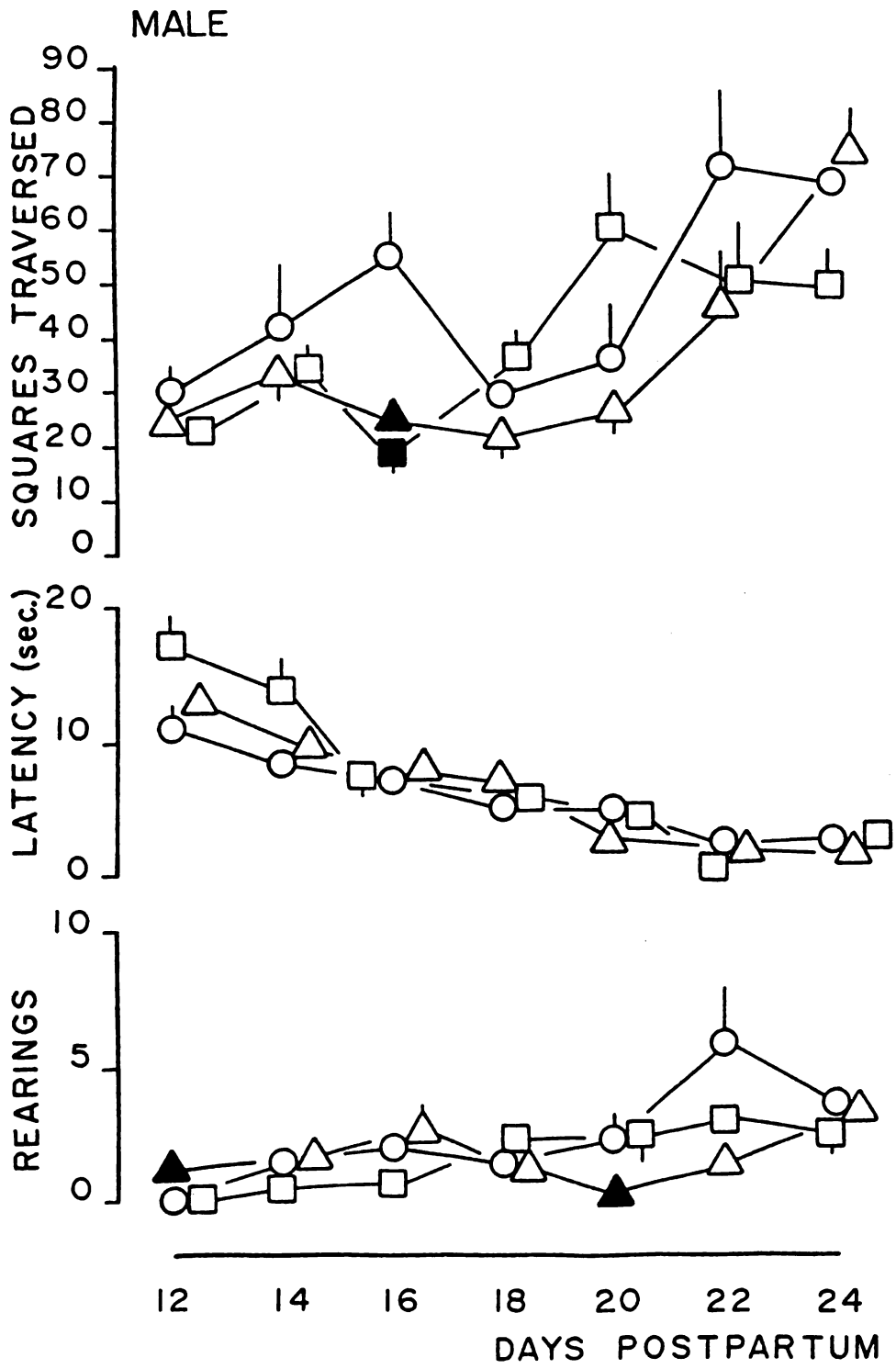

MALE
SQUARES TRAVERSED
90
80
70
60
50
40
30
20
10
0
LATENCY (sec.)
20
10
0
REARINGS
10
5
0
12
14
16
18
20
22
24
DAYS POSTPARTUM

Figure 5

Figure 6. Open field activity of female offspring from dams administered PBB during lactation. Dams received 0 (○), 0.6 (△), or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of individual values of all female offspring from four litters per dose. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

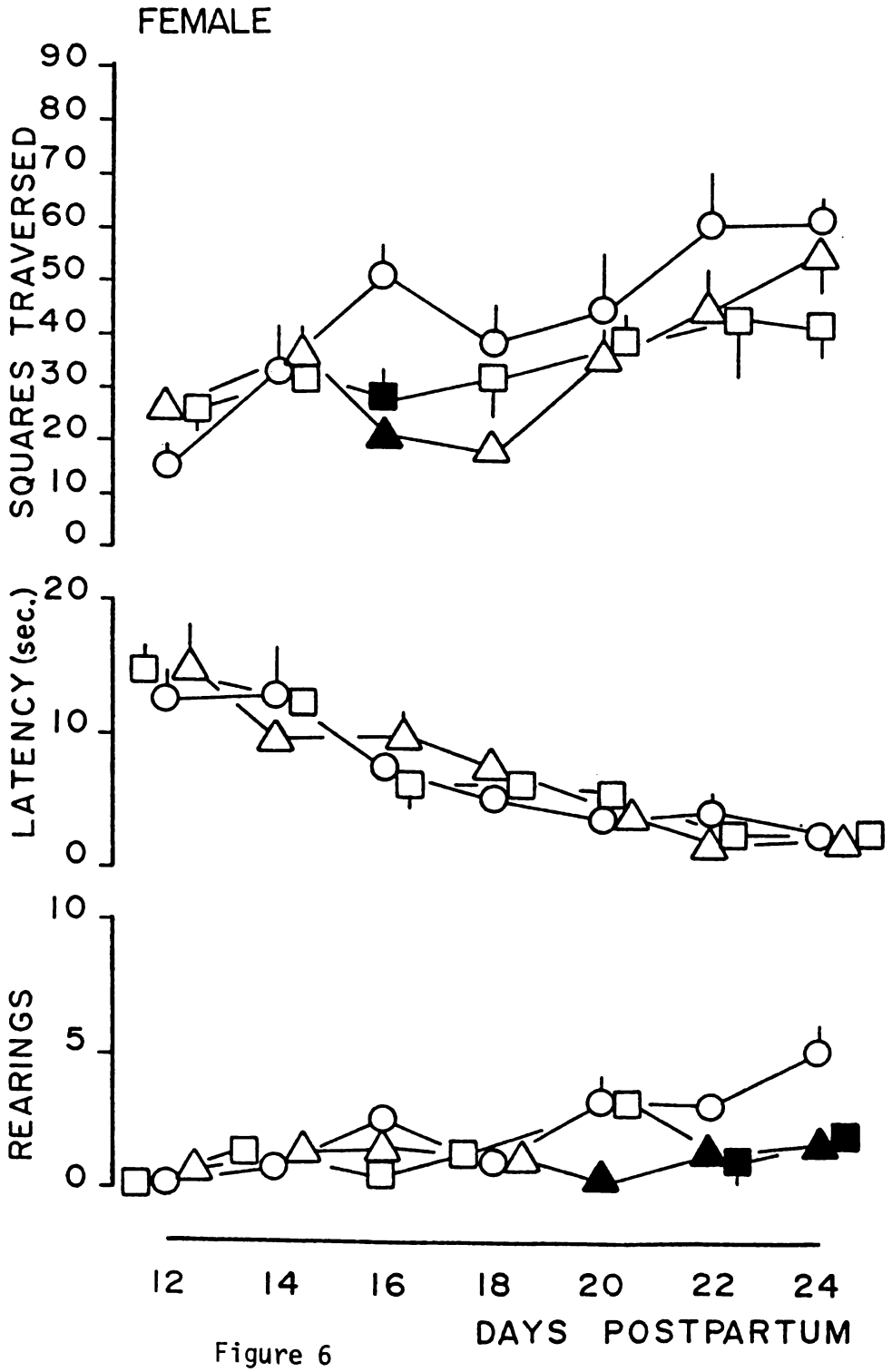

FEMALE
SQUARES TRAVERSED
0
10
20
30
40
50
60
70
80
90
LATENCY (sec.)
0
10
20
REARINGS
0
5
10
12
14
16
18
20
22
24
DAYS POSTPARTUM

Figure 6

Figure 7. Accommodated locomotor activity following distilled water injection (1 ml/kg) in male and female offspring from dams administered PBB during gestation and lactation. Dams received 0 (○), 0.12 (⬡), 0.6 (△) or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from four litters per dose. The point of injection of distilled water is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

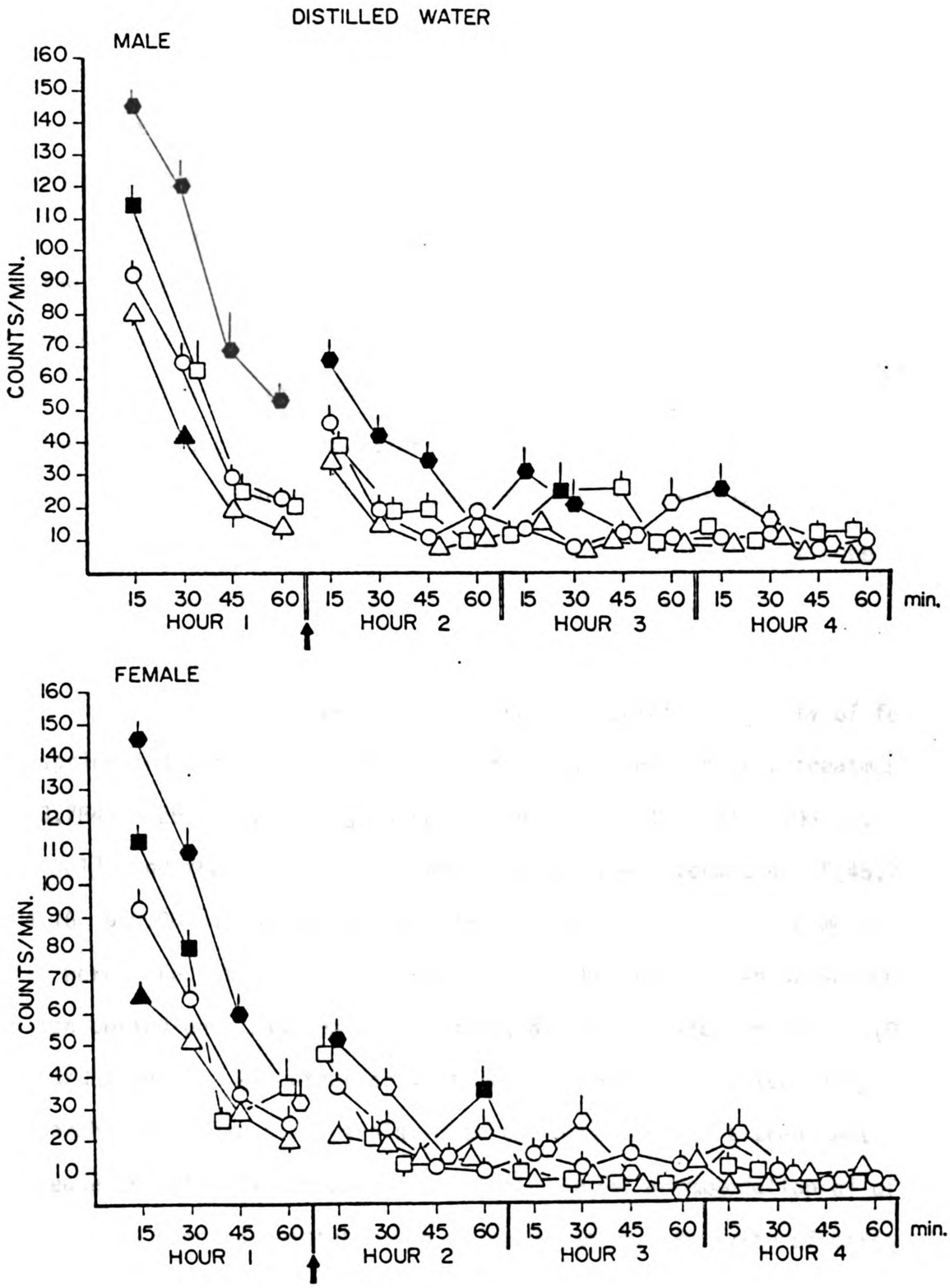

DISTILLED WATER
MALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
FEMALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 7

activity of rats is often stimulated following injection of a test compound, and remains stimulated until the animal once again habituates to the test chamber. Analysis of the significant treatment versus observation time interaction indicated that the offset of this stimulation (defined as lack of significance from counts per minute obtained during the last 15 minutes of the accommodation period) was shifted due to BP-6 exposure. Offset occurred at 30 minutes following distilled water injection for offspring from dams administered 0, 0.6 or 6 mg/kg/day BP-6. Offset of stimulation did not occur during the entire 3-hour observation period for male offspring from dams administered 0.12 mg/kg/day BP-6, indicating that in general these animals were more active than controls and stimulated to a much greater degree by the environment.

Analysis of spontaneous locomotor activity of female offspring indicated significant effects with regard to BP-6 treatment [$F(3,784) = 26.16$, $p<0.001$], observation time [$F(15,784) = 103.54$, $p<0.001$], and the treatment and observation time interaction [$F(45,784) = 4.16$, $p<0.001$]. Offspring from dams administered 0.12 or 6 mg/kg/day BP-6 were more active than controls during the initial 1-hour accommodation period and transiently following distilled water injection (0.12 mg/kg/day only). All other activity measurements were essentially comparable to those of controls, with the exception of a transient decrease in activity compared to controls during accommodation of offspring from dams administered 0.6 mg/kg/day BP-6. Activity of offspring exposed to 0, 0.6 or 6 mg/kg/day BP-6 was in general not stimulated following distilled water injection, while that of offspring exposed to 0.12 mg/kg/day BP-6 was stimulated for 30 minutes only.

ii. d-Amphetamine. d-Amphetamine-induced stimulation of spontaneous locomotor activity in male and female offspring of dams administered BP-6 during gestation and lactation is presented in Figure 8. ANOVA revealed significant effects for BP-6 treatment [$F(3,740) = 55.32$, $p<0.001$], observation time [$F(15,740 = 89.66$, $p<0.001$], and the treatment and observation time interaction in males [$F(45,740) = 2.37$, $p<0.001$]. Offspring from dams administered 0.12 mg/kg/day BP-6 were transiently more active than controls during accommodation and following d-amphetamine injection. Offspring from dams administered 0.6 mg/kg/day BP-6 were transiently less active than controls, while the activity of those from dams administered 6 mg/kg/day BP-6 was essentially comparable to that of controls. Analysis of the significant treatment versus observation time interaction indicated that the offset of d-amphetamine stimulation (defined as lack of significance from counts per minute obtained during the last 15 minutes of the accommodation period) was shifted in males due to BP-6 exposure. Offset times were: 105 min (control), 150 min (0.12 mg/kg/day BP-6), 105 min (0.6 mg/kg/day BP-6), and 135 min (6 mg/kg/day BP-6).

Analysis of spontaneous locomotor activity of female offspring revealed significant effects with regard to BP-6 treatment [$F(3,800 = 88.43$, $p<0.001$], observation time [$F(15,800 = 58.97$, $p<0.001$], and the treatment and observation time interaction [$F(45,800 = 1.92$, $p<0.001$]. No treatment-related effects were observed during the initial 1-hour habituation period. However, following d-amphetamine injection, activity of female offspring from dams administered 0.12 mg/kg/day BP-6 was stimulated to a greater degree than that of controls, while activity of offspring from dams administered 0.6 or 6 mg/kg/day BP-6 was

Figure 8. d-Amphetamine-induced stimulation of accommodated locomotor activity in male and female offspring from dams administered PBB during gestation and lactation. Dams received 0 (○), 0.12 (▽), 0.6 (△), or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from four litters per dose. The symbol X represents activity of control rats following distilled water injection. The point of injection of distilled water (1 ml/kg) or d-amphetamine (2 mg/kg/day) is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

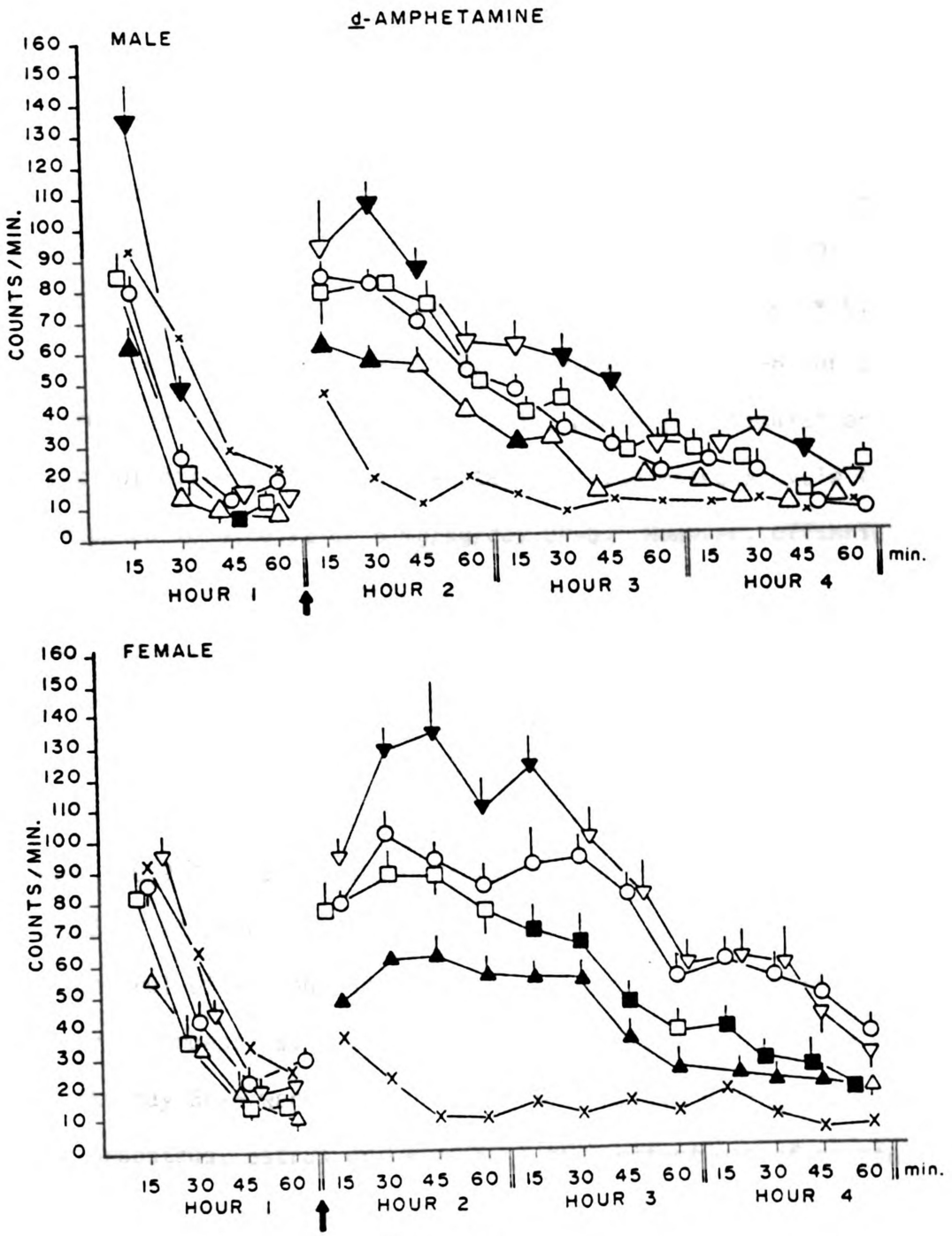

d-AMPHETAMINE
MALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
FEMALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 8

stimulated less. Offset of stimulation was shifted due to BP-6 exposure; it occurred at 180 min for offspring from dams administered 0, 0.12, or 0.6 mg/kg/day BP-6, but did not occur during the 3-hour observation period for offspring exposed to 6 mg/kg/day BP-6.

Activity at each stage of the estrous cycle is presented in Figure 9. Sufficient data were not available for females exposed to 0.12 mg/kg BP-6 for metestrus. Significant treatment and estrous cycle stage interactions were observed upon comparison of unaccommodated locomotor activity of offspring exposed to 0, 0.6 or 6 mg/kg/day BP-6 for all 4 stages of the estrous cycle [$F(6,328) = 6.31$, $p<0.001$] or those exposed to 0, 0.12, 0.6 or 6 mg/kg/day BP-6 for proestrus, estrus, or diestrus [$F(6,296) = 3.45$, $p<0.01$]. Stimulation of activity was not different across the stages of the estrous cycle for offspring exposed to 0, 0.12 or 6 mg/kg/day BP-6. However, offspring exposed to 0.6 mg/kg/day BP-6 exhibited enhanced activity during diestrus when compared to controls and to activity generated by these animals during proestrus, estrus or metestrus. A significant treatment and estrous cycle stage interaction was also observed following injection with d-amphetamine [for comparison of 0, 0.6 or 6 mg/kg/day BP-6 at all 4 stages $F(6,984) = 9.55$, $p<0.001$; for comparison of 0, 0.12, 0.6 or 6 mg/kg/day BP-6 at proestrus, estrus or diestrus $F(6,888) = 4.20$, $p<0.001$]. Control offspring were transiently less active during metestrus than during proestrus, estrus or diestrus. Offspring exposed to 0.6 or 6 mg/kg/day BP-6 were significantly more active during diestrus than during proestrus, estrus or metestrus; this effect was of greater magnitude and longer duration for offspring exposed to 0.6 mg/kg/day BP-6. No estrous cycle-related changes in activity were observed for

Figure 9. d-Amphetamine-induced stimulation of accommodated locomotor activity in female offspring from dams administered PBB during gestation and lactation. Dams received 0 (◯), 0.12 (▽), 0.6 (△) or 6 mg/kg/day (□) fireMaster BP-6. Data are presented according to stage of the estrous cycle. Each data point and vertical line represents the mean and standard error of female offspring from four litters per dose. The point of injection of d-amphetamine (2 mg/kg/day) is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$). Data were not available for metestrus in offspring exposed to 0.12 mg/kg/day fireMaster BP-6.

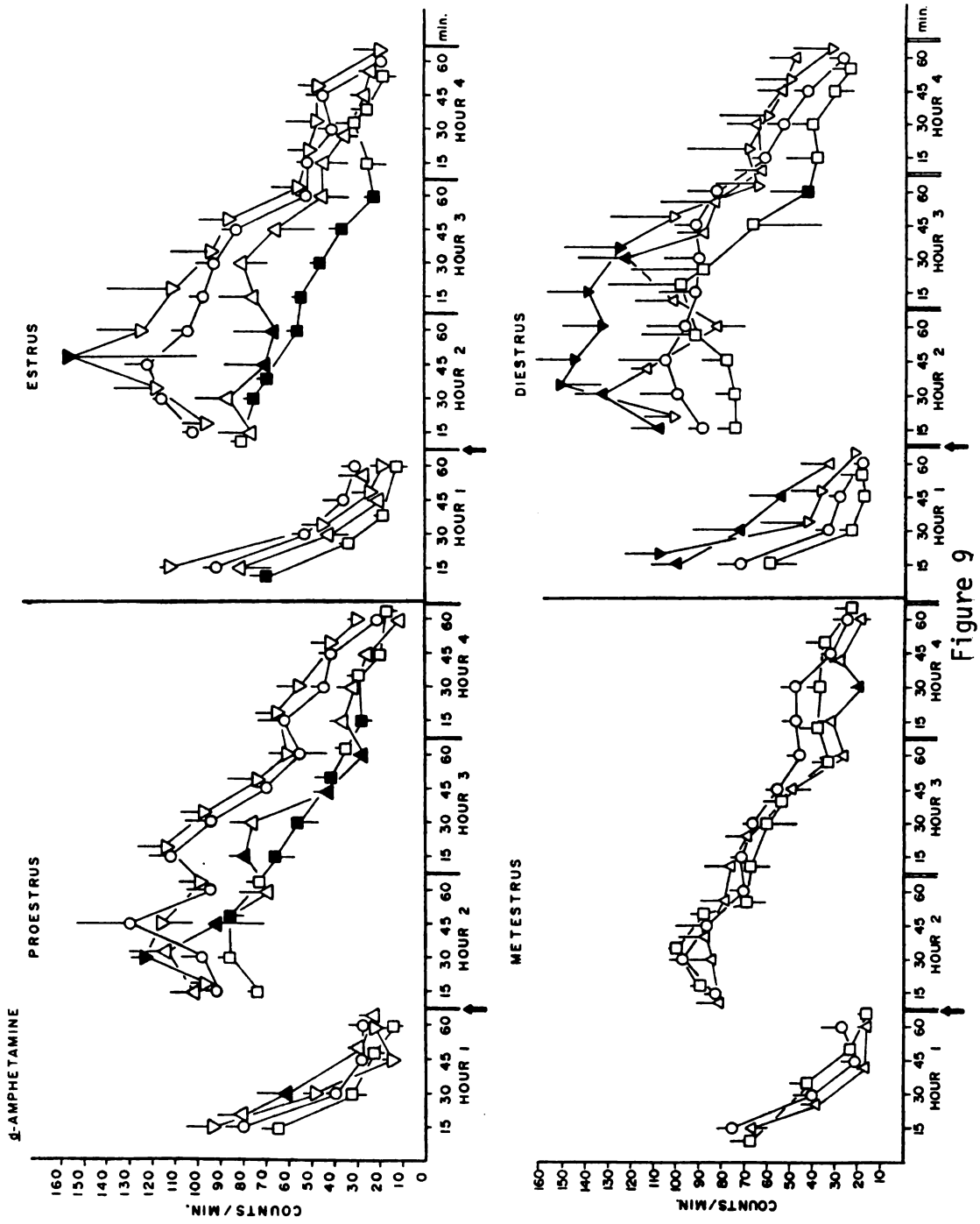

d-AMPHETAMINE
PROESTRUS
ESTRUS
METESTRUS
DIESTRUS
COUNTS/MIN.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
min.

Figure 9

offspring exposed to 0.12 mg/kg/day BP-6. Activity of offspring exposed to 0.6 or 6 mg/kg/day BP-6 was stimulated significantly less than that of controls during proestrus and estrus in a dose-related manner. Activity was stimulated to a greater extent than that of controls in offspring exposed to 0.12 mg/kg/day BP-6, and transiently in those exposed to 0.6 mg/kg/day BP-6, during diestrus only. Essentially no PBB-related effects were observed during metestrus.

iii. Scopolamine. Scopolamine-induced stimulation of spontaneous locomotor activity in male and female offspring of dams administered BP-6 during gestation and lactation is presented in Figure 10. ANOVA revealed significant effects for treatment [$F(3,784 = 26.66$, $p<0.001$] and observation time [$F(15,784) = 38.97$, $p<0.001$]; no interaction was observed between the 2 factors. Offspring from dams administered 0.12 or 0.6 mg/kg/day BP-6 were significantly more active than controls during the habituation period, and those from dams administered 0.12 mg/kg/day BP-6 were transiently more active following scopolamine injection.

Significant effects for treatment [$F(3,774) = 46.40$, $p<0.001$] and observation time [$F(15,774) = 32.36$, $p<0.001$] were observed following assessment of motor activity in female rats at 9 months of age. As with males, no interaction was observed between the two factors. Following scopolamine injection, offspring from dams administered 0.6 or 6 mg/kg/day BP-6 were significantly more active than controls; this effect was of greater magnitude and longer duration in offspring from dams administered 0.6 mg/kg/day BP-6.

Figure 10. Scopolamine-induced stimulation of accommodated locomotor activity in male and female offspring from dams administered PBB during gestation and lactation. Dams received 0 (○), 0.12 (▽), 0.6 (△) or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from four litters per dose. The point of injection of scopolamine (0.5 mg/kg, male; 1 mg/kg, female) is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

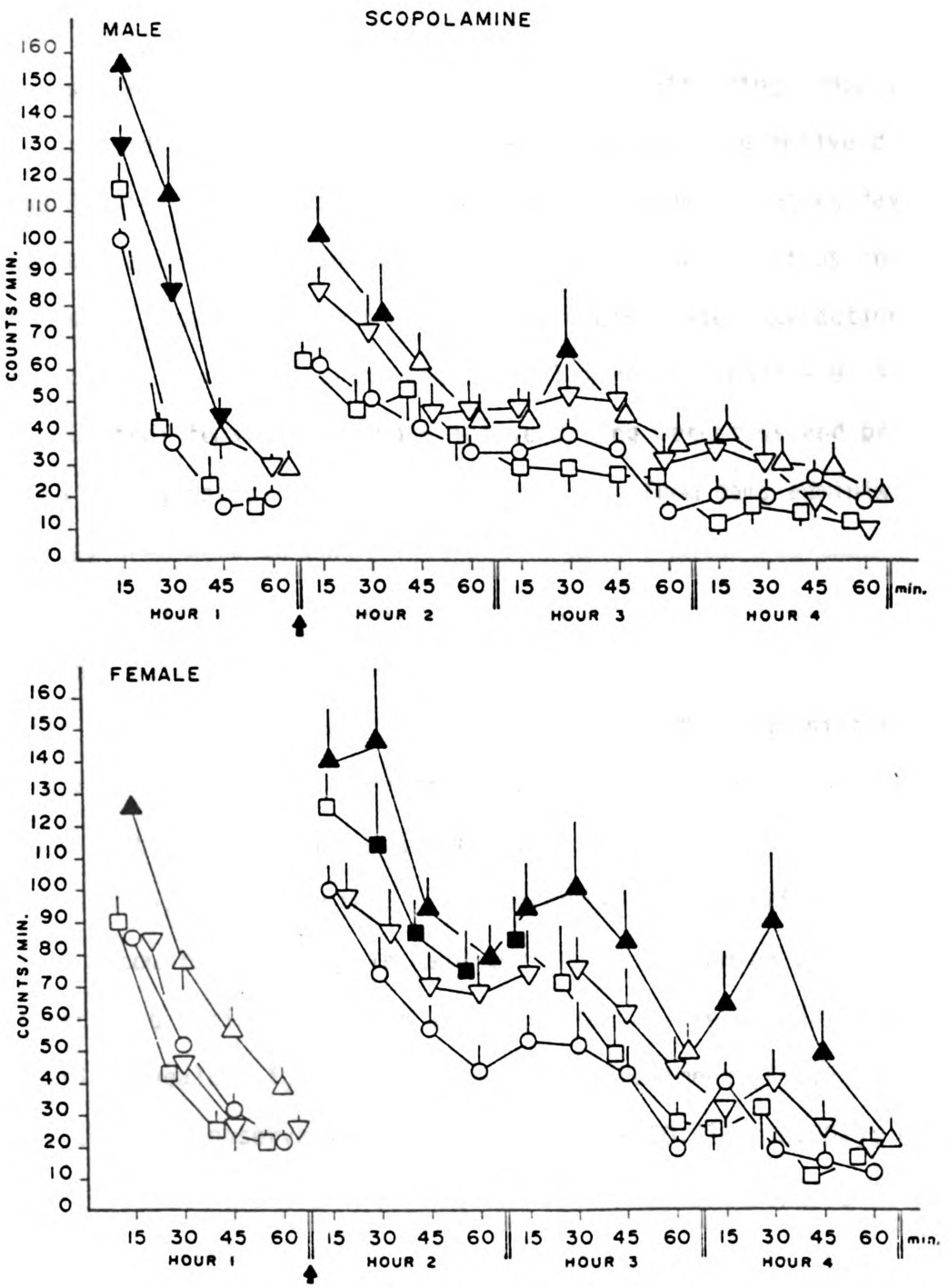

SCOPOLAMINE
MALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
FEMALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 10

Activity of each stage of the estrous cycle is presented in Figure 11. Data were available for females exposed to 0.12 mg/kg/day BP-6 for estrus only. Significant treatment and estrous cycle stage interactions were observed upon comparison of unaccommodated locomotor activity of offspring exposed to 0, 0.6 or 6 mg/kg/day BP-6 [$F(6,286) = 3.01$, $p<0.01$]. Enhancement of activity was not different across the stages of the estrous cycle for control offspring. However, offspring exposed to 0.6 or 6 mg/kg/day were somewhat more active during estrus and diestrus. Offspring from dams administered 0.6 mg/kg/day BP-6 were more active than controls during habituation in estrus and diestrus. A significant treatment and estrous cycle stage interaction was also observed following injection with scopolamine [$F(6,864) = 9.74$, $p<0.001$]. Control females were more active during metestrus and proestrus than during estrus or diestrus. Offspring from dams administered 0.6 mg/kg/day BP-6 were somewhat less active during metestrus than during the other stages of the estrous cycle, while those from dams administered 6 mg/kg/day BP-6 were somewhat more active during diestrus than during the other three stages. Offspring from dams administered 0.6 or 6 mg/kg/day BP-6 were significantly more active than controls during some portion of estrus or diestrus.

iv. Fenfluramine. Fenfluramine-induced stimulation of spontaneous locomotor activity in male and female offspring of dams administered BP-6 during gestation and lactation is presented in Figure 12. Analysis revealed significant effects for treatment [$F(3,656) = 18.64$, $p<0.001$] and observation time [$F(15,656) = 30.91$, $p<0.001$]; no interaction was observed between the two factors. All offspring from dams administered BP-6 exhibited decreased activity compared to controls

Figure 11. Scopolamine-induced stimulation of accommodated locomotor activity in female offspring from dams administered PBB during gestation and lactation. Dams received 0 (◯), 0.12 (▽), 0.6 (△) or 6 mg/kg/day (□) fireMaster BP-6. Data are presented according to stage of the estrous cycle. Each data point and vertical line represents the mean and standard error of female offspring from four litters per dose. The point of injection of scopolamine (1 mg/kg) is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$). Data were not available for proestrus, metestrus or diestrus in offspring exposed to 0.12 mg/kg/day fireMaster BP-6.

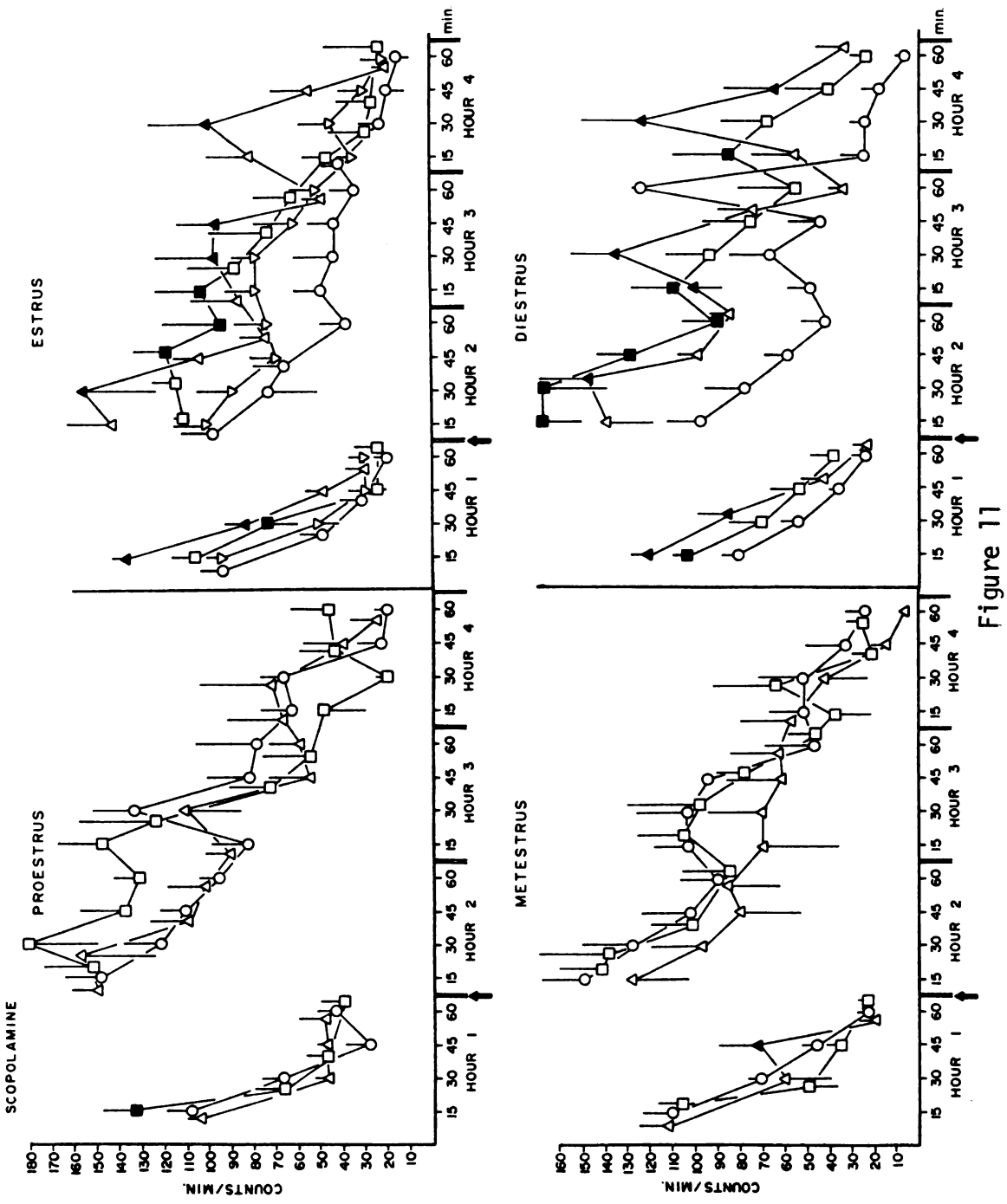

SCOPOLAMINE
PROESTRUS
ESTRUS
METESTRUS
DIESTRUS
COUNTS/MIN.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
min

Figure 11

Figure 12. Fenfluramine-induced stimulation of accommodated locomotor activity in male and female offspring from dams administered PBB during gestation and lactation. Dams received 0 (○), 0.12 (▽), 0.6 (△) or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from four litters per dose. The point of injection of fenfluramine (10 mg/kg) is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

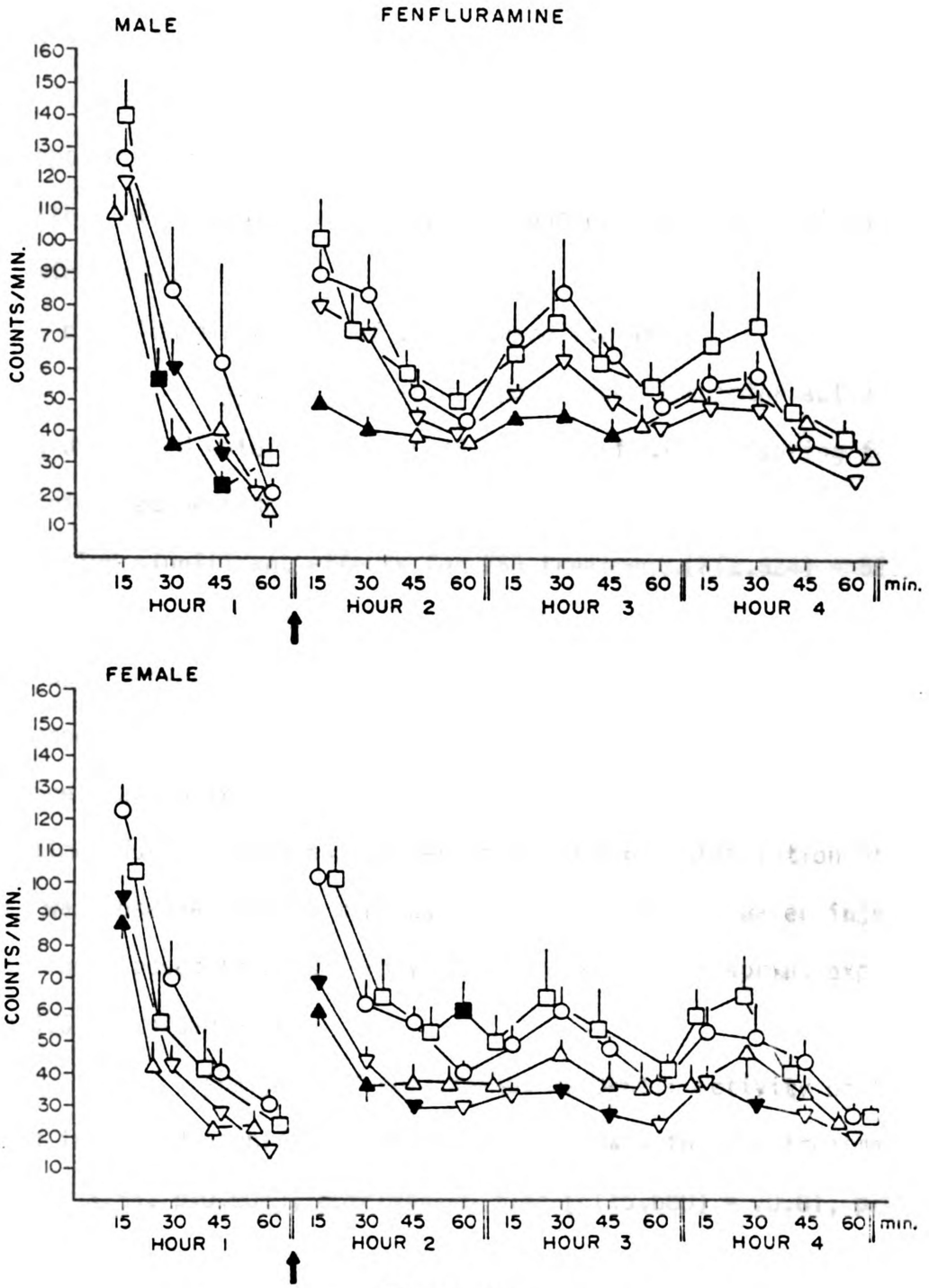

MALE
FENFLURAMINE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
FEMALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 12

during some portion of the initial 1-hour acclimation period. Activity of male offspring exposed to 0.6 mg/kg/day BP-6 was enhanced significantly less following fenfluramine injection than was that of controls or offspring exposed to 0.12 or 6 mg/kg/day BP-6.

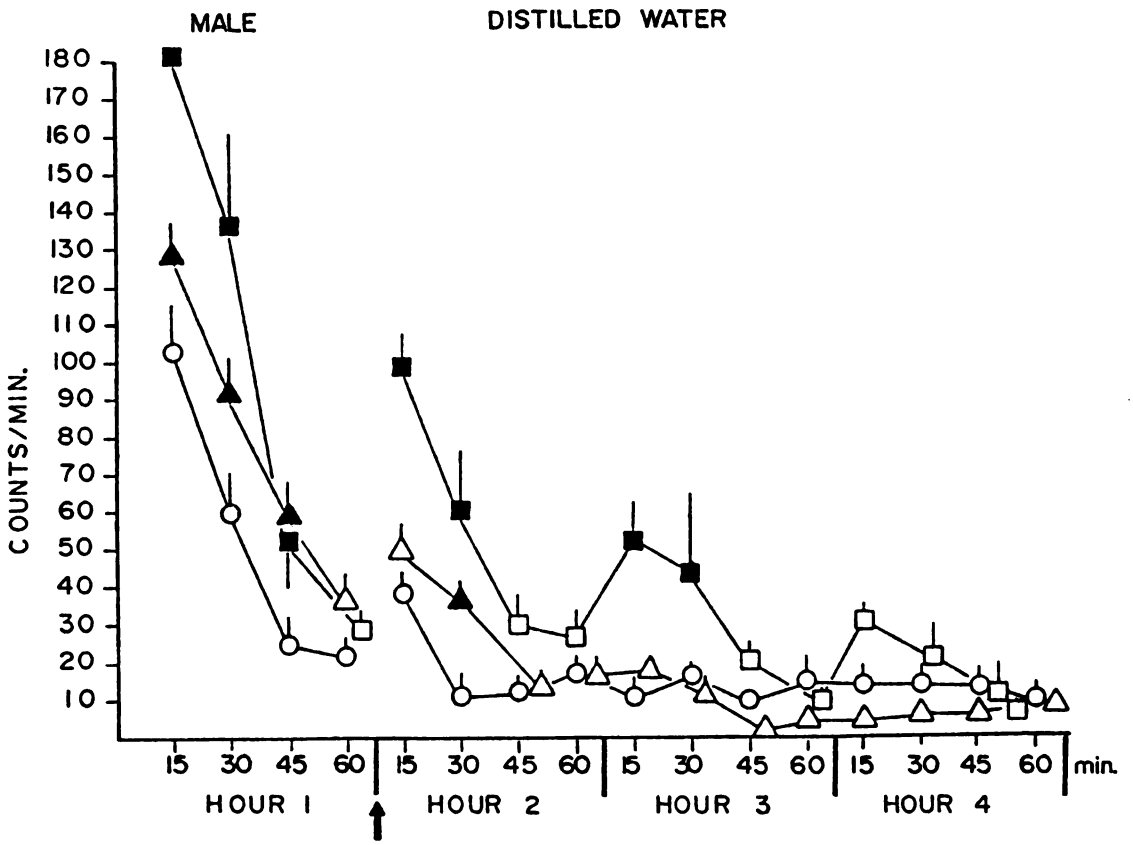
Significant effects were also observed for treatment [$F(3,704) = 34.13$, $p<0.001$] and observation time [$F(15,704) = 32.84$, $p<0.001$] for female offspring at 12 months of age. Activity of offspring exposed to 0.12 or 0.6 mg/kg/day was transiently decreased compared to that of controls during the habituation period and following fenfluramine injection.

b. Administration of BP-6 during lactation

i. Distilled water. Spontaneous locomotor activity following distilled water injection of male and female offspring from dams administered BP-6 during lactation is presented in Figure 13. ANOVA revealed significant effects for PBB treatment [$F(2,624) = 55.37$, $p<0.001$], observation time [$F(15,624) = 69.06$, $p<0.001$], and the treatment and observation time interaction in males. Offspring from dams administered 0.6 or 6 mg/kg/day BP-6 during lactation were significantly more active than controls during the accommodation period, and at various times during the 3-hour observation period. Stimulation of activity was observed for 45 minutes following distilled water injection in offspring exposed to 6 mg/kg/day BP-6, but not in offspring exposed to 0 or 0.6 mg/kg/day BP-6.

