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Whole Body Inhalation Exposure to 1-Bromopropane Suppresses the IgM Response to Sheep Red Blood Cells in Female B6C3F1 Mice and Fisher 344/N Rats

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Abstract

1-Bromopropane (1-BP) is categorized as a high-production volume chemical currently used in the manufacture of pharmaceuticals, pesticides and other chemicals. Its usage is estimated to be around 5 million pounds/year resulting in the potential for widespread exposure in the workplace. Case reports and animal studies have suggested exposure to this compound may cause adverse reproductive and neurological effects. Using a battery of immunological assays, the immunotoxicity of 1-BP after whole body inhalation exposure in both mice and rats was evaluated. Significant decreases in the spleen IgM response to SRBC were observed in both mice (125-500 ppm) and rats (1000 ppm) after exposure to 1-BP for 10 weeks. In addition, total spleen cells and T-cells were significantly decreased after approximately 4 weeks of 1-BP exposure in both mice (125-500 ppm) and rats (1000 ppm). No change in natural killer (NK) cell activity was observed. The observed alterations in spleen cellularity, phenotypic subsets and impairment of humoral immune function across species, raises further concern about human exposure to 1-BP and demonstrates the need for additional investigations into potential adverse health effects.

INTRODUCTION

Prior to 1998, 1-bromopropane (1-BP) was primarily used to manufacture pharmaceuticals, pesticides and other chemicals. More recently it has been introduced as an alternative to ozone-depleting halogenated solvents in industry, especially in Asia and Europe, because of its relatively short atmospheric lifetime (2 to 3 weeks) and lower ozone-depletion potential. In addition, the United States Environmental Protection Agency (EPA) is considering 1-BP as a potential substitute for ozone-depleting solvents in the non-aerosol solvent cleaning sector for general metals and electronics cleaning applications, in spray adhesives and coatings applications, and aerosol propellant and solvent applications (EnviroTech 2001), which could potentially enhance the exposure incidence to this chemical.

1-BP is considered by the EPA to be a high production volume chemical with 1.5 million pounds produced and 2.8 million pounds imported for use in the United States between 1999 and 2000 (OSHA, 1999). Estimated global sales of 1-BP for solvent and adhesives applications are 4,839, 3,152, and 3,736 metric tons for the years 2000, 2001, and 2002, respectively (NTP

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2002). Estimated usage of 1-BP was estimated to be around 5 million pounds/year (EnviroTech 2001) in 2001 but this could increase if it is used as a substitute for hydrofluorocarbons and methylene chloride.

Occupational exposure limits are not available at this time. However the National Institute for Occupational Safety and Health (NIOSH) has estimated the levels of 1-BP in the breathing zones of workers in three plants where a spray adhesive containing 1-BP was used in the manufacturing of seat cushions for aircrafts or furniture. The 8-hour time weighted average (TWA) exposure ranged from 18-381 ppm for the three plants (Trout 1999). At this time, exposure data are limited to the inhalation route. Dermal exposure to 1-BP is very likely since many of the products containing 1-BP come in contact with the skin; however, no data on this exposure route are available.

There are limited human and animal toxicity data for 1-BP. Current research suggests that exposure to 1-BP may effect the nervous and reproductive systems (Raymond and Ford 2007; WIL Research Laboratories 2001). A case report of a 19 year old male exposed to 1-BP through the use of a cleaning and degreasing solvent describes weakness of the lower extremities and right hand, numbness and difficulty swallowing and urinating after the 2 month exposure period (Sclar 1999). In addition, four workers were reported to develop serious systemic illnesses soon after the introduction of 1-BP as a furniture adhesive into their workplace (Raymond and Ford 2007). Studies have also been conducted on factory workers exposed to 1-BP. Although these effects were not found to be statistically significant, symptoms associated with exposure to 1-BP at concentrations greater than 170 ppm included irritation of the mucus membranes, headache, and vertigo (Ichihara *et al.* 2004). In addition to human case studies, a 12-week inhalation (200 – 800 ppm) exposure study conducted in rats (Ichihara *et al.* 2000), suggests an effect of 1-BP on the central nervous system.

