Neurotoxicity Research, Vol. 3, pp. 557–579
Reprints available directly from the publisher
Photocopying permitted by license only

Behavioral Effects Induced by Acute Exposure to Benzo(*a*)pyrene in F-344 Rats

CRYSTAL R. SAUNDERS, DOLORES C. SHOCKLEY* and MAURICE E. KNUCKLES

Department of Pharmacology, Meharry Medical College, 1005 D.B. Todd Blvd, Nashville, TN 37208, USA

Polycyclic aromatic hydrocarbons (PAHs) are highly persistent environmental pollutants which pose potential adverse effects on human health. Benzo(*a*)-pyrene (B(*a*)P) is the prototypical representative of these widely dispersed lipophilic contaminants. (B(*a*)P) exposure in experimental animals results in an array of tissue- and organ-specific responses including carcinogenicity, teratogenicity, reproductive and immunotoxicity. However, no previous studies have examined the potential neurobehavioral toxicity of B(*a*)P *in vivo*. The present study was conducted to investigate the behavioral effects induced by single oral doses of (B(*a*)P) in 8-week-old male and female F-344 rats. Rats were exposed to 0, 12.5, 25, 50, 100 and 200 mg/kg of B(*a*)P by oral gavage. Motor activity measurements and the functional observational battery (FOB) were used to assess behavioral changes induced by B(*a*)P at 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h post treatment. Statistical analyses revealed significant ($p < 0.001$) dose, sex and time interactions. (B(*a*)P) doses ranging from 25 to 200 mg/kg produced a significant suppression (up to 60%) in four motor activity parameters: horizontal activity, total distance, stereotype and vertical activity in both sexes within 2 and 4 h of dosing. B(*a*)P treated male and female animals also showed significant ($p < 0.001$) changes in neuromuscular, autonomic, sensorimotor and physiological functions within 2 and 4 h post B(*a*)P administration except in the 12.5 mg/kg treatment group. The 12.5 mg/kg dose did not produce significant ($p > 0.05$) behavioral toxicity in either males or females. All treated animals (25–200 mg/kg) recovered from the toxic effects of B(*a*)P by 72 h. Significant ($p < 0.05$) gender differences were noted in FOB tests measures with males displaying greater sensitivity to B(*a*)P. These data suggest that motor activity and FOB measurements can be used as indices to detect B(*a*)P neurotoxicity.

Keywords: Benzo(*a*)pyrene (B(*a*)P); locomotor activity; functional observational battery (FOB); neurotoxicity; behavior

INTRODUCTION

Benzo(*a*)pyrene (B(*a*)P) is a member of the polycyclic aromatic hydrocarbon (PAH) family of compounds, of which there are more than some 100 different chemicals (ATSDR, 1995). Of all the PAHs, B(*a*)P has been studied most extensively for its toxic effects. B(*a*)P is released into the environment from natural sources such as volcanoes and forest fires, from industrial and vehicle exhaust fumes and from manmade sources including the manufacturing of products such as coal, tar, asphalt, petroleum and

*Corresponding author. Tel.: +1-615-327-6510. Fax: +1-615-327-6632.

aluminum (Darby and Willis, 1986; Davies *et al.*, 1986; Knecht and Elliehausen, 1987; Lesage *et al.*, 1987; Lioy *et al.*, 1988; Menzie and Santodonato, 1992; ATSDR, 1995). B(*a*)P originating from manmade sources is quantitatively the most significant (ATSDR, 1995). Nearly all release of B(*a*)P into the environment is into the air, water and soil (ATSDR, 1995). PAHs including B(*a*)P are present at high concentrations at hazardous waste sites and at least 600 waste sites have been identified and are on the national priorities list for clean up (ATSDR, 1995). This information is important for humans who live near these sites as they are highly susceptible to exposure to these compounds (EPA, 1985).

The greatest release to the residential environment has been attributed to the combustion of coal and wood heating (Freeman and Cantell, 1990; ATSDR, 1995). Other sources of B(*a*)P exposure may result from consuming contaminated food and water (Hecht *et al.*, 1979; Grimmer, 1983; Lioy *et al.*, 1988). Meats cooked at high temperatures or vegetables and fruits grown near areas with high vehicular traffic can increase the levels of B(*a*)P in food (Hecht *et al.*, 1979; Wang and Meresz, 1982; Grimmer, 1983; Lioy *et al.*, 1988). Cigarette smoking or tobacco chewing also represent sources of potential B(*a*)P exposure (Hoffman *et al.*, 1986; Adams *et al.*, 1987; ATSDR, 1995). For some people, the primary exposure to B(*a*)P occurs in the workplace (Darby and Willis, 1986; Lesage *et al.*, 1987). Workers may be exposed to B(*a*)P in industries involved in the production of petroleum, aluminum, asphalt, tar or coal (Lloyd, 1971; Hammond *et al.*, 1976; Davies *et al.*, 1986; Lesage *et al.*, 1987; ATSDR, 1995). Human exposure to B(*a*)P from the ingestion of unprocessed grain and from charcoal-cooked or smoked meats was estimated to be between 0.001 and 0.9 μg/day (Hecht *et al.*, 1979; Grimmer, 1983; Lioy *et al.*, 1988; ATSDR, 1995). The range of ambient air exposures was reported to be between 0.02 and 3 μg/day (Lloyd, 1971; Hammond *et al.*, 1976; EPA, 1985; Davies *et al.*, 1986; Lesage *et al.*, 1987; Menzie and Santodonato, 1992; ATSDR, 1995). Ingestion of B(*a*)P from contaminated drinking water was estimated to be between 0.002 and 0.12 mg/day (ATSDR, 1995). Exposure from the inhalation of tobacco smoke, depending on the number of cigarettes smoked, was estimated to be between 0.5 and 7.8 μg/day (Hammond *et al.*, 1976; Hoffman *et al.*, 1986; Adams *et al.*, 1987; ATSDR, 1995). Occupational exposure levels were estimated to be between 19.4 and 25.0 mg/day via the skin (Darby and Willis, 1986; Davies *et al.*, 1986; Lesage *et al.*, 1987; Lioy *et al.*, 1988). Due to the widespread availability of B(*a*)P in the environment and the great potential for human exposure, the adverse effects of thin compounds merits investigation.

Chronic exposure to B(*a*)P can cause damage to organs such as the lung, skin, ovary, testes, kidney, stomach and colon in animals (Lloyd, 1971; Jerina *et al.*, 1980; Maclure and MacMahon, 1980; Legraverend *et al.*, 1983; Iwagawa and Maeda, 1992; ATSDR, 1995). In addition, B(*a*)P has induced cancer in many of these animal tissues (Jerina *et al.*, 1980; Lavoie *et al.*, 1987; Iwagawa and Maeda, 1992; Likhachev *et al.*, 1992). Epidemiological studies have reported increased incidences of lung and skin cancers in humans occupationally exposed to B(*a*)P (Lloyd, 1971; Hammond *et al.*, 1976; Redmond *et al.*, 1976; Jerina *et al.*, 1980; Maclure and MacMahon, 1980). Many toxic effects induced by B(*a*)P are mediated through the aryl hydrocarbon (Ah) receptor (Sutter and Greenlee, 1992; Eaton, 1995; Nebert *et al.*, 2000). Binding of B(*a*)P to the Ah receptor results in their translocation to the nucleus and is the initial step that results in altered gene expression including the CYP1A1 gene (Sutter and Greenlee, 1992; Eaton, 1995; Nebert *et al.*, 2000). Induction of CYP1A1 is relevant to human health because B(*a*)P requires metabolic activation to 7,8-diol-9,10-epoxide before eliciting many of its toxic effects (Sutter and Greenlee, 1992; Eaton, 1995; Salas and Burchiel, 1998; Nebert *et al.*, 2000). The 7,8-diol-9,10-epoxide metabolite is believed to be responsible for carcinogenicity, teratogenicity, immune

and developmental toxicity in rats (Redmond *et al.*, 1976; Maclure and MacMahon, 1980; Lavoie *et al.*, 1987; Eaton, 1995; Salas and Burchiel, 1998). Levels of activating enzymes, however, are not the sole determinants of B(*a*)P toxicity; sufficient quantities of detoxifying enzymes are also critical. The conjugation of bioactivated B(*a*)P with glutathione is a critical detoxifying pathway (Nebert *et al.*, 2000). If bioactivated B(*a*)P is not detoxified, the highly reactive diol-epoxide intermediates may oxidize macromolecules such as DNA, protein, and lipid resulting in cell injury, neurotoxicity and other deleterious effects (Lebel, 1991; Likhachev *et al.*, 1992; Nebert *et al.*, 2000).

While the carcinogenicity of B(*a*)P is well established, there is little published information regarding the neurotoxic effects of B(*a*)P. B(*a*)P is highly lipophilic and easily crosses the blood–brain barrier (ATSDR, 1995). Some of the neurotoxic effects that have been reported following acute B(*a*)P exposure include a decrease in levels of catecholamines (dopamine (DA) and norepinephrine (NE)) and antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in the lung, liver and kidney (ATSDR, 1995; Kim and Lee, 1997; Stephanou, 1998; Nebert *et al.*, 2000). Acetylcholinesterase inhibition, elevations in intracellular calcium and decreased levels of Sp1 binding to DNA in the developing rat brain have also been reported to following acute administration of B(*a*)P (Mounho and Burchiel, 1997; Jett *et al.*, 1999; Hood *et al.*, 2000). Workers chronically exposed to B(*a*)P have shown neurological deficits and a loss of short-term memory (Majachrzak *et al.*, 1990). Due to the high lipid content, rapid metabolic rate, and decreased levels of antioxidant enzymes in the nervous system, we hypothesize that the nervous system may be highly susceptible to damage by B(*a*)P.

Behavioral changes are often the first indicators of exposure to a neurotoxicant (Adler *et al.*,1986; Tilson, 1987a,b; 1990; MacPhail *et al.*, 1989), and are recommended to be among the first tier of tests that should be conducted to assess the neurotoxic potential of compounds (Tilson, 1987a,b; 1990; Haggerty, 1989; MacPhail *et al.*, 1989; Moser, 1990; Crofton and Howard, 1991). Motor activity is used extensively in toxicology to screen for chemically induced changes in behavior and is a rapid, reliable and sensitive behavioral endpoint in evaluating the neurotoxic potential of chemicals (Tilson, 1987a,b; MacPhail *et al.*, 1989; Crofton and Howard, 1991). Since motor activity encompasses motor, sensory and associative processes of the nervous system, alterations in motor activity may serve as an indicator of nervous system dysfunction (MacPhail *et al.*, 1989; Schulze, 1990; Tilson, 1990; Crofton and Howard, 1991). Another method to assess neurological function is the functional observational battery (FOB), which is a subjective hands-on, semi-quantitative assessment. The FOB is useful to detect gross signs of neurotoxicity (Moser *et al.*, 1988; Moser, 1990). It has been suggested that motor activity and FOB evaluations together present a comprehensive assessment of a chemical's potential for neurotoxicity and allow for early detection of neurotoxic effects (Tilson, 1987a,b; Moser *et al.*, 1988; MacPhail *et al.*, 1989; Schulze and Boysen, 1991). Neurotoxicity testing has increased given the possible link between neurodegenerative diseases and exposure to environmental chemicals (Spencer, 1992). The objective of the present study was to determine whether single oral doses of B(*a*)P can induce behavioral changes as measured by motor activity and the FOB. Further, this study was also undertaken to characterize acute behavioral toxicity that may result from accidental exposures to B(*a*)P and to establish the onset and duration of B(*a*)P-induced behavioral effects.