Analysis of spontaneous locomotor activity of female offspring indicated significant effects with regard to BP-6 treatment [$F(2,688) = 3.54$, $p<0.001$], observation time [$F(15,688) = 70.01$, $p<0.001$], and the treatment and observation time interaction [$F(30,688) = 1.92$,

Figure 13. Accommodated locomotor activity following distilled water injection (1 ml/kg) in male and female offspring from dams administered PBB during lactation. Dams received 0 (○), 0.6 (△), or 6 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from four litters per dose. The point of injection of distilled water is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).


MALE
DISTILLED WATER
COUNTS/MIN.
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

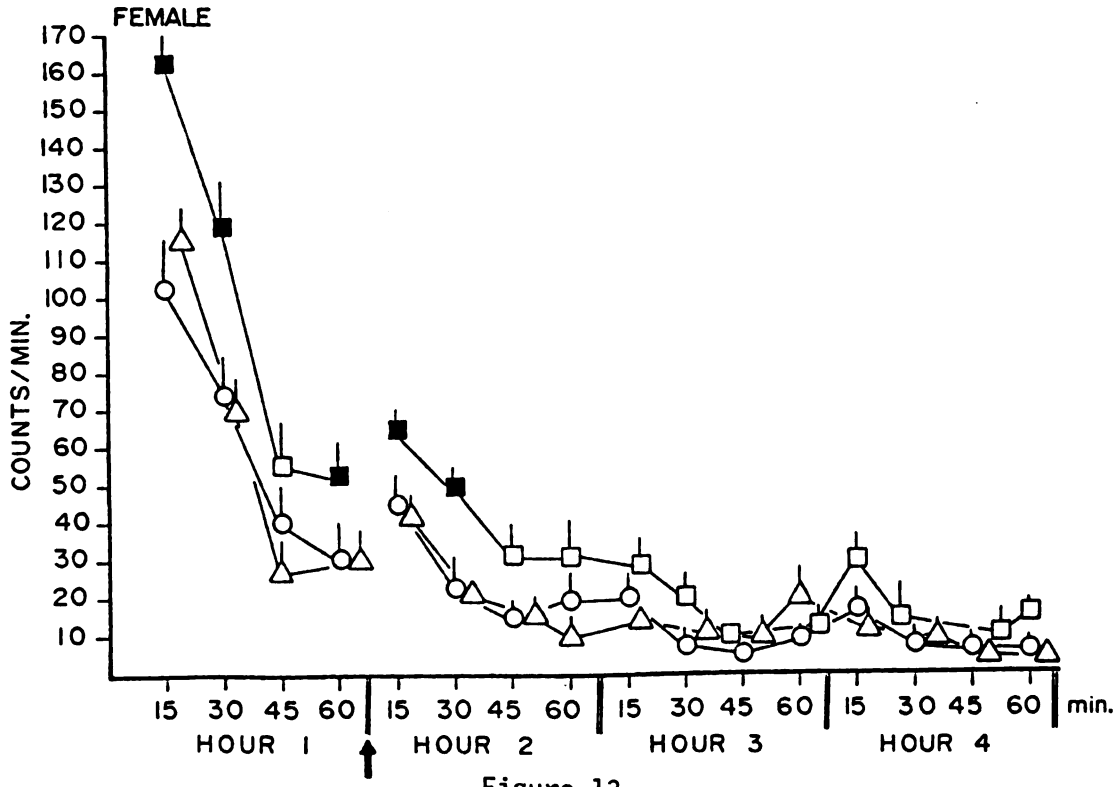

FEMALE
COUNTS/MIN.
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 13

$p<0.01$]. Offspring from dams administered 6 mg/kg/day BP-6 were significantly more active than controls during the accommodation period and for the first 45 minutes following distilled water injection. PBB-related effects were not observed in offspring exposed to 0.6 mg/kg/day BP-6. The activity of control and PBB-exposed offspring was not stimulated following distilled water injection.

ii. d-Amphetamine. d-Amphetamine-induced stimulation of spontaneous locomotor activity in male and female offspring of dams administered BP-6 during lactation is presented in Figure 14. ANOVA revealed significant effects for BP-6 treatment [$F(2,624) = 61.39$, $p<0.001$] and observation time [$F(15,624) = 44.91$, $p<0.001$] in males. No significant effect was observed for the treatment and observation time interaction, indicating that the pattern of stimulation was similar for control and PBB-exposed male offspring. Offspring from dams administered 6 mg/kg/day BP-6 were transiently more active than controls during the accommodation period. They were more active than controls for the majority of the 3-hour observation period following d-amphetamine injection. Activity of offspring exposed to 0.6 mg/kg/day BP-6 was comparable to that of controls. Offset of d-amphetamine-induced stimulation occurred at the following times: control, 105 min; 0.6 mg/kg/day, 120 min; and 6 mg/kg/day, 105 min.

Analysis of spontaneous locomotor activity of female offspring revealed significant effects with regard to BP-6 treatment [$F(2,688) = 4.18$, $p<0.025$], observation time [$F(15,688) = 25.29$, $p<0.001$], and the treatment and observation time interaction [$F(30,688) = 1.71$, $p<0.025$]. Offspring from dams administered 6 mg/kg/day BP-6 were

Figure 14. d-Amphetamine-induced stimulation of accommodated locomotor activity in male and female offspring from dams administered PBB during lactation. Dams received 0 (○), 0.6 (△) or 6 mg/kg/day (□) fire-Master BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from four litters per dose. The symbol X represents activity of control rats following distilled water injection. The point of injection of distilled water (1 ml/kg) or d-amphetamine (2 mg/kg) is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.05$).

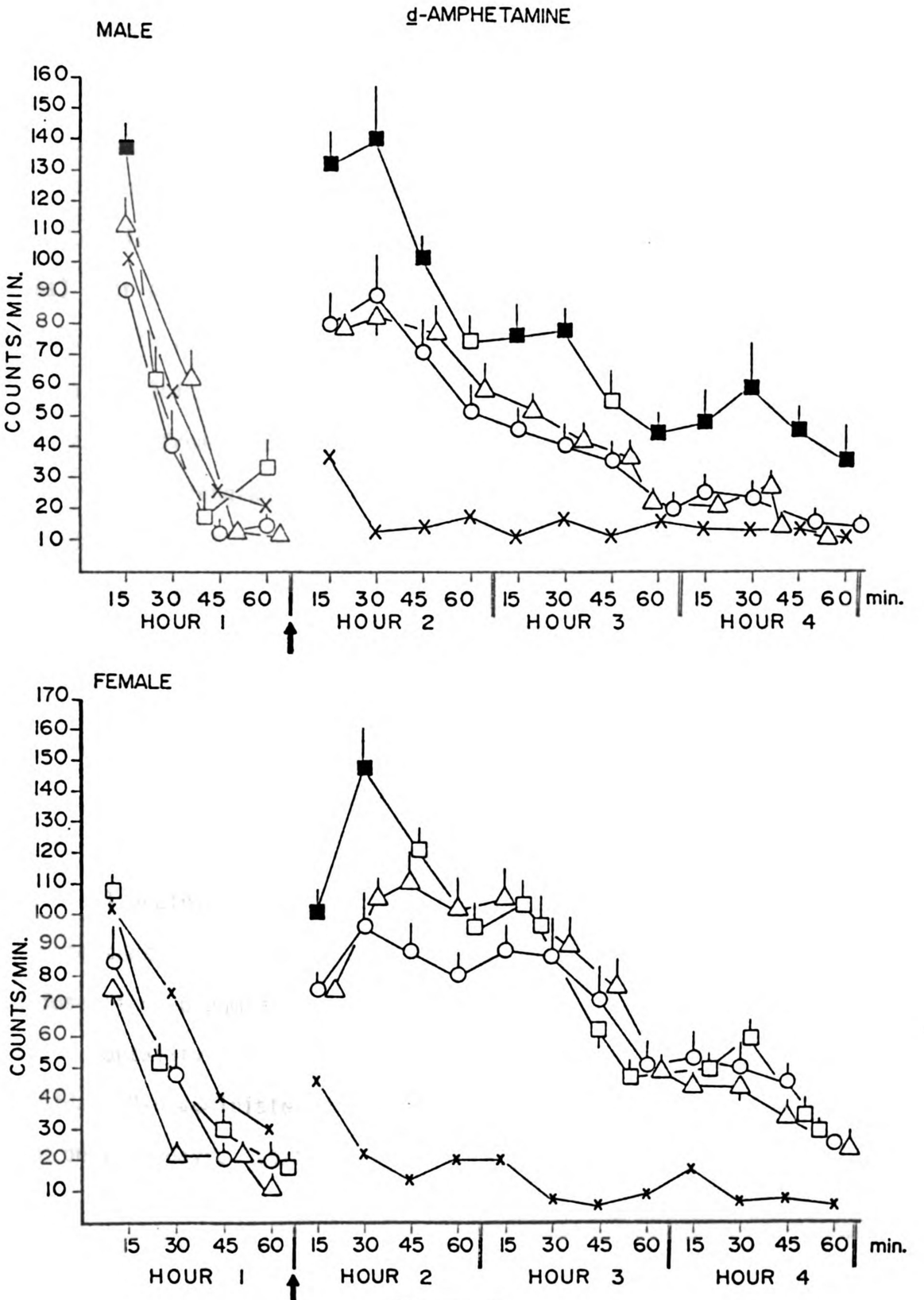

MALE
d-AMPHETAMINE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60
HOUR 1
15 30 45 60
HOUR 2
15 30 45 60
HOUR 3
15 30 45 60
HOUR 4
min.
FEMALE
COUNTS/MIN.
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60
HOUR 1
15 30 45 60
HOUR 2
15 30 45 60
HOUR 3
15 30 45 60
HOUR 4
min.

Figure 14

transiently more active than controls following d-amphetamine injection. Activity of offspring exposed to 0.6 mg/kg/day BP-6 was comparable to that of controls. Offset of stimulation occurred at 165 min for control and PBB-exposed offspring.

D. Discussion

Maternal toxicity (as assessed by body weight) occurred at a lower BP-6 dose following administration during gestation and lactation (40 doses) than during gestation alone (12 doses) or lactation alone (24 doses). With regard to cumulative PBB dose, the no-effect level for maternal toxicity achieved in this study was 43 mg (6 mg/kg/day BP-6 administered from days 0 through 24 of lactation).

Gestation length was increased by prenatal administration of 60 mg/kg/day BP-6. This phenomenon has not been reported previously for rodents administered PBB, but is similar to the extended gestation which occurred as a result of PBB ingestion by cows (Jackson and Halbert, 1974). Calves born to these cows were dead or died shortly after birth, as did rat pups born to dams administered 60 mg/kg BP-6. Birth defects observed in these pups included hydrocephaly and underdeveloped limbs. Administration of 12 mg/kg/day BP-6 to pregnant rats also resulted in late fetal death, but no malformations were observed. These results indicate that the extent of damage to the developing organism depends on the dose of BP-6 administered. Both doses acted late in development to produce mortality and, in fact, it was further demonstrated that 60 mg/kg/day BP-6 administered to dams during lactation was lethal to pups after only 12 days. It is of interest that pups exposed to 60 mg/kg/day

BP-6 during either gestation or lactation survived approximately 12 days of exposure. In the case of those exposed in utero, survival may have been compromised by the cranial and limb defects. Therefore, exposure during lactation did not appear to result in a decreased chance of survival relative to that observed in pups exposed during gestation.

In general, surviving offspring appeared at first to thrive. However, those from dams administered 6 mg/kg/day BP-6 during gestation and lactation had a 19% preweaning mortality rate, while that of all other treatment groups was negligible. Mortality data would thus indicate enhanced toxicity due to PBB exposure during both gestation and lactation.

This conclusion is supported by offspring body weight data. No body weight changes were observed in offspring from dams administered 0.6 or 6 mg/kg/day BP-6 during gestation only, while a deficit in body weight gain was observed following 6, but not 0.6, mg/kg/day BP-6 administered during lactation only. Body weight deficits were observed in offspring from dams administered either 0.6, 2, or 6, but not 0.12, mg/kg/day BP-6 during gestation and lactation. Thus, exposure during gestation and lactation appeared to be the most detrimental in terms of body weight deficits, with exposure during gestation being the least detrimental. These results are supported by the cross-fostering studies of Harris and coworkers (1978), who observed body weight deficits in offspring from dams administered 10 mg BP-6 daily during gestation and lactation or lactation alone, but not during gestation alone.

Assessment of physical and behavioral developmental endpoints was difficult due to the small number of litters tested and thus the

enhanced possibility of statistical error. Precocious eye opening observed in offspring exposed to 6 mg/kg/day BP-6 during gestation, as well as the precocious acquisition of locomotor activity in offspring exposed to 0.6 or 6 mg/kg/day during lactation, were felt to be artifactual due to anomalous data obtained for controls. Results of sensory function testing were also highly variable. Acquisition of cliff avoidance and forward locomotion was delayed in pups exposed to 0.6, 2 or 6 mg/kg/day BP-6 during gestation and lactation. Although interpretation of these effects is difficult, the data obtained provided an indication of which parameters should be assessed in the confirmatory study. These included time to eye opening and acquisition of cliff avoidance and forward locomotion.

Probe studies revealed a shift in the normal pattern of acquisition of open field activity at all doses administered during gestation and lactation or lactation alone. By 22 (females) or 24 (males and females) days of age, offspring exposed to 6 mg/kg/day BP-6 were less active than controls. Although open field activity was only assessed for a 3-day period for offspring exposed to PBB during gestation alone, a shift in the pattern of development was nevertheless still observed. However, the greatest degree of disruption of acquisition of locomotor activity was observed in offspring exposed to PBB during gestation and lactation.

Examination of drug-induced stimulation of locomotor activity was conducted in these offspring as adults in an attempt to clarify the nature of the open field deficit (including possible neurotransmitters involved) and to evaluate the persistence of effects on motor activity. Testing involved a one-hour habituation period which was followed by drug injection (distilled water, d-amphetamine, scopolamine or fenfluramine) and subsequent monitoring for three hours.

Probe studies indicated that male offspring exposed perinatally to 0.12 mg/kg/day BP-6 (cumulative dose = 4.8 mg) were significantly more active than controls following introduction into the locomotor activity testing apparatus prior to injection with distilled water, d-amphetamine or scopolamine. Transient enhancement of unaccommodated locomotor activity was also observed at 6 months of age in female offspring from dams administered 0.12 or 6 mg/kg/day BP-6 (cumulative dose = 240 mg) during gestation and lactation. In addition, offspring from dams administered 0.6 (cumulative dose = 14.4 mg; males only) or 6 mg/kg BP-6 (cumulative dose = 144 mg) during lactation were significantly more active than controls during accommodation.

Injection of distilled water following the accommodation period would be expected to result in transient excitation followed by activity levels corresponding to those observed due to habituation. However, a significantly longer duration of stimulation than that observed for controls was noted in probe study offspring exposed to 0.12 (males and females), 0.6 (males) or 6 mg/kg/day BP-6 (males and females) during gestation and lactation or to 6 mg/kg/day BP-6 (males and females) during lactation.

By contrast, d-amphetamine-induced stimulation of accommodated locomotor activity was affected by prior PBB exposure. Challenge with 2 mg/kg d-amphetamine to probe study offspring from dams administered BP-6 during gestation and lactation resulted in less stimulation and a shorter duration of action (females only) in those exposed to 0.6 (males and females) or 6 mg/kg/day BP-6 (females only) than in controls. Activity of offspring from dams administered 0.12 mg/kg/day BP-6 was stimulated

to a greater degree and for a longer period of time (males only) than was activity of controls. Activity of male and female offspring from dams administered 6 mg/kg/day BP-6 during lactation was significantly greater than that of controls following d-amphetamine injection while activity of offspring exposed to 0.6 mg/kg/day BP-6 was comparable to that of controls. These data indicate lack of a monophasic response to PBB exposure. While this phenomenon is not unusual among behavioral toxicants, clarification of these effects is required.

Stimulation of locomotor activity by d-amphetamine has been shown to result primarily from enhanced release of dopamine and inhibition of dopamine reuptake (Moore, 1978). The present results, therefore, indicate that BP-6 exposure may result in modulation of dopaminergic systems.

Analysis of d-amphetamine-induced stimulation of locomotor activity in female offspring according to stage of the estrous cycle revealed that the greatest BP-6-induced deficits occurred during proestrus and estrus, while enhanced activity observed in females exposed to 0.12 mg/kg/day BP-6 was greatest during diestrus. These results suggest the presence of an estrogen deficit in females of the two higher dose levels, as estrogen levels are normally high during proestrus and estrus (Feder, 1981), and estrogen levels have been shown to be positively correlated with motor activity (Norton, 1984). The relationship of estrogen titer to the effects observed in female offspring exposed to 0.12 mg/kg/day BP-6 is unclear, as estrogen levels are low during diestrus.

Brain cholinergic influences on locomotion are in general inhibitory (Swonger and Rech, 1972). Therefore, the antimuscarinic drug

scopolamine stimulates locomotion. Activity of male probe study animals exposed to 0.12 mg/kg/day BP-6 during gestation and lactation was transiently increased over that of controls following scopolamine injection. Scopolamine stimulated activity to the same degree in controls and in male offspring from dams administered 0.6 or 6 mg/kg/day BP-6. In contrast, scopolamine stimulated activity of female rats exposed to 0.6 or 6, but not 0.12 mg/kg/day, BP-6; this response was greatest in females exposed to 0.6 mg/kg/day BP-6, again suggesting a multiphasic effect. Although the data indicate that a sex-related difference in response exists, it should be emphasized that males and females were administered 0.5 and 1 mg/kg scopolamine, respectively. These doses were chosen because pilot studies indicated that the response of males to 1 mg/kg scopolamine was too variable. It has been demonstrated that scopolamine produces an inverted U dose-response effect on motor activity in female rats, with peak activity observed at a dose of 1 mg/kg (Stewart and Blain, 1975). The dose of scopolamine required to elicit a maximum response in male rats may not have been achieved in this study. These results indicate, however, that with regard to females, PBB may disrupt cholinergic systems. These results require confirmation.

Analysis of scopolamine-induced stimulation of locomotor activity in female offspring according to stage of the estrous cycle revealed a high degree of variability. The origin of this variability is unclear because little work has been done on the relation of cholinergic systems and estrogen with regard to locomotor activity. However, diestrus (the stage with lowest estrogen titer) appeared to be the only stage at which activity was significantly increased in PBB-treated rats.

Locomotor activity of probe study animals was stimulated following injection of fenfluramine. This is contrary to previously published reports which indicate that fenfluramine decreases locomotor activity in doses ranging from 2 to 30 mg/kg (LeDouarec and Neveau, 1970; Maickel and Johnson, 1973; Garattini et al., 1978; Rech et al., 1984). However, most of these tests were conducted within 1 hour of fenfluramine administration: a time during which fenfluramine acts to release serotonin (5HT). This initial release phenomenon is followed by 5HT depletion; maximum depletion is achieved between 2 and 4 hours following intraperitoneal administration (Costa et al., 1971). During this time of maximum depletion, fenfluramine has been shown to produce an increase in intertrial responses in a conditioned avoidance paradigm (McElroy et al., 1982). In addition, fenfluramine has been demonstrated to enhance confinement motor activity 60-180 minutes following administration (Clineschmidt et al., 1975). In the present study male offspring from dams administered 0.6 mg/kg/day BP-6 exhibited less increase in motor activity following fenfluramine injection than did controls or other BP-6 exposed offspring. A similar effect was observed in female offspring exposed to 0.12 or 0.6 mg/kg/day BP-6. Thus, PBB modulation of serotonergic systems may occur. However, further investigation of these effects is required.

Results of these probe studies indicate exposure to PBB during gestation and lactation produces behavioral deficits of the greatest magnitude in comparison to exposure during gestation alone or lactation alone. This regimen was, therefore, chosen for confirmatory studies to maximize the behavioral toxicity observed.

A dose of 2 mg/kg/day administered during gestation and lactation appeared to represent a no-effect level with regard to maternal toxicity or developmental parameters. These offspring exhibited a slight decrease in body weight gain beginning at weaning compared to controls and a delay in acquisition of forward locomotion and cliff avoidance. Spyker (1975) has suggested that the insidious nature of behavioral teratogens lies in the fact that their effects may occur at exposure levels not toxic to the dam and at levels which do not cause malformations in the offspring. It was important to choose doses for confirmatory studies with this premise in mind. Therefore, 2 mg/kg/day was chosen as the high dose and 0.2 mg/kg/day was chosen as the low dose.

Testing of many different behavior parameters provided a means to select those tests which appeared most sensitive. This approach has been suggested by Muller and coworkers (1984) to reduce the Type I error rate (probability of a false positive).

Results of these studies were sufficient for power analysis for the confirmatory study. The method of Ott (1977) was used to estimate the sample size required to produce a minimum difference of 20% between sample means.

Accommodated locomotor activity testing with psychoactive agents revealed possible effects of PBB exposure on dopaminergic, cholinergic, and serotonergic neuronal systems. Confirmatory studies were, therefore, conducted to clarify these effects.

CHAPTER 2

CONFIRMATORY ASSESSMENT OF BEHAVIORAL CHANGES EARLY IN LIFE RESULTING FROM PERINATAL PBB EXPOSURE

A. Introduction

Results of probe studies indicated that PBB administration to pregnant and/or lactating rats results in behavioral deficits in their offspring which may be manifested at an early age, particularly in those exposed during both gestation and lactation. Confirmatory studies were, therefore, conducted to test the hypothesis that perinatal PBB exposure produces behavioral changes early in life.

Additional exploratory studies were conducted to determine if short-term memory processes show signs of premature aging. These studies were conducted under the premise of Spyker (1975) that functional impairment caused by a behavioral toxicant may not appear until the central nervous system is stressed by age-related changes.

Chronic assessment of estrous cycle length was also conducted because perinatal PBB exposure has been shown to result in delayed vaginal opening as well as lengthened estrous cycles in rats and menstrual cycles in monkeys (Lambrecht et al., 1978; Harris et al., 1980; Johnston et al., 1980; McCormack et al., 1981).

Animals used for these studies were obtained from a large colony of control or PBB-exposed dams. The nature of that colony will be described subsequently.

B. Materials and Methods

1. Experimental Subjects and Dosing Procedure

Virgin female Sprague-Dawley rats, 6-8 weeks of age, were obtained from the Sendai virus-free colony of Harlan Sprague-Dawley, Madison, WI. Sendai virus was suspected to be present in the animal care facility in which these animals were to be housed, as well as in the rat colony from which the probe study animals were obtained. To ensure the quality of the final study, all subjects were, or were derived from, Sendai virus-free rats and were housed in a different animal care facility. All personnel working with this new colony were required to wear protective clothing, shoe covers, and particle filter masks. The animal care facilities were temperature and humidity-controlled and had a 12-hour light-dark cycle (lights on 1100-2300 hours daily). Food (Wayne Lab Blox, Chicago, IL) and water were supplied ad libitum.

Following at least one week of acclimation to the laboratory environment, vaginal smears were obtained from each female. The presence of cornified cells or cornified and nucleated cells in the vaginal lavage indicated that the female rat was in estrus and, therefore, receptive. Each receptive female was placed overnight in the cage of two non-dosed male Sprague-Dawley rats from the same Sendai virus-free colony.

It is of particular importance in confirmatory studies to control for type I errors (α - the probability of a false positive) as well as type II errors (β - the probability of a false negative response). One of the ways in which type II error can be minimized and

power (β-1) increased is by using an adequate sample size (Muller et al., 1984). The method of Ott (1977) was used to estimate the sample size required to produce a minimum difference of 20% between sample means. Using data obtained from the probe study, and setting α = .05 and β = .05, it was estimated that a sample size of at least 16 litters was needed. Therefore, 54 dams were randomly assigned to 1 of 3 dose groups, each group consisting of 18 dams. These dams were administered 0, 0.2 or 2 mg/kg/day fireMaster BP-6 (lot #6244-A) orally in a peanut butter "treat", as described previously (Chapter 1), daily from day 6 of gestation through day 24 of lactation. This exposure regimen was chosen because probe study data indicated that it resulted in maximal disruption of offspring behavior. It is also a regimen simulating that to which human infants have frequently been exposed.

Body weights and general appearance of all dams were monitored throughout gestation and lactation. Gestation length, number of pups born alive, sex ratio of the pups, and mortality from birth through weaning were also assessed. To facilitate comparison with future studies in which BP-6 will be administered during gestation alone or lactation alone, offspring from each litter were cross-fostered as soon after birth as possible to a dam of the same dose group. At 3 days of age all litters were culled to 4 pups per sex where possible. At this time indelible ink was injected subcutaneously into the paws of the pups in a specific pattern for the purpose of identification. Females were randomly assigned numbers 1 through 4, while males were randomly assigned numbers 5 through 8. Pups remained with their foster mother until weaning on postpartum day 24 and were housed 2 or 3 per cage thereafter according to BP-6 treatment and sex.

All pups were assigned to testing groups according to their randomly designated identification numbers. Female 1 and male 5 were assigned to Group 1, female 2 and male 6 to Group 2, female 3 and male 7 to Group 3, and female 4 and male 8 to Group 4. Each group was tested for a variety of physical and behavioral developmental endpoints to assess deficits from birth through adulthood. Assignment of pups to 4 groups minimized the litter interaction, as only one male and one female from each litter were subjected to any particular test.

A value of 0.05 was assigned to α for the overall study. Partitioning of offspring into 4 testing groups resulted in the partitioning of α according to a modification of the Bonferroni correction. For 4 tests, the probability of type I error according to the Bonferroni correction is $\frac{\alpha}{\#\text{ of tests (n)}}$ (Muller et al., 1984), or $\frac{.05}{4}$. This is a highly conservative approach to data analysis leading to decreased power and an inflated type II error rate, particularly with a large number of tests. It has, therefore, been suggested that a more rational approach to α correction for multiple tests would be $\frac{\alpha}{\sqrt{n}}$ (Mattsson, personal communication). This correction will be used in the present study in all cases.

2. Analysis of PBB Levels in Body Fat

At 2 months of age, rats which were not assigned to a testing group but nevertheless were from dams administered 0, 0.2 or 2 mg/kg BP-6 from day 6 of gestation through day 24 of lactation, were killed and the abdominal fat was removed and analyzed for PBB content.

The extraction and analysis procedures of McCormack and co-workers (1981) were utilized. Samples were weighed and ground with anhydrous sodium sulfate until they were dry and pulverized. The

powdered tissue was extracted with four separate 30-ml portions of hexane. Approximately 1-2 ml of the pooled extracts was placed on a Florisil (60-100 mesh) column (500 x 200 mm). PBB were eluted with 200 ml hexane; this mixture was evaporated to dryness and brought up to the desired volume with hexane.

Quantitation was by gas-liquid chromatography using a Shimadzu GC-4BM Series PF (Shimadzu Seisakusho, Ltd., Kyoto, Japan) gas chromatograph with a ^{63}Ni electron capture detector. A 3.0 m x 2.5 mm - i.d. column packed with 1% OV-101 was used. Carrier gas (N_2) flow and column temperature were 30 ml/min and 260°C, respectively. PBB levels were expressed as μg 2,2',4,4',5,5'-hexabromobiphenyl per gram wet tissue weight.

3. Assessment of Physical and Behavioral Developmental Endpoints

As mentioned previously, male number 5 and female number 1 were assigned to Group 1. The members of this group were evaluated for behavioral changes early in life as well as age-related changes. Table 16 lists all parameters measured in Group 1 animals, the method of statistical analysis used in each case, and the partitioning of α according to groups of studies.

a. "Incidental" data. These data were considered incidental to, but supportive of, the primary goal of testing in Group I animals: assessment of behavioral changes early in life. The level of significance chosen for each test was 0.025 (see Table 16).

All group members were weighed on postpartum days 0, 3, 7, 14, 21, 28 and 60. Body weight data were analyzed by a repeated measures analysis of variance (ANOVA) nested for sex and litter. Two

TABLE 16

Parameters Measured in Group 1 Animals, Including Method of Statistical Analysis and Partitioning of α

A. "Incidental" data

Parameter	Statistical Analysis	α[a]
1. Body Weight	Nested repeated measures ANOVA	0.025
2. Crown-rump length	ANOVA with mean comparisons	0.025
3. Eye Opening	ANOVA with mean comparisons	0.025
4. Vaginal opening or testes descent	ANOVA with mean comparisons	0.025

B. Exploratory Studies

Parameter	Statistical Analysis	α
1. Y-maze		
a. Entries (6 tests)	ANOVA with mean comparisons	0.0144[b]
b. % Alternations (6 tests)	ANOVA with mean comparisons	0.0144[b]
2. Estrous cycle (5 tests)	ANOVA with mean comparisons	0.0224[a]

C. Confirmatory Studies - Analyzed by overall study MANOVA with α = 0.025[c]

Parameter	Statistical Analysis	α
1. Locomotion (4 tests)		
a. Collapsed data ($\bar{X}$ 4 tests)	Multifactorial ANOVA	0.0125[d]
b. Individual test values	MANOVA	0.0062[e]
c. Individual test values	Multifactorial ANOVA	0.0062[b]
2. Cliff avoidance	Multifactorial ANOVA	0.0125[d]
3. Cage emergence (ranked values)	Multifactorial ANOVA	0.0125[d]
4. Open field (collapsed data)	Nested repeated measures ANOVA	0.0125[d]

[a] α (.05) partitioned among 4 tests; therefore, $\alpha = \frac{.05}{\sqrt{4}} = 0.025$.

[b] α (.05) partitioned among 12 tests; therefore, $\alpha = \frac{.05}{\sqrt{12}} = 0.0144$.

[c] α (.05) partitioned among Groups 1-4; therefore, $\alpha = \frac{.05}{\sqrt{4}} = 0.025$.

[d] α (.025) partitioned among 4 tests; therefore, $\alpha = \frac{.025}{\sqrt{4}} = 0.0125$.

[e] α (.0125) partitioned among 4 tests; therefore, $\alpha = \frac{.0125}{\sqrt{4}} = 0.0062$.

ANOVA were conducted: one to compare results of 0, 0.2 and 2 mg/kg/day offspring and one to compare results of 0 and 0.2 mg/kg/day offspring.

Distance from the crown of the head to the base of the tail was measured on postpartum day 0 using a vernier caliper calibrated to 0.1 mm. Although crown-rump length was not measured in the probe study, it is a standard measure of physical development used in teratology studies and therefore was assessed in the final study. Results were evaluated by ANOVA; means were compared using the least significant difference test.

Postpartum day of the opening of both eyes was assessed for each group member and analyzed by ANOVA with mean comparisons using the least significant difference test. The same form of analysis was used for latency to vaginal opening and testes descent. Although these indicators of sexual maturation were not assessed in the probe study, they were measured in the final study because PBB exposure has been correlated with delays in sexual maturation (Harris et al., 1980; McCormack et al., 1981).

b. Confirmatory studies. These data were representative of the primary goal of Group 1 testing and were, therefore, analyzed using an aggregatory measure. Multivariate analysis of variance (MANOVA, SAS Institute, Inc., Cary, NC) was used to test overall differences among 4 behavioral parameters due to PBB exposure. All Group 1 pups were subjected to all procedures. Two MANOVA were conducted: one to compare results from animals exposed to 0, 0.2 or 2 mg/kg/day BP-6 and one to compare results from those exposed to 0 or 0.2 mg/kg/day BP-6.

One of the behavioral parameters comprising the MANOVA was acquisition of various forms of neonatal forward locomotion. The 4 phases of acquisition were pivoting (the most primitive), forward locomotion with the head and body low, forward locomotion with the body raised and head low, and forward locomotion with the head and body raised (adult-type locomotion).

Animals were monitored from day 1 postpartum through acquisition of adult locomotion. Testing was conducted between 1100 and 1300 hours in the same room in which the animals were housed. The value used for each experimental subject for the study-wide MANOVA was the mean day of acquisition across all four phases of locomotion, as this was felt to represent maturational continuity. Treatment effects among these means were analyzed by multifactorial ANOVA (with sex and litter as factors). In addition, MANOVA was conducted using the four phases of acquisition as variables; the values used for each subject were the days of acquisition of each phase of locomotion. Treatment effects for each phase were assessed by multifactorial ANOVA (with sex and litter as factors). Two analyses were conducted in all cases: one to compare results from offspring exposed to 0, 0.2 or 2 mg/kg/day BP-6 and one to compare results from those exposed to 0 or 0.2 mg/kg/day BP-6.

Acquisition of cliff avoidance was also assessed. Each pup was placed on the edge of a tabletop in the same room in which it was housed, with forepaws and nose protruding over the edge. Observed daily from day 2 postpartum between 1100 and 1300 hours, acquisition was considered to be when each pup showed retraction and/or sideward movement beginning within 10 seconds and requiring no longer than 30 seconds

to complete. The actual day of acquisition was utilized for the study-wide MANOVA, and treatment effects within this variable were analyzed using multifactorial ANOVA (with sex and litter as factors). As with locomotion, two comparisons were conducted.

Cage emergence behavior was evaluated in probe study animals, but the data were not presented in Chapter 1 due to inconsistencies in testing methodology. These inconsistencies were resolved prior to the final study. On day 60 postpartum, each Group 1 animal was placed inside a stainless steel mouse cage turned on its side to provide easy access to the tabletop on which it was placed. Testing was conducted between 1400 and 1600 hours in a dimly lighted room separate from that in which the animals were housed. They were allowed to acclimate to the room at least 5 minutes prior to testing. The latency to emerge from mouse cage to tabletop was measured in seconds, with a maximum of 900 seconds being allowed. Because of the 900-second ceiling, cage emergence data were ranked, and these ranks were utilized for the study-wide MANOVA. Treatment effects among means were analyzed by multifactorial ANOVA (with sex and litter as factors). Two comparisons were conducted.

Open field activity was analyzed for all Group 1 offspring according to the method outlined in Chapter 1. Testing was conducted on postpartum days 12, 14, 15, 16, 17, 18, 19, 20, 22 and 24 to provide a complete characterization of the pattern of development of open field activity. The open field apparatus consisted of a 50 (L) x 50 (W) x 16 (H) cm plexiglas enclosure marked off into 10 x 10 cm squares. Each test session was 3 minutes in duration; the number of squares traversed during this period, as well as latency to move from

the center square, and number of rearings were recorded. Testing was conducted between 1100 and 1300 hours in the same room in which the animals were housed. Squares traversed, rearings, and latency to move from the center square were tabulated; however, only the number of squares traversed was included in the overall study MANOVA due to an obvious lack of effect with regard to the other two parameters. The value used for each subject for MANOVA was the mean number of squares traversed across all time points. This collapsing of data was legitimate due to the lack of a group versus day interaction when group means across days were compared using a repeated measures ANOVA nested for litter and sex.

c. Exploratory studies. The behavioral tests described below had not been addressed in the probe study and were therefore considered to be exploratory in nature.

Beginning at 2 months of age all Group 1 offspring were tested for spontaneous alternation using a Y-maze. This procedure is thought to be an excellent test for short-term memory, is intimately involved with cholinergic systems, and is disrupted by the aging process in rats (Swonger and Rech, 1972; Zornetzer et al., 1982).

Each rat was subjected to one 8-minute test session at 2, 3, 4, 6, 9 and 12 months of age. Testing was conducted in a quiet, dimly-lit testing room in a Y-shaped maze constructed of wood and painted black. Hinged doors covered with wire mesh allowed access to the maze through the top. The start and choice alleys of the maze measured 13 (W) x 15 (H) x 60 (L) cm.

Testing was conducted according to the method of Swonger and Rech (1972). The test rat was placed in the start alley with its

nose pointed toward the choice point located at the entrance to the two choice alleys. An entry was defined as the complete entry of the rat, up to the base of the tail, into a choice alley. The first alley entered by the rat was not considered as an entry because no choice was involved. An alternation was defined as an entry into the alley visited least recently by the rat. The first 2 entries were not considered in the tabulation of alternations because at least 2 entries would have to be made at the start of a session before an alley could be designated as that entered least recently. Percentage alternation (P) was calculated as follows: $P = 100 \times A/(N-2)$, where A = number of alternations and N = number of entries.

Treatment effects at each time point were assessed by ANOVA with mean comparisons using the least significant difference test.

Johnston et al. (1980) demonstrated that perinatal administration of 100 ppm fireMaster to rat dams resulted in increased length of the estrous cycle of offspring, indicating an estrogen deficit. Because these researchers only measured length of the estrous cycle at one time point (2 months of age), it was considered useful to assess estrous cycle length from puberty through adulthood. Female rats in general are anestrous by the end of their first year; i.e., they no longer exhibit regular estrous cycles (Lu et al., 1979). This phenomenon is due to either decreased production of steroid hormones or constant production of estrogen with decreased modulation of trophic hormones, and its onset may be affected by substances which disrupt the hormonal milieu.

To evaluate the effect of perinatal fireMaster exposure on estrous cyclicity, all female rats of Group 1 were tested for estrous

cycle duration at 2, 4, 6, 8, 10, and 12 months of age using the technique described in Chapter 1. The vaginal lavage was examined for at least 4 days, and up to 2 weeks if cycling was irregular to ensure that the animal was not pseudopregnant. An entire estrous cycle was considered to be from the first appearance of cornified cells in the lavage to the next. Constant estrus, metestrus, or diestrus for 2 weeks was considered to be an indication of the onset of anestrous.

Treatment effects for estrous cycle length at each time point were assessed by ANOVA with paired comparisons using the least significant difference test. Incidence of females anestrous at each time point was compared among doses using Fisher's exact probability test (Gad and Weil, 1982).

C. Results

1. Developmental Parameters

Dams were administered 0, 0.2 or 2 mg/kg/day BP-6 from day 6 of gestation through day 24 of lactation; their body weights are presented in Table 17. ANOVA revealed no PBB-related differences in body weights of natural mothers during gestation or foster mothers during lactation. Appearance of PBB-treated dams was comparable to that of controls throughout gestation and lactation.

Developmental parameters are presented in Table 18. No PBB-related differences were observed for gestation length, litter size, sex ratio of the offspring, or pup mortality between birth and weaning.

Concentration of the major BP-6 congener 2,2',4,4',5,5'-hexabromobiphenyl in the abdominal fat of 2 month-old offspring exposed to PBB perinatally is presented in Table 19. Few animals were available

TABLE 17

Mean (± S.E.M.) Body Weights of Female Rats Administered fireMaster BP-6 (PBB) During Gestation and Lactation

GESTATION (NATURAL MOTHER)

Day of Gestation	Body Weights (grams)		
	0 mg/kg/day	0.2 mg/kg/day	2 mg/kg/day
0	248.2±2.9	244.4±1.8	251.9±2.7
3	261.9±2.7	256.7±2.4	263.8±2.9
6	271.2±2.8	267.5±2.3	272.9±2.8
9	280.6±2.6	274.4±2.6	281.0±3.0
12	293.1±2.5	289.1±2.7	293.4±3.4
15	309.6±3.0	306.5±2.9	309.7±3.2
18	349.4±3.7	350.2±3.7	347.9±4.6
21	393.8±4.6	394.8±5.0	390.6±5.5

LACTATION (FOSTER MOTHER)

Day of Lactation	Body Weights (grams)		
	0 mg/kg/day	0.2 mg/kg/day	2 mg/kg/day
0	296.2±3.3	293.6±4.1	298.9±4.7
3	301.6±3.7	297.3±4.1	298.6±4.3
6	304.3±3.5	305.3±4.0	303.0±4.3
9	314.6±2.9	312.0±4.5	309.1±4.4
12	317.8±2.5	311.6±4.0	311.4±4.3
15	322.1±3.3	316.4±4.1	314.5±4.6
18	322.1±4.3	318.8±4.5	314.9±4.4
21	315.6±3.2	314.5±4.6	308.4±4.4
24	306.3±3.2	304.7±5.0	303.2±5.8

All mean body weights of PBB-exposed animals were comparable to those of controls.

TABLE 18

Mean (± S.E.M.) Developmental Parameters for Offspring from Dams Administered FireMaster BP-6 (PBB) During Gestation and Lactation

Parameter	0 mg/kg	0.2 mg/kg	2 mg/kg
Length of Gestation (days)	21.8±0.1[a]	21.9±0.1	21.5±0.1
Number of pups born			
Male	5.8±0.4	6.0±0.6	5.2±0.6
Female	6.1±0.4	5.9±0.5	5.1±0.7
Number of pups born dead	0.7±0.3	1.5±0.4	0.6±0.2
Number of pups dead by weaning	0.2±0.1	0±0	0.1±0.1

[a] n=18 litters in all cases.

TABLE 19

Mean (± S.E.M.) Concentration of 2,2',4,4',5,5'-Hexabromobiphenyl (HBB) in Abdominal Fat of Two-Month-Old Offspring from Dams Administered FireMaster BP-6 During Gestation and Lactation

Concentration of 2,2',4,4',5,5'-HBB (μg/g tissue)					
0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day	
Male	Female	Male	Female	Male	Female
0.017±0.004 (n=4)	0.384±0.351 (n=2)	13.36±3.77 (n=4)	8.62±5.19 (n=3)	36.30±10.25 (n=4)	35.83±4.13 (n=4)

for testing at this age; however, these data provide an indication of PBB levels at each BP-6 dose as compared to controls. The relatively high mean PBB concentration observed for control females was felt to be due to one contaminated sample.

2. Assessment of Physical and Behavioral Developmental Endpoints

a. "Incidental data". Repeated measures ANOVA nested for sex and litter revealed a significant difference ($p<0.025$) in body weight between offspring exposed to 2 mg/kg/day BP-6 and controls (Table 20). This difference was manifested as a decreased body weight gain compared to controls throughout the 60-day observation period. No PBB-related effect on body weight was observed in offspring exposed to 0.2 mg/kg/day BP-6. Significant effects were observed for sex ($p<0.025$ for both groups), litter ($p<0.025$ for both groups), day ($p<0.025$ for both groups), and the following interactions: BP-6 group and day ($p<0.025$ for offspring exposed to 2 mg/kg/day only) and sex and day ($p<0.025$ for both groups). Significant effects for sex and day are not surprising, as body weights were measured over a 60-day time period. Of interest is the significant litter effect, which was initially assessed because one male and one female from each litter were tested. The significant interaction terms suggest that PBB affected the pattern of weight gain over the 60-day observation period and that the pattern of weight gain was different between males and females.

Crown-rump length was significantly decreased compared to control ($p<0.025$) for male offspring from dams administered either 0.2 or 2 mg/kg/day BP-6 (Table 21), indicating that perinatal PBB exposure was overtly toxic to these pups. PBB-related effects on crown-rump length were not observed in female offspring. In addition, no

TABLE 20

Mean (± S.E.M.) Body Weights of Offspring from Dams Administered FireMaster BP-6 (PBB) During Gestation and Lactation

Day Postpartum	Body Weight (grams)[a]					
	0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day*	
	Male	Female	Male	Female	Male	Female
0	6.4±0.1	5.9±0.1	6.2±0.1	5.9±0.1	5.9±0.1	5.7±0.1
3	8.8±0.3	8.3±0.3	8.8±0.3	8.4±0.3	7.8±0.3	7.4±0.3
7	16.0±0.4	15.1±0.5	15.3±0.6	14.7±0.5	13.2±0.5	12.7±0.6
14	30.5±0.8	28.9±0.9	29.9±1.4	28.3±1.3	26.4±0.8	25.7±0.9
21	48.3±1.5	44.7±1.7	47.6±2.2	45.2±2.0	41.6±1.3	40.4±1.4
28	79.6±2.2	70.4±2.5	79.5±3.6	71.2±3.2	68.1±2.2	62.4±2.1
60	276.0±5.7	187.8±3.5	267.6±6.3	184.3±3.5	253.7±4.0	171.4±4.1

[a]Mean comprised of 1 individual from each of 18 litters in all cases.

STATISTICAL ANALYSIS continued on next page....

TABLE 20 (continued)

STATISTICAL ANALYSIS (α set equal to 0.025)

Effects	Level of Significance (α)	
	0 mg/kg vs. 0.2 mg/kg	0 mg/kg vs. 0.2 mg/kg vs. 2 mg/kg
GROUP	Not Significant	0.0037
Sex	0.0001	0.0001
Litter	0.0001	0.0001
Group x Sex	Not Significant	Not Significant
Litter x Sex	Not Significant	Not Significant
Day	0.0001	0.0001
Group x day	Not Significant	0.0001
Sex x day	0.0001	0.0001
Group x sex x day	Not Significant	Not Significant

TABLE 21

Mean (+ S.E.M.) Values for Physical Developmental Parameters for Offspring from Dams Administered fireMaster BP-6 (PBB) During Gestation and Lactation

Parameter[a]	DOSE GROUP					
	0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Crown-rump length (mm)	39.4±0.6	36.3±0.8	37.2±0.6*	35.9±0.6	36.0±0.5*	35.4±0.5
Eye opening (day)	15.0±0.2	15.2±0.2	14.9±0.2	14.8±0.3	14.7±0.2	15.0±0.3
Testes descent (day)	28.0±0.6	---	29.0±0.8	---	28.2±1.0	---
Vaginal opening (day)	---	33.9±1.0	---	35.1±1.0	---	35.1±0.6

[a]Mean comprised of 1 individual from each of 18 litters in all cases.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.025$.