In addition to the possible adverse neurological health effects associated with 1-BP exposure, surveys done on factory workers exposed to the structurally similar chemical 2-bromopropane (2-BP) have identified adverse reproductive effects associated with exposure to this chemical (Ichihara *et al.* 1999; Kim *et al.* 1996). Animal studies conducted in rats exposed by whole body inhalation to concentrations of 1-BP for 7 weeks support these findings (WIL Research Laboratories 2001; Yu *et al.* 1997). Reproductive toxicity, including an exposure-related increase in estrous cycle length and ovarian follicular cysts, was observed in female Sprague-Dawley rats exposed to 250 ppm-750 ppm 1-BP (WIL Research Laboratories 2001). Female rats were also observed to have increased amounts of glycogen in the liver. Repeated inhalation exposure of males to 1-BP resulted in decreased prostate weight and exposure concentration-related decreases in percentages of normal sperm and motile sperm when exposed to 200 ppm and higher. 1-BP exposure-related effects in the liver, kidney and spleen of the F1 generation after exposure of the parents to 250 and 500 ppm have also been observed.

Studies investigating the immune-related effects of 1-BP exposure are lacking. However, studies have demonstrated that exposure to 2-BP significantly suppresses the spleen antibody response to sheep red blood cells (SRBC) in rats after oral exposure to 1000 ppm of 2-BP (Jeong *et al.* 2002). Due to the potential for occupational exposure and human and rodent studies suggesting the adverse health effects associated with exposure to this compound, 1-BP was nominated for immunotoxicological evaluation by the National Institute for Occupational Safety and Health (NIOSH) and the National Toxicology Program (NTP). The present study was undertaken to evaluate immunotoxicity in rodents following whole-body inhalation exposure of 1-BP. The antibody response to sheep red blood cells, splenocyte phenotyping and natural killer cell activity were selected as endpoint for the evaluation of chemical induced immunotoxicity.

MATERIALS AND METHODS

Test Material

1-Bromopropane (1-BP, CAS# 106-94-5) was obtained from Albemarle PCC (Thann, France). The purity of 1-BP was determined by elemental analyses and gas chromatography to be 99.5% or greater. The bulk chemical was stored at room temperature under a nitrogen headspace; no degradation was detected using gas chromatography.

Animals

Female B6C3F1 mice and F344/N rats (~4 weeks old) were obtained from Taconic Laboratory Animals and Services (Germantown, NY). Animals were quarantined for 14 days, and were ~6 weeks old on the first day of the study. Animals were housed individually in wire-mesh units in inhalation chambers (Hazelton 2000, Lab Products, Inc., Aberdeen, MD), maintained at $75 \pm 3^\circ\text{F}$, $55 \pm 15\%$ relative humidity, with 15 air changes per hour, in a 12-h light/dark cycle. Food (NTP-2000, Zeigler Bros., Inc., Gardners, PA) and water were available *ad libitum*, except during exposures when food was withheld. Animals were randomized and identified by tail tattoo. The Batelle Animal Facility is an environmentally controlled barrier facility fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Vapor Generation, Monitoring and Exposure of 1-BP

Groups of up to 8 rats or mice were exposed in each of four chambers by whole-body inhalation to 1-BP at concentrations of 0, 125 (mice only), 250, 500, or 1000 ppm (rats only) for 6 h plus T_{90} (10 min) per day, 5 days per week (excluding holidays) for approximately 4 or 10 weeks (Table 1). Control animals were exposed to filtered conditioned air, which was also provided to exposed animals. All animals were observed twice daily for clinical signs. 1-BP vapor was generated by passage of liquid 1-BP from a generator reservoir onto a heated glass column (300°F) where it was vaporized. Chamber concentrations of 1-BP were monitored continuously during the exposure period using an on-line Gas Chromatograph with a Flame Ionization Detector.

Toxicological studies

Animal exposures took place at Battelle Memorial Institute (Richland, WA). At the end of the study [approximately day 28 (4 weeks) and day 72 (10 weeks)], the animals were sacrificed by inhalation CO_2 asphyxiation. Peripheral blood was collected by cardiac puncture and spleens were weighed and collected in Hank's Buffered Salt Solution (HBSS). For immunotoxicological analysis, spleens and sera (isolated from peripheral blood) were shipped overnight on ice, on the day of collection, to the Health Effects Laboratory Division of NIOSH (NIOSH/HELD) in Morgantown, WV. Studies by McCay *et al.* have demonstrated the ability to transport the tissue in this manner and successfully conduct the plaque forming cell (PFC) and natural killer (NK) assays (McCay 1995). PFC and NK responses were shown to be within historical control ranges for both assays when whole spleens had been held on ice for up to 24 hours prior to evaluation. Spleens from two sets of experiments were collected. Immunized spleens (see below) were collected for analysis of the IgM response to sheep red blood cells (SRBC) and unimmunized spleens were collected for analysis of splenocyte immune phenotyping and the NK assay.