MATERIALS AND METHODS

Animals

Male and female Fisher F-344 rats, weighing approximately 120–160 g, were obtained from Harlan Laboratory, Indianapolis, IN. Animals

were housed individually in plastic cages. The vivarium was maintained under a timed 12 h/12 h light/dark cycle (lights on at 0600 h) with a temperature range of 22 ± 6°C and a relative humidity range of 47 ± 15%. Rats were given free access to food and water, but on test days and 24 h before B(*a*)P administration the animals were fasted. All animals were given a 7-day acclimation period to the animal care facilities and were eight weeks of age at the beginning of behavioral testing.

Test Compound

B(*a*)P (>97% purity) as specified by the supplier was obtained from Sigma Chemical Company (St. Louis, MO). The purity was confirmed before experiments by gas chromatography–mass spectrometry. The dosing solution was prepared fresh each day by dissolving a desired quantity of B(*a*)P in peanut oil (Sigma Chemical). Wrapping in aluminum foil protected the dosing solution from photolysis. Handling of B(*a*)P, a known carcinogen, was in accordance with OSHA Regulations 29-CFR-1910-1450, requiring protective equipment: gloves, mask and laboratory clothing. All handling of B(*a*)P in the powder form occurred under an exhaust hood and unused solutions were stored in a hazardous waste container for proper disposal.

Benzo(a)pyrene Administration

Rats were randomly assigned to one of the following six treatment groups (n = 10/sex/dose): 0, 12.5, 25, 50, 100 and 200 mg/kg of B(*a*)P. (B(*a*)P) was dissolved in peanut oil and administered by oral gavage, as a single dose in a volume of 10 ml/kg body weight. Control animals received 10 ml/kg of peanut oil only. The oral route was chosen because it mimics human exposure conditions.

Motor Activity Measurements

Animals were individually placed (at 1715–1745 h) in activity cages (42 cm × 42 cm × 30 cm, Digiscan Animal Activity System, Omnitech Electronics, Columbus, OH), for a 30-min habituation period. B(*a*)P was administered by oral gavage, and animals were immediately returned to activity cages for monitoring. Activity measurements (counts) were conducted at 2 hour intervals during the 12 h nocturnal phase of the animal's light/dark cycle. The apparatus measured the following motor activities: horizontal activity: the total number of beam interruptions in the horizontal sensors (corresponds to gross motor activity); total distance: the total path the animal has traveled in cm (a more accurate measure of ambulatory activity); vertical activity: the number of beam interruptions in the vertical sensor (corresponds to the number of rears the animal has made during the sampling period); and stereotypic activity: the total number of beam interruptions resulting from repetitive non locomotor small body movements that may be associated with toxicity such as grooming, head bobbing, sniffing and tremors. Observational scores from the FOB confirmed stereotypic activity, as the Digiscan activity system does not discriminate between the various types of stereotypic behaviors. This device only measures repeated photobeam breaks resulting from small body movements. Once placed in the activity cages, the animals were left undisturbed. Animals were returned to home cages each morning at 0700 where food and water were made available. The number of beam interruptions (counts) during 2 h and 12 h intervals quantified locomotor activities. Rats were monitored for five consecutive days following B(*a*)P administration on day 1.

Functional Observational Battery

Dosages and housing conditions were identical to those used in the motor activity studies;

however, new groups of animals (n = 10/sex/dose) were used for FOB measurements. The FOB includes 29 individual tests grouped into six functional domains: autonomic, neuromuscular, CNS excitability, CNS activity, sensorimotor and physiological (Moser *et al.*, 1988; Moser, 1990). Test measures and respective functional domains of the FOB are listed in Table I. The FOB tests were conducted before dosing (time 0) to obtain baseline values for all animals. The animals were then dosed with B(*a*)P and FOB tests were conducted at 2, 4, 6, 12, 24, 48, 72, 96 h post treatment. The same recorder and observer who were blind to the treatment status of the test subjects conducted all tests. The FOB was conducted as described by Moser *et al.* (1988) beginning with home cage observations where descriptions of the rat's posture, home cage activity, and the presence of any abnormal muscle movements were made. Open-field observations were made next by placing the animal on an open cart for 3 min. The open field observations included the animal's response to being handled; counting the number of fecal boluses and urine pools and examining the eyes and mouth for wetness measured toxicant-induced changes in autonomic function (i.e. lacrimation, salivation, urination and defecation). Assessments of the animals' gait, the presence of abnormal muscle movements and stereotypic activity were also evaluated during the 3 min observational period. At the end of the observational period, the righting reflex was measured by determining the ability of the rat to flip over in midair and land on its feet. Alterations to sensory stimuli (i e. touch, sight, sound and pain) were assessed measuring the animal's response to the touch of a pencil, the pinch of a tail, response to a flashlight and the snap of a metal clicker. Measuring both forelimb and hindlimb grip strengths assessed toxicant-induced muscle weakness, which was quantified by measuring the force required to free the animal's grip from a wire mesh cage. Landing foot splay was measured by dipping the hind feet on an inkpad then dropping the animal 20 cm prone position on absorbent paper. Measuring the distance between digits determined hind-limb foot-splay. At the end of the sequence of tests, B(*a*)P effects on body temperature and weight were recorded.

TABLE I Summary of FOB parameters affected by acute administration of B(*a*)P in F-344 rats (at 6 h time point) (– no statistically significant effects; FOB, functional observational battery; *, significantly affected ($p < 0.05$) in all B(*a*)P treatment groups except in the 12.5 mg/kg treatment group; ↑ increase and ↓ decrease indicates direction of change)

Parameter	*M*	*F*
Neuromuscular		
Rearing	↓*	↓*
Gait	↓*	↓*
Forelimb grip strength	↓*	↓*
Hindlimb grip strength	↓*	↓*
Landing foot splay	↓*	↓*
Aerial righting	↓*	↓*
Mobility	↓*	↓*
Sensorimotor		
Approach response	–	–
Touch response	↓*	↓*
Tail pinch	↓*	↓*
Click response	↓*	↓*
Autonomic		
Urination	(↑*	↑*
Defecation	↑*	↑*
Lacrimation	–	–
Salivation	–	–
Pupil response	–	–
Physiological		
Body weight	↓*	↓*
Excitability		
Arousal	↓*	↓*

Pilot Range Finding Study

A pilot range finding study was conducted to choose suitable doses of B(*a*)P that would produce behavioral alterations without producing sickness or malaise. Rats (n = 5/sex/dose) were administered 0, 100 and 200 mg/kg of B(*a*)P/kg of body weight. Since a dose of 200 mg/kg produced behavioral alterations (up to 60%) in motor activity and the FOB without resulting in malaise, this became the highest dose for all subsequent behavioral studies. Additional

animals ($n = 10$/sex) were dosed with 200 mg/kg and tested to determine if a 200 mg/kg dose would produce consistent and reliable behavioral changes. Doses of 12.5, 25, 50 and 100 mg/kg were also used to establish dose-response and time course relationships for B(*a*)P-induced behavioral toxicity. A 12.5 mg/kg dose of B(*a*)P produced no behavioral alterations in motor activity or the FOB in the pilot range finding studies. To establish the 12.5 mg/kg dose as the no-observed-adverse-effect-level (NOAEL), this dose was administered to an additional group ($n = 5$/sex) of animals. Again, no behavioral changes were noted at the 12.5 mg/kg dose in either the motor activity or FOB endpoints.

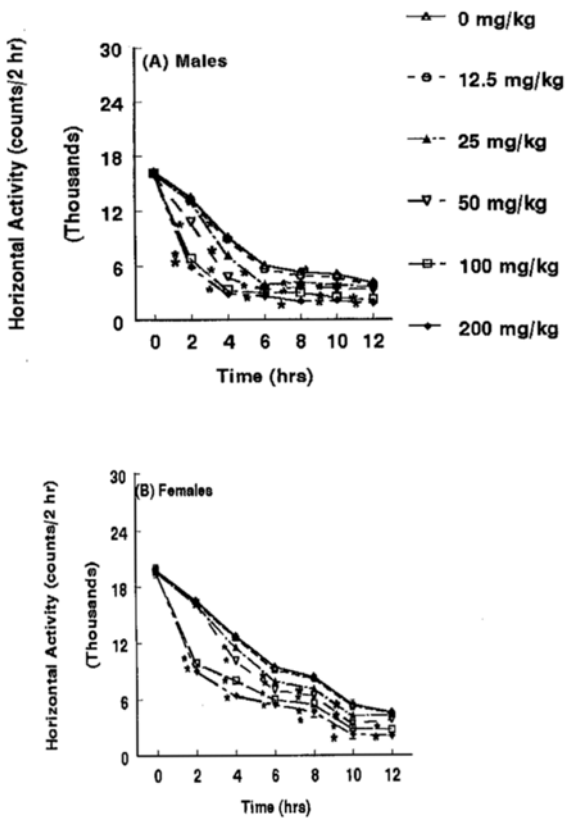


FIGURE 1 Effects of B(*a*)P on horizontal activity in (A) male and (B) female rats measured at 2 h intervals on day 1. Values represent mean ± SE for 10 rats/sex/treatment group at each time point. $*p < 0.05$ between vehicle control and B(*a*)P-exposed rats.

Statistical Analysis

Motor activity data were initially analyzed in a three-way repeated analysis of variance (ANOVA) with dose and sex as factors at each time point. As ANOVA revealed significant gender differences, motor activity data for male and female animals were analyzed separately. Using the Bonferroni multiple range tests made controls versus treatment group comparisons. Motor activity data are reported in Figs. 1–4 as counts (horizontal activity, stereotype and vertical activity) or centimeters traveled (total distance) per 2 h for 12 h on day 1 to show the onset of B(*a*)P effects and reported as group means the standard error. The total 12 h motor activity counts or centimeters traveled are reported in Figs. 5 and 8 for days 1 through 5 following B(*a*)P administration and are expressed as percent of vehicle control values (corresponding controls for each day). The criterion for statistical significance was $p < 0.05$ for all statistical analysis.

The FOB test scores (1 = no effect 5 = severe effect) were recorded for each animal. Rank data including home cage, handling reactivity, gait, autonomic and stimulus reactivity responses were analyzed, using the Mann and Whitney rank sum test to compare male and female groups at each time point. As significant gender differences were noted in the FOB, data for male and female animals were reanalyzed separately. The Kruskal Wallis test was used to analyze FOB data for each sex followed by the Newman Keuls *post hoc* test to further compare treated groups vs. the vehicle control group (Tables II and III). A three-way repeated measures ANOVA was used to analyze continuous data including grip strengths, rearing, landing foot splay and body weight gain with dose, time and sex as factors. Body weight data were analyzed separately for male and female animals as a weight differential existed between sexes at the start of behavioral testing. Group comparisons were made using the Bonferroni multiple range *post hoc* tests. The

criterion for statistical significance was $p < 0.05$ for all FOB tests.

RESULTS

Two Hour Motor Activity Measurements

The time course of B(*a*)P effects on nocturnal motor activity were analyzed by a three-way repeated measures ANOVA with dose, sex and time as factors on day 1 at each 2 h time point for male and female treated animals. As ANOVA revealed significant ($p < 0.001$) time, dose and gender interactions in motor activity parameters, data for males and females are plotted separately in Figs. 1–4. Motor activities on day 1 are presented as group means ($n = 10$ rats/sex/dose; Total $N = 120$) and are compared with the corresponding vehicle control group at each 2 h time point (Fig. 1: horizontal activity; Fig. 2: total distance; Fig. 3: stereotypic activity and Fig. 4: vertical activity).