PBB-related effects were observed for time to eye opening, testes descent, or vaginal opening.

b. Confirmatory studies. Table 22 provides mean values for acquisition of forward locomotion, cliff avoidance and cage emergence, three of the behavioral parameters comprising the Group 1 overall study MANOVA. The analysis of these data is found in Table 23. Aggregatory analysis of the entire data set by MANOVA revealed an overall PBB treatment effect ($p<0.025$) for offspring from dams administered 2 mg/kg/day BP-6. A significant sex effect ($p<0.025$) was observed for offspring exposed to either 0.2 or 2 mg/kg/day BP-6, indicating a sexually dimorphic component to these behaviors.

ANOVA revealed a significant difference ($p<0.0125$) in acquisition of forward locomotion in offspring from dams administered 2 mg/kg/day BP-6. A highly significant litter effect ($p<0.0125$) was observed for offspring exposed to either 0.2 or 2 mg/kg/day BP-6; however, no sex effect was observed. MANOVA of the 4 stages of acquisition of forward locomotion (Table 24) revealed a significant PBB treatment effect ($p<0.0062$) for offspring from dams administered 2 mg/kg/day BP-6. Separate ANOVA of the 4 different stages indicated that these treatment-related effects occurred for stages 3 (forward locomotion with the head low and body raised; $p<0.0062$) and 4 (forward locomotion with the head and body raised; $p<0.0062$). Thus, the more advanced stages of locomotion were affected to the greatest degree by perinatal PBB exposure. Litter was a significant variable for stages 1, 2 and 3, but not for stage 4, indicating that litter effects depend on the tests conducted.

TABLE 22

Mean (± S.E.M.) Values for Behavioral Parameters for Offspring from Dams Administered fireMaster BP-6 (PBB) During Gestation and Lactation

Parameter[a]	DOSE GROUP					
	0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Locomotion (day)[b]						
a) Pivoting	3.9± 0.2	3.9±0.2	3.6± 0.3	3.6± 0.3	3.8± 0.3	3.8± 0.3
b) Forward locomotion, body low, head low	6.1± 0.4	6.3±0.5	5.7± 0.4	6.3± 0.5	6.8± 0.5	7.0± 0.6
c) Forward locomotion, body raised, head low	11.4± 0.4	11.2±0.5	12.2± 0.3	12.3± 0.3	12.5± 0.4	13.0± 0.4
d) Forward locomotion, body raised, head raised	18.1± 0.5	18.3±0.5	18.8± 0.5	18.4± 0.5	20.7± 0.4	20.8± 0.6
Cliff avoidance (day)	7.6± 0.4	8.0±0.4	8.1± 0.4	8.5± 0.5	8.7± 0.6	8.8± 0.5
Cage emergence (sec)	254.2±76.4	25.2±6.1	341.9±96.8	36.4±15.4	136.1±59.1	97.5±49.1

[a]Mean comprised of 1 individual from each of 18 litters in all cases.

[b]For statistical analysis of locomotion, see Table 23.

TABLE 23

Overall Statistical Analysis for Acquisition of Behavior by Offspring from Dams Administered fireMaster BP-6 (PBB) During Gestation and Lactation

Type of Analysis	Test Parameter	Level of Significance (α)	
		0 mg/kg vs. 0.2 mg/kg	0 mg/kg vs. 0.2 mg/kg vs. 2 mg/kg
Overall MANOVA (α = 0.025)	Group	N.S.[a]	0.0243
	Sex	0.0006	0.0006
	Group x sex	N.S.	N.S.
ANOVA[b] Locomotion (α = 0.0125)	Group	N.S.	0.0017
	Litter	0.0001	0.0001
	Sex	N.S.	N.S.
	Group x sex	N.S.	N.S.
ANOVA Cliff avoidance (α = 0.0125)	Group	N.S.	N.S.
	Litter	N.S.	N.S.
	Sex	N.S.	0.0001
	Group x sex	N.S.	N.S.
ANOVA[c] Cage emergence (α = 0.0125)	Group	N.S.	N.S.
	Litter	N.S.	N.S.
	Sex	0.0001	0.0001
	Group x sex	N.S.	N.S.
Repeated measures[b] ANOVA with split-split Plot design Open field (α = 0.0125)	Group	N.S.	0.0068
	Litter	0.0001	0.0001
	Sex	N.S.	N.S.
	Litter x sex	0.0004	0.0002
	Group x sex	N.S.	N.S.
	Day	0.0001	0.0001
	Group x day	N.S.	N.S.
	Sex x day	N.S.	N.S.
	Group x sex x day	N.S.	N.S.

[a]Not significant.

[b]Data collapsed to mean value for MANOVA.

[c]ANOVA conducted on ranked data.

TABLE 24

Statistical Analysis for Acquisition of Locomotion by Offspring from Dams Administered fireMaster BP-6 (PBB) During Gestation and Lactation

Type of Analysis	Test Parameter	Level of Significance (α)	
		0 mg/kg vs. 0.2 mg/kg	0 mg/kg vs. 0.2 mg/kg vs. 2 mg/kg
Overall MANOVA (α = 0.025)	Group	N.S.[a]	0.0002
	Sex	N.S.	N.S.
	Group x sex	N.S.	N.S.
ANOVA Pivoting (α = 0.0062)	Group	N.S.	N.S.
	Litter	0.0001	0.0001
	Sex	N.S.	N.S.
	Group x sex	N.S.	N.S.
ANOVA Forward locomotion, head, low, body low (α = 0.0062)	Group	N.S.	N.S.
	Litter	0.0001	0.0001
	Sex	N.S.	N.S.
	Group x sex	N.S.	N.S.
ANOVA Forward locomotion, head low, body raised (α = 0.0062)	Group	N.S.	0.0060
	Litter	N.S.	0.0015
	Sex	N.S.	N.S.
	Group x sex	N.S.	N.S.
ANOVA Forward locomotion, head raised, body raised (α = 0.0062)	Group	N.S.	0.0001
	Litter	N.S.	N.S.
	Sex	N.S.	N.S.
	Group x sex	N.S.	N.S.

[a]Not significant.

Acquisition of cliff avoidance and time of cage emergence did not exhibit any PBB treatment-related effects. However, a trend toward delayed acquisition of cliff avoidance (male and female offspring) and time of cage emergence (female offspring only) was observed. Sexual dimorphism was apparent for both behaviors at the 2 mg/kg/day BP-6 level and for time to cage emergence at the 0.2 mg/kg/day BP-6 level ($p<0.0125$ in all cases). Significant litter effects were not observed for these two behaviors.

Repeated measures ANOVA nested for sex and litter revealed a significant difference ($p<0.0125$) in number of squares traversed in an open field by male and female offspring from dams administered 2 mg/kg/day BP-6. Significant effects for litter ($p<0.0125$ for both dose groups) and the litter by sex interaction ($p<0.0125$ for all comparisons) were observed, indicating that litter effects were more prominent in one sex than the other. Which sex is affected to the greater degree cannot be determined from this analysis. No significant differences were observed for the group by sex, group by day, sex by day, or group by sex by day interactions, although a significant effect was observed for both groups for day. The lack of interaction indicates a trend toward a PBB effect across the entire 12 day observation period, rather than a shift in the pattern of development of open field activity. Observation of graphical representations of these data (Figures 15 and 16) indicates that the normal pattern of acquisition of open field activity such as that observed in probe study animals was not observed in this study; no peak in activity was observed at approximately 15 days of age in either control or PBB-exposed offspring. The

Figure 15. Open field activity of male offspring from dams administered fireMaster BP-6 (PBB) during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of 18 litters represented by one individual per litter. Analysis of variance revealed a significant difference ($p=0.0068$) between controls and offspring exposed to 2 mg/kg/day fireMaster with regard to squares traversed. Analysis of variance was not conducted on data obtained for number of rearings or latency to move from the center square because lack of PBB effect could be ascertained observationally.

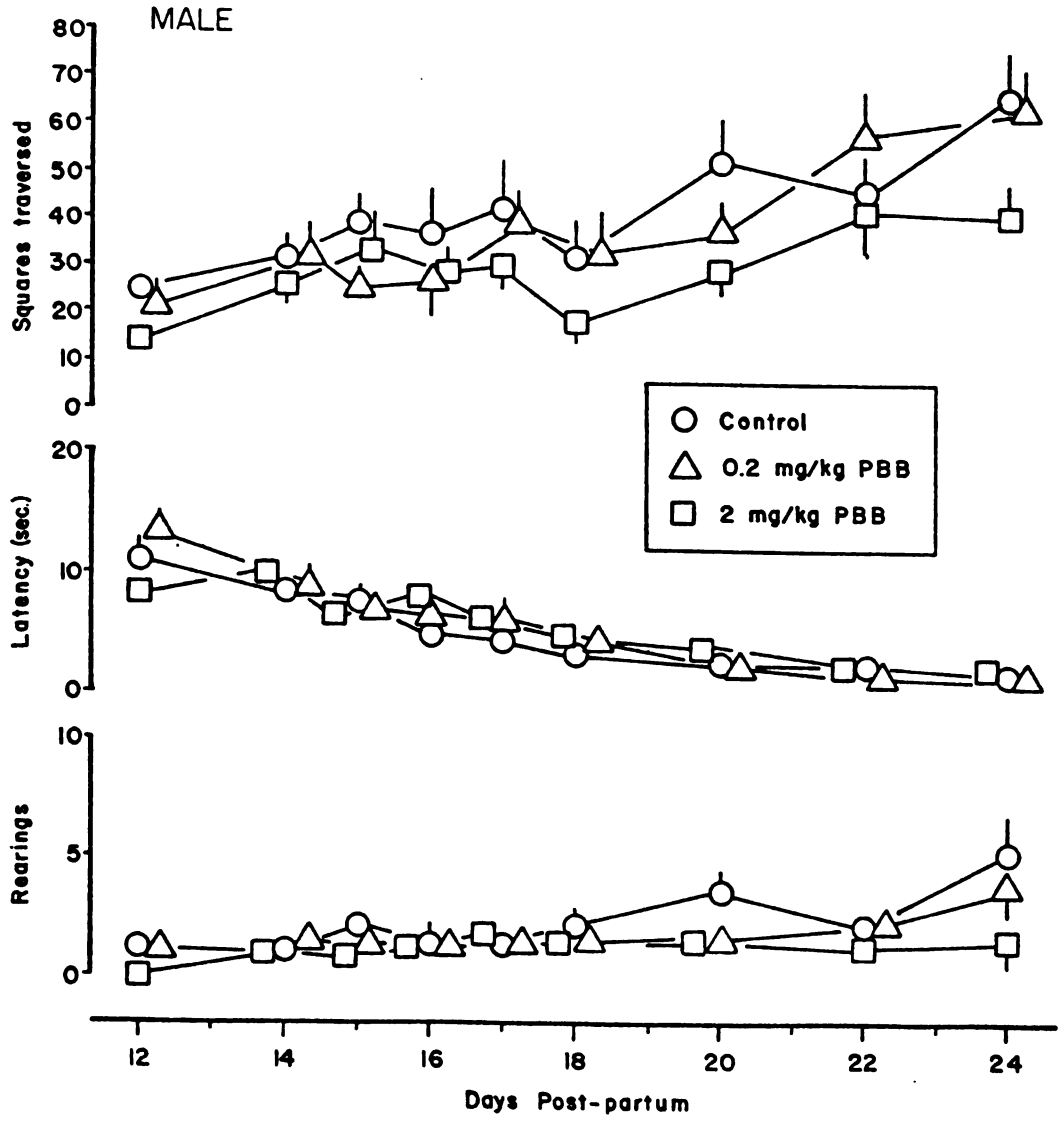

MALE
Squares traversed
80
70
60
50
40
30
20
10
0
Latency (sec.)
20
10
0
Rearings
10
5
0
Control
0.2 mg/kg PBB
2 mg/kg PBB
12
14
16
18
20
22
24
Days Post-partum

Figure 15

Figure 16. Open field activity of female offspring from dams administered fireMaster BP-6 (PBB) during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of 18 litters represented by one individual per litter. Analysis of variance revealed a significant difference ($p=0.0068$) between controls and offspring exposed to 2 mg/kg/day fireMaster with regard to squares traversed. Analysis of variance was not conducted on data obtained for number of rearings or latency to move from the center square because lack of PBB effect could be ascertained observationally.

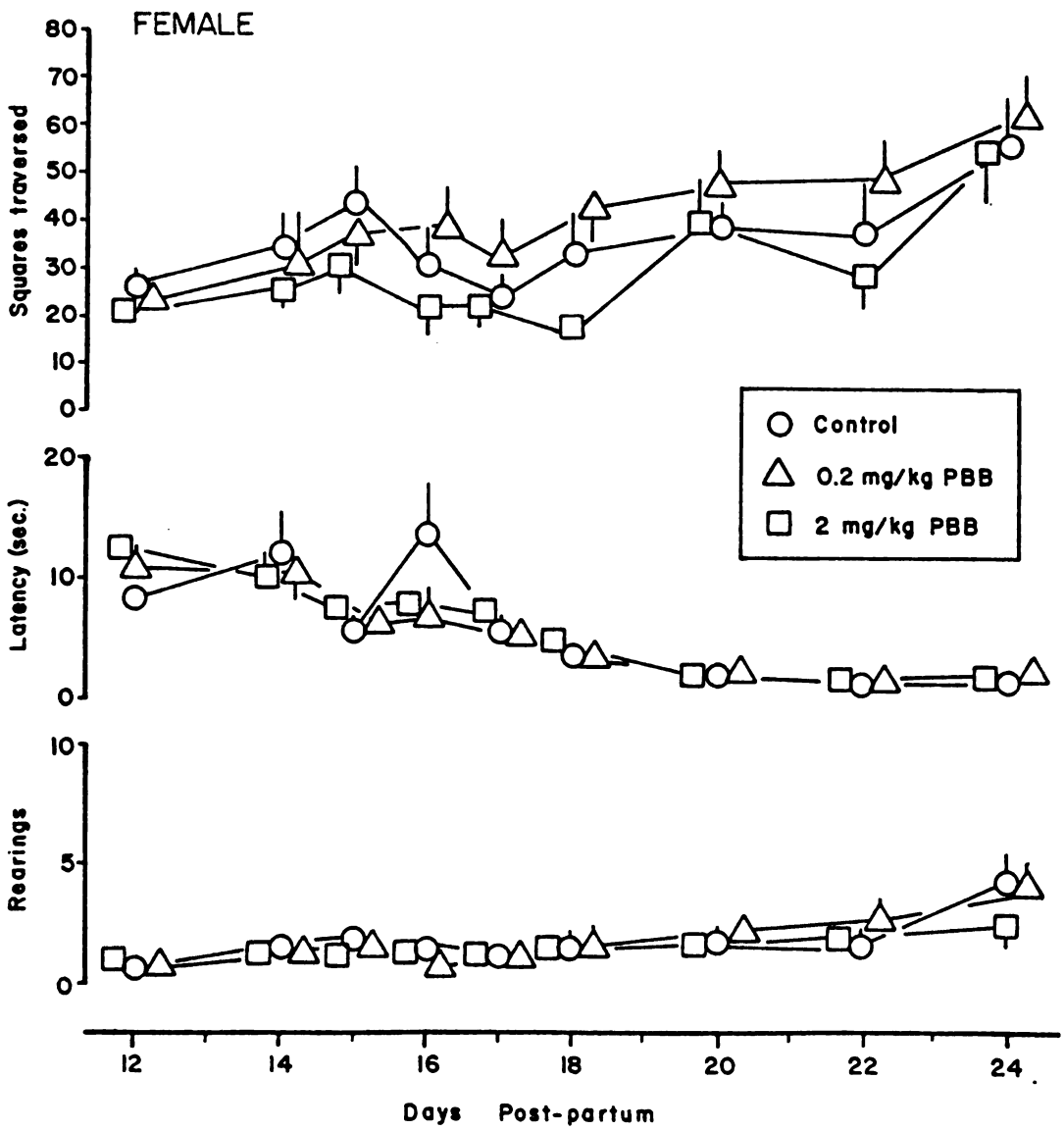

FEMALE
Squares traversed
80
70
60
50
40
30
20
10
0
Latency (sec.)
20
10
0
Rearings
10
5
0
Control
0.2 mg/kg PBB
2 mg/kg PBB
12
14
16
18
20
22
24
Days Post-partum

Figure 16

explanation for this may be in the fact that open field testing was conducted in a room separate from the home environment for probe study animals, but for final study animals was conducted in the room in which the pups had been raised, with the dam in close proximity. Similar results were obtained by Randall and Campbell (1976), who demonstrated that developing rats tested for locomotor activity in an isolated situation demonstrated a peak in activity on day 15, while those observed in the context of a normal litter showed no such peak.

c. Exploratory studies. Table 25 presents body weights of Group 1 animals from 3 to 12 months of age. A significant deficit in body weight was observed at 3 months of age only in female offspring from dams administered 2 mg/kg/day BP-6 during gestation and lactation. These data indicate that the body weight deficits observed early in life of offspring exposed to 2 mg/kg/day BP-6 (see Table 20) were recovered later in life, although a trend still existed up to 12 months of age for a deficit in body weight of males and females exposed to 2 mg/kg/day BP-6.

Table 26 presents results obtained from testing spontaneous alternation in Group 1 offspring. Entry into a novel arm of the Y-maze, which may be considered as a generalized measure of locomotor activity, decreased by nearly one-half from 2 to 12 months of age. At each of the 6 time points assessed, female offspring made a greater number of entries than did males. ANOVA revealed no PBB treatment-related effects at any of the time points.

Spontaneous alternation (as measured by % alternation) also tended to decrease with time, although not to as great an extent as

TABLE 25

Mean (+ S.E.M.) Body Weights of Rats Tested Chronically for Spontaneous Alternation and Estrous Cycle Length Following Perinatal Exposure to fireMaster BP-6 (PBB)

Months Postpartum	Body Weight (grams)					
	0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day	
	Male	Female	Male	Female	Male	Female
3	371.9+ 8.1 (18)	231.3+4.3 (16)	362.5+ 8.1 (15)	234.9+4.5 (15)	343.1+ 5.8 (17)	214.1+4.5* (17)
4	410.4+ 8.8 (15)	245.0+4.1 (16)	394.1+ 7.0 (16)	251.2+4.2 (16)	379.4+ 6.6 (17)	230.8+4.7 (16)
6	447.5+ 9.3 (17)	267.6+3.7 (16)	431.2+ 8.7 (16)	266.5+4.1 (16)	421.8+ 5.8 (17)	258.6+6.9 (16)
9	475.7+13.3 (15)	280.0+5.0 (15)	466.8+10.0 (15)	281.1+4.8 (15)	449.8+10.5 (16)	273.4+7.5 (15)
12	489.6+11.7 (11)	290.3+7.2 (9)	489.9+12.2 (11)	287.9+8.8 (10)	484.8+16.0 (9)	277.9+9.7 (9)

*Significantly different from control mean by ANOVA and least significant differences test, $p<0.0144$.

Numbers in parentheses = N

TABLE 26

Spontaneous Alternation of Offspring from Dams Administered fireMaster BP-6 (PBB) During Gestation and Lactation

ENTRIES

Months Postpartum	Dose (mg/kg/day BP-6)					
	0		0.2		2	
	Male	Female	Male	Female	Male	Female
2	26.4+1.9[a] (17)	30.5+1.2 (17)	28.1+0.9 (16)	33.7+1.6 (15)	27.1+1.8 (16)	33.1+1.3 (16)
3	28.1+1.5 (17)	29.9+1.4 (17)	27.7+0.7 (15)	28.1+1.3 (15)	25.7+2.0 (17)	31.5+1.5 (17)
4	21.0+2.0 (17)	29.9+1.7 (17)	21.6+1.7 (16)	26.8+1.0 (16)	24.1+1.4 (17)	30.4+1.3 (16)
6	21.1+1.5 (17)	28.6+1.7 (15)	18.4+1.6 (16)	24.0+0.9 (16)	21.9+1.6 (17)	27.2+1.4 (16)
9	16.5+1.8 (16)	22.9+1.5 (16)	16.9+1.3 (16)	19.0+0.9 (16)	17.4+1.4 (15)	21.8+1.2 (14)
12	12.1+2.0 (11)	19.6+1.1 (10)	12.3+1.7 (13)	19.9+1.8 (11)	14.6+1.6 (15)	20.1+1.0 (14)

Numbers in parentheses = N

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TABLE 26 (continued)

% ALTERNATIONS

Months Postpartum	Dose (mg/kg/day)					
	0		0.2		2	
	Male	Female	Male	Female	Male	Female
2	70.1±3.2[a]	67.8±2.9	67.8±3.4	62.0±3.5	65.3±2.6	70.8±2.7
3	69.9±3.8	66.3±2.2	71.9±2.4	68.1±2.8	65.8±3.1	62.1±2.1
4	60.7±5.8	66.6±2.6	66.3±2.9	59.3±3.6	66.1±2.4	62.8±3.0
6	59.1±3.7	55.0±3.9	53.9±5.3	56.9±3.9	67.8±3.3	61.8±3.0
9	62.1±2.8	58.4±2.5	67.9±3.3	60.5±3.5	54.2±3.4	55.8±3.2
12	56.1±6.7	59.9±4.1	55.0±7.0	62.1±3.1	62.4±5.8	69.0±3.1

[a]Mean ± S.E.M. All means from PBB-exposed animals were comparable to those of controls.

did entries. No obvious sexual dimorphism was observed. As with entries, no PBB treatment-related effects were observed.

Analysis of estrous cycle length (Table 27) revealed no PBB treatment-related effects from 2 through 12 months of age. Some control and PBB-exposed females had irregular estrous cycles indicative of the onset of the anestrous state as early as 6 months of age. Although not significant by Fisher's exact probability test, the number of females with irregular cycles in the 2 mg/kg/day BP-6 exposure group was double that of control females and females in the 0.2 mg/kg/day BP-6 exposure group at 8 months of age and nearly double at 10 months of age. A plateau appeared to be achieved by 12 months of age.

D. Discussion

No PBB-related effects were observed for maternal body weight, gestation length, litter size, sex ratio of the offspring, or pup mortality between birth and weaning in either dose group examined. Thus, the behavioral and physical effects observed in the present study occurred in the absence of overt toxicity to the dams or mortality to the embryo or fetus.

Administration of 0.2 or 2 mg/kg/day BP-6 to dams during gestation and lactation resulted in decreased crown-rump length of male offspring, while administration of 2, but not 0.2, mg/kg/day BP-6 resulted in decreased body weight gain in male and female offspring compared to controls. In addition, the pattern of body weight gain for the first 60 days of life was altered by BP-6 exposure. Monitoring of body weights of these animals to 1 year of age revealed that the body weight effects tended to diminish with time.

TABLE 27

Length of the Estrous Cycle and Onset of Anestrous in Female Offspring from Dams Administered fireMaster BP-6 (PBB) During Gestation and Lactation

ESTROUS CYCLE LENGTH (DAYS)

Months Postpartum	Dose (mg/kg BP-6)		
	0	0.2	2
2	4.4+0.2[a] (T7)	4.2+0.2 (T6)	4.4+0.1 (T7)
4	4.2+0.2 (T7)	4.1+0.2 (T6)	4.4+0.2 (T7)
6	4.1+0.3 (T5)	4.4+0.2 (T5)	4.5+0.2 (T5)
8	4.5+0.3 (T3)	4.0+0.2 (T4)	4.4+0.3 (T9)
10	4.6+0.2 (T9)	4.1+0.4 (T7)	4.3+0.3 (T4)
12	---[b]	---[b]	---[b]

% OF ANIMALS WITH IRREGULAR ESTROUS CYCLES

Months Postpartum	Dose (mg/kg BP-6)		
	0	0.2	2
2	0	0	0
4	0	0	0
6	6.3	6.3	6.3
8	12.5	12.5	25.0
10	40.0	41.7	71.4
12	93.3	80.0	90.9

[a]Mean ± S.E.M.

[b]No mean available due to irregularity of estrous cycles.

All values from PBB-exposed animals were comparable to those of controls.

Numbers in parentheses = N.

PBB levels in abdominal body fat of exposed offspring at 2 months of age are probably very close to peak levels, as exposure terminated at 24 days of age and the half-life of PBB in the fat of rats after a single intraperitoneal dose was determined to be 69.3 weeks (Miceli and Marks, 1981). It is difficult to compare tissue analysis obtained in the present study with results obtained by other authors. McCormack and coworkers (1981) analyzed tissue levels of PBB at 28 days of age in offspring from dams administered 10 or 100 ppm BP-6 during gestation and lactation. Levels of PBB in liver were 18 (10 ppm group) or 410 μg/g wet tissue (100 ppm group) and in fat were 162 (10 ppm group) or 1693 μg/g wet tissue (100 ppm group). By contrast, rats killed at 14 days of age following perinatal exposure to 50 ppm BP-6 had liver PBB levels of only 10 μg/g wet tissue (Rickert et al., 1978).

In the present study the highest dose of 2 mg/kg/day was equivalent to 40 ppm (based on a daily food consumption of 15 g), while the lowest dose of 0.2 mg/kg/day was equivalent to 4 ppm. Based on the McCormack et al. (1981) study, PBB levels in fat were four to ten times that in liver; thus, it could be postulated that PBB levels in fat in the Rickert et al. (1978) study might have been in the range from 40 to 100 μg/g wet tissue. Therefore, the values of 36.3 and 35.8 μg/g wet tissue obtained for offspring in the present study exposed to 2 mg/kg/day BP-6 are in line with the Rickert et al. analysis.

The PBB fat levels observed in the present study are well within the range of levels observed in highly exposed farmers (4.92 ppm) and Michigan Chemical Company production workers (196.5 ppm; Wolff et al., 1979). In addition, 2 of the 18 children reported as having possible

learning disabilities due to PBB exposure had fat levels of 13.27 and 20.96 ppm (Schwartz and Rae, 1983).

No PBB-related effects were observed in the latency to eye opening, testes descent, or vaginal opening.

Confirmatory analysis of acquisition of locomotion, cliff avoidance, cage emergence, and open field activity revealed a significant overall study effect. MANOVA revealed a significant effect overall for the acquisition of locomotion by pups from dams administered 2 mg/kg BP-6. ANOVA of each stage revealed a significant delay in acquisition of forward locomotion with the head low and body raised and forward locomotion with the head and body raised. Thus, a deficit was observed as locomotion became more complex. Significant effects for litter were observed with the more primitive stages of locomotion. However, these effects disappeared as locomotion became more complex, indicating that the litter effects observed in this paradigm were overcome with increasing age and behavioral complexity.

A trend toward delayed acquisition of cliff avoidance was observed for male and female PBB-exposed offspring which was not in itself significant, but rather contributed to the significance of the overall study MANOVA. A significant sex effect revealed the presence of a sexually dimorphic component to this behavior. No significant litter effects were observed.

Cage emergence is a relatively simple test which measures the response of an animal to a novel environment. In the present study, a marked difference in the willingness to enter a novel environment (i.e., emerge from the cage to the tabletop) was observed between male and female rats. Males were quite hesitant to emerge, while females emerged

readily. These data are consistent with those obtained by Ader and Conklin (1963) in offspring from dams handled frequently during gestation. Although no statistically significant PBB-related effects were observed, male offspring exposed to 2 mg/kg BP-6 tended to emerge more readily than did controls, while females exposed to 2 mg/kg BP-6 tended to emerge less readily than did controls. These data would indicate a possible release of inhibitions in male offspring exposed to PBB, and a heightening of inhibitions in females. No significant litter effects were observed.

Confirmatory studies were conducted to further characterize the PBB-related changes in acquisition of open field activity observed in the probe study. Results of these studies were inconsistent with probe study results. Activity levels were reduced and no clear-cut activity peak was observed on day 15 postpartum. These discrepancies were thought to relate to methodology. Probe study offspring were tested in a room far removed from their dams and home environment, while final study animals were tested only a few feet from their dams in the room in which they were raised. Randall and Campbell (1976) demonstrated that developing rats tested alone attained peak locomotor activity at 15 days of age, while no such peak was observed for developing rats tested in the presence of the normal litter environment, four siblings, an anesthetized lactating female, or an anesthetized adult male. These authors postulated that peak activity observed in the rat at 15 days of age was due in part to isolation distress, rather than entirely to maturation of different neurotransmitter systems. Thus, isolated neonates responded to the novel environment of the open field with hyperactivity. It may be postulated that since final study animals were tested in an

environment with familiar sounds and smells, their activity response was unlike that of the probe study animals who were tested in a totally novel environment. Despite their lack of response to the novel environment, however, offspring from dams administered 2 mg/kg BP-6 traversed significantly fewer squares than did controls across the entire 12-day observation period. Lack of significance in the interaction term of the ANOVA revealed that this was a generalized depression of activity compared to controls, rather than a shift in the development of acquisition. However, significant effects were observed for litter, days, and the litter and sex interaction for offspring exposed to either dose of BP-6. These results underscore the complexity of this behavioral response.

The significance of the overall study MANOVA revealed that the original hypothesis advanced was true; perinatal PBB exposure resulted in behavioral changes early in life.

Exploratory locomotor activity tests revealed a possible cholinergic deficit in PBB-exposed animals. Therefore, additional animals exposed to PBB perinatally were monitored periodically for spontaneous alternation in a Y-maze for one year. Number of entries into each arm of the maze during the test session decreased over the course of one year, an habituation phenomenon observed previously by Swonger and Rech (1972) upon repeated exposure to the testing apparatus. This activity may be considered a generalized locomotor response to a novel environment and would be expected to decrease as the novelty faded. No differences were observed between control and PBB-exposed animals with regard to number of entries at any time during the one-year monitoring period.

Females of all groups made slightly more entries than did males, but this is in keeping with the fact that activity levels are higher in females than in males (Norton, 1982).

Spontaneous alternation, which requires the animal to remember the arm of the maze visited least recently, did not decrease to an appreciable extent in any animals on test over the one-year period; the small decrement observed was similar to that observed by Swonger and Rech (1972) over a 5-week period of testing. These authors attributed the decrease to habituation; therefire, it may be concluded that age-related changes in cholinergic systems did not produce deficits in spontaneous alternation up to one year of age in animals of the current study. No PBB treatment-related effects were observed for spontaneous alternation at any time point assessed, indicating absence of a deficit in short-term memory.

In the present study, female offspring from dams administered 2 mg/kg/day BP-6 displayed irregular estrous cycles at an earlier age than did controls or offspring exposed to 0.2 mg/kg/day BP-6; many of these animals, as well as controls, displayed persistent estrus. However, both controls and PBB-exposed females with irregular cycles displayed a variety of abnormalities in addition to persistent vaginal estrus. This is contrary to the viewpoint expressed by Johnston et al. (1980) and Lambrecht et al. (1978) that reproductive abnormalities observed in female rats following PBB exposure are due to enhanced metabolism of steroids. Therefore, the mechanism of action of enhanced reproductive aging due to perinatal PBB exposure is unclear and requires further elucidation.

CHAPTER 3

EFFECTS OF PERINATAL PBB EXPOSURE ON LOCOMOTOR ACTIVITY

A. Introduction

Testing of drug-induced stimulation of accommodated locomotor activity during the probe study revealed possible PBB interaction with dopaminergic, cholinergic or serotonergic neuronal systems. The interaction with dopaminergic systems was the most clear-cut. To clarify these effects confirmatory studies were conducted on locomotor activity following distilled water, d-amphetamine, scopolamine or fenfluramine. Experimental subjects were offspring from dams administered 0, 0.2 or 2 mg/kg/day BP-6 from day 6 of gestation through day 24 of lactation.

The hypothesis tested in the present study was that perinatal PBB exposure results in disruption of drug-induced accommodated locomotor activity. In addition to the study designed to test this hypothesis, exploratory studies were conducted to define the exact nature of the interaction of PBB with dopaminergic systems.

Probe studies revealed that PBB-induced deficits in responding to d-amphetamine and scopolamine by females was related to stage of the estrous cycle. Therefore, all females of the confirmatory study were tested in this regard following d-amphetamine, scopolamine or fenfluramine. Open field activity following distilled water or d-amphetamine

was assessed during estrus or following ovariectomy with or without hormone replacement to further characterize estrous cycle effects.

B. Materials and Methods

1. Experimental Subjects

Experimental subjects were offspring from a colony of female Sprague-Dawley rats administered 0, 0.2 or 2 mg/kg/day BP-6 in a peanut butter "treat" from day 6 of gestation through day 24 of lactation. The source of this colony, housing conditions, and breeding regimen are described in Chapter 2.

Body weights and general appearance of all dams were monitored throughout gestation and lactation. Gestation length, number of pups born alive, sex ratio of the pups, and mortality from birth through weaning were also assessed. All maternal and developmental parameters were comparable between controls and PBB-exposed animals, as reported in Chapter 2.

Offspring from each litter were cross-fostered as soon after birth as possible with a dam of the same dose group. At 3 days of age all litters were culled to four pups per sex where possible. At this time indelible ink was injected subcutaneously into the paws of the pups in a specific pattern for the purpose of identification. Females were randomly assigned numbers 1 through 4, while males were randomly assigned numbers 5 through 8. Pups remained with their foster mother until weaning on postpartum day 24 and were housed two or three per cage thereafter according to BP-6 treatment and sex.

Male number 6 and female number 2 were assigned to Group 2: the group tested for PBB-related effects on locomotor activity. Table 28 lists all parameters measured in Group 2 animals, the method of statistical analysis used in each case, and the partitioning of α according to groups of studies.

2. Assessment of Locomotor Activity

a. "Incidental" data. These data were considered incidental to, but supportive of, the primary goal of testing in Group 2 animals: assessment of BP-6 effects on accommodated locomotor activity with drug challenge.

All group members were weighed immediately prior to testing at 3, 5 and 7 months of age. Body weight data were analyzed by a repeated measures ANOVA nested for sex and litter. Two ANOVA were conducted: one to compare results of 0, 0.2 and 2 mg/kg/day offspring and one to compare results of 0 and 0.2 mg/kg/day offspring.

b. Exploratory studies. Accommodated locomotor activity with l-DOPA. Data from probe study animals indicated that accommodated locomotor activity was stimulated less by d-amphetamine in PBB-exposed offspring than in controls. d-Amphetamine has been shown to stimulate motor activity by acting primarily on dopaminergic neurons to enhance release of dopamine and to inhibit dopamine reuptake, resulting in decreased inactivation (Rech and Stolk, 1970; Iversen and Iversen, 1981). Therefore, it could be postulated that perinatal PBB exposure results in deficits in dopaminergic systems by affecting dopamine synthesis, release, inactivation or receptor activation. Because of the indirect mechanism of d-amphetamine action, it could further be

TABLE 28

Parameters Measured in Group 2 Animals, Including Method of Statistical Analysis and Partitioning of α

Parameter	Statistical Analysis	α
A. "Incidental" data		
1. Body Weight	Nested repeated measured ANOVA	0.025[a]
B. Exploratory Studies		
1. Accommodated locomotor activity		
a. l-DOPA	Multifactorial ANOVA with mean comparisons	0.025[b]
2. Dopaminergic neuronal function		0.025[b]
a. Drug challenge A	Multifactorial ANOVA with mean comparisons	0.0177[c]
b. Drug challenge B	Multifactorial ANOVA with mean comparisons	0.0177[c]
3. Ovariectomy Study	Multifactorial ANOVA with mean comparisons	0.025[b]
4. Drug challenge estrous cycle study		0.025[b]
a. d-Amphetamine	Multifactorial ANOVA	0.0144[d]
b. Scopolamine	Multifactorial ANOVA	0.0144[d]
c. Fenfluramine	Multifactorial ANOVA	0.0144[d]

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TABLE 28 (continued)

C. Confirmatory Studies - Accommodated locomotor activity with drug challenge, $\alpha = 0.025$[e]

Parameter	Statistical Analysis	α
1. Distilled water	Nested repeated measures ANOVA	0.0125[f]
2. d-Amphetamine	Same as 1.	0.0125[f]
3. Scopolamine	Same as 1.	0.0125[f]
4. Fenfluramine	Same as 1.	0.0125[f]

[a] α arbitrarily assigned as 0.025 to correspond with overall group level.

[b] α partitioned among 4 tests; therefore, $\alpha = \frac{.05}{\sqrt{4}} = 0.025$.

[c] α partitioned among 2 tests; therefore, $\alpha = \frac{.025}{\sqrt{2}} = 0.0177$.

[d] α partitioned among 3 tests; therefore, $\alpha = \frac{.05}{\sqrt{3}} = 0.0144$.

[e] α partitioned among Groups 1-4; therefore, $\alpha = \frac{.05}{\sqrt{4}} = 0.025$.

[f] α partitioned among 4 tests; therefore, $\alpha = \frac{.025}{\sqrt{4}} = 0.0125$.

postulated that PBB exerts its effects on dopaminergic neurons presynaptically. To investigate the possibility of postsynaptic disruption, accommodated locomotor activity was assessed following injection of 250 mg/kg of the dopamine precursor l-dihydroxyphenylalanine (l-DOPA). l-DOPA is converted centrally and peripherally to dopamine via the enzyme l-amino acid decarboxylase ("DOPA decarboxylase"). Peripheral conversion to dopamine is prevented by pretreatment with DOPA decarboxylase inhibitors such as Ro4-4602 (also known as benserazide). This compound does not cross the blood brain barrier when administered in small doses and therefore allows for a much larger proportion of a dose of l-DOPA to act centrally (Thut and Rech, 1972). l-DOPA (Hoffman-LaRoche, Nutley, NJ) was suspended in 0.5% methyl cellulose, while Ro4-4602 (Hoffmann-LaRoche, Inc., Nutley, NJ) was dissolved in distilled water. Due to difficulty in suspending l-DOPA, it was administered as a 125 mg/ml solution. Ro4-4602 was administered as a 50 mg/ml solution to provide a dose of 50 mg/kg.

Eight male and 8 female Group 2 offspring were tested at a mean age of 9 months for accommodated locomotor activity according to the method outlined in Chapter 1. All drugs were dissolved in distilled water and administered intraperitoneally. Each rat was placed in a 30 (L) x 35 (W) x 16 (H) cm plastic cage containing a cylinder 15 cm in diameter, around which activity was linearly directed. The motor activity cage contained bedding of the same type as that found in the home cage (Bed O'Cobs, The Andersons, Maumee, OH). Activity was monitored by a Stoelting activity meter, model 31400, which records the number of times the animal disrupts the electromagnetic field generated over the surface of the apparatus. The entire motor activity apparatus was

located in a sound-attenuating box to eliminate environmental disruptions. All experiments were controlled by electromechanical programming circuits. The number of activity counts per minute was recorded for each animal. It is well known that female rats tend to be more active than males (Norton, 1982) and that nocturnal activity exceeds diurnal activity. In order to equalize activity as much as possible, males were tested from 0800 to 1300 hours (at the end of the nocturnal phase), while females were tested from 1200 to 1700 hours (during the diurnal phase).

Motor activity was measured for each animal for 1 hour to allow the animal to acclimate to its surroundings. At the end of 1 hour the rat was quickly removed from the motor activity cage and injected intraperitoneally with the selected drug.

An exception to this methodology in the present experiment was that each animal was injected intraperitoneally with 50 mg/kg Ro4-4602 immediately prior to initial introduction into the locomotor activity cage. Following a 1-hour period of accommodation, each rat was quickly removed from the apparatus, injected intraperitoneally with 250 mg/kg l-DOPA, and its activity monitored for an additional 3 hours. Data were analyzed by multifactorial ANOVA with paired comparisons using the least significant difference test. Factors were BP-6 dose and observation time.

Dopaminergic neuronal function. To further investigate the dopaminergic disruption potentially caused by PBB exposure, eight male Group 2 offspring from each dose group were tested for unaccommodated locomotor activity at approximately 10 months of age. Each animal

was placed in the motor activity apparatus described previously. No acclimation period was provided, thus giving higher baseline activity due to the novelty of the environment. The total activity session was 15 minutes in length, with monitoring occurring in 5-minute intervals. All drugs used for this study were dissolved in distilled water with the exception of α-methyltyrosine, which was suspended in 0.5% methyl cellulose. All drugs were administered intraperitoneally in equal volumes. Testing was conducted from 0900 to 1300 hours.

Drug challenge A was designed to determine if BP-6 exposure resulted in disruption of dopamine synthesis. α-Methyltyrosine (αMT) inhibits tyrosine hydroxylase: the enzyme responsible for conversion of the amino acid tyrosine to l-DOPA. This conversion is the rate-limiting step in the synthesis of catecholamines (Cooper et al., 1979). Thus, inhibition of this enzyme eliminates dopaminergic influence and has been shown to cause prominent behavioral depression (Rech et al., 1966). Rats were administered the following drugs intraperitoneally and tested once weekly: distilled water (15-minute pretreatment), 2 mg/kg d-amphetamine (15-minute pretreatment), 100 mg/kg αMT (Merck Institute for Therapeutic Research, West Point, PA; 120-minute pretreatment), and a combination of 2 mg/kg d-amphetamine and 100 mg/kg αMT.

Drug challenge B was conducted to determine if perinatal BP-6 exposure resulted in disruption of the storage capacity of catecholaminergic neurons. Reserpine is the drug normally used to deplete storage granules of catecholamines. However, this depletion and the resultant behavioral depression are extremely long-lasting. Rech and coworkers (1968) determined that Ro4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydrobenzo-(α)-quinolizine) depletes

brain catecholamines and induces behavioral depression of very short duration. In addition, these researchers determined that the behavioral depression induced by a combination of Ro4-1284 and α-methyltyrosine was greater than that induced by either drug alone. These drug interactions were investigated in control and PBB-exposed offspring. Rats were administered the following drugs intraperitoneally and tested once weekly: distilled water (15-minute pretreatment), 100 mg/kg αMT (120-minute pretreatment), 1 mg/kg Ro4-1284 (30-minute pretreatment; Hoffman-LaRoche, Nutley, NJ), and a combination of 100 mg/kg αMT and 1 mg/kg Ro4-1284. Comparison of control and PBB-exposed groups was conducted by multifactorial ANOVA (factors were BP-6 dose, observation time, and challenge drug dose); means were compared using the least significant difference test.

Drug challenge - estrous cycle. Results of accommodated locomotor activity testing of female probe study offspring revealed a selective PBB effect following d-amphetamine or scopolamine drug challenge according to stage of the estrous cycle. It was postulated that enhanced PBB effects during stages of the estrous cycle characterized by high estrogen titer might be due to enhanced metabolism of steroid hormones by PBB-induced mixed function oxidases. To investigate the role of estrogen in this behavioral modification, six (control) or eight (0.2 and 2 mg/kg BP-6) Group 2 females were tested no more than once weekly at approximately 10 months of age for open field activity in the manner described in Chapter 1 under the following drug and hormonal conditions: 1) distilled water during estrus (30-minute pretreatment), 2) 2 mg/kg d-amphetamine (30-minute pretreatment) during estrus, 3)

distilled water 2 weeks following ovariectomy, 4) 2 mg/kg d-amphetamine 3 weeks following ovariectomy, 5) distilled water following ovariectomy and subsequent injection with estrogen and progesterone, and 6) 2 mg/kg d-amphetamine following ovariectomy and subsequent injection with estrogen and progesterone.

Prior to testing, vaginal smears were obtained from all females according to the method described in Chapter 1 for at least 1 week to ensure that all had normal cycles. One week following the first dose of d-amphetamine, each rat was anesthetized with a combination of ketamine and acepromazine (5:1), and ovariectomy was performed. One week after surgery, vaginal smears were obtained from all rats for 1 week to ensure that ovariectomy was successful. Behavioral testing was initiated the following week.

When testing of ovariectomized animals was complete, each was injected subcutaneously with 0.1 mg/kg estradiol benzoate (Sigma Chemical Company, St. Louis, MO) as a suspension in 0.5% methyl cellulose at 0800 hours. Forty-eight hours later, each rat received an injection of 1 mg/kg progesterone (Sigma Chemical Company, St. Louis, MO) as a suspension in 0.5% methyl cellulose at 0800 hours. Estrogen and progesterone administered at these appropriate time intervals have been shown to produce sexual behavioral estrus (Telegdy and Stark, 1973). At 1400 hours of the same day as the progesterone injection, each rat was injected with distilled water and tested for open field activity 30 minutes later. This same procedure was followed 1 week later prior to testing with d-amphetamine.

Numbers of squares traversed and rearings were evaluated for treatment effects via multifactorial ANOVA (factors were BP-6 dose, estrous cycle condition, and drug challenge); mean comparisons were made by the least significant difference test.

Female rats tested for d-amphetamine, scopolamine and fenfluramine-induced stimulation of accommodated locomotor activity in the confirmatory studies of Group 2 offspring (to be addressed subsequently) were retested 2 weeks later at a different stage of the estrous cycle than that at which they were tested originally. Equipment and procedures were the same as those outlined in Chapter 1. Treatment effects for each drug were analyzed by multifactorial ANOVA. Factors were BP-6 dose, estrous cycle stage, and observation time.

c. Confirmatory studies. Beginning at 3 months of age, all Group 2 offspring were assessed for drug-induced stimulation of spontaneous locomotor activity. This testing was conducted because PBB-related effects were observed for all drug challenges conducted in probe study animals. Equipment and methodology were the same as those described in section b of this chapter.