IgM Plaque Forming Cell (PFC) response to a T-dependent antigen

The primary IgM response to SRBC was enumerated using a modified hemolytic plaque assay of Jerne and Nordin (Jerne and Nordin 1963) after intravenous challenge with SRBC. On days 25 (4 week study) and 67 (10 week study) for mice and day 26 (4 week study) and day 68 (10

week study) for rats, animals were immunized with SRBC (7.5×10^7 SRBC/mouse and 2×10^8 SRBC/rat) in a 200 μ L volume by intravenous injection. The spleen plaque forming cell (PFC) assay was performed as previously described (Anderson *et al.* 2007). The results were expressed as specific activity (IgM PFC per 10^6 spleen cells) and total activity (IgM PFC per spleen). Due to a shipping error, the PFC assay was not conducted on rat spleens from the 4 week exposure study.

Serum IgM antibody titers to the T-dependent antigen, SRBC

Sera collected from the same animals evaluated for the PFC response were used to determine the serum titers of the primary IgM response to SRBC. An indirect enzyme-linked immunosorbent assay (ELISA) system, as described by Temple *et al.* (1993), was used to determine the serum titers of the primary IgM response to SRBC. The SRBC membrane preparation was applied to Immulon II microtiter plates (Dynatech Labs, Chantilly, VA). Horseradish peroxidase-conjugated anti-mouse IgM antibody (Southern Biotech, Birmingham, Alabama) was used as the detection antibody and peroxidase (2, 2'-azino-di[3-ethyl-benzthiazoline-6-sulfonic acid]) as the substrate (Sigma, St Louis, MO). Analysis was performed on a Spectromax 340PC (Molecular Devices, Sunnyvale, CA) plate reader.

Splenocyte Immune Phenotyping

Single cell suspensions of the spleens from the unimmunized animals were prepared by disrupting the spleen between the frosted ends of microscopic slides. Following lysis of red blood cells using Zap-o-globin (Beckman Coulter, France), the total number of nucleated cells was counted using an electronic cell counter (Coulter Electronics Inc., Hialeah, FL). Spleen cells were centrifuged and re-suspended (cell density of 1.5×10^6 /mL) in phosphate buffered saline (PBS) containing 1% bovine serum albumin and 0.1% sodium azide, pH 7.4. To prevent non-specific binding, the cells were incubated with a mouse Fc Block (clone 2.4G2, Pharmingen, San Diego, CA) or a rat Fc block (clone D34-485, Pharmingen, San Diego, CA) for 5 minutes. Cells were then labeled with the appropriate fluorescently conjugated monoclonal antibody (mAb) (Table 2) for visualization using flow cytometry. An isotype specific negative control was used for each antibody. Cell suspensions were incubated with labeled antibodies on ice in the dark for at least 30 min, washed, and then resuspended in 0.1 mL of a 1 μ g/mL solution of propidium iodide (PI). After 5 min incubation with PI, cells were washed, resuspended in PBS, and enumerated using a FACS Vantage Flow Cytometer (Becton Dickinson, San Jose, CA). PI-stained cells (dead cells) were eliminated from the analysis. The forward scatter threshold was set to eliminate red blood cells. The percentage of gated live cells with the corresponding cell surface marker minus the isotype control was converted to absolute numbers of cells using the measured total numbers of nucleated cells.

Natural Killer Cell Assay

An *in vitro* cytotoxicity assay using ^{51}Cr -labeled Yac-1 cells (ATCC, TIB 160, Manassas, VA) was used as described previously (Holsapple *et al.* 1988). Splenocytes (obtained as described above) were adjusted to 2×10^7 cells per mL in complete media (RPMI, 10% fetal calf serum, 50 IU penicillin and 50 μ g streptomycin). Splenocyte and Yac-1 cells were prepared in ratios of 200:1, 100:1, 50:1, 25:1, 12.5:1, 6.25:1 and replicate wells plated for each concentration. After 4-hour incubation at 37°C and 5% CO₂, 100 μ L of supernatant was transferred to tubes and counted on a gamma counter (Packard Cobra II Auto-Gamma). Maximum release was determined by lysing ^{51}Cr -labeled Yac-1 cells with 0.1% Triton X in complete media. Spontaneous release was determined by incubating Yac-1 tumor cells in complete media. The results were expressed in lytic units (LU) (Bryant *et al.* 1992) for each of the effector to target cell ratios.

Statistical Analysis

The design structure of these experiments was a completely randomized design, and the treatment structure utilized a one-way layout with animals randomly assigned to a vehicle control or test article. Comparisons of endpoints between the control group and each treatment level were carried out using a one-way ANOVA with Dunnett's test (Dunnett and Crisafio 1955). If the assumptions for parametric analysis were not met, then the nonparametric Kruskal-Wallis k-sample test was used followed by the Mann-Whitney U test for pairwise comparisons with the control. Linear trend analysis was performed to determine if 1-BP exposure had exposure concentration-related effects for the specified endpoints. Differences were considered significant if $p < 0.05$ as compared to the vehicle control.

