

a structural analog of nPB, was associated with significant reproductive effects in both male and female workers (Kim, 1996; Park, 1997; Ichihara, 1997). In animal studies, iPB has been shown to induce estrous cycle alterations, decreases in accessory sex gland weights (e.g. seminal vesicle, prostate), reductions in sperm counts and sperm motility, and changes in sperm morphology (Yu, 1997; Ichihara, 1997; Kamijima, 1997). Results presented by Ichihara and colleagues indicated that nPB exerts some level of reproductive toxicity in rats (Ichihara *et al.*, 1998, 1999; Wang, 1999).

More recently, two studies have reported effects of nPB on the female reproductive system in rats. In the first study, female rats were dosed at 0, 200, 400, and 800 ppm for eight hours a day for 7 weeks. Tests of vaginal smears showed a significant increase in the number of irregular estrous cycles with extended diestrus⁶ in the 400 and 800 ppm dose groups, and dose dependent reduction of the number of normal antral follicles in the 400 ppm group (Yamada, 2003). In the second study, female rats were exposed to 1000 ppm nPB for 7 days per week for three weeks. The ratio of the number of estrous cycles of 6 days or longer to the total number of estrous cycles was calculated for the 1000 ppm exposure group and the control group. This ratio was two times higher in the exposed animals than controls, however, this difference was not statistically significant (Sekiguchi, 2002).

In 1999, the Brominated Solvents Consortium (BSOC), a group of several nPB manufacturers, initiated a two-generation study (WIL, 2001) designed to investigate thoroughly the reproductive toxicity of nPB, as well as to provide additional information on other toxic endpoints of concern, including liver effects, and effects on the central nervous system (CNS). In this study, groups of 25 male and female rats were exposed to nPB via whole-body inhalation. The F0, or first generation, animals were exposed to target air concentrations of 0, 100, 250, 500, or 750 parts per million (ppm) of nPB for 6 hours/day, 7 days/week for at least 70 days prior to mating. The F1, or second generation, animals were exposed to 0, 100, 250, or 500 ppm nPB (infertility in the F0 750 ppm group precluded having an F1 750 ppm group). Exposure of male animals in both generations continued throughout mating to the day prior to study termination. Exposure for female

animals in both generations continued throughout mating and gestation through gestation day 20. After birth of the pups, the females' exposure continued on lactation day 5 through the day prior to study termination.

In this study, fertility was compromised significantly at 500 ppm, and no live offspring were produced at 750 ppm. There was strong evidence of dose-response in both the parent (F0) and offspring (F1) generations for a constellation of reproductive effects in both males and females, including decreases in sperm motility and changes in sperm morphology, reduced numbers of implantation sites and changes in estrous cycles, and reduced litter size. There were slight decreases (only some of which were statistically significant) at 250 ppm, and even 100 ppm for some reproductive endpoints. Statistically significant effects were observed at 250 ppm for reduced prostate weight in F0 males and increased estrous cycle length F1 females. Sperm motility in the 250 ppm group of F1 males was slightly reduced (84.8%) compared to the control group (88.9%). The difference was statistically significant ($p < 0.05$). The study authors noted, however, that the sperm motility percentage for F1 males was slightly higher than the mean value in the WIL Research Laboratories historical control data (83.2%). Therefore, the authors did not attribute the reduction in sperm motility to exposure to nPB at 250 ppm. Male reproductive effects were consistent with those identified in the Japanese studies previously cited (Ichihara *et al.*, 1998, 1999, 2000a; Wang, 1999).

Liver effects similar to those reported in the ClinTrials (1997b) 90-day inhalation study were observed in males and females in both generations. Increases in liver weights occurred in both sexes following exposure to 500 ppm; corresponding increases in the incidence of minimal to mild hepatocellular vacuolation were observed at 250 ppm in males and 500 ppm in females. The adverse effects on the central and peripheral nervous system reported by Yu (2001) and Ichihara (1999, 2000b) occurred at higher doses than those associated with reproductive and liver effects in the two-generation study.

Carcinogenicity/Mutagenicity. Limited *in vitro* screening assays testing for mutagenicity and potential carcinogenicity have been conducted on nPB. Two studies have been performed investigating the potential mutagenicity of nPB in bacterial strains. Barber *et al.* (1981) exposed five *S. typhimurium* strains (TA98, TA100, TA1535, TA1537 and TA1538) to five different vapor

concentrations of nPB ranging from 1.1 to 20.3 $\mu\text{mol}/\text{plate}$ (135–2497 $\mu\text{g}/\text{plate}$). Exposures were performed in a closed incubation system in the presence and absence of liver S9 fraction (from Arochlor-induced rats). Increases in revertants were observed in only strains TA100 and TA1535 in both the absence and presence of S9; increases were not reported in the other strains. Elf Atochem (1994) exposed the same bacterial strains to nPB concentrations of 100 to 100,000 $\mu\text{g}/\text{plate}$ in both the absence and presence of liver S9 (from male Sprague-Dawley rats induced with Arochlor 1254). This protocol also used a closed system (closed stainless-steel vessels). The highest concentration was slightly cytotoxic; however, this assay did test up to the limit dose (5,000 $\mu\text{g}/\text{plate}$) recommended for bacterial reversion assays. Appropriate positive and negative controls were used to determine spontaneous background revertant frequency. No increases in revertants were reported in any strain or condition. Given these conflicting studies, the current data regarding mutagenicity of nPB in bacterial strains are equivocal. Unpublished studies of *in vivo* micronucleus formation (Elf Atochem 1995a) indicate that nPB is not clastogenic, and a published dominant lethal assay with NPB was negative (Saito-Suzuki *et al.* 1982).

In a cell death bioassay using cultured human liver cells (HepG2 hepatoma), the cytotoxicity of nPB was evaluated at concentrations ≤ 500 ppm (SLR 2001a). Results of the bioassay indicated that nPB was cytotoxic (measured as decreased cell viability) at the highest concentration tested (500 ppm). There were no positive responses reported at any concentration for tests that evaluated enzyme function, DNA damage, or DNA damage and repair when tested at concentrations up to 500 ppm. A closely related compound, ethyl bromide, is weakly carcinogenic in rodents (Haseman and Lockhart 1994), and iPB has been shown to induce reverse mutations in bacteria (Maeng and Yu 1997). Results from these screening assays for short-term genotoxicity do not suggest significant concerns regarding nPB's potential carcinogenicity, although more data are needed.

The National Institute of Environmental Health Sciences' National Toxicology Program (NTP) is planning to conduct carcinogenicity studies in both sexes of rats and mice, which will allow for more definitive conclusions. To date, the NTP has not initiated new experimental studies on nPB, and the data will not be available for several years.

⁶ Diestrus is a period of sexual inactivity during the estrous cycle.