TABLE II Summary of effects of B(*a*)P on sensorimotor function in males and females Fisher F-344 rats

Parameter	*Treatment group (mg/kg)*	*Time point (h)*	*Mean severity scores ± S.E.* *Males*	*Females*
		4†		
Click response	Control		2.3±0.153	2.4±0.163
	12.5		2.3±0.153	2.3±0.153
	25.0		2.2±0.143	2.3±0.153
	50.0		2.2±0.143	2.3±0.153
	100.0		1.8±0.133*	2.0±0.000
	200.0		1.5±0.167*	1.7±0.153*
		6‡		
	Control		2.5±0.167	2.4±0.167
	12.5		2.3±0.153	2.4±0.163
	25.0		1.9±0.153*	2.1±0.100
	50.0		1.7±0.153*	1.9±0.100*
	100.0		1.4±0.163*	1.7±0.153*
	200.0		1.2±0.133*	1.5±0.167*
		12		
	Control		2.5±0.167	2.5±0.167
	12.5		2.4±0.167	2.4±0.167
	25.0		2.4±0.000	2.4±0.167
	50.0		1.9±0.000*	2.1±0.100*
	100.0		1.6±0.163*	1.9±0.100*
	200.0		1.5±0.167*	1.8±0.133*
		24		
	Control		2.4±0.163	2.5±0.167
	12.5		2.3±0.153	2.4±0.163
	25.0		2.3±0.153	2.4±0.163
	50.0		2.0±0.100*	2.2±0.167*
	100.0		1.8±0.167*	2.0±0.000*
	200.0		1.6±0.167*	1.9±0.167*
		48¶		
	Control		2.4±0.163	2.4±0.163
	12.5		2.3±0.153	2.4±0.163
	25.0		2.3±0.153	2.3±0.153
	50.0		2.3±0.153	2.3±0.153
	100.0		2.0±0.000*	2.2±0.000*
	200.0		1.8±0.133*	2.1±0.153*

† First time point to show behavioral changes.
‡ Peak effects observed at this time point.
¶ Last time point at which treatment groups were significantly different from controls. Data assessed for statistical significance by Kruskal Wallis Analysis *$p < 0.05$ as compared to controls.

Horizontal Activity

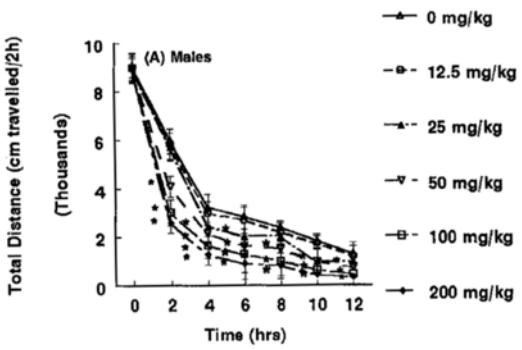
Post hoc analysis revealed that the onset of B(*a*)Ps suppressive effects on horizontal activity occurred within 2 h ($p < 0.001$) and persisted at 4 h ($p < 0.001$); at 6 h ($p < 0.026$); at 8 h ($p < 0.028$); at 10 h; ($p < 0.05$) and 12 h ($p < 0.05$) in the 50, 100, and 200 mg/kg male treated groups on day 1 (Fig. 1A). The 25 mg/kg dose produced a significant ($p < 0.01$) decrease in horizontal activity in males at 4 h post B(*a*)P administration on day 1 (Fig. 1A).

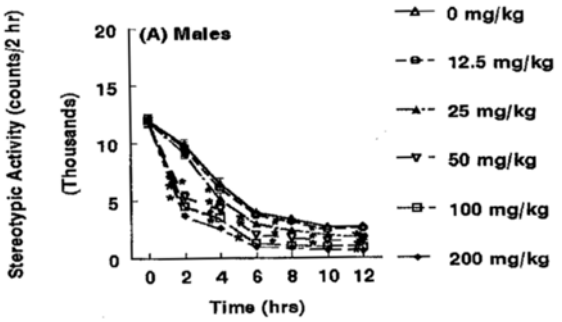
A similar pattern of effects was observed in female treated animals on day 1 (Fig. 1B). Suppression of horizontal activity in the 50, 100 and 200 mg/kg female treatment groups occurred at 2 h ($p < 0.008$); 4 h ($p < 0.01$); 6 h ($p < 0.02$); 8 h ($p < 0.03$); 10 h ($p < 0.03$) and 12 h ($p < 0.05$) (Fig. 1B).

The 25 mg/kg female exposed group did not show decreased motor activity until 4 h post treatment. Reductions in horizontal activity up to 79% were observed in male treated animals. Decreases (up to 65%) in horizontal activity were also noted in female B(*a*)P exposed animals. The 12.5 mg/kg dose did not suppress any of the motor activity endpoints measured at 2 h intervals on day 1 in either male or female treatment groups (Fig. 1).

Total Distance

The effects of B(*a*)P on total distance in male and female animals are presented in Fig. 2. The 50,





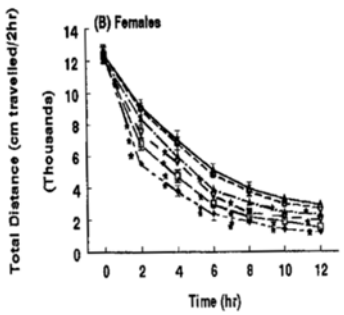


FIGURE 2 Effects of B(*a*)P on total distance in (A) male and (B) female rats measured at 2 h intervals on day 1. Values represent mean ± SE for 10 rats/sex/treatment group at each time point. $*p < 0.05$ between vehicle control and B(*a*)P-exposed rats.

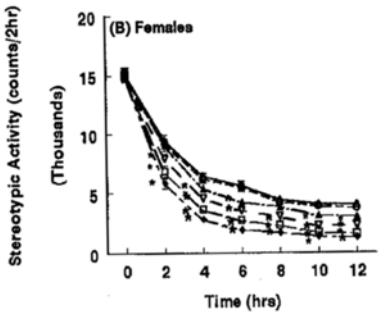


FIGURE 3 Effects of B(*a*)P on Stereotypic Activity in (A) male and (B) female rats measured at 2 h intervals on day 1. Values represent mean ± SE for 10 rats/sex/treatment group at each time point. $*p < 0.05$ between vehicle control and B(*a*)P-exposed rats.

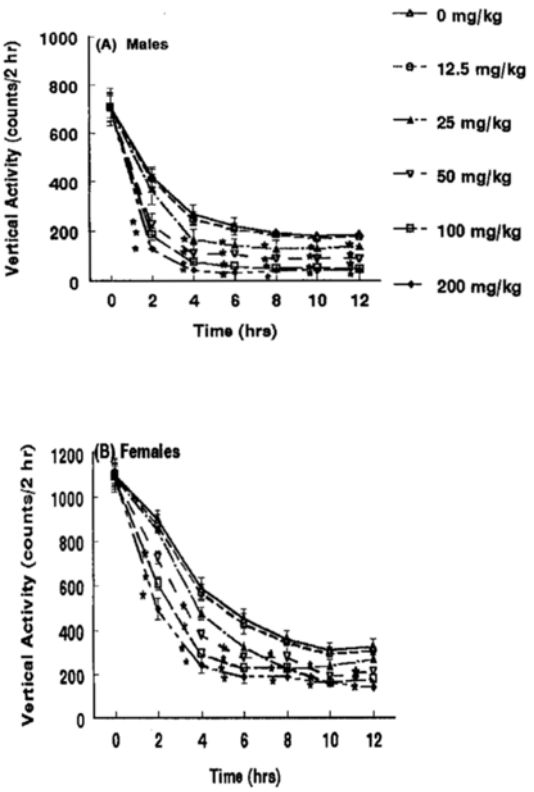


FIGURE 4 Effects of B(*a*)P on vertical activity in (A) male and (B) female rats measured at 2 h intervals on day 1. Values represent mean ± SE for 10 rats/sex/treatment group at each time point. $*p < 0.05$ between vehicle control and B(*a*)P-exposed rats.

100 and 200 mg/kg male treatment groups showed significant reductions in total distance compared to the vehicle control group at 2 h ($p < 0.001$); 4 h ($p < 0.008$); 6 h ($p < 0.26$); 8 h ($p < 0.03$); 10 h ($p < 0.04$) and 12 h ($p < 0.05$) (Fig. 2A). The onset of B(*a*)Ps suppressive effects in the 25 mg/kg male treatment group, however, did not occur until 4 h post treatment (Fig. 2A). The later onset of effects produced by the 25 mg/kg dose was consistent in all motor activity parameters and in both sexes.

Statistically significant differences in total distance between B(*a*)P treated females and controls were observed at 2 h ($p < 0.001$); 4 h ($p < 0.004$); 6 h ($p < 0.040$); 8 h ($p < 0.036$); 10 h ($p < 0.040$) and 12 h ($p < 0.05$) in the 50, 100 and 200 mg/kg B(*a*)P exposed females (Fig. 2B). Suppression of total distance in the 25 mg/kg female treated group occurred at 4 h. B(*a*)P reduced total distance by 81% in males and lowered by 70% in females (Fig. 2).

Stereotypic Activity

Analysis of stereotypic activity showed a pattern of effects (Fig. 3). The 50, 100 and 200 mg/kg treated male animals showed significant decreases in stereotypic activity at 2 h ($p < 0.001$); 4 h ($p < 0.001$); 6 h ($p < 0.01$); 8 h ($p < 0.030$); 10 h ($p < 0.04$) and 12 h ($p < 0.05$). However, male animals administered a 25 mg/kg dose of B(*a*)P did not show statistically significant reductions in stereotypic activity until 4 and 6 h post treatment (Fig. 3A).

Similar reductions were observed in B(*a*)P treated female animals. Significant dose dependent decreases in stereotypic activity occurred at 2 h ($p < 0.001$); 4 h ($p < 0.005$); 6 h ($p < 0.028$); 8 h ($p < 0.03$); 10 h ($p < 0.04$) and 12 h ($p < 0.05$) (Fig. 3B) in the 50, 100 and 200 mg/kg exposed females (Fig. 3B). Stereotypic activity was decreased approximately 73% in males and lowered up to 64% in females compared to their respective controls (Fig. 3).

Vertical Activity

Vertical activity data were consistent with the effects of B(*a*)P on horizontal activity, total distance and stereotypic activity. Significant differences in the vertical activity of male treated animals (50–200 mg/kg) were noted at 2 h ($p < 0.001$); 4 h ($p < 0.009$); 6 h ($p < 0.01$); 8 h ($p < 0.03$); 10 h ($p < 0.041$); 12 h ($p < 0.05$) in the 50, 100 and 200 mg/kg treated males (Fig. 4A). The 25 mg/kg dose, however, did not decrease vertical activity in males until 4 h post treatment.

Female treated groups (50–200 mg/kg) showed similar reductions in vertical activity at

2 h ($p < 0.008$); 4 h ($p < 0.01$); 6 h ($p < 0.02$); 10 h ($p < 0.04$); 12 h ($p < 0.04$) (Fig. 4B).

Vertical activity was decreased up to 83% in males and 66% in females.