RESULTS

Toxicity

Statistically significant but not exposure concentration-related decreases in body weights were observed following exposure of mice to all concentrations of 1-BP at 4 but not 10 weeks (Table 3). Three deaths occurred in the 500 ppm group during the first week of exposure. At 4 weeks, a decreasing trend in spleen weights was observed with a significant decrease in spleen weight observed only in mice exposed to 250 ppm 1-BP. In contrast to body weight data, a significant decrease in spleen weights was observed following exposure to 250 and 500 ppm 1-BP at 10 weeks. In rats, no significant changes in body or spleen weight were observed following exposure to any concentration of 1-BP at either time point (data not shown).

Suppression of IgM PFC response to SRBC in both mice and rats

In mice, no statistically significant changes were observed in PFC/ 10^6 cells for any 4 week exposure concentration although a decreasing trend was observed (Figure 1A). A decrease in PFC/spleen was also observed in mice exposed to 1-BP for 4 weeks with a 35% decrease ($p < 0.05$) observed at the 250 ppm dose (Figure 1B). By 10 weeks, statistically significant, as well as exposure concentration-related decreases in both PFC/spleen and PFC/ 10^6 cells were observed for all 1-BP exposure concentrations (Figure 1C and D). A dose responsive decrease in spleen cellularity was also observed by week 10 reaching statistical significance at both the 250 ppm and 500 ppm doses (Figure 2). No significant changes were observed at either time point in the mouse serum SRBC IgM levels (data not shown).

Exposure of rats to 1000 ppm 1-BP by inhalation for 10 weeks resulted in a significant suppression of PFC/spleen (68%) and PFC/ 10^6 cells (61%) (Figure 3). A decreasing trend was identified for both PFC/spleen and PFC/ 10^6 cells. No change in spleen cellularity was observed after exposure to any concentration (data not shown). Due to problems during shipping, the spleens collected from the rats exposed to 1-BP for 4 weeks were not analyzed. No statistically significant changes were observed at either time point in the rat serum when SRBC IgM levels were analyzed (data not shown).

Splenocyte Immune Phenotyping

Analysis of mouse spleens showed an exposure concentration-related decrease in number of total spleen cells and T-cells (CD3+) reaching significance at all concentrations of 1-BP for the 4 week time point. This trend was not observed at the 10 week time point (Table 4). Exposure to 1-BP for either 4 or 10 weeks did not affect the number of B-cells, macrophages, or the T-cell subsets that were analyzed. An exposure concentration-related elevation in murine NK cell (NK1.1+) numbers was observed after 10 weeks of exposure reaching statistical significance at 500 ppm 1-BP. A similar trend in increasing NK cell numbers was observed at both the 4 and 10 week time points in the rats exposed to 500 ppm 1-BP (Table 5). Comparable

to the mouse data, a significant decrease in total spleen cells was observed at the 4 week but not the 10 week time point in the rat. Analysis of rat spleens also identified a significant decrease in total T-cells after exposure to the high concentrations of 1-BP (1000 ppm) at both time points (Table 5). A significant decrease in the CD4+/CD8- T-cell subsets was observed after exposure to 1-BP (500 and 1000 ppm) for the 10 week time point. No biological alterations in B-cell (CD45/B220+) or macrophage (CD11b+) numbers were observed after exposure to 1-BP at either 4 or 10 weeks in either species.

Natural Killer Cell Activity

There was no consistent modulation of NK cell function at either time point in rats or mice (data not shown).

DISCUSSION

Results of the present investigation identify quantitative changes in spleen cellularity and impairment of cell function as indicated by suppression of the PFC IgM response to SRBC following exposure to 1-BP. These results are consistent with those from another study investigating immunotoxicity associated with oral administration of the structurally-related 2-BP in rats, in which the spleen IgM response to SRBC was significantly reduced after a 28 day oral exposure to 1000 mg/kg 2-BP (Jeong *et al.* 2002). In addition, 2-BP exposure resulted in a reduction in the total number of spleen cells, B-cells/spleen, T-cells/spleen and CD4+ and CD8+ cells/spleen.