The first drug challenge was with intraperitoneally injected distilled water (day 1), followed by 2 mg/kg d-amphetamine (day 2). At 5 months of age, all Group 2 animals were tested for scopolamine-induced stimulation of locomotor activity. In contrast to the probe study, a dose of 1 mg/kg scopolamine was tested in males and females. This was deemed necessary because the 0.5 mg/kg dose tested in probe study males produced little stimulation of activity. At 7 months of age, all Group 2 animals were challenged with 10 mg/kg fenfluramine.

Treatment-related effects for each drug challenge were analyzed by a repeated measures ANOVA nested for sex.

C. Results

1. "Incidental Data"

Repeated measures ANOVA nested for sex and litter revealed a significant difference ($p<0.025$) in body weight between offspring exposed to 2 mg/kg/day BP-6 and controls (Table 29). This difference was manifested as a decreased body weight gain compared to controls throughout the 4-month observation time. No PBB-related effect on body weight was observed in offspring exposed to 0.2 mg/kg/day BP-6. Significant ($p<0.025$) effects for litter (sex age), and age and sex interaction were observed. When sexes were analyzed separately, it was discovered that the PBB treatment-related effect resided with the females only, although no group by sex interaction was observed. A trend for body weight deficit existed in males exposed to 2 mg/kg/day BP-6.

2. Exploratory Studies

a. Accommodated locomotor activity with l-DOPA. Activity of control and PBB-exposed rats upon introduction to the testing chamber (unaccommodated locomotor activity) was less (Figure 17) than that observed in all other experiments of this nature. Therefore, the peripheral decarboxylase inhibitor benserazide acted to decrease spontaneous locomotor activity. Subsequent injection of l-DOPA resulted in stimulation of accommodated locomotor activity. Peak stimulation was observed 90 to 105 minutes following injection. The degree and pattern of stimulation observed for males and females was essentially comparable for PBB-exposed offspring and controls.

TABLE 29

Mean (± S.E.M.) Body Weights of Rats Tested for Drug-Induced Stimulation of Spontaneous Locomotor Activity Following Perinatal Exposure to fireMaster BP-6 (PBB)

Months of Age	Body Weight (grams)					
	0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day	
	Male	Female	Male	Female	Male	Female
3	367.3+ 7.2 (17)	227.3+3.8 (17)	367.0+5.8 (17)	227.4+5.9 (17)	350.6+7.7 (17)	210.0+5.2 (17)
5	420.9+ 8.9 (17)	260.5+4.0 (17)	424.6+6.8 (17)	256.6+6.2 (17)	405.3+9.4 (16)	245.5+5.1 (17)
7	454.1+10.5 (16)	272.3+4.5 (16)	456.4+9.8 (17)	270.1+6.8 (17)	435.9+9.7 (17)	254.4+5.3 (17)

Numbers in parentheses = N.

[a]Significantly different from control mean by repeated measures ANOVA ($p<0.025$).

Figure 17. Accommodated locomotor activity following l-DOPA and benserazide in male and female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (◯), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point represents the mean and standard error of 8 litters represented by one male or one female per litter. Benserazide (50 mg/kg) was injected immediately prior to the initial 1-hour accommodation period. The point of injection of l-DOPA (250 mg/kg) is indicated by the arrow. Shaded symbols indicate difference from control mean by analysis of variance and the least significant difference test ($p<0.025$).

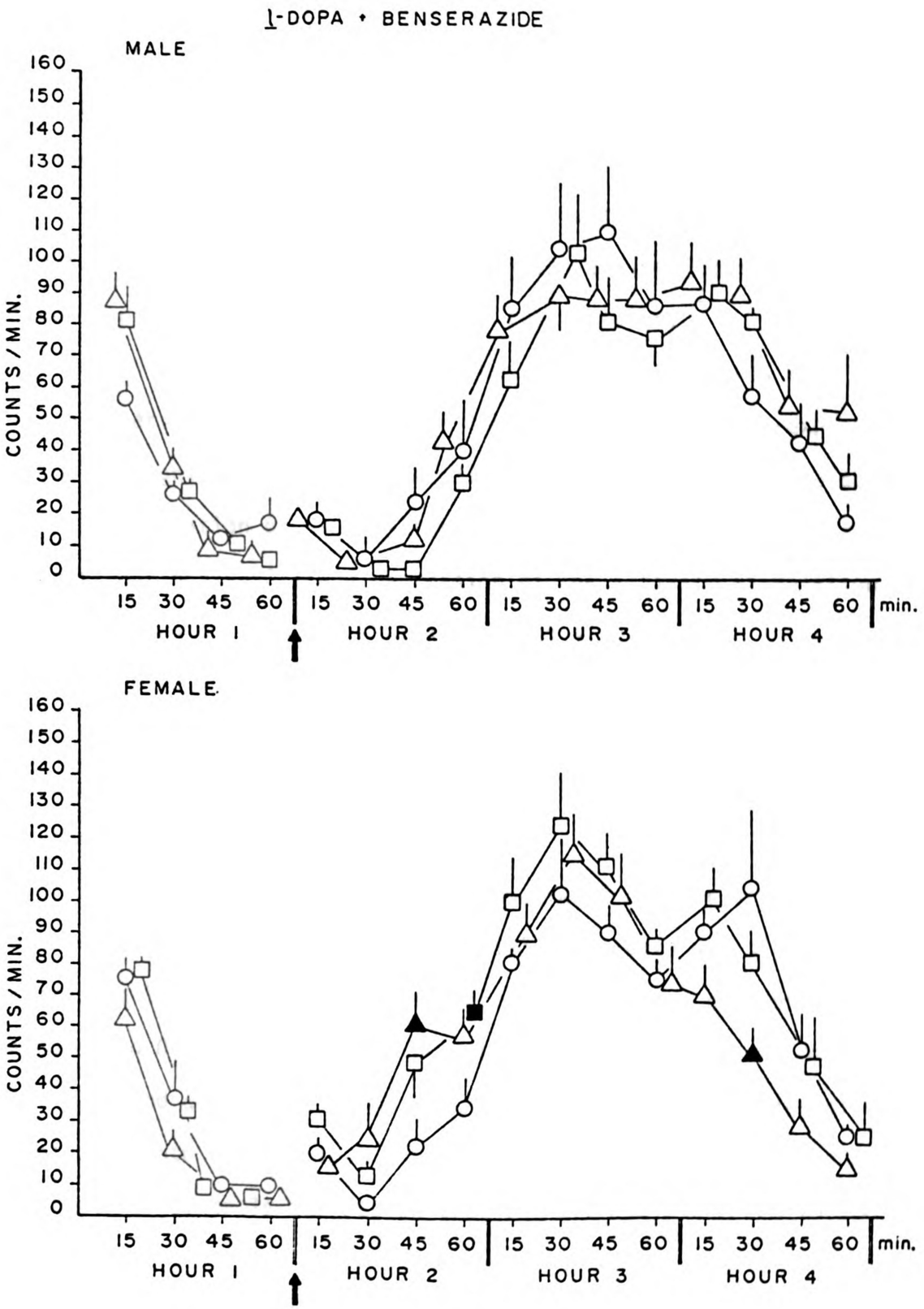

L-DOPA + BENSERAZIDE
MALE
COUNTS / MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
FEMALE
COUNTS / MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 17

b. Dopaminergic neuronal function. Locomotor response 10 minutes after introduction to the novel environment was significantly greater in offspring from dams administered 0.2 mg/kg/day BP-6 than in controls (Figure 18). A similar trend was observed in offspring from dams administered 2 mg/kg/day BP-6, resulting in a high baseline level of responding. Administration of d-amphetamine to control animals resulted in a significant increase in activity over baseline after 10 minutes. Activity of PBB-exposed animals remained comparable to baseline levels throughout the 15-minute test session.

α-Methyltyrosine depressed activity levels well below those observed with d-amphetamine and, in the case of PBB-exposed animals, significantly below baseline levels at the 10-minute observation. Combination of α-methyltyrosine and d-amphetamine resulted in a slight, but nonsignificant depression of the activity stimulation observed following d-amphetamine alone, and a reversal of the activity depression following α-methyltyrosine. This reversal was significant for control offspring at the 15-minute observation and for offspring exposed to 0.2 mg/kg/day BP-6 at 10 and 15 minutes, but not for offspring exposed to 2 mg/kg/day BP-6.

Depression of unaccommodated locomotor activity was also observed following Ro4-1284 (Figure 19). Activity was significantly decreased 10 minutes after introduction to the test chamber for animals exposed to 0.2 or 2 mg/kg/day BP-6 but not for controls. Combination of Ro4-1284 and α-methyltyrosine resulted in further depression of activity 5 minutes into testing than that observed with either drug alone. The activity of controls and PBB-exposed animals was decreased to the same

Figure 18. Unaccommodated 15-minute locomotor activity following distilled water, d-amphetamine, α-methyltyrosine, or a combination of d-amphetamine and α-methyltyrosine in male offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of eight litters represented by one male per litter. Distilled water (DW, 1 ml/kg) and d-amphetamine (d-A, 2 mg/kg) were injected 15 minutes prior to testing, while α-methyltyrosine (αMT, 100 mg/kg) was injected 120 minutes prior to testing. Asterisk indicates difference from control mean by analysis of variance and the least significant difference test ($p<0.0177$). Symbol shaded on right half is significantly different from distilled water, while symbol shaded on left half is significantly different from d-amphetamine. Symbol shaded on bottom half is significantly different from α-methyltyrosine.

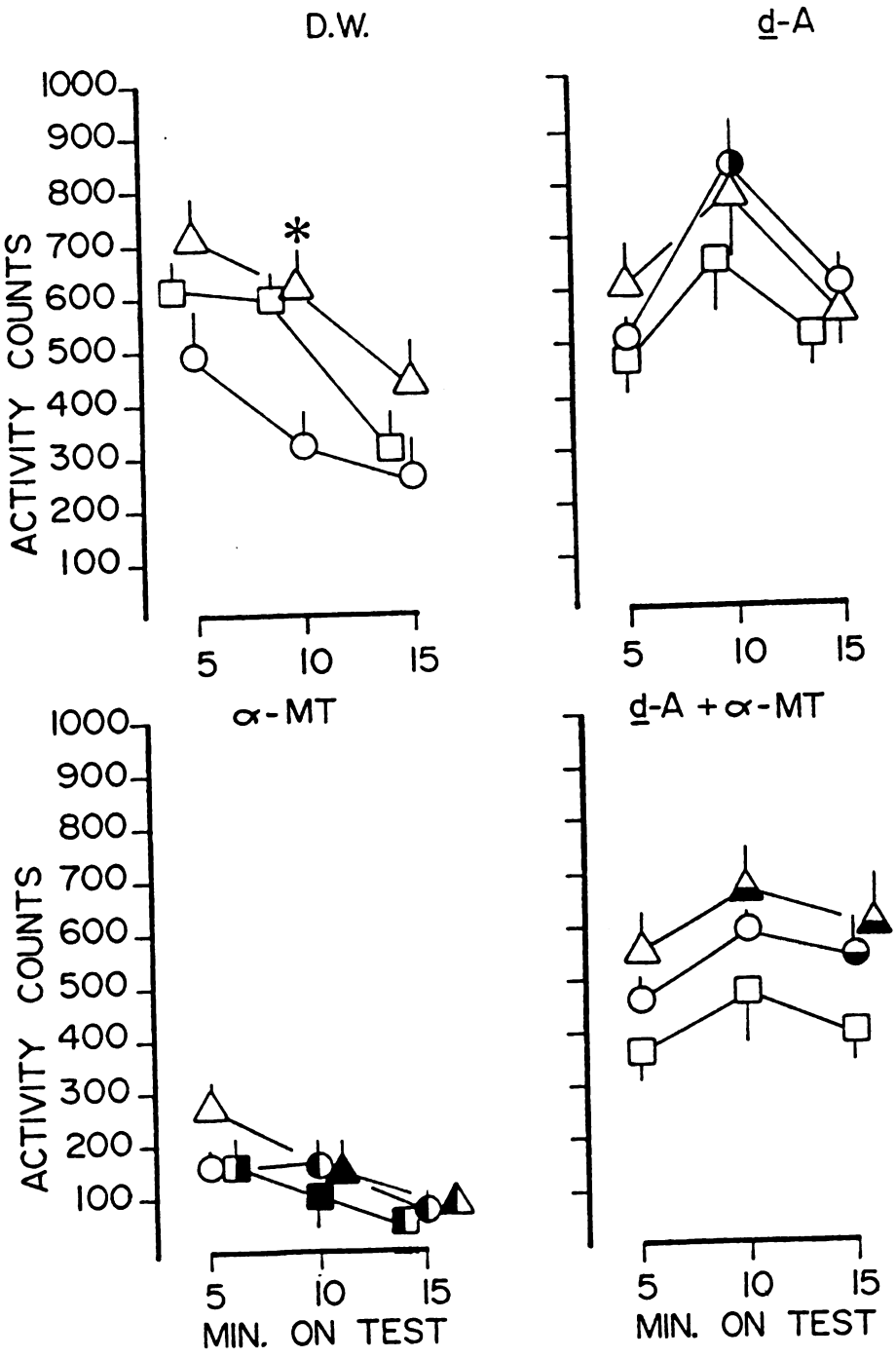

D.W.
d-A
1000
900
800
700
600
500
400
300
200
100
ACTIVITY COUNTS
*
5
10
15
α-MT
d-A + α-MT
MIN. ON TEST

Figure 18

Figure 19. Unaccommodated 15-minute locomotor activity following distilled water, α-methyltyrosine, RO4-1284, or a combination of α-methyltyrosine and RO4-1284, in male offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (◯), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of eight litters represented by one male per litter. Distilled water (DW, 1 ml/kg) was injected 15 minutes prior to testing, RO4-1284 (1 mg/kg) 30 minutes and α-methyltyrosine (αMT, 100 mg/kg) 120 minutes. Asterisk indicates difference from control mean by analysis of variance and the least significant difference test ($p<0.0177$). Symbol shaded on right half is significantly different from control mean.

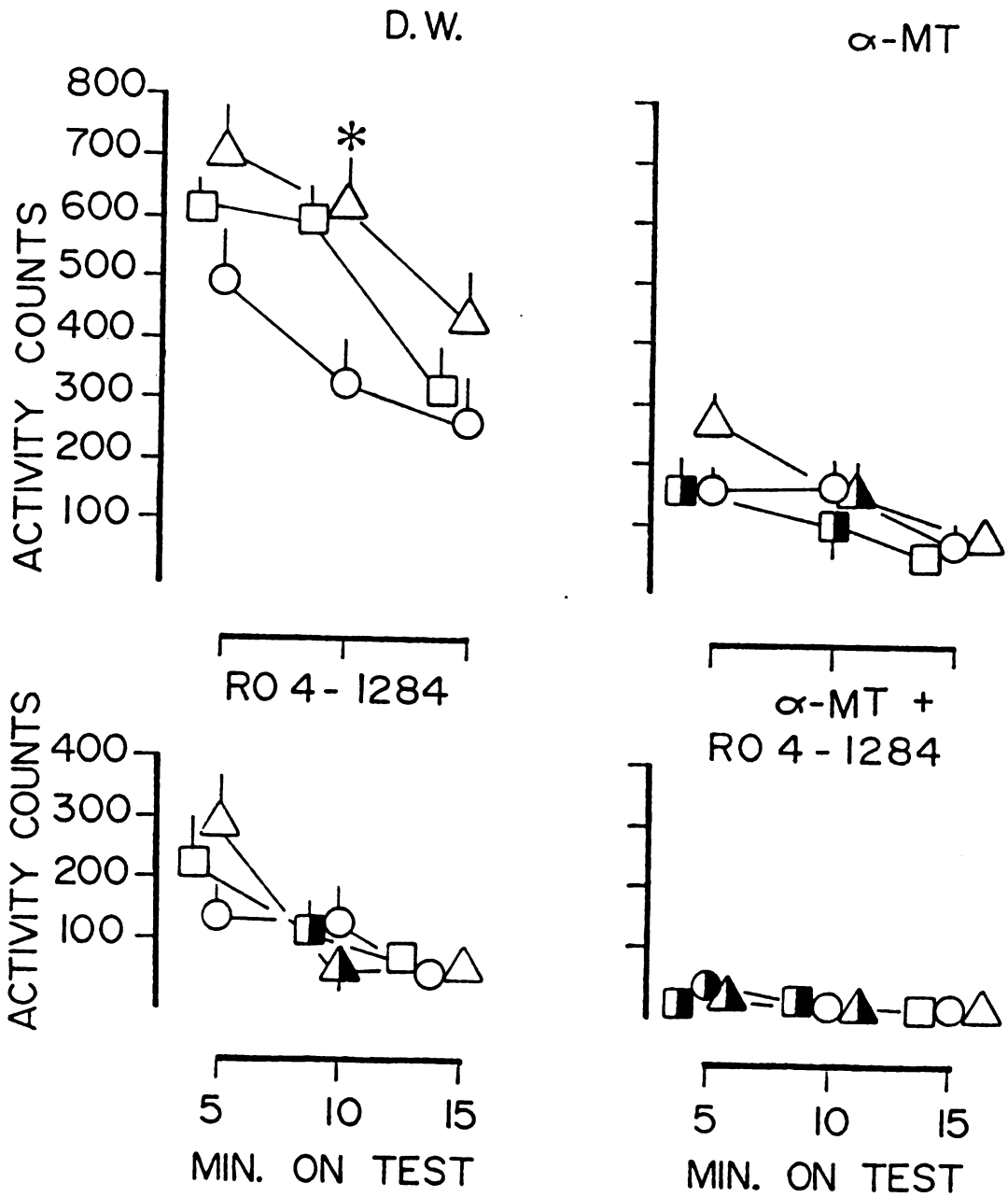

D.W.
α-MT
800
700
600
500
400
300
200
100
ACTIVITY COUNTS
*
RO 4 - 1284
α-MT +
RO 4 - 1284
400
300
200
100
ACTIVITY COUNTS
5
10
15
MIN. ON TEST
5
10
15
MIN. ON TEST

Figure 19

extent. The degree of activity suppression at 10 and 15 minutes was the same as that observed with either drug alone.

c. Drug challenge - estrous cycle. Figure 20 presents the number of squares traversed in an open field by female rats following manipulations of the estrous cycle. Although the number of squares traversed following distilled water injection was somewhat greater for PBB-exposed animals than for controls, it was not statistically significant. No change in baseline activity was observed due to ovariectomy or to ovariectomy followed by replacement of estrogen and progesterone.

Activity of control and PBB-exposed females was comparable following injection of d-amphetamine, and was significantly greater than that following distilled water. Although activity remained above baseline following d-amphetamine injection in ovariectomized control animals, it was significantly less than that observed in intact animals administered d-amphetamine during estrus. d-Amphetamine-induced stimulation of activity of PBB-exposed animals was comparable for both conditions. Replacement of estrogen and progesterone in ovariectomized animals did not reverse the effects observed following ovariectomy, with the exception that the number of squares traversed by females exposed to 2 mg/kg/day BP-6 was significantly greater than that of controls.

Number of rearings during the open field session was also assessed in these animals; these data are presented in Figure 21. No PBB treatment-related effects were observed following distilled water injection in intact, ovariectomized, or ovariectomized females with hormone replacement. d-Amphetamine enhanced the number of rearings observed in females under all 3 conditions of estrous cycle manipulation;

Figure 20. Squares traversed in an open field and response to d-amphetamine according to estrous cycle condition in female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (open bars), 0.2 (striped bars) or 2 mg/kg/day (shaded bars) fireMaster BP-6. Testing was conducted 30 minutes after distilled water (DW, 1 mg/kg) or d-amphetamine (d-A,2 mg/kg) injection under the following conditions: 1) during estrus, 2) following ovariectomy (+OVX), or 3) following ovariectomy (+OVX) and subsequent subcutaneous replacement injections of 0.1 mg/kg estradiol (+EST, 52-hour pretreatment) and 1 mg/kg progesterone (+PROG, 8-hour pretreatment). Each bar represents the mean, while each vertical line represents the standard error of 6 (control) or 8 (0.2 and 2 mg/kg/day BP-6) litters represented by one female per litter. Asterisk indicates difference from control mean by analysis of variance and the least significant difference test ($p<0.025$). Symbol "a" indicates difference from distilled water, while "b" indicates difference from d-amphetamine administered during estrus and following ovariectomy.

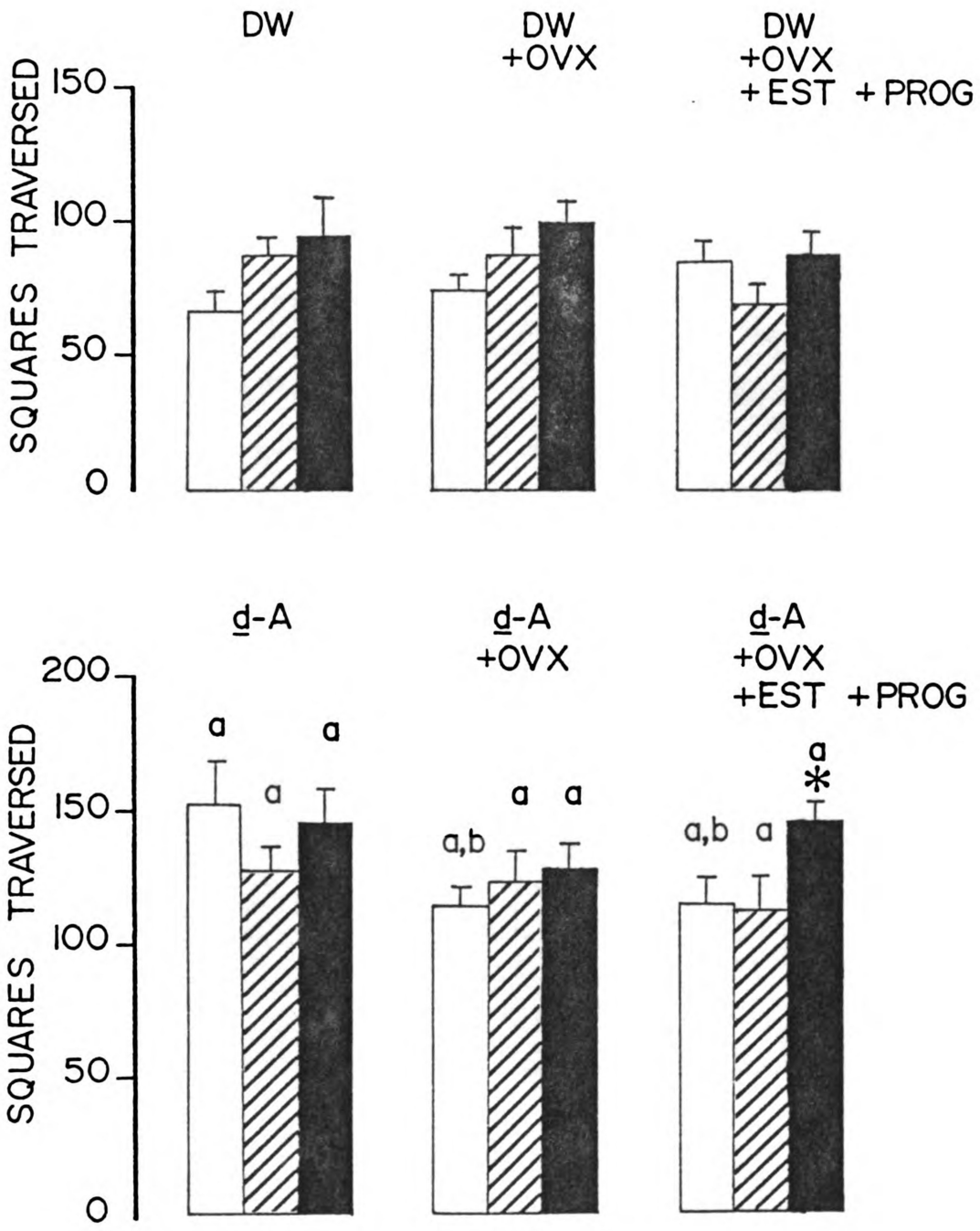

DW
DW
+OVX
DW
+OVX
+EST +PROG
SQUARES TRAVERSED
150
100
50
0
d-A
d-A
+OVX
d-A
+OVX
+EST +PROG
200
150
100
50
0
a
a
a
a
a
a
a,b
a,b
a
*

Figure 20

Figure 21. Number of rearings in an open field and response to d-amphetamine according to estrous cycle condition in female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (open bars), 0.2 (striped bars) or 2 mg/kg/day (shaded bars) fireMaster BP-6. Testing was conducted 30 minutes after distilled water (DW, 1 mg/kg) or d-amphetamine (d-A, 2 mg/kg) injection under the following conditions: 1) during estrus, 2) following ovariectomy (+OVX), or 3) following ovariectomy (+OVX) and subsequent subcutaneous replacement injections of 0.1 mg/kg estradiol (+EST, 52-hour pretreatment) and 1 mg/kg progesterone (+PROG, 8-hour pretreatment). Each bar represents the mean, while each vertical line represents the standard error of 6 (control) or 8 (0.2 and 2 mg/kg/day BP-6) litters represented by one female per litter. Symbol "a" indicates difference from distilled water by analysis of variance and the least significant difference test ($p<0.025$), while "b" indicates difference from d-amphetamine administered during estrus and following ovariectomy.

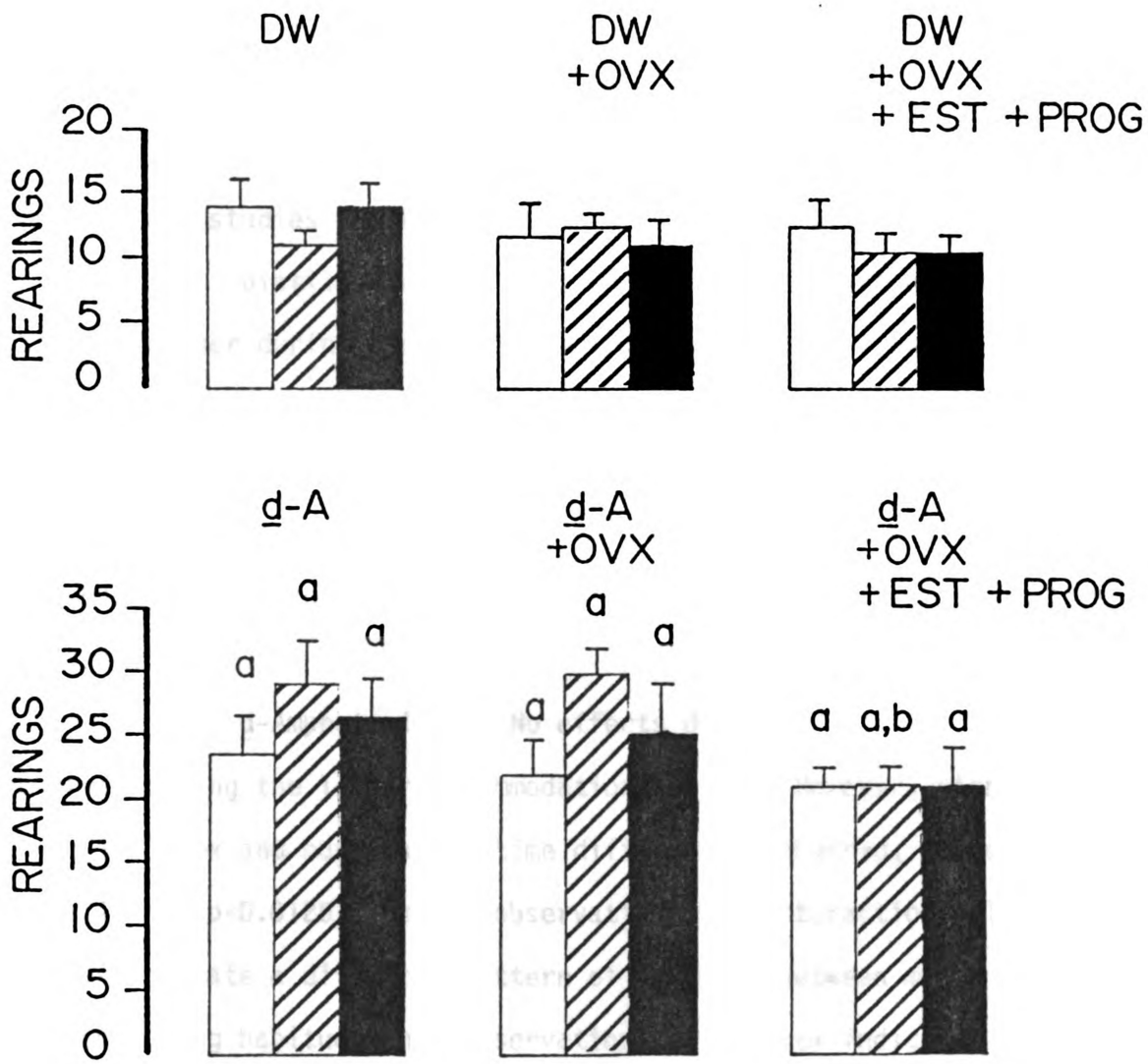

DW
DW
+OVX
DW
+OVX
+ EST + PROG
REARINGS
20
15
10
5
0
d-A
d-A
+OVX
d-A
+OVX
+ EST + PROG
REARINGS
35
30
25
20
15
10
5
0
a
a
a
a
a
a
a
a,b
a

Figure 21

this enhancement was comparable for intact and ovariectomized animals. A similar degree of enhancement was also observed for controls and females exposed to 2 mg/kg BP-6 following ovariectomy and hormone replacement. However, females exposed to 0.2 mg/kg BP-6 had significantly fewer rearings following ovariectomy and hormone replacement than in the intact state or following ovariectomy alone.

3. Confirmatory Studies

Although the drug challenge estrous cycle study was considered to be exploratory in nature, results are presented with those of the confirmatory studies due to their close relationship.

a. Distilled water. No PBB treatment-related effects were observed either during the initial accommodation period or following distilled water injection for either sex (Figure 22). Substantial sex differences in responding were observed during accommodation ($p<0.0125$) and following distilled water (<0.0125); observation time differences ($p<0.0125$) were also observed. No interactions among these parameters occurred.

b. d-Amphetamine. No effects due to PBB treatment were observed during the 1-hour accommodation period. However, significant ($p<0.0125$) sex and observation time differences occurred, as well as a significant ($p<0.0125$) sex and observation time interaction. These results indicate a different pattern of response between males and females during habituation. Observation of the data indicates that males were more active upon introduction to the novel environment than were females.

Figure 22. Accommodated locomotor activity following distilled water injection (1 ml/kg) in male and female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (◯), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from the number of litters indicated in Table 30, represented by one individual per litter. The point of injection of distilled water is indicated by the arrow. Analysis of variance revealed no significant difference between the activity of control and PBB-exposed offspring during either the accommodation period or following distilled water injection.

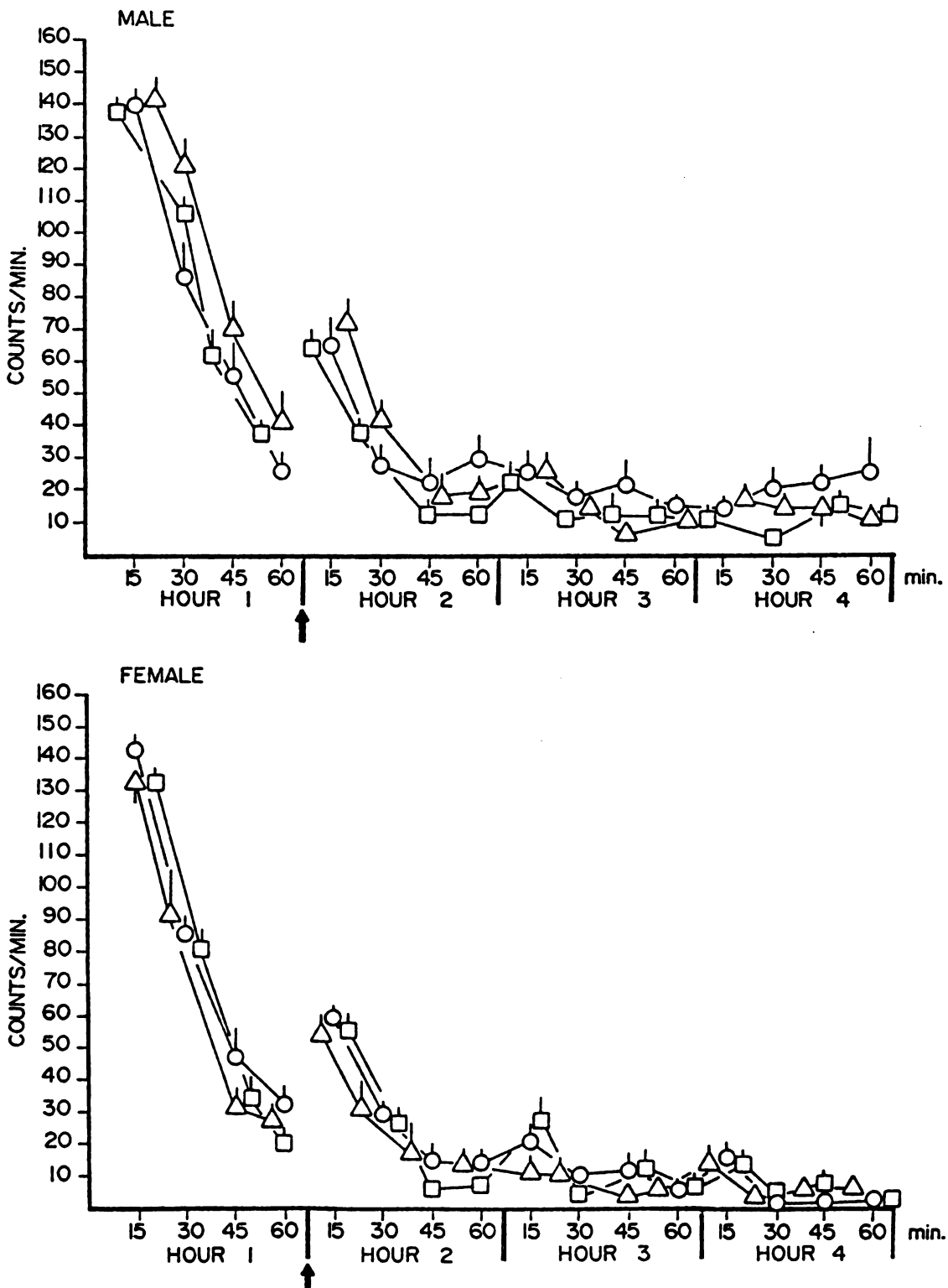

MALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
FEMALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 22

Following d-amphetamine injection, offspring exposed to 2 mg/kg/day BP-6 were significantly ($p<0.0125$) less active than controls when assessed by repeated measures ANOVA nested for sex (Figure 23). This deficit was observed to the greatest degree in male offspring. Significant ($p<0.0125$) effects for sex and observation time were observed, as well as significant ($p<0.0125$) interactions between observation time and BP-6 dose and observation time and sex. These results suggest that the pattern of stimulation observed following d-amphetamine is different between the sexes and is altered by PBB exposure. Separate analysis of the sexes revealed that the significant interaction between observation time and BP-6 dose occurred with males only. This is probably due to the fact that response to d-amphetamine with time resulted in roughly parallel dose-related curves for control and PBB-exposed females throughout most of the 4-hour observation period, while the response for males was attenuated at the 2 mg/kg/day dose only and all response curves joined approximately 2 hours after injection of d-amphetamine. Activity of control offspring and those exposed to 0.2 mg/kg/day BP-6 was comparable following d-amphetamine injection. The p-value for females was 0.0504, however, and may have reached significance had a greater number of animals been tested.

Activity at each stage of the estrous cycle is presented in Figure 24. Significant BP-6 treatment [$F(3,356) = 7.584$, $p<0.001$], and observation time [$F(3,356) = 197.104$, $p<0.001$], effects were observed during the accommodation period. Lack of significant interaction among these factors and observation of the data revealed that for all four stages of the estrous cycle, activity of PBB-exposed females was somewhat less than that of controls. Observation of the data revealed

Figure 23. d-Amphetamine-induced stimulation of accommodated locomotor activity in male and female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from the number of litters indicated in Table 30, represented by one individual per litter. The symbol X represents activity of control rats following distilled water injection. The point of injection of distilled water (1 ml/kg) or d-amphetamine (2 mg/kg) is indicated by the arrow. Analysis of variance revealed no significant difference between the activity of control and PBB-exposed offspring during the accommodation period. Following d-amphetamine injection, a significant difference ($p=0.0031$) was observed between controls and offspring exposed to 2 mg/kg/day BP-6. A significant ($p=0.0038$) observation time and BP-6 dose interaction was also observed, indicating a PBB-related shift in the pattern of response to d-amphetamine.

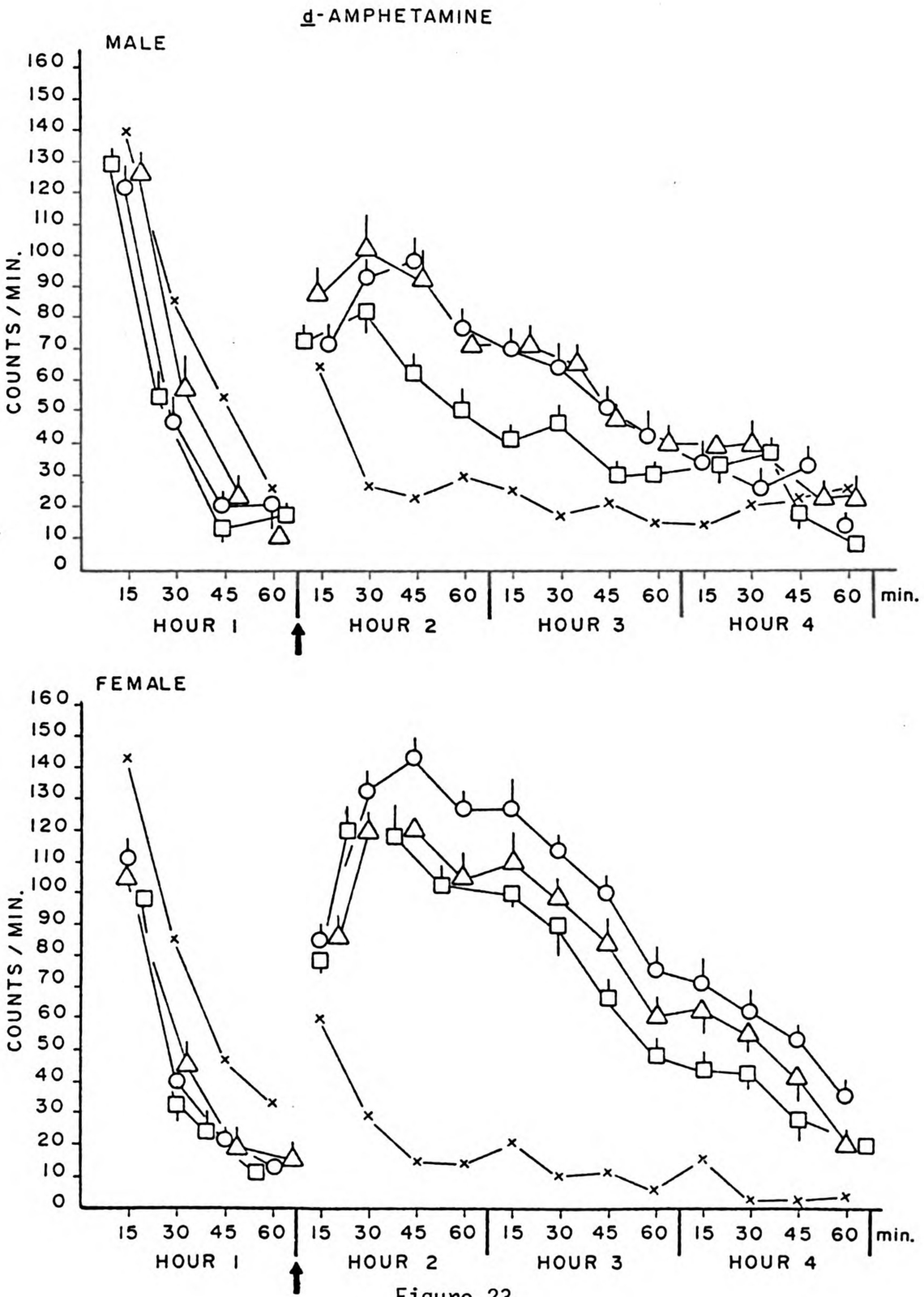

d-AMPHETAMINE
MALE
COUNTS / MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
FEMALE
COUNTS / MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 23

Figure 24. d-Amphetamine-induced stimulation of accommodated locomotor activity according to stage of the estrous cycle in female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of female offspring from at least 5 litters per dose. The point of injection of d-amphetamine (2 mg/kg) is indicated by the arrow. Analysis of variance revealed a significant difference between controls and offspring exposed to 2 mg/kg BP-6 with regard to activity during proestrus, metestrus, estrus, and diestrus ($p<0.001$ in all cases). Effects of the greatest magnitude were observed during metestrus and proestrus.

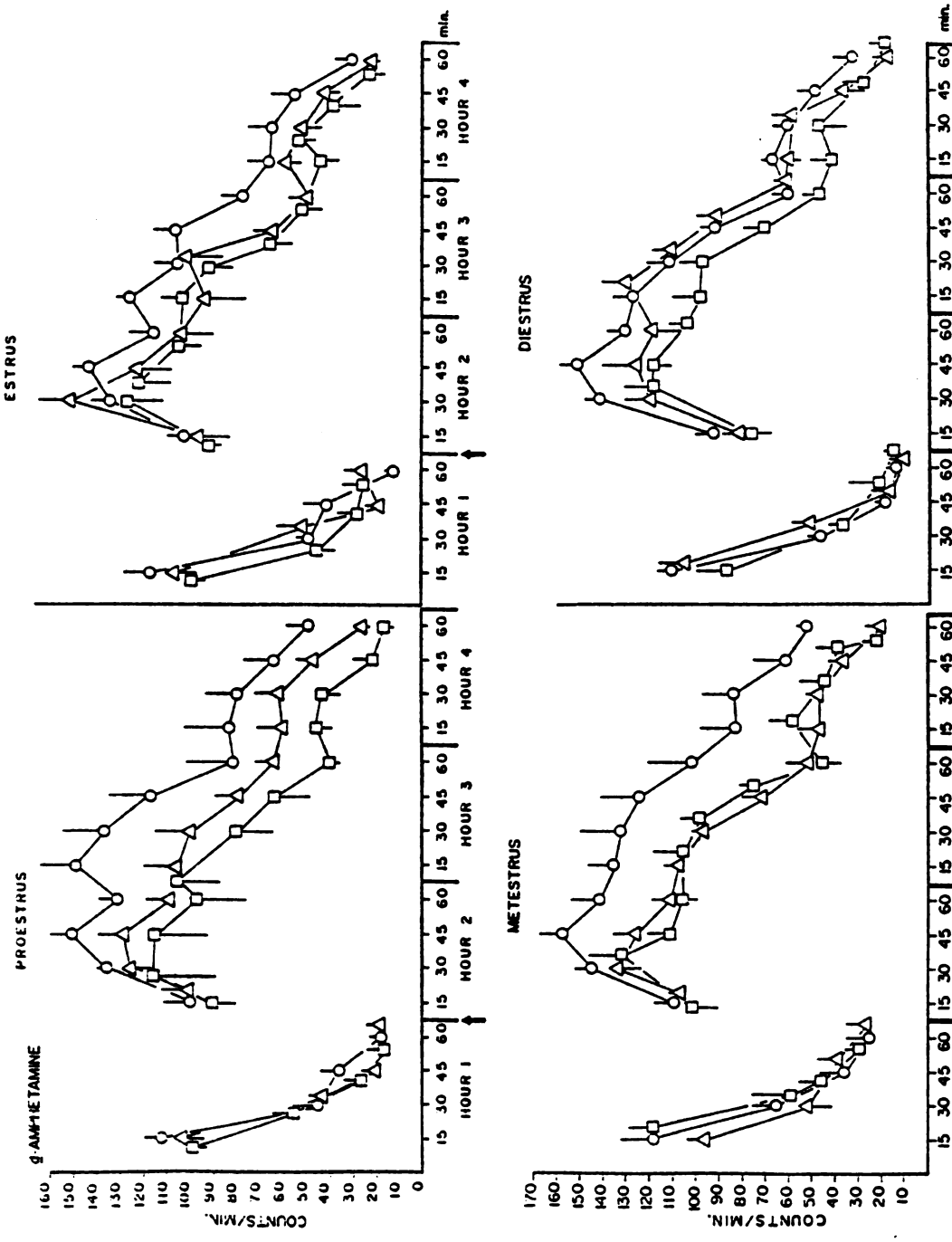

d-AMPHETAMINE
PROESTRUS
ESTRUS
METESTRUS
DIESTRUS
COUNTS/MIN.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
min.

Figure 24

that the significant estrous cycle factor may have resulted from the fact that activity decreased less during the accommodation period during metestrus as compared to the other three stages of the estrous cycle.

Significant effects for BP-6 treatment [$F(2,1068) = 77.840$, $p<0.001$] and observation time [$F(11,1068) = 126.36$, $p<0.001$] were observed following injection with d-amphetamine. In addition, a significant interaction occurred between BP-6 treatment and estrous cycle stage [$F(6,1068) = 3.777$, $p=0.001$], indicating that the PBB effect was different across the stages of the estrous cycle. ANOVA was conducted at each stage of the estrous cycle. Significant BP-6 treatment effects were observed during proestrus [$F(2,216) = 21.734$, $p<0.001$], estrus [$F(2,324) = 8.758$, $p<0.001$], metestrus [$F(2,240) = 41.273$, $p<0.001$] and diestrus [$F(2,288) = 17.634$, $p<0.001$]. A significant observation time effect ($p<0.001$) was observed for all four stages; however, no interaction occurred between BP-6 treatment and observation time. Observation of the data reveal that the PBB-related deficit in response to d-amphetamine was of the greatest magnitude during proestrus and metestrus. Females exposed to either dose of PBB appeared to be affected.

c. Scopolamine. PBB treatment-related effects were not observed during the initial acclimation period (Figure 25). However, as described previously, significant ($p<0.0125$) sex and observation time differences were observed, as well as a significant ($p<0.0125$) sex and observation time interaction. Males were again more active than females upon introduction to the novel environment.