Total 12 h Activity Measurements

The histograms in Figs. 5–8 represent total (12 h) nocturnal activity measured for five consecutive days' post B(*a*)P administration. Motor activity is expressed as a percent of the vehicle control group on each day tested. B(*a*)P induced significant ($p < 0.001$) reductions in total 12 h nocturnal motor activity: (Fig. 5) horizontal activities (Fig. 6) total distance (Fig. 7) stereotype and (Fig. 8) vertical activity.

Total (12 h) Horizontal Activity

Horizontal activity of all male groups were comparable controls on day 0 before B(*a*)P administration on day 1 (Fig. 5A). Following B(*a*)P administration, the horizontal activity of the 50, 100 and 200 mg/kg groups were significantly ($p < 0.001$) lower than the vehicle control group on days 1 and 2. Reductions in motor activity ranging between 20 and 62% were noted on day 1 in the 25–200 mg/kg treated males and reductions between 20 and 35% were noted on day 2 in the 50, 100 and 200 mg/kg B(*a*)P exposed males. Rats administered a 12.5 mg/kg dose of B(*a*)P did not display statistically significant ($p < 0.05$) reductions in horizontal activity or in any of the other total (12 h) motor activity parameters measured on

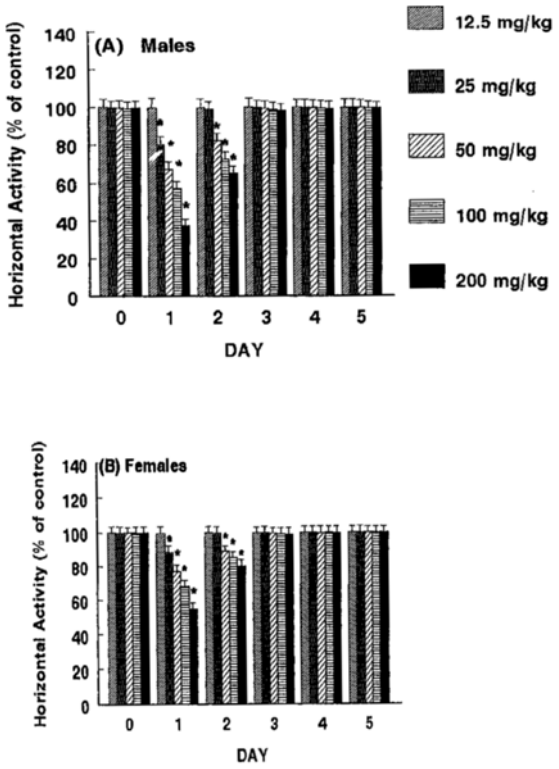


FIGURE 5 Effects of B(*a*)P on horizontal activity in (A) male and (B) female rats. Histograms represent total (12 h) horizontal for five consecutive days post-B(*a*)P administration. The data (% control/12 h) are means ± SE for 10 rats/dosage group.

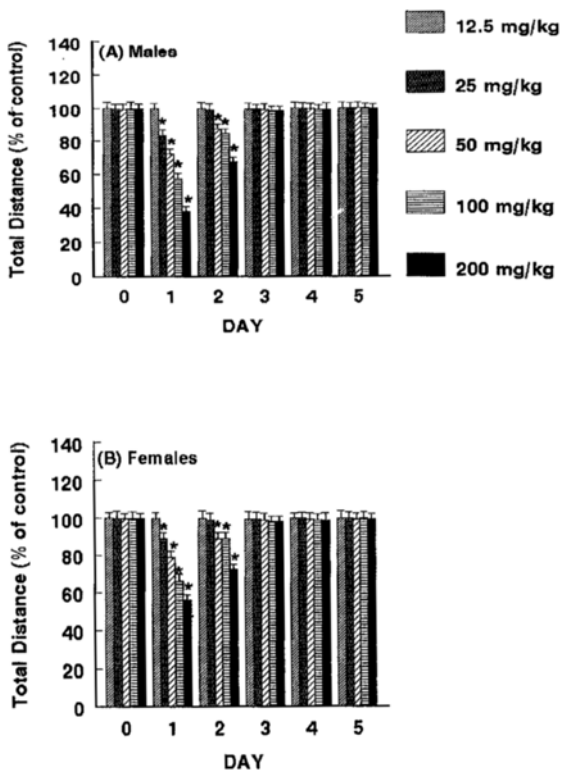
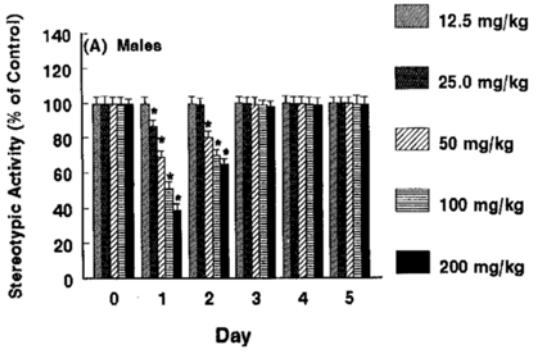


FIGURE 6 Effects of B(*a*)P on total distance in (A) male and (B) female rats. Histograms represent total (12 h) horizontal for five consecutive days post-B(*a*)P administration. The data (% control/12 h) are means ± SE for 10 rats/dosage group.



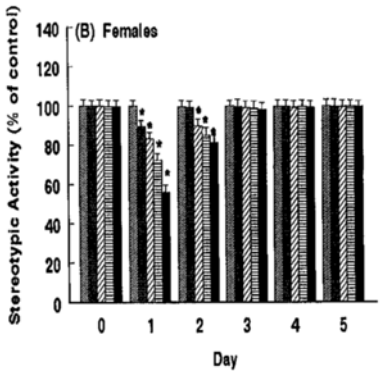
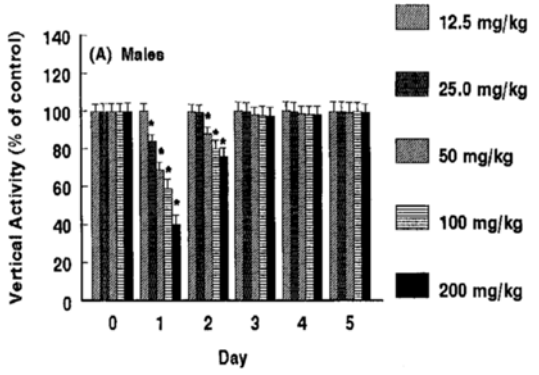


FIGURE 7 Effects of B(*a*)P on stereotypic activity in (A) male and (B) female rats. Histograms represent total (12 h) horizontal for five consecutive days post-B(*a*)P administration. The data (% control/12 h) are means ± SE for 10 rats/dosage group.



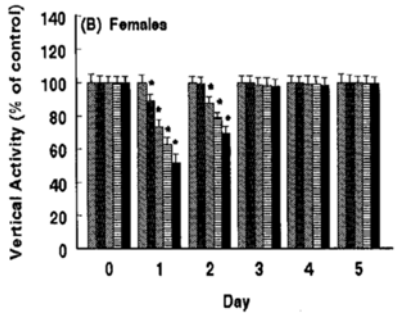


FIGURE 8 Effects of B(*a*)P on vertical activity in (A) male and (B) female rats. Histograms represent total (12 h) horizontal for five consecutive days post-B(*a*)P administration. The data (% control/12 h) are means ± SE for 10 rats/dosage group.

days 1–5. Total 12 h horizontal activity was comparable to controls by day 3 in all B(*a*)P treated males and remained comparable to controls through day 5 (Fig. 5A).

Total (12 h) horizontal activity was also suppressed in female treated animals compared to female controls with high doses (50–200 mg/kg) producing suppression of horizontal activity on days 1 and 2 (Fig. 5B). However, the 25 mg/kg dose of B(*a*)P decreased total (12 h) horizontal activity in females for only one day. B(*a*)P decreased horizontal activity in females between 12 and 45% on day 1. Reductions ranging between 15 and 21% were noted on day 2 in only the 50, 100 and 200 mg/kg female treatment groups. Total (12 h) horizontal activity in females was comparable to controls from days 3 through 5.

Total Distance

An analysis of total (12 h) distance also revealed statistically significant ($p < 0.001$) dose×sex×time effects in B(*a*)P exposed animals. Total distance was decreased approximately 21 to 63% in male treated groups (25–200 mg/kg) and decreased between 22 and 30% on day 2 in the 50, 100 and 2000 mg/kg groups (Fig. 6A).

B(*a*)P administration produced similar effects in female treated animals (Fig. 6B). Reductions in total distance ranged between 12 and 43% on day 1 in the 25, 50, 100 and 200 mg/kg groups and

was lowered between 10 and 20% in the 50, 100 and 200 mg/kg female groups on day 2 (Fig. 6B).

Total Stereotypic Activity

The histograms in Fig. 7 represent the effects of B(*a*)P on stereotypic activity in both males (Fig. 7A) and females (Fig. 7B). B(*a*)P dosing (25–200 mg/kg) produced significant decreases in stereotypic activity in males and females on days 1 and 2. Only the 50, 100 and 200 mg/kg treated male and female groups showed decreased stereotypic activity on day 2. All male and female treated animals recovered from the suppressive effects of B(*a*)P on day 3 (Fig. 7). Reductions in stereotypic activity ranging between 18 and 62% were observed in males (25–200 mg/kg) on day 1 and reductions between 18 and 30% were noted in male treated animals (50–200 mg/kg) on day 2 (Fig. 7A). Total 12 h stereotypic activity was also decreased (14–42%) in females on day 1 and lowered between 10 and 21% on day 2 in only the 50, 100 and 200 mg/kg female treatment groups (Fig. 7B).

TABLE III Summary of effects of B(*a*)P on sensiroimotor function in male and female Fisher F-344 rats

Parameter	*Treatment group (mg/kg)*	*Time point (h)*	*Mean severity scores*	
			Males	*Females*
Tail Pinch		4†		
	Control		2.5±0.167	2.6±0.156
	12.5		2.4±0.167	2.6±0.156
	25.0		2.4±0.153	2.5±0.167
	50.0		2.4±0.153	2.5±0.167
	100.0		2.0±0.167*	2.1±0.133*
	200.0		1.7±0.167*	1.8±0.143*
		6‡		
	Control		2.6±0.167	2.6±0.156
	12.5		2.5±0.167	2.6±0.156
	25.0		1.8±0.143*	2.2±0.167*
	50.0		1.6±0.156*	2.0±0.000*
	100.0		1.5±0.167*	1.8±0.143*
	200.0		1.3±0.133*	1.5±0.167*
		12		
	Control		2.5±0.167	2.5±0.167
	12.5		2.5±0.167	2.5±0.167
	25.0		2.0±0.000*	2.2±0.153*
	50.0		2.0±0.000*	2.2±0.153*
	100.0		1.9±0.167*	2.1±0.143*
	200.0		1.6±0.167*	1.9±0.143*
		24		
	Control		2.5±0.167	2.6±0.167
	12.5		2.5±0.167	2.5±0.167
	25.0		2.0±0.000*	2.2±0.153*
	50.0		2.0±0.167*	2.1±0.143*
	100.0		2.1±0.167*	2.2±0.167*
	200.0		1.8±0.167*	2.0±0.167*
		48¶		
	Control		2.5±0.167	2.6±0.167
	12.5		2.4±0.167	2.6±0.167
	25.0		2.4±0.167	2.5±0.167
	50.0		2.4±0.167	2.5±0.167
	100.0		2.2±0.153*	2.4±0.167*
	200.0		2.1±0.167*	2.3±0.153*

† First time point to show behavioral changes.
‡ Peak effects observed at this time point.
¶ Last time point at which treatment groups were significantly different from controls. Data assessed for statistical significance by Kruskal Wallis Analysis *$p < 0.05$ as compared to controls.