NIOSH estimated occupational exposure to 1-BP measure over a period of 8-hours in three different manufacturing plants, ranged from 18-381 ppm (Reh, 1999; Reh, 2000; Reh, 2001). Until occupational exposure limits are set the EPA is recommending an 8-hour TWA exposure limit of 50-100 ppm (USEPA, 2000). The exposure concentrations used in the studies presented in this manuscript fall within the range of actual human exposure data. Differences in 1-BP toxicity have been demonstrated between species. For example, exposure of mice to the high dose (500 ppm) of 1-BP caused mortality in several of the animals but this was not observed when rats were exposed to 1000 ppm 1-BP. 1-BP biological half life differences and rate of metabolism are possible explanations for this phenomenon. Risk assessment will need to be conducted to determine the exact correlation between the animal and human exposure concentrations.

In the current studies, the significant morbidity and mortality observed in mice was apparent by 2 weeks although the surviving animals in this dose group appeared normal at 4 weeks and by the end of the 10 week exposure period (data not shown). Phenotypic analysis of the murine spleens also revealed an exposure concentration-related decrease in total cells and T-cells at 4 weeks with an apparent recovery by the 10 week time point. This decrease in total cells was similar in the exposed rats. One possible explanation for the observed recovery with respect to morbidity and splenocyte number by the end of the 10 week exposure period would be the ability of 1-BP to induce its own metabolism. Cytochrome P450 is the major enzyme system responsible for metabolism of 1-BP (Garner *et al.* 2006). Inhalation exposure of 1-BP has been found to elevate the cytochrome P450 isoenzyme (CYP2E1) and other hepatic related enzymes in rat microsomes suggesting that 1-BP can induce its own metabolism (Kim *et al.* 1999). Repeated exposure to 1-BP resulting in the induction of P450 enzymes may result in a greater capacity to metabolize 1-BP to CO₂ and other non-toxic metabolites. This phenomenon has been observed in other immunosuppressive chemicals including benzo(a)pyrene (Kawabata and White 1989) (Gelboin 1980). Other toxic oxidative metabolites of 1-BP may be conjugated with glutathione (GSH) and eliminated as N-acetyl-S-propylcysteine compounds (Garner *et al.* 2006; Lee *et al.* 2007). Lee *et al.* (2007) reported GSH depletion in spleen cells after oral

treatment with 1-BP and suggested that GSH conjugation may play a role in 1-BP induced immunosuppression.

The results from these studies demonstrate inhalation exposure to 1-BP suppresses the IgM response to SRBC, and impacts spleen cellularity and function. The T-cell dependent antibody response is one of the most sensitive indicators of immune integrity because it relies on an organized immune response that is dependent on the functional capacity and cooperation of numerous cell types including B-cells, T-cells and macrophages (Anderson 2006). Immunosuppression determined by the spleen IgM response to SRBC (plaque assay) was observed for both species after exposure to 1-BP however, the serum IgM response to SRBC was not decreased in either species. Although the mechanism underlying the contradictory results between the splenic PFC response and serum IgM titers to SRBC is unknown, it has been observed following exposure to other chemicals (Johnson *et al.* 2000; Temple *et al.* 1993). The differences in sensitivities may be attributed to the specific endpoints that each assay measures. The plaque assay enumerates antibody producing cells in the spleen while the ELISA method measures IgM levels in the serum which may be produced in the spleen as well as the lymph nodes and bone marrow. It is conceivable that the sensitivities of each assay can vary depending on the compound being tested. It has also been determined that the murine spleen PFC response to SRBC peaks on day four after immunization while the serum IgM response peaks between days five and six (Loveless *et al.* 2007). It should also be noted that in our studies, the spleen and serum were both collected on the same day after immunization.

The reduced number of T-cells may contribute to the observed decreases in IgM response to SRBC, especially when evaluating PFC/spleen. Although T-cell function was not specifically evaluated, exposure to 1-BP caused a significant decrease in PFC/10⁶ cells for both species at 10 weeks suggesting not only a decrease in cell number but also a decrease in immune function. These results suggest the T-cell as a possible target for 1-bromopropane induced immunotoxicity. A reduction in or impairment of T-cells would depress the ability of the immune system to mount a normal response to pathogens which could result in an increased risk of infection.

Short term whole body inhalation exposure to 1-BP, at concentrations similar to what has been measured in occupational environments, was identified to affect key elements of splenocyte cellularity and humoral immunity in both rats and mice. The suspected neurological, reproductive, and immunological effects coupled to the potential increase in usage and exposure clearly demonstrates the need for more intense studies evaluating the potential adverse human health effects caused by exposure to 1-BP.

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Figure 1. Inhalation Exposure to 1-BP Suppresses the Spleen IgM Response to SRBC in B6C3F1 Mice

The IgM response to SRBC/10⁶ splenocytes (A, C) and IgM response to SRBC/spleen (B, D) is illustrated after exposure to 1-bromopropane for 4 and 10 weeks. Values represent the mean $\pm$ SE derived from eight animals (5 animals for 500 ppm dose) in each group. *p < 0.05 vs. air control.