Figure 25. Scopolamine-induced stimulation of accommodated locomotor activity in male and female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (◯), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from the number of litters indicated in Table 30, represented by one individual per litter. The point of injection of scopolamine (1 mg/kg) is indicated by the arrow. Analysis of variance revealed no significant difference between the activity of control and PBB-exposed offspring during either the accommodation period or following scopolamine injection. A significant ($p<0.0125$) effect was observed for the observation time, sex, and BP-6 dose interaction, indicating a PBB-related shift in the pattern of response to scopolamine according to sex.

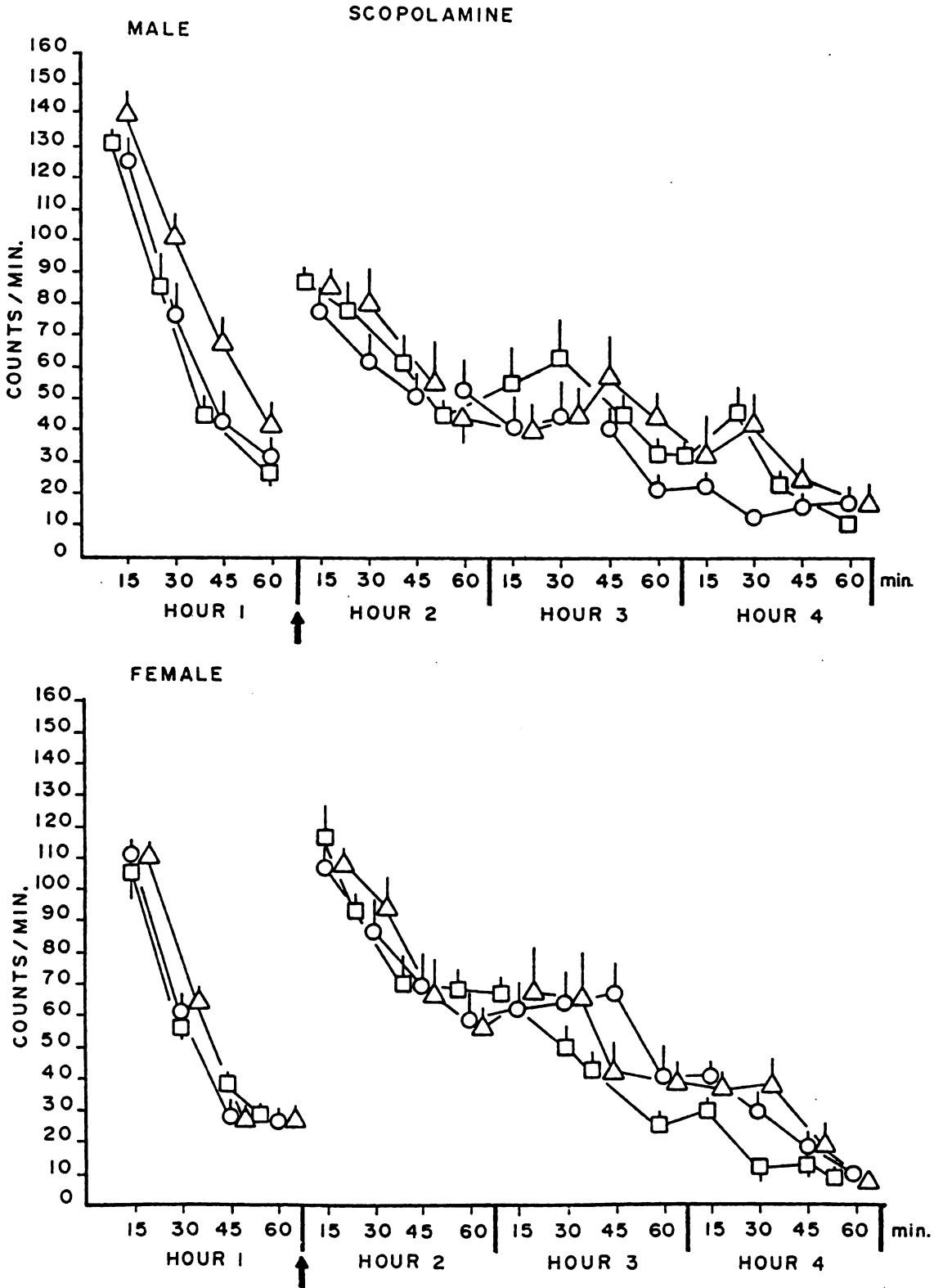

SCOPOLAMINE
MALE
COUNTS / MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 30 45 60
HOUR 1
HOUR 2
HOUR 3
HOUR 4
min.
FEMALE
COUNTS/MIN.

Figure 25

No PBB-related effects were observed following scopolamine injection. However, significant ($p<0.0125$) effects were observed for observation time, the observation time and sex interaction, and the observation time, sex, and BP-6 dose interaction. Observation of the data reveal that females were initially stimulated to a greater extent following scopolamine injection than were males; their activity was comparable by 1 hour post-administration, however. The significant observation time, sex, and BP-6 dose interaction may have occurred because male PBB-exposed rats were more active than controls during the last hour of observation, while females exposed to 2, but not 0.2 mg/kg/day BP-6, were less active than controls during the same time period.

Analysis of the response of female rats by stage of the estrous cycle (Figure 26) revealed no significant effects related to PBB treatment during the accommodation period or following scopolamine injection. However, a significant ($p<0.0125$) interaction was observed between BP-6 treatment and estrous cycle stage, indicating that in general, PBB treatment may have affected the response to scopolamine differently across the stages of the estrous cycle. This was most apparent during estrus; offspring exposed to 0.2 mg/kg/day BP-6 were somewhat less active than controls, while those exposed to 2 mg/kg/day BP-6 were transiently more active.

d. Fenfluramine. No PBB treatment-related effects were observed during acclimation or following fenfluramine injection (Figure 27). Significant ($p<0.0125$) effects were observed during accommodation for sex, observation time, and the observation time and sex interaction.

Figure 26. Scopolamine-induced stimulation of accommodated locomotor activity according to the stage of the estrous cycle in female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of female offspring from at least five litters per dose. The point of injection of scopolamine (1 mg/kg) is indicated by the arrow. Analysis of variance revealed no significant difference between the activity of controls and PBB-exposed offspring during any stage of the estrous cycle.

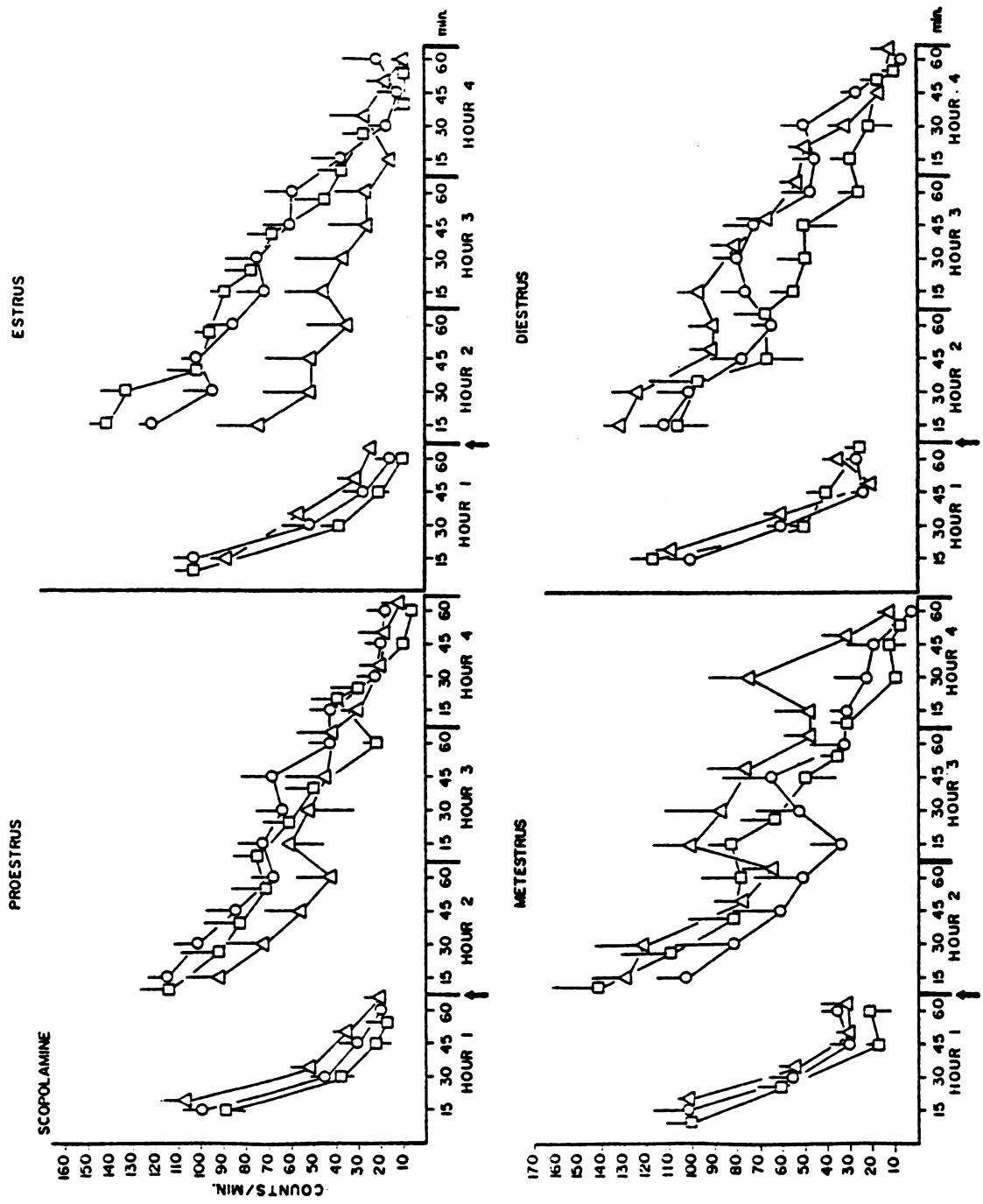

SCOPOLAMINE
PROESTRUS
ESTRUS
METESTRUS
DIESTRUS
COUNTS/MIN.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
min.

Figure 26

Figure 27. Fenfluramine-induced stimulation of accommodated locomotor activity in male and female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (◯), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of all male or female offspring from the number of litters indicated in Table 30, represented by one individual per litter. The point of injection of fenfluramine (10 mg/kg) is indicated by the arrow. Analysis of variance revealed no significant difference between the activity of controls and PBB-exposed offspring. However, a significant ($p<0.0125$) interaction occurred between BP-6 dose, sex, and observation time. Separate analysis of males and females indicated that the pattern of response to fenfluramine was altered by PBB exposure in males only during either the accommodation period or following fenfluramine injection.

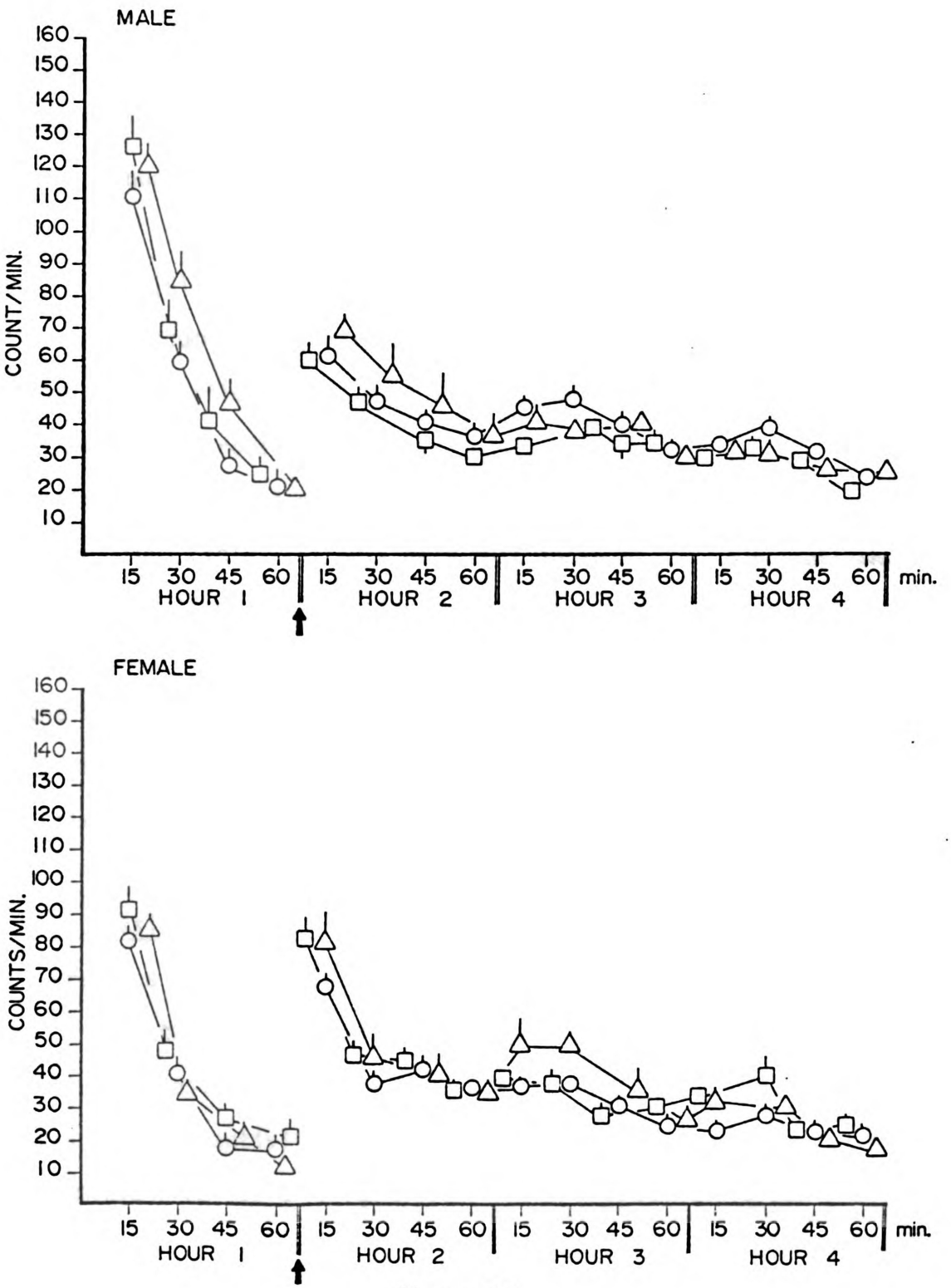

MALE
COUNT/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4
FEMALE
COUNTS/MIN.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
15 30 45 60 15 30 45 60 15 30 45 60 15 30 45 60 min.
HOUR 1
HOUR 2
HOUR 3
HOUR 4

Figure 27

As observed previously, males were more active upon introduction to the novel environment than were females.

Fenfluramine injection resulted in significant ($p<0.0125$) differences for observation time and the observation time and sex interaction. Females were initially more active than males following fenfluramine injection, while males were more active than females approximately 1 hour after injection.

Analysis of fenfluramine response according to estrous cycle stage (Figure 28) revealed no PBB treatment-related effects during either the accommodation period or following fenfluramine injection. However, a significant ($p=0.001$) interaction was observed between BP-6 treatment and estrous cycle stage, indicating that PBB treatment may have affected the response to fenfluramine differently across the stages of the estrous cycle. Effects were most apparent during estrus, when activity was stimulated more in offspring exposed to 2 mg/kg/day BP-6 than in controls. The estrous cycle term was significant ($p<0.001$) due to the fact that activity was stimulated more by fenfluramine during diestrus than during the other stages of the estrous cycle. Activity was stimulated least during proestrus.

D. Discussion

Previous reports indicated that prenatal or perinatal PBB exposure produces spontaneous hyperactivity (Schantz and Bowman, 1983; Gause et al., 1984). Results of the present confirmatory analyses revealed no spontaneous hyperactivity at 3, 5 or 7 months of age in offspring from dams administered 0.2 or 2 mg/kg/day BP-6 during gestation and

Figure 28. Fenfluramine-induced stimulation of accommodated locomotor activity according to stage of the estrous cycle in female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg (□) fireMaster BP-6. Each data point and vertical line represents the mean and standard error of female offspring from at least five litters per dose. The point of injection of fenfluramine (10 mg/kg) is indicated by the arrow. Analysis of variance revealed no significant difference between the activity of controls and PBB-exposed offspring during any stage of the estrous cycle.

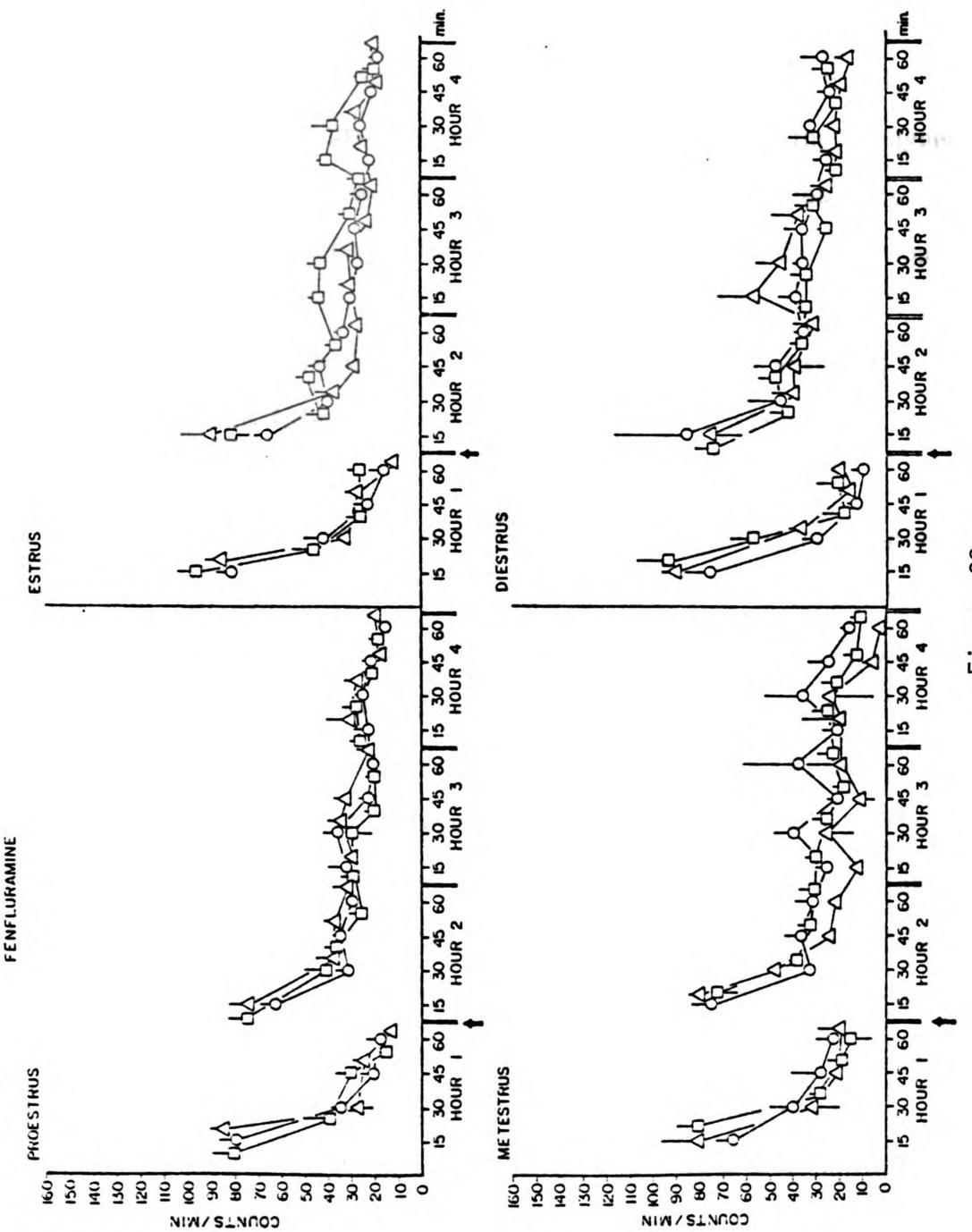

FENFLURAMINE
PROESTRUS
ESTRUS
METESTRUS
DIESTRUS
COUNTS / MIN
HOUR 1
HOUR 2
HOUR 3
HOUR 4
min.

Figure 28

lactation. In contrast, testing of males at approximately 10 months of age for 15-minute unaccommodated locomotor activity revealed hyperactivity of PBB-exposed offspring (particularly those exposed to 0.2 mg/kg/day) 10 minutes after introduction into the test chamber. This was also true for open field testing of females exposed to 0.2 or 2 mg/kg/day BP-6. It could be postulated that a certain minimum tissue level of PBB is required for the production of disinhibition leading to hyperactivity in these paradigms. However, these latter studies were exploratory in nature and therefore clarification of this hypothesis requires further testing.

No PBB-related effects on locomotor activity were observed following distilled water injection, indicating a lack of effect on baseline activity.

By contrast, d-amphetamine-induced stimulation of locomotor activity was affected by prior PBB exposure. d-Amphetamine stimulated activity of offspring from dams administered 2 mg/kg/day BP-6 during gestation and lactation significantly less than that of controls. Furthermore, interactions between BP-6 dose and observation time and between BP-6 dose and sex indicated that the pattern of stimulation observed following d-amphetamine is different between the sexes and is altered by PBB exposure. The PBB-induced deficit in response to d-amphetamine was greater for males than for females. No treatment-related effect was observed for offspring exposed to 0.2 mg/kg/day BP-6.

Stimulation of locomotor activity by d-amphetamine has been shown to result primarily from enhanced release of dopamine and inhibition of dopamine reuptake (Moore, 1978). The present results indicate that BP-6

exposure may result in modulation of dopaminergic systems and since d-amphetamine acts indirectly to enhance dopaminergic-mediated responses, that the effect may be presynaptic rather than postsynaptic. To investigate this possibility, male rats were tested for l-DOPA-induced stimulation of locomotor activity to identify possible postsynaptic effects. Pretreatment with the peripheral decarboxylase inhibitor benserazide resulted in comparable suppression of unaccommodated locomotor activity in control and PBB-exposed rats. This dose of benserazide (50 mg/kg) is thought to penetrate the blood-brain barrier very little (Thut and Rech, 1972); thus, this may represent a peripheral effect, although the exact mechanism is unclear because benserazide also inhibits the activity of transaminases (Rauws et al., 1982) and oxidases (Andree and Clarke, 1982).

l-DOPA (l-3,4-dihydroxyphenylalanine) is the precursor of the neurotransmitter dopamine and, unlike dopamine, crosses the blood-brain barrier (Bianchine, 1980). Within the body l-DOPA is converted to dopamine via l-amino acid decarboxylase; this occurs primarily in the central nervous system when peripheral decarboxylase is inhibited by compounds such as benserazide. Dopamine binds to postsynaptic dopaminergic receptors in the nucleus accumbens and thus produces enhanced locomotor activity (Pijnenburg and Van Rossum, 1973). In the present study essentially no difference was observed in the amount of activity stimulation elicited by l-DOPA between control and PBB-exposed animals. Results of these studies thus suggest that PBB disruption of dopaminergic synapses is primarily presynaptic in nature and probably does not involve a substantial postsynaptic component.

Two aspects of presynaptic dopaminergic function which may be disrupted are synthesis and release of dopamine. Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of dopamine; inhibition of this enzyme by α-methyltyrosine results in decreased dopamine content in tissues of the central nervous system (Moore and Wuerthele, 1979). This decrease in dopaminergic influence has been shown to produce profound behavioral depression (Rech et al., 1966). Stolk and Rech (1970) determined that d-amphetamine-induced stimulation of behavior is antagonized by doses of α-methyltyrosine devoid of behavioral effects, providing evidence that newly-synthesized catecholamines are important to the stimulant action of amphetamine. In order to assess the role of synthesis in PBB-mediated disruption of d-amphetamine stimulation of behavior, male rats were tested for unaccommodated locomotor activity. Following distilled water injection, PBB-exposed animals were more active than controls. Thus, it is not surprising that their response to d-amphetamine was less than that of controls, based on the premise of Dews and Morse (1961) that low levels of baseline behavior are stimulated more by amphetamine than are high rates. It is therefore unclear if stimulation of unaccommodated activity following d-amphetamine was depressed in PBB-exposed animals due to a dopaminergic deficit or to the initial disinhibition of response to a novel environment.

A dose of α-methyltyrosine (100 mg/kg) demonstrated to produce moderate behavioral depression in a shuttle box avoidance paradigm (Rech et al., 1966) produced marked suppression of activity in control and PBB-exposed rats. Behavioral depression was somewhat greater in PBB-exposed animals, which again may have been related to the high baseline

activity of these animals or to the fact that they already had lowered synthesis rates. An unexpected result was that observed upon combination of d-amphetamine and α-methyltyrosine: α-methyltyrosine did not completely antagonize d-amphetamine-induced stimulation of locomotor activity in control or PBB-exposed animals. Activity tended to be attenuated, however, and to a greater extent in rats exposed to 2 mg/kg/day BP-6 than in controls or those exposed to 0.2 mg/kg/day BP-6. It could be postulated that under normal conditions, animals exposed to 2 mg/kg/day BP-6 can compensate for reduced dopamine synthesis, but that upon the stress exerted on reserves that is produced by d-amphetamine, a deficit occurs. It must be emphasized that this study was of the hypothesis-generating type and that the suggestion of effects on dopamine synthesis needs confirmation.

The drug Ro4-1284 reduces brain catecholamines by depleting storage granules of the large reserve pool of these neurotransmitters (Rech et al., 1968). This depletion results in behavioral depression; this depression is potentiated by α-methyltyrosine due to elimination of both stored and newly synthesized catecholamines (Rech et al., 1978). Ro4-1284 (50 mg/kg) produced behavioral depression in control and PBB-exposed animals of the same degree as that produced by α-methyltyrosine. Again, behavioral depression was somewhat greater in PBB-exposed animals. Further depression of behavior was produced upon combination of the two drugs. No PBB-related effects which could be related to catecholamine depletion were observed, however, following Ro4-1284 alone or upon combination of Ro4-1284 with α-methyltyrosine due to the extremely low levels of activity observed in all groups.

Thus, confirmatory studies revealed the presence of a PBB-related deficit in d-amphetamine-induced stimulation of locomotor activity. This decreased responding is most likely due to a dopaminergic deficit which exploratory studies suggested may be presynaptic in origin. The exact nature of this deficit requires further clarification.

PBB effects on cholinergic systems were investigated by administration of the anticholinergic drug scopolamine. Although females were initially more active than males following scopolamine injection (resulting in a significant sex and session interaction), no PBB-related effects were observed which could be interpreted as affecting the stimulation produced by the antimuscarinic drug. Thus, the suspected imbalance in cholinergic systems raised by the probe study was not confirmed in more extensive testing. No PBB-related effects were observed following fenfluramine injection. However, analysis of the results of both sexes in the confirmatory study revealed a significant observation time by sex by BP-6 dose interaction. Separate analysis of the sexes revealed that this was due to an effect in males administered 2 mg/kg BP-6. These animals were somewhat less active than controls during the peak stimulatory effect of fenfluramine. These studies suggested that perinatal PBB exposure may result in a serotonergic imbalance in male, but not female rats, which is manifested as a decrease in fenfluramine-induced stimulation of locomotor activity. However, the magnitude of the response was not great and the exact significance is unclear.

Results of these confirmatory studies support the hypothesis that perinatal PBB exposure results in disruption of drug-induced accommodated locomotor activity. This disruption was most apparent following

drug challenge with d-amphetamine, indicating a possible disruption of dopaminergic systems. Challenge with the indirect-acting serotonergic agonist fenfluramine revealed possible disruption of serotonergic systems in male, but not female, rats. No evidence was found for disruption of cholinergic systems.

Sexual dimorphism was observed in most of the behavioral tests conducted. In addition, males and females appeared to respond differently to perinatal PBB exposure. To assess the influence of varying hormonal titer throughout the estrous cycle, females were tested for drug-induced stimulation of locomotor activity at various stages.

Analysis of motor activity data revealed greater deficits in response to d-amphetamine during proestrus and metestrus than during estrus and diestrus.

To clarify the estrogen and d-amphetamine interaction, females were retested for open field activity at approximately 10 months of age. All were established as having normal estrous cycles prior to testing and were initially tested in estrus. No differences were observed in the activity of control and PBB-exposed animals during estrus, following ovariectomy, or following ovariectomy with hormone replacement. These data are consistent with those obtained by Telegdy and Stark (1973). No PBB-related differences in activity were observed in estrus following d-amphetamine injection. However, d-amphetamine enhanced activity (as measured by squares traversed) significantly less in control animals following ovariectomy, thus supporting the theory of endogenous estrogen enhancement of catecholamine release as advanced by Fludder and Tonge (1976). This was not the case for PBB-exposed animals; activity was

stimulated to the same extent during estrus and following ovariectomy. It is possible that these animals had already adapted to low levels of estrogen due to enhanced metabolism and therefore ovariectomy was not as great a challenge as it was to controls.

Estrogen and progesterone replacement did not reverse the diminished response to d-amphetamine observed in ovariectomized controls. This may have resulted from priming doses of estrogen and progesterone insufficient to reinstate control behavior, as other researchers have used multiple injections (Telegdy and Stark, 1973; Earley and Leonard, 1978) rather than one injection of each hormone, as in the present study. Hormone replacement exerted no additional effects on PBB-exposed animals with regard to squares traversed, with the exception that low control values resulted in a significantly greater number of squares traversed by females exposed to 2 mg/kg/day BP-6. Number of rearings was diminished following hormone replacement compared to the estrus or ovariectomized conditions in females exposed to 0.2 mg/kg/day BP-6. The exact mechanism of this effect is unknown, but appears to be different from that affecting squares traversed.

No general PBB treatment-related effects were observed across the stages of the estrous cycle following either scopolamine or fenfluramine injection. However, significant BP-6 treatment and estrous cycle stage interactions indicated that PBB affected the response to each drug differently across the stages of the estrous cycle. This was most apparent during estrus. These results indicate that although no PBB-related effects were observed in females tested regardless of estrous cycle stage, that these effects may be unmasked under conditions of differing hormone titer. This requires further investigation.

CHAPTER 4

EFFECTS OF PERINATAL PBB EXPOSURE ON CONDITIONED SUPPRESSION OF DRINKING

A. Introduction

Conditioned suppression of drinking is a conflict paradigm used to assess the anxiolytic potential of drugs (Kilts et al., 1981). It is a learned behavior and therefore is also useful to assess the effects of an environmental contaminant or drug on learning and memory.

In the present group of experiments (see Chapters 2 and 3), PBB-exposed rats appeared to respond differently to a novel environment in cage emergence and unaccommodated locomotor activity situations than did controls. Male PBB-exposed rats appeared to be more willing to enter a novel environment compared to controls than were female PBB-exposed rats. In addition, although epidemiologic data were subjective in nature, cognitive deficits were reported in PBB-exposed children and older men (Valciukas et al., 1978; Schwartz and Rae, 1983; Seagull, 1983).

These lines of evidence led to the hypothesis that perinatal PBB exposure affects conditioned suppression of drinking. This hypothesis was tested using the psychoactive agent d-amphetamine and diazepam to uncover subtle behavioral effects which might not be manifested during baseline responding.

B. Materials and Methods

1. Experimental Subjects

Experimental subjects were offspring from a colony of female Sprague-Dawley rats administered 0, 0.2 or 2 mg/kg/day BP-6 in a peanut butter "treat" from day 6 of gestation through day 24 of lactation. The source of this colony, housing conditions, and breeding regimen are described in Chapter 2.

Body weights and general appearance of all dams were monitored throughout gestation and lactation. Gestation length, number of pups born alive, sex ratio of the pups, and mortality from birth through weaning were also assessed. All maternal and developmental parameters were comparable between controls and PBB-exposed animals, as reported in Chapter 2.

Offspring from each litter were cross-fostered as soon after birth as possible with a dam of the same dose group. At 3 days of age all litters were culled to four pups per sex where possible. At this time indelible ink was injected subcutaneously into the paws of the pups in a specific pattern for the purpose of identification. Females were randomly assigned numbers 1 through 4, while males were randomly assigned numbers 5 through 8. Pups remained with their foster mother until weaning on postpartum day 24 and were housed 2 or 3 per cage thereafter according to BP-6 treatment and sex.

Male number 7 and female number 3 were assigned to Group 3: the group tested for conditioned suppression of drinking. Table 30 lists all parameters measured in Group 3 animals, the method of statistical analysis used in each case, and the partitioning of α according to groups of studies.

TABLE 30

Parameters Measured in Group 3 Animals, Including Method of Statistical Analysis and Partitioning of α

A. "Incidental" data

Parameter	Statistical Analysis	α
1. Body Weight	ANOVA with mean comparisons	0.025[a]

B. Confirmatory Studies - Conditioned suppression of drinking with drug challenge, $\alpha = 0.025$[b]

Parameter	Statistical Analysis	α
1. d-Amphetamine	Multifactorial ANOVA	0.018[c]
2. Diazepam	Multifactorial ANOVA	0.018[c]

[a] Arbitrarily assigned as 0.025 to correspond with overall group level.

[b] α partitioned among Groups 1-4; therefore, $\alpha = \frac{.05}{\sqrt{4}} = 0.025$.

[c] α partitioned among 2 tests and 4 drug doses; therefore, $\alpha = \frac{.025}{\sqrt{8}} = 0.088$.

2. Assessment of Punished Responding

a. "Incidental" data. These data were considered incidental to, but supportive of, the primary goal of testing in Group 3 animals: assessment of the effects of perinatal BP-6 exposure on conditioned suppression of drinking.

All animals were weighed each day of testing. For the sake of clarity, only body weights obtained at the following time points were included in statistical analysis: immediately prior to training, the first day of shock and tone, before the first dose of d-amphetamine, and before the first dose of diazepam. Body weight data were analyzed by ANOVA. Mean comparisons were conducted using the least significant difference test.

b. Confirmatory studies. Each animal was trained to a conditioned suppression of drinking paradigm, beginning at approximately 6 months of age. Males were tested from 0800 to 1100 hours, while females were tested from 1000 to 1400 hours.

The experimental chamber consisted of a rectangular box (45 (L) x 30 (W) x 30 (H) cm) consisting of plexiglas sides and a stainless steel floor and ceiling, as described by Ford et al. (1979). A metal drinking tube protruded from one wall; it was attached to a calibrated (± 0.5 ml units) length of polyethylene tubing used to measure the volume of fluid consumed. The reinforcer was water in all cases. The ceiling of the box contained a speaker attached to a tone generator. Seven-second tone periods were produced intermittently (variable interval 21 seconds) throughout the 10-minute testing session. During the latter 5 seconds of the tone, contact between the floor and the drinking

tube closed a circuit which resulted in the delivery of a shock (via a BRS/ Foringer shock generator, model S6-901) to the rat. This punishing stimulus was a 110 volt, 60 Hz alternating electric current of a chosen intensity. An optical lickometer (model E24-01, Coulbourn Instruments, Lehigh Valley, PA) was used to measure number of licks during the following 3 time periods: 1) nontone periods (unpunished licks), 2) the first 2 seconds of the tone (prepunished licks), and 3) the last 5 seconds of the tone (punished licks). Electromechanical programming equipment was used to control experimental contingencies and to record the number of licks occurring at the 3 time periods. In addition to licks, the volume of water consumed during the 10-minute test session was monitored.

Prior to training, all Group 3 animals were deprived of water overnight. For the next 3 days, each animal was placed singly into the testing chamber for 10 minutes with no shock and no tone to allow the animal to find the tube and drink freely from it. At the end of the acclimation period, the tone and shock were introduced. Each rat received the majority of its daily water intake during the test session. However, this was not sufficient and therefore each received 5 minutes of free water no less than 1 hour after the test session. The shock level was initially set at 0.3 mA and was increased daily until punished responding was approximately 20-30% of unpunished responding. The mean shock intensity required to produce this level of responding was 0.53 mA, and did not differ among control and PBB-exposed offspring. McMillan (1973) observed that punishment intensity is a determinant of drug effect with regard to punished responding. Ford and coworkers (1979) demonstrated a dose-related decrease in punished responding with

d-amphetamine in the conditioned suppression of drinking paradigm at 0.03, but not 0.1 mA. Therefore, a relatively high rate of baseline responding during the punished period was desired to clearly demonstrate the rate decreasing effects of d-amphetamine, as this was the first drug to be tested.

Stable baseline responding was achieved in approximately 2 weeks of testing each animal 6 days per week. At this time, each animal received an intraperitoneal injection of d-amphetamine (dissolved in distilled water; the solutions were made to deliver an equal volume at all dose levels) 15 minutes prior to testing. All rats received each dose of d-amphetamine: 0.25, 0.5, 1 and 2 mg/kg. The order of dosing was randomized and at least 2 drug-free testing days intervened between each drug testing day.

Following the final dose of d-amphetamine, each rat was trained to a new shock level to facilitate testing of diazepam. Diazepam has been shown to increase punished responding in a conditioned suppression of drinking paradigm in a dose-related manner (Ford et al., 1979; Commissaris and Rech, 1982). A better dose-response relationship was achieved with a shock level of 0.1, rather than 0.03 mA (Ford et al., 1979). This was supported by the work of McMillan (1973) in pigeons; he determined that diazepam exerted the greatest increase in punished responding when baseline rates of responding were low. Therefore, in the current study rats were trained to a level of punished responding 10% or less than the rate of unpunished responding. The mean shock intensity required to produce this level of responding was 0.93 mA, and did not differ among control and PBB-exposed offspring.

Stable baseline responding was achieved in approximately 2 weeks. At this time, each rat received an intraperitoneal injection of 1.8 mg/kg diazepam (suspended in 0.5% methyl cellulose; diazepam suspensions were made to deliver an equal volume at all dose levels) 15 minutes prior to testing. Kilts and coworkers (1981) demonstrated an "initial treatment" phenomenon for diazepam in the conditioned suppression of drinking paradigm; i.e., the anticonflict activity of this drug increased over the first few exposures and then stabilized. Therefore, all animals received 4 consecutive daily doses of 1.8 mg/kg diazepam (demonstrated to be an effective dose to overcome the initial treatment phenomenon) prior to testing of the dose-response curve. Two testing days after the last initial diazepam dose, each rat received 1 of 4 diazepam doses (1.8, 3, 5.6 or 10 mg/kg) each drug dose day. All rats received all doses in random order. At least 2 drug-free days intervened between each drug testing day.

Unpunished licks, prepunished licks, punished licks and water consumed were expressed as percent of baseline responding under the same condition. These data were analyzed for each drug by multifactorial ANOVA; factors were BP-6 dose, sex and drug dose.

C. Results

1. "Incidental" Data

Body weights of male offspring from dams administered 2 mg/kg/day BP-6 were significantly less than those of controls when measured prior to injection with d-amphetamine (Table 31). Although a trend toward decreased body weight gain was observed in male offspring exposed

TABLE 31

Mean (± S.E.M.) Body Weights of Rats Trained to a Conditioned Suppression of Drinking Paradigm Following Perinatal Exposure to fireMaster BP-6 (PBB)

Time of Measurement	Body Weight (grams)					
	0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Day 1 (no shock, no tone)	392.5+12.0 (10)	232.5+5.5 (11)	378.5+ 8.7 (12)	247.9+3.0 (12)	371.2+8.4 (11)	222.5+6.2 (11)
First day of shock and tone	371.2+11.3 (11)	219.7+6.5 (11)	348.9+ 9.4 (12)	231.8+5.8 (12)	345.8+8.8 (12)	209.1+5.2 (13)
Immediately prior to d-amphetamine	371.8+10.2 (11)	226.7+5.3 (11)	349.7+ 9.9 (12)	233.9+4.6 (12)	344.3+9.6* (12)	215.5+5.4 (13)
Immediately prior to diazepam	379.0+ 9.9 (11)	230.8+5.4 (10)	360.3+10.2 (12)	237.6+4.4 (12)	356.8+7.5 (12)	219.9+4.3 (13)

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.025$.

Numbers in parentheses = N.

to 2 mg/kg/day BP-6 at other time points, they were not significantly less as assessed by ANOVA. Body weights of PBB-exposed females were comparable to those of controls throughout the test period.

2. Confirmatory Studies

Observation of baseline data (not shown) indicated no difference in performance in the conditioned suppression of drinking paradigm (CSD) between control and PBB-exposed male or female offspring. Administration of 0.25 to 2 mg/kg d-amphetamine to all animals on test resulted in a dose-related decrease in the number of unpunished, prepunished, and punished licks, as well as in the amount of water consumed during the 10-minute test session (Figures 29 and 30). No PBB-related effects were observed with regard to unpunished or prepunished responding or the amount of water consumed, for any dose of d-amphetamine administered to either sex. However, punished responding was diminished for male and female offspring following injection of 0.25 mg/kg d-amphetamine. This PBB treatment-related effect was not observed with higher doses of d-amphetamine. Sexual dimorphism was observed for this response ($p<0.018$ for sex). No interactions between BP-6 and d-amphetamine, BP-6 and sex, d-amphetamine and sex, or among BP-6, d-amphetamine and sex were observed.

Unlike d-amphetamine, doses of diazepam ranging from 1.8 to 10 mg/kg had no effect on unpunished responding or the amount of water consumed by male or female rats during the 10-minute test period (Figures 31 and 32). No PBB-related effects were observed with regard to these parameters. Prepunished responding was enhanced over baseline to an equal degree for control and PBB-exposed male rats in a

Figure 29. Effect of d-amphetamine on conditioned suppression of drinking in male offspring from dams administered FireMaster BP-6 during gestation and lactation. Dams received 0 (◯), 0.2 (△) or 2 mg/kg (□) FireMaster BP-6. Each data point represents the mean and standard error of all male offspring from the number of litters indicated in Table 31, represented by one individual per litter. Responses measured during the 10-minute test session included the number of licks obtained during unpunished, prepunished, and punished periods, as well as water consumed following a 15-minute pretreatment with 0.25, 0.5, 1 or 2 mg/kg d-amphetamine.

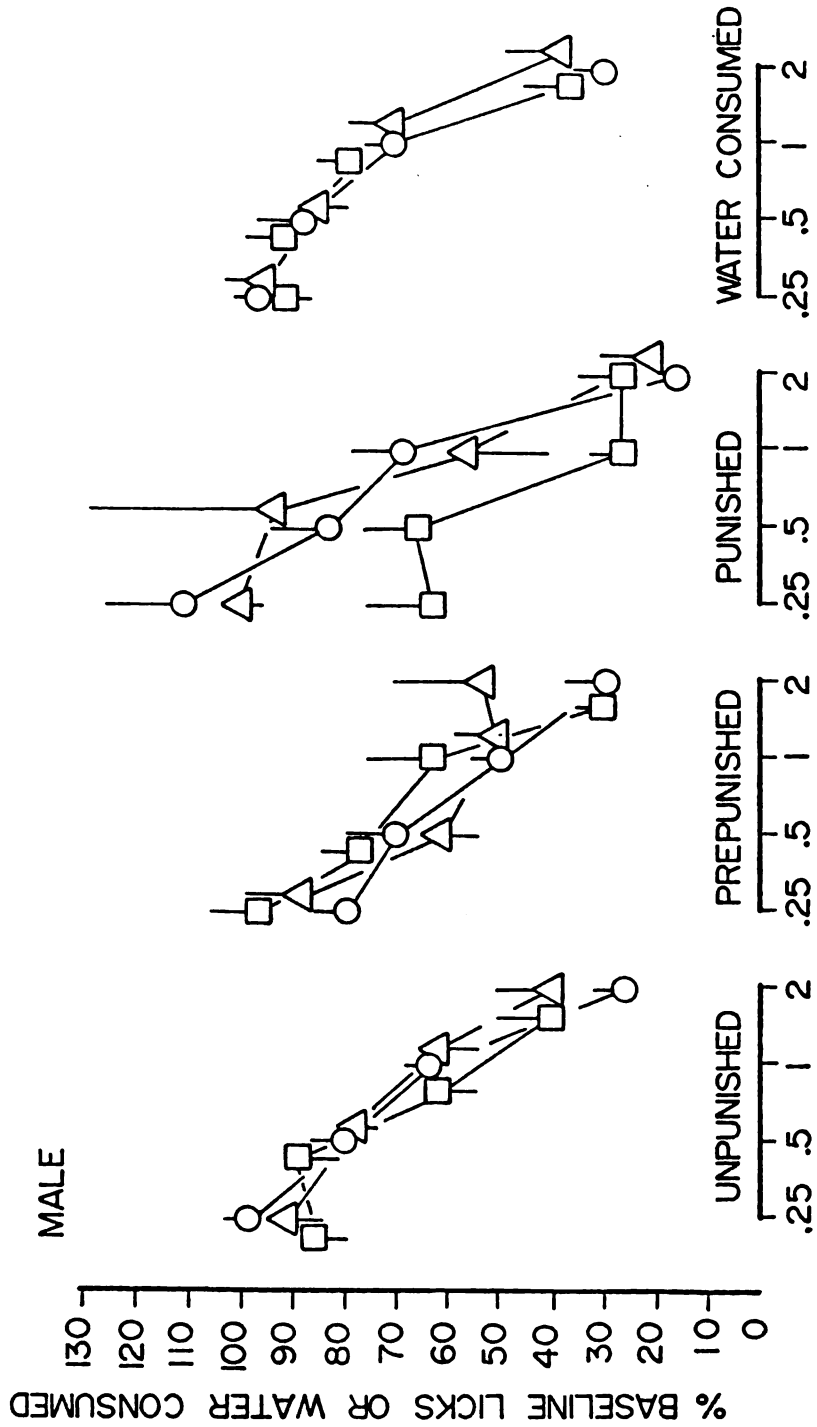

% BASELINE LICKS OR WATER CONSUMED
130
120
110
100
90
80
70
60
50
40
30
20
10
0
MALE
UNPUNISHED
PREPUNISHED
PUNISHED
WATER CONSUMED
.25 .5 1 2
mg/kg d-AMPHETAMINE

Figure 29

Figure 30. Effect of d-amphetamine on conditioned suppression of drinking in female offspring from dams administered FireMaster BP-6 during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg (□) FireMaster BP-6. Each data point represents the mean and standard error of all female offspring from the number of litters indicated in Table 31, represented by one individual per litter. Responses measured during the 10-minute test session included the number of licks obtained during unpunished, prepunished, and punished periods, as well as water consumed following a 15-minute pretreatment with 0.25, 0.5, 1 or 2 mg/kg d-amphetamine.