Total Vertical Activity

A similar pattern of effects was observed in the vertical activity of B(*a*)P treated rats (Fig. 8). Vertical activity decreased between 18 and 63% in all treated males except the 12.5 mg/kg treatment group on day 1. Decreases ranging between 10 and 23% were also observed on day 2 in only the 50, 100 and 200 mg/kg male exposed groups (Fig. 8A). Total (12 h) vertical activity in females was inhibited by approximately 10–47% on day 1 in the 25–200 mg/kg groups and inhibited total distance by 11, 19 and 27% in the 50, 100 and 200 mg/kg groups, respectively on day 2 (Fig. 8B).

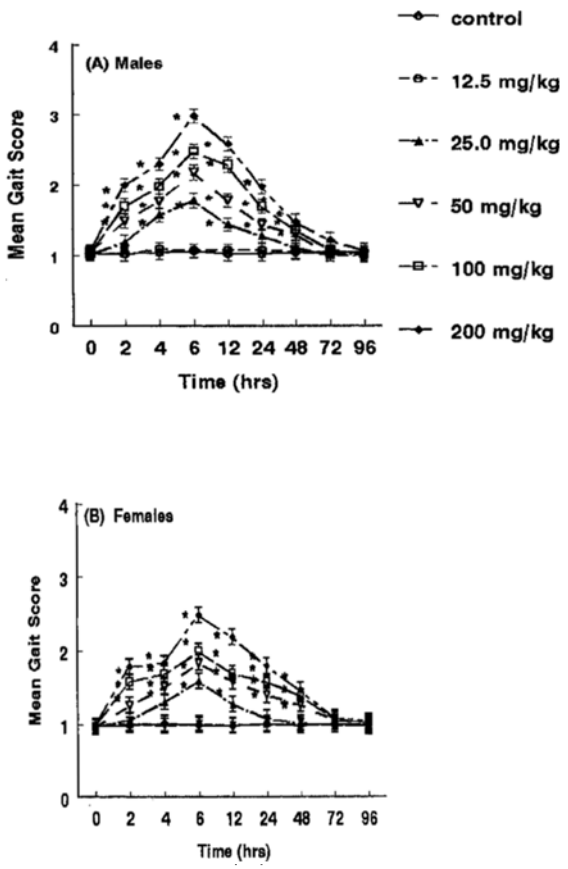


FIGURE 9 Effects of single oral doses of B(*a*)P on gaiting in (A) male and (B) female rats. Values represent mean ± SE for 10 rats/sex/ treatment group at each time point. $*p < 0.05$ between vehicle control and (B(*a*)P)-exposed rats.

Functional Observational Battery

The effects of B(*a*)P on FOB functional endpoints are shown in Tables II, III and IV and Figs. 9–12 as mean severity scores±standard error of the mean. Rats (n = 10/sex/dose) were administered B(*a*)P and FOB tests were conducted at time 0 (pretreatment period), 2, 4, 6, 12, 24, 48, 72 and 96 h. The principal functions altered by B(*a*)P were neuromuscular (i.e. decreased mobility, grip strength and activity, abnormal gaiting and loss of the righting reflex), autonomic (i.e. increased defecation and urination), sensorimotor (i.e. decreased responses sound, touch, pain and approach) and physiological (decreased body weight gain) functions.

Neuromuscular Function

Gaiting

Abnormal gaiting occurred within 2 h following B(*a*)P administration in the 50 (5/10), 100 (6/10) and 200 (6/10) mg/kg treated males. Abnormal gaiting was also observed in three out of 10 rats in the 25 mg/kg treated group. However, the 25mg/kg dose did not produce significant alterations in gaiting in males until 4 h post treatment. Gaiting abnormalities peaked at the 6 h time point, where all male treatment groups exhibited abnormal gaiting except the 12.5 mg/kg treated males. This dose did not produce statistically significant effects in any of the FOB endpoints in either sex. Males (25–200 mg/kg) also exhibited abnormal gaits at 12 h. At 24 and 48 h, mean severity scores increased in only the 50, 100 and 200 mg/kg treated males. By 72 h, gaiting returned to vehicle control levels in all male treatment groups (Fig. 9A).

Similar patterns of effects were noted in female animals. Comparable doses of B(*a*)P (25–200 mg/kg) produced alterations in gaiting in females. The onset of effects, time to peak effects and the duration of effects were identical in female animals also. However, the magnitude of

B(*a*)P effects was greater in males and statistically significant ($p < 0.05$), as evidenced by their higher mean severity gait scores in all FOB test measures (Figs. 9–12; Tables II–V). In female F-344 rats, the 50 (4/10), 100 (5/10) and 200 (6/10) mg/kg groups exhibited abnormal gaits at 2 h of treatment compared with the vehicle control group (0/10) (Fig. 9B). Abnormal gaiting was not observed in the 25 mg/kg exposed females (6/10) until 4 h after B(*a*)P administration. As observed in males, the toxic effects of B(*a*)P on gaiting were greatest at the 6 h time point than any other time point investigated. At 24 h, abnormal gaiting was observed only in the 50, 100 and 200 mg/kg females. Only the two highest doses (100 and 200 mg/kg) produced abnormalities in gaiting at 48 h. This was the last time point at which treatment groups showed statistically significant effects.

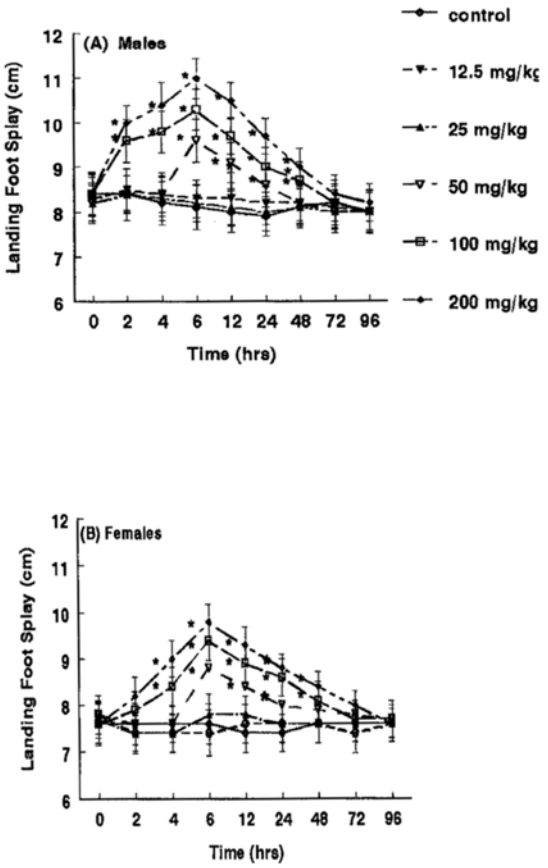


FIGURE 10 Effects of single oral doses of B(*a*)P on landing footsplay in (A) male and (B) female rats. Values represent mean ± SE for 10 rats/sex/treatment group at each time point. $*p < 0.05$ between vehicle control and B(*a*)P-exposed rats.

Landing Foot Splay

The effects of B(*a*)P on landing foot splay are presented in Fig. 10. Only the two highest treatment groups (100 and 200 mg/kg) exhibited increased landing foot splays in males at 2 and 4 h post treatment. At 6 h, increased landing foot splays were observed in the 50, 100 and 200 mg/kg exposed males. This remained true at the 12 and 24 h time points. By 48 h, only males in the 100 and 200 mg/kg exposed groups exhibited increased landing foot splays. All treated males displayed landing foot splays comparable to controls by 72 h. The two lowest doses of B(*a*)P (12.5 and 25 mg/kg) did not cause significant alterations in landing foot splay at any of the time points investigated.

The effects of B(*a*)P on landing foot splay in female treated animals are presented in Fig. 10B. Female animals in the 100 and 200 mg/kg treatment groups did not display significant increases in landing foot splay until 4 h post treatment. The toxic effects of B(*a*)P on landing foot splay peaked at 6 h in the 50–200 mg/kg B(*a*)P exposed females. Dysfunctional landing foot splays were also observed at 12 and 24 h. At 48 h, only the 100 and 200 mg/kg treated females displayed increased landing foot splays compared to vehicle controls. Female treated animals recovered from the toxic effects of B(*a*)P by 72 h. Landing foot splay severity scores were highest in B(*a*)P exposed males, indicating greater dysfunction in male animals.

Forelimb Grip Strength

The effects of B(*a*)P on forelimb grip strength in males and females are presented in Fig. 11.

TABLE IV Summary of effects of B(a)P on autonomic function in male and female Fisher F-344 rats

Parameter	Treatment group (mg/kg)	Time point (h)	Mean severity scores Males	Females
Urination		2†		
	Control		1.5±0.167	1.5±0.167
	12.5		1.5±0.167	1.5±0.167
	25.0		1.5±0.167	1.5±0.167
	50.0		1.5±0.167	1.5±0.167
	100.0		2.0±0.000*	1.7±0.167*
	200.0		2.3±0.133*	2.0±0.167*
		4		
	Control		1.5±0.167	1.5±0.167
	12.5		1.5±0.167	1.5±0.167
	25.0		1.5±0.133	1.5±0.167
	50.0		2.0±0.000*	1.7±0.167*
	100.0		2.3±0.153*	2.1±0.167*
	200.0		2.7±0.163*	2.3±0.167*
		6‡		
	Control		1.5±0.167	1.5±0.167
	12.5		1.5±0.167	1.5±0.167
	25.0		2.2±0.133*	1.8±0.133*
	50.0		2.5±0.167*	2.1±0.133*
	100.0		2.8±0.167*	2.4±0.167*
	200.0		3.2±0.133*	2.7±0.133*
		12		
	Control		1.6±0.167	1.5±0.167
	12.5		1.6±0.167	1.6±0.167
	25.0		1.7±0.133	1.6±0.167
	50.0		2.1±0.000*	1.9±0.143*
	100.0		2.4±0.167*	2.0±0.133*
	200.0		2.7±0.167*	2.3±0.167*
		24		
	Control		1.5±0.167	1.5±0.167
	12.5		1.5±0.167	1.5±0.167
	25.0		1.5±0.167	1.5±0.167
	50.0		2.0±0.167*	1.7±0.167*
	100.0		2.3±0.167*	2.0±0.167*
	200.0		2.5±0.167*	2.2±0.167*
		48¶		
	Control		1.6±0.156	1.5±0.167
	12.5		1.5±0.167	1.5±0.167
	25.0		1.7±0.167	1.6±0.167
	50.0		1.7±0.167	1.6±0.167
	100.0		2.0±0.167*	1.8±0.167*
	200.0		2.2±0.167*	1.9±.167*

† First time point to show behavioral changes.
‡ Peak effects observed at this time point.
¶ Last time point at which treatment groups were significantly different from control. Data assessed for statistical significance by Kruskal Wallis Analysis *$p < 0.05$ as compared to controls.