Figure 2. Inhalation Exposure to 1-BP Decreases Spleen Cellularity in B6C3F1 Mice

Spleen cellularity is illustrated after exposure of female B6C3F1 to 1-bromopropane for 4 (A) and 10 weeks (B). Values represent the mean $\pm$ SE derived from eight animals (5 animals for 500 ppm dose) in each group. * $p < 0.05$ vs. air control

Figure 3. Inhalation Exposure to 1-BP Suppresses the IgM Response to SRBC in Fisher 344 Rats
The IgM response to SRBC/10⁶ splenocytes (A) and IgM response to SRBC/spleen (B) is illustrated after exposure to 1-bromopropane for 10 weeks. Values represent the mean $\pm$ SE derived from eight animals in each group. * $p < 0.05$ vs. air control.

Table 1

Animal Allocation for 1-Bromopropane Studies

Study 1-BP (ppb)	Mouse														Rat			
	4 Weeks					10 Weeks					4 weeks*				10 Weeks			
	0	125	250	500	0	125	250	500	0	250	500	1000	0	250	500	1000		
Immunized (Spleen PFC assay and by ELISA)	8	8	8	5	8	8	8	5	8	8	8	8	8	8	8	8		
Unimmunized (NK cell assay and spleen phenotyping)	8	8	8	5	8	8	8	5	8	8	8	8	8	8	8	8		

* 4-week immunized rats pleens were not analyzed due to a shipping error

Table 2

Markers and Clones used for Splenocyte Immune Phenotyping

Cell Type	Mouse		Rat	
	Marker	Clone	Marker	Clone
B-cell	CD45/B220+	RA3-6B2-FITC	CD45RA+	OX33-PE
T-cell	CD3+	145-2C11-FITC	CD3+	G4.18-FITC
Natural Killer Cell	NK1.1+	PK136-PE	NK+	10/78-PE
Macrophage	Cd11b+	M1/70-PE	Cd11b+	WT.5-FITC
CD4+	CD4+	H129.19-FITC	CD4+	OX-35-FITC
Cd8+	CD8+	53-6.7-PE	CD8+	OX-8-PE

Conjugate fluorescent probes: phycoerythrin (PE) and fluorescein isothiocyanate (FITC)

Table 3

Spleen and Body Weights of B6C3F1 Mice after 1-BP Exposure

Exposure	N	4 Weeks		10 Weeks	
		Body Weights (gm)	Spleen Weight (mg)	Body Weights (gm)	Spleen Weight (mg)
Control	8	23.7 ± 0.6	134.0 ± 40 [#]	25.3 ± 0.5	135.5 ± 7.6 [#]
125 ppm	8	21.6 ± 0.4 [*]	129.0 ± 8.6	26.2 ± 0.7	125.4 ± 5.5
250 ppm	8	20.8 ± 0.3 [*]	110.9 ± 4.4 [*]	24.2 ± 0.7	113.8 ± 5.0 [*]
500 ppm	5 [^]	22.1 ± 0.3 [*]	123.4 ± 0.3	24.5 ± 0.3	114.6 ± 2.6 [*]

Values represent the mean ± SE derived from the number in each group

* p? 0.05

[#] Trend Analysis is significant at? 0.05

[^] Exposure to 500 ppm resulted in 3 deaths

Table 4
Splenocyte Immune Phenotyping in Female B6C3F1 Mice after Exposure to 1-BP