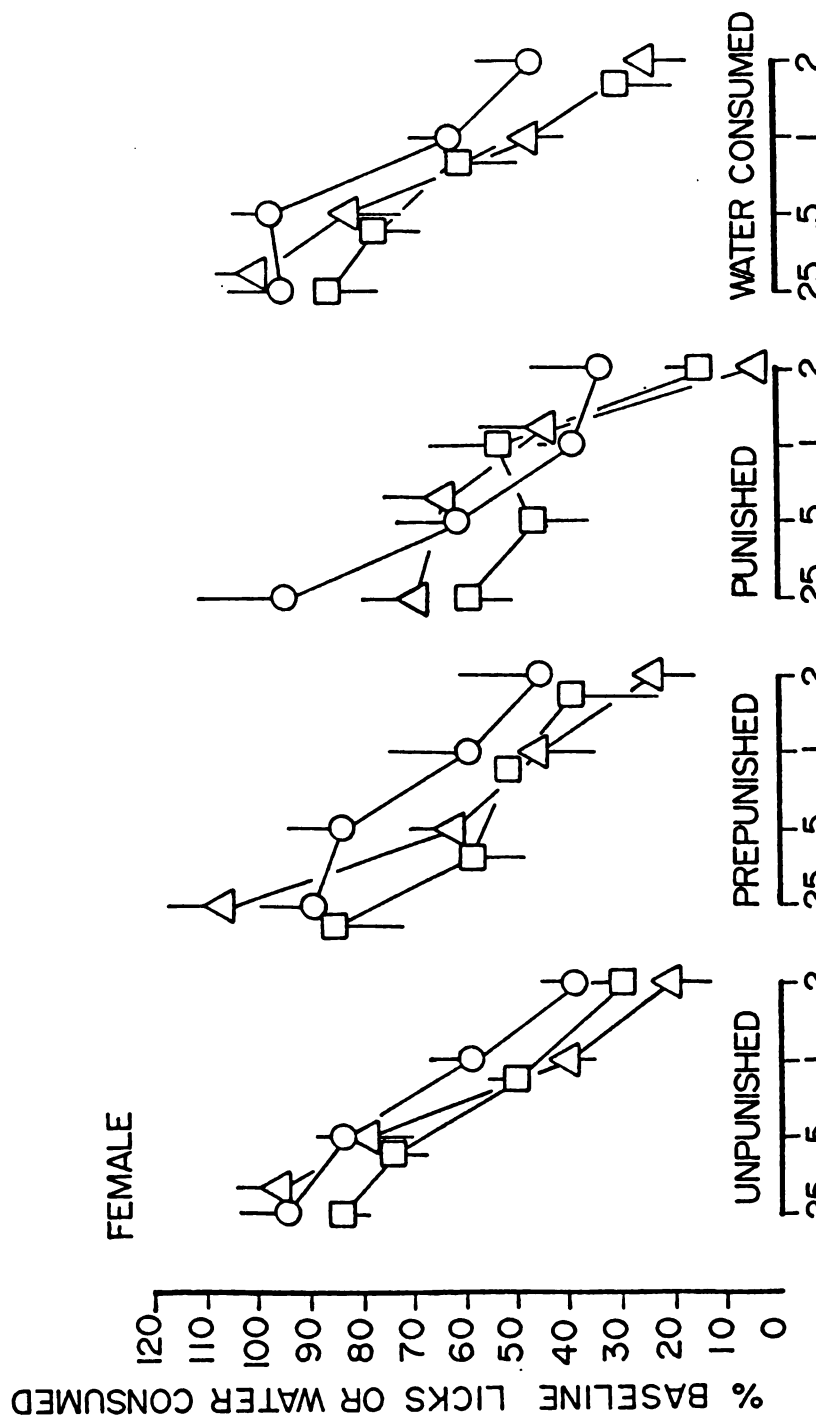

% BASELINE LICKS OR WATER CONSUMED
120
110
100
90
80
70
60
50
40
30
20
10
0
FEMALE
UNPUNISHED
PREPUNISHED
PUNISHED
WATER CONSUMED
.25
.5
1
2
mg/kg d-AMPHETAMINE

Figure 30

Figure 31. Effect of diazepam on conditioned suppression of drinking in male offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (○), 0.2 (△) or 2 mg/kg (□) fireMaster BP-6. Each data point represents the mean and standard error of all male offspring from the number of litters indicated in Table 31, represented by one individual per litter. Responses measured during the 10-minute test session included the number of licks obtained during unpunished, prepunished, and punished periods, as well as water consumed following a 15-minute pretreatment with 1.8, 3, 5.6 or 10 mg/kg diazepam. Analysis of variance revealed no significant difference between control and PBB-exposed offspring.

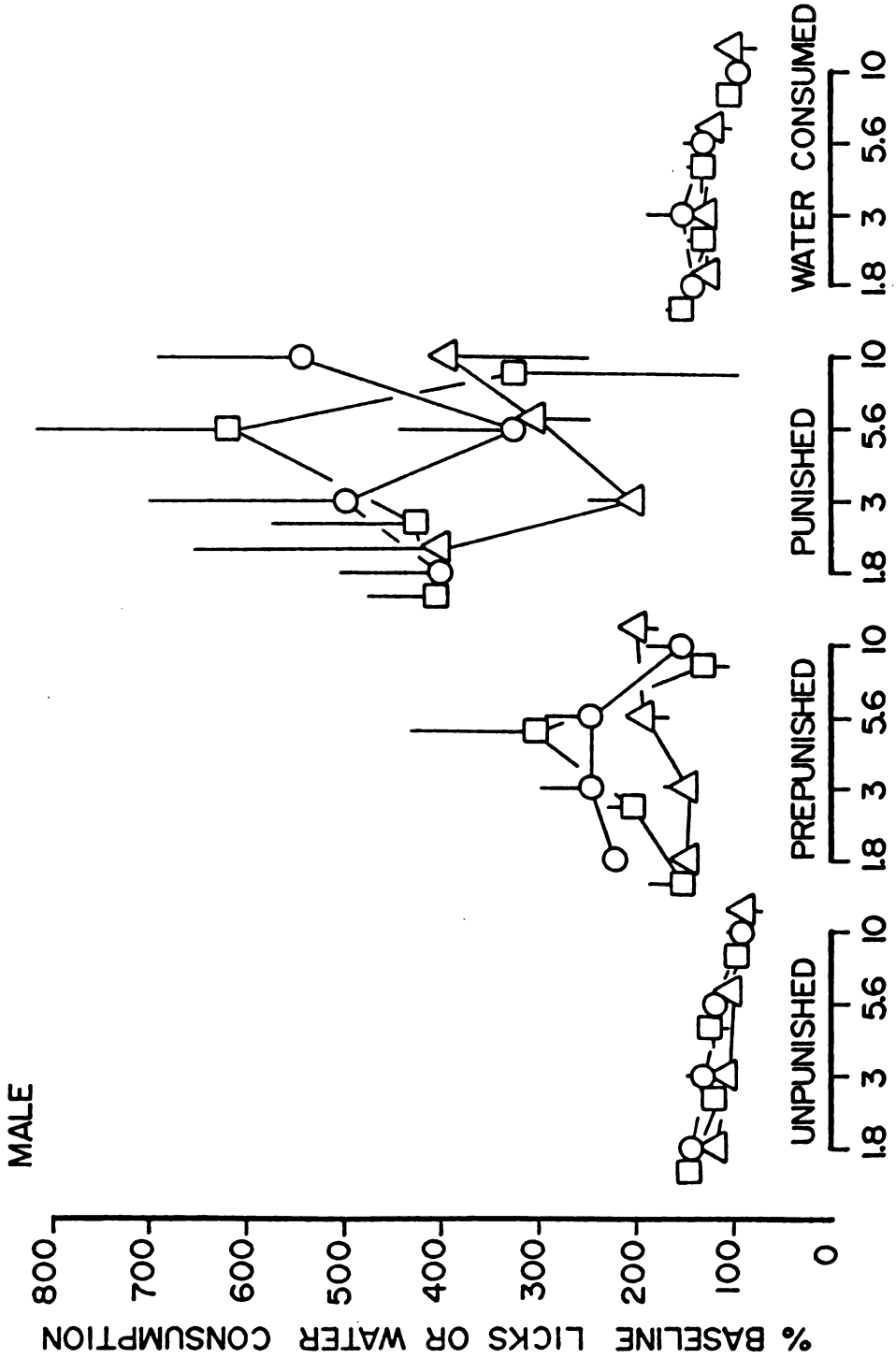

MALE
% BASELINE LICKS OR WATER CONSUMPTION
800
700
600
500
400
300
200
100
0
UNPUNISHED
PREPUNISHED
PUNISHED
WATER CONSUMED
1.8 3 5.6 10
mg/kg DIAZEPAM

Figure 31

Figure 32. Effect of diazepam on conditioned suppression of drinking in female offspring from dams administered fireMaster BP-6 during gestation and lactation. Dams received 0 (◯), 0.2 (△) or 2 mg/kg (□) fireMaster BP-6. Each data point represents the mean and standard error of all female offspring from the number of litters indicated in Table 31, represented by one individual per litter. Responses measured during the 10-minute test session included the number of licks obtained during unpunished, prepunished, and punished periods, as well as water consumed following a 15-minute pretreatment with 1.8, 3, 5.6 or 10 mg/kg diazepam. Analysis of variance revealed no significant difference between control and PBB-exposed offspring.

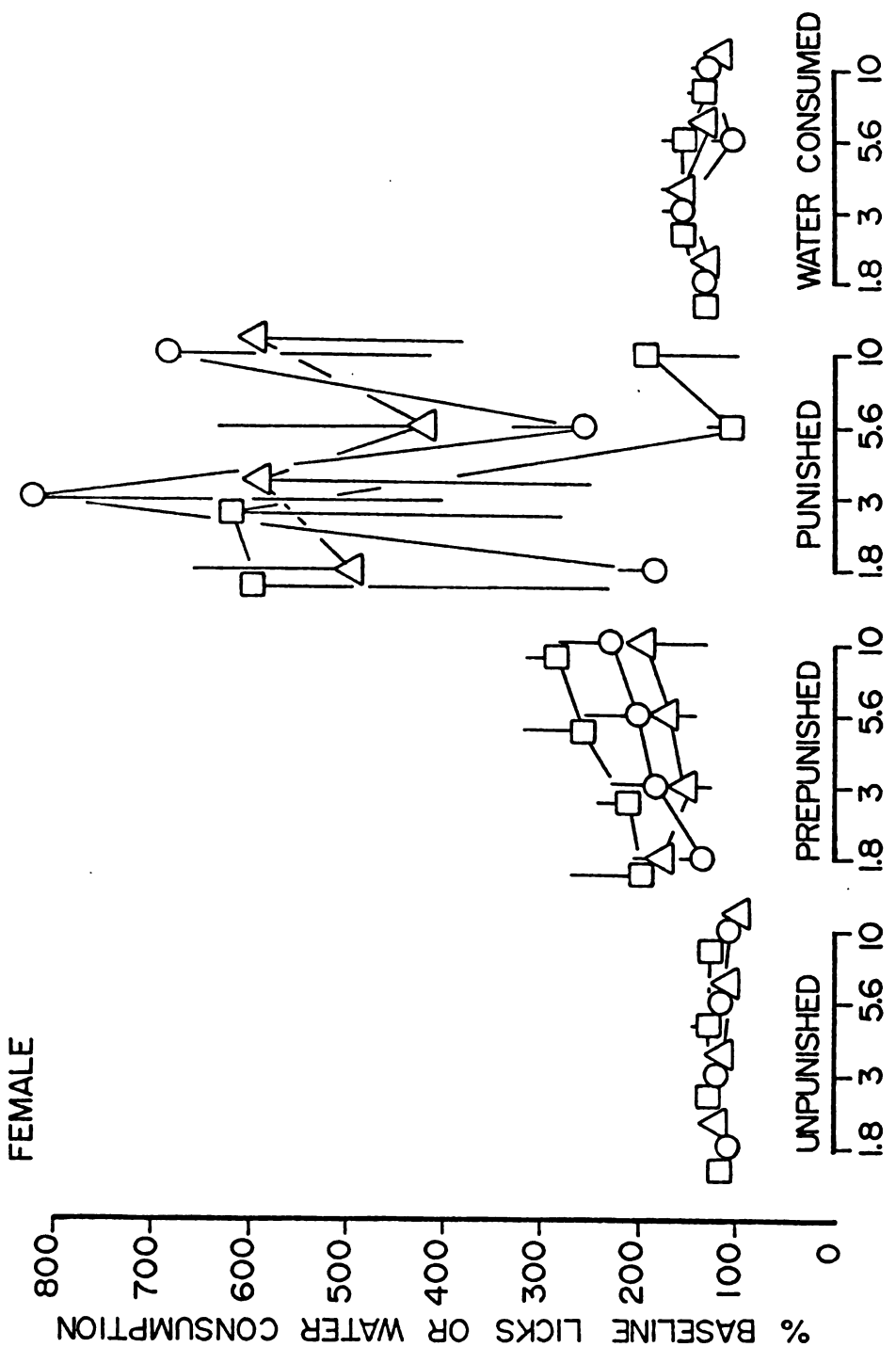

FEMALE
% BASELINE LICKS OR WATER CONSUMPTION
800
700
600
500
400
300
200
100
0
UNPUNISHED
PREPUNISHED
PUNISHED
WATER CONSUMED
1.8 3 5.6 10
mg/kg DIAZEPAM

Figure 32

dose-related manner from 1.8 to 5.6 mg/kg diazepam. However, prepunished responding was not enhanced to as great a degree following 10 mg/kg diazepam as following 5.6 mg/kg. Prepunished responding was enhanced following diazepam in a dose-related manner for all females on test; no PBB-related differences were observed.

Release of suppressed responding during the punished period was observed following all doses of diazepam in male and female rats; the magnitude of this release was in general greater than that observed during the prepunished period. Responding during this period following diazepam injection was highly variable, and no dose-response relationship was observed due to a depression of responding in control animals following administration of 5.6 mg/kg diazepam. A trend toward greater release of punished responding was observed in PBB-exposed female offspring than in control females following 1.8 mg/kg diazepam. This effect was not observed at 3 mg/kg diazepam, and in fact, punished responding of female offspring exposed to either 0.2 or 2 mg/kg/day BP-6 was somewhat less than that of controls following 5.6 or 10 mg/kg diazepam. Punished responding of PBB-exposed males was comparable to that of controls following administration of 1.8 or 5.6 mg/kg diazepam. However, males exposed to 0.2 mg/kg/day BP-6 responded less than did controls following 3 mg/kg diazepam, while males exposed to either PBB dose responded less following 10 mg/kg diazepam. These mean differences in responding were not statistically significant, but rather represent a trend in responding.

D. Discussion

Drug challenge was initiated in an attempt to uncover subtle behavioral effects not revealed during baseline responding for the conditioned suppression of drinking paradigm. Baseline punished responding was maintained at a relatively high level during the initial portion of the study, resulting in a clear-cut amphetamine dose-related decrease of unpunished, prepunished, and punished responding in all animals on test. This dose-related suppression of unpunished and punished responding has been reported previously and is independent of species or testing paradigm (Geller and Seifter, 1960; Miczek, 1973; Ford et al., 1979; Lazareno, 1979; Spealman, 1979; de Carvalho et al., 1981). No PBB-related effects were observed during unpunished or prepunished components of this paradigm.

A low dose of d-amphetamine (0.25 mg/kg) produced suppression of responding during the punished period only in animals exposed to 0.2 (females only) or 2 mg/kg BP-6 (males and females), but not in controls or males exposed to 0.2 mg/kg BP-6. This PBB-related effect was not observed at higher doses of d-amphetamine which resulted in enhanced suppression in all animals on test. Enhanced suppression of drinking during the punished period was not observed in controls following 0.25 mg/kg d-amphetamine; however, this dose appeared to strip away functional reserves in PBB-exposed animals, resulting in diminished punished responding. This PBB-related effect was eliminated at doses of amphetamine which also produced behavioral deficits in controls.

In contrast to results observed following d-amphetamine, increasing doses of diazepam exerted no effect on unpunished licks or water

consumed for either control or PBB-exposed rats. This lack of effect by diazepam on unpunished responding has been demonstrated over the dose range used in the present study previously (Kilts et al., 1981). Number of licks incurred during the prepunished period was increased slightly by diazepam, indicating that baseline responding during this period was suppressed due to anticipation of punishment. No PBB-related effects on prepunished responding were observed.

Punished responding was markedly enhanced by diazepam treatment. The most effective doses were 3 and 10 mg/kg for control animals; diazepam effects were diminished at 5.6 mg/kg, a phenomenon which has been observed previously (Commissaris and Rech, 1982). The reason for the extreme variability in responding during the punished period is unknown. A variable response to diazepam has been observed in the punished component of this behavior previously, and was found to increase with increasing diazepam dose (Ford et al., 1979; Kilts et al., 1981; Commissaris and Rech, 1982). Because of the high degree of variability, it was difficult to identify PBB-related effects. However, the pattern of enhanced punished responding for each dose of diazepam appeared to differ somewhat between control and PBB-exposed animals. In addition, punished responding was diminished following 10 mg/kg diazepam for animals exposed to 0.2 (females only) or 2 mg/kg BP-6 (males and females).

Statistical analysis of these results suggests rejection of the hypothesis that perinatal PBB exposure affects conditioned suppression of drinking. However, a trend for PBB-related effects following d-amphetamine or diazepam was observed. Power analysis conducted on probe

study data revealed that a sample size of at least 16 was required for each dose group to detect a minimum difference of 20% between sample means. Fewer animals were tested in the present study because not all could be trained to the conditioned suppression of drinking paradigm. Therefore, PBB-related effects might be more clear-cut upon repetition of the study with a larger sample size.

CHAPTER 5

ACQUISITION AND PERFORMANCE OF AN AUTOSHAPING PARADIGM BY RATS EXPOSED TO PBB PERINATALLY

A. Introduction

Cognitive deficits have been reported in epidemiologic studies of PBB-exposed children and older men (Valciukas et al., 1978; Schwartz and Rae, 1983; Seagull, 1983). Although the interpretation of these studies is difficult due to the subjective nature of the data, lack of correlation of effects with PBB levels, and failure to take into account the environmental factors surrounding the experimental subjects, it is still of great importance to determine experimentally if PBB exposure does affect cognition.

Hughes and Sparber (1978) developed an autoshaping operant paradigm which they felt to be a straightforward, objective measure of learning. Acquisition and performance of various aspects of autoshaping have been demonstrated to be affected by methylparathion (Gupta et al., 1985) and methylmercury (Hughes and Sparber, 1978).

Rats have been shown to be sensitive to deficits in operant responding produced by oral administration of PBB (Geller et al., 1979). Therefore, they were tested for cognitive deficits in the present study. The hypothesis tested was that perinatal PBB exposure affects the acquisition and performance of an autoshaping paradigm.

B. Materials and Methods

1. Experimental Subjects

Experimental subjects were offspring from a colony of female Sprague-Dawley rats administered 0, 0.2 or 2 mg/kg BP-6 in a peanut butter "treat" from day 6 of gestation through day 24 of lactation. The source of this colony, housing conditions, and breeding regimen are described in Chapter 2.

Body weights and general appearance of all dams were monitored throughout gestation and lactation. Gestation length, number of pups born alive, sex ratio of the pups, and mortality from birth through weaning were also assessed. All maternal and developmental parameters were comparable between controls and PBB-exposed animals, as reported in Chapter 2.

Offspring from each litter were cross-fostered as soon after birth as possible with a dam of the same dose group. At 3 days of age all litters were culled to four pups per sex where possible. At this time indelible ink was injected subcutaneously into the paws of the pups in a specific pattern for the purpose of identification. Females were randomly assigned numbers 1 through 4, while males were randomly assigned numbers 5 through 8. Pups remained with their foster mother until weaning on postpartum day 24 and were housed 2 or 3 per cage thereafter according to BP-6 treatment and sex.

Male number 8 and female number 4 were assigned to Group 4: the group tested for acquisition and performance of the autoshaping paradigm. Table 32 lists all parameters measured in Group 4 animals, the method of statistical analysis used in each case, and the partitioning of α according to groups of studies.

TABLE 32

Parameters Measured in Group 4 Animals, Including Method of Statistical Analysis and Partitioning of α

A. "Incidental" data

Parameter	Statistical Analysis	α
Body weight	ANOVA with mean comparisons	0.025[a]

B. Confirmatory Studies

Parameter	Statistical Analysis	α
Overall study	No aggregated analysis	0.025[b]
1. Phase I		0.0144[c]
a. Day of acquisition	ANOVA with mean comparisons	0.0102[d]
b. Latency to lever press	ANOVA with mean comparisons	0.0102[d]
2. Phase II Acquisition	Repeated measures ANOVA	0.0144[c]
3. Phase III		0.0144[c]
a. Acquisition	Repeated measures ANOVA	0.0083[e]
b. d-Amphetamine challenge	Multifactorial ANOVA	0.0083[e]
c. Chloral hydrate challenge	Multifactorial ANOVA	0.0083[c]

[a] α arbitrarily assigned as 0.025 to correspond with overall group level.

[b] α (0.05) partitioned among Groups 1-4; therefore, $\alpha = \frac{.05}{\sqrt{4}} = 0.025$.

[c] α (0.025) partitioned among Phases 1-3; therefore, $\alpha = \frac{.025}{\sqrt{3}} = 0.0144$.

[d] α (0.0144) partitioned among 2 tests; therefore, $\alpha = \frac{.0144}{\sqrt{2}} = 0.0102$.

[e] α (0.0144) partitioned among 3 tests; therefore, $\alpha = \frac{.0144}{\sqrt{3}} = 0.0083$.

2. Acquisition and Performance of Operant Responses

a. "Incidental" data. These data were considered incidental to, but supportive of, the primary goal of testing in Group 4 animals: assessment of the effect of perinatal PBB exposure on the acquisition and performance of an autoshaping paradigm.

All animals were weighed each day of testing. For the sake of clarity, only body weights obtained at the following time points were included in statistical analysis: immediately prior to training, before the first dose of d-amphetamine, and before the first dose of chloral hydrate. Body weight data were analyzed by ANOVA. Mean comparisons were conducted using the least significant difference test.

b. Confirmatory studies. At approximately 6 months of age, Group 4 rats were food deprived to 80% of their free-feeding weights and subjected to an autoshaping procedure 6 days per week in a modification of the method of Hughes and Sparber (1978). This procedure is designed to measure acquisition of a series of operant responses of increasing difficulty.

The testing apparatus consisted of two standard Coulbourn model E99-12 operant test systems (Coulbourn Instruments, Lehigh Valley, PA) located in a sound-attenuating enclosure. Each operant cage contained a retractable lever on the right front panel. To the left of the lever was a food presentation chute, and above the lever was an amber arc light. All phases of testing, as well as data acquisition, were controlled by solid-state programming circuits (Coulbourn Instruments, Lehigh Valley, PA). Group 4 males were tested from 0800 to 1200 hours, while females were tested from 1200 to 1600 hours.

The first phase of testing, referred to hereafter as Phase I, involved acquisition of lever pressing behavior. One rat was placed in each experimental chamber for a period of approximately one hour. The cue light was illuminated on a variable interval 90-second schedule and the lever was extended for 15 seconds. Each extension constituted a trial and 32 trials comprised a session. Contact with the lever resulted in presentation of a 45 mg BioServe food pellet (Bio-Serve, Inc., Frenchtown, NJ), immediate withdrawal of the lever, extinguishing of the cue light, and initiation of a new trial. If the rat failed to respond during the 15-second period, no food pellet was delivered, but a new trial was initiated. The number of responses emitted and time required to make contact with the lever (latency to respond) were recorded daily. Day of acquisition of 32 successful trials and the latency to lever press on the day of acquisition were analyzed statistically by ANOVA. Mean comparisons were conducted using the least significant difference test.

Following acquisition of Phase I, Group 4 rats were trained to Phase II (fixed interval 90-second responding) in the same operant cages. The lever remained extended during the 1-hour test period. The cue light, however, was presented on a fixed interval 90-second response contingency. All lever presses within the 15-second period of cue light illumination were reinforced by a food pellet. Acquisition of asymptotic responding was measured. Mean number of bar presses for each treatment group across 24 days of training were compared via repeated measures ANOVA. Two analyses were conducted: one to compare the results of offspring exposed to 0, 0.2 or 2 mg/kg/day BP-6

and one to compare the results of offspring exposed to 0 or 0.2 mg/kg/day BP-6.

Following acquisition of Phase II, all rats were trained to a fixed-ratio 20 (FR-20) paradigm in the same operant cages. Each set of twenty lever presses elicited a food pellet reinforcement during the 30-minute test period. Acquisition of asymptotic responding was measured. Mean number of reinforcements obtained across 14 days of training were compared via repeated measures ANOVA. Two analyses were conducted: one to compare the results of offspring exposed to 0, 0.2 or 2 mg/kg/day BP-6 and one to compare the results of offspring exposed to 0 or 0.2 mg/kg/day BP-6.

When asymptotic responding on the FR-20 contingency was achieved, drug challenge was initiated. All rats received all doses of 0.5, 1 or 2 mg/kg d-amphetamine. Doses were administered in random order, and at least 2 drug-free testing days intervened between drug testing days. When a dose-response curve for d-amphetamine had been obtained, all rats then received chloral hydrate (20, 40, 80 or 120 mg/kg). Again, all rats received all doses, doses were administered in random order, and at least two drug-free days were followed by a drug treatment day. Effects related to BP-6 exposure for both drug challenges were evaluated by multifactorial ANOVA. Factors were BP-6 dose and drug dose.

C. Results

1. "Incidental" Data

Body weights of all offspring tested in the autoshaping paradigm are presented in Table 33. All mean body weights of PBB-exposed

TABLE 33

Mean (± S.E.M.) Body Weights of Rats Tested in an Autoshaping Paradigm Following Perinatal Exposure to fireMaster BP-6 (PBB)

Time of Measurement	Body Weight (grams)					
	0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Day 1 of training	330.8+15.8 (6)	260.1+6.5 (8)	362.9+16.8 (8)	240.3+10.3 (7)	406.5+10.2 (4)	242.0+8.1 (5)
Immediately prior to d-amphetamine	367.3+13.1 (6)	261.1+6.5 (8)	395.0+11.1 (8)	243.1+ 8.3 (7)	408.5+ 6.1 (4)	240.8+5.4 (5)
Immediately prior to chloral hydrate	392.2+ 8.9 (6)	259.5+5.0 (8)	411.8+11.0 (8)	246.9+ 5.3 (7)	424.8+ 9.6 (4)	242.2+5.8 (5)

All mean body weights of PBB-exposed animals were comparable to those of controls.

Numbers in parentheses = N.

males and females were comparable to those of controls throughout the autoshaping test period.

2. Confirmatory Studies

a. Phase I - VI-90 seconds. Data for acquisition and performance of Phase I of the autoshaping paradigm are presented in Table 34. In this phase a lever was presented 32 times on a VI-90 second schedule; contact with the lever within 15 seconds of presentation resulted in a food pellet reinforcement and retraction of the lever. Acquisition of this behavior required several days, as test animals were initially apprehensive of the novel environment of the testing chamber and of the noise made upon lever presentation. Successful completion of 32 trials was significantly ($p<0.0102$) delayed for female offspring from dams administered 2 mg/kg/day BP-6; acquisition by all other PBB-exposed animals was comparable to that of controls, although a trend toward delayed acquisition was observed in all PBB-exposed animals. Latency to press the lever during each 15-second period was also measured at the time of successful completion of 32 trials; no PBB-related differences were observed.

b. Phase II - FI-90 seconds. During Phase II, animals were trained to press the lever on a FI-90 second schedule in response to a cue light. All lever presses during the 15-second period of cue light illumination were reinforced with a food pellet. No significant effects due to PBB treatment were observed for either sex when analyzed by repeated measures ANOVA. However, the p-value for males was 0.0278, indicating that testing of a larger number of animals independently (thus allowing for less rigid statistical criteria) would reveal a

TABLE 34

Acquisition and Performance by Rats of Phase I of an Autoshaping Paradigm Following Perinatal Exposure to fireMaster BP-6 (PBB)

NUMBER OF DAYS REQUIRED TO ACHIEVE 32 LEVER PRESSES (DAY OF ACQUISITION)

Number of Days					
0 mg/kg/day		0.2 mg/k/day		2 mg/kg/day	
Male	Female	Male	Female	Male	Female
6.0±0.6[a] (7)	4.4±0.8 (8)	8.5±1.0 (8)	7.1±0.9 (7)	7.7±1.1 (7)	10.8±2.5* (5)

MEAN LATENCY TO LEVER PRESS ON DAY OF ACQUISITION

Latency (sec)					
0 mg/kg/day		0.2 mg/kg/day		2 mg/kg/day	
Male	Female	Male	Female	Male	Female
2.31±0.16[a]	2.45±0.47	1.71±0.34	2.81±0.38	1.99±0.75	2.32±0.79

[a]Mean ± S.E.M.

*Significantly different from control mean by ANOVA and least significant difference test, $p<0.0102$.

Numbers in parentheses = N.

significant effect. Observation of data for the males (Figure 33) reveals that males exposed to either 0.2 or 2 mg/kg/day BP-6 had a greater number of successful bar presses during the 60-minute test session than did controls; those exposed to 2 mg/kg/day had the greatest number. This trend was apparent from day 4 through day 20 of training. Responses were not measured beyond 24 days because animals were trained to a new paradigm.

In contrast, female rats exposed to 2 mg/kg BP-6 exhibited a trend for fewer lever presses from training days 8 through 14 when compared to controls (Figure 34). Acquisition of FI-90 second responding was comparable between controls and females exposed to 0.2 mg/kg/day BP-6. The opposite response observed in males was of greater magnitude than that observed in females. A significant ($p=0.0001$) training day effect was observed for males and females.

Following acquisition of Phase II, all Group 4 animals were trained to respond on a FR-20 contingency. Each 20 lever presses during the 30-minute test session resulted in presentation of a food pellet reinforcement. No PBB-related significant effects were observed for acquisition of FR-20 responding. However, significant effects were observed for training day for both sexes ($p=0.0001$) and for the training day and BP-6 dose interaction for females (for those exposed to 0.2 mg/kg/day BP-6, $p=0.004$; for those exposed to 2 mg/kg/day BP-6, $p=0.0001$). This significant training day and BP-6 dose interaction occurred because females exhibited a PBB-related deficit in FR-20 responding (Figure 36) from day 9 of training through the final training

Figure 33. Acquisition and performance by male rats of Phase II (FI-90 seconds) of an auto-shaping paradigm following perinatal exposure to fireMaster BP-6 (PBB). Dams of these males were administered 0 (○), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6 during gestation and lactation. Each data point and vertical line represents the mean and standard error of all male offspring from the number of litters indicated in Table 32 represented by one individual per litter. Acquisition of this food-rewarded FI-90 seconds paradigm over a period of 24 days is presented. Each test session was 60 minutes in duration. A trend is apparent for enhanced acquisition and performance by male offspring exposed to either 0.2 or 2 mg/kg/day fireMaster BP-6.

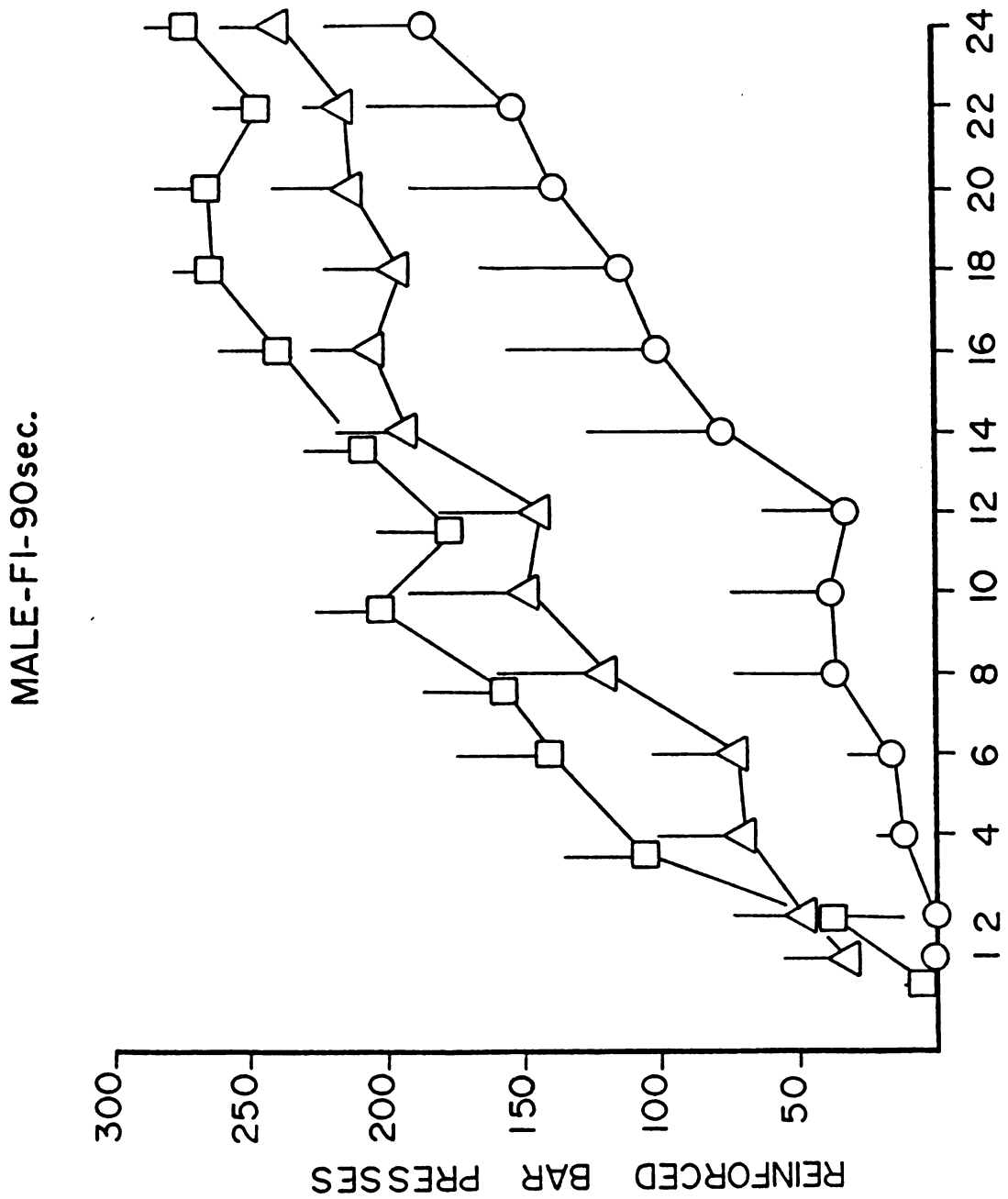

MALE-FI-90sec.
REINFORCED BAR PRESSES
300
250
200
150
100
50
1 2 4 6 8 10 12 14 16 18 20 22 24
DAYS ON TEST

Figure 33

Figure 34. Acquisition and performance by female rats of Phase II (FI-90 seconds) of an autoshaping paradigm following perinatal exposure to fireMaster BP-6 (PBB). Dams of these females were administered 0 (◯), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6 during gestation and lactation. Each data point and vertical line represents the mean and standard error of all female offspring from the number of litters indicated in Table 32, represented by one individual per litter. Acquisition of this food-rewarded FI-90 seconds paradigm over a period of 24 days is presented. Each test session was 60 minutes in duration. A trend is apparent for a transient delay in acquisition by offspring exposed to 2 mg/kg/day fireMaster BP-6.

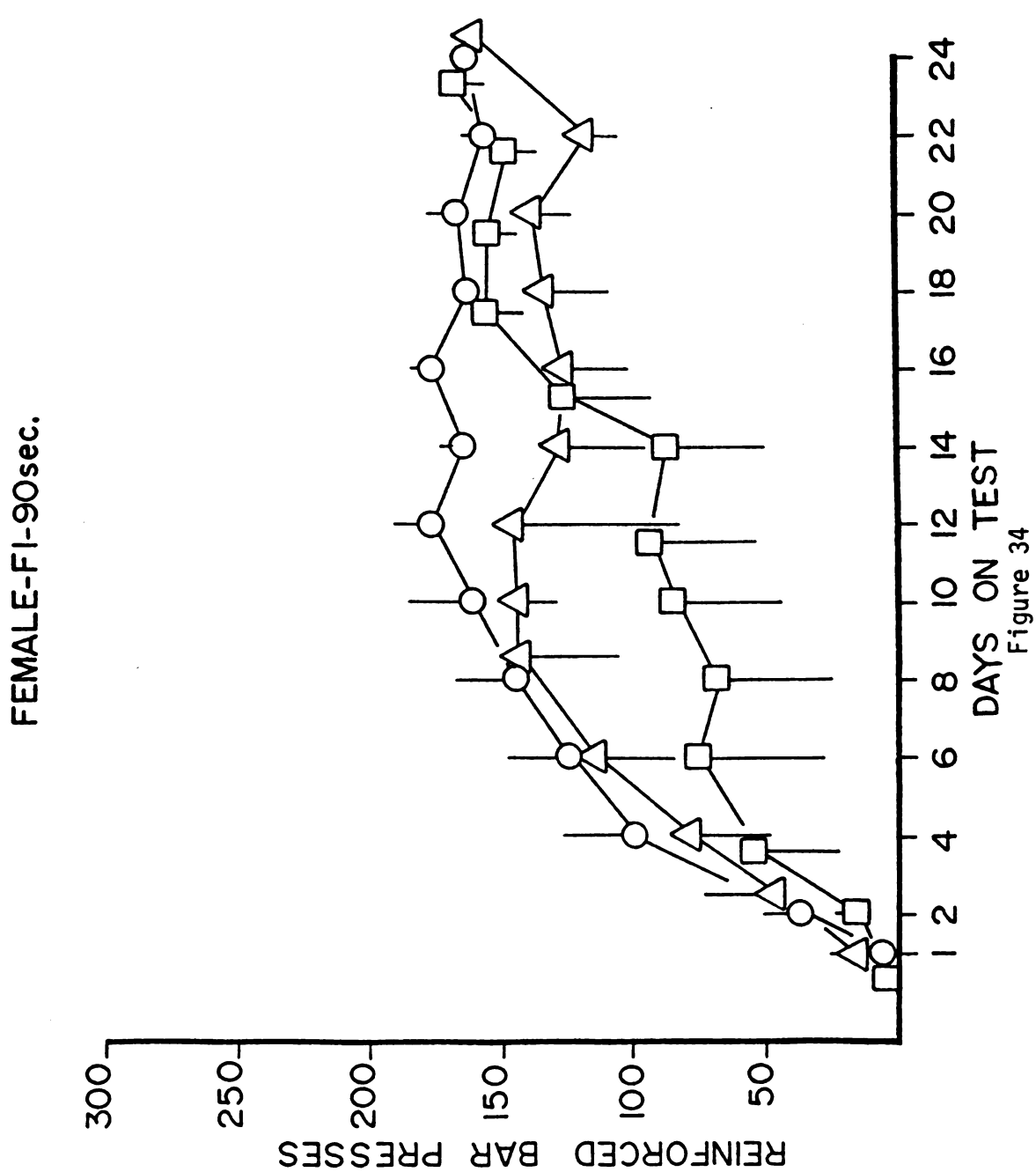

FEMALE-FI-90sec.
REINFORCED BAR PRESSES
300
250
200
150
100
50
1 2 4 6 8 10 12 14 16 18 20 22 24
DAYS ON TEST

Figure 34

Figure 35. Acquisition and performance by male rats of Phase III (FR-20) of an autoshaping paradigm following perinatal exposure to fireMaster BP-6 (PBB). Dams of these males were administered 0 (◯), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6 during gestation and lactation. Each data point and vertical line represents the mean and standard error of all male offspring from the number of litters indicated in Table 32, represented by one individual per litter. Acquisition of this food-rewarded FR-20 paradigm over a period of 14 days is presented. Each test session was 30 minutes in duration. A trend is apparent for transient enhanced acquisition by offspring exposed to 0.2 or 2 mg/kg/day fireMaster BP-6.

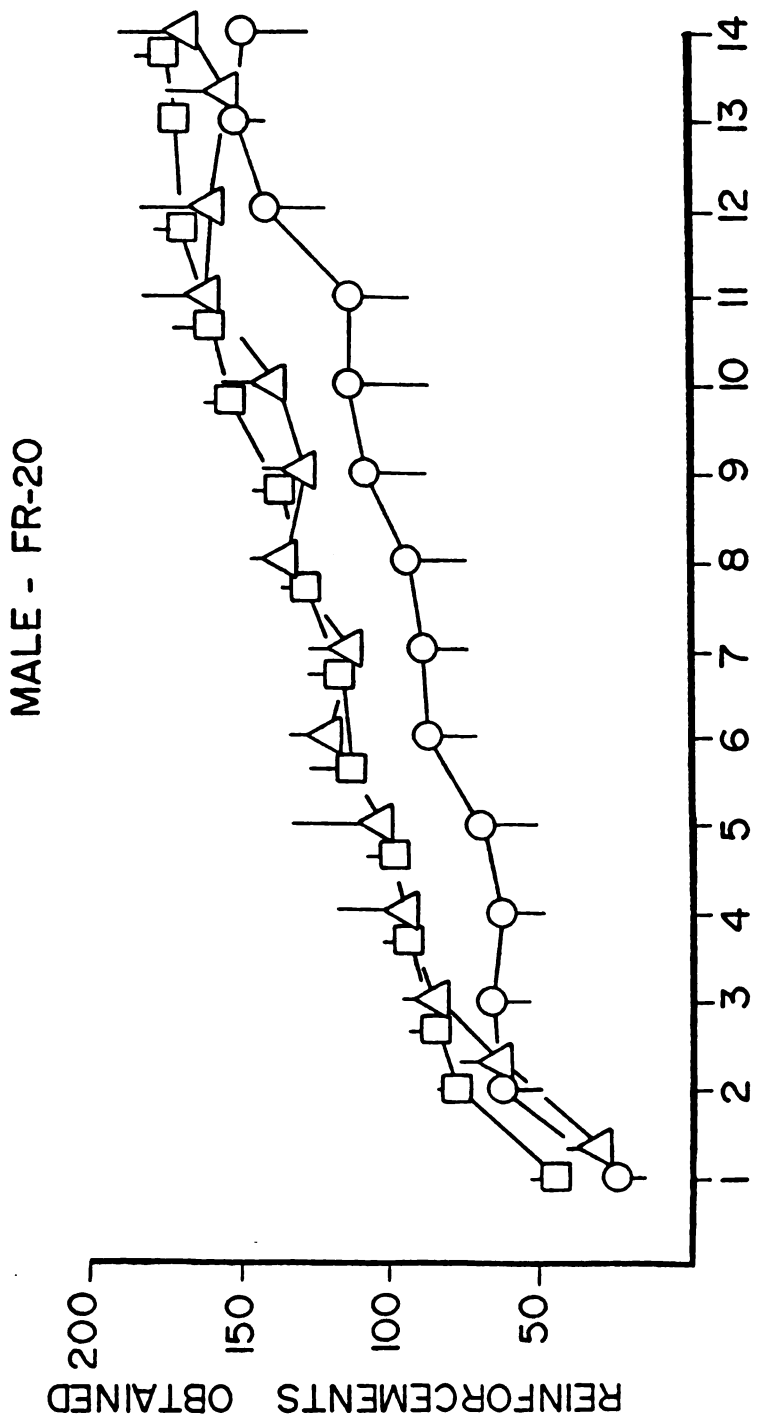

MALE - FR-20
REINFORCEMENTS OBTAINED
200
150
100
50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS ON TEST

Figure 35

day (day 14). These results would indicate a deficit in the performance, rather than the acquisition, of the learned behavior.

Although no significant PBB-related effects were observed for male rats on the FR-20 contingency, PBB-exposed animals exhibited a trend to press the lever a significantly greater number of times during the test session (Figure 35). This trend was transient, and no difference in rate of responding was observed with regard to BP-6 dose.