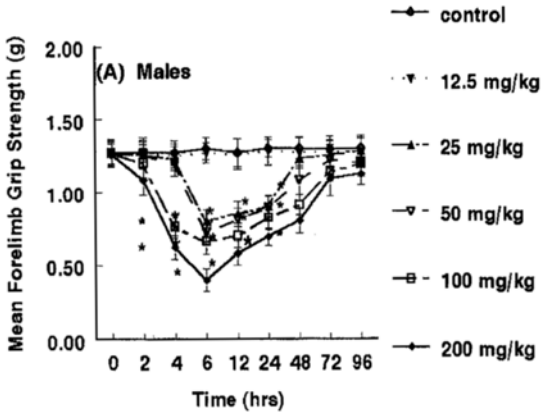
Males (6/10) in the two highest treatment groups (100 and 200 mg/kg) showed significant decreases in forelimb grip strength at 4 h (Fig. 11A). At 6 h, all male exposed groups showed decreases in forelimb grip strength except the 12.5 mg/kg exposed males. Significant decreases in forelimb grip strengths were also observed at 12, 24, and 48 h in the 50, 100 and 200 mg/kg exposed males. No significant changes ($p < 0.05$) in forelimb grip strengths were noted at 72 h in male treated animals.

Female rats in the 100 and 200 mg/kg treated groups showed decreased forelimb grip strengths at 4 h following B(*a*)P administration. All female treated animals showed decreased forelimb grip strengths at 6 and 12 h, except the 12.5 mg/kg B(*a*)P exposed females. Decreased grip strengths were observed in only the 50, 100 and 200 mg/kg females at 24 h. As observed in males, only the two highest doses of B(*a*)P produced decreases in forelimb grip strengths at 48 h.

Hindlimb Grip Strength

Significant decreases in hindlimb grip strengths were also noted in B(*a*)P treated male and female animals (Fig. 12). At 4 h male animals in the 100 and 200 mg/kg groups exhibited decreased hindlimb grip strengths (Fig. 12A). This effect peaked at the 6 h time point in all treated males except the 12.5 and 25 mg/kg treatment groups. Reduced hindlimb grip strengths were also observed at the 12 and 24 h time points in the 50, 100 and 200 mg/kg treatment groups. At 48 h, only the 100 and 200 mg/kg males displayed reduced hindlimb grip strengths.

Although the decreases in hindlimb grip strengths were greater in males, a similar pattern of effects was noted in females (Fig. 12B). At 4 h, females in the 100 and 200 mg/kg exposed groups showed decreased hindlimb grip strengths compared to female control animals.



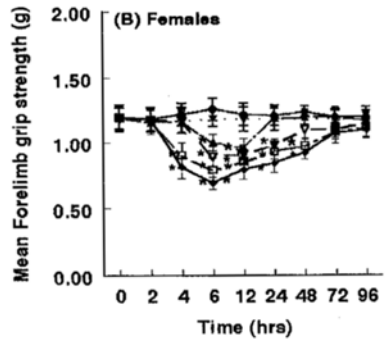
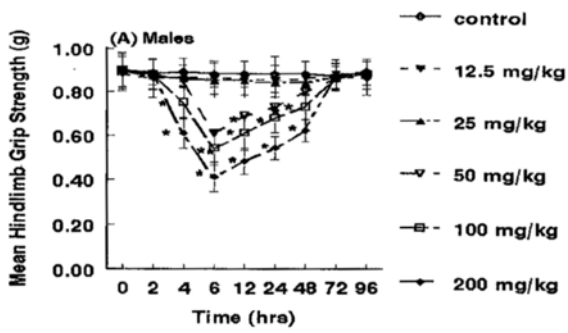


FIGURE 11 Effects of single oral doses of B(*a*)P on forelimb grip strength in (A) male and (B) female rats. Values represent mean ± SE for 10 rats/sex/treatment group at each time point. $*p < 0.05$ between vehicle control and B(*a*)P-exposed rats.



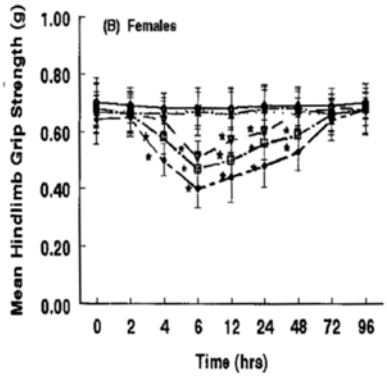


FIGURE 12 Effects of single oral doses of B(*a*)P on hindlimb grip strength in (A) male and (B) female rats. Values represent mean ± SE for 10 rats/sex/treatment group at each time point. $*p < 0.05$ between vehicle control and B(*a*)P-exposed rats.

Decreases in hindlimb grip strengths were greatest at 6 and 12 h in females (50–200 mg/kg) than any other time point. Reduced grip strengths were also noted at 24 h in the same treatment groups. Only the 100 and 200 mg/kg treated females showed decreases in hindlimb grip strengths compared to controls at 48 h. This was the last time point to show significant decreases in hindlimb grip strength.

Sensorimotor Function

Male animals in the 25 (3/10) and 50 (4/10) mg/kg treatment groups displayed intense

TABLE V Summary of effects of B(*a*)P on autonomic function in male and female Fisher F-344 rats

Parameter	*Treatment group (mg/kg)*	*Time point (h)*	*Mean severity scores*	
			Males	*Females*
Defecation		2†		
	Control		1.6±0.156	1.6±0.156
	12.5		1.7±0.133	1.6±0.156
	25.0		1.7±0.133	1.6±0.156
	50.0		1.7±0.100	1.7±0.167
	100.0		2.1±0.153*	1.9±0.143*
	200.0		2.4±0.167*	2.0±0.000*
		4		
	Control			
	12.5		1.7±0.143	1.6±0.167
	25.0		1.8±0.143	1.6±0.167
	50.0		2.3±0.133*	1.9±0.167*
	100.0		2.6±0.163*	2.2±0.167*
	200.0		2.8±0.133*	2.4±0.167*
		6‡		
	Control		1.6±0.156	1.6±0.156
	12.5		1.7±0.133	1.6±0.156
	25.0		2.1±0.000*	1.9±0.167*
	50.0		2.6±0.163*	2.2±0.133*
	100.0		2.9±0.100*	2.5±0.163*
	200.0		3.3±0.143*	2.9±0.100*
		12		
	Control		1.6±0.156	1.5±0.167
	12.5		1.7±0.133	1.6±0.167
	25.0		1.9±0.111*	1.8±0.133*
	50.0		2.4±0.167*	2.1±0.133*
	100.0		2.7±0.167*	2.3±0.153*
	200.0		2.9±0.100*	2.5±0.167*
		24		
	Control		1.6±0.156	1.6±0.156
	12.5		1.6±0.156	1.7±0.100
	25.0		1.7±0.156	1.7±0.100
	50.0		2.1±0.167*	1.9±0.111*
	100.0		2.3±0.167*	2.0±0.167*
	200.0		2.4±0.100*	2.1±0.100*
		48¶		
	Control			
	12.5		1.6±0.156	1.6±0.156
	25.0		1.7±0.133	1.6±0.156
	50.0		1.7±0.143	1.6±0.156
	100.0		1.7±0.133*	1.7±0.153
	200.0		2.1±0.100*	1.9±0.167*
			2.1±0.167*	2.0±0.167*

† First time point to show behavioral changes.
‡ Peak effects observed at this time point.
¶ Last time to observe behavioral changes. Data assessed for statistical significance by Kruskal Wallis Analysis *$p < 0.05$ as compared to controls.

reactions to touch and sound at the 2 and 4 h time points. One male animal in the 25 mg/kg treated group displayed an exaggerated response at 4 h (i.e. jumped) at the sudden sound of a metal clicker. Male treated animals (100 and 2000 mg/kg) showed decreased responsiveness to sensory stimuli (click response, tail pinch and approach) at 4 h after B(*a*)P dosing (Tables II and III). Sensorimotor dysfunction peaked for all treatment groups at 6 h except the 12.5 and 25 mg/kg treatment groups. Only males in the 50 (5/10), 100 (6/10) and 200 (7/10) mg/kg treatment groups showed decreased responsiveness to sensory stimuli at 24 h. At 48 h, only males in the 100 and 200 mg/kg groups displayed alterations in sensorimotor function compared with the vehicle control group (0/10).

Female treated animals (100 and 200 mg/kg) also exhibited decreased responsiveness to sensorimotor stimuli at 4 h (Tables II and III; tail pinch and click response, respectively). Alterations in sensorimotor function (decreased responses to sensory stimuli) peaked at 6 h in the 50, 100 and 2000 treated females and persisted at 24 h. After 48 h, only the 100 and 200 mg/kg female treated groups showed decreased responsiveness to approach, touch and pain compared to female controls. As observed in male animals, the behavioral changes induced by B(*a*)P in females were reversible after 72 h.

Autonomic Function

Male rats displayed significant changes ($p < 0.001$) in autonomic function (Tables IV and V). Increased urination and defecation were noted after 2 h following B(*a*)P administration in the 100 (4/10) and 200 mg/kg (6/10) treated males. At 4 h, increased urination and defecation were observed in the 50, 100 and 200 mg/kg treatment groups. Autonomic dysfunction (urination and defecation) peaked in all male treated animals at 6 h, except the 12.5 mg/kg male treatment group. Autonomic changes (urination and defecation) were still evident after 24 h in the 50 (5/10), 100 (6/10) and 200 (7/10) mg/kg B(*a*)P treatment groups. At 48 h post B(*a*)P dosing, only the high dose male groups (100 and 200 mg/kg) exhibited changes in autonomic function. Autonomic function in all male treated groups returned to control levels by 72 h. Although slight increases in lacrimation and salivation were observed in the 50 (1/10), 100 (2/10) and 200 (3/10) mg/kg treated males, these effects were not statistically significant.

Significant increases in autonomic dysfunction (urination and defecation) were also noted in the 200 mg/kg female treated at 2 h following B(*a*)P administration (Tables IV and V). At 4 h, female animals in the 100 and 200 mg/kg groups exhibited increased urination and defecation. Autonomic effects peaked at 6 h in all female treated animals, except the 12.5 mg/kg treated group. The 12.5 mg/kg dose did not produce significant alterations in any autonomic functions. By 24 h, only treated females in the 50, 100 and 200 mg/kg treatment groups displayed increased urination and defecation. Only the 100 and 200 mg/kg treated females showed significant changes in autonomic function at 48 h. After 72 h, autonomic function in all treated female animals was comparable to controls.

Physiological Function

Body weight gain data for male rats are presented in Fig. 13A. Body weight gains in all male B(*a*)P treated groups (25, 50, 100 and 200 mg/kg) were significantly ($p < 0.001$) lower than the control group on day 5 following B(*a*)P administration. Body weight gains in the 25 and 50 mg/kg groups returned to control levels by day 6. The 100 and 200 mg/kg B(*a*)P exposed groups, however, continued to exhibit significant decreases in body weight gain on day 5 ($p < 0.001$), day 6 ($p < 0.001$), day 7 ($p < 0.001$), day 8 ($p < 0.005$) and day 9 ($p < 0.0048$). Male rats in

the 100 and 200 mg/kg treatment groups displayed a 23–26% reduction in body weight gain compared to male control animals on day 9. Body weight gain in males was comparable to vehicle controls after two weeks following acute dosing with B(*a*)P.