		Absolute values $\times 10^6$ (Percentage)							
Exposure	Weeks Exposed	Total Cells	CD45/B220+	CD3+	CD4+CD8-	CD4-CD8+	NK1.1+	CD11b+	
Control (8)	4	209.69 $\pm$ 7.19 [#]	70.78 $\pm$ 4.94 (36.65 $\pm$ 2.56)	42.87 $\pm$ 1.69 [#] (20.56 $\pm$ 0.96)	27.68 $\pm$ 2.28 (14.33 $\pm$ 1.18)	15.39 $\pm$ 1.68 (7.97 $\pm$ 0.87)	4.25 $\pm$ 0.33 (2.20 $\pm$ 0.17)	10.01 $\pm$ 0.35 (5.18 $\pm$ 0.18)	
125 ppm (8)	4	176.14 $\pm$ 6.27 [*]	72.16 $\pm$ 4.71 (37.36 $\pm$ 2.44)	33.74 $\pm$ 1.61 [*] (19.29 $\pm$ 1.05)	25.84 $\pm$ 1.89 (13.38 $\pm$ 0.98)	14.66 $\pm$ 1.01 (7.59 $\pm$ 0.53)	4.50 $\pm$ 0.34 (2.33 $\pm$ 0.18)	10.26 $\pm$ 0.51 (5.31 $\pm$ 0.26)	
250 ppm (8)	4	170.42 $\pm$ 2.25 [*]	66.54 $\pm$ 3.17 (34.45 $\pm$ 1.64)	30.88 $\pm$ 1.76 [*] (18.11 $\pm$ 0.97)	26.81 $\pm$ 2.08 (13.88 $\pm$ 1.08)	15.32 $\pm$ 1.34 (7.93 $\pm$ 0.69)	4.97 $\pm$ 0.44 (2.57 $\pm$ 0.23)	9.59 $\pm$ 0.47 $\pm$ (4.96 $\pm$ 0.25)	
500 ppm (5)	4	142.52 $\pm$ 4.39 [*]	64.91 $\pm$ 3.43 (33.60 $\pm$ 1.78)	28.52 $\pm$ 1.30 [*] (20.03 $\pm$ 0.80)	30.21 $\pm$ 3.68 (15.64 $\pm$ 1.90)	16.83 $\pm$ 2.17 (8.71 $\pm$ 1.12)	4.28 $\pm$ 0.55 (2.22 $\pm$ 0.29)	11.03 $\pm$ 0.82 (6.49 $\pm$ 0.32)	

		Absolute values $\times 10^6$ (Percentage)							
Exposure	Weeks Exposed	Total Cells	CD45/B220+	CD3+	CD4+CD8-	CD4-CD8+	NK1.1+	CD11b+	
Control (8)	10	188.30 $\pm$ 7.37	101.88 $\pm$ 1.23 (59.62 $\pm$ 0.72)	54.74 $\pm$ 2.65 (29.03 $\pm$ 0.60)	29.30 $\pm$ 0.75 (17.15 $\pm$ 0.44)	19.00 $\pm$ 0.37 (11.12 $\pm$ 0.22)	1.11 $\pm$ 0.12 [#] (0.65 $\pm$ 0.07)	6.99 $\pm$ 0.30 (4.09 $\pm$ 0.18)	
125 ppm (8)	10	183.92 $\pm$ 7.48	102.18 $\pm$ 0.73 (59.79 $\pm$ 0.43)	50.47 $\pm$ 2.63 (27.45 $\pm$ 0.83)	29.37 $\pm$ 0.76 (17.19 $\pm$ 0.45)	18.68 $\pm$ 0.41 (10.93 $\pm$ 0.24)	1.52 $\pm$ 0.15 (0.89 $\pm$ 0.09)	6.29 $\pm$ 0.23 (3.68 $\pm$ 0.13)	
250 ppm (8)	10	188.98 $\pm$ 17.99	98.04 $\pm$ 1.43 (57.37 $\pm$ 0.84)	53.97 $\pm$ 4.24 (28.94 $\pm$ 0.97)	31.67 $\pm$ 0.58 (18.53 $\pm$ 0.34)	19.23 $\pm$ 0.34 (11.25 $\pm$ 0.20)	1.55 $\pm$ 0.15 (0.91 $\pm$ 0.09)	7.12 $\pm$ 0.56 (4.17 $\pm$ 0.33)	
500 ppm (5)	10	215.13 $\pm$ 5.53	98.20 $\pm$ 1.49 (57.46 $\pm$ 0.87)	64.42 $\pm$ 3.06 (29.91 $\pm$ 0.98)	30.39 $\pm$ 1.52 (17.78 $\pm$ 0.89)	20.17 $\pm$ 0.46 (11.80 $\pm$ 0.27)	2.09 $\pm$ 0.13 [*] (1.22 $\pm$ 0.08 [*])	5.66 $\pm$ 0.34 (3.10 $\pm$ 0.20)	

Splenocytes were prepared from groups of animals that have been exposed to 1-BP. Values representing the mean $\pm$ SE derived from the number in each group are expressed as the percentage of total spleen cells and the absolute cell number per spleen.

* Represent p ? 0.05.