After 14 days of training, drug challenge was initiated. Administration of 0.5 to 2 mg/kg d-amphetamine to all animals on test resulted in a dose-related decrease in the percent baseline day reinforcers obtained (Figure 37). Overall rate of responding was less for females than for males, and no PBB treatment-related effects were observed among females. Male offspring from dams administered 2 mg/kg/day BP-6 exhibited a trend to obtain significantly fewer reinforcements than did controls following 0.5, 1, or 2 mg/kg d-amphetamine. Number of reinforcers obtained by male offspring exposed to 0.2 mg/kg/day BP-6 was comparable to that of controls.

Administration of 20 to 120 mg/kg chloral hydrate resulted in a dose-related decrease in percent baseline day reinforcers obtained by male and female control offspring or offspring exposed to 0.2 mg/kg/day BP-6 (Figure 38). Male rats exposed to 2 mg/kg/day BP-6 exhibited a trend to obtain a significantly greater number of reinforcers following 20 or 40 mg/kg chloral hydrate; i.e., their responding was actually stimulated by these doses. By contrast, responding of female rats exposed to 2 mg/kg/day BP-6 was less than that of controls

Figure 36. Acquisition and performance by female rats of Phase III (FR-20) of an autoshaping paradigm following perinatal exposure to fireMaster BP-6 (PBB). Dams of these females were administered 0 (◯), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6 during gestation and lactation. Each data point and vertical line represents the mean and standard error of all female offspring from the number of litters indicated in Table 32, represented by one individual per litter. Acquisition of this food-rewarded FR-20 paradigm over a period of 14 days is presented. Each test session was 30 minutes in duration. A trend was apparent for a deficit in performance by offspring exposed to 0.2 or 2 mg/kg/day fireMaster BP-6.

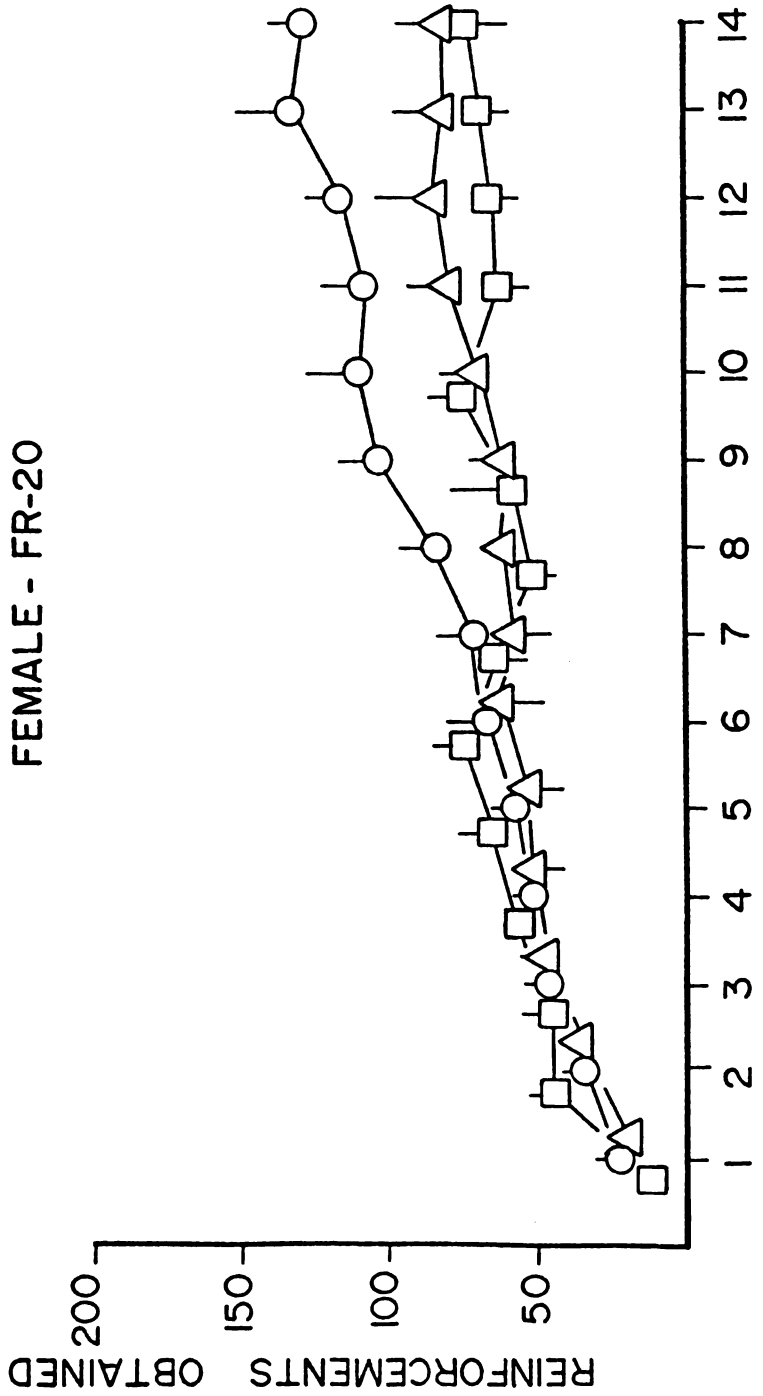

FEMALE - FR-20
REINFORCEMENTS OBTAINED
200
150
100
50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS ON TEST

Figure 36

Figure 37. Effect of d-amphetamine on FR-20 responding in male and female rats following perinatal exposure to fireMaster BP-6 (PBB). Dams of these offspring were administered 0 (○), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6 during gestation and lactation. Each data point represents the mean and standard error of all male or female offspring from the number of litters indicated in Table 32, represented by one individual per litter. Data are presented as percent of baseline day food pellet reinforcers obtained during the 30-minute test session following a 15-minute pretreatment with 0.5, 1 or 2 mg/kg d-amphetamine. A trend was apparent for a decrease in reinforcers obtained following d-amphetamine in male offspring exposed to 2 mg/kg/day fireMaster BP-6.

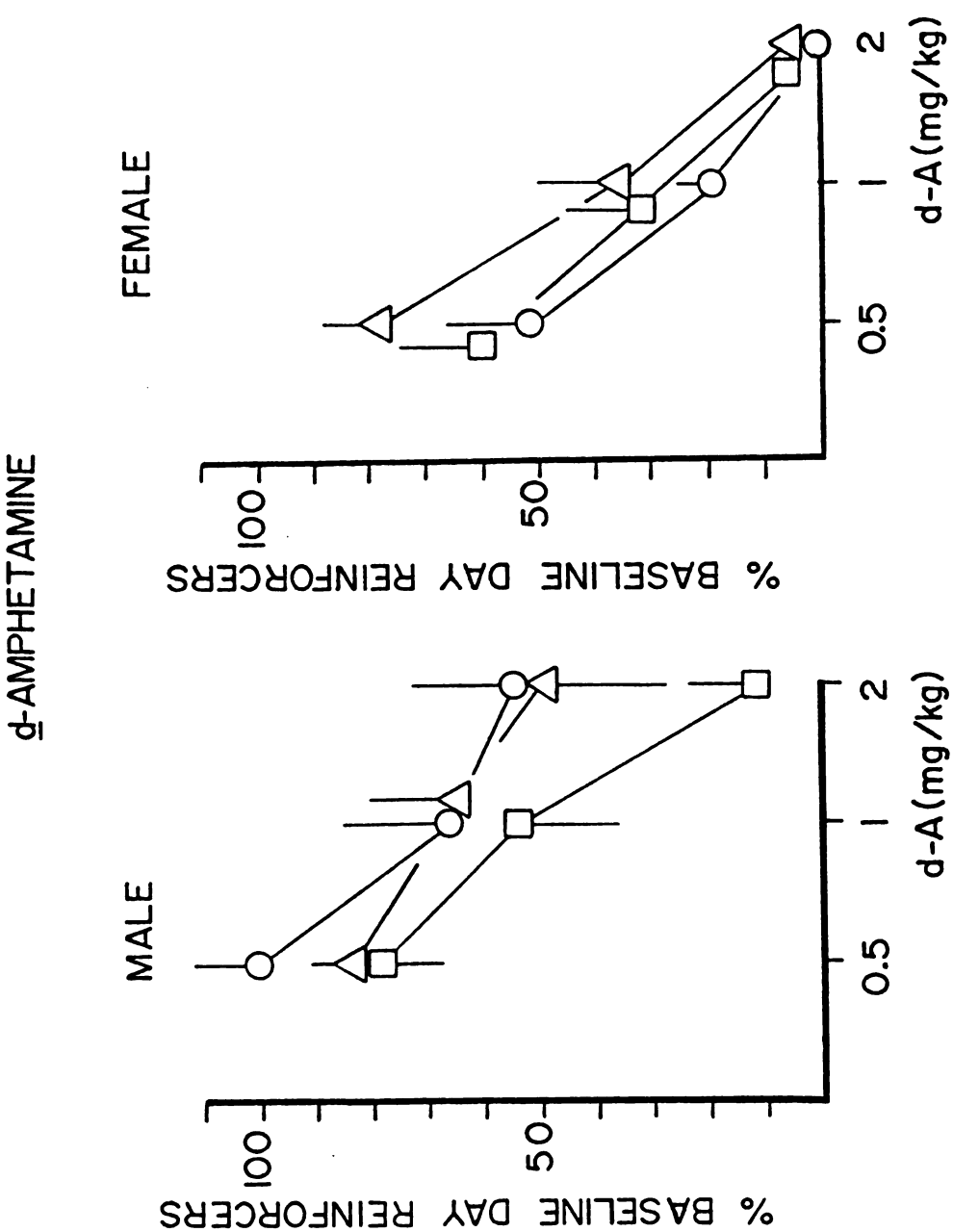

d-AMPHETAMINE
MALE
FEMALE
% BASELINE DAY REINFORCERS
100
50
0.5
1
2
d-A(mg/kg)

Figure 37

Figure 38. Effect of chloral hydrate on FR-20 responding in male and female rats following perinatal exposure to fireMaster BP-6 (PBB). Dams of these offspring were administered 0 (○), 0.2 (△) or 2 mg/kg/day (□) fireMaster BP-6 during gestation and lactation. Each data point represents the mean and standard error of all male or female offspring from the number of litters indicated in Table 32, represented by one individual per litter. Data are presented as percent of baseline day food pellet reinforcers obtained during the 30-minute test session following a 15-minute pretreatment with 20, 40, 80 or 120 mg/kg chloral hydrate. A trend was apparent for an increase in reinforcers obtained by male rats exposed to 2 mg/kg/day fireMaster BP-6 and a decrease in reinforcers obtained by female rats exposed to 2 mg/kg/day fireMaster BP-6, following administration of 20 mg/kg chloral hydrate.

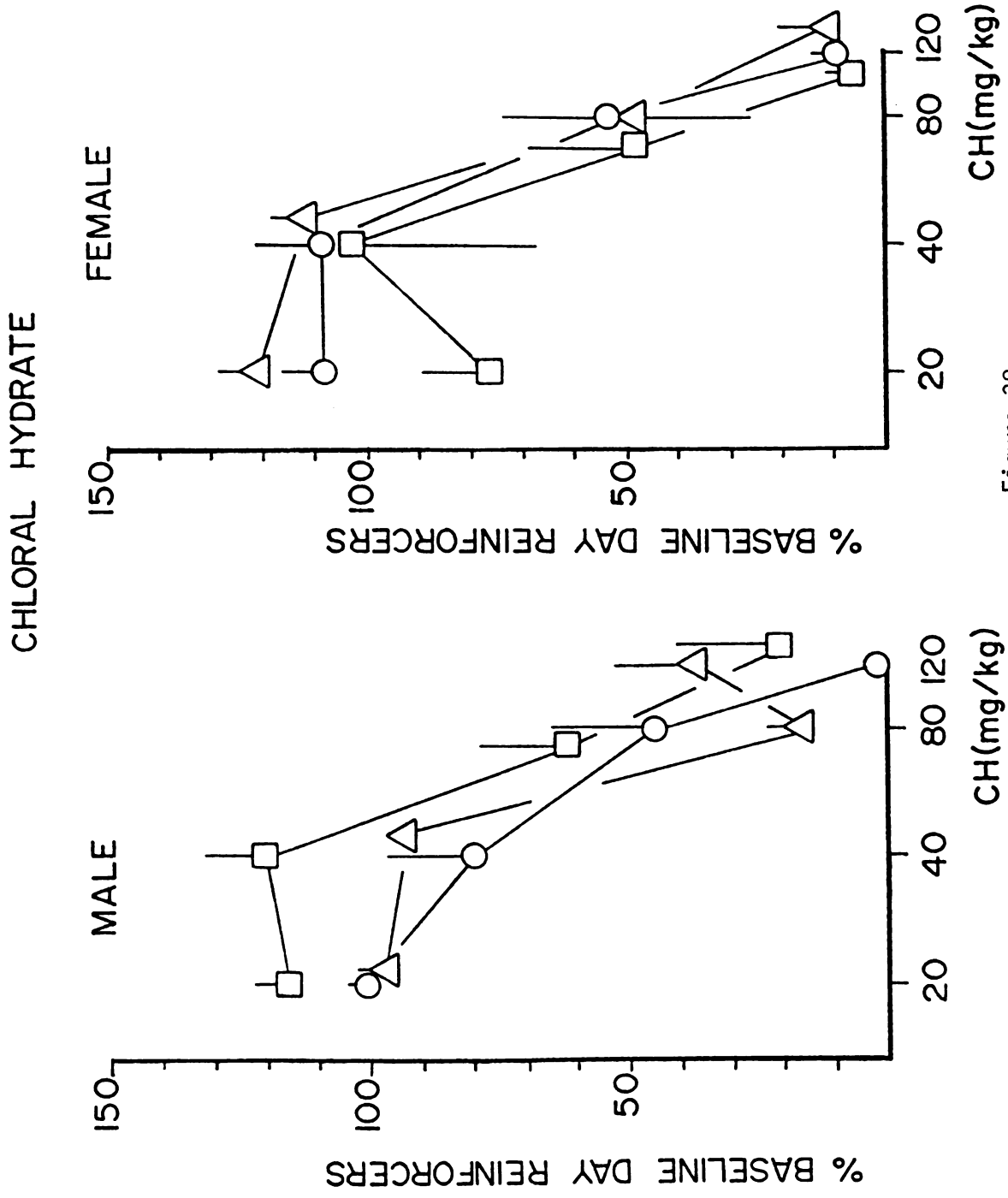

CHLORAL HYDRATE
MALE
FEMALE
% BASELINE DAY REINFORCERS
150
100
50
20
40
80
120
CH(mg/kg)

Figure 38

following 20 mg/kg chloral hydrate, but was comparable to that of controls following 40, 80 or 120 mg/kg chloral hydrate.

D. Discussion

In the present study, acquisition of VI-90 second responding in female offspring exposed to 2 mg/kg BP-6 was delayed when compared to controls. Although not statistically significant, a trend toward delayed acquisition was also observed in male and female offspring exposed to 0.2 mg/kg/day BP-6 and in male offspring exposed to 2 mg/kg/day BP-6. This was the first operant paradigm to which these animals were exposed and was characterized by a very loud presentation of the exteroceptive stimulus (the lever) to which the animals cued. Delayed acquisition may not have been due entirely to a lack of association of lever extension with food reward, but rather to an enhanced sensitivity to the loud noise. Once acquired, latency to press the lever once it was extended was comparable for control and PBB-exposed animals, indicating no deficit in performance of the task.

The VI-90 second schedule was replaced by a FI-90 second schedule of reinforcement. In this schedule the exteroceptive stimulus was a cue light located above the lever; the operant task was to associate the cue light (presented at a fixed interval) with the reinforcement period. This is considered to be the simplest type of operant procedure used in behavioral toxicology testing (Norton, 1982). In the present study, the FI responding of male rats exposed to either 0.2 or 2 mg/kg/day BP-6 was significantly greater than that of controls for the majority of the testing period. Control males exhibited an extremely low level of

responding; thus, the stimulant or disinhibitory effects exerted by BP-6 were readily apparent. Sexual dimorphism in this response was observed: female rats exposed to 2 mg/kg/day BP-6 made significantly fewer responses than did controls for approximately half of the test sessions. Responding of control females was much greater than that of control males. The PBB-related effects observed in animals with low levels of responding would not be expected to be identical to those in animals with higher rates of responding. Results of female rats indicated that the effects of PBB exposure on acquisition of FI-90 second responding were in fact inhibitory, but the magnitude of these effects was far less than that observed in males.

Animals were subsequently trained to a FR-20 contingency in which they were required to press a lever 20 times to receive a food pellet reinforcement. As with the fixed-interval contingency, responding of control male rats was less than that of control females. Males exposed to 0.2 or 2 mg/kg/day BP-6 pressed the lever significantly more times than did controls during acquisition of the behavior, but not once asymptotic levels of responding had been achieved. In contrast, acquisition of FR-20 responding was unaffected by PBB exposure in female rats. However, as performance increased with time to asymptotic levels, a deficit was revealed in females exposed to either 0.2 or 2 mg/kg/day BP-6. This deficit was still present at a time when asymptotic levels of responding were achieved by control females.

To identify further deficits for which compensation may have occurred, drug challenge with a stimulant or a depressant was initiated during the FR-20 phase. Following injection of d-amphetamine, the

overall rate of responding was less in females than in males, a situation different from that observed with spontaneous locomotor activity. However, with regard to operant responding, a stimulant such as d-amphetamine would not be expected to generate as much activity in high responders (females) as in low responders (males). Because PBB appear to act in general as depressants of behavior in females, it is not surprising that the dose-related rate-decreasing effects of amphetamine were comparable for control and PBB-exposed females.

A decreased number of reinforcements following amphetamine injection was obtained by male rats exposed to 2 mg/kg BP-6. If these animals were already stimulated or their behavior disinhibited by BP-6, it is possible that the behavioral disruption produced by d-amphetamine would be enhanced in PBB-exposed animals. This enhanced responsiveness to d-amphetamine is in contrast to the decreased sensitivity to d-amphetamine-induced stimulation of locomotor activity, indicating a different mechanism of brain locus of action.

The action of chloral hydrate on the central nervous system is similar to that of barbiturates: it is a central depressant. Like barbiturates, low doses would be expected to increase behavioral output (Iversen and Iversen, 1981). This was indeed the case in the present study for male rats at doses of 20 and 40 mg/kg chloral hydrate. Males exposed to 2 mg/kg/day BP-6 responded significantly more than did controls following injection of these low doses. This would seem to represent a potentiation of the disinhibition of behavior experienced by these animals. In contrast, females exposed to 2 mg/kg/day BP-6 pressed the lever significantly less than controls following 20 mg/kg chloral

hydrate, indicating that the disinhibition observed following this low dose was counteracted by PBB exposure. All other animals on test showed a decline in operant responding with increasing chloral hydrate dose.

Results of these studies indicate the proposed hypothesis is true. PBB does affect the acquisition and performance of an autoshaping paradigm. As with the conditioned suppression of drinking paradigm, however, effects would be expected to be more clear-cut if a larger number of subjects was tested.

DISCUSSION

A. Developmental Changes

Maternal toxicity (as assessed by body weight) occurred at a lower BP-6 dose following administration during gestation and lactation (40 doses) than during gestation alone (12 doses) or lactation alone (24 doses). With regard to cumulative PBB dose, the no-effect level for maternal toxicity achieved in this study was 43 mg (6 mg/kg/day BP-6 administered from days 0 through 24 of lactation).

Gestation length was increased by prenatal administration of 60 mg/kg/day BP-6. This phenomenon has not been reported previously for rodents administered PBB, but is similar to the extended gestation which occurred as a result of PBB ingestion by cows (Jackson and Halbert, 1974). Calves born to these cows were dead or died shortly after birth, as did rat pups born to dams administered 60 mg/kg/day BP-6. Birth defects observed in these pups included hydrocephaly and underdeveloped limbs. Administration of 12 mg/kg/day BP-6 to pregnant rats also resulted in late fetal death, but no malformations were observed. These results indicate that the extent of damage to the developing organism depends on the dose of BP-6 administered. Both doses acted late in development to produce mortality and, in fact, it was further demonstrated that 60 mg/kg/day BP-6 administered to dams during lactation was lethal to pups after only 12 days. It is of interest that pups exposed

to 60 mg/kg/day BP-6 during either gestation or lactation survived approximately 12 days of exposure. In the case of those exposed in utero, survival may have been compromised by the cranial and limb defects. Therefore, exposure during lactation did not appear to result in a decreased chance of survival relative to that observed in pups exposed during gestation. Administration of 800 mg/kg BP-6 to pregnant Wistar rats on one day of pregnancy only resulted in cleft palate and diaphragmatic hernia in the pups when given on gestation days 11, 12 or 13 (Beaudoin, 1977), indicating that a high dose of PBB administered during a crucial period of development can adversely affect development.

In the present study anomalies were produced at a BP-6 dose roughly equivalent to 1000 ppm administered in the diet. Mice administered 1000 ppm fireMaster in the diet during gestation gave birth to pups exhibiting cleft palate, exencephaly, hydronephrosis, and cystic malformations of the midbrain and adjacent structures (Corbett et al., 1975; Welsch and Stedman, 1984). The fact that these types of anomalies, as well as those observed in the Beaudoin (1977) study, were not observed in the present study is not surprising. Many malformations, including cleft palate and exencephaly, have been shown to be genetically linked. As an example, A/J mice are more susceptible to cortisone-induced cleft palate than are C57BL/6J mice due to later palate closure in the former strain (Biddle, 1981). There is, therefore, no reason to believe that PBB-induced malformations would be identical among species of rodents or strains of rats. The similarity in the response of rodents to prenatal administration of PBB lies in the fact that malformations have always been observed at levels which are maternally toxic. This fact, coupled

with the wide array of anomalies observed, indicates that hazard for these effects to the embryo and/or fetus is not exclusive of hazard to the dam.

In general, surviving offspring appeared at first to thrive. However, those from dams administered 6 mg/kg/day BP-6 during gestation and lactation had a 19% pre-weaning mortality rate, while that of all other treatment groups was negligible. Mortality data would thus indicate enhanced toxicity due to PBB exposure during both gestation and lactation.

This conclusion is supported by offspring body weight data. No body weight changes were observed in offspring from dams administered 0.6 or 6 mg/kg/day BP-6 during gestation only, while a deficit in body weight gain was observed following 6, but not 0.6 mg/kg/day BP-6 administered during lactation only. Body weight deficits were observed in offspring from dams administered either 0.6 or 6, but not 0.12, mg/kg/day BP-6 during gestation and lactation. Confirmatory studies revealed a body weight deficit in offspring following 2, but not 0.2, mg/kg/day BP-6 administered during gestation and lactation, indicating that 0.2 mg/kg/day was a no-effect level for offspring body weight changes in the present study. These results are supported by the cross-fostering studies of Harris and coworkers (1978), who observed body weight deficits in offspring from dams administered 10 mg BP-6 daily during gestation and lactation or lactation alone, but not during gestation alone.

Analysis of body weights from the four groups of final study animals revealed a trend toward decreased body weight gain from 3 to 12 months of age in offspring from dams administered 2 mg/kg/day BP-6.

However, this trend was significant in only a few instances, thus indicating a degree of recovery in these animals. The biological half-life of the congener 2,2',4,4',5,5'-hexabromobiphenyl (PBB) was determined to be 145 days in lean rats, but 311 days in obese rats (Domino et al., 1982). While the rats in the present study were not necessarily obese, they were fed ad libitum, which would indicate a growing adipose tissue compartment into which PBB would partition. This phenomenon has been described by Tuey and Matthews (1980) for growing rats. A 6-fold and 4.5-fold increase in body weight was observed for male and female offspring, respectively, from weaning to 12 months of age. McCormack and coworkers (1980) determined that the resultant increased organ mass and redistribution of PBB to developing fat deposits contributed to reduction of PBB concentration in tissues with increasing age and to diminished toxicity.

Many authors have advocated the inclusion of acquisition of physical landmarks in behavioral teratology test batteries to assess overt toxicity (Spyker, 1975; Vorhees et al., 1979; Zbinden, 1981). However, Adams and Buelke-Sam (1981) have questioned the functional relevance of this assessment with regard to central nervous system function. In the present study, pinna detachment, incisor eruption, and eye opening were assessed in probe study animals. Precocious eye opening observed in offspring exposed to 6 mg/kg/day BP-6 during gestation was felt to be artifactual due to delayed eye opening of controls. However, because probe studies were conducted to identify potential effects, eye opening was assessed in the final study; in this latter case, no treatment-related effects were observed. Table 35 presents a comparison of

TABLE 35

A Comparison of Behavioral Testing Conducted in Probe Study and Final Study Animals

Exposure Regimen	Probe Study											Final Study		
	Gestation			Gestation and Lactation					Lactation			Gestation and Lactation		
BP-6 Dose (mg/kg/day)	0	0.6	6	0	0.12	0.6	2	6	0	0.6	6	0	0.2	2
Crown-rump length												X	X	X
Pinna detachment	X	X	X	X	X	X	X	X	X	X	X			
Incisor eruption	X	X	X	X	X	X	X	X	X	X	X			
Locomotion														
a) Pivoting	X	X	X	X	X	X	X	X	X	X	X	X	X	X
b) Forward locomotion, body low, head low	X	X	X	X	X	X	X	X	X	X	X	X	X	X
c) Forward locomotion, body raised, head low	X	X	X	X	X	X	X	X	X	X	X	X	X	X
d) Forward locomotion, body raised, head raised	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Surface Righting	X	X	X	X	X	X	X	X	X	X	X			
Cliff avoidance	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Negative geotaxis	X	X	X	X	X	X	X	X	X	X	X			
Swimming	X	X	X	X	X	X	X	X						

CONTINUED ON NEXT PAGE....

TABLE 35 (continued)

Exposure Regimen	Probe Study											Final Study		
	Gestation			Gestation and Lactation					Lactation			Gestation and Lactation		
BP-6 Dose (mg/kg/day)	0	0.6	6	0	0.12	0.6	2	6	0	0.6	6	0	0.2	2
Auditory startle	X	X	X	X	X	X	X	X						
Eye opening	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Visual placing	X	X	X	X	X	X	X	X	X	X	X			
Testes Descent												X	X	X
Vaginal opening												X	X	X
Cage Emergence												X	X	X
Open field activity	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Accommodated motor activity														
a) Distilled water				X	X	X		X	X	X	X	X	X	X
b) d-Amphetamine				X	X	X		X	X	X	X	X	X	X
c) Scopolamine				X	X	X		X				X	X	X
d) Fenfluramine				X	X	X		X				X	X	X

CONTINUED ON NEXT PAGE....

TABLE 35 (continued)

Exposure Regimen	Probe Study											Final Study		
	Gestation			Gestation and Lactation					Lactation			Gestation and Lactation		
BP-6 Dose (mg/kg/day)	0	0.6	6	0	0.12	0.6	2	6	0	0.6	6	0	0.2	2
Estrous Cycle - Motor Activity														
a) d-Amphetamine				X		X		X				X	X	X
b) Scopolamine				X		X		X				X	X	X
c) Fenfluramine														
Conditioned suppression of drinking												X	X	X
Autoshaping operant behavior												X	X	X

testing conducted in probe study and final study animals to clarify the nature of testing in each case.

Due to body weight deficits observed in exploratory studies, crown-rump length of final study pups was measured at birth. In accordance with decreased body weights, crown-rump lengths of PBB-exposed male pups were significantly less than those of controls; a similar trend was observed with females.

Previous studies indicated a delay in sexual maturation of female offspring exposed to PBB perinatally (Harris et al., 1978; Johnston et al., 1980; McCormack et al., 1981). Therefore, time to testes descent and vaginal opening was determined. No delays were observed, but this is not surprising in view of the fact that PBB-induced hormonal changes which could lead to delayed sexual maturation were observed at perinatal doses of 100 ppm and greater (Harris et al., 1978; Johnston et al., 1980; McCormack et al., 1981; Newton et al., 1982; Wilke and Braselton, 1984). In the present final study the highest dose of 2 mg/kg/day was equivalent to 40 ppm (based on a daily food consumption of 15 g).

Tests of reflex development included surface righting, cliff avoidance, and negative geotaxis. Acquisition of cliff avoidance was delayed for probe study offspring exposed to 0.6 or 2 mg/kg/day during gestation and lactation. Confirmatory studies revealed a trend toward delayed acquisition for male and female PBB-exposed offspring which was not in itself significant, but rather contributed to the significance of the overall study MANOVA.

Sensory function tests included visual placing and auditory startle. Results obtained for these tests in the probe study were highly variable,

and the validity of both tests has been questioned (Vorhees et al., 1979; Adams and Buelke-Sam, 1981). Therefore, sensory evaluation was not conducted in the final study.

B. Effects on Locomotor Activity

1. Acquisition of Locomotion

Neonatal rats acquire locomotion in a series of stages which can be easily observed, as proposed by Vorhees and coworkers (1979). Probe studies indicated that PBB exposure affected acquisition of these various stages. Acquisition was unaffected by exposure to 0.6 or 6 mg/kg/day BP-6 during gestation alone. However, exposure to 0.6, 2 or 6 mg/kg/day BP-6 during gestation and lactation resulted in a trend toward delayed acquisition of forward locomotion with the body and head low and forward locomotion with the body and head raised. These delays were not dose-related and, as a further complication, acquisition of the stage characterized by forward locomotion with the body raised and head low was actually enhanced in pups exposed to 0.6 or 6 mg/kg/day BP-6 during lactation.

These results were clarified by confirmatory testing of pups exposed to 0.2 or 2 mg/kg/day BP-6 during gestation and lactation. MANOVA revealed a significant effect overall for the acquisition of locomotion by pups from dams administered 2 mg/kg/day BP-6. ANOVA of each stage revealed a significant delay in acquisition of forward locomotion with the head low and body raised and forward locomotion with the head and body raised. Thus, a deficit was observed as locomotion became more complex.

2. Response to a Novel Environment

Open field activity is one of the most frequently used tests in behavioral toxicology (Zbinden, 1981). Unlike assessment of simple acquisition of ambulation, measures of locomotor activity, such as those obtained with the open field, may also reflect changes in sensory or motor functions, alterations in reactivity or motivational states, or perturbations of a variety of regulatory states (Reiter, 1978). Tilson and Mitchell (1984) have suggested that motor activity changes should be used as evidence of an effect, but that the exact nature of the effect must be identified by further testing.

Neonatal rats separated from the home cage (Randall and Campbell, 1976) exhibit a characteristic pattern of the development of motor activity: an initial increase from postnatal days 1 through 15 or 16, followed by a decline and subsequent increase at approximately day 20 to adult levels of activity (Campbell et al., 1969; Mabry and Campbell, 1974; Melberg et al., 1976; Lucot et al., 1981; Lucot and Seiden, 1982; Rech et al., 1983). The initial rise in activity is thought to be due to maturation of developing excitatory catecholaminergic systems (Campbell et al., 1969; Mabry and Campbell, 1974; Melberg et al., 1976), while the decline has been attributed to maturation of the inhibitory serotonergic (Mabry and Campbell, 1974; Lucot et al., 1981; Lucot and Seiden, 1982), cholinergic (Fibiger et al., 1970; Melberg et al., 1976), and possibly dopaminergic systems (Melberg et al., 1976). Administration to neonates of neurotoxins or compounds known to act through these neuronal systems has been shown to result in a shift or deficit in development of locomotor activity (Fibiger et al., 1970; Mabry and Campbell, 1974; Shaywitz et al., 1978; Lucot et al., 1981; Lucot and Seiden, 1982; Rech et al., 1983).

Exploratory studies revealed a shift in the normal pattern of acquisition of open field activity at all doses administered during gestation and lactation or lactation alone. Although open field activity was only assessed for a 3-day period for offspring exposed to PBB during gestation alone, a shift in the pattern of development was nevertheless still observed. Confirmatory studies were conducted to further characterize the PBB-related changes in acquisition of open field activity. Results of these studies were inconsistent with probe study results and with those from the literature discussed previously. Activity levels were reduced and no clear-cut activity peak was observed on day 15 postpartum.

These discrepancies were thought to relate to methodology. Probe study offspring were tested in a room far removed from their dams and home environment, while final study animals were tested only a few feet from their dams in the room in which they were raised. Final study animals were tested in the room in which they were raised to maintain the colony as virus-free as possible. Randall and Campbell (1976) demonstrated that developing rats tested alone attained peak locomotor activity at 15 days of age, while no such peak was observed for developing rats tested in the presence of the normal litter environment, four siblings, an anesthetized lactating female, or an anesthetized adult male. These authors postulated that peak activity observed in the rat at 15 days of age was due in part to isolation distress, rather than entirely to maturation of different neurotransmitter systems. Thus, isolated neonates responded to the novel environment of the open field with hyperactivity.

It may be postulated that since final study animals were tested in an environment with familiar sounds and smells, their activity response was unlike that of the probe study animals who were tested in a totally novel environment. Despite their lack of response to the novel environment, however, offspring from dams administered 2 mg/kg/day BP-6 traversed significantly fewer squares than did controls across the entire 12-day observation period. Lack of significance in the interaction term of the ANOVA revealed that this was a generalized depression of activity compared to controls, rather than a shift in the development of acquisition. These data collectively indicate that PBB exposure disrupts open field activity of neonates. However, as suggested by Tilson and Mitchell (1984), they do not indicate which neurotransmitters may be involved or the role which the body weight deficits observed in these animals may have played.

One component of open field activity is the emotional response to, or fear of, the novel environment. The frequency of urination and defecation in the open field is felt to be a response to this novelty, i.e., a measure of "emotionality" (Zbinden, 1981). This has been questioned by Archer (1973), who found little correlation between open field defecation and measures of emotionality obtained from other tests, such as active avoidance learning and cage emergence. Cage emergence is a relatively simple test which measures the response of an animal to a novel environment. In the present study a marked difference in the willingness to enter a novel environment (i.e., emerge from the cage to the tabletop) was observed between male and female rats. Males were quite hesitant to emerge, while females emerged readily. These data are consistent with those obtained by Ader and Conklin (1963) in offspring

from dams handled frequently during gestation. Although no statistically significant PBB-related effects were observed, male offspring exposed to 2 mg/kg/day BP-6 tended to emerge more readily than did controls, while females exposed to 2 mg/kg/day BP-6 tended to emerge less readily than did controls. These data would indicate a possible release of inhibitions in male offspring exposed to PBB, and a heightening of inhibitions in females.

Unaccommodated locomotor activity provides yet another measure of response to a novel environment. Probe studies indicated that male offspring exposed to 0.12 mg/kg/day BP-6 (cumulative dose = 4.8 mg) during gestation and lactation were significantly more active than controls following introduction into the locomotor activity testing apparatus prior to injection with distilled water, d-amphetamine or scopolamine. Transient enhancement of unaccommodated locomotor activity was also observed at 6 months of age in female offspring from dams administered 0.12 or 6 mg/kg/day BP-6 (cumulative dose = 240 mg) during gestation and lactation. In addition, offspring from dams administered 0.6 (cumulative dose = 14.4 mg; males only) or 6 mg/kg/day BP-6 (cumulative dose = 144 mg) during lactation were significantly more active than controls during accommodation. These results demonstrated no dose-related pattern of spontaneous hyperactivity, although this behavioral response to prenatal or perinatal PBB exposure had been demonstrated previously in rats and monkeys (Schantz and Bowman, 1983; Gause et al., 1984). To clarify these effects, confirmatory data were collected. These analyses of unaccommodated locomotor activity revealed no spontaneous hyperactivity at 3, 5 or 7 months of age in offspring from dams

administered 0.2 (cumulative dose = 8 mg) or 2 mg/kg/day BP-6 (cumulative dose = 80 mg) during gestation and lactation. ANOVA of locomotor activity data revealed a substantial litter effect; thus, the effects observed in probe study animals may have been unduly influenced by one litter, as all individuals of a dose group (regardless of litter) were treated individually in the analysis. It is of interest, however, that re-testing of males at approximately 10 months of age for 15-minute unaccommodated locomotor activity revealed hyperactivity of PBB-exposed offspring (particularly those exposed to 0.2 mg/kg/day) 10 minutes after introduction into the test chamber. This was also true for open field testing of females exposed to 0.2 or 2 mg/kg/day BP-6. It may be postulated that a certain minimum tissue level of PBB is required for the production of disinhibition leading to hyperactivity in these paradigms. Clarification of this hypothesis requires further testing.

3. Accommodated Locomotor Activity

a. Baseline performance. Injection of distilled water following the accommodation period would be expected to result in transient excitation followed by activity levels corresponding to those observed due to habituation. However, a significantly longer duration of stimulation than that observed for controls was noted in probe study offspring exposed to 0.12 (males and females), 0.6 (males) or 6 mg/kg/day BP-6 (males and females) during gestation and lactation or to 6 mg/kg/day BP-6 (males and females) during lactation. As with unaccommodated activity, confirmatory studies failed to reveal PBB-related effects for accommodated activity following distilled water injection.

b. Dopaminergic influence. By contrast, d-amphetamine-induced stimulation of accommodated locomotor activity was affected by prior PBB exposure. Challenge with 2 mg/kg d-amphetamine to probe study offspring from dams administered BP-6 during gestation and lactation resulted in less stimulation and a shorter duration of action (females only) in those exposed to 0.6 (males and females) or 6 mg/kg/day BP-6 (females only) than in controls. Activity of offspring from dams administered 0.12 mg/kg/day BP-6 was stimulated to a greater degree and for a longer period of time (males only) than was activity of controls. Activity of male and female offspring from dams administered 6 mg/kg/day BP-6 during lactation was significantly greater than that of controls following d-amphetamine injection, while activity of offspring exposed to 0.6 mg/kg/day BP-6 was comparable to that of controls. These data indicate lack of a monophasic response to PBB exposure. While this phenomenon is not unusual among behavioral toxicants, confirmatory studies were conducted at doses designed to clarify the multiphasic nature of the activity response. d-Amphetamine stimulated activity of offspring from dams administered 2 mg/kg/day BP-6 during gestation and lactation significantly less than controls. No treatment-related effect was observed for offspring exposed to 0.2 mg/kg/day BP-6. Thus, while final studies confirmed that PBB exposure disrupts d-amphetamine-induced stimulation of locomotor activity, they failed to clarify the exact nature of the dose-response curve.

A similar biphasic response to d-amphetamine was observed in offspring from dams administered 0.5 or 5 mg/kg fireMaster FF-1 for 20 days prior to conception (Gause et al., 1984). d-Amphetamine disrupted operant responding more in controls and high-dose than in

low-dose animals. These authors postulated that since PBB induce liver microsomal enzymes, the rate of metabolism of d-amphetamine would be enhanced, resulting in diminished effects in low-dose animals. Few effects were observed in high-dose animals because PBB were competing with d-amphetamine for "active sites" on microsomal enzymes. While it is true that the primary route of metabolism for d-amphetamine in the rat is p-hydroxylation (Axelrod, 1970), the nature of the degradation is different from that observed for many other substrates of liver mixed function oxidases. Groppetti and Costa (1969) demonstrated that neither phenobarbital nor 3-methylcholanthrene change the rate of amphetamine disappearance in the rat. Since PBB produce a mixed type of microsomal enzyme induction with inducing properties of both phenobarbital and 3-methylcholanthrene (Dent et al., 1976a,b), it may be inferred that PBB-mediated effects on d-amphetamine-modulated behaviors may involve a substantial non-metabolic component.

Stimulation of locomotor activity by d-amphetamine has been shown to result primarily from enhanced release of dopamine and inhibition of dopamine reuptake (Moore, 1978). The present results indicate that BP-6 exposure may result in modulation of dopaminergic systems and, since d-amphetamine acts indirectly to enhance dopaminergic-mediated responses, that the effect may be presynaptic rather than postsynaptic. To investigate this possibility, male rats were tested for l-DOPA-induced stimulation of locomotor activity to identify possible postsynaptic effects. Pretreatment with the peripheral decarboxylase inhibitor benserazide resulted in comparable suppression of unaccommodated locomotor activity in control and PBB-exposed rats. This dose of

benserazide (50 mg/kg) is thought to penetrate the blood-brain barrier very little (Thut and Rech, 1972); thus, this may represent a peripheral effect, although the exact mechanism is unclear because benserazide also inhibits the activity of transaminases (Rauws et al., 1982) and oxidases (Andree and Clarke, 1982).

l-DOPA (l-3,4-dihydroxyphenylalanine) is the precursor of the neurotransmitter dopamine and, unlike dopamine, crosses the blood-brain barrier (Bianchine, 1980). Within the body l-DOPA is converted to dopamine via l-amino acid decarboxylase; this occurs primarily in the central nervous system when peripheral decarboxylase is inhibited by compounds such as benserazide. Dopamine binds to postsynaptic dopaminergic receptors in the nucleus accumbens and thus produces enhanced locomotor activity (Pijnenburg and Van Rossum, 1973). In the present study essentially no difference was observed in the amount of activity stimulation elicited by l-DOPA between control and PBB-exposed animals. Results of these studies thus indicate that PBB disruption of dopaminergic synapses is primarily presynaptic in nature and probably does not involve a substantial postsynaptic component.

Two aspects of presynaptic dopaminergic function which may be disrupted are synthesis and release of dopamine. Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of dopamine; inhibition of this enzyme by α-methyltyrosine results in decreased dopamine content in tissues of the central nervous system (Moore and Wuerthele, 1979). This decrease in dopaminergic influence has been shown to produce profound behavioral depression (Rech et al., 1966). Stolk and Rech (1970) determined that d-amphetamine-induced stimulation

of behavior is antagonized by doses of α-methyltyrosine devoid of behavioral effects, providing evidence that newly-synthesized catecholamines are important to the stimulant action of amphetamine. In order to assess the role of synthesis in PBB-mediated disruption of d-amphetamine stimulation of behavior, male rats were tested for unaccommodated locomotor activity. Following distilled water injection, PBB-exposed animals were more active than controls. Thus, it is not surprising that their response to d-amphetamine was less than that of controls, based on the premise of Dews and Morse (1961) that low levels of baseline behavior are stimulated more by amphetamine than are high rates. It is, therefore, unclear if stimulation of unaccommodated activity following d-amphetamine was depressed in PBB-exposed animals due to a dopaminergic deficit or to the initial disinhibition of response to a novel environment.

A dose of α-methyltyrosine (100 mg/kg) demonstrated to produce moderate behavioral depression in a shuttle box avoidance paradigm (Rech et al., 1966) produced marked suppression of activity in control and PBB-exposed rats. Behavioral depression was somewhat greater in PBB-exposed animals, which again may have been related to the high baseline activity of these animals or to the fact that they already had lowered synthesis rates. An unexpected result was that observed upon combination of d-amphetamine and α-methyltyrosine: α-methyltyrosine did not completely antagonize d-amphetamine-induced stimulation of locomotor activity in control or PBB-exposed animals. Activity tended to be attenuated, however, and to a greater extent in rats exposed to 2 mg/kg BP-6 than in controls or those exposed to 0.2 mg/kg BP-6. It could be postulated that under normal conditions, animals exposed to 2

mg/kg BP-6 can compensate for reduced dopamine synthesis, but that upon the stress exerted on reserves that is produced by d-amphetamine, a deficit is produced. It must be emphasized that this study was of the hypothesis-generating type and that the suggestion of effects on dopamine synthesis need confirmation.

The drug Ro4-1284 reduces brain catecholamines by depleting storage granules of the large reserve pool of these neurotransmitters (Rech et al., 1968). This depletion results in behavioral depression; this depression is potentiated by α-methyltyrosine due to elimination of both stored and newly synthesized catecholamines (Rech et al., 1968). Ro4-1284 (50 mg/kg) produced behavioral depression in control and PBB-exposed animals of the same degree as that produced by α-methyltyrosine. Again, behavioral depression was somewhat greater in PBB-exposed animals. Further depression of behavior was produced upon combination of the two drugs. No PBB-related effects which could be related to catecholamine depletion were observed, however, following Ro4-1284 alone or upon combination of Ro4-1284 with α-methyltyrosine due to the extremely low levels of activity observed in all groups.

Thus, confirmatory studies revealed the presence of a PBB-related dopaminergic deficit which appeared to be presynaptic in origin. The exact nature of this deficit requires further clarification.

c. Cholinergic influence. Brain cholinergic influences on locomotion are in general inhibitory (Swonger and Rech, 1972). Therefore, the antimuscarinic drug scopolamine stimulates locomotion. Activity of male probe study animals exposed to 0.12 mg/kg/day BP-6 during

gestation and lactation was transiently increased over that of controls following scopolamine injection. Scopolamine stimulated activity to the same degree in controls and in male offspring from dams administered 0.6 or 6 mg/kg BP-6. In contrast, scopolamine stimulated activity of female rats exposed to 0.6 or 6, but not 0.12, mg/kg/day BP-6; this response was greatest in females exposed to 0.6 mg/kg/day BP-6, again suggesting a multiphasic effect. Although the data indicate that a sex-related difference in response exists, it should be emphasized that males and females were administered 0.5 and 1 mg/kg scopolamine, respectively. These doses were chosen because pilot studies indicated that the response of males to 1 mg/kg scopolamine was too variable. It has been demonstrated that scopolamine produces an inverted U dose-response effect on motor activity in female rats, with peak activity observed at a dose of 1 mg/kg (Stewart and Blain, 1975). The dose of scopolamine required to elicit a maximum response in male rats may not have been achieved in this study.