Single oral doses of B(*a*)P produced significant ($p < 0.01$) reductions in body weight gain in female F-344 rats (Fig. 12B). Body weight gain in all female exposed groups, but the 12.5 mg/kg treated group was significantly ($p < 0.01$) lower than the vehicle control group on days 4 and 5. Body weight gains in the 25 and 50 mg/kg groups, however, returned to control levels by day 6 and comparable to that of control rats through day 9. The 100 and 200 mg/kg doses of B(*a*)P significantly ($p < 0.05$) suppressed body weight gain on day 5 ($p < 0.05$), day 6 ($p < 0.001$), day 7 ($p < 0.001$), day 8 ($p < 0.005$) and day 9 ($p < 0.080$). Female rats in the 100 and 200 mg/kg treatment groups displayed between a 21 and 25% reduction in body weight gain compared with vehicle control animals on day 9. Body weight gain in females was comparable to vehicle controls after two weeks following acute dosing with B(*a*)P.

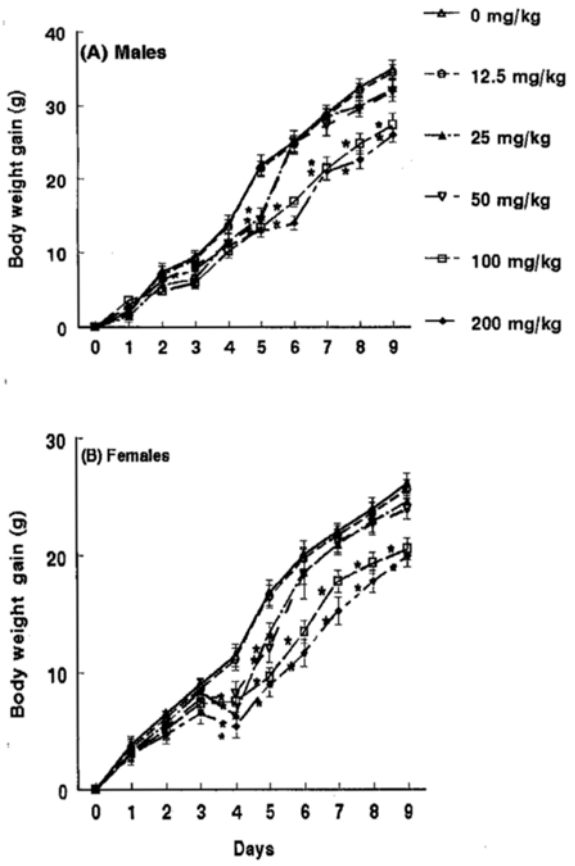


FIGURE 13 Effects of single oral doses of B(*a*)P on body weight gain in (A) male and (B) female rats. Values represent mean ± SE for 10 rats/sex/treatment group at each time point. $*p < 0.05$ between vehicle control and B(*a*)P-exposed rats.

DISCUSSION

To our knowledge, this is the first report documenting the behavioral effects of single oral doses of B(*a*)P. Our findings suggest that B(*a*)P can cause behavioral toxic effects, as evidenced by decreased nocturnal motor activity and alterations in neuromuscular, autonomic and physiological functions. The suppressive effects of B(*a*)P on nocturnal motor activities (horizontal activity, total distance, stereotype and vertical activity) and neurotoxic effects on FOB neurobiological endpoints began 2 h post B(*a*)P administration. The onset and duration of behavioral changes are consistent with oral pharmacokinetic data of B(*a*)P in the rat.

In oral bioavailability studies conducted in our laboratory, approximately 10% of the administered B(*a*)P (100 mg/kg) was recovered from the plasma within 2 h and the plasma concentration peaked at 8 h. The plasma levels of B(*a*)P decreased from 70% at 8 h to 29% at 24 h to 5% and at 48 and at 72 h, only trace levels of B(*a*)P were detectable (Ramesh *et al.*, 1999). Our bioavailability studies are consistent with the findings of Rahmann (1986). The results show biological plausible effects based on plasma levels of B(*a*)P over time that are consistent with the observed behavioral changes. Higher doses of B(*a*)P produced an earlier onset (2 h) and a longer duration (48 h) of action than lower

doses. There are several possible explanations why higher doses of B(*a*)P (50, 100 and 200 mg/kg) would induce an earlier onset and exert longer-lasting effects on behavioral endpoints than lower doses. This may be due to pharmacokinetic factors such as higher plasma concentrations, increase in storage of fat depots, differences in plasma protein binding characteristics and slower rates of metabolism and elimination at higher doses. As mentioned in Materials and Methods, the test compound was dissolved in peanut oil, which facilitates absorption and promotes the bioavailability of B(*a*)P (Laher *et al.*, 1984). It has been reported that B(*a*)P elimination is rapid following low-level exposure and is eliminated within 2 days following high-level exposure in rats and humans (Hecht *et al.*, 1979; Lioy *et al.*, 1988; Likhachev *et al.*, 1992).

Males were more sensitive to B(*a*)P toxicity than females as indicated by the greater severity scores in FOB measurements and the greater reductions in motor activity endpoints. The reduced effects in females may be due to more rapid metabolism of B(*a*)P as higher levels of aryl hydrocarbon hydroxylase have been reported in females (Ramesh *et al.*, 2000). Gender-related differences have also been reported in the regulation of antioxidant systems and phase II detoxification pathways that may account for gender differences (Waxman *et al.*, 1985). Differences in metabolic enzyme levels regulated by hormones (Waxman *et al.*, 1985) may also account for the greater sensitivity of males in behavioral endpoints. Hood *et al.* (2000) have reported that female animals cleared 42% of the parent B(*a*)P (100 $\mu g/m^3$; nose only particulate: carbon black aerosol) from the blood compared with 15% cleared by male animals after 1 h post treatment. A slower rate of clearance may also contribute to the greater sensitivity of males following B(*a*)P administration.

The mechanisms underlying B(*a*)P induced neurobehavioral toxicity are not yet known. Studies using radiolabeled B(*a*)P (^{3}HB(*a*)P; 15 nM; i.p.) revealed that this chemical crosses the blood–brain barrier and was distributed to the olfactory lobes, cerebellum, striatum, hippocampus, cortex and hypothalamus in the rat brain within 1 h and reached maximum concentrations at 24 h post treatment followed by a significant decline at 48, 72 and 96 h, respectively (Das *et al.*, 1985). The highest levels of radiolabeled B(*a*)P were found in the olfactory lobes, cerebellum and striatum. The effects of B(*a*)P on behavioral endpoints were reversible after 48 h and are consistent with the toxicokinetics of B(*a*)P reported by Das *et al.* (1985).

We believe that B(*a*)P induced behavioral toxicity may be caused by CNS depression possibly prolonged by the peanut oil vehicle. Chlorinated hydrocarbons such as halothane and chloroform, are structurally similar to B(*a*)P and can produce CNS suppression at equivalent doses. Aromatic hydrocarbons including toluene and benzene display anesthetic actions at comparable doses, which have been attributed to interferences with ion channels; however, such effects may also lead to changes in neurotransmitter output and enzyme activity, which may also account for the observed behavioral changes (Stengard, 1994). Occupational exposure to benzene has been shown to produce neurological deficits including somnolence and incoordination, which are associated with CNS suppression (Varona *et al.*, 1998).

Suppression of motor activity may be due to decreased levels of striatal dopamine, which has been reported by Stephanou (1998). Autonomic effects including increased urination and defecation observed in the FOB may occur as a result of cholinergic overstimulation as B(*a*)P is a potent inhibitor of acetylcholinesterase (Jett *et al.*, 1999). Bioactivation of the parent B(*a*)P compound to highly reactive diol epoxide intermediates and decreased levels of antioxidant enzymes may lead to oxidative stress (Nebert *et al.*, 2000).

Behavioral changes were accompanied by losses in body weight gain in both male and female animals. Although changes in body

weight gain may suggest B(*a*)P toxicity, these measurements are of limited neurotoxic value as these changes may be an unrelated non-specific effect. Single oral doses of 25 and 50 mg/kg of B(*a*)P caused significant alterations in behavior but did not cause significant decreases in body weight gain at the same time point as the behavioral changes, suggesting that B(*a*)P is producing behavioral toxic effects by direct mechanisms. In addition, the 100 and 200 mg/kg doses of B(*a*)P did not reduce body weight gain at the same time points as the behavioral changes.

In summary, B(*a*)P produced a variety of behavioral effects that are specific for nervous system dysfunction including decreased motor activity, neuromuscular, physiological and autonomic deficits and decreased responsiveness to sensory stimuli. The monitoring of motor activity with FOB analyses can serve as endpoints to screen a chemical for its neurotoxic potential (Moser, 1990; Tilson, 1990). The observed effects of B(*a*)P on behavioral endpoints were reversible. This negated the need for an expanded neurotoxicity battery. These data are of limited help in understanding the potential for neurotoxicity from long-term low-level exposure to B(*a*)P such as might occur in environmental settings or near hazardous waste sites, since the doses used in this study are significantly higher than normal ambient levels. Furthermore, there were no observed effects at the lowest dose given. However, in special situations such as the ingestion of contaminated food and water, in industrial settings and people living in close proximity to hazardous waste sites, these doses are toxicologically relevant as PAH concentrations encountered under these conditions are approximately 1000–10,000 times higher than normal ambient levels (ATSDR, 1995). Analyses of PAH concentrations from aircraft exhaust fumes, during flight-related activities and ground support activities have been estimated to be as high as 4 mg/m^3 (Childers *et al.*, 2000). There is a great potential for flight personnel, maintenance crews and passengers to be exposed to high levels of PAHs. It has been estimated that industrial workers are typically exposed to PAH concentrations ranging from 0.1 to 0.6 mg/m^3, which includes 0.2 mg/m^3 of B(*a*)P (Haugen *et al.*, 1988; ATSDR, 1995; Burstyn *et al.*, 2000). The concentrations of PAHs in food products range from 0.002 to 0.9 mg/kg (Gammage, 1983; Adams *et al.*, 1987; ATSDR, 1995). PAH levels exceeding 4 mg/kg have been reported at Superfund hazardous waste sites (ATSDR, 1995). It has been estimated that approximately 11 million individuals live within a 1 mile radius of Superfund hazardous waste sites (ATSDR, 1995). Behavioral effects following single acute exposure have prompted studies in our laboratory to examine the neurobehavioral effects of B(*a*)P after repeated low-level administration. Future studies will also include neurochemical and histological methods to further characterize the neurotoxicity of B(*a*)P after chronic low-dose administration of B(*a*)P.

Acknowledgements

Grants MHPF/ATSDR U50-ATU398948, NHLBIT32-HL07809 supported this research. The authors wish to thank Dr Aramandla Ramesh and Dr Alfred Nyanda for their help in preparing this manuscript and special gratitude to Dr Jethro Ekuta and Dr Ansah for their technical assistance with the behavioral studies

References

Adams, J.O., Mara, K. and Hoffman, D. (1987) "Toxic and carcinogenic agents in undiluted mainstream smoke and side stream smoke of different types of cigarettes", *Carcinogenesis* **8**, 729–731.

Adler, S., Candrian, R., Elsner, J. and Zbinden, G. (1986) "Neurobehavioral screening in rats: validation study", *Methods Fund. Exp. Clin. Pharmacol.* **8**, 279–289.

ATSDR (1995) Toxicological profile for Polycyclic Aromatic Hydrocarbons (PAHs) (Agency for Toxic Substances and Disease Registry, Atlanta, GA).

Burstyn, I., Kromhout, H. and Boffetta, P. (2000) "Literature review of levels and determinants of exposure to

potential carcinogens and other agents in the road construction industry", *AIHAJ* **5**, 715–726.

Childers, J.W., Witherspoon, C.L., Smith, L.B. and Pleil, J.D. (2000) "Real-time and integrated measurement of potential human exposure to particle bound polycyclic aromatic hydrocarbons from aircraft exhaust", *Environ. Health Perspect.* **108**, 853–862.

Crofton, K. and Howard, J. (1991) "Interlaboratory comparison of motor activity experiments: implications for neurotoxicological assessments", *Neurotoxicol. Teratol.* **13**, 599–609.

Darby, F. and Willis, A. (1986) "Occupational health hazards from road construction and sealing work", *Ann. Occup. Hgy.* **30**, 445–454.

Das, M., Mukhtar, H. and Seth, P. (1985) "Distribution of benzo(*a*)pyrene in discrete regions of the rat brain", *Bull. Environ. Contam. Toxicol.* **35**, 500–504.

Davies, G., Hodkinson, A. and Divetta, C. (1986) "Measurement and analysis of occupational exposures to coke oven emissions", *Ann. Occup. Hyg.* **30**, 51–62.

Eaton, D. (1995) "Role of cytochrome P4501A2 in chemical carcinogenesis: implications for human variability in expression and enzyme activity", *Pharmacogenetics* **5**, 259–274.

Environmental Protection Agency (EPA) (1985) An exposure and risk assessment for benzo(*a*)pyrene and other polycyclic aromatic hydrocarbons (Office of Water, Washington, DC) **Vol. IV**, EPA 440/4-85-020-V4.

Freeman, D.J. and Cantell, C.R. (1990) "Woodburning as a source of atmospheric polycyclic aromatic hydrocarbons", *Environ. Sci. Technol.* **24**, 1581–1585.

Gammage, R. (1983) In: Bjouseth, ed, Handbook of PAHs (Dekker, New York, NY), pp 653–707.

Grimmer, G. (1983) In: Grimmer, G., ed, Polycyclic Aromatic Hydrocarbons (CRC Press, Boca Raton, FL).

Haggerty, G.C. (1989) "Development of a tier neurobehavioral testing capabilities for incorporation into pivotal rodent safety assessment studies", *J. Am. Coll. Toxicol.* **8**, 53–70.

Hammond, E.D., Selikoff, J.J. and Lawther, P.O. (1976) "Inhalation of B(*a*)P and cancer in man", *Ann. NY. Acad. Sci.* **271**, 116–124.

Haugen, A., Becher, G., Benestad, K.V., Trivers, G.E., Newman, M.J. and Harris, C.C. (1988) Biomonitoring of individuals exposed to high levels of PAH in the work environment. Polynuclear Aromatic Hydrocarbons: A decade of progress (Batelle Press, Columbus, Ohio).

Hecht, S., Grabowski, W. and Groth, K. (1979) "Analysis of feces for B(*a*)P after consumption of charcoal-broiled beef by rats and humans", *Food Cosmet. Toxicol.* **17**, 223–227.

Hoffman, D., Harley, N., Fisenne, I. and Adams, J. (1986) "Carcinogenic agents in snuff", *J. Natl.Cancer Inst.* **76**, 435–437.

Hood, D.B., Nayyar, T., Ramesh, A., Greenwood, A. and Inyang, F. (2000) "Modulation in the developmental expression profiles of sp1 subsequent to transplacental exposure of fetal rats to desorbed benzo(*a*)pyrene following maternal inhalation", *Inhalation Toxicol.* **12**, 101–125.

Iwagawa, M. and Maeda, T. (1992) "Comparative dose-response study on the pulmonary carcinogenicity of 1,6-dinitropyrene and benzo(*a*)pyrene in F-344 rats", *Carcinogenesis* **10**, 1285–1290.

Jerina, D., Sayer, J. and Thakker, D. (1980) "Carcinogenicity of polycyclic aromatic hydrocarbons. The bay region theory", In: Gelboin, H., ed, Carcinogenesis: Fundamental mechanisms and environmental effects (Reidel Publishing, Massachusetts), pp 1–12.

Jett, D., Navoa, R.V. and Lyons, M. (1999) "Additive inhibitory action of chlorpyrifos and PAHs on acetylcholinesterase activity in vitro", *Toxicol. Lett.* **105**, 223–229.

Kim, K.B. and Lee, B.M. (1997) "Oxidative stress to DNA, protein and antioxidant enzymes (superoxide dismutase and catalase) in rats treated with Benzo(*a*)pyrene", *Cancer Lett.* **113**, 205–212.

Knecht, U. and Elliehausen, H. (1987) "Polycyclic aromatic hydrocarbons (PAH) in abraded particles of brake and clutch linings", *Int. J. Environ Occup. Soc. Med.* **28**, 227–236.

Laher, J.M., Rigler, M.W. and Vetter, R.D. (1984) "Similiar bioavailability and lymphatic transport of benzo(*a*)pyrene when administered to rats in different amounts of dietary fat", *J. Lipid Res.* **12**, 1337–1342.

Lavoie, E., Bradley, J. and Rice, J. (1987) "Tumorigenic activity of nonalternant polynuclear aromatic hydrocarbons in newborn mice", *Cancer Let.* **34**, 15–20.

Lebel, C. (1991) "Oxygen radicals: common mediators of neurotoxicity", *Neurotox. Teratol.* **13**, 341–346.

Legraverend, C., Harrison, D. and Ruscetti, W. (1983) "Bone marrow toxicity induced by oral benzo(*a*)pyrene", *Toxicol. Appl. Pharmacol.* **70**, 390–410.

Lesage, J., Perrault, G. and Durand, P. (1987) "Evaluation of worker exposure to polycyclic aromatic hydrocarbons", *Am. Ind. Hyg. Assoc. J* **48**, 753–759.

Likhachev, A., Beniashvili, D. and Bykov, V. (1992) "Relevance of quantitation of benzo(*a*)pyrene metabolites in animal excretes to evaluate individual human cancer risk", *Prig. Cain. Biol. Res.* **374**, 435–452.

Lioy, P., Waldman, J. and Greenberg, A. (1988) "The total human exposure study to benzo(*a*)pyrene: Comparison of the food and inhalation pathway", *Arch. Environ. Health* **43**, 304–312.

Lloyd, J.W. (1971) "Long-term mortality study of steelworkers: V. Respiratory cancer in coke plant workers", *J. Occup. Med.* **13**, 53–68.

Maclure, K.M. and MacMahon, B. (1980) "An epidemiologic perspective of environmental carcinogenesis", *Epidemiol. Rev.* **2**, 19–48.

MacPhail, R., Peele, R. and Crofton, K. (1989) "Motor activity and screening for neurotoxicity", *J. AM. Coll. Toxicol.* **10**, 661–669.

Majachrzak, R., Sroczynski, J. and Chelmeska, E. (1990) "Evaluation of the nervous system in workers in the furnace and coal divisions of the coke-producing plants", *Med. Pr.* **2**, 108–113.

Menzie, C. and Santodonato, J. (1992) "Ambient concentrations and exposure to carcinogenic PAHs in the environment", *Environ. Sci. Technol.* **26**, 1278–1284.

Moser, V., McCormick, J., Creason, J. and MacPhail, R. (1988) "Comparison of chlordimeform and carbaryl using a functional observational battery", *Fundam. Appl. Toxicol.* **11**, 189–216.

Moser, V. (1990) "Approaches to assessing the validity of a functional observational battery", *Neurotoxicol. Teratol.* **12**, 483–488.

Mounho, B. and Burchiel, S. (1997) "Alterations in human B cell calcium homeostasis by PAH: Possible associations with CYP1A1 metabolism and increased protein tyrosine phosphorylation", *Toxicol. Appl. Pharmacol.* **148**, 80–89.

Nebert, D., Roe, A. and Dieter, M. (2000) "Role of the aromatic hydrocarbon and the Ah gene battery in the oxidative stress response, cell cycle control and apoptosis", *Biochem. Pharmacol.* **59**, 65–85.

Rahmann, A. (1986) "The influences of bile in the bioavailability of PAHs from rat intestine", *J. Physiol. Pharmacol.* **64**, 1214–1248.

Ramesh, A., Inyang, F. and Hood, D. (1999) "Oral bioavailability and metabolic profile of Benzo(*a*)pyrene in F-344 rats", *ISSX Proc.* **15**, 236.

Ramesh, A., Inyang, F. and Hood, D. (2000) "Aryl hydrocarbon hydroxylase (AHH) activity in F-344 rats subchronically exposed to Benzo(*a*)pyrene (B(*a*)P) and Fluoranthene (FLA) through the diet", *J. Biochem. Mol. Toxicol.* **14**, 155–161.

Redmond, E., Strobina, B. and Cypress, R. (1976) "Cancer experience among coke by-product workers", *Ann. NY Acad. Sci.* **2**, 102–111.

Salas, V. and Burchiel, S. (1998) "Apoptosis in the daudi human B cells in reponse to benzo(*a*)pyrene and benzo(*a*)pyrene-7,8-dihydrodiol", *Toxicol. Appl. Pharmacol.* **151**, 367–376.

Schulze, G.E. and Boysen, B.G. (1991) "A neurotoxicity screening battery for use in safety evaluations: Effects of acrylamide and 3′, 3′-iminodipropionitrile", *Fund. Appl. Toxicol.* **16**, 602–615.

Schulze, G.E. (1990) "Large scale assessment of motor activity in rodents: Procedure for routine use in toxicology studies", *J. AM. Coll. Toxicol.* **9**, 455–463.

Spencer, P. (1992) "Are human neurodegenerative disorders linked to environmental chemicals with excitotoxic properties?", *Ann. NY. Acad. Sci.* **648**, 154–160.

Stengard, K. (1994) "The effects of toluene on striatal dopamine and acetylcholinesterase activity", *Pharmacol. Toxicol.* **75**, 115–118.

Stephanou, P. (1998) "Alterations in central monoaminergic neurotransmission induced by polycyclic aromatic hydrocarbons in rats", *Europ. J. Drug Metab.* **23**, 475–481.

Sutter, T.R. and Greenlee, W.F. (1992) "Classification of members of the Ah gene battery", *Chemosphere* **25**, 223–226.

Tilson, H. (1990) "Neurotoxicology in the 1990s", *Neurotoxicol. Teratol.* **12**, 293–300.

Tilson, H. (1987a) "Behavioral indices of neurotoxicity: What can be measured?", *Neurotoxicol. Teratol.* **9**, 427–448.

Tilson, H. (1987b) "Neurobehavioral techniques to assess the effects of chemicals on the nervous system", *Annu. Rev. Pharmacol. Toxicol. Appl.* **24**, 425–450.

Varona, A., Echevarria, E., Irazusta, J., Serrano, R., Gil, J. and Casia, L. (1998) "Effects of acute benzene on brain enkephalin immunostaining and degradation", *Neurotoxicol. Teratol.* **20**, 611–616.

Wang, D. and Meresz, O. (1982) "Occurrence and potential uptake of polynuclear aromatic hydrocarbons of highway traffic origin by proximally grown food crops", In: Cook, M., Dennis, A. and Fisher, G, eds, Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry Sixth International Symposium, (Battelle Press, Columbus, Ohio), pp 885–896.

Waxman, D.J., Dannan, G.A. and Guengerich, F.P. (1985) "Regulation of rat hepatic cytochrome P- 450: Age-dependent expression, hormonal imprinting and xenobiotic inducibility of sex-specific isoenzymes", *Biochemistry* **24**, 4409–4417.