[#] Trend Analysis is significant at ? 0.05

Table 5

Splenocyte Immune Phenotyping in Female Fisher 344 Rats after Whole Body Exposure to 1-BP

Absolute Values $\times 10^6$ (Percentage)						
Total Cells	CD45/B220+	CD3+	CD4+CD8-	CD4-CD8+	NK+	CD11b+
435.79 $\pm$ 26.52 [#]	225.75 $\pm$ 2.15 (53.40 $\pm$ 0.51)	129.77 $\pm$ 7.84 (29.82 $\pm$ 0.69)	91.82 $\pm$ 2.39 (21.72 $\pm$ 0.57)	67.92 $\pm$ 1.06 (16.07 $\pm$ 0.25)	28.42 $\pm$ 0.97 (6.72 $\pm$ 0.23)	16.99 $\pm$ 0.89 (4.02 $\pm$ 0.21)
403.01 $\pm$ 16.75	228.82 $\pm$ 4.07 (54.13 $\pm$ 0.96)	118.31 $\pm$ 7.23 (29.50 $\pm$ 0.90)	91.56 $\pm$ 1.78 (21.66 $\pm$ 0.42)	72.51 $\pm$ 1.74 (17.15 $\pm$ 0.41)	31.02 $\pm$ 1.27 (7.34 $\pm$ 0.30)	16.77 $\pm$ 0.85 (3.97 $\pm$ 0.20)
390.80 $\pm$ 9.97	211.28 $\pm$ 6.29 [*] (49.98 $\pm$ 1.49 [*])	121.12 $\pm$ 5.24 (30.94 $\pm$ 0.89)	92.96 $\pm$ 2.19 (21.99 $\pm$ 0.52)	77.06 $\pm$ 2.17 [*] (18.24 $\pm$ 0.51 [*])	35.55 $\pm$ 2.66 [*] (8.41 $\pm$ 0.63 [*])	16.41 $\pm$ 1.51 (3.88 $\pm$ 0.36)
359.85 $\pm$ 9.77 [*]	227.06 $\pm$ 1.71 (53.40 $\pm$ 0.51)	97.84 $\pm$ 2.92 [*] (27.22 $\pm$ 0.58)	88.13 $\pm$ 2.47 (20.85 $\pm$ 0.59)	71.88 $\pm$ 2.44 (17.00 $\pm$ 0.58)	33.77 $\pm$ 1.76 (7.99 $\pm$ 0.42)	16.54 $\pm$ 0.77 (3.91 $\pm$ 0.18)

Total Cells	CD45/B220+	CD3+	CD4+CD8-	CD4-CD8+	NK+	CD11b+
653.20 $\pm$ 24.30 [#]	326.06 $\pm$ 3.28 (53.08 $\pm$ 0.53)	192.58 $\pm$ 6.35 [#] (29.55 $\pm$ 0.58)	132.87 $\pm$ 2.56 (21.63 $\pm$ 0.42)	115.96 $\pm$ 1.98 (18.88 $\pm$ 0.32)	56.59 $\pm$ 0.66 [#] (9.21 $\pm$ 0.11)	12.03 $\pm$ 0.99 (1.96 $\pm$ 0.16)
638.86 $\pm$ 46.17	325.82 $\pm$ 2.31 (53.04 $\pm$ 0.38)	187.18 $\pm$ 14.38 (29.25 $\pm$ 0.36)	133.13 $\pm$ 1.77 (21.67 $\pm$ 0.29)	118.79 $\pm$ 1.92 (19.34 $\pm$ 0.31)	57.96 $\pm$ 0.98 (9.43 $\pm$ 0.16)	11.56 $\pm$ 0.59 (1.88 $\pm$ 0.10)
625.94 $\pm$ 25.66	308.20 $\pm$ 4.36 [*] (50.17 $\pm$ 0.71 [*])	164.57 $\pm$ 5.02 (26.43 $\pm$ 0.77 [*])	122.22 $\pm$ 2.67 [*] (19.89 $\pm$ 0.44 [*])	116.36 $\pm$ 1.51 (18.94 $\pm$ 0.25)	65.30 $\pm$ 1.86 [*] (10.63 $\pm$ 0.30 [*])	10.24 $\pm$ 0.67 (1.67 $\pm$ 0.11)
600.00 $\pm$ 8.26	333.76 $\pm$ 5.43 (54.33 $\pm$ 0.88)	152.00 $\pm$ 4.86 [*] (25.34 $\pm$ 0.74 [*])	118.62 $\pm$ 2.32 [*] (19.31 $\pm$ 0.38 [*])	109.19 $\pm$ 2.24 (17.77 $\pm$ 0.37)	65.61 $\pm$ 2.65 [*] (10.68 $\pm$ 0.43 [*])	12.25 $\pm$ 1.07 (1.99 $\pm$ 0.17)

Splenocytes were prepared from groups of animals that have been exposed to 1-BP. Values representing the mean $\pm$ SE derived from the number in each group are expressed as the percentage of total spleen cells and the absolute cell number per spleen.

* Represent p ? 0.05.

Trend Analysis is significant at ? 0.05