This question was addressed in the confirmatory study, in which both males and females received 1 mg/kg scopolamine. Although females were initially more active than males following scopolamine injection (resulting in a significant sex and session interaction), no PBB-related effects were observed which could be interpreted as affecting the stimulation produced by the anti-muscarinic drug. Thus, the suspected imbalance in cholinergic systems raised by the probe study was not confirmed in more extensive testing.

d. Serotonergic influence. Locomotor activity of probe study animals was stimulated following injection of fenfluramine. This is contrary to previously published reports which indicate that fenfluramine decreases locomotor activity in doses ranging from 2 to 30 mg/kg (LeDouarec and Neveau, 1970; Maickel and Johnson, 1973; Garattini et al., 1978; Rech et al., 1984). However, most of these tests were conducted within 1 hour of fenfluramine administration: a time during which fenfluramine acts to release serotonin (5HT). This initial release phenomenon is followed by 5HT depletion; maximum depletion is achieved between 2 and 4 hours following intraperitoneal administration (Costa et al., 1971). During this time of maximum depletion, fenfluramine has been shown to produce an increase in intertrial responses in a conditioned avoidance paradigm (McElroy et al., 1982). In addition, fenfluramine has been demonstrated to enhance confinement motor activity 60-180 minutes following administration (Clineschmidt et al., 1975). In the present study male offspring from dams administered 0.6 mg/kg/day BP-6 exhibited less increase in motor activity following fenfluramine injection than did controls or other BP-6 exposed offspring. A similar effect was observed in female offspring exposed to 0.12 or 0.6 mg/kg/day BP-6. Analysis of the results of both sexes in the confirmatory study revealed a significant observation time by sex by BP-6 dose interaction. Separate analysis of the sexes revealed that this was due to an effect in males administered 2 mg/kg/day BP-6. These animals were somewhat less active than controls during the peak stimulatory effect of fenfluramine. These studies suggested that perinatal PBB exposure may result in a serotonergic imbalance in male, but not female rats, which is

manifested as a decrease in fenfluramine-induced stimulation of locomotor activity. However, the magnitude of this response was not great and the exact significance is unclear.

C. Contribution of Estrous Cycle Stage to Behavioral Effects

1. Time-related Estrous Cycle Changes

Latency to vaginal opening is assessed in neonatal rats to determine the onset of puberty, as vaginal patency and first ovulation occur on approximately the same day (Parker and Mahesh, 1976). Latency to opening may be either precocious or delayed. Precocious vaginal opening is thought to result from alteration of the steroid environment of the female neonate toward a predominantly androgenic pattern (Gellert et al., 1971) and has been observed following exposure of developing rats to estrogens and androgens, or to the following environmental agents: polychlorinated biphenyls of low chlorine content, chlordecone, aldrin, and DDT (Gellert et al., 1971, 1974, 1978; Gellert, 1978). Conversely, vaginal opening is delayed in neonatal rats by decreased estrogen levels and by environmental contaminants such as polybrominated biphenyls (Harris et al., 1978; McCormack et al., 1981).

The hormonal changes which accompany puberty in the female rat are essentially the same as those which facilitate ovulation in the adult: estrogen-positive feedback is facilitated by progesterone (Parker and Mahesh, 1976; Arneric et al., 1980). It has been postulated that since PBB induce the enzymes which metabolize estrogen and progesterone, sexual development may be delayed (Arneric et al., 1980; Johnston et al., 1980; Bonhaus et al., 1981).

In the present study a delay in vaginal opening was not observed in PBB-exposed animals. This is not in keeping with the Harris et al. (1978) and McCormack et al. (1981) studies which indicated a delay in vaginal opening following perinatal exposure to PBB. However, rats of the former study were exposed via the dam to a cumulative dose of 80 mg, while those of the latter were exposed via the dam to a cumulative dose of approximately 63 mg. In the present study, dams received cumulative doses of either 2.4 or 24 mg; thus, lack of effect on vaginal opening time is not entirely unexpected. In addition, vaginal opening data obtained in the Harris and McCormack studies were pooled from offspring of from 5 to 8 litters; thus, results from members of only one litter could have a profound effect on the outcome of the study. No statistical analysis was conducted in the Harris study. However, in the McCormack study, α was chosen as 0.05, even though the offspring assessed were subjected to additional testing. Thus, the statistically significant effect as reported (which was not marked) may have been a product of Type I error, rather than a truly significant effect.

Another aspect of PBB effects on steroids was reported by Lambrecht and coworkers (1978), who observed an increase in menstrual cycle length in female monkeys administered PBB chronically. This increase was associated with flattened serum estrogen and progesterone peaks and decreased fertility. Johnston and coworkers (1980) observed increased estrous cycle length in female offspring from dams administered 100 ppm BP-6 during gestation and lactation: a treatment regimen demonstrated to result in enhanced metabolism of estrogen and progesterone (Arneric et al., 1980; Bonhaus et al., 1981). No PBB-related

changes in estrous cycle length were observed from 2 to 10 months of age in the present study, even though enhanced metabolism of steroids has been demonstrated at a cumulative dose of only 6 mg BP-6 administered perinatally (McCormack et al., 1979). A similar dissociation of estrous cycle length and serum progesterone levels was reported by Jonsson et al. (1976), who observed a marked decrease in serum progesterone in rats following DDT or PCB (Aroclor 1242) but no change in estrous cycle length with time compared to controls. Control estrous cycle length increased slightly from 2 to 9 months of age in the Jonsson et al. study, but serum progesterone levels remained the same.

The effects observed in the Lambrecht et al. (1978) and Johnston et al. (1980) studies may not have been due entirely to enhanced steroid metabolism, but also could have involved a precocious reproductive aging component. Old rats rarely have regular estrous cycles. Instead, the vaginal smear shows one of 3 patterns: 1) constant vaginal cornification (constant estrus), 2) repeated irregular pseudopregnancies (constant diestrus), or 3) no predictable pattern (Clemens and Meites, 1971). Spontaneous persistent estrus is of particular interest, because it occurs prematurely in neonatal rats administered polycyclic aromatic hydrocarbons (Heinrichs et al., 1971; Gellert, 1978; Gellert et al., 1974, 1978; Jonsson et al., 1976). The onset of this syndrome is also enhanced by neonatal administration of estrogen or androgen, nonsteroidal estrogenic compounds, or continuous illumination (Kumagai and Shimizu, 1982; Takeo, 1984).

Spontaneous persistent estrus is a presenile condition characterized by failure of spontaneous ovulation and luteinization and thus

infertility (Everett and Tyrey, 1983). The constant estrous state is thought to be a dysfunction which arises in the hypothalamus, resulting from a deficiency of catecholamines which normally stimulate neurons to release luteinizing hormone releasing factor (Clemens and Meites, 1971; Everett and Tyrey, 1983). The other irregularities which occur in the estrous cycle due to aging have not been as extensively investigated (Gellert, 1978).

In the present study, female offspring from dams administered 2 mg/kg/day BP-6 displayed irregular estrous cycles at an earlier age than did controls or offspring exposed to 0.2 mg/kg/day BP-6; many of these animals, as well as controls, displayed persistent estrus. However, both controls and PBB-exposed females with irregular cycles displayed a variety of abnormalities in addition to persistent vaginal estrus. This is contrary to the viewpoint expressed by Johnston et al. (1980) and Lambrecht et al. (1978) that reproductive abnormalities observed in female rats following PBB exposure are due to enhanced metabolism of steroids.

These apparently opposite effects were also observed with polychlorinated biphenyls. Treatment with polychlorinated biphenyls results in enhanced metabolism of steroids, yet also results in precocious reproductive aging (Jonsson et al., 1976; Gellert, 1978). Increasing the dose of polychlorinated biphenyls administered to neonates resulted in an increased number of animals with persistent vaginal estrus at an earlier age of onset (Gellert, 1978). Thus, the increased incidence of 5-day and 6-day estrous cycles over control 4-day cycles

observed by Johnston et al. (1980) at 2 months of age in female rats exposed to 100 ppm PBB perinatally may have been the beginning of irregularities resulting from premature reproductive aging. This was not observed until 8 months of age in the present study, but the dose administered here was less than that observed by Johnston et al. (1980). Although these data might indicate that PBB are estrogenic, it has been shown that o,p'-DDD induces premature anovulation, despite the fact that it is devoid of estrogenic activity (Gellert and Heinrichs, 1975). In addition, unlike BP-6, PAH which are estrogenic appear to be those which are metabolized (Gellert, 1978; Jonsson et al., 1976). Therefore, the mechanism of action by which PBB induces premature reproductive aging requires further study.

2. Effects on Locomotor Activity Related to Stage of the Estrous Cycle

a. Response to d-amphetamine. Sexual dimorphism was observed in most of the behavioral tests conducted in this study. In addition, males and females appeared to respond differently to perinatal PBB exposure. To assess the influence of varying hormonal titer throughout the estrous cycle, females were tested for drug-induced stimulation of locomotor activity at various stages.

The greatest difference in responding due to PBB dose occurred following injection with d-amphetamine. Schneider and Norton (1979) have demonstrated that the activity of female rats is stimulated following d-amphetamine to a greater extent than is the activity of males. These authors reported that this enhanced activity was due not only to sex differences in the metabolism of d-amphetamine, but also to sex-related differences in the central nervous system.

The baseline activity of female rats has been reported to depend on stage of the estrous cycle under certain environmental conditions. Female rats show hyperactivity during the estrus phase when assessed in a running wheel, but not a residential maze (Bolles, 1967). The estrus phase is characterized by high estrogen and progesterone titers; however, exogenously administered estradiol or progesterone have no excitatory effect on the baseline exploratory behavior of rats (Telegdy and Stark, 1973).

Interaction of steroids with d-amphetamine has been reported. Fludder and Tonge (1978) examined catecholamine release and turnover following d-amphetamine administration during the 4 stages of the rat estrous cycle. Their results led them to postulate that amphetamine effects would be potentiated during proestrus when estrogen titers are highest, not affected during estrus when both estrogen and progesterone titers are high, potentiated somewhat during metestrus when progesterone predominates, and not affected during the "resting" state of diestrus. Indeed, amphetamine effects have actually been shown to be attenuated during estrus and following exogenous estradiol due to a depletion of catecholamines by estrogens (Fludder and Tonge, 1976; Earley and Leonard, 1978).

Analysis of motor activity data from probe study females revealed greater amphetamine-induced stimulation of activity during proestrus and estrus than during diestrus. This was not supported by final study results, however; an equal amount of stimulation was observed during all stages. The true nature of this response requires further testing with a larger number of animals.

Female offspring exposed to 0.2, 0.6, 2 or 6 mg/kg/day BP-6 were stimulated less by amphetamine during proestrus than were controls. Proestrus is the stage characterized by highest estrogen titer (Adler, 1981), and therefore it appears that the estrogen interaction with d-amphetamine postulated by Fludder and Tonge (1978) is diminished in PBB-exposed animals. This diminished effect may occur due to enhanced metabolism of estrogen by induced mixed function oxygenases, resulting in less estrogen available to facilitate catecholamine release. This phenomenon may be coupled with the previously postulated decrease in catecholamine release resulting from PBB exposure.

Decreased sensitivity to d-amphetamine was observed in probe study animals during estrus exposed to 0.6 or 6 mg/kg/day BP-6, and to a much lesser extent in final study animals. The diminished effect observed in final study animals is not surprising due to lack of either estrogen or progesterone dominance during estrus and is in keeping with the results of Fludder and Tonge (1976, 1978).

Progesterone titer is dominant during metestrus (Adler, 1981). Enhanced metabolism of progesterone may have been responsible for the diminished response to d-amphetamine observed in females exposed to 0.2 or 2 mg/kg/day BP-6. This phenomenon was not observed in probe study animals; this result may have been due to the low response rate of these controls. Activity of females exposed to 0.12 mg/kg/day BP-6 in the probe study was stimulated more by d-amphetamine during diestrus than was that of controls. This is in all probability related to enhanced baseline activity in these animals.

b. Ovariectomy and estrogen replacement. To clarify the estrogen and d-amphetamine interaction, females were retested for open field activity at approximately 10 months of age. All were established as having normal estrous cycles prior to testing and were initially tested in estrus. No differences were observed in the activity of control and PBB-exposed animals during estrus, following ovariectomy, or following ovariectomy with hormone replacement. These data are consistent with those obtained by Telegdy and Stark (1973). No PBB-related differences in activity were observed in estrus following d-amphetamine injection. However, d-amphetamine enhanced activity (as measured by squares traversed) significantly less in control animals following ovariectomy, thus supporting the theory of endogenous estrogen enhancement of catecholamine release as advanced by Fludder and Tonge (1976). This was not the case for PBB-exposed animals; activity was stimulated to the same extent during estrus and following ovariectomy. It is possible that these animals had already adapted to low levels of estrogen due to enhanced metabolism and therefore ovariectomy was not as great a challenge as it was to controls.

Estrogen and progesterone replacement did not reverse the diminished response to d-amphetamine observed in ovariectomized controls. This may have resulted from priming doses of estrogen and progesterone insufficient to reinstate control behavior, as other researchers have used multiple injections (Telegdy and Stark, 1973; Earley and Leonard, 1978) rather than one injection of each hormone, as in the present study. Hormone replacement exerted no additional effects on PBB-exposed animals with regard to squares traversed, with the exception

that low control values resulted in a significantly greater number of squares traversed by females exposed to 2 mg/kg/day BP-6. Number of rearings was diminished following hormone replacement compared to the estrus or ovariectomized conditions in females exposed to 0.2 mg/kg/day BP-6. The exact mechanism of this effect is unknown, but appears to be different from that affecting squares traversed.

c. Response to scopolamine. Information is not available pertaining to cholinergic modulation of behavior according to stage of the estrous cycle. It is known that estradiol induces acetylcholinesterase (Moudgil and Kanungo, 1973), the enzyme responsible for metabolism of acetylcholine (Cooper et al., 1982). Acetylcholinesterase activity in the rat has been reported to be significantly higher during proestrus than during diestrus (Owasoyo et al., 1980). Cholinergic output tends to inhibit behavior (Swonger and Rech, 1972); therefore, it may be postulated that estradiol would return behavior to baseline levels by increasing metabolism of acetylcholine.

In the present study, response to the anticholinergic drug scopolamine was quite variable in females and the effects were not consistent across stages of the estrous cycle when comparing results of probe and final study animals. Animals exposed to 0.6 and 6 mg/kg/day were more active than controls during diestrus. Since this is the stage of the estrous cycle characterized by lowest hormonal titer, it would indicate that the PBB-related effect may be due to a cholinergic deficit in exposed animals or to diminished estrogen modulation of cholinergic output. These effects were not observed in final study animals, however. No PBB-related effects were observed when all females were

analyzed regardless of estrous cycle stage, which indicates lack of a clear-cut cholinergic deficit. However, when tested according to stage of the estrous cycle, females exposed to 0.2 mg/kg/day BP-6 were less active during estrus than controls or females exposed to 2 mg/kg/day BP-6. These results are in keeping with the fact that estrogen titer is high during estrus, which would mean enhancement of acetylcholinesterase activity and thus diminished cholinergic influence that would be exacerbated by scopolamine. By enhancing estrogen metabolism, PBB might diminish induction of acetylcholinesterase, enhance cholinergic tone, and attenuate the behavioral response to scopolamine. It is unclear, however, why such a phenomenon would not be observed during proestrus (when estrogen titer is greatest) or why there is no clear-cut dose-response for PBB. This requires further investigation, as does the general area of estrus cycle modulation of cholinergically-mediated behaviors.

d. Response to fenfluramine. Although 5HT neurons are involved in the secretion of gonadotropins and mating behavior, modulation of serotonergic output by varying steroid levels throughout the estrous cycle of the rat has not been thoroughly investigated. DiPaolo and coworkers (1983) determined that exogenous estradiol can modulate serotonergic systems, but only under precisely defined conditions and only in the dorsal raphe nucleus and substantia nigra. No change in sensitivity to the indirect-acting 5HT agonist fenfluramine was observed across the stages of the estrous cycle in the present study. In addition, no PBB-related effects were observed, indicating the lack of a deficit in serotonergic neurons resulting from PBB exposure, and

suggesting that there is a lack of effect of decreased steroid titer on serotonergic output under the conditions of the study.

Fenfluramine-induced stimulation of locomotor activity was greatest during diestrus and was almost negligible during proestrus, indicating an interaction between steroid hormones and serotonergic systems. Although no PBB-related effects were observed, a significant interaction occurred between BP-6 dose and estrous cycle stage, indicating a differential effect of PBB across the stages. This phenomenon requires further investigation.

D. Effects on Memory and Learning

1. Short-term Memory

Spyker (1975) has suggested that the value of behavioral teratology studies is enhanced by long-term monitoring of offspring (i.e., a "longitudinal" research design). She applied this concept to the study of methyl mercury and uncovered subtle, delayed consequences of perinatal exposure. Short-term memory (i.e., retention of recently-acquired information) has been shown to decay more rapidly in aged than in young rats when tested for spontaneous alternation (Zornetzer et al., 1982). This test measures the ability of an animal to inhibit responses to the most recently visited environment and instead choose the novel environment (Swonger and Rech, 1972). This requires remembrance of the most recently visited arm of the maze. Central inhibitory control of this exploratory behavior is mediated primarily by cholinergic systems interacting predominantly with the hippocampus (Olton, 1983). Anti-cholinergic drugs and procedures which disrupt the integrity of the

hippocampus (including aging) disrupt maze performance (Swonger and Rech, 1972; Zornetzer et al., 1982; Olton, 1983; Wirsching et al., 1984). It could, therefore, be postulated that a toxic agent which causes a cholinergic deficit would disrupt spontaneous alternation at an earlier age than that seen with control animals.

Exploratory locomotor activity tests revealed a possible cholinergic deficit in PBB-exposed animals. Therefore, additional animals exposed to PBB perinatally were monitored periodically for spontaneous alternation in a Y-maze for one year. Number of entries into each arm of the maze during the test session decreased over the course of one year, an habituation phenomenon observed previously by Swonger and Rech (1972) upon repeated exposure to the testing apparatus. This activity may be considered a generalized locomotor response to a novel environment and would be expected to decrease as the novelty faded. No differences were observed between control and PBB-exposed animals with regard to number of entries at any time during the one-year monitoring period. Females of all groups made slightly more entries than did males, but this is in keeping with the fact that activity levels are higher in females than in males (Norton, 1982).

Spontaneous alternation, which requires the animal to remember the arm of the maze visited least recently, did not decrease to an appreciable extent in any animals on test over the one-year period; the small decrement observed was similar to that observed by Swonger and Rech (1972) over a 5-week period of testing. These authors attributed the decrease to habituation; therefore, it may be concluded that age-related changes in cholinergic systems did not produce deficits in

spontaneous alternation up to one year of age in animals of the current study. No PBB treatment-related effects were observed for spontaneous alternation at any time point assessed, indicating absence of a deficit in short-term memory. It is of interest, however, that the degree of habituation observed over the one-year period was greater in control than in PBB-exposed animals. This would indicate that perinatal PBB exposure exerts a disinhibitory effect on behavior.

2. Acquisition of Food-Rewarded Operant Behavior

Tests such as spontaneous alternation uncover deficits in short-term, or "working"" memory, while operant paradigms assess deficits in cognition, or "reference" memory (Wirsching et al., 1984). Hughes and Sparber (1978) developed an autoshaping operant paradigm which they felt to be a straightforward, objective measure of learning. Acquisition and performance of various aspects of autoshaping have been demonstrated to be affected by methylparathion (Gupta et al., 1985) and methylmercury (Hughes and Sparber, 1978).

In the present study, acquisition of VI-90 second responding in female offspring exposed to 2 mg/kg/day BP-6 was delayed when compared to controls. Although not statistically significant, a trend toward delayed acquisition was also observed in male and female offspring exposed to 0.2 mg/kg/day BP-6 and in male offspring exposed to 2 mg/kg/day BP-6. This was the first operant paradigm to which these animals were exposed and was characterized by a very loud presentation of the exteroceptive stimulus (the lever) to which the animals cued. Delayed acquisition may not have been due entirely to a lack of association of lever extension with food reward, but rather to an enhanced

sensitivity to the loud noise. Once acquired, latency to press the lever once it was extended was comparable for control and PBB-exposed animals, indicating no deficit in performance of the task. VI responding is characterized by a very high and sustained rate of responding, due to the uncertainty of reinforcement (Iversen and Iversen, 1981). These data indicate that PBB-exposed animals were as capable of high rates of responding as were controls.

The VI-90 second schedule was replaced by a FI-90 second schedule of reinforcement. In this schedule the exteroceptive stimulus was a cue light located above the lever; the operant task was to associate the cue light (presented at a fixed interval) with the reinforcement period. This is considered to be the simplest type of operant procedure used in behavioral toxicology testing (Norton, 1982). This schedule produces intermittent or low rates of responding characterized by enhanced responding immediately prior to anticipated reinforcement (Iversen and Iversen, 1981). Because stimulants are best detected with low rates of responding, while depressants are best detected with high rates (Dews and Morse, 1961), it follows that FI responding should be sensitive to CNS stimulation. In the present study, the FI responding of male rats exposed to either 0.2 or 2 mg/kg/day BP-6 tended to be greater than that of controls for the majority of the testing period. Control males exhibited an extremely low level of responding; thus, the stimulant or disinhibitory effects exerted by BP-6 were readily apparent. Sexual dimorphism in this response was observed: female rats exposed to 2 mg/kg/day BP-6 made fewer responses than did controls for approximately half of the test sessions. Responding of control females was much

greater than that of control males. The PBB-related effects observed in animals with low levels of responding would not be expected to be identical in animals with higher rates of responding. Results of female rats indicated that the effects of PBB exposure on acquisition of FI-90 second responding were in fact inhibitory, but the magnitude of these effects was less than that observed in males.

Animals were subsequently trained to a FR-20 contingency in which they were required to press a lever 20 times to receive a food pellet reinforcement. In contrast to fixed-interval responding, fixed ratio schedules result in high, regular response rates with only a short post-reinforcement pause (Iversen and Iversen, 1981). According to Dews and Morse (1961), this type of paradigm is best suited to identify depressants, rather than stimulants. As with the fixed-interval contingency, responding of control male rats was less than that of control females. Males exposed to 0.2 or 2 mg/kg/day BP-6 tended to press the lever more times than did controls during acquisition of the behavior, but not once asymptotic levels of responding had been achieved. This may have been due to the fact that higher levels of responding with time masked the stimulant or disinhibitory effects observed more readily with low rates of responding on the fixed-interval contingency.

In contrast, acquisition of FR-20 responding was unaffected by PBB exposure in female rats. However, as performance increased with time to asymptotic levels, a deficit was revealed in females exposed to either 0.2 or 2 mg/kg/day BP-6. This deficit was still present at a time when asymptotic levels of responding were achieved by control females.

To identify further deficits for which compensation may have occurred, drug challenge with a stimulant or a depressant was initiated during the FR-20 phase. Following injection of d-amphetamine, the overall rate of responding was less in females than in males, a situation different from that observed with spontaneous locomotor activity. However, with regard to operant responding, a stimulant such as d-amphetamine would not be expected to generate as much activity in high responders (females) as in low responders (males). Because PBB appear to act in general as depressants of behavior in females, it is not surprising that the dose-related rate-decreasing effects of amphetamine were comparable for control and PBB-exposed females.

A decreased number of reinforcements following amphetamine injection was obtained by male rats exposed to 2 mg/kg/day BP-6. If these animals were already stimulated or their behavior disinhibited by BP-6, it is possible that the behavioral disruption produced by d-amphetamine would be enhanced in PBB-exposed animals. This enhanced responsiveness to d-amphetamine is in contrast to the decreased sensitivity to d-amphetamine-induced stimulation of locomotor activity, indicating a different mechanism of action.

The action of chloral hydrate on the central nervous system is similar to that of barbiturates: it is a central depressant. Like barbiturates, low doses would be expected to increase behavioral output (Iversen and Iversen, 1981). This was indeed the case in the present study for male rats at doses of 20 and 40 mg/kg chloral hydrate. Males exposed to 2 mg/kg/day BP-6 responded more than controls following injection of these low doses. This would seem to represent a potentiation of the disinhibition of behavior experienced by these animals.

In contrast, females exposed to 2 mg/kg/day BP-6 pressed the lever less than controls following 20 mg/kg chloral hydrate, indicating that the disinhibition observed following this low dose was counteracted by PBB exposure. All other animals on test showed a decline in operant responding with increasing chloral hydrate dose.

3. Punished Responding

Suppression of baseline behavior is observed following presentation of an aversive stimulus along with a positively reinforcing stimulus (Geller and Seifter, 1960). This premise is the basis of various conflict procedures utilized to assess the anxiolytic potential of drugs (Kilts et al., 1981). In the present experiment PBB-exposed rats responded differently to a novel environment in cage emergence and unaccommodated locomotor activity situations than did controls. Male PBB-exposed rats appeared to be more willing to enter a novel environment compared to controls than were female PBB-exposed rats compared to controls. To investigate the level of anxiety in control and PBB-exposed animals, a conditioned suppression of drinking paradigm was utilized which enabled a comparison of unpunished and punished responding.

McMillan (1973) has suggested that the effect of drugs on punished responding depends on the level of baseline responding, which in turn is determined by the intensity of the punishing stimulus. To facilitate drug challenge with the central stimulant d-amphetamine and the central depressant diazepam, shock levels were varied. Baseline responding with shock levels designed to suppress punished responding to

levels approximately 20-30% or to 10% of unpunished responding was comparable between control and PBB-exposed animals.

Drug challenge was initiated in an attempt to uncover subtle behavioral effects not revealed during baseline responding. Baseline punished responding was maintained at a relatively high level during the initial portion of the study, resulting in a clear-cut amphetamine dose-related decrease of unpunished, prepunished, and punished responding in all animals on test. This dose-related suppression of unpunished and punished responding has been reported previously and is independent of species or testing paradigm (Geller and Seifter, 1960; Miczek, 1973; Ford et al., 1979; Lazareno, 1979; Spealman, 1979; de Carvalho et al., 1981). No PBB-related effects were observed during unpunished or prepunished components of this paradigm.

A low dose of d-amphetamine (0.25 mg/kg) produced suppression of responding during the punished period only in animals exposed to 0.2 (females only) or 2 mg/kg/day BP-6 (males and females), but not in controls or males exposed to 0.2 mg/kg/day BP-6. This PBB-related effect was not observed at higher doses of d-amphetamine which resulted in enhanced suppression in all animals on test. Enhanced suppression of drinking during the punished period was not observed in controls following 0.25 mg/kg d-amphetamine; however, this dose appeared to strip away functional reserves in PBB-exposed animals, resulting in diminished punished responding. This PBB-related effect was eliminated at doses of amphetamine which also produced behavioral deficits in controls.

The type of enhanced behavioral deficit observed in PBB-exposed animals following d-amphetamine in the conditioned suppression

of drinking paradigm is similar to that observed in the FR-20 paradigm, but is opposite that observed for d-amphetamine-induced stimulation of spontaneous locomotor activity. The effect of d-amphetamine on either punished responding or locomotor activity has been attributed to activation of dopaminergic systems (Moore, 1978; Lazareno, 1979). Enhancement of locomotor activity by d-amphetamine occurs via nigrostriatal dopaminergic systems (Pijnenberg and Van Rossum, 1973). However, high doses of amphetamine or lower doses coupled with stress such as footshock lead to characteristic, repetitive behavior known as stereotypy which is mediated by mesolimbic and mesocortical dopaminergic systems (Pijnenberg and Van Rossum, 1973; MacLennan and Maier, 1983; Carr and White, 1984). Ford and coworkers (1979) demonstrated that combination of d-amphetamine and diazepam in a conditioned suppression of drinking paradigm resulted in stereotyped behavior, which they attributed in part to the additive effect of diazepam and d-amphetamine on dopaminergic neurons. This drug interaction may have resulted from stimulation of mesolimbic dopaminergic neurons to produce stereotypy, with possible enhancement due to the stress of punishment (MacLennan and Maier, 1983). Thus, depending on the behavioral paradigm assessed, PBB may act on different dopaminergic systems to exert disruptive effects.

In contrast to results observed following d-amphetamine, increasing doses of diazepam exerted no effect on unpunished licks or water consumed for either control or PBB-exposed rats. This lack of effect by diazepam on unpunished responding has been demonstrated over the dose range used in the present study previously (Kilts et al., 1981). Number of licks incurred during the prepunished period was

increased slightly by diazepam, indicating that baseline responding during this period was suppressed due to anticipation of punishment. No PBB-related effects on prepunished responding were observed.

Punished responding was markedly enhanced by diazepam treatment. The most effective doses were 3 and 10 mg/kg for control animals; diazepam effects were diminished at 5.6 mg/kg, a phenomenon which has been observed previously (Commissaris and Rech, 1982). The reason for the extreme variability in responding during the punished period is unknown. A variable response to diazepam has been observed in the punished component of this behavior previously, and was found to increase with increasing diazepam dose (Ford et al., 1979; Kilts et al., 1981; Commissaris and Rech, 1982). Because of the high degree of variability, it was difficult to identify PBB-related effects. However, the pattern of enhanced punished responding for each dose of diazepam appeared to differ between control and PBB-exposed animals. In addition, punished responding was diminished following 10 mg/kg diazepam for animals exposed to 0.2 (females only) or 2 mg/kg/day BP-6 (males and females). The mechanism of action by which PBB exerts this effect is unknown, as benzodiazepine release of punished responding has been purported to involve GABA-ergic and possibly dopaminergic components (Guidotti, 1978; Ford et al., 1979; Lazareno, 1979).

Results of these studies indicate that perinatal PBB exposure can affect learning and memory. Although psychoactive agents helped to unmask these PBB-related effects, they were apparent without pharmacologic intervention in the acquisition and performance of operant behavior.

SUMMARY AND CONCLUSIONS

The purpose of this series of experiments was to characterize the neurobehavioral toxicity of perinatally administered polybrominated biphenyls. Studies were conducted in rats using the 'leapfrog' experimental design suggested by Muller and coworkers (1984). Pilot studies (designed to evaluate methodology), exploratory studies (designed to evaluate data analysis; hypothesis generating) and confirmatory studies (designed to confirm an hypothesis) were conducted to extract the maximum amount of information from the animals tested.

Hypothesis-generating studies were conducted in which female Sprague-Dawley rats were administered orally 0, 0.6, 6, 12, or 60 mg/kg/day fireMaster BP-6 from days 6 through 18 of gestation; 0, 0.12, 0.6, 2, or 6 mg/kg/day BP-6 from day 6 of gestation through day 24 of lactation, or 0, 0.6, 6, or 60 mg/kg BP-6 from days 0 through 24 of lactation. These were followed by hypothesis-testing studies in which female Sprague-Dawley rats received 0, 0.2 or 2 mg/kg BP-6 orally from day 6 of gestation through day 24 of lactation. Offspring of these dams were subjected to behavioral testing.

Maternal toxicity (as assessed by body weight) occurred at a lower BP-6 dose following administration during gestation and lactation (40 doses) than during gestation alone (12 doses) or lactation alone (24 doses). With regard to cumulative PBB dosage, the no-effect level for

maternal toxicity achieved in this study was 43 mg (6 mg/kg/day administered from days 0 through 24 of lactation).

Gestation length was increased by prenatal administration of 60 mg/kg/day BP-6. Pups from this group were born dead and exhibited birth defects which included hydrocephaly and underdeveloped limbs. Administration of 12 mg/kg/day BP-6 to pregnant rats also resulted in late fetal death, but no malformations were observed, indicating that the extent of damage to the developing organism depends on the dose of BP-6 administered. Postnatal mortality was also observed; a dose of 60 mg/kg/day BP-6 administered to dams during lactation was lethal to pups after only 12 days. Although no fetal mortality was observed in offspring from dams administered 6 mg/kg/day BP-6 during gestation and lactation, this group had a 19% preweaning mortality rate, while that of all other treatment groups was negligible. Doses at which failure to thrive, mortality and/or malformations occurred were also shown to be maternally toxic.

No body weight changes were observed in offspring from dams administered BP-6 during gestation or those administered 0.6 mg/kg/day BP-6 during lactation. Decreased body weight gain compared to controls was observed in offspring exposed to 6 mg/kg/day BP-6 during lactation or to 0.6, 2 or 6 mg/kg/day during gestation and lactation. An approximate cumulative dose of 4 mg (0.2 mg/kg/day during gestation and lactation or 0.6 mg/kg/day during lactation) appeared to be the no-effect level for offspring body weight changes in this study. Body weight deficits appeared to recover with age. This observation is of interest because behavioral deficits observed later in life occurred in the absence of significant body weight deficits.

Analysis of probe study data revealed deficits in acquisition of various developmental and behavioral endpoints. Confirmatory studies revealed decreased crown-rump length in male offspring from dams administered 0.2 or 2 mg/kg/day BP-6, indicating an effect of these doses on physical development. No PBB-related effects were observed, however, on time to eye opening, testes descent or vaginal opening.

Effects on the acquisition of forward locomotion, cliff avoidance, cage emergence, and open field activity were analyzed in a confirmatory study. An overall study MANOVA revealed a significant effect for offspring from dams administered 2 mg/kg/day BP-6. Individual ANOVA was conducted for each of the four behavioral tests and revealed that significant effects were most apparent for acquisition of forward locomotion and open field activity. Acquisition of forward locomotion was affected to a greater extent as locomotion became more complex. A significant contribution of litter to the overall effect was observed with the most primitive forms of locomotion but disappeared as locomotion became more complex.

Offspring from dams administered 2 mg/kg/day BP-6 traversed significantly fewer squares in the open field behavior than did controls throughout the observation period (days 12 to 24 postpartum). Lack of significance in the interaction term of the repeated measures ANOVA revealed that this was a generalized depression of activity compared to controls, rather than a shift in the development of acquisition, as had been observed previously in probe studies. The difference between probe and confirmatory studies was that confirmatory study animals were tested in the room in which they were housed, while probe study animals were tested in a novel environment. The latter exposure condition may have

resulted in a pattern of acquisition of open field activity different from that of the former, due to isolation distress.

This series of studies revealed that perinatal PBB exposure resulted in behavioral changes early in life. These changes were observed in offspring exposed to a level of PBB which produced concentrations in abdominal fat (approximately 36 μg/g wet tissue weight) within the range of those observed in highly exposed humans.

Although no significant effects were observed for cage emergence, a trend occurred which contributed to the overall study MANOVA. Overall, males were quite hesitant to enter a novel environment, while females emerged into the novel environment quite readily. However, male offspring exposed to 2 mg/kg/day BP-6 tended to emerge more readily than controls, while females exposed to 2 mg/kg/day BP-6 tended to emerge less readily than did controls. These data would indicate a possible PBB-mediated release of inhibitions in male offspring and a heightening of inhibitions or loss of exploratory motivation in females.

Analysis of unaccommodated locomotor activity in a confirmatory study conducted at 3, 5 and 7 months of age revealed no differences in response to a novel environment between control and PBB-exposed offspring. However, retesting of males at approximately 10 months of age for 15-minute unaccommodated locomotor activity revealed possible hyperactivity of PBB-exposed offspring. This was also true for open field testing of females, thus revealing an effect opposite to that observed for cage emergence behavior.

Accommodated locomotor activity following distilled water injection was relatively unaffected by PBB exposure. By contrast, d-amphetamine-induced stimulation of accommodated locomotor activity was in general

attenuated in PBB-exposed offspring. However, activity of offspring exposed to 0.6 or 6 mg/kg/day BP-6 during lactation or to 0.12 mg/kg/day BP-6 during gestation and lactation was actually stimulated more than that of controls. The phenomenon of a biphasic effect is not unusual among behavioral toxicants. Confirmatory studies were conducted to clarify this phenomenon. A PBB-related deficit in response to d-amphetamine was observed for offspring exposed to 2, but not 0.2, mg/kg/day BP-6. Thus, while final studies confirmed that PBB exposure disrupts d-amphetamine-induced stimulation of locomotor activity, they failed to clarify the exact nature of the dose-response curve.

The disrupted response to d-amphetamine indicated possible interaction of PBB with dopaminergic systems. Stimulation of accommodated locomotor activity by the dopamine precursor l-DOPA was unaffected by prior PBB exposure, indicating lack of a postsynaptic effect. The nature of the postulated presynaptic effect was therefore investigated by pharmacologic challenge designed to determine if dopamine synthesis or release were deficient. These studies revealed that α-methyltyrosine attenuation of d-amphetamine-induced stimulation of motor activity was greater in offspring exposed to 2 mg/kg/day BP-6 than in those exposed to 0 or 0.2 mg/kg/day BP-6. These results indicate that dopamine synthesis may be somewhat impaired due to exposure to higher doses of PBB. Further confirmation is required, however. Studies with Ro4-1284, a substance which depletes storage granules of dopamine, were inconclusive due to the marked behavioral depression observed in all animals tested; this merits further investigation.

Probe studies suggested that the accommodated locomotor activity of PBB-exposed female rats was stimulated more than that of controls following injection with the anticholinergic drug scopolamine. These results would indicate a possible cholinergic deficit. However, the activity of final study PBB-exposed offspring was comparable to that of controls, indicating lack of a substantial cholinergic deficit.

Injection of the indirect-acting serotonergic agonist fenfluramine resulted in stimulation of locomotor activity. This stimulation was somewhat attenuated by prior PBB exposure, particularly in males. Effects were not marked, but indicate the possibility of a PBB-related serotonergic imbalance in male rats.

PBB exposure has been reported to result in compromised reproductive abilities. In the present study, the onset of puberty in male and female rats was unaffected by prior PBB exposure. Estrous cycle length was unaffected through 10 months of age in PBB-exposed females; however, these animals demonstrated accelerated reproductive aging. This phenomenon has been reported for animals exposed to polychlorinated biphenyls and merits further investigation.

Sexual dimorphism was observed in most of the behavioral tests conducted in this study. In addition, males and females appeared to respond differently to perinatal PBB exposure. Analysis of the PBB-related disruption of d-amphetamine-induced stimulation of locomotor activity according to stage of the estrous cycle revealed that disruption was greatest during proestrus, the stage characterized by highest estrogen titer. This may have resulted from enhanced metabolism of estrogen (which normally stimulates locomotor activity) due to PBB

induction of mixed function oxygenases interacting with the previously postulated decrease in catecholamine release resulting from PBB exposure.

Attenuation of d-amphetamine-induced stimulation was pronounced in final study females tested during metestrus. This may have resulted from enhanced metabolism of progesterone, the hormone which predominates during this stage and has also been shown to enhance d-amphetamine-induced stimulation (Fludder and Tonge, 1978).

PBB-related effects observed during estrus and diestrus were less clear-cut, which is not surprising in view of the fact that neither estrogen nor progesterone predominate during estrus and the titer of both is low during diestrus.

Ovariectomy had no effect on the stimulation produced by d-amphetamine in PBB-exposed animals, but resulted in attenuation of stimulation in controls as compared to that observed during estrus. These results indicate that PBB-exposed animals may have compensated for low levels of estrogen due to enhanced metabolism and therefore ovariectomy was not as great a challenge as it was to controls. Results obtained following estrogen and progesterone injections in ovariectomized animals were equivocal, possibly because the doses of hormones administered were designed to render the females sexually receptive but may not have been sufficient for behavioral testing.

Response to the anticholinergic drug scopolamine was somewhat variable for females. Results obtained during the various stages of the estrous cycle were not consistent between probe and final study animals. However, a significant interaction observed in final study animals

between BP-6 dose and estrous cycle stage indicates that the response to scopolamine across the stages of the estrous cycle was changed by prior PBB exposure. Thus, PBB may exert subtle effects on cholinergic systems which are modulated by hormone titer. However, the characterization of these effects requires further investigation.

The same type of response was observed following injection with fenfluramine: lack of a significant PBB effect but presence of a significant interaction between BP-6 dose and estrous cycle stage. Thus, PBB may also exert subtle effects on serotonergic systems which are modulated by hormone titer.

Because probe studies revealed a possible PBB-related deficit in the locomotor response of offspring to scopolamine, they were tested in a spontaneous alternation paradigm up to one year of age. Performance in this test has been shown to be highly dependent on cholinergic systems, particularly in the hippocampus, and is considered to represent short-term memory. In the present study, no clear-cut PBB-related effects were observed with regard to spontaneous alternation, indicating little disruption of cholinergic systems by PBB.

Disruptions in cognition were conducted using conditioned suppression of drinking and autoshaping operant paradigms. In the former case, PBB exposure had no effect on baseline behavior with regard to punished or unpunished responding. Challenge with 0.25 mg/kg d-amphetamine resulted in a trend toward enhanced suppression of responding during the punished period in animals exposed to 2 mg/kg/day BP-6 as compared to controls. This PBB-related deficit was not observed at higher doses of d-amphetamine which resulted in enhanced suppression of punished

responding in all animals on test. It was of interest that the PBB-related effect on punished responding observed following d-amphetamine was opposite that observed for motor activity. Dopaminergic systems have been implicated in both behaviors, but it may be that PBB exerts disruptive effects on these behaviors by interacting with different dopaminergic systems in each case.

The anxiolytic drug diazepam released suppression of punished responding in all animals on test; no dose-response relationship was established, however. Responding was extremely variable during the punished period and thus it was difficult to identify PBB-related effects. However, the pattern of enhanced punished responding for each dose of diazepam appeared to differ between control and PBB-exposed animals.

More clear-cut PBB-related effects on cognition were observed in an autoshaping paradigm designed to measure acquisition and performance of several operant procedures. Acquisition of a VI-90 second schedule of responding was significantly delayed, compared to controls, for female offspring exposed to 2 mg/kg/day BP-6; a similar trend was observed in all other PBB-exposed animals. Acquisition and/or performance of a FI-90 second or a FR-20 schedule for reinforcement was affected by prior PBB exposure. Acquisition of responding appeared to be enhanced in PBB-exposed male offspring but suppressed in female offspring. These results are similar to those obtained for cage emergence, in which male PBB-exposed offspring entered a novel environment more readily than did controls, while females entered a novel environment less readily than

did controls. These data would indicate a general PBB-related trend toward stimulation or disinhibition in males and inhibition in females.

Drug challenge with d-amphetamine under a FR-20 schedule of responding revealed a trend toward decreased reinforcements obtained by male rats exposed to 2 mg/kg/day BP-6. This enhanced responsiveness to d-amphetamine is similar to that observed in the conditioned suppression of drinking paradigm but opposite that observed for locomotor activity, again indicating a different mechanism of action.

Male rats exposed to 2 mg/kg/day BP-6 exhibited enhanced FR-20 responding compared to controls following injection of low doses of the central depressant chloral hydrate. This may be due to a potentiation of the disinhibition postulated to occur with these animals. In contrast, females exposed to 2 mg/kg/day BP-6 pressed the lever fewer times than did controls following a low dose of chloral hydrate. These results indicate that the disinhibition observed following a low dose was counteracted by PBB exposure.

The following conclusions may be reached from the present study:

1. Administration of BP-6 to female rats during both gestation and lactation results in a greater number of developmental and behavioral deficits than does administration during gestation alone or lactation alone.
2. A dose of 2 mg/kg/day BP-6 represents a no-effect level for maternal body weight changes and most developmental parameters. However, crown-rump length was decreased in male offspring exposed to this dose, and offspring body weights were decreased from 0 to 60 days postpartum. These body weight changes appeared to diminish

with time, however, indicating that many of the behavioral changes observed later in life occurred in the absence of adult body weight deficits.

3. A study-wide MANOVA of behavioral tests conducted early in life revealed a significant PBB-related effect on acquisition of forward locomotion, cliff avoidance, cage emergence and open field activity in offspring from dams administered 2 mg/kg/day BP-6. ANOVA conducted for individual behavioral tests revealed significant delays in acquisition of forward locomotion (the more advanced stages of development were affected to the greatest degree) and a significantly fewer number of squares traversed in an open field during the 12-day observation period.
4. Behavioral deficits observed in young animals occurred at PBB tissue levels within the range of those observed in highly exposed humans.
5. Locomotor deficits observed in young animals were retained in adults, depending on the drug challenge administered. The indirect-acting dopaminergic agonist d-amphetamine stimulated accommodated locomotor activity less in offspring exposed to 2 mg/kg/day BP-6 than in controls. This phenomenon was not observed following injection of the dopamine precursor l-DOPA, indicating that PBB may exert presynaptic, rather than postsynaptic effects. Exploratory studies indicated that these presynaptic effects may involve decreased dopamine synthesis. Clear-cut PBB-related effects were not observed following injection of drugs acting through cholinergic or serotonergic systems.

6. Although no PBB-related effects were observed with regard to the onset of puberty or length of the estrous cycle, reproductive aging was accelerated in females exposed to 2 mg/kg/day.
7. Sexually dimorphic responding was observed in most of the behavioral tests; the sexes also responded differently as a result of PBB exposure. The PBB-related deficit in response to d-amphetamine in females appeared to be modulated by hormonal titer. This deficit was most apparent during the proestrus and metestrus stages of the estrous cycle (stages characterized by high estrogen and high progesterone titers, respectively) and least apparent during diestrus (the stage characterized by low hormonal titer). A significant interaction between BP-6 dose and estrous cycle stage following scopolamine or fenfluramine injection was observed, indicating a PBB-mediated difference in pattern of responding across the stages of the estrous cycle.
8. No PBB-related effects were observed on short-term memory (as tested by spontaneous alternation) or on baseline unpunished or prepunished responding. Challenge with d-amphetamine revealed suppression of punished responding in PBB-exposed animals at low amphetamine doses which did not produce suppression in controls. These PBB-related effects disappeared with higher d-amphetamine doses which also produced suppressed punished responding in controls. Attempts to demonstrate differences in the effects of the anxiolytic drug diazepam on punished responding of control and PBB-exposed offspring yielded equivocal results.

9. Acquisition and performance of the three phases of an operant autoshaping paradigm were impaired to varying degrees by PBB exposure. This presented in the form of a stimulatory or disinhibitory effect in male PBB-exposed offspring, but an inhibitory effect in female PBB-exposed offspring.

Results of this study indicate that perinatal exposure of rats to levels of PBB which produce tissue concentrations in the range of those observed in humans produces neurobehavioral toxicity. This toxicity is manifested from birth to adulthood, has a sexually dimorphic component, and involves cognition as well as locomotion.

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