Attachment 3 (8-25-93)

Risk Assessment Issue Paper for:
Derivation of a Provisional Subchronic RfC for
Di(2-ethylhexyl)phthalate (CASRN 117-81-7)

Pregnant Wistar rats (25/group) were exposed to aerosol concentrations of 0, 0.01, 0.05, and 0.3 mg/L di(2-ethylhexyl) phthalate (DEHP) (0, 10, 50, and 300 mg/m^3), 6 hours/day, on gestational days 6–15, by head/nose exposure (Merkle et al., 1988). An MMAD 50% of <1.2 μm and slope factors of 7.3, 16.8, and 5.8 were determined for 10, 50, and 300 mg/m^3 concentrations, respectively. These animals were acclimatized by sham exposure to air without DEHP during days 0–6 of gestation prior to the exposure period. At the end of the exposure period, 20 animals/group were subjected to cesarean section on gestational day 20. The remaining 5 rats/group were allowed to deliver and rear the pups until weaning (postnatal day 21).

No difference in maternal body weight gain or behavior was seen between controls and exposed groups except for a significant reduction in body weight (9%) in the dams exposed to 300 mg/m^3 DEHP at postnatal day 21 compared to controls. This effect on body weight was not concentration-dependent. Furthermore, the food consumption was not measured. Macroscopic examination revealed no treatment-related toxic effects in exposed animals.

The conception rate was 90%, 95%, 85%, and 80% with increasing exposure to DEHP, but there was no significant difference among the groups. The number of corpora lutea and mean uterine weights among groups was similar. Early resorptions were reportedly seen throughout all groups, but there was no concentration-response trend. In the 50 mg/m^3 group, a statistically significant decrease in the number of mean live fetuses/dam (11.68 in the 50 mg/m^3 group vs. 12.0 in controls) and live implantations/dam (94.74% vs. 92.37%) was observed. Skeletal examination showed a low incidence of anomalies, variations, and retardations in exposed animals. The incidence of fetuses with retardations (gross and skeletal)/litter was 18.83%, 23.22%, 26.46%, and 32.63% (primarily due to renal pelvis dilatations) with increasing concentration, but it was not significant for any exposure level. The investigators considered the renal pelvic dilatations unrelated to DEHP exposure because it is common for this strain of rat and was observed at a high incidence in historical controls (no data reported). The incidence of litters with gross retardations was 16.67%, 33.33%, 31.25%, and 56.25% for the control, 10-, 50-, and 300-mg/m^3 groups, respectively; this finding was significant at the high concentration. There was a slight concentration-related increase, although not significant, in the percentage of litters with fetuses having skeletal abnormalities; 16.67%, 21.05%, 25%, and 37.5% for 0, 10, 50, and 300 mg/m^3 DEHP, respectively. The litters with skeletal retardations were not significantly different from controls. A NOAEL of 10 and a LOAEL of 50 mg/m^3 DEHP was determined based on developmental effects, specifically increased incidence of litters with gross retardations.

Although the effect was not significant in the 10- and 30-mg/m^3 groups, the incidence was higher than the controls and showed an increasing trend.

Physical development was assessed in the pups who were raised until postnatal day 21. Survival rate, viability and lactation indices, righting test (postnatal day 6), gripping reflex (day 13), pupillar reflex (day 20), hearing test (day 21), eye/ear auricle, incisivi, fur development, and body weight gain were evaluated. Exposed animals did not show alterations for any of these parameters. Therefore, DEHP did not affect the development of the offspring (postexposure and lactation period) of the exposed rats.

Male Wistar rats (27/group) were exposed in a head-nose inhalation system to 0, 0.01, 0.05, or 1.0 mg/L DEHP (0, 10, 50, and 100 mg/m^3), 6 hours/day, 5 days/week, for 4 weeks (Klimisch et al., 1991). The particle size of the aerosols had a MMAD of ≤ 1.2 μm. Another group (15/sex/group) was exposed and observed for a longer period to evaluate reversibility of effects. Some of the exposed males were mated with untreated female rats 2 and 6 weeks after the end of exposure. No clinical effects were observed in any group. A significant increase in the relative lung weight, accompanied by foam cell proliferation and thickening of alveolar septi, was observed in the two high-concentration males groups compared to the control group. Relative liver weights were increased in both sexes, but there was no corresponding histopathologic changes. Effects were reversible. No reproductive effects were observed. A NOAEL of 50 mg/m^3 and a LOAEL of 100 mg/m^3 was determined for increased lung weight and changes to the alveoli septum. The study is limited because it is reported in an abstract and details are lacking.

Male ICR mice (20/group) were exposed to air saturated with unspecified amounts of DEHP vapors at room temperature, 2 hours/day, 3 days/week, for 4–16 weeks (Lawrence et al., 1975). Mice (5/group) were sacrificed at the end of 4, 8, 12, and 16 weeks. The lungs and several other tissues were examined for histopathologic changes. There were no consistent lung abnormalities attributed to the DEHP exposure. The investigators reported that preliminary work in the laboratory had suggested a toxic effect on the lungs of mice exposed to phthalate vapors; however, this study failed to confirm the preliminary findings. No other details were reported regarding this study.

Pregnant Fischer 344 rats (23–26/group) received 0%, 0.25%, 0.5% or 1% DEHP (equivalent to 0, 164, 313 or 573 mg/kg-day) in the diet during gestational days 0–20 (Price et al., 1986). Doses were based on the Wolkowski-Tyl et al. (1983a) study on Fischer 344 rats. Maternal food consumption and weight gain during the treatment period were reduced in a dose-related manner; decreased food consumption was significant in the 0.5%- and 1%-DEHP exposed groups and reduced weight gain was significant in the 1%-dosed group. There were no statistically significant DEHP-related effects observed for the percent of fertile matings, live litters, and viable litters. A significant increase in postimplantation mortality (7.8%, 8.57%, 21.4%, 19.52% with increasing doses) occurred only in the intermediate dose. Dose-related decreases in average litter size (9.47, 9.3, 8.18, and 8 per litter, respectively) and average pup weight per litter (4.91, 4.86, 4.75, and 4.52 g, respectively) occurred; the effects were significant only in the high-dose group. No major incidence of

malformations in the fetuses of exposed animals were evident although significance was not reported. A NOAEL of 0.5% DEHP (313 mg/kg-day) for reproductive effects was determined based on decreased average litter size and pup weight per litter.

Tomita et al. (1982) and Yagi et al. (1980) conducted a study in which pregnant mice (3–8 females/group) were administered a single oral dose of 0.05, 0.1, and 1 mL/kg DEHP (99% purity) on gestational day 6, 7, 8, 9 or 10. Maternal and reproductive parameters were evaluated. The animals receiving 10 mL/kg DEHP on gestational day 7 or 8 had decreased body weight, although significance was not reported. Exposure on day 9 or 10 did not affect body weights. The average body weight of fetuses was significantly reduced in all dose levels and for all gestational days of exposure except for the 10-mL/kg DEHP group exposed on gestational day 9. Resorption occurred in exposed animals depending on the dose and day of exposure. However, no statistical analyses were given. High incidences of gross and skeletal abnormalities were observed for exposures on gestational day 7 or 8. Exposure to 2.5 mL/kg DEHP on day 7 caused 80% gross and 60% skeletal anomalies and exposure to 7.5 mL/kg on day 8 caused 65.5% gross and 82.8% skeletal anamolies, respectively. For day 9 or 10, gross abnormalities were 20% and 0%, respectively, with exposure to 10 mL/kg DEHP. No statistical analyses were conducted for these values. Common malformations reported were exencephaly, open eyelid, club foot and bent or no tail, and abnormal thoracic lumbar, sacral and caudal vertebrae.

In a study by Tyl et al. (1988) (unpublished report by Wolkowski-Tyl et al., 1983b), pregnant CD-1 mice were exposed to 0, 0.025%, 0.05%, 0.1%, and 0.15% DEHP (0, 250, 500, 1,000, and 1,500 ppm) in the diet during gestational days 0-17. Treatment-related clinical signs included piloerection, lethargy, and rough coat in the 0.05%-, 0.1%-, and 0.15%-DEHP groups. Food consumption was significantly higher in the 0.15% group relative to controls. A significant dose-related decrease in maternal body weight was measured on gestational days 12, 16, and 17, with significantly lower weights in the two highest dose levels compared to the controls. Maternal weight gain was also significantly lower with these dose levels. A significant dose-related increase in relative liver weight was exhibited with significance in the 0.1%- and 0.15%-exposed groups.

The percentages of resorption, non-live and affected implants/litter were increased, in a dose-dependent manner, with values significant for the 0.1%- and 0.15%-exposed mice (Tyl et al., 1988; Wolkowski-Tyl et al., 1983b). Decreased fetal body weights/litter was dose-related; values were significant for the high-dose group relative to controls. A significant dose-related increase in the percentage of malformed fetuses/litter was also reported; significant for 0.05%, 0.1%, and 0.15% groups. There was an increased incidence of external malformations (e.g., exophthalmia, exencephaly), visceral malformations (e.g., malformed arteries, aorta), and skeletal defects (e.g., fused and branches ribs), however, statistical analyses were not conducted. It was concluded that DEHP produced maternal toxicity and fetotoxicity in CD-1 mice exposed to 0.1% and 0.15% diets during gestation. Teratogenicity was observed in animals at these doses, as well as with 0.05%-exposed animals. Therefore, a NOAEL of 0.025% DEHP for teratogenicity and a NOAEL of 0.05% DEHP for maternal and reproductive toxicity were determined. The study was limited

because only the liver weight was measured and histopathological examinations were not conducted in the dams.

Shiota and Nishimura (1982) evaluated pregnant ICR mice given a diet containing 0.05%, 0.1%, 0.2%, 0.4%, and 1 wt-% DEHP throughout gestation. Maternal weight gain decreased and percentage of fetal resorptions increased significantly for the 0.2%-, 0.4%-, and 1%-DEHP groups compared to the controls. All implanted ova died early in the rats exposed to 0.4% and 1% DEHP. Therefore, no fetuses were available for examination. A significant decrease in fetal weight and an increase in fetal malformations were reported for the 0.2%-DEHP exposed mice. These effects were not dose-related. A NOAEL of 0.1% DEHP for maternal, reproductive, and teratogenic effects was determined in this study.

DERIVATION OF A PROVISIONAL SUBCHRONIC RfC

A NOAEL of 10 mg/m^3 was determined for developmental toxicity (increased incidence of litters with gross retardations) from an inhalation rat study by Merkle et al. (1988). Therefore, this study was used to derive the provisional subchronic RfC. Calculation of the human equivalent concentration for the NOAEL is as follows:

$NOAEL_{HEC}$ = NOAEL x RDDR(ER)

where: the $NOAEL_{HEC}$ was calculated for a particle:extrarespiratory effect and

RDDR(ER) = 0.0089 x 70/0.297 [$(BW)_H/(BW)_A$, female Wistar rats] for MMAD = 1.0 μm and sigma g = 2.4, based on dosimetric modeling as described in U.S. EPA (1989),

RDDR(ER) = 2.1.

$NOAEL_{HEC}$ = 10 mg/m^3 x 2.1 = 21 mg/m^3

Subchronic RfC = $NOAEL_{HEC}$/(UF x MF)
= 21 mg/m^3 / (100 x 1)
= 2E-1 mg/m^3

An uncertainty factor of 100 reflects 3 to extrapolate from rats to humans, 10 to protect sensitive human subpopulations, and 3 for a deficient database. The resulting provisional subchronic RfC is 2E-1 mg/m^3.

The study is given a medium confidence rating because the sample size was adequate and statistical significance was reported.

Although this specific end point was not observed in any other studies, this finding was corroborated by oral mice reproductive studies that reported teratogenic effects, primarily skeletal malformations. The database was given low confidence because there were no human toxicity studies and limited chronic and subchronic inhalation animal studies. A low confidence in the RfC follows